Mechanisms determining the coexistence of open- and closed-canopy biomes

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A patch of forest surrounded by fynbos in the Jonkershoek Valley, South Africa.

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

D.T Clarkson

Declaration

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

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Chapter 2: Power, S.C., Verboom, G.A., Bond, W.J. & Cramer, M.D. (2017) Environmental correlates of biome-level floristic turnover in South Africa. *Journal of Biogeography*, **44**, 1745-1757.

Simon C Power

Abstract

Open- (e.g. grassland, savanna, shrubland) and closed-canopy (e.g. forest) biomes frequently coexist in the same landscape, where open environments tend to be fire-prone with higher light, but lower nutrient and water availability than closed environments. Environmental heterogeneity could select for divergent floristic assemblages and adaptive traits, from which emergent differences in resource availability and fire incidence contribute to excluding species from the alternate habitat. In this thesis, I investigated whether the coexistence of open–closed canopy biomes, such as forest and fynbos in the Cape Floristic Region, is contingent on environmental heterogeneity coupled with contrasting species traits. Given the heterogeneity in multiple environmental properties between open- and closed-canopy biomes, I hypothesized that boundaries between open- and closed-canopy biomes will display greater floristic turnover compared to boundaries between structurally similar biomes (e.g. open- and open-canopy biomes). To explore this, genus- and family-level turnover were correlated with climate, fire, leaf area index (LAI: proxy for understorey light) and soil properties across biome boundaries in South Africa. Both genus- and family-level turnovers were highest across open–closed boundaries and most strongly predicted by increased differences in LAI, suggesting that contrasting light regimes provide significant adaptive challenges for plants. The potential effect of contrasting light regimes is highlighted by the absence of open-canopy species from forest understoreys, where low, dynamic light could limit the ability of plants to acquire sufficient carbon. This apparent shade intolerance led to the hypothesis that open-canopy species lack the traits to maintain a positive carbon balance under low and dynamic light. To test this, leaf traits and photosynthetic response to continuous or dynamic light were compared between forest and fynbos species grown under three light treatments. Fynbos species experienced high mortality under shade treatments, produced leaves that were thicker, up to 1000 times smaller, had lower photosynthetic rates (0.8 versus 3.4μmol m^{-2} s^{-1}) under continuous low light (400 μmol m^{-2} s^{-1}) and lower light-use efficiency during dynamic light sequences than forest species. These differences imply that shade intolerance in fynbos species is associated with traits that are inefficient at harvesting light and require relatively continuous high intensity light for carbon assimilation. Moreover, these inefficiencies would make it difficult to support the carbon intensive traits (e.g. cluster roots, lignotubers, sclerophyllous leaves) that facilitate fire survival and nutrient acquisition/conservation in open habitats. In contrast, forest species are able to colonize open habitats during the long-term absence of fire, implying that they are able to tolerate high light and low nutrient conditions. Given that plants frequently cope with contrasting conditions through the expression of phenotypic plasticity, it was hypothesized that closed-canopy species possess greater plasticity than open-canopy species. To assess this, the response of leaf traits and foliar nutrition to changes in LAI and soil nutrition were compared between forest and fynbos species in the field. Leaf size and specific

leaf area in forest species correlated positively with LAI and soil nutrition, whereas fynbos species response was weak, suggesting that forest species are more plastic. This plasticity may be realised by the variable light conditions forest species experience through their canopy and the occupation of higher nutrient soils, which alleviate belowground constraints. By comparison, the occupation of low nutrient soils by fynbos may inhibit plasticity given the selection of inflexible, conservative leaves. Consequently, I propose that the coexistence of open- and closed-canopy biomes arises from the steep turnover in selective regimes, which together with the contrasting adaptive traits and degrees of phenotypic plasticity they require, act together to competitively exclude species from the alternate habitat.

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Chapter 1

General Introduction

A patchwork of vegetation complexes (e.g. desert, forest, grassland, savanna) known as biomes, cover the Earth's terrestrial surface. Biomes are historically defined by their dominant growth-forms (e.g. grasses, shrubs, trees) and the climate regimes across which they are distributed because of the importance of precipitation and temperature in determining plant growth (Schimper, 1903; Holdridge, 1947; Whittaker, 1975). For example, tall, closed-canopy tropical forests tend to occur where rainfall is aseasonal and exceeds 2000 mm, whereas neighbouring savannas consisting of a continuous grass layer and sparsely distributed trees tend to occur below this threshold, where rainfall is seasonal (Whittaker, 1975; Woodward *et al*., 2004). While the broad distribution of these two biomes may correlate with rainfall gradients, in landscapes where they co-occur both receive the same rainfall (Staver *et al*., 2011). This lack of fine-scale heterogeneity raises the question, what environmental factors other than climate may support the coexistence of contrasting biomes within the same landscape?

Recent studies investigating the distributions of biomes have emphasized the link between biome occurrence and the variation in edaphic properties (e.g. Lloyd *et al*., 2015; Veenendal *et al*., 2015) and the incidence of fire (e.g. Bond *et al*., 2005). Turnover in edaphic properties is evident across multiple biome boundaries, including between the sclerophyllous (e.g. fynbos) and xerophytic (e.g. succulent karoo) shrubland biomes in South Africa. Here, the sclerophylls occupy sandstone-derived soils, which have a lower nutrient availability and higher water holding capacity compared to the shale-derived soils occupied by the xerophytes (Lechemere-Oertel & Cowling, 2001). However, the boundary between sclerophyllous shrublands and other biomes across the globe such as forest is commonly associated with a difference in the incidence of fire, given its occurrence in shrublands but not forests (Manders, 1990a; Enright *et al*., 2001; Wood & Bowman, 2012). Differences in the incidence of fire between biomes may be a consequence of topographical features such as ravines and gullies, which can act as barriers to the spread of fire and provide refuge for fire sensitive species (Geldenhuys, 1994, Bowman, 2000; Wood *et al*., 2011). Furthermore, topographic variability can result in the accumulation of greater soil moisture availability along drainage lines compared to adjacent areas (Western *et al*., 1999), which could support more water demanding species. Thus, imposed variations in topography can affect biome coexistence through its influence on the distribution of fire and hydrological niches.

Patterns of environmental heterogeneity may not only be driven by imposed factors but also emerge from the vegetation, through a process of 'niche construction' (i.e. active modification of an environment, *sensu* Odling-Smee *et al*., 1996). Emergent heterogeneities are most notable across structurally divergent boundaries such as forest and maquis, a sclerophyllous shrubland, in New Caledonia. Both biomes occur on serpentine soils, yet the soils they inhabit differ in nutrient availability (Enright *et al*., 2001). The higher nutrient availability in forest soils may result from the high tree cover, which promotes greater litterfall and nutrient accumulation (Belsky *et al*., 1989; Paiva *et al*., 2015). In contrast, the occurrence of fire in maquis may act to reduce nutrient availability through pyro-mineralization (Stock & Lewis, 1986; Neary *et al*., 1999). Moreover, the presence of fire in maquis may emerge from the vegetation. Species in fire-prone vegetation types such as grasslands, savannas and sclerophyllous shrublands actively encourage fire through the retention of dead branches and leaves that contain higher concentrations of secondary compounds compared to forest trees, which retard fire (van Wilgen *et al*., 1990; Schwilk, 2003; Bowman *et al*., 2014). While differences in flammability may affect soil nutrient availability, its combination with divergent vegetation structure can also generate large differences in light availability. Fire in low-stature pyrophytic vegetation acts to keep the vegetation open and exposed to saturating light (Warman & Moles, 2009; Lehmann *et al*., 2011). By comparison, light availability in the understorey of tall pyrophobic forests, with their dense canopy cover, tends to be between 70 and 95% lower than in open habitats, and also temporally dynamic both in the long- (e.g. seasonal) and short-term (e.g. daily/hourly variations) (Manders, 1990a; Chazdon & Pearcy, 1991; Enright *et al*., 2001; Hoffmann *et al*., 2009). Moreover, persistence in low light (i.e. shade tolerance), as opposed to light saturated environments, is dependent on the maintenance of a positive carbon balance (Givnish, 1988; Walters & Reich, 2000; Valladares & Niinemets, 2008). While a difference in light availability may not occur across all biome boundaries, the environmental heterogeneity that occurs between any coexisting biomes, whether it be imposed, emergent or a combination of both, is likely to present species with contrasting challenges for persistence.

Species typically respond to environmental heterogeneity through the expression of alternative ecophysiological characteristics, depending on the spatial/temporal scale and severity of variation in environmental factors. One such mechanism is 'phenotypic plasticity', referring to the expression of alternative phenotypes by a single genotype under different environmental conditions (Bradshaw, 1965; West-Eberhard, 1989; Sultan, 2000; Callaway *et al*., 2003). For example, in soil columns where there are variable patches of nutrient availability, roots frequently proliferate in nutrient-rich patches and increase their uptake capacity compared to roots in adjacent oligotrophic patches (Hodge, 2004). Similarly, in environments where light is spatially and temporally variable such as tropical forests,

species generally display leaves that are thinner with a lower photosynthetic capacity in shade compared to full sun (Valladares *et al*., 2000; Goulart *et al*., 2011). However, in environments where there are multiple and severe abiotic constraints, phenotypic plasticity is often limited (Lortie & Aarssen, 1996; van Kleuen & Fischer, 2005; Portsmuth & Niinemets, 2007) because traits that are advantageous for one abiotic factor, could be maladaptive for another (Valladares *et al*., 2007). Instead, under unfavourable conditions, species are expected to specialize adaptively to their environment (Lortie & Aarsen, 1996; Grime & Mackey, 2002). Evidence for such specialization is apparent in species that inhabit extremely nutrient poor soils, such as those in sclerophyllous shrublands (e.g. fynbos, kwongan) in the southern hemisphere. Here adaptive traits such as cluster roots, parasitism and carnivory are employed to acquire nutrients, particularly P, that are otherwise scarce (Lamont, 1982; Lambers *et al*., 2006). By comparison, in the xerophytic shrubland (succulent karoo) mentioned above, where nutrient availability is high but water is scarce, species rely on the production of succulent leaves and stems for water storage (Eller & Ruess, 1982; Ripley *et al*., 2013). Hence, a strong turnover in environmental regimes with extreme abiotic constraints is likely to be accompanied by an adaptive divergence in traits with low plasticity.

A potential consequence of environmental heterogeneity (imposed and emergent) and the concomitant turnover in plant adaptive traits is the formation of strong floristic patterning between environments. For instance, members of Asteraceae are present in multiple environments; however, their greatest diversity occurs within arid environments (Donoghue, 2008). Such patterns of diversity are commonly considered as evidence for phylogenetic niche conservatism (PNC), which is the tendency for closely related species to occupy similar environments (Donoghue, 2008; Wiens *et al*., 2010; Crisp & Cook, 2012). PNC is considered to arise from multiple processes that limit the movement of species into different environments, including trade-offs between adaptive traits and the requirement for co-adapted traits in certain environments (Crisp & Cooke, 2012). For example, species occupying soils with a low nutrient and moisture content tend to allocate relatively more resources to root development for nutrient and water acquisition (Hoffmann *et al*., 2004; Lambers *et al*., 2008; Poorter *et al*., 2012). This allocation bias could limit aboveground growth and the ability of such species to compete in low light environments where large leaf areas and stem elongation are required to maximize light interception (Givnish, 1988; Valladares & Niinements, 2008). In addition to traits associated with nutrient acquisition, nutrient poor environments select for traits that promote nutrient conservation such as sclerophyllous leaves (Eckstein *et al*., 1999; Wright & Westoby, 2003). Therefore, for species to move into such nutrient poor conditions, they require the production of multiple co-occurring adaptive traits, which may not readily be achieved evolutionary (Crisp & Cooke, 2012; Edwards & Donoghue, 2013; Donoghue & Edwards, 2014). It is thus unsurprising that phylogenetic turnover between habitats is

frequently and positively correlated with turnover in environmental conditions (e.g. Buckley & Jetz, 2008; Sander & Wardell-Johnson, 2011; Hardy *et al*., 2012).

In landscapes where contrasting assemblages of species coexist and form sharp boundaries under the same climatic regime, they are commonly interpreted to represent alternative stable states (ASS; Warman & Moles, 2009; Staver *et al*., 2011; Hoffmann *et al*., 2012; Tng *et al*., 2013; Dantas *et al*., 2015). According to ASS theory, a switch between states is possible if one of the states experiences a disturbance that is sufficient to shift it to the alternate state (Lewontin, 1969; Petraitis & Latham, 1999; Scheffer *et al*., 2001; Beisner *et al*., 2003). A switch between states is, however, also dependent on 'ecological resilience', which is the magnitude of disturbance a state can tolerate before switching (Holling, 1973; Peterson *et al*., 1998; Carpenter *et al*., 2001). This resilience is determined by the size of each state together with the environmental turnover between states and plant-environment feedback loops (Beisner *et al*., 2003; Scheffer & Carpenter, 2003). In states that cover a large area, contain strong feedback loops and an environment that is dramatically different to the alternative state, once-off perturbations are unlikely to cause a switch and instead stability between states shall be maintained. A frequently reported example of ASS are coexisting closed-canopy forest and open-canopy, relatively nutrient poor savanna biomes, where switches between the biomes are reported to be mediated by alterations in fire regime (Warman & Moles, 2009; Staver *et al*., 2011; Hoffmann *et al*., 2012; Murphy & Bowman, 2012; Tng *et al*., 2013). Given that savannas are fire-prone whereas forests are fire sensitive, an increase in fire frequency and/or intensity in savanna could lead to fire penetrating forest, with the resulting tree death being followed by a possible colonization by open-canopy species. Conversely, if fire is suppressed for extended periods, forest species begin to encroach into open-canopy vegetation. Fire suppression, however, does not always result in a rapid shift from open- to closed-canopy vegetation (McCoy *et al*., 1999; Bond *et al*., 2003; Higgins *et al*. 2007; Wood & Bowman, 2012), implying that savannas can resist a switch to forest possibly because low nutrient savanna soils can limit forest growth (e.g. Hoffmann *et al*., 2009).

In addition to forest and savanna, there are numerous examples of coexisting open- versus closed-canopy biomes with contrasting nutrient availability and the incidence of fire, such as forest–grassland (Gray & Bond, 2015) and various forest–sclerophyllous shrublands (Enright *et al*., 2001; Wood & Bowman, 2012) including forest–fynbos in the hyper-diverse Cape Floristic Region of South Africa. Here the coexistence of forest and fynbos is considered to represent ASS (Coetsee *et al*., 2015) with patches of tall, closed-canopy forest (commonly < 10 ha) in ravines and gullies surrounded by low-stature fynbos (Mucina & Rutherford, 2006). Coupled to the structural turnover between these biomes is a large difference in understorey light availability, with light extinction levels in forests (84-96%)

significantly higher than below fynbos (61-77%) vegetation (Manders, 1990a). Although both biomes frequently co-occur on the same soil-type (i.e. sandstone-derived soils, Mucina & Rutherford, 2006), the soils occupied by fynbos tend to have a lower nutrient availability compared to those occupied by adjacent forest (van Daalen, 1984; Manders, 1990a; Cramer, 2010; Coetsee *et al*., 2015; Cowling & Potts, 2015). The nutrient levels of the sandstone soils occupied by fynbos are so impoverished that many species rely on specialized structures such as cluster roots and symbiotic associations (e.g. N_2-fixation, mycorrhizae) to aid nutrient acquisition (Lamont, 1982; Allsopp & Stock, 1992; Lambers *et al*., 2008). Furthermore, the prevalence of small, low specific leaf area (SLA) leaves and use of serotinuous cones for seed storage in some species (e.g. Proteaceae) also aids nutrient conservation (Cramer *et al*., 2014). By comparison, forest species produce broad leaves with high SLA's (Midgley *et al*., 1995; Lamont *et al*., 2002) and rely on fleshy fruits for seed storage (Manders, 1990b; Cowling et al., 1997). In addition to the differences in resource availability, fynbos is fire-prone (*ca*. 10-15 year interval) and actively encourages fire, whereas fire is absent from forest with the structure and foliar chemistry of trees actively retarding its intrusion (van Wilgen *et al*., 1990; Kraaij & van Wilgen, 2014). Similar to the reports for other open–closed biome boundaries (e.g. Brown & Podger, 1982; McCoy *et al*., 1999; Hoffmann *et al*., 2009), in the long-term absence of fire, forest trees do begin to colonize adjacent fynbos, however, fynbos species are absent from forest understoreys (Phillips, 1931; Manders & Richardson, 1992; Cowling *et al*., 1997). While this asymmetrical colonization suggests a difference in resilience between forest and fynbos, the adaptive mechanisms, which support their coexistence, are yet to be established.

For the coexistence of open- and closed-canopy biomes such as forest–fynbos to have initially established within a landscape, some degree of historical environmental heterogeneity (e.g. climate, geology, topography) is likely to have been present. Environmental heterogeneity could select for different floristic elements, each displaying their own suite of traits, depending on the severity and number of environmental axes separating the vegetation types. Moreover, the expression of divergent traits may feedback into the environment and induce further environmental heterogeneity by altering resource availability (e.g. soil nutrients) and the incidence of fire. This could strengthen the resilience of each vegetation type and reinforce their coexistence. I thus hypothesized that the coexistence of open- and closed-canopy biomes is contingent on environmental heterogeneity that is both imposed and emerges from the floristic components of each biome. Furthermore, contrasting environments and the divergent ecophysiological traits they require act together to limit the establishment of the alternate state, thus maintaining the abrupt boundary between open- and closed-canopy biomes. To test this hypothesis I set out three research chapters that broadly explored environmental heterogeneity between

biomes and how species from each biome may respond to changes in environmental conditions, with a special focus on the forest–fynbos boundary.

Given the tendency for related species to occupy similar environments (i.e. phylogenetic niche conservatism) and that different environments require different adaptive traits, the first research chapter compared the environmental and floristic turnover between multiple biome boundaries that varied in structural turnover. With open–closed canopy boundaries displaying environmental turnover along multiple axes (light, fire, nutrients) that require contrasting adaptive traits, I hypothesized that open- versus closed-canopy biomes will show greater floristic turnover compared to structurally similar ones. To test this hypothesis, I used the flora of South Africa to correlate genus- and family-level turnover with heterogeneity in climate, light availability, soil properties and fire frequency across biome boundaries.

With the reported absence of open-canopy species from closed-canopy understoreys where low, dynamic light could constrain the ability of plants to maximize light capture and carbon assimilation, the second research chapter compared shade tolerance between open- and closed-canopy species. I hypothesized that high light and low nutrient conditions in open habitats select for traits (e.g. small, sclerophyllous leaves), which are unable to support a positive carbon balance in low light compared to broad-leaved forest species. My experimental approach included a comparison of leaf/branch traits and photosynthetic response to continuous and dynamic light between forest and fynbos species grown under three different light treatments.

Finally, building on the absence of open-canopy species from forests in contrast to the ability of forest species to colonize open habitats during the absence of fire, the third research chapter determined whether this asymmetrical colonization is linked to differences in phenotypic plasticity. Given that forest species experience contrasting light availabilities through their development (low light understorey versus high light upper canopy) coupled with their capacity to establish on soils with a lower nutrient status than their own, I hypothesized that forest species possess greater phenotypic plasticity to light and nutrients compared to open habitat species. To test this hypothesis, I investigated the plasticity in leaf traits and foliar nutrition of selected forest and fynbos species in relation to changes in light and nutrient availability across their common boundary in the field.

In addition to the three research chapters, I have included a paper under review of which I am a co-author and consider relevant to the thesis (see Appendix S5 in Supporting Information). My contributions included data collection and analysis, and writing. I cite the paper in the thesis discussion.

Chapter 2

Environmental correlates of biome-level floristic turnover in South Africa

Abstract

Aim Biomes are defined according to the growth-forms of their dominant species but also contrast in floristic composition. Biome boundaries thus represent areas of taxonomic turnover. The degree of turnover is dependent on the difference in environmental conditions between biomes and the ability of lineages to evolve adaptive traits. Open- and closed-canopy biomes differ in structure, disturbance and resource availability. I aimed to determine whether these boundaries impose greater adaptive challenges to the movement of floristic lineages than do structurally similar ones.

Location South Africa.

Methods I determined genus- and family-level dissimilarity between neighbouring biomes, and tested their correlations with differences in climate and soil properties, fire frequency and leaf area index (LAI: a measure of canopy cover used as a proxy for understorey light availability).

Results Genus- and family-level dissimilarities between biomes were positively correlated with differences in LAI. High taxonomic turnover was also associated with differences in fire frequency and soil organic carbon. LAI was strongly predicted by differences in precipitation in the driest quarter. Although environmental correlates with floristic turnover varied, the taxonomically dissimilar Fynbos boundaries were linked to differences in cation exchange capacity.

Main conclusions Biome boundaries with edaphic contrasts or emergent differences in vegetation structure caused by plant-water-fire interactions present possibly the hardest boundaries for floristic transitions. The infrequency of transitions across these boundaries is probably a consequence of environments contrasting in light and moisture availability, fire frequency and soil properties, requiring co-adaptation across multiple traits. Variation in floristic turnover between boundaries, however, implies that adaptive challenges and the ease with which they are overcome varies with biome.

Introduction

Terrestrial biomes are defined in terms of the physiognomy of their dominant species (Woodward *et al*., 2004). Where the tropical forest biome is characterized by the prevalence of tall, evergreen trees, for example, savanna is characterized by a continuous graminoid layer with a scattering of mainly deciduous trees (Whittaker, 1975; Scholes & Archer, 1997). Although biomes have been physiognomically defined, many are also floristically distinct, such that their boundaries invariably reflect high levels of taxonomic and phylogenetic turnover. For example, with the exception of Fabaceae which is common to both, Apocynaceae, Rubiaceae and Moraceae characterize the African tropical forest biome, whereas Burseraceae, Combretaceae and Poaceae are more prominent in African savanna systems (White, 1983; Gentry, 1988). This floristic turnover is likely a consequence of environmental filtering, with differences in the abiotic and biotic conditions of each biome favouring the establishment of different suites of plant lineages. Thus, the level of floristic turnover across a biome boundary likely reflects the degree to which environmental conditions differ, and the ease with which this difference can be traversed adaptively (Wiens *et al*., 2010; Crisp & Cook, 2012; Donoghue & Edwards, 2014).

Open- versus closed-canopy biome boundaries (e.g. forest–grassland, forest–savanna, forest–shrubland boundaries) constitute a striking example of contrasting adaptive environments. A prominent feature at such boundaries is the emergent difference in light availability, with a switch from light saturation in open canopies to *ca.* 30% incident light in some closed-canopy forest understories (Hoffmann *et al*., 2009). The reduced light levels are a direct consequence of greater canopy cover, a vegetation characteristic commonly expressed as leaf area index (LAI: leaf area per unit ground area). Differences in light absorption and canopy structure likely contribute towards differences in microclimate and soil moisture with open-canopy vegetation being warmer, less humid and windier (Little *et al*., 2012; Ibanez *et al*., 2013) with drier soils (Manders, 1990a) compared to neighbouring closed canopies. Furthermore, soils of open-canopy vegetation generally have lower nutrient availability (Hoffmann *et al*., 2009; Wood & Bowman, 2012; Coetsee *et al*., 2015), possibly as a consequence of interactions between plants, soil and fire (Wood & Bowman, 2012). Disturbance in the form of fire is also a general feature of open-canopy biomes (e.g. grassland, savanna), although the fire regimes in these systems may vary greatly among biomes (Archibald *et al*., 2013). Nonetheless, strong differences in resource availability and disturbance regime present sharply contrasting environmental challenges for plant species associated with open- versus closed-canopy biomes.

Contrasting patterns of resource limitation (light is more limiting in closed-canopy biomes; water and nutrients more so in open-canopy biomes), coupled with differences in disturbance regime, imply that

species of open- and closed-canopy biomes should differ in their adaptive traits and patterns of biomass allocation (Bloom *et al*., 1985). For example, increased allocation to height growth and leaf area is a common feature of shade-tolerant, closed-canopy species (Givnish, 1988; Westoby *et al*., 2002), as is the possession of large but thin, chlorophyll-rich leaves whose photosynthetic systems maximize carbon gain under low, often dynamic light conditions (Valladares & Niinemets, 2008). In contrast, open-canopy species have small, thick leaves that enhance heat dissipation and tolerance of dry, commonly oligotrophic conditions (Niinemets, 2001; Lamont *et al*., 2002; McDonald *et al*., 2003). Moreover, plants experiencing moisture and nutrient stress tend to allocate relatively more resources below than aboveground (Hoffmann *et al*., 2004; Poorter *et al*., 2012). For nutrients, this allocation may include the construction of structures such as cluster roots and/or symbiotic associations (e.g. N_2-fixation, mycorrhizae) which enhance nutrient acquisition (Lambers *et al*., 2008). In addition, many species in fire-prone open-canopy biomes rely on investment in belowground structures such as lignotubers for persistence (Bond & Midgley, 2001). Thus, for a lineage to move between open- and closed-canopy biomes, it will commonly require significant shifts in multiple adaptive traits.

Differences in the types of adaptations required for successful persistence coupled with the strong differences in selective regime, leads to the prediction that open- and closed-canopy environments will be characterized by dissimilar taxonomic lineages (Donoghue, 2008; Crisp *et al*., 2009). Thus, I expect floristic turnover between open- and closed-canopy biomes to be high compared to open–open and closed–closed biome boundaries. Moreover, assuming that the trait divergence responsible for these patterns is ancient, and that the evolutionary conservatism of these traits has been sufficient to prevent frequent transitions across open–closed canopy biome boundaries, I predict that high floristic turnover should be evident at both low and high taxonomic levels. To test my predictions, I use the flora of South Africa to correlate genus- and family-level turnover across biome boundaries (Fig. 2.1a) with changes in LAI and a range of environmental variables, including fire frequency, climate and soil properties. I also explored the importance of environmental variables in characterising each biome boundary. An explicitly phylogenetic approach to quantify levels of floristic turnover and estimate rates of transition between biomes is not possible because of the lack of a comprehensive genus-level phylogenetic hypothesis for the South African flora, and a regional bias (mostly Cape lineages) in the lineages for which species-level phylogenetic hypotheses are available. Our use of floristic turnover as a measure of differential environmental filtering is potentially confounded by the effect of differential speciation/extinction between the biomes under comparison. However, influence of the latter is likely to be most pronounced at the species-level, diminishing at high taxonomic levels as examined here.

Materials and Methods

Units of analysis

Our broad units of analysis are the nine South African biomes, namely Albany Thicket, Desert, Forest, Fynbos, Grassland, Indian Ocean Coastal Belt, Nama-Karoo, Succulent Karoo and Savanna (Mucina & Rutherford, 2006). I have, however, elected to split the Fynbos Biome into its three major vegetation types/sub-biomes (Fynbos, Renosterveld and Strandveld) because a review by Bergh *et al*. (2014) revealed these to be floristically disparate. Each South African biome, which is broadly defined by its climatic conditions and dominant plant growth-forms (Rutherford & Westfall, 1986), has been classified into vegetation units (Mucina & Rutherford, 2006) which do not necessarily correspond to floristic associations (see Bergh *et al.,* 2014), but are defined as 'a complex of plant communities ecologically and historically occupying habitat complexes at the landscape level' (Mucina & Rutherford 2006). Upper case biome names (e.g. Forest) strictly refer to those defined by Mucina and Rutherford (2006) including our split of the Fynbos biome, whereas lower case (e.g. forest) refers to the global name given to a biome.

Genus- and family-level turnover

In the absence of appropriate phylogenetic data, I used genus- and family-level composition to estimate floristic turnover between biomes. For this purpose, I made use of the lists of 'important taxa' for each vegetation unit provided by Mucina and Rutherford (2006), to quantify between-biome turnover as the pairwise floristic dissimilarities between adjoining vegetation units representing the biomes under comparison along their common boundary. 'Important taxa' represent species which have 'a frequent occurrence or are prominent in the landscape' (Mucina & Rutherford, 2006) and, although they have been assembled from diverse sources and are not comprehensive, they do describe the floristics of spatially defined units in a consistent manner (Bergh *et al.*, 2014). Using these lists to examine floristic turnover assumes a broadly consistent approach to family and genus delimitation across biomes since any bias, which systematically increases the number of genera or families that are endemic to a particular biome will tend to exaggerate the floristic distinctness of the latter. To some extent the possibility of such bias is controlled for by our analysis of patterns at two different taxonomic levels. In addition, I note that the 'important taxa' lists used here show a tendency to capture a broad phylogenetic spread of taxa at the expense of closely-related elements.

Using the 'important taxa' lists, genus- (GD) and family-level (FD) dissimilarity between neighbouring vegetation units representing different biomes was quantified using Jaccard indices as

determined using 'vegan' 2.3-5 (Oksanen *et al*., 2016) in R 3.2.3 (R development core team 2015). Although, the Jaccard index is considered to be sensitive to differences in richness (Koleff *et al*., 2003), I found its relationship with differences in genus-/family-level richness to be weak (Genus-level: R^2 = 0.01; Family-level: R^2 = 0.17). Prior to the determination of FD, the list of families was updated according to Angiosperm Phylogeny Group III system using the 'apgFamilies' function from 'taxize' 0.7.9 in R. To graphically represent the differences in GD and FD between and within biomes, a principal co-ordinates analysis using the Jaccard distances was conducted. Furthermore, a pairwise permutational analysis of variance (PERMANOVA), as implemented in the 'RVAideMemoire' 0.9-55 (Hervé, 2016) in R was used to assess the significance of taxonomic turnover between biomes. Finally, the times of divergence between families sampled in this study were determined by using the R package 'ape' 3.4 (Paradis *et al*., 2004) to extract a cophenetic matrix from the dated phylogenetic tree of Qian and Zhang (2014), pruned to contain only the sampled families. The Qian and Zhang (2014) family-level tree is an expansion of the Zanne *et al*. (2014) species-level tree with equivalent dating but with the addition of six families.

LAI and environmental variables

To assess the significance of LAI, fire frequency, climate and soil properties (Table 2.1) as correlates of floristic turnover across biome boundaries, I quantified between-biome differences in each variable as the pairwise contrast between neighbouring vegetation units representing different biomes. Given the size and geographical scope of the analysis, a geographic information system (GIS) approach was employed to quantify these variables for each vegetation unit. The relevant GIS layers were point-sampled by placing a grid of points spaced 1 km apart across the whole of South Africa. Points that fell within a vegetation unit were then averaged and the absolute difference between neighbouring units representing different biomes quantified to determine 'contrast' values. I excluded transformed areas by masking them out using a land transformation layer. The layer was derived from the multi-class Land Cover 2013-14 map for South Africa (http://egis.environment.gov.za, accessed 16 February 2016), a product originating form LANDSAT 8 images. Pixels from the Land Cover layer were reclassified as either natural (e.g. forest, grasslands, shrublands) or not (e.g. plantations, cultivated land, settlements, mining). Only points falling in natural areas and not water bodies or vegetation unit borders were retained for averaging.

Table 2.1 List of environmental variables used, with sampling periods as follows: climate variables 1950-2005, mean annual fire frequency 2000-2014, LAI 2005-2015. The resolution of the source layers was: climate and soil variables *ca.* 1 km, mean annual fire frequency and LAI 500 m.

Environmental Predictor	Abbreviation (Unit)	Source
Mean Annual Precipitation	MAP (mm)	Hijmans *et al*. (2005), www.worldclim.org
Mean Annual Temperature	MAT (°C)	
Mean Temperature Driest Quarter	(°C)	
Precipitation Driest Quarter	(mm)	
Precipitation Wettest Quarter / MAP		
Clay Fraction	Clay (%, w/w)	Hengl *et al*. (2014) http:// http://soilgrids.org/
Organic Carbon Content	Organic carbon (%, w/w)	
Cation Exchange Capacity	CEC (cmol kg^{-1})	
pH	pH	
Mean Annual Fire Frequency	(year-1)	MODIS Burned Area Product 5.1 (MCD45), http://modis-fire.umd.edu
Leaf Area Index	LAI (m^2 m^{-2})	MODIS Leaf Area Index/FPAR global 500 m product (MOD15A2H), https://lpdaac.usgs.gov/

Nine climate and soil properties were included in the analysis (Table 2.1) with their selection based on data availability and what I considered to be biologically relevant for plants in South Africa. These properties were sampled directly from their source GIS layers. Leaf area index was determined by sampling eight-day composite images from the MODIS C6 LAI/FPAR product, which were then averaged to determine month-averages. To improve data reliability, the quality control layer was used to reclassify any pixel that contained interfering factors such a clouds and/or aerosols as missing data. Fire frequency was determined by sampling monthly layers of the burn date band from the MODIS burned area product. To further improve the reliability of both the LAI and fire frequency layers, any pixel in a given month that was reported as invalid for more than half of the sample period (Table 2.1) was reclassified as missing data. Following this, monthly layers were averaged to determine the mean annual LAI or fire frequency. Prior to sampling, the resolution of the LAI and fire frequency layers were adjusted to *ca.* 1 km to match the climate and soil layers using bilinear interpolation. Resolution adjustments, monthly/annual averaging and sampling of GIS layers were conducted using the 'raster' 2.5-2 (Hijmans, 2015) package in R.

Boosted regression tree analyses

Boosted regression tree (BRT) models were used to determine whether and how differences in environmental conditions influence GD, FD and contrast in LAI (log transformed) across biome

boundaries. BRT analysis is a form of non-linear modelling that uses machine learning (De'ath, 2007; Elith *et al*., 2008). The modelling entails decision trees recursively splitting (i.e. boosting) the data into two increasingly homogenous groups to produce a bifurcating tree. The speed at which trees are built, the number of nodes they contain and the proportion of data that is selected for each tree is controlled by the learning rate, tree complexity and bag fraction. The prediction of GD, FD or contrast in LAI was optimized by adjusting these parameters so as to minimize the predictive deviance of each model (Elith *et al*., 2008). Model performance was evaluated by expressing the predictive deviance of 10-fold cross-validation as a percentage of the null deviance and examining the cross-validation correlation between observed and fitted values.

GD, FD and contrast in LAI models were optimised with a learning rate of 0.005 and bag fraction of 0.5. Tree complexity varied between models: GD = 8, FD = 8 and contrast in LAI = 5. Prior to running the models, all predictor variables were evaluated for collinearity using the 'select07' (Dormann *et al*., 2013) procedure implemented in R, with variables forming strong relationships ($r > 0.7$) being excluded. Following an initial model run, a simplification method (Elith *et al*., 2008) was implemented to further improve predictive performance. Simplified GD, FD and contrast in LAI models were each run ten times using the 'gbm' 2.1.1 (Ridgeway, 2015) and 'dismo' 1.0-15 (Hijmans *et al*., 2016) packages in R. Model results were assessed by examining the relative influence of predictors and their relationships with the three response variable: GD, FD or contrast in LAI. Relative influence quantifies the importance of a predictor in modelling the response. The influence is calculated from the number of times the predictor is selected for splitting during tree building, weighted by the square improvement the model gains from each split, and then averaged over all trees (Friedman & Meulman, 2003). Relationships between predictors and response variables were visualized using partial dependence plots. These plots show the dependence of the response variable on a specific predictor once the average effects of all other predictors have been accounted for (Friedman & Meulman, 2003).

Boundary correlates

Biome boundaries are likely to vary in the type and degree of environmental turnover that characterizes them. To investigate this variation, I performed discriminant functions analyses on boundaries which had been sampled more than 30 times, using 'MASS' 7.3-45 (Venables & Ripley, 2002) in R. Prior to analysis, variables were tested for collinearity as outlined above, log transformed where necessary and standardized by subtracting the mean and dividing by the standard deviation.

Results

Floristic turnover between biomes

Despite some overlap (Fig. 2.1b & c, see Tables S2.1 & S2.2 in Appendix S2 in Supporting Information for shared genera and families), the data reveal limited generic sharing (GD: mean ± 95% confidence interval = 0.88 ± 0.01) between neighbouring biomes across the 771 unique boundaries examined. This pattern is similarly replicated at the family-level (FD: 0.70 ± 0.01), where the median divergence time for families was 119.2 Ma (maximum divergence: all families = 352.2 Ma, angiosperms = 188.3; minimum divergence = 29.2 Ma). Floristic dissimilarity at both the genus (pairwise PERMANOVA: $P < 0.01$) and family ($P < 0.05$) levels was significant between all boundaries, with boundaries involving Forest or Fynbos showing the highest dissimilarities, and those involving Nama-Karoo or Albany Thicket the lowest (Fig. 2.2).

Correlates of floristic and LAI turnover

Across all biome boundaries, genus- and family-level turnover correlated in a similar manner to contrasts in LAI and other environmental variables (Fig. 2.2 & 2.3, Table 2.2). Biome comparisons reflecting strong LAI differences (e.g. Forest–Fynbos, Forest–Grassland) were consistently associated with high floristic turnover (Fig. 2.2), with BRT models identifying contrast in LAI as the strongest predictor of both GD and FD given its higher relative influence compared to other variables (Table 2.2). While the remotely sensed LAI values used here are a measure of effective LAI and are higher than ground measurements (see Fig. 4.1a in chapter 4), the turnover in LAI between biomes, such as Forest and Fynbos, is consistent between measurement techniques. The partial dependence plots showed that following an initial lag, GD and FD between biomes became greater with an increase in LAI differences of up to 4.3 and 4.5 $m^2 m^{-2}$, once the average effects of all other variables in the models were accounted for (Fig. 2.3a & e). For both taxonomic levels, contrast in mean annual fire frequency was the next strongest predictor (Table 2.2). Small increases in the contrast in fire frequency (GD and FD: 0 to 2 fires per 50 years) were correlated with sharp increases in floristic turnover, although any further changes in fire frequency had little effect (Fig. 2.3b & f). Amongst the remaining variables, the combined influence of contrasts in soil properties on GD (Table 2.2: 35.6%) was greater than climatic contrasts (16.0%), although the opposite was recorded for FD (Table 2.2: soil variables = 28.3%; climatic variables = 31.1%). Nonetheless, both GD and FD were positively correlated with differences in soil organic carbon of up to 4.8 and 10.3%, respectively (Fig. 2.3c & g). Furthermore, higher GD was associated with increased differences in clay, however, when differences exceeded 7.9%, GD

declined (Fig. 2.3d). Similarly, when differences in mean temperature in the driest quarter increased over the range of 1.2 to 9.8 °C, FD generally declined (Fig. 2.3h).

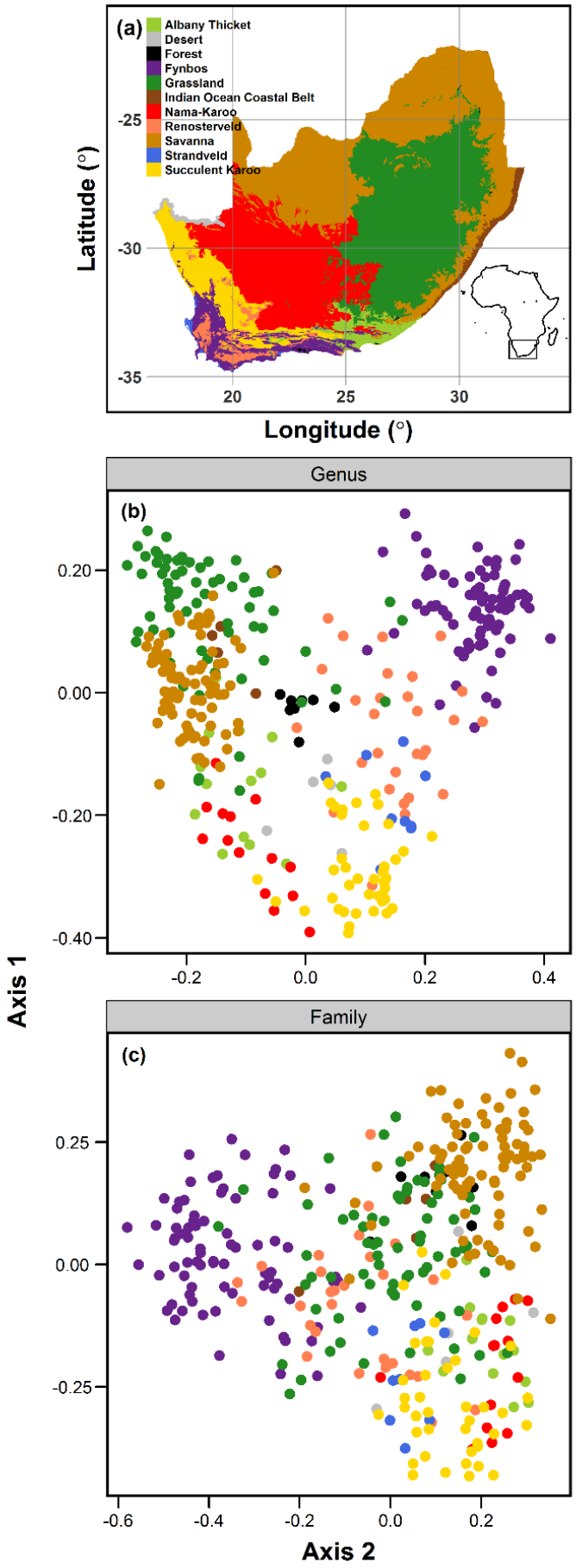

Figure 2.1 (a) Biomes of South Africa, modified from Mucina and Rutherford (2006). (b) and (c) Principal coordinates analysis showing the variation in genus- and family-level dissimilarities between biomes based on Jaccard distances determined using 'stats' and 'vegan' 2.3-5 (Oksanen, 2016) packages in R. Each point represents a vegetation unit within a biome with colours corresponding to map legend.

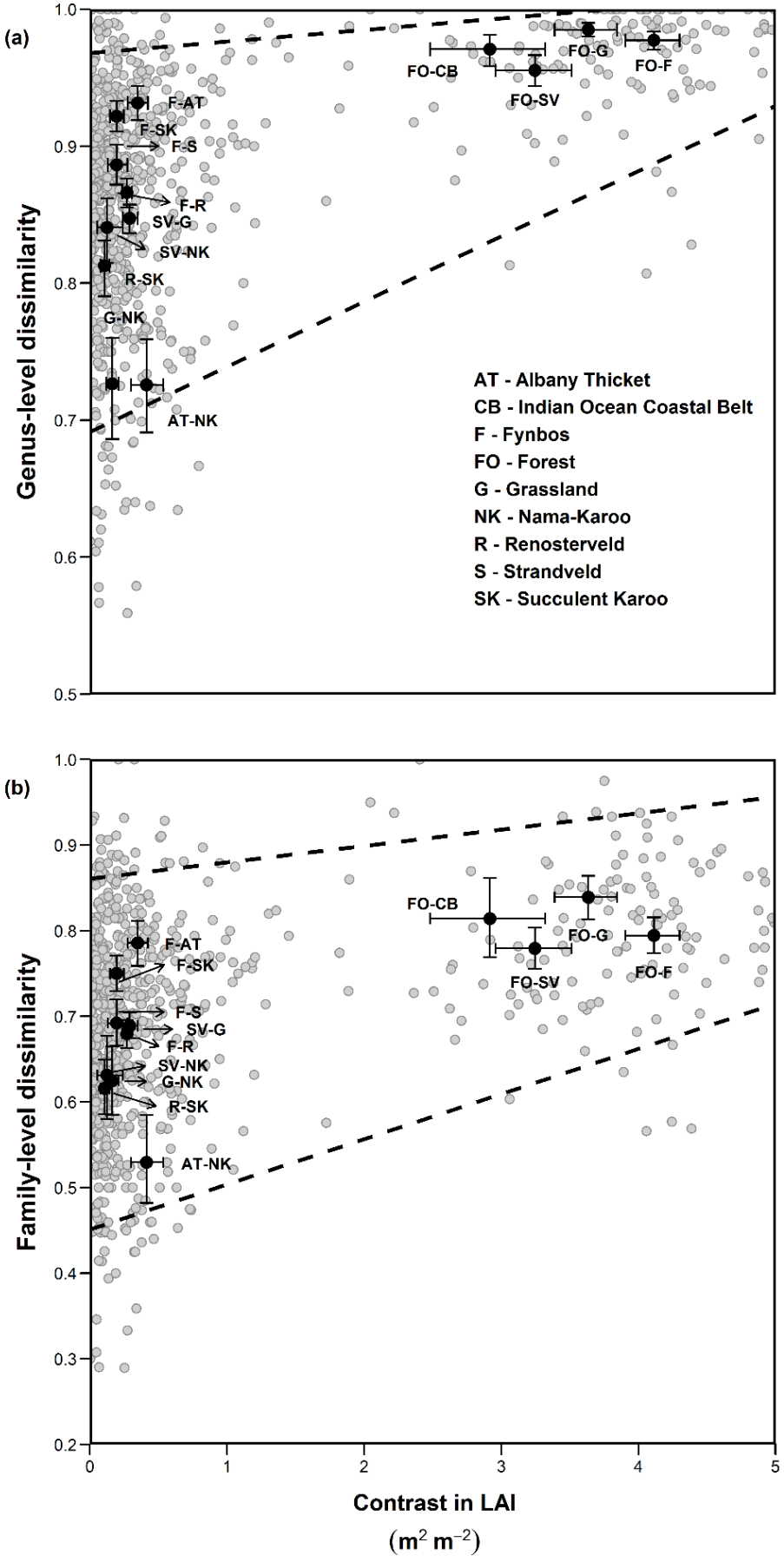

Figure 2.2 Changes in (a) genus- and (b) family-level turnover as a function of differences in LAI across all boundaries between South African biomes. Grey circles indicate individual boundaries; while black indicate the mean ± 95% confidence interval for selected boundaries. Broken lines represent the significant ($P < 0.001$) 5th and 95th linear quantiles, calculated using 'quantreg' 5.21 (Koenker, 2016) in R.

Table 2.2 The relative influence of variables in predicting turnover in floristics and LAI across biome boundaries in South Africa. Values are the mean ± 95% confidence interval of 10 runs of each model. Where values are missing, variables were dropped during model simplification (see Elith *et al.* 2008) or excluded from the start. CEC was excluded from all models given its collinearity with clay ($r > 0.7$). CV deviance explained and correlation provide an evaluation of each models performance.

Contrast	**Relative Influence (%)**		
	Genus-level dissimilarity	**Family-level dissimilarity**	**Contrast in LAI**
Leaf Area Index	32.6 ± 0.3	21.8 ± 0.2	-
Mean Annual Fire Frequency	15.8 ± 0.1	18.8 ± 0.1	13.4 ± 0.0
Mean Annual Precipitation	-	7.4 ± 0.1	5.7 ± 0.0
Precipitation in the Driest Quarter	8.6 ± 0.1	7.5 ± 0.1	22.6 ± 0.1
Precipitation in the Wettest Quarter / Mean Annual Precipitation	-	-	7.2 ± 0.0
Mean Annual Temperature	7.4 ± 0.1	6.7 ± 0.1	9.1 ± 0.1
Mean Temperature Driest Quarter	-	9.5 ± 0.1	5.3 ± 0.0
pH	8.9 ± 0.1	8.2 ± 0.1	20.2 ± 0.1
Organic Carbon Content	15.2 ± 0.1	12.0 ± 0.1	10.6 ± 0.1
Clay Fraction	11.5 ± 0.1	8.1 ± 0.1	6.0 ± 0.0
CV Deviance Explained (%)	37.5 ± 0.5	25.3 ± 0.3	42.5 ± 0.2
CV Correlation	0.61 ± 0.0	0.51 ± 0.0	0.65 ± 0.0

Of all biome boundaries investigated, differences in dry season moisture availability and soil fertility were identified as the strongest predictors of contrasts in LAI, given the relative influence of precipitation in the driest quarter (22.6%) and soil pH (20.2%). Differences in LAI between biomes rose with increasing differences in precipitation in the driest quarter, over the range of 0 to 108 mm (Fig. 2.4a). Similarly, neighbouring biomes with large contrasts in soil properties such as pH (> 0.4) and organic carbon (between 2.4 and 11.3%) were associated with a higher contrast in LAI (Figs. 2.4b & d). The generally positive response of the contrast in LAI to increased differences in environmental predictors was also evident with fire frequency. Differences of ≤ 2.3 fires every ten years equated to larger contrasts in LAI between neighbouring biomes, although for any greater difference in fire frequency, contrasts in LAI did not show any change (Fig. 2.4c).

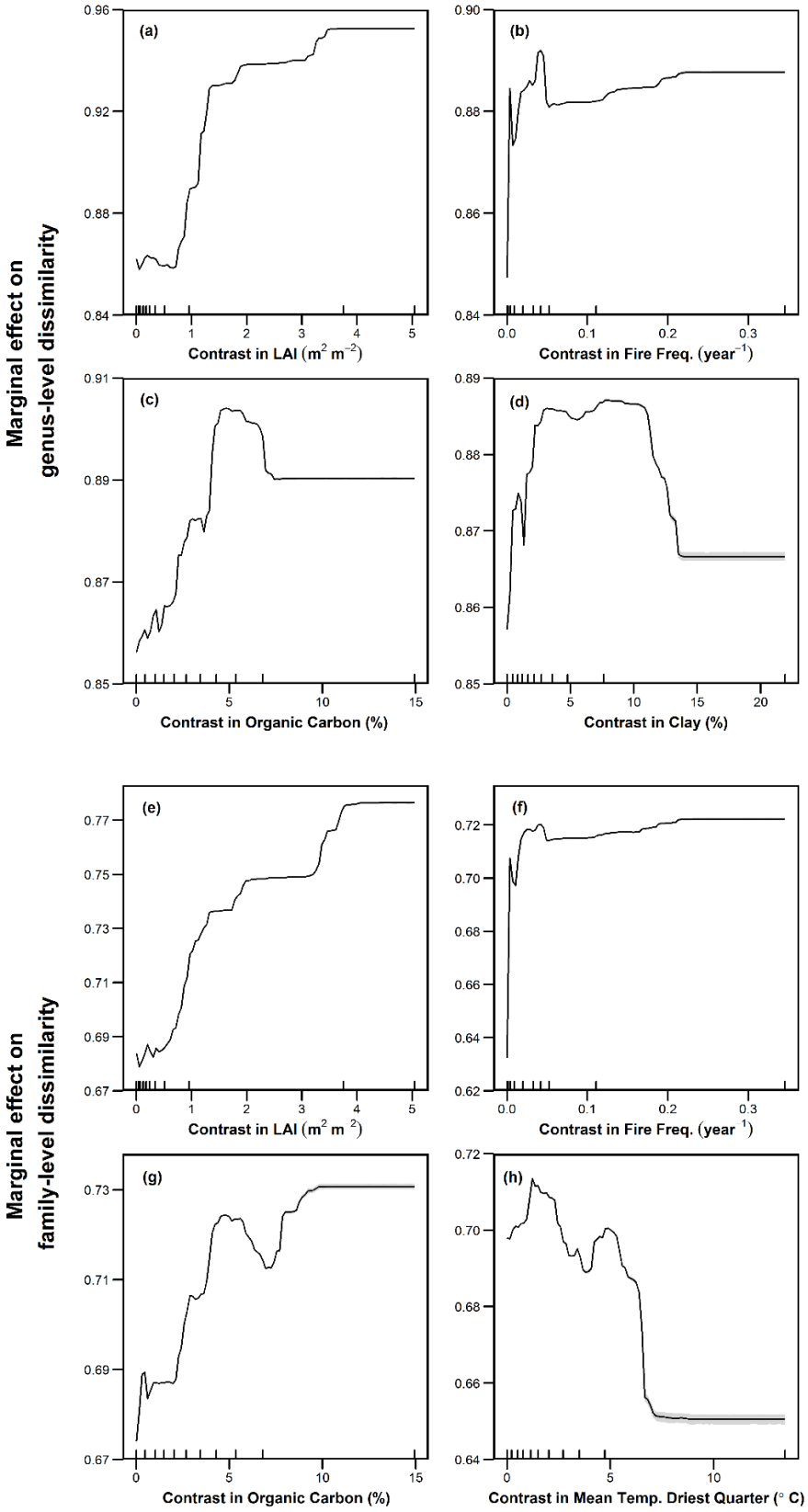

Figure 2.3 The partial dependence of genus- (a – d) and family-level (e – h) turnover on the four most influential environmental predictors between South African biomes. The marginal effect represents the modelled relationship between GD or FD and an environmental predictor once the average effects of all other predictors were accounted for. Solid line represents the mean of 10 runs of the final, simplified BRT model. Grey ribbon represents the 95% confidence interval based on 1000 bootstrap replicates. A flattening of the line indicates that an increase in the predictor has no further effect on the modelled response. Variables are plotted in descending order of relative influence (see Table 2.1). Bars on the x-axis represent deciles of the raw data. Freq. = frequency and Temp. = temperature.

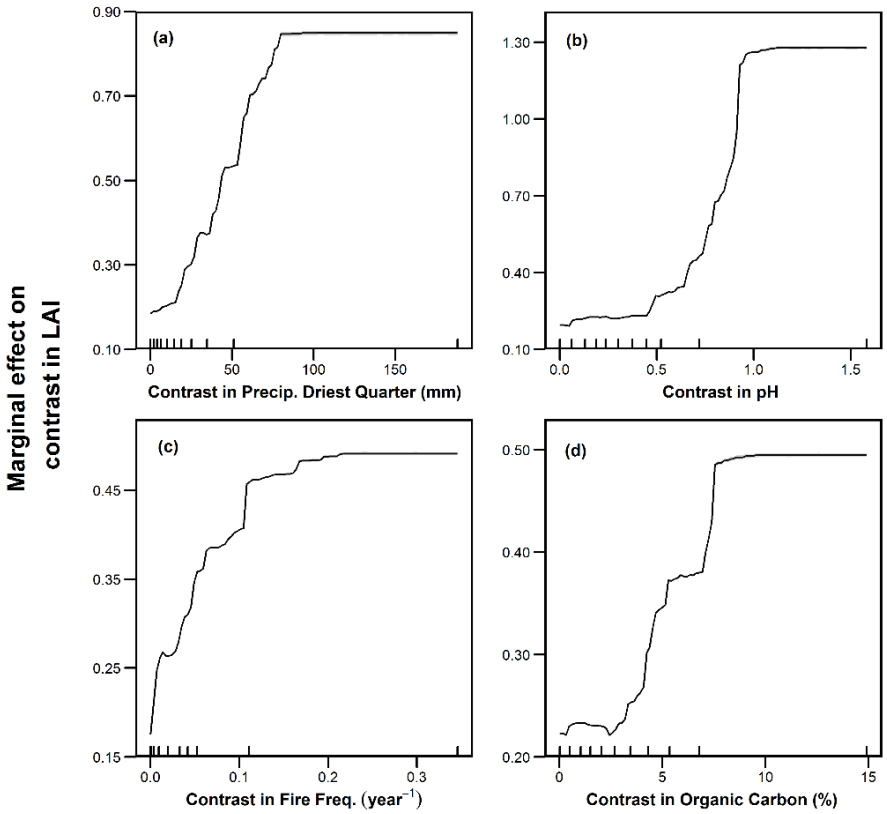

Figure 2.4 The partial dependence of turnover in LAI on the four most influential environmental predictors. The marginal effect represents the modelled relationship between contrasts in LAI and an environmental predictor once the average effects of all other predictors were accounted for. Solid line represents the mean of 10 runs of the final, simplified BRT model. Grey ribbon represents the 95% confidence interval based on 1000 bootstrap replicates. A flattening of the line indicates that an increase in the predictor has no further effect on the modelled response. Variables are plotted in descending order of relative influence (see Table 2.1). Bars on the x-axis represent deciles of the raw data. Freq. = frequency and Precip. = precipitation.

Environmental correlates of individual biome boundaries

The nine most frequently sampled boundaries (n > 30) varied considerably with regard to their most influential environmental predictors (Figs. 2.5). There were, however, some consistent patterns. All three Forest boundaries (Forest–Grassland, Forest–Fynbos and Forest–Savanna), for example, were most strongly predicted by differences in LAI (Fig. 2.5a – c). This is not unexpected given the 70-78% greater canopy cover of Forest compared to its neighbours (see Fig. S2.1a). Although the consistently strong difference in LAI across Forest boundaries is potentially attributable to the relatively higher mean annual precipitation (MAP = 908 ± 31 versus 775 ± 35 mm, mean ± 95% confidence interval) and precipitation in the driest quarter (114 ± 10 versus 70 ± 6 mm) received, and near absence of fire in Forest relative to its neighbours, I note that the importance of climatic variables and fire frequency varied considerably between these three boundaries (Fig. 2.5a – c). Fire frequency and precipitation in

the driest quarter were, however, the most important predictors of the Fynbos–Albany Thicket boundary (Fig. 2.5d), with fire-prone Fynbos receiving more precipitation during the driest quarter (mean ± 95% confidence interval: 102 ± 8 versus 75 ± 6 mm).

Soils properties, especially soil cation exchange capacity (CEC), were identified as more important correlates of Fynbos boundaries (Fig. 2.5d – g) compared to other boundaries, with Fynbos soils generally having lower CEC relative to neighbouring biomes (Strandveld is an exception; see Fig. S2.1k). Furthermore, soil properties frequently combined with other factors such as mean annual temperature (MAT, Fynbos–Succulent Karoo), LAI and mean temperature driest quarter (Fynbos–Strandveld), and fire frequency (Fynbos–Renosterveld) to best predict several Fynbos boundaries. Similarly, boundaries not involving Forest or Fynbos, such as Savanna–Grassland, were commonly influenced by multiple variables. The Savanna–Grassland boundary was best predicted by differences in LAI and MAT, with the higher elevation Grassland biome being 2°C cooler on average than Savanna (see Fig. 2.4h & S2.1f). In comparison, contrast in fire frequency was the strongest predictor of the boundary where the fire-prone Renosterveld neighboured the fire-free and relatively drier, and hotter Succulent Karoo (see Fig. 2.4i, S2.1c & f).

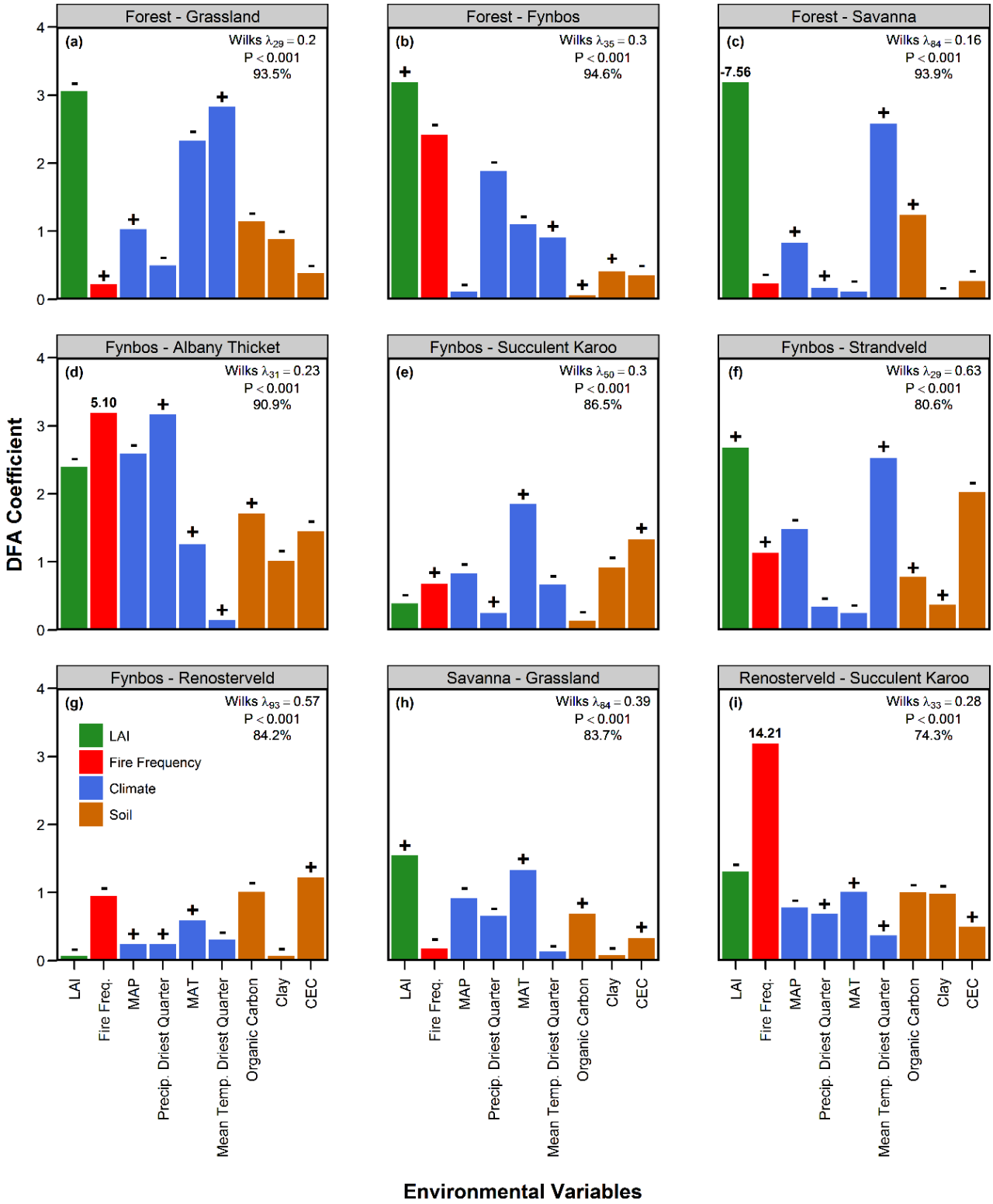

Figure 2.5 Relative importance of LAI, fire frequency and climate and soil properties in characterizing biome boundaries. Values represent the standardized coefficients from a linear discriminant functions analysis (DFA) conducted using 'MASS' 7.3-45 (Venables & Ripley, 2002) in R. Wilks lambda multivariate analysis of variance (MANOVA) was used to test significance of DFA. Predictive accuracy (%) indicates the probability of the model correctly characterizing the boundary with the given variables. ± indicates the sign of DFA coefficient. Where a coefficient value exceeds the y-axis maximum, the value is reported above the specific variable's column. Soil pH and Precipitation Wettest Quarter/MAP were excluded from the analysis given their collinearity ($r > 0.7$) with Precipitation Driest Quarter and MAP, respectively. CEC = cation exchange capacity, Freq. = frequency and Temp. = temperature.

Discussion

In addition to their climatic-physiognomic basis (Rutherford & Westfall, 1986), my analyses show that South African biomes display a high level of floristic distinctiveness, a pattern which I interpret as an expression of phylogenetic niche conservatism on a heterogeneous landscape. This conservatism is likely a consequence of functional attributes having deep historical origins given the high turnover at both genus and family levels (Fig. 2.2), and the antiquity of family-level divergences (median age: 119.2 Ma). Moreover, the generally positive relationship between floristic and environmental turnover (Fig. 2.3), implies that the environmental differences associated with the biome boundaries, present significant adaptive challenges that are not easily overcome, evolutionarily (Edwards & Donoghue, 2013). This is consistent with a growing emphasis on the influence of phylogenetic trait and niche conservatism in contributing to gradients of biodiversity (e.g. Donoghue, 2008; Crisp *et al*., 2009; Wiens *et al*., 2010; Kerkhoff *et al*., 2014). The data also shows, however, that floristic turnover varies between different boundaries (Fig. 2.2), implying variation in the adaptive challenges boundaries present. The relatively higher GD and FD of all Forest boundaries compared to the Renosterveld–Succulent Karoo boundary, for example, suggests that differences in fire frequency which characterize the latter boundary, present an easier adaptive challenge for plant genera and families than do differences in canopy cover based on LAI across Forest boundaries (Fig. 2.5a – c & i). This variation in floristic turnover highlights the importance of developing a better mechanistic understanding of the relative strength of specific environmental filters as barriers for adaptive evolution.

The consistently high floristic turnover associated with open- versus closed-canopy biome boundaries (Fig. 2.3: e.g. Forest–Grassland) identifies such boundaries as possibly one of the hardest adaptive boundaries for plants across South Africa. Moreover, the positive relationship that emergent differences in canopy cover has with floristic turnover (Fig. 2.3a & e), coupled with its strong and consistent association with Forest boundaries (Fig. 2.5a – c); imply a role for light as a potential mechanistic explanation. While high- and low-light environments are a product of differing environmental conditions and vegetation, they do impose contrasting adaptive challenges. For example, where forest trees are required to invest heavily in aboveground biomass to facilitate tall growth and enhance the competitive ability to capture light (Givnish, 1988; Hoffmann *et al*., 2004), the presence of fire and greater seasonal moisture stress in neighbouring open-canopy biomes (Fig. S2.1b & d) may favour increased belowground investment to enhance persistence and resource acquisition (Coughenour, 1985; Bond & Midgley, 2001; Hoffman *et al*., 2004). Furthermore, where low light conditions generally favour the evolution of large leaves which maximize carbon gain under low light (Valladares & Niinemets, 2008; Onstein *et al*., 2014), high light environments select for small

leaves which enhance heat dissipation and improve tolerance of low water availability (Thuiller *et al*., 2004; Yates *et al*., 2010). Tolerance of seasonal moisture availability in open-canopy biomes may also require the evolution of co-adapted stomata and xylem systems. (Brodribb *et al*., 2014). Thus, the evolutionary transition between contrasting light environments is likely constrained by the need for a shift in biomass and multiple co-adapted traits, resulting in open- and closed-canopy biomes in South Africa being floristically distinct.

Given the correlation between forest distribution and seasonal moisture stress (Schimper, 1903; Murphy & Bowman, 2012) it is not surprising that contrast in LAI was positively related to contrasts in precipitation in the driest quarter (Table 2.2, Fig. 2.4a). Moreover, this correlation might indicate that precipitation in the driest quarter has more of a direct role than light availability in determining floristic turnover. The relatively higher precipitation that Forests receive during the driest quarter compared with their neighbours (Fig. S2.1d) facilitates the establishment of tall, closed canopies and, in so doing, drives the exclusion of shade-intolerant lineages (Ratnam *et al*., 2011) such as those, which are prevalent in Fynbos (Manders & Richardson, 1992), grasslands and savannas (Sage *et al*., 1999; Hoffmann, 2000). Furthermore, the exclusion of shade-intolerant, pyrophytic growth-forms (e.g. Hennenberg *et al*., 2007), coupled with the absence of a continuous biomass layer (van Wilgen *et al*., 1990) and an unsuitable microclimate (lower temperature, higher humidity, reduced wind speed; Little *et al*., 2012; Ibanez *et al*., 2013), allows forests to actively retard the intrusion of fires from open-canopy biomes (Fig. S2.1b) through 'niche construction' (*sensu* Odling-Smee *et al*., 1996). The lower precipitation in the driest quarter experienced by open canopies (Fig. S2.1d) could further stimulate fire (Lehmann *et al*., 2011), preventing the establishment of fire sensitive forest species and thus maintaining low, open vegetation. Consequently, the turnover in floristics and LAI between open- and closed-canopy biomes likely emerges more as a consequence of interactions between vegetation structure, seasonal moisture availability and fire than individual physical site constraints.

Although floristic turnover is highest across biome boundaries characterized by strong differences in canopy cover, high turnover is also a feature of some open–open canopy biome boundaries, notably those involving Fynbos (Fig. 2.2). This result is not surprising as it has long been appreciated that Fynbos is floristically distinct from its neighbouring vegetation (e.g. Renosterveld, Succulent Karoo), a pattern traditionally attributed to the extreme nutrient impoverishment (Lechmere-Oertel & Cowling, 2001; Cowling & Potts, 2015) of the soils on which Fynbos occurs (Goldblatt, 1978; Linder, 2003; Verboom *et al*., 2014). While the coarseness of our soil data does not allow for a fair assessment of soil properties as correlates of biome boundaries, our results do indicate a role for them, particularly clay and organic carbon, in defining high floristic turnover (Fig. 2.3c, d & g). This pattern is

highlighted further by the consistent association of several Fynbos boundaries with CEC (Fig. 2.5d – g). As is the case for the adaptive transition between open- and closed-canopy biomes, the transition to the generally lower CEC Fynbos soils (Fig. S2.1k) may require the evolution of multiple co-adapted traits such as sclerophyllous leaves, high rates of nutrient reabsorption and specialized structures such as cluster roots that enhance nutrient conservation and acquisition (Lambers *et al.*, 2008; Cramer *et al*., 2014). Specialization to stressful environments may, however, constrain trait plasticity (Lortie and Aarsen, 1996). For example, *Protea compacta*, a species that occurs on Fynbos's oligotrophic soils, has a weak capacity to down-regulate phosphorus uptake (Shane *et al*., 2008), which at elevated supply can lead to phosphorus-toxicity (Shane *et al*., 2004). This trait specialization could thus limit the species ability to adapt to more fertile soils and so contribute to strong floristic turnover between Fynbos and its neighbouring biomes.

A common feature of boundaries involving either Forest or Fynbos is that they exhibit large differences in resource availability. For Forest, the difference relates to fire, light and seasonal moisture stress, whereas for Fynbos it relates to soil nutrients. Furthermore, both boundaries are spatially abrupt, the Forest boundary on account of its emergent nature (Manders, 1990; Gray & Bond, 2015) and the Fynbos boundary on account of its geological basis (Lechmere-Oertel, 2001; Cowling & Potts, 2015). I propose that the large environmental turnover across these boundaries coupled with their abruptness act together to present plant genera and families with an adaptive challenge in which multiple traits are required to undergo co-adaptation to a dramatically different environment. Although the environmental data I used only reflect a fraction of evolutionary time and may be influenced by humans (e.g. fire frequency), evidence of only three family-level (Diosmeae, Penaeaceae and Phyliceae) transitions from Forest to Fynbos since the origin of their boundary in the Paleocene-Miocene (Onstein *et al*., 2014), indicates that transitions by plant families between these biomes are infrequent, with the consequence that this boundary describes strong floristic turnover. Predicting transition rates between biomes using dated phylogenies (e.g. Kerkhoff *et al*., 2014; Zanne *et al*., 2014, Daru *et al*., 2016), in comparison to the taxonomic approach followed here, is appealing because in principle it allows historical biome occupancy and rates of transition between biomes to be explicitly estimated. Taking such an approach, however, has several limitations. Firstly, there is a shortage of comprehensive, species-level phylogenies for lineages occupying biomes across South Africa. Secondly and perhaps more critical, is that the incomplete phylogenetic sampling associated with a regionally focussed study of this type is likely, in many instances, to result in biome transitions being mapped incorrectly.

Chapter 3

Why are species from open-canopy biomes relatively shade-intolerant compared to their closed-canopy neighbours?

Abstract

Aim The absence of open-canopy species from closed-canopy understoreys, where light availability is relatively low and dynamic, suggests they are shade-intolerant. I investigated whether this apparent shade intolerance is a result of open-canopy species inability to maintain a positive carbon balance under low light relative to closed-canopy species.

Location Cape Floristic Region, South Africa.

Methods I quantified light availability and sunfleck properties below forest (closed-canopy) and fynbos (open-canopy) vegetation. Forest and fynbos species were grown under three light treatments (24%, 54%, 100% irradiance) and their photosynthetic response to continuous and fluctuating light was quantified. Furthermore, dark respiration, root respiration, growth and leaf traits were measured under each treatment.

Results Light availability was significantly lower and more dynamic under forest than fynbos understoreys. Although leaf and root respiration rates were similar between forest and fynbos, forest species showed higher light-use efficiency under fluctuating light and higher rates of photosynthesis (54% irradiance: 3.4 versus 0.8 μmol CO_2 m^{-2} s^{-1}) under low light intensities compared to fynbos. Fynbos species experienced some mortality under 24 and 54% irradiance. They also displayed smaller, thicker leaves that were erect and tightly aggregated along branches compared to forest species.

Main conclusions Shade intolerance in open-canopy species is probably brought about by their inability to efficiently harvest light and assimilate carbon, and thus maintain a positive carbon balance under low, dynamic light compared to closed-canopy species. These inefficiencies will compromise their ability to establish under closed canopies and are likely driven by the contrasting selective regimes open- (high light, low nutrients, fire-prone) and closed-canopy (low light, high nutrients, no fire) species experience, which require opposing trait expressions.

Introduction

Globally, open- and closed-canopy biomes are commonly juxtaposed, often forming sharp boundaries (e.g. forest–maquis in New Caledonia, Enright *et al*., 2001; forest–moorland in Tasmania, Wood & Bowman, 2012; forest–grassland in South Africa, Gray & Bond, 2015; forest–savanna in Brazil, Hoffmann *et al*., 2009) that are characterized by strong turnover in fire occurrence and the availability of light, nutrients and water. Open-canopy species are largely absent from neighbouring low light forest understoreys and tend to die out as forest species colonize their habitat, for example, in the long-term absence of fire (Brown & Podger, 1982; Manders & Richardson, 1992; McCoy *et al*., 1999; Hoffmann *et al*., 2009). This inability to exist below overtopping forest trees suggests that open-canopy species are shade-intolerant. Growth and reproduction in low light conditions (i.e. shade tolerance) is largely dependent on the maintenance of a positive carbon balance by maximising light capture and photosynthetic efficiency whilst minimising respiratory costs (Bjorkman, 1981; Pearcy, 1988; Givnish, 1988; Valladares & Niinemets, 2008). Therefore, open-canopy species potentially lack the structural and physiological adaptations to tolerate shade in forest understoreys.

The most striking feature of open–closed biome boundaries is the turnover in vegetation structure, with closed canopies dominated by tall trees in contrast to open canopies, which are dominated by low-stature shrubs, graminoids, and sparsely distributed trees in the case of savannas. Open canopies are largely exposed to saturating light intensities with low levels of light extinction in the understorey compared to forests where understorey light is 1-5% of that intercepted by the upper canopy (Manders, 1990a; Enright *et al*., 2001; Hoffmann *et al*., 2009), with intensities sometimes lower than 50 μmol m^{-2} s^{-1} (Chazdon & Pearcy, 1991). Inter-dispersed within the diffuse understorey light, however, are sunflecks, which are spatially and temporally dynamic patches of higher intensity light. Sunflecks predominantly exist for less than 10 s, yet they are potentially important, contributing between 10-90% of total daily photosynthetic photon flux density in forest understoreys (Pearcy *et al*., 1990; Chazdon & Pearcy, 1991; Sims & Pearcy, 1993; Leakey *et al*., 2004). Despite being short-lived, sunflecks are often vital to understorey carbon budgets as flecks with intensities greater than 50 μmol m^{-2} s^{-1} can drive up to 65% of daily photosynthesis (Pearcy & Calkin, 1983; Pfitsch & Pearcy, 1989; Chazdon & Pearcy, 1991). Thus in order for plants to persist in low light understoreys, it may be of critical importance to intercept and use sunflecks efficiently.

Contrasting light regimes imply that species from open- and closed-canopy habitats are likely to invest in different structural and physiological traits. For instance, at the whole-plant level species inhabiting shaded environments tend allocate more resources to height growth and leaf area than those inhabiting high light habitats, in order to maximize light interception (Givnish, 1988; Lusk, 2002; Westoby *et al*.,

2002). Together with greater aboveground investment, shade-tolerant species also produce large, thin leaves that are able to maximize the excitation of chlorophyll under low light (Givnish, 1988; Valladares & Niinemets, 2008). By comparison, plants in open habitats tend to have smaller, thicker leaves that can, among other functions, aid heat dissipation (Givnish, 1979; Niinemets, 2001; Yates *et al*., 2010). Furthermore, such leaves are often spirally arranged with a high inclination angle and level of aggregation along branches, whereas shade leaves follow an alternate or opposite arrangement with a low inclination angle and level of leaf aggregation (Falster & Westoby, 2003; Niinemets, 2010; Valladares & Niinemets, 2007). While leaf display patterns in shade-adapted species increase the potential for light interception, the light harvesting efficiency of small, erect and clumped leaves by comparison, can be substantially lower (e.g. 10-40%; Valladares & Niinemets, 2007).

Efficient light interception under low light conditions does not necessarily translate into increased growth in shade-tolerant species; however, as their photosynthetic rates are generally lower than those of shade-intolerant species, regardless of light availability (Kitajima, 1994; Walters & Reich, 1996; Valladares *et al*., 2000; Walters & Reich, 2000). This difference in photosynthesis is probably a consequence of the trade-off between maximising photosynthesis versus minimising respiratory costs (Waters & Reich, 1996, 2000; Craine & Reich, 2005). In high light, shade-intolerant species can achieve high photosynthetic rates and growth rates, but in low light, their elevated photosynthesis is tied to higher respiratory costs, given that photosynthesis and dark respiration are positively correlated (Bazzaz, 1979; Givnish, 1988; Reich *et al*., 1998; Wright *et al*., 2004). By contrast, while the relatively lower rates of photosynthesis in shade-tolerant species under low light may lead to slower growth, they have low respiratory costs, which are critical for maintaining a positive carbon balance in forest understoreys (Walters & Reich, 2000).

Higher sunfleck-use efficiency of shade-tolerant species compared to intolerant species potentially contributes to the positive carbon balance of shade-tolerant species in low light (Pearcy *et al*., 1996; Valladares *et al*., 1997; Montgomery & Givnish, 2008; Valladares & Niinemets, 2008). To achieve this efficiency, shade-tolerant species rely on their ability to: (1) rapidly induce photosynthesis, (2) maintain induction between successive sunflecks and (3) reduce the rate of induction-loss post-illumination (Chazdon & Pearcy, 1986a, b; Pearcy, 1990). The physiological efficiency of induction gain and loss is largely dependent on the light regulation of photosynthetic enzymes and stomatal conductance (Pearcy *et al*., 1996; Way & Pearcy, 2012; Kaiser *et al*., 2015; Vialet-Chabrand *et al*., 2017). Although stomatal response to a step increase in light is generally slow (Vico *et al*., 2011; Kaiser *et al*., 2018) and thus unlikely to influence induction during short sunflecks (<10 s), initial steady-state conductance is important to the early stages of induction gain in non-induced leaves, given

its role in the diffusion of gases in and out of leaves (Allen & Pearcy, 2000; Wachendorf & Küppers, 2017). In addition to stomatal conductance, the induction of photosynthesis during illumination from a sunfleck is dependent on the rapid activation of Rubisco (Sassenrath-Cole & Pearcy, 1992; Allen & Pearcy, 2000). The slow deactivation of Rubisco post-illumination, because of a large pool of intermediates and RuBP in the Calvin cycle (Kaiser *et al*., 2018), can increase carbon gain by 150-200% (Way & Pearcy, 2012) when balanced with the post-illumination CO_2 burst (Pearcy *et al*., 1996). While keeping Rubisco active consumes energy (Portis, 2003), its elevated activity and the maintenance of open stomata are critical to greater sunfleck-use efficiency in shade-tolerant species.

Whole-plant carbon balance is determined by both leaf-level CO_2 exchange and belowground respiratory costs, considering that root respiration can account for 10-50% of daily-assimilated carbon (Lambers *et al*., 2008). Similar to the pattern observed for aboveground respiration, previous studies have found that shade-intolerant species tend to express higher rates of root respiration relative to tolerant species (Reich *et al*., 1998; Walters & Reich, 1999, 2000). Variations in root respiration may, however, not only be correlated with the degree of shade tolerance but may also be a consequence of differences in belowground resource availability (Givnish, 1988). This is highly relevant in the context of open- and closed-canopy vegetation, given that the former tends to inhabit soils of lower nutrient availability than closed canopies (Enright *et al*., 2001; Hoffmann *et al*., 2009; Wood & Bowman, 2012; Gray & Bond, 2015). Species occupying low nutrient soils frequently show greater allocation to roots, including specialized roots such as cluster roots (e.g. Proteaceae, Cyperaceae, Restionaceae), to enhance nutrient acquisition (Lambers *et al*., 2006). Cluster roots, however, have high carbon costs, consuming 52-100% of daily photosynthate (Lambers *et al*., 2006). Thus edaphic conditions in open-canopy vegetation potentially select for greater belowground respiration than closed-canopy species, a trait that is incompatible with the low respiratory costs required in low light environments.

The absence of open-canopy species from neighbouring forest understoreys coupled with the contrasting trait adaptations required by high and low light environments, leads to the hypothesis that, relative to closed-canopy species, species from open canopy environments are unable to maintain a positive carbon balance under low light availability. Thus, I predict that open-canopy species have a lower capacity to harvest light, displaying small, thick leaves coupled with a low overall investment in leaf area. Furthermore, I predict that rates of CO_2 exchange aboveground and belowground will be higher in open-canopy species while rates of photosynthetic induction and sunfleck-use efficiency will be higher in closed-canopy species. To test these predictions, I quantified the growth, leaf/branch traits and gaseous exchange properties of open- and closed-canopy species across a light gradient. For this study, I examined species from forest and fynbos vegetation in South Africa. Fynbos is an open-

canopy, fine-leaved, sclerophyllous shrubland in which patches of tall, broad-leaved, closed-canopy forest vegetation are inter-dispersed (Mucina & Rutherford, 2006). During reciprocal transplants fynbos species are able to germinate in forest understoreys, however, they die rapidly and are completely absent from forest vegetation (Manders & Richardson, 1992). While previous studies indicate that the ranges of light saturated photosynthesis of forest (9-16 μmol CO_2 m^{-2} s^{-1}; Richardson & Kruger, 1990) and fynbos (2.8-14.0 μmol CO_2 m^{-2} s^{-1}; Mooney *et al*., 1983; Herppich *et al*., 2002) species tend to overlap, the effects of reduced and fluctuating light availability on gaseous exchange have not been investigated. Hence, forest and fynbos species provide an ideal situation to investigate differences in shade-tolerance between open- and closed-canopy biomes.

Materials and Methods

Characterization of field light regimes

To determine overall light conditions and sunfleck properties in the field, I measured daily fluctuations in photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) below forest and fynbos vegetation using photodiodes connected to a data-logger (Campbell Scientific CR-10X). Sampling was conducted in Orangekloof (33.995° S, 18.394° E), a protected valley in Table Mountain National Park. Vegetation consists of patches of Southern Afrotemperate Forest (hereafter 'forest') inter-dispersed within Peninsula Granite Fynbos and Sandstone Fynbos (hereafter 'fynbos'), all vegetation types within the Forest and Fynbos biomes of South Africa (Mucina & Rutherford, 2006). Along the margin of forest–fynbos boundaries (*ca.* 10-200 m) exists a mix of species from both vegetation types, known as 'scrub/transition' vegetation (McKenzie *et al*., 1977). I sampled PPFD at a single site in the valley in adjacent patches of forest, transition and fynbos vegetation. Sampling in each vegetation type was conducted for *ca.*15 hours on separate days by placing nine photodiodes (Silicon PIN Photodiode BPW 34 with no filter, OSRAM Opto Semiconductors GmbH & Co.) 5 cm aboveground and one in an unobstructed area above the sampled vegetation type. The photodiodes were calibrated by placing them next to a calibrated quantum sensor attached to a LI-6400 Portable Photosynthesis System (Li-Cor Inc. Lincoln, Nebraska, USA) and exposing them to varying natural light conditions. I then correlated the results from the photodiodes with those from the Li-Cor quatum sensor to obtain PPFD values for each photodiode. In forest, photodiodes were placed along a transect 1 m apart while in fynbos and transition, they were placed directly below individual plants. The logger recorded intensities every second between 05h30 and 20h30 (dawn until dusk) on cloud-free days during the summer month of January. Data from each vegetation type was then averaged and empirical characteristics including total daily PPFD, and the intensity and relative contribution of sunflecks of

different lengths to the understorey light environment were calculated. I classified sunflecks as any period during which the light intensity exceeded 50 μmol m^{-2} s^{-1}.

Experimental design, study species and growth conditions

I conducted two glasshouse experiments on forest and fynbos species at the University of Cape Town (33.956° S; 18.462° E). The first experiment was designed to determine species photosynthetic capacity and response to flashing light under varying degrees of light availability. For this, I evaluated species light response curves, their photosynthetic induction response to rapid increase in light and their response to sequences of lightflecks under three different light treatments. The second experiment aimed to determine leaf-level rates of CO_2 gain/loss, rates of root respiration and traits that may influence the harvesting of light under varying degrees of light availability. For this, I measured species leaf gaseous exchange rates *in situ* under each light treatment, determined root respiration rates and quantified leaf traits and growth. In both experiments, I placed all species under three light treatments: 100% light (i.e. no shade) and two shade cloth enclosures (2.5 x 3.4 m), intercepting 54 and 24% of light relative to that in the 'no shade' treatment. To determine the light intensity within each treatment, I mapped a grid of 1 x 1m cells across each enclosure. Using a hand-held quantum sensor (SKP210; Skye Instruments, UK) I recorded the light intensity in each cell 1.5 m aboveground (height of the trolley in which plants were grown) at midday when the sky was clear. The mean intensity in the 24, 54 and 100% treatments was 87, 198 and 366 μmol m^{-2} s^{-1} at midday, respectively.

In the first experiment, I selected five forest (*Diospyros whyteana* (Hiern); *Kiggelaria africana* L.; *Olea capensis* L. ssp. *macrocarpa*; *Olea europaea* L. ssp. *africana* (Mill.) P.S.Green; *Rapanea melanophloeos* (L.) Mez) and four fynbos (*Berzelia lanuginosa* (L.) Brongn.; *Erica versicolor* Wendl.; *Phylica ericoides* L.; *Searsia lucida* (L.) F.A.Barkley) species (n = 5). The plants were grown in 10 L potting bags containing locally derived soil with the addition of slow release fertilizer (see Appendix S3 in Supporting Information for details) and watered daily to ensure that moisture was not limiting. I rearranged pots and trolleys every week to reduce the effect of shade patches created by the superstructure of the glasshouse. Plants were exposed to the light treatments for 11 weeks (May – July) before I commenced the evaluation of light response curves and the induction and sunfleck responses.

In the second experiment, five forest (*D. whyteana; K. africana*; *Chionanthus foveolatus* (E.Mey.) Steam; *O. europaea* ssp. *africana*; *R. melanophloeos*) and four fynbos (*E. versicolor*; *P. ericoides*; *Protea repens* (L.) L; *S. lucida*) species were grown in 20 L pots (n=5). The growth medium consisted of sand supplemented with slow release fertilizer and gypsum (see Appendix S3 for details). Plants

were watered and rearranged as per experiment one. The measurement of steady-state rates of assimilation and dark respiration, and root respiration began two weeks after exposure to the treatments. After one month (March) of exposure, the plants were harvested to quantify structural leaf traits.

Light response curves

All plant gaseous exchange measurements were conducted on fully expanded leaves using a LI-6400 Portable Photosynthesis System equipped with 2 x 3 mm broadleaf cuvette and an integrated red/blue LED light source (LI-6400-02B). I set the [CO_2] in the cuvette to 400 ppm, maintained relative humidity between 55-65% and controlled leaf temperature at 25°C (estimated from energy balance using LI-6400 software). I performed all measurements in a laboratory (25°C) with the plant illuminated from three sides, using dichroic halogen spotlights (ECO-3000, Eurolux) to ensure that all leaf surfaces outside of the cuvette received a PPFD > 1500 µmol m^{-2} s^{-1}. For the light response curves (assimilation versus PPFD), photosynthesis was recorded at 11 light intensities: 2000, 1500, 1000, 500, 300, 200, 150, 100, 50, 25, 0 µmol m^{-2} s^{-1}. Leaves were exposed to each light intensity for a minimum of 120 s and a maximum of 240 s before the photosynthetic system 'matched' (performed to remove differences between reference and sample infrared gas analyzers in the LI-6400) and the measurement recorded. Prior to the initiation of the light sequence, I exposed the leaf in the cuvette to 1500 µmol m^{-2} s^{-1} of light until steady-state photosynthesis was attained. After the sequence, the leaf was removed and the area exposed in the cuvette scanned (CanoScan 4200F, Canon Inc. Japan) with a suitable reference area (1 cm^2) and analysed using ImageJ (https://imagej.nih.gov/ij/) to calculate leaf area. Net photosynthetic rates were then expressed per the corrected leaf area and fitted to a non-rectangular hyperbola model:

$$A_n = \frac{\phi I + A_{max} - \sqrt{(\phi I + A_{max})^2 - 4 \phi \theta A_{max} I}}{2\theta} - R_d$$

where θ is the curvature factor, ϕ is the quantum yield (efficiency with which light is converted into fixed carbon), I is the light intensity (µmol m^{-2} s^{-1}), A_{max} (µmol CO_2 m^{-2} s^{-1}) is the light saturated rate of gross CO_2 assimilation and R_d (µmol CO_2 m^{-2} s^{-1}) is the dark respiration during photosynthesis (Lambers *et al*., 2008). Fitting of the curve was optimized using differential evolution using 'DEoptim' 2.4-4 (Adria *et al*., 2016) package in R 3.2.3 (R development core team 2015). Following the recommendations of Chen *et al*. (2016), the lower and upper limits of θ, ϕ, A_{max} and R_d were set at 0, 0, 0, 0 and 1, 1, 100, 100, respectively, during the optimization process. From each curve, I obtained

the A_{max}, R_d, quantum yield (ϕ) and light compensation point (LCP: light intensity where photosynthesis = respiration).

Induction and sunfleck response

To determine the photosynthetic induction response to a rapid increase in light intensity, leaves were exposed to 20 μmol m^{-2} s^{-1} of light in the LI-6400 cuvette until photosynthesis and conductance readings stabilised. An induction sequence was initiated in which light conditions were set at 20 μmol m^{-2} s^{-1} (low) for 10 minutes followed by 30 minutes of 1000 μmol m^{-2} s^{-1} (high) and then returned to 20 μmol m^{-2} s^{-1} (low) for 20 minutes. Readings were taken every 10 s with the photosynthetic system 'matched' at 5, 20, 25, 35, 50 and 55 minutes during the induction sequence. The induction sequence was performed directly after the light response curve (see above) and on the same leaf. A locally weighted scatterplot smoother (LOESS) curve was fitted to the data using the 'loess.as' function from 'fANCOVA' 0.5-1 (Wang, 2010) in R. I used the generalized cross-validation method to select the optimal span value. With the curve, I was able to determine the time taken to reach 50 and 90% ($T_{50\%A}$ and $T_{90\%A}$, respectively) of the maximum photosynthetic rate recorded between the start and end of the high light period. The induction state of photosynthesis 30 s into the high light period was calculated using:

$$\text{Induction State 30s (\%)} = \frac{P_{LF} - P_L}{P_H - P_L} \times 100$$

where P_{LF} is the photosynthetic rate 30 s into high light period of the induction sequence, P_L and P_H are steady-state rates of photosynthesis at low (20 μmol m^{-2} s^{-1}) and high (1000 μmol m^{-2} s^{-1}) light intensities determined from the light response curves, following Chazdon and Pearcy (1986a).

After the induction sequence, leaves were exposed to two different lightfleck sequences composed of alternating periods of high and low light intensity in the LI-6400 cuvette. In the first sequence (5 s), leaves were subjected to 23 periods of 5 s flashes of 1000 μmol m^{-2} s^{-1} high light, followed by 5 s of 20 μmol m^{-2} s^{-1} low light. The second sequence (30 s) followed a similar pattern; however, the low light period between flashes was 30 s long and there were 20 high/low light periods in total. Prior to the initiation of each sequence, leaves were exposed to 20 μmol m^{-2} s^{-1} of light until steady-state conditions were achieved. After correcting the net photosynthetic rates for leaf area, I fitted a LOESS curve to the data, following the same procedure as for the induction response. Using the curve I

calculated the total amount of carbon gained (area under the curve; mmol m^{-2}) and the light-use efficiency (LUE) in the sequences using:

$$\text{Light-Use Efficiency} = \frac{\text{carbon gain during lightfleck sequence}}{\text{predicted carbon gain under steady-state conditions}}$$

Where predicted carbon gain refers to the amount of carbon assimilated under steady-state conditions Chazdon and Pearcy (1986b). I calculated this potential carbon gain using the photosynthetic rates attained at 20 and 1000 μmol m^{-2} s^{-1} light intensities during the light response curve. Upon the initiation of a lightfleck sequence, gas exchange values recorded by the LI-6400 Portable Photosynthesis System were unstable. Consequently, the last 8 and 10 light pulses during the 5 s and 30 s sequences, respectively, where values between successive flecks were more stable were used to calculate carbon gain and LUE.

Steady-state gaseous exchange and fluorescence

Potential *in situ* rates of photosynthesis and light-adapted chlorophyll fluorescence (Φ_{PSII}) achieved under the three light treatments (24, 54, 100% irradiance) were measured at the maximum light intensities observed in each treatment (i.e. for 24% irradiance at 120 μmol m^{-2} s^{-1}, 54% irradiance at 400 μmol m^{-2} s^{-1} and 100% irradiance at 1000, μmol m^{-2} s^{-1}). Prior to taking the measurements, I recorded the rates of dark respiration 1-2 hours before sunrise. Photosynthetic CO_2 assimilation and ΦPSII were measured using a leaf chamber fluorometer (LI-6400-40) attached to a LI-6400. CO_2, relative humidity and leaf temperature conditions in the cuvette were maintained at the same levels as all previous gaseous exchange measurements (see above). After each measurement, I harvested the leaf and determined its area (for method see above) to express the corrected net photosynthetic rate per leaf area.

Root respiration

Root respiration was measured in the pots using a LI-6400 gas analyser equipped with a soil CO_2 flux chamber (LI-6400-09). Two weeks prior to measurements; a polyvinyl chloride collar (diameter: 100 mm; height: 45 mm) was inserted into the surface layer of the pot. During the measurements, the chamber was inserted into the collar and sealed with a foam gasket. The target [CO_2] was set to *ca.* 400 ppm and the ΔCO_2 limit set to 5 μmol m^{-2} s^{-1}.

Plant structural traits

After one month of exposure to the three light treatments, I harvested the plants in the second experiment. Initially, a single branch was cut from the plant to determine the investment in leaf area/stem cross-sectional area (m^2 m^{-2}), hereafter 'leaf area/stem area', and leaf area/stem length (m^2 m^{-1}). I removed all leaves from the branch and recorded its basal stem diameter, and total length. The leaves were weighed and scanned to determine their area using ImageJ as described above. To determine specific leaf area (SLA: m^2 kg^{-1}), the leaves were dried at 60°C for 48 h and reweighed. The bulk of the remaining plant was separated into leaves, stems and roots, with the root system gently excavated under running water. Using the fresh weight of the bulk leaves and the leaf area of the subsampled branch, I was able to determine the total leaf area of the plant. Once all the plant organs had been dried, I quantified leaf area ratios (m^2 kg^{-1}) and shoot:root ratios.

Statistical analysis

Prior to analysing the data statistically, I conducted a preliminary analysis to determine whether the experimental species could be classified as, "forest" or "fynbos" based on their distributions across forest, transition and fynbos vegetation in the field. For this, I extracted abundance data for each species from a phytosociological study (McKenzie *et al*., 1977) conducted in the Orangekloof valley of Table Mountain National Park. Vegetation in the valley consists of a mosaic of forest and fynbos vegetation. McKenzie *et al*. recorded species abundance in 78 plots, which they designated as either forest, fynbos or transition vegetation. To determine the preference for each species to occur in each vegetation type, I first established a linear sequence of scores for the three vegetation types (fynbos = 1, transition = 2, forest = 3). Following this, a species habitat preference was determined as the sum of scores across all plots, with the score of each plot (1, 2 or 3) weighted by the relative abundance of the species in that plot. If a species only occurred in forest, transition or fynbos, its score would be one, two or three, respectively. Results indicated that the species separated out into two distinct groups "forest" (Vegetation score: *C. foveolatus* = 3.00, *K. africana* = 2.68, *D. whyteana* = 2.96, *O. capensis* = 2.80, *O. europaea* = 3.00, *R. melanophloeos* = 2.91) and "fynbos" (*B. lanuginose* = 1.00, *E. versicolor* = 1.08, *P. ericoides* = 1.00, *S. lucida* = 1.38). Thus, I decided to perform all comparative analyses as "forest" versus "fynbos" species.

All statistical analyses were conducted in R. I performed a two-way linear mixed effects model using restricted maximum likelihood to test the differences between vegetation types (forest and fynbos) and the three light treatments in both pot experiments. The fixed factor was a two-way interaction between vegetation type and irradiance level (24, 54, and 100%). The random factor was species with the model

performed using 'lme4' 1.1-13 (Bates *et al*., 2015). Following this, I ran an ANOVA of the linear mixed effects model to determine the significance of the main effects and/or interaction using 'car' 2.1-4 (Fox & Weisberg, 2011). The ANOVA used the Kenward-Roger approximation for the degrees of freedom. Where significant differences ($P < 0.05$) between vegetation types and/or light treatments were found, I computed the least-squares means on the linear mixed effects model using 'lsmeans' 2.26-3 (Lenth, 2016). Before running any analysis, response variables were log transformed to ensure data were normally distributed.

Results

Light regimes

Understorey light availability and its dynamic tendencies varied substantially between the three vegetation types. In forest, the daily light accumulation in the understorey was significantly ($P < 0.001$) lower than in either transition or fynbos vegetation (Fig. 3.1a). Although the mean maximum sunfleck intensity recorded on forest floor was 1060 µmol m^{-2} s^{-1}, the dense canopy intercepted 96% of light it received, casting deep shade in the understorey. By contrast, the mean maximum light intensities in transition (1698 µmol m^{-2} s^{-1}) and fynbos (1710 µmol m^{-2} s^{-1}) were similar, with understorey light extinction levels significantly lower than those in forest (Fig. 3.1b). In addition to greater levels of shade, the forest light environment was more dynamic relative to neighbouring vegetation. The intensity of short sunflecks with a duration between 5 s and 7 minutes was generally low across all three vegetation types; however, their cumulative PPFD was consistently higher in forest vegetation (Figs. 3.2a & b). Furthermore, sunflecks that were seven minutes long, showed the greatest contribution to the total amount of light received from all dynamic light events in the forest understorey (Fig. 3.2c). In comparison, the contribution of short sunflecks in transition and fynbos vegetation was low with the majority of light in their understoreys accumulated during long, uninterrupted periods (Figs. 3.2b & c).

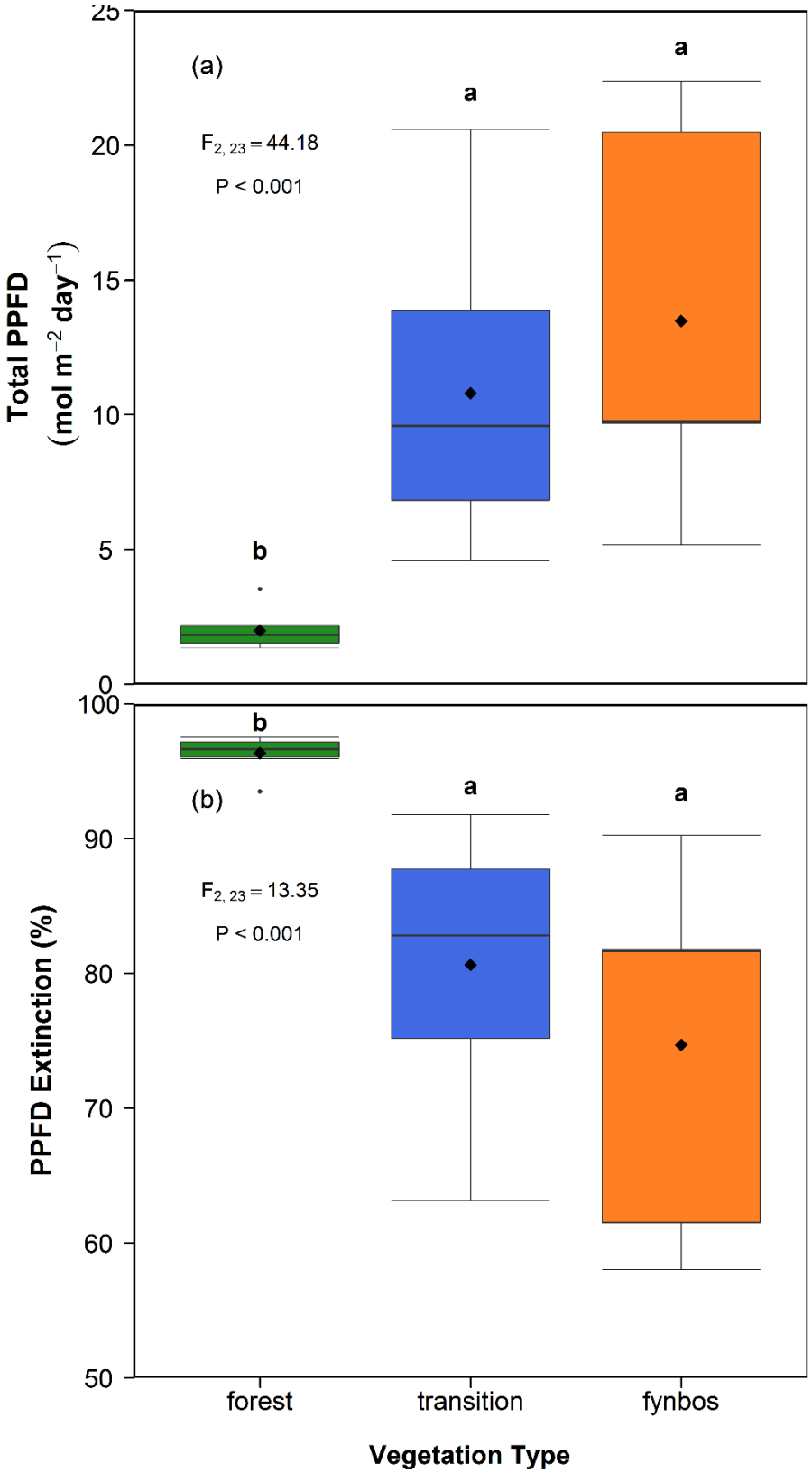

Figure 3.1 Variations in (a) total daily photosynthetic photon flux density (PPFD, mol m^{-2} day^{-1}) and (b) percentage light extinction relative to unshaded conditions, in the understorey of forest, transition and fynbos vegetation. Box corresponds to interquartile range (IQR: 25th and 75th percentiles). Whiskers extend from the box to the lowest value within 1.5*IQR, with circles representing outliers. Diamonds represent means with letters indicating significant differences between vegetation types determined from a one-way ANOVA followed by a post-hoc Tukey HSD using 'agricolae' (de Mendibru, 2016) in R.

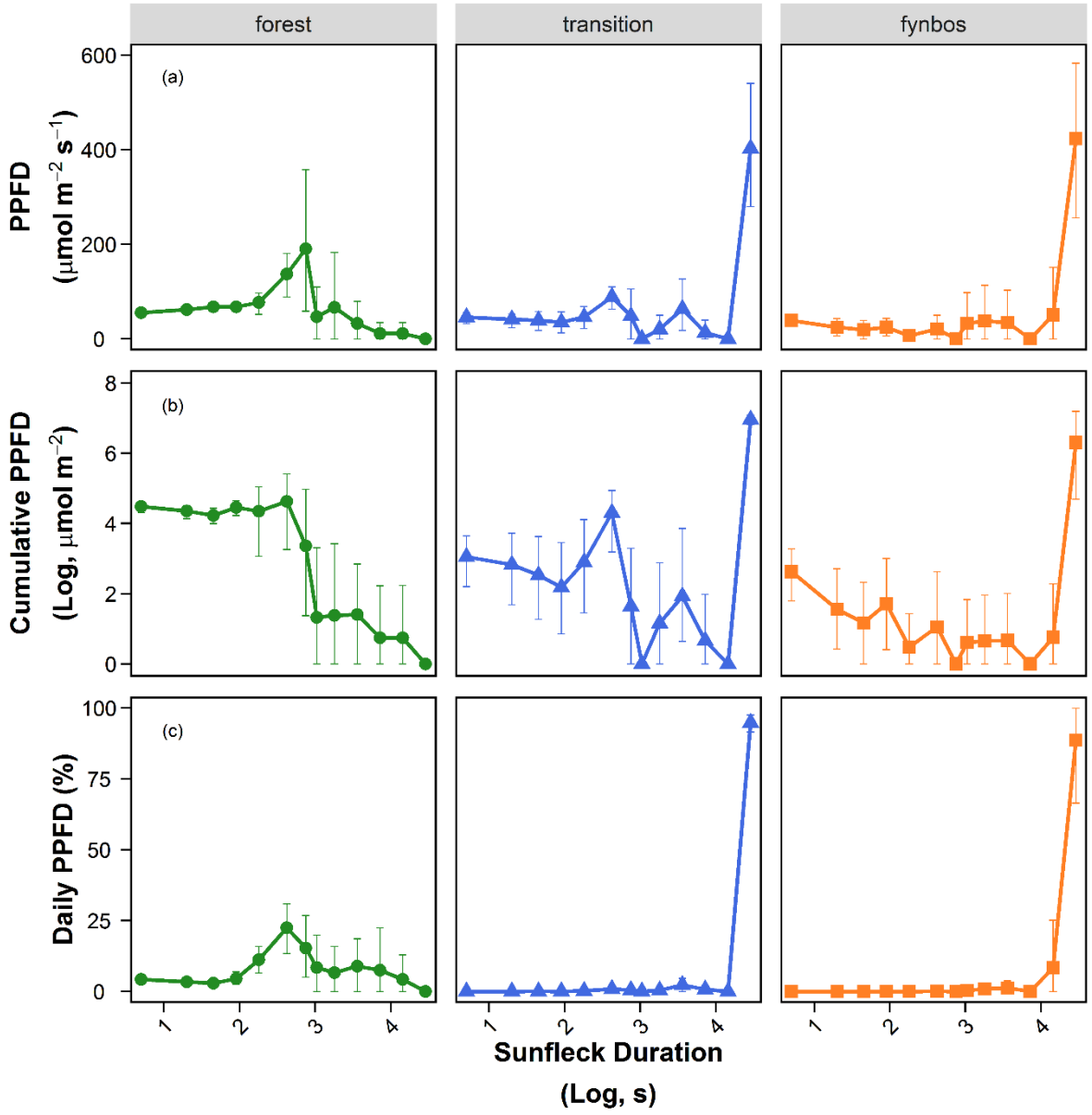

Figure 3.2 Differences in (a) intensity, (b) cumulative PPFD and (c) relative contribution to total daily PPFD of sunflecks with varying duration below the understorey of forest, transition and fynbos vegetation. Points and error bars represent the mean ± 95 % confidence interval of probes. Confidence intervals were calculated using non-parametric bootstrapping with 5000 replicates from the 'Hmisc' 4.0-0 (Harrell & Dupont, 2016) package in R.

Variation in growth and leaf traits with light

Overall, plant biomass at the end of the second experiment did not differ between forest and fynbos species in any light treatment. However, biomass for all species was significantly ($P < 0.001$) lower in the 24% relative to the 100% irradiance treatment (Fig. 3.3a, see Table S3.2 in Appendix S3 for species values). Moreover, in both shade treatments, all *Protea repens* (24%: n=5, 54%: n=5 dead individuals)

and some *Erica versicolor* (24%: n=3, 54%: n=1), *Phylica ericoides* (24%: n=3, 54%: n=4) and *Searsia lucida* (54%: n=1) replicates died prior to harvest whereas there was no mortality among forest species. All dead replicates were excluded from the analysis of morphological data, while *P. repens* was not included in the physiological data given the lack of replicates. Despite the change in total biomass with light there was no change in allocation as the shoot:root ratio did not differ significantly between vegetation types or light treatments (Fig. 3.3b). Leaf area ratio (LAR), however, did show a sharp drop from the 54% to 24 % irradiance treatments in fynbos compared to forest species (Fig. 3.3c). Although the linear mixed model indicated a weak significant interaction between vegetation type and irradiance level for LAR, pairwise post-hoc analysis showed no significant differences. Nonetheless, the drop in LAR under the 24% treatment was likely a result of *P. ericoides*, as it shed leaves under the low light treatment.

Forest species had significantly larger leaves and greater investment in leaf area per stem area than fynbos species (Figs. 3.4, 3.5a, d & Table S3.2). At the species level, this striking difference is illustrated by the over 1000-fold difference in individual leaf size and 30-fold difference in leaf area per stem length between *Rapanea melanophloeos* (leaf size: mean ± 95% confidence interval = 5645 ± 1534 mm^2; leaf area/stem length: 0.134 ± 0.052 m^2 m^{-1}) and *P. ericoides* (5 ± 1; 0.004 ± 0.002). In spite of SLA being consistently higher in forest than fynbos species, there was no significant difference between treatments (Fig. 3.5b). The SLA of all species, however, was significantly lower when grown under 100% relative to 54% irradiance (Figs. 3.5b & c).

Gaseous exchange characteristics under continuous light

Photosynthetic responses to light intensity varied between vegetation types and light treatments (See Fig. S3.1 and Table S3.1 for species light response curves and their relevant properties). Maximum assimilation rate (A_{max}) per unit leaf area was significantly higher in fynbos than in forest species, regardless of the growth irradiance level (Fig. 3.6a). Contributing to this pattern was the approximately four-fold difference in A_{max} between the fynbos shrub *Berzelia lanuginosa* (mean ± 95% confidence interval = 20.1 ± 3.8 µmol m^{-2} s^{-1}) and the forest tree *R. melanophloeos* (5.1 ± 2.0). Reduced light availability resulted in a significantly lower A_{max} under 24% and 54% compared to 100% irradiance for both vegetation types (Fig. 3.6a). The adverse effects of shade on photosynthesis, however, were more dramatic in fynbos species (Fig. S3.1), whose A_{max} declined by 39% compared to a 21% decline in forest species in response to the 24% shading treatment. The response of dark respiration (R_d) and light compensation point (LCP) to light availability was similar to that of photosynthesis; however, a significant decline in R_d and LCP with shading was apparent at 54% irradiance in fynbos species,

whereas in forest, a significant decline only became apparent at 24% irradiance (Fig. 3.6b & d). While the apparent quantum yield (ϕ) was not different between forest and fynbos, it did show a significant decrease with increased shading (Fig. 3.6c).

Although the rate of photosynthesis under saturating light conditions in the light response curves was higher in fynbos compared to forest, the opposite occurred when they were exposed to the steady-state maximum light intensities associated with their growth conditions (Fig. 3.7a & Table S3.4). This difference in photosynthesis was most notable at 54% irradiance, where rates were significantly higher in forest compared to fynbos species. As in light response curves, the photosynthetic rate of all species declined with increased shading (Fig. 3.7a). However, there were no differences in dark respiration measured before sunrise (Figs. 3.7a & b). Light-adapted fluorescence (Φ_{PSII}) showed little variation between forest and fynbos species; nonetheless, both vegetation types exhibited a significant decline with increased light availability (Fig. 3.7c). Similar to the pattern observed in aboveground CO_2 losses, rates of root respiration in pots did not differ between vegetation types or irradiance levels (Fig. 3.7d). Although the CO_2 efflux measured may have multiple sources aside from the plants roots, the plants were grown in sand with very little organic matter. Thus, I consider the efflux measurements to be a reasonable representation of root respiration.

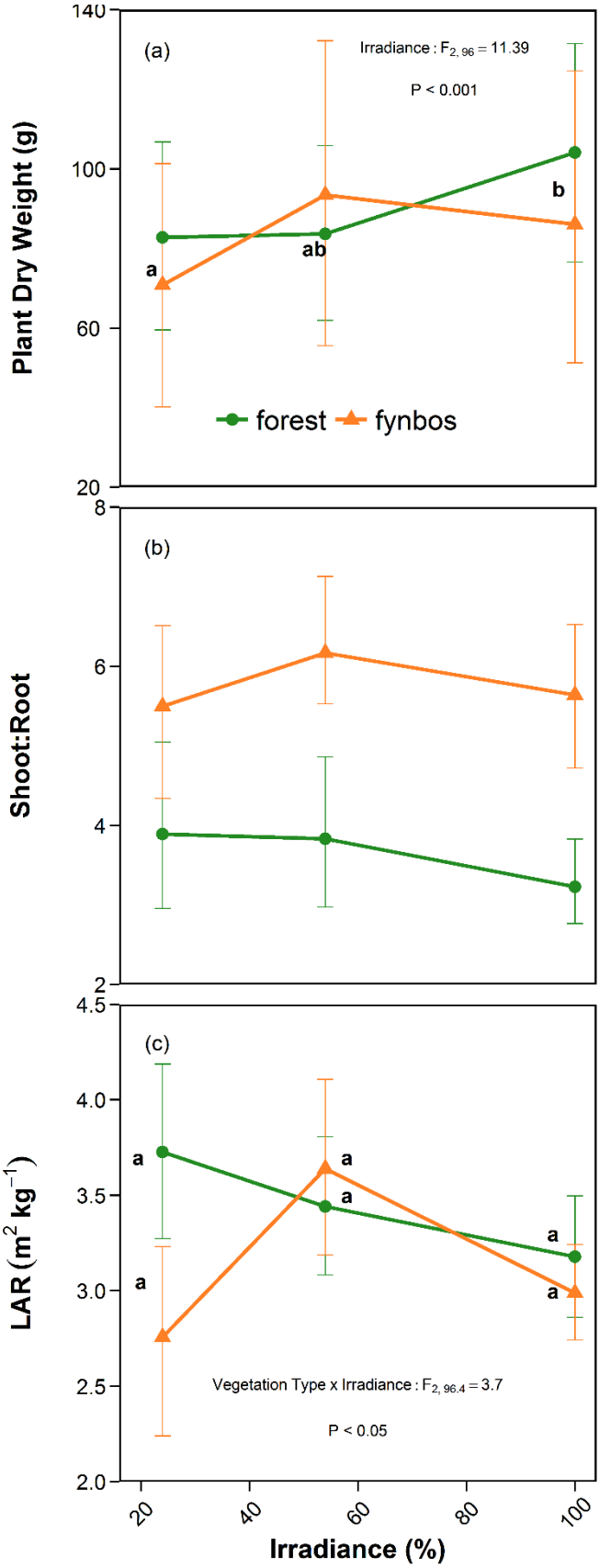

Figure 3.3 Biomass accumulation and allocation in forest and fynbos species grown under 24%, 54% and 100% irradiance. Points and error bars represent the mean ± 95 % confidence interval of replicates. Letters denote significant differences between vegetation types and/or light treatments (P < 0.05) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

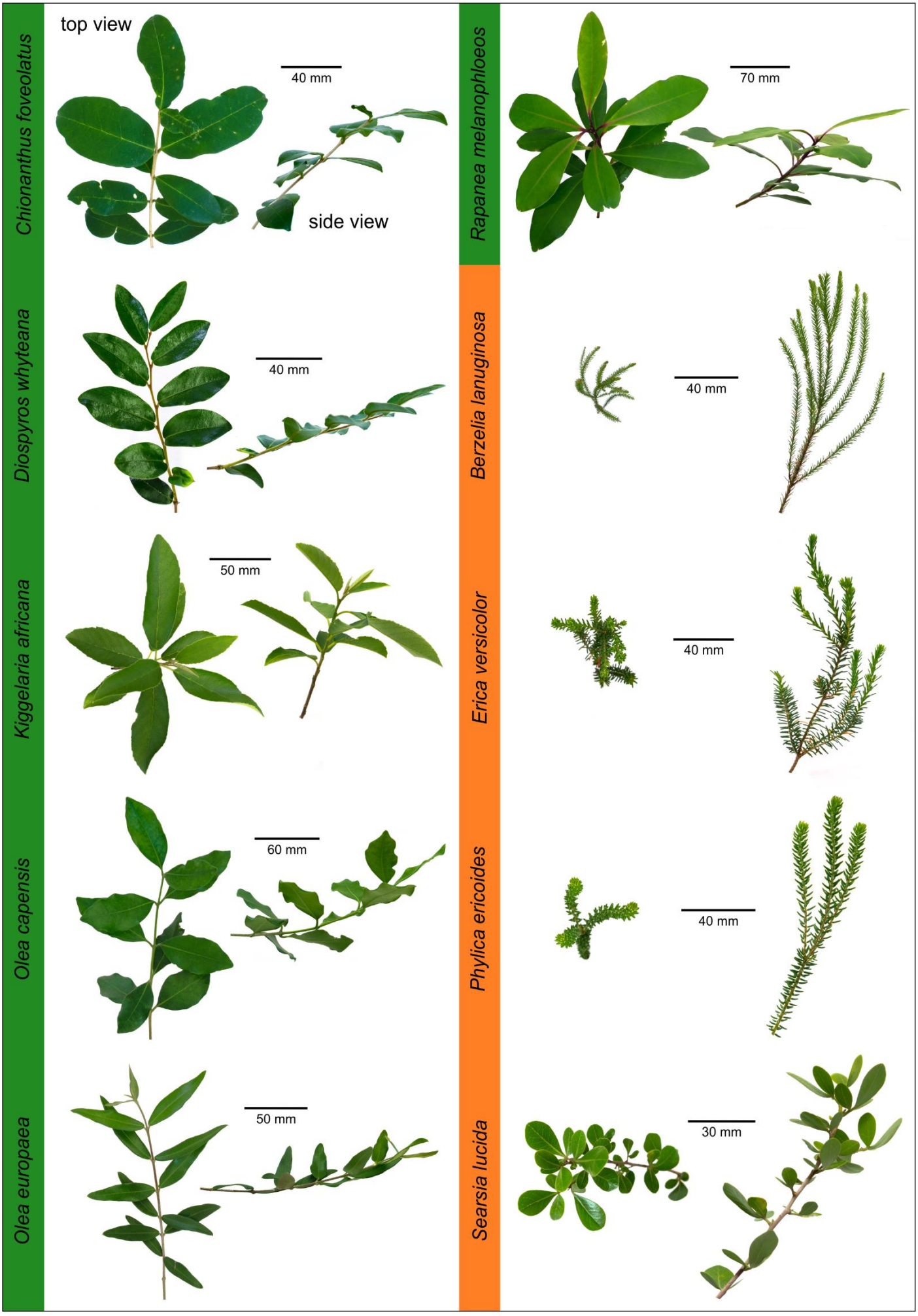

Figure 3.4 Overhead and side-on view of forest and fynbos species branches sampled. (Photos: KF Packer)

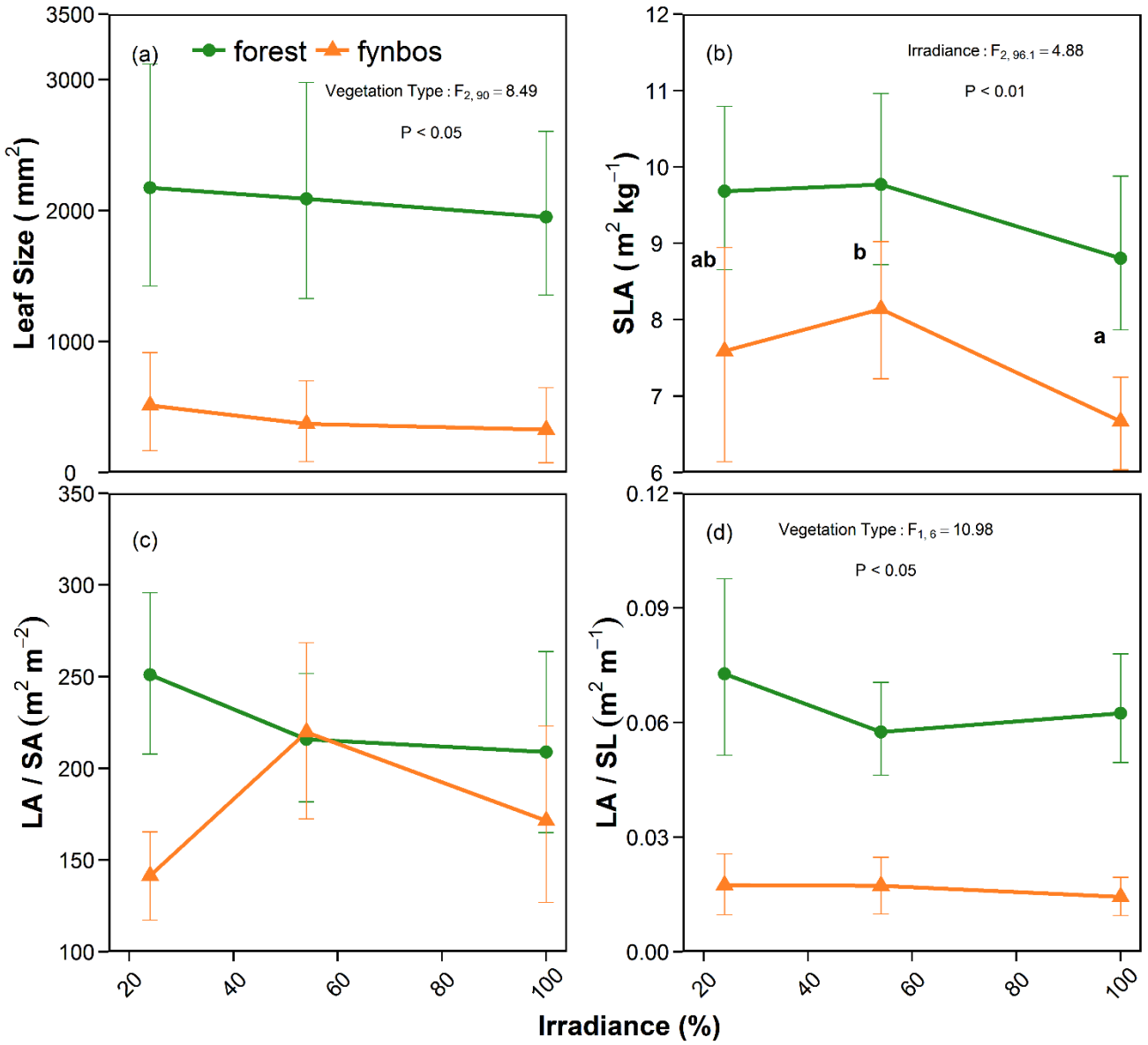

Figure 3.5 Leaf and branch traits of forest and fynbos species grown under 24%, 54% and 100% irradiance. Points and errors bars represent the mean ± 95% confidence interval of replicates. Letters denote significant differences between vegetation types and/or light treatments ($P < 0.05$) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

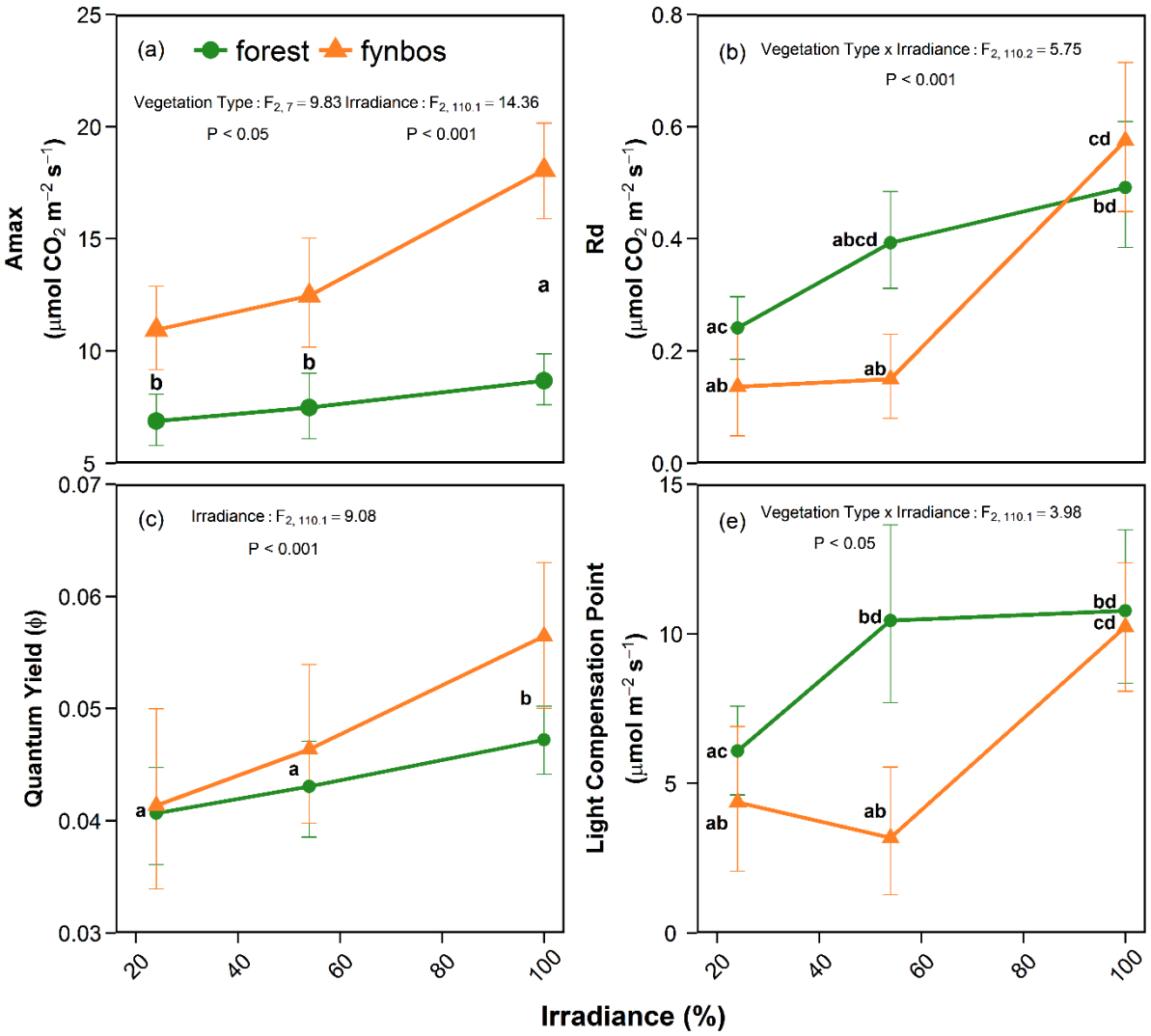

Figure 3.6 Gaseous exchange properties of light response curves measured on forest and fynbos species grown under 24%, 54% and 100% irradiance. Measurements were conducted in a laboratory (25°C) with leaves outside the cuvette receiving a PPFD > 1500 µmol m^{-2} s^{-1}. Points and error bars represent the mean ± 95% confidence interval of replicates. Letters denote significant differences between vegetation types and/or light treatments (P < 0.05) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

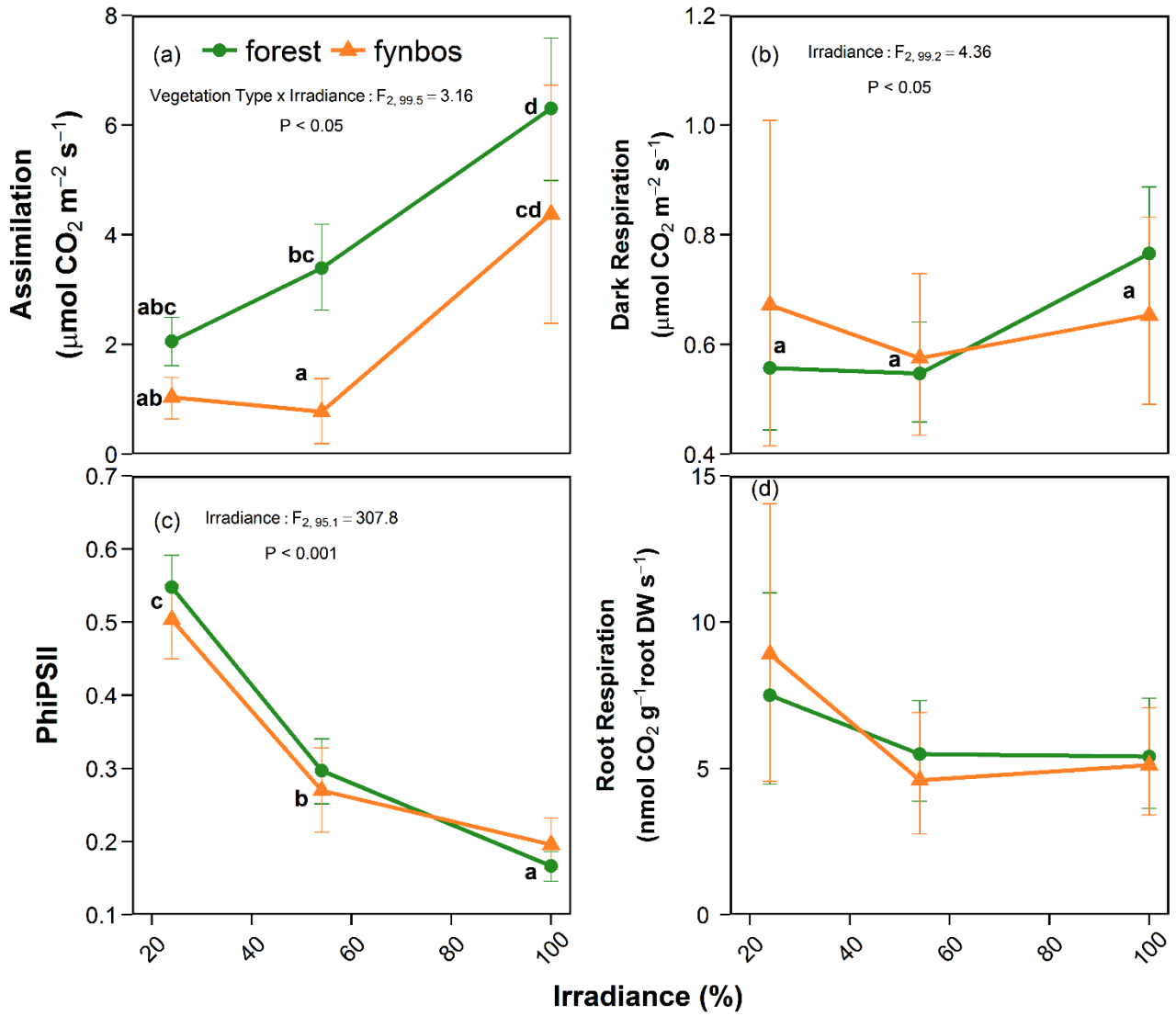

Figure 3.7 Gaseous exchange properties (a–c) and root respiration (d) of forest and fynbos species under contrasting light availabilities. In the 24%, 54% and 100% irradiance treatments, steady-state assimilation, dark respiration and light adapted fluorescence (ΦPSII) were measured at the maximuim light intensities within each treatment: 120, 400 and 1000 µmol m^{-2} s^{-1}, respectively. Measurements were conducted in the glasshouse shade enclosures. Points and error bars represent the mean ± 95% confidence interval of replicates. Letters denote significant differences between vegetation types and/or light treatments (P < 0.05) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

Photosynthetic response to dynamic light

The photosynthetic induction responses to a rapid increase in light intensity (20 to 1000 µmol m^{-2} s^{-1}) were similar between forest and fynbos species, with very little difference in the time taken to reach either 50% ($T_{50\%A}$) or 90% ($T_{90\%A}$) of the assimilation rates achieved during the 30 minutes of elevated illumination (Figs. 3.8a & b, see Fig. S3.3 and Table S3.5 for each species response). After 30 s of illumination, however, the relative induction state of all species from the 100% treatment was significantly higher than in plants grown under 54% but not 24% irradiance (Fig. 3.8c). During sequences of fluctuating light intensities (high = 1000 µmol m^{-2} s^{-1}, low = 20 µmol m^{-2} s^{-1}) there was little difference in carbon gain between forest and fynbos species, regardless of the length of low light period (5 s or 30 s; Figs. 3.9a & b, see Figs. S3.4 & 2.5 and Table S3.5, for each species response). Although the LI-6400 Portable Photosynthesis System is not ideally suited for high-resolution measurements (i.e. every 1 second), it does allow for a broad assessment of carbon gain during lightfleck sequences as indicated by the significant decrease in carbon gain with increased shading in species from both vegetation types (Fig. 3.9a &b). Indeed, while the rate of photosynthesis in some fynbos species, including *B. lanuginosa*, *E. versicolor* and *P. ericoides* was positive after the initial flecks during the 5 s high – 30 s low light sequence, by the end it was negative in plants grown under 24% and 54% irradiance treatments (Fig. S3.5). Moreover, during this lightfleck sequence the light-use efficiency (carbon gain relative to steady-state conditions) was significantly higher in forest compared to fynbos species (Fig. 3.9d).

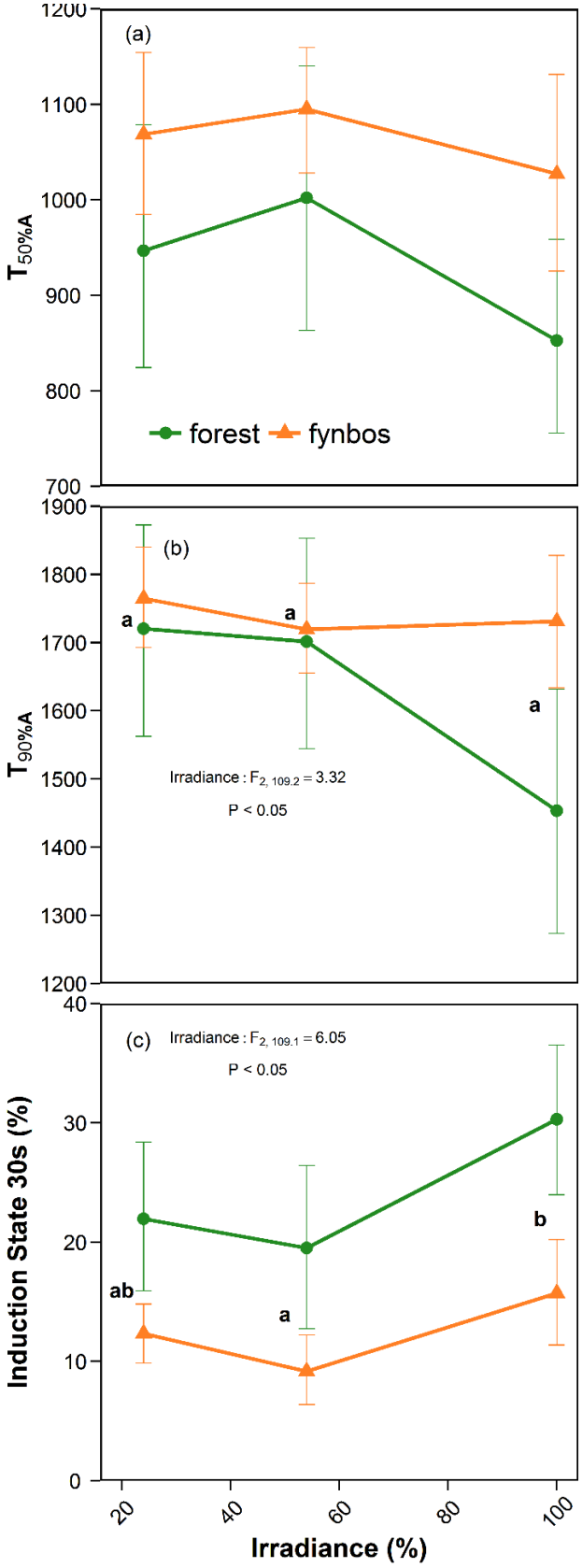

Figure 3.8 Time required to reach 50% (a) and 90% (b) of steady state rates of photosynthesis and the relative induction state (% full induction) after 30s (c) when exposed to 20 µmol m^{-2} s^{-1} of light for 10 minutes followed by 30 minutes of 1000 µmol m^{-2} s^{-1} and then 20 µmol m^{-2} s^{-1} for 20 minutes. Points and error bars represent the mean ± 95% confidence interval of forest and fynbos species grown under 24%, 54% and 100% irradiance. Letters denote significant differences between vegetation types and/or light treatments ($P < 0.05$) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

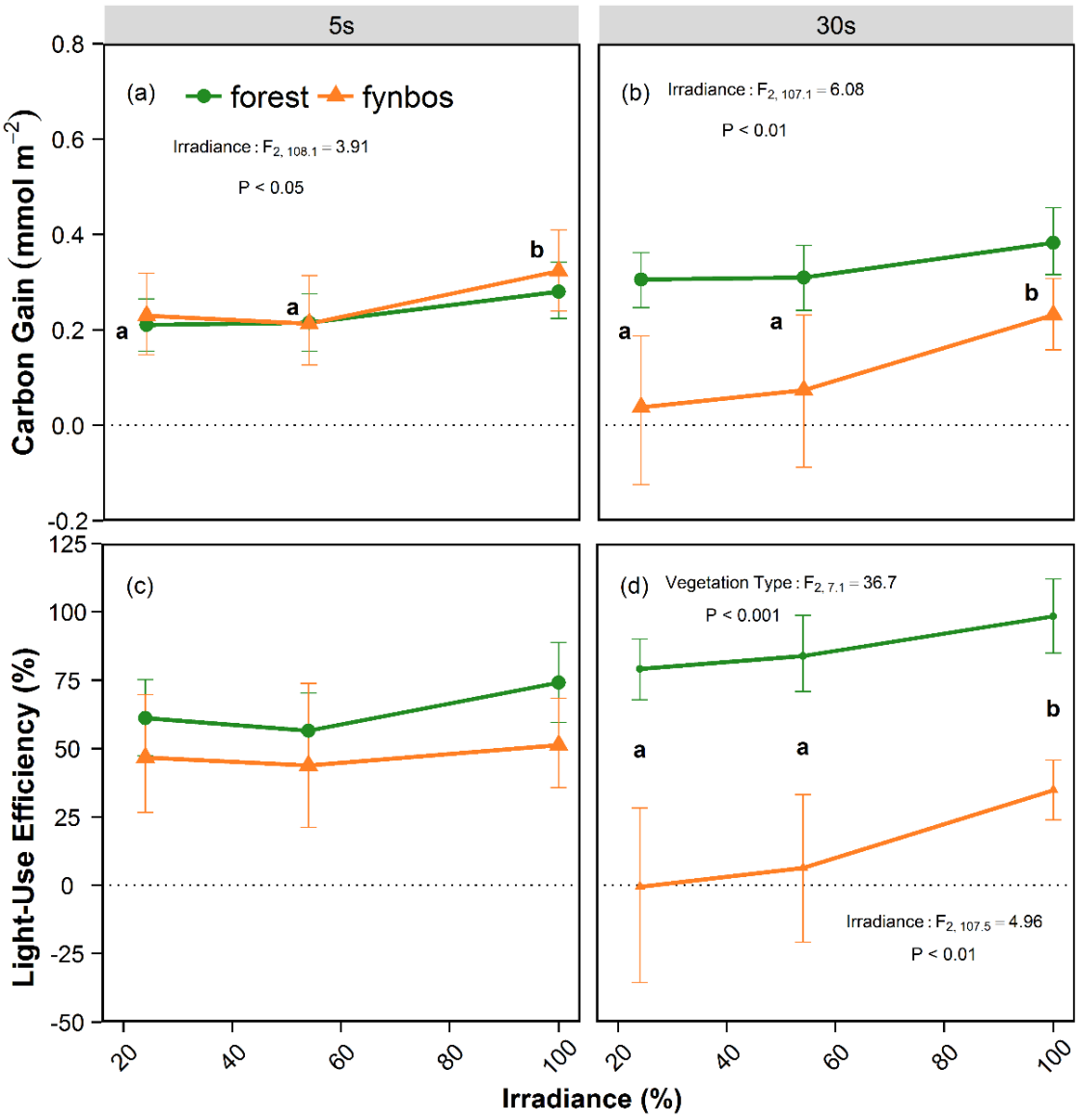

Figure 3.9 Net carbon gain (a – b) and light-use efficiency (c – d) of leaves during a sequence of lightflecks where 5s flashes (1000 µmol m^{-2} s^{-1}) were separated by either 5 s or 30 s of low light (20 µmol m^{-2} s^{-1}). Points and error bars represent the mean ± 95% confidence interval of forest and fynbos species grown under 24%, 54% and 100% irradiance. Letters denote significant differences between vegetation types and/or light treatments ($P < 0.05$) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

Discussion

High mortality in shaded conditions coupled with poor photosynthetic performance under continuously low or fluctuating light conditions in open-canopy species (Figs. 3.7a, 3.9d & S3.2a) supports the assertion that species from open-canopy vegetation are relatively shade-intolerant, unable to maintain a positive carbon balance in low light, compared to neighbouring forest (Manders & Richardson, 1992; McCoy *et al*., 1999; Hoffmann *et al*., 2009, Bowman, 2000; Bond & Parr, 2010). This shade intolerance is potentially a consequence of open-canopy environments selecting for leaf traits (Figs. 3.4 & 3.5: small, erect, sclerophyllous leaves) and photosynthetic properties (Fig. 3.6: high CO_2 exchange) that support persistence in high light, hot and nutrient constrained conditions but on the other hand are inefficient at light capture and the assimilation of carbon under low, dynamic light (Falster & Westoby, 2003; Niinemets, 2010; Walters & Reich, 2000; Craine & Reich, 2005). By comparison, species that inhabit closed-canopy understoreys appear shade-tolerant given their lack of mortality under shade coupled with the possession of large, flat leaves, and the expression of high photosynthetic efficiency and utilization of sunflecks (Figs. 3.4 – 3.6 & 3.9) that can support a positive carbon balance in low light (Givnish, 1988; Valladares & Niinemets, 2008). Contrasting selective regimes and the opposing traits they require may thus explain the inability of open-canopy species to exist below overtopping forest trees (Brown & Podger, 1982; Manders & Richardson, 1992; McCoy *et al*., 1999; Hoffmann *et al*., 2009).

Of all the morphological and physiological properties examined, leaf and branch traits showed the greatest distinction between forest and fynbos species across all light treatments (Figs. 3.4 & 3.5). The production of larger, thinner leaves coupled with a greater proportional display of leaf area per stem length in forest species (Figs. 3.5a, b & d) indicates that in low and often dynamic light environments (Figs. 3.1 & 3.2), forest species are better equipped to maximize light capture. By comparison, the tight spatial aggregation, high angles and whorled arrangements of leaves along fynbos species branches, with the exception of *S. lucida* (Fig. 3.4), is expected to reduce light harvesting efficiency (Falster & Westoby, 2003; Niinemets, 2010; Valladares & Niinemets, 2007) and thus hinder their ability to survive in forest understoreys. Poor light harvesting efficiency, however, is unlikely to be a disadvantage in the high light, relatively warmer and low nutrient conditions that dominate open-canopy vegetation (Manders, 1990a; Enright *et al*., 2001; Hoffmann *et al*., 2009; Little *et al*., 2012). The small, thick and erect leaves commonly possessed by fynbos species can reduce the absorption of excess, potentially harmful light (Demmig-Adams & Adams III, 1992), increase heat shedding (Parkhurst & Loucks, 1992), facilitate nutrient conservation (Eckstein *et al*., 1999; Wright & Westoby, 2003) and potentially enhance nutrient acquisition via mass-flow (Yates *et al*., 2010). Hence, the

environmental constraints in fynbos select for canopy traits that contrast strongly with forest species and are unlikely to intercept sufficient light in low light environments.

The higher photosynthetic capacity of fynbos relative to forest species (Fig. 3.6a), a pattern consistent with previous studies investigating species adapted to sun versus shade conditions (e.g. Kitajima, 1994; Walters & Reich, 1996; Valladares *et al*., 2000; Walters & Reich, 2000), may have contributed to the poor performance of fynbos species under shade. Under high light, a higher photosynthetic capacity and the concomitant ability to maximize carbon gain may be critical to fynbos species persistence in a resource-constrained and fire-prone environment. The oligotrophic soils that fynbos species occupy select for multiple carbon intensive structures, including sclerophyllous leaves (Wright *et al*., 2002), serotinous cones (e.g. Proteaceae; Cramer & Midgley, 2009) and cluster roots (e.g. Proteaceae, Cyperaceae, Restionanceae; Lambers *et al*., 2006), all important in conserving and acquiring nutrients in a regularly burnt environment. While higher rates of photosynthesis may support these traits, increased photosynthesis comes with elevated respiratory costs (Bazzaz, 1979; Givnish, 1988; Reich *et al*., 1998; Wright *et al*., 2004). A high respiration rate is contrary to the carbon conservative lifestyle required under low light conditions (Walters & Reich, 2000; Craine & Reich, 2005) and is thus likely to inhibit the ability of fynbos species to maintain a positive carbon balance under forest canopies. Moreover, under low light availability, the lower rates of photosynthesis in fynbos relative to forest species combined with similar rates of dark respiration expressed per area or mass (Figs. 3.7a, b & S3.6a, b) imply that it is difficult, if not impossible for fynbos species to fix enough carbon to support their carbon intensive lifestyle in forest understoreys.

Despite similarities in photosynthetic induction properties (Fig. 3.8), the greater light-use efficiency observed in forest relative to fynbos species during lightfleck sequences (Fig. 3.8d; 30 s 20 µmol m^{-2} s^{-1} - 5 s 1000 µmol m^{-2} s^{-1}), implies that fynbos species have an inferior capacity to utilize understorey sunflecks. It may, however, be argued that the high intensity flashes I administered during sequences were shorter and brighter compared to the fairly low intensity (200 µmol m^{-2} s^{-1}) but long-lived (7 min) sunflecks that made the greatest contribution to light in the forest understorey I sampled (Fig. 3.2). Nonetheless, the results do indicate the inability of fynbos species to reach and maintain rates of photosynthesis during periods of intermittent light that are equivalent to those reached under continuous light conditions. Given this incapacity was most apparent in fine-leaved species (*B. lanuginosa*, *E. versicolor*, *P. ericoides*, Table S3.5); poor sunfleck-use may be linked to the structural canopy traits of fynbos species. Small, erect and clumped leaves have high levels of self-shading (Falster & Westoby, 2003; Valladares & Niinemets, 2007; Niinemets, 2010), with the result that the probability of maximizing leaf exposure during short and rapid increases in light intensity is low.

Furthermore, while investment in thick, carbon rich leaves in fynbos species may facilitate higher photosynthetic capacity, this capacity is likely to be under-utilized, with low intensity forest understorey sunflecks unable to fully penetrate the leaves and activate Rubisco (Way & Pearcy, 2012). Consequently, the carbon costs of supporting thick leaves are unlikely to be met during intermittent light as evidenced by their negative assimilation rates at the end of a lightfleck sequence (except *S. lucida*, Fig. S3.5). A lack of light penetration thus provides further support for my assertion that adaptations required for survival in resource constrained fynbos environments are inappropriate for the efficient harvesting of light and assimilation of carbon in forest understoreys, leading to shade intolerance.

Although the morphological and physiological traits in forest species may facilitate shade tolerance in low light, they are not necessarily favourable in neighbouring open-canopy vegetation. For instance, the low photosynthetic capacity in forest species may lead to slow growth rates. Given forest species sensitivity to fire (van Wilgen *et al*., 1990; McCoy *et al*., 1999; Hoffmann *et al*., 2009; Wood & Bowman, 2012), slow growth rates may limit their ability to rapidly develop height and/or belowground storage organs for fire survival, thus limiting their potential colonization of open habitats. Conversely the thick bark for fire protection (Pausas, 2015; Ondei *et al*., 2016) and belowground storage organs (i.e lignotubers/rhizomes) for post-fire resprouting (Bond & Midgley, 2001), that fire-prone ecosystems commonly select for, may incur a carbon cost that open-canopy species are unable to support under low light availability. Fire may therefore not only limit tree encroachment into open habitats but also add to the carbon cost of nutrient adaptations that limit the ability of open-canopy species to exist below forest trees, given the carbon intensive adaptive traits required for fire survival.

Traits required for high versus low light environments are frequently considered incompatible, making it difficult to compete across light gradients (Valladares & Niinemets, 2008). Notwithstanding the observed reponses to light were from juveniles and could well differ from those of adults, I propose that in context of open- versus closed-canopy biomes, this trait incompatibility is exacerbated by the low nutrient supply and occurrence of fire in open habitats. Although supported by a high photosynthetic capacity in high light, the small, sclerophyllous leaves and investment in belowground structures that aid tolerance of saturating light, nutrient acquisition and conservation, and fire survival in open habitats are the opposite of what it is required for persistence in low light environments. Hence, the difference in shade tolerance between open- versus closed-canopy species and the concomitant absence of open-canopy species from closed-canopy understoreys, is a result of the contrasting selective regimes they experience (low light, high nutrients versus high light, low nutrients, fire-prone), which leads to a fundamental trade-off in photosynthetic properties (low A_{max}, high sunfleck-use

efficiency versus high A_{max}, low sunfleck-use efficiency), leaf traits (large, thin, erect versus small, thick, flat) and biomass allocation (stem elongation, leaf area versus lignotubers, cluster roots, cones).

Chapter 4

Does phenotypic plasticity differ between open- and closed-canopy biomes?

Abstract

Aim In the absence of fire, closed-canopy species may colonize open habitats (high light, low nutrient) but open-canopy species do not generally colonize closed canopies (low light, high nutrient). I determined whether the asymmetrical colonization across open-closed boundaries is supported by greater plasticity in closed- compared to open-canopy species, the expression of which facilitates persistence in contrasting conditions.

Location Cape Floristic Region, South Africa.

Methods Leaf area index (LAI: proxy for understorey light) and soil nutrition were sampled across forest (closed-canopy) – fynbos (open-canopy) boundaries, including transition vegetation running parallel to the boundary. I quantified leaf traits and foliar nutrition of species occurring across the boundary. To evaluate plasticity, I compared the correlations of species traits and LAI/nutrient availability between forest, transition and fynbos habitats.

Results Forest had higher LAI and nutrient availability than either transition or fynbos. Forest species response to LAI and soil nutrients was significantly greater than either fynbos or transition species, implying greater plasticity. Forest species tended to increase their leaf size, specific leaf area (SLA) and leaf area/stem length but decrease foliar nutrient concentrations with an increase in LAI and soil nutrition. Fynbos species response to environmental heterogeneity was generally weak, suggesting a lack of plasticity.

Main conclusions Plasticity in closed-canopy species may be a consequence of the greater environmental heterogeneity they experience through their lives coupled with the occupation of high nutrient soils, which alleviate belowground constraints. This plasticity may support closed-canopy species colonization of open habitats as their ability to reduce leaf size and SLA will aid the tolerance of oligotrophic soils. Conversely, specialization to oligotrophic soils may compromise plasticity in open-canopy species given the selection for conservative and inflexible leaves.

Introduction

Open- versus closed-canopy biome boundaries (e.g. forest–maquis in New Caledonia, Enright *et al*., 2001; forest–moorland in Tasmania, Wood & Bowman, 2012; forest–grassland in South Africa, Gray & Bond, 2015; forest–savanna in Brazil, Hoffmann *et al*., 2009) are characterized by sharp changes in resource availability and disturbance regime. In the absence of fire, closed-canopy species may colonize open habitats but open-canopy species do not generally colonize closed canopies (Brown & Podger, 1982; Manders & Richardson, 1992; McCoy *et al*., 1999; Hoffmann *et al*., 2009). This asymmetry in boundary movement suggests that closed-canopy species may be better equipped to cope with contrasting resource conditions than open-canopy species. Plants cope with resource variations by adapting to the alternative conditions or through the expression of phenotypic plasticity, thus allowing them to maintain growth and reproduction across ecological gradients (Bradshaw, 1965; West-Eberhard, 1989; Sultan, 2000; Callaway *et al*., 2003). Difference in phenotypic plasticity between open- and closed-canopy vegetation may therefore arise from the exposure to contrasting degrees and types of environmental heterogeneities.

The most striking shift in resource availability between open- and closed-canopy boundaries is the emergent difference in light availability. Where open canopies are light saturated with low levels of light extinction, closed canopies are characterized by strong light gradients that culminate in high levels of light extinction in the understorey (Chapter 3; Manders, 1990a; Enright *et al*., 2001; Hoffmann *et al*., 2009). These contrasting light conditions require the expression of very different traits. For example, species growing under low light conditions commonly increase their allocation to aboveground structures, thus facilitating stem elongation to enhance light capture (Givnish, 1988; Valladares & Niinemets 2008), a strategy not required in full sun. Furthermore, shade leaves tend to be larger, thinner and more chlorophyll-rich than those in sun, to maximize carbon gain under the low, often dynamic light conditions they experience (Valladares & Niinemets, 2008). In contrast, smaller and thicker sun leaves with higher Rubisco and lower chlorophyll contents (Givnish, 1988; Valladares & Niinemets 2008) may help in the alleviation of stress (Givnish, 1979; Niinemets, 2001) from the light saturating, hot and dry conditions encountered in open-canopy environments and the upper storey of closed canopies (Little *et al*., 2012; Ibanez *et al*., 2013). Thus, for species to persist in the diverse light conditions associated with closed-canopy environments there is selection for phenotypic plasticity.

In addition to light, open- and closed-canopy environments differ in nutrient availability, with soils under closed canopies tending to be relatively nutrient-rich compared to those of open habitats (Enright *et al*., 2001; Hoffmann *et al*., 2009; Wood & Bowman, 2012; Gray & Bond, 2015). Higher nutrient

availability in closed-canopy soils may support the higher demand for foliar nutrients in sun-exposed, more photosynthetically active leaves than in shaded leaves (Givnish, 1988; Kitajima, 1994; Valladares & Niinemets, 2008). Moreover, soils in closed canopies may facilitate the vertical growth in height given the competitive pressure associated with such environments (Bloom *et al*., 1985; Givnish, 1988; Cramer, 2012). In contrast, species on nutrient poor soils tend to allocate relatively more resources to roots, including specialized structures such as cluster roots (e.g. Proteaceae, Cyperaceae) and/or symbiotic associations (e.g. N_2-fixation, mycorrhizae) to enhance N and P acquisition (Lambers *et al*., 2008). Cluster root bearing species, however, readily express symptoms of P toxicity with increasing supply (Hawkins *et al*., 2008), apparently because they lack the ability to regulate P uptake (Shane *et al*., 2008). Species that are specialized to nutrient poor soils are thus unlikely to cope with eutrophic soil conditions and consequently exhibit low nutritional plasticity (Lortie & Aarsen, 1996). Plasticity may also be less in plants that promote nutrient conservation traits such as sclerophyllous leaves and serotiny (Niinemets, 2001; McDonald *et al*., 2003; Cramer *et al*., 2014). Although such traits may aid nutrient conservation under impoverished conditions, a conservative strategy generally shows low plasticity with altered resource supply to avoid unsustainable trait production even when conditions are favourable (Chapin, 1980; Valladares *et al*., 2000, 2007). Underlying oligotrophic soil conditions may therefore contribute to low phenotypic plasticity in open-canopy vegetation (Verboom *et al*., 2017).

Asymmetry in species' movements across ecological boundaries during fire intervals coupled with contrasting selective pressures (light gradient and high nutrients versus saturating light and low nutrients) led me to hypothesize that closed-canopy species possess greater phenotypic plasticity than open-canopy species. Consequently, I predict that traits important to light acquisition such as leaf size, specific leaf area (SLA) and the proportional investment in leaf area will respond to changes in light availability to a greater extent in closed- but not open-canopy species. Furthermore, given their occupation of both nutrient-rich and -poor soils (i.e. during colonization of open habitats), coupled with the contrasting photosynthetic demands that dynamic light environments require, I expect the foliar nutrition of closed-canopy species to show a greater responsiveness to changes in both nutrients and light than open-canopy species. To test these predictions, I quantified levels of variation in leaf traits of open- and closed-canopy species, and assessed the extent to which this variation is correlated with light and nutrient availability. Our experimental approach included the exploration of the forest-fynbos biome boundary in South Africa. Fynbos is an open-canopy, fire-prone (*ca.* every 10-15 years) sclerophyllous shrubland in which patches of closed-canopy forest vegetation are inter-dispersed (Mucina & Rutherford, 2006). In the absence of fire (*ca.* > 30 years), forest species begin to colonize fynbos along boundary margins to form a 'scrub' vegetation (McKenzie *et al*., 1977). This scrub

vegetation acts as a transition between forest and fynbos, where light and nutrient availability tend to be intermediate relative to that of its neighbours (Manders, 1990a; Coetsee & Wigley, 2013). The forest–transitional scrub–fynbos vegetation gradient thus provides an ideal location to determine the response of open- and closed-canopy species leaf traits to light and nutrient availability.

Materials and Methods

Study Site

The study was conducted in Orangekloof (33.995° S, 18.394° E), a protected valley in Table Mountain National Park. Vegetation consists of patches of Southern Afrotemperate Forest (hereafter 'forest') inter-dispersed within Peninsula Granite Fynbos and Sandstone Fynbos (hereafter 'fynbos'), all vegetation types within the Forest and Fynbos biomes, respectively, of South Africa (Mucina & Rutherford, 2006). The transition scrub vegetation (*ca.* 10-200 m in width), which I refer to as 'transition', frequently intersects forest–fynbos boundaries in the valley. Management in the valley has actively excluded fire from any of the fynbos vegetation in close proximity to the forest patches since 1972. Rainfall in the valley is predominantly received during winter, culminating in a mean annual rainfall of 1 227 mm (McKenzie *et al*., 1977). The geology consists of Basement Granite at the bottom of the valley, topped by Table Mountain Group sandstones with occasional intrusions of Table Mountain Group shales between the two (McKenzie *et al*., 1977). All sampling was conducted on vegetation inhabiting sandstone-derived soils.

Environmental Heterogeneity

I sampled four sites across the valley to quantify variations in light and nutrient availability between neighbouring patches of forest, transition and fynbos vegetation. In each patch, I set out a 10 x 5 m plot. For an integrated measure of understorey light availability, I quantified leaf area index (LAI: m^2 m^{-2}). LAI was measured using a CI-110 digital canopy imager (CID Bio-Sciences, Washington, USA) that captures hemispherical photographs using a gimballed fish-eye lens. At each plot, I captured 30 images; approximately 1 m aboveground along three evenly spaced 10 m transects to attain a mean plot LAI. In forest vegetation, I considered the recorded LAI value to represent the understorey, while assuming the LAI at the top of the canopy to be zero.

For soil nutrient availability, I collected three surface soil samples within a 50 m radius of the centre of each plot. Prior to analysis, samples were air-dried and passed through a 1 mm sieve. Each sample was then split by repeatedly quartering the soil and taking opposite quarters for each successive

subsample to obtain samples of appropriate volume for each analysis (Gerlach *et al*., 2002). For the determination of total P, K, Ca and Mg, a subsample was dry-ashed at 450°C for 12 h and then analysed using X-Ray fluorescence (XRF) spectrometry. Samples were placed in polypropylene cups and analysed in a Spectro Xepos X-Ray Fluorescence (XRF) analyzer (Spectro, Amatek materials analysis division, Kleve, Germany). All measurements were conducted in a helium atmosphere using a silicon drift detector. I calibrated the results using a certified standard (National Research Center for Certified References Materials, Beijing, China), for which elemental concentrations were obtained from NOAA Technical memorandum NOS ORCA 68 (1992).

For the analysis of total N and C, pH, available P and exchangeable cations, I used samples that were not dry-ashed. Total N and C were determined using mass spectrometry. Approximately 40 mg of soil was placed in a 9 x 5 mm tin capsule (Säntis Analytical, Teufen, Switzerland). Samples were then combusted in a Flash 2000 organic elemental analyzer and the gasses passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all from Thermo Scientific, Bremen, Germany). Results were calibrated using two in-house and one IAEA standard. Soil pH, available P and exchangeable cation concentrations were analysed at the Institute for Plant Production (Department of Western Cape, South Africa). Soil pH was determined by shaking 2 g of material in 20 ml 1 M KCl at 180 rpm for 60 min, centrifuging at 10 000 g for 10 min and measuring the pH of the supernatant. Plant available P was determined according to Olsen (1954). Exchangeable cations (K, Ca and Mg) were extracted with ammonium acetate and EDTA at pH 4.65 and their concentrations determined using a Thermo ICP iCAP 6000 Series Spectrometer (Thermofisher Scientific, Surrey, UK).

Leaf traits and foliar nutrition

Before selecting species for detailed sampling, I recorded the species composition within each plot. Using these data, I selected three forest (*Cassine peragua* L., *Olea capensis* L., *Rapanea melanophloeos* (L.) Mez) and five fynbos (*Anthospermum aethiopicum* L., *Erica hirtiflora* Curtis, *Myrsine africana* L., *Searsia lucida* (L.) F.A.Barkley, *Widdringtonia nodiflora* (L.) Powrie) species that were common to forest and transition or to fynbos and transition. I collected terminal branches from five individuals of each species either in the plot or within 50 m of it, taking care not to sample in the neighbouring vegetation types. Within forest patches, I sampled both understorey (‘shade’) and canopy (‘sun’) branches.

From each branch, five structural leaf traits were measured: leaf size (diameter of the largest circle within the leaf: mm), specific leaf area (SLA: $m^2\ kg^{-1}$), internode length (m), leaf area/stem cross-section area ($m^2\ m^{-2}$), hereafter 'leaf area/stem area', and leaf area/stem length ($m^2\ m^{-1}$). Branch basal diameter was measured 2 cm below the first basal leaf using digital callipers. Once all leaves were removed and weighed, the total length of the branch, including lateral branches, was recorded. For internode length, I measured the distance between the third and second last nodes of the terminal branch. A subsample of five or more leaves coupled with a suitable reference area (1 cm^2) were digitally scanned (CanoScan 4200F, Canon Inc. Japan) to calculate area and size using ImageJ (https://imagej.nih.gov/ij/). Using the area of the subsampled leaves and the total leaf fresh weight, I was able to determine the leaf area of the entire branch. For nutritional analysis, leaves were dried at 60°C for 48 h and milled. Samples were analysed for P, K, Ca and Mg using XRF (see above). For leaf N, between 1 – 2 mg of sample was passed through a mass spectrometer (see above).

Statistical Analysis

All statistical analyses were conducted in R 3.2.3 (R development core team 2015). To investigate the phenotypic plasticity of forest and fynbos species in relation to light and nutrient availability I took a three-fold approach. Firstly, I examined the relative variability of a trait within a species across plots by calculating the coefficient of variation (CV: standard deviation/mean). I chose CV as measure of variation given the large differences in mean trait values between species. Following this, I investigated the responsiveness ('reaction norm'; Sultan, 2000) of species traits to changes in light and nutrient availability using linear regression. The slope and Pearson correlation coefficient of the regression were extracted to determine the magnitude, direction and strength of the relationship. Finally, I compared the differences in trait–environment responses across the forest–transition–fynbos vegetation gradient by relating species trait variation (CV) and the predictive strength (correlation coefficient) of their reaction norms to their relative habitat preference (forest, transition or fynbos). This assessment of phenotypic plasticity between species across forest–fynbos vegetation gradients by comparing species reaction norms to their relative habitat preference differs from the traditional expression of plasticity in which phenotypic responses of a single genotype are compared (e.g. Bradshaw, 1965). Comparison of phenotypic plasticity between species across contrasting niches is appropriate for such vegetation gradients, as suggested by Gianoli and Valladares (2012).

To determine relative habitat preference, I extracted abundance data for each species from a phytosociological study conducted in the valley by McKenzie *et al*. (1977) as per chapter 3. These authors recorded species abundance in 78 plots, which they designated as either forest, fynbos or

transition vegetation. For the purpose of species habitat preference, I first established a linear sequence of scores for the three vegetation types (fynbos = 1, transition = 2, forest = 3). Following this, a species habitat preference was determined as the sum of scores across all plots, with each plots' score (1, 2 or 3) weighted by the relative abundance of the species in that plot. I termed this value a species 'Vegetation Index' score. If a species only occurred in forest, transition or fynbos, its score would be one, two or three, respectively.

Prior to performing the trait–environment linear regressions, all traits and LAI data were log transformed. Given that I was interested in the responsiveness of species to overall change in soil nutrient availability, I simplified the 11 soil nutrient variables using principal components analysis (PCA) to derive a single Soil Nutrient Index (SNI) using the 'prcomp' function in R. For the regressions of CV or correlation coefficient on Vegetation Index I performed a phylogenetic least squares regression (PGLS) to account for the phylogenetic non-independence of the species sampled (Felsenstein, 1985). For the phylogeny, I pruned the Qian and Jin (2016) species-level tree to contain only the species sampled, using 'ape' 3.4 package (Paradis *et al*., 2004) in R. Although *Rapanea melanophloeos* was absent from the published phylogeny, other *Rapanea* species were present and so I randomly selected *Rapanea salicina* as a proxy. The PGLS models were run using 'nlme' 3.1-131 (Pinheiro *et al*., 2017), assuming Brownian motion for trait evolution. To assess the variation in LAI and soil properties between the three vegetation types, I conducted a one-way ANOVA followed by a post-hoc Tukey HSD using 'agricolae' 1.2-4 (de Mendibru, 2016) in R.

Results

Environmental heterogeneity

There was substantial variation in light and nutrient availability across the three vegetation types, with forest tending to have significantly ($P < 0.01$) higher understorey LAI and soil nutrient concentrations than both transition and fynbos (Figs. 4.1a & Fig. S4.1 in Appendix S4 in Supporting Information). LAI within transition vegetation was variable ($0.3 – 2.3$ m^2 m^{-2}) but significantly higher than in fynbos (Fig. 4.1a). In contrast, the nutritional status of transition and fynbos soils were similar (except for Total K and P-Olsen, see Fig. S4.1), a pattern supported by the low explanatory power of the PCA axis (PC 2 = 19.3%) along which fynbos and transition samples were distributed (Fig. 4.1b). Conversely, PC 1, accounting for 68.1% of the total variance strongly reflects the nutritional separation of forest from other vegetation types and was negatively correlated with most soil nutrient properties except total K and Mg (Fig. 4.1b). Given the variation captured by PC 1, I considered this axis to be a good

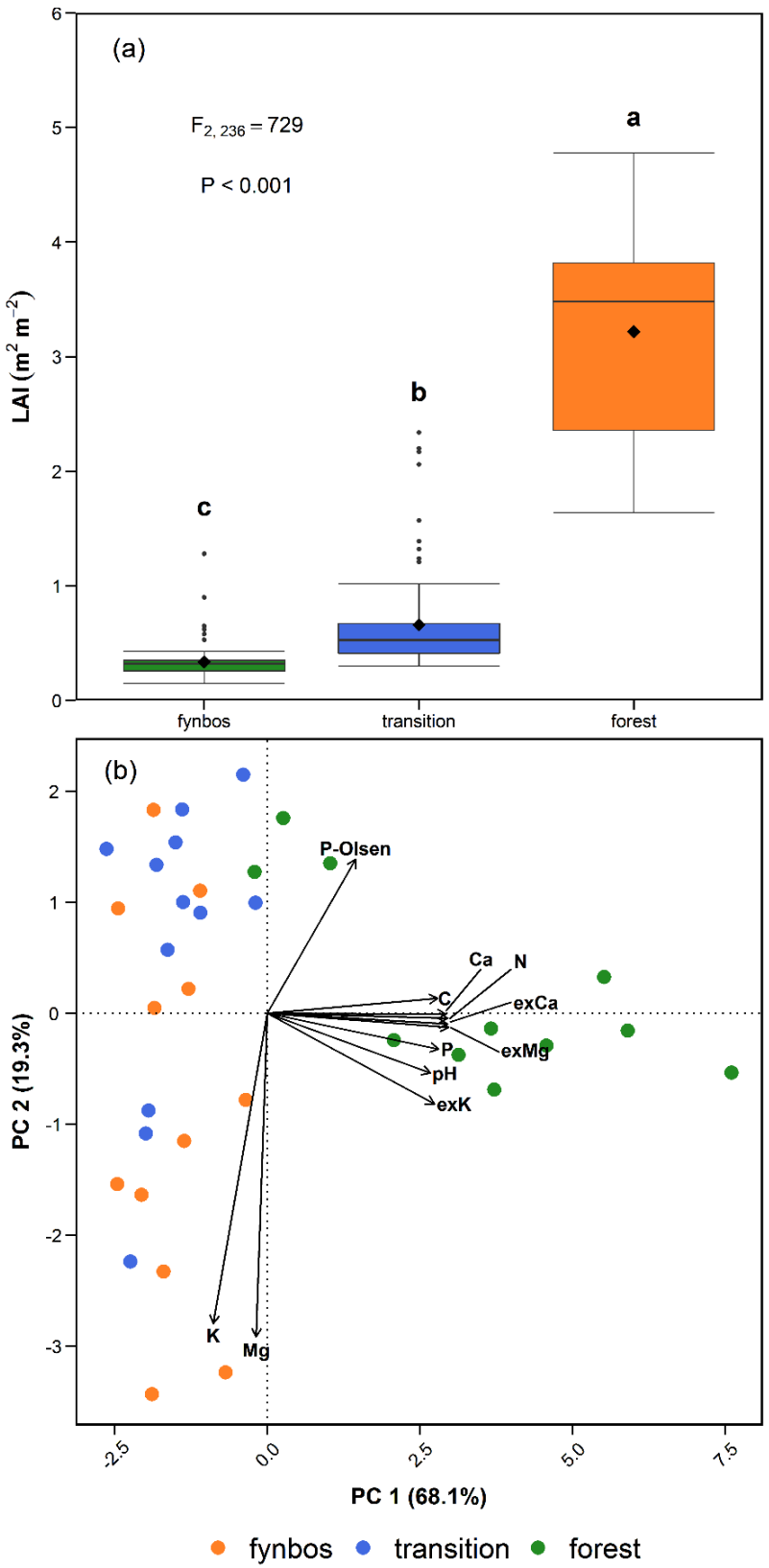

Figure 4.1 (a) Variations in LAI across sampled vegetation types. Box corresponds to interquartile range (IQR: 25th and 75th percentiles). Whiskers extend from the box to the lowest value within 1.5*IQR, with circles representing outliers. Diamonds represent means with letters indicating significant differences between vegetation types determined from a one-way ANOVA. Forest values refer to the understorey LAI as I assumed canopy LAI to be zero. (b) Principal components analysis biplot describing the variation in 11 soil properties between forest, transition and fynbos. Each point represents a sampled plot, while arrows indicate the direction and strength of soil vectors. PC 1 explained the greatest variation in soil properties and was consequently used as the Soil Nutrient Index (SNI).

indicator of changes in nutrient availability and thus used it as a 'Soil Nutrient Index' (SNI). High and low SNI refer to high and low nutrient availability, respectively.

Trait variation

Sampling across the forest–fynbos boundary revealed that species occupying forest tended to have significantly larger leaves and a greater investment in leaf area/stem area and length relative to species common in either fynbos or transition vegetation (Figs. 4.2a, d & e). This striking pattern is illustrated by the *ca.* 41-fold difference in leaf size and two-fold difference in leaf area/stem area between the forest tree *Cassine peragua* (leaf size: mean ± 95% confidence interval = 38.4 ± 4.5 mm; leaf area/stem area: 3130 ± 76 m^2 m^{-2}) and the fynbos shrub *Anthospermum aethiopicum* (0.9 ± 0.1; 1769 ± 3.92). In addition, the positive correlation between the coefficient of variation (CV) and Vegetation Index (Figs. 4.3 a – d) indicated that the relative variance of structural traits, including leaf size, SLA, internode length and leaf area/stem area, was significantly greater in forest than in either fynbos or transition species. A similar pattern was evident for foliar nutrient concentrations, with the CV of [N] and [P] per area being 50% and 36% higher, respectively, in forest than either fynbos or transition species (Figs. 4.3f & g, see Fig. S4.2 for foliar nutrition expressed per mass). Furthermore, forest species had significantly higher foliar [P], [Mg] and [K] than species from the other two vegetation types (Figs. 4.2g, h & j).

Phenotypic responsiveness to light and nutrients

Overall, the responsiveness of structural leaf traits to changes in LAI (excluding internode length) and SNI (excluding internode length and leaf area/stem area) was significantly (phylogenetically least squares regression, $P < 0.01$) stronger and more positive in forest than either fynbos or transition species (Fig. 4.4). Contributing to this pattern was the significant (reaction norm, $P < 0.05$) increase in trait values with LAI and SNI among all three broad-leaved tree species. In the forest tree *Olea capensis*, for example, leaf size and SLA were 1.7 times greater under high relative to low LAI (according to untransformed data). Furthermore, all three forest species increased their investment in leaf area per stem length with increasing LAI (low LAI: mean ± 95% confidence interval = 0.09 ± 0.05 versus high LAI: 0.12 ± 0.09 m^2 m^{-1}) and SNI (0.09 ± 0.07 versus 0.17 ± 0.14). By comparison, the response of fynbos and transition species to LAI was weak to neutral with most species exhibiting correlation coefficients close to zero (Figs. 4.4a – e). Exceptions to this pattern were *A. aethiopicum* (SLA: $r = 0.62$, $P < 0.05$) and *Searsia lucida* (SLA: $r = 0.37$, $P < 0.05$) whose leaves tended be thinner under higher LAI (Fig. 4.4b). Although the response of fynbos and transition species leaf traits to SNI was also weak, *S. lucida* and *Widdringtonia nodiflora*, did significantly increase their leaf area per stem area with SNI (Fig. 4.4j & Table S4.1).

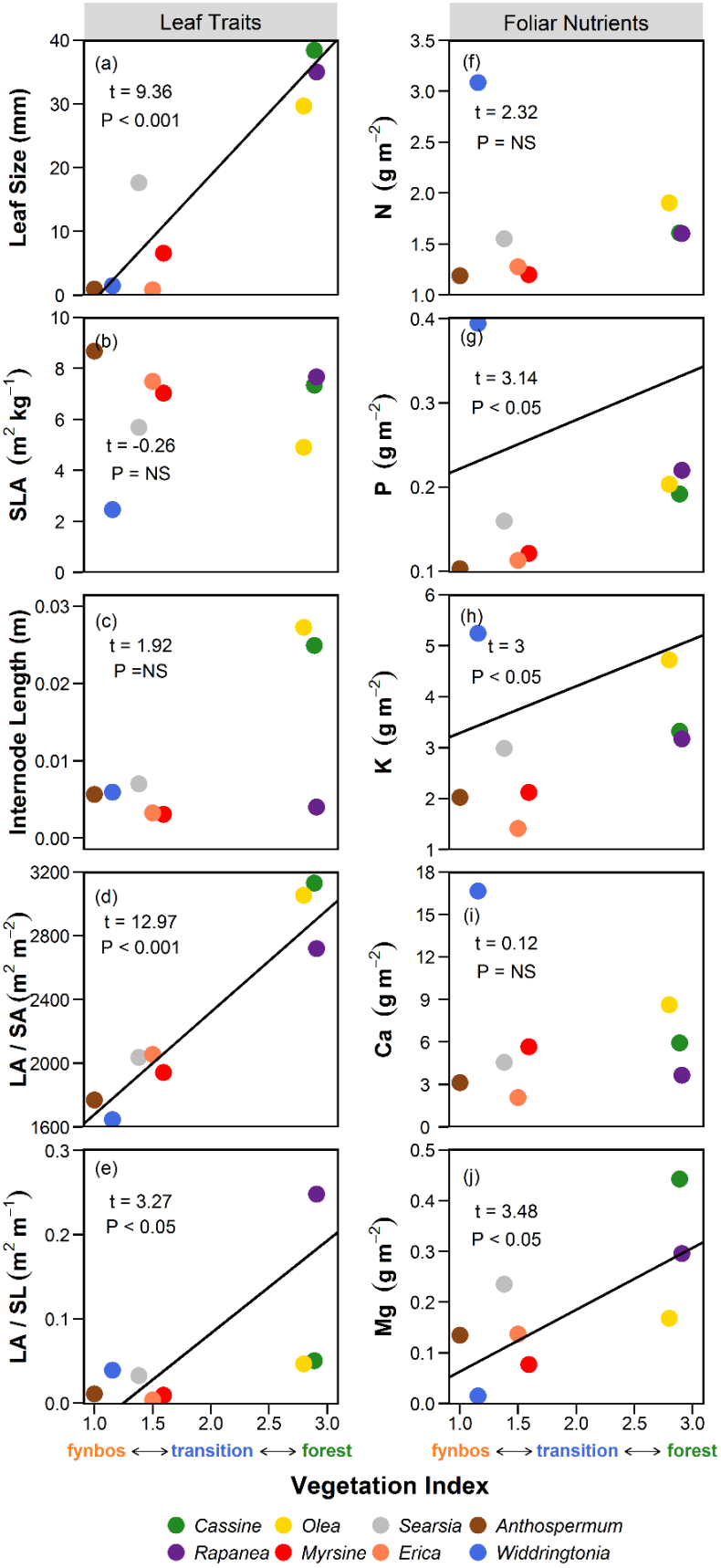

Figure 4.2 Differences in mean species trait values across the forest–fynbos habitat gradient. Regression lines and corresponding statistics derived from PGLS, using 'nlme' 3.1 (Pinheiro *et al*., 2017) in R.

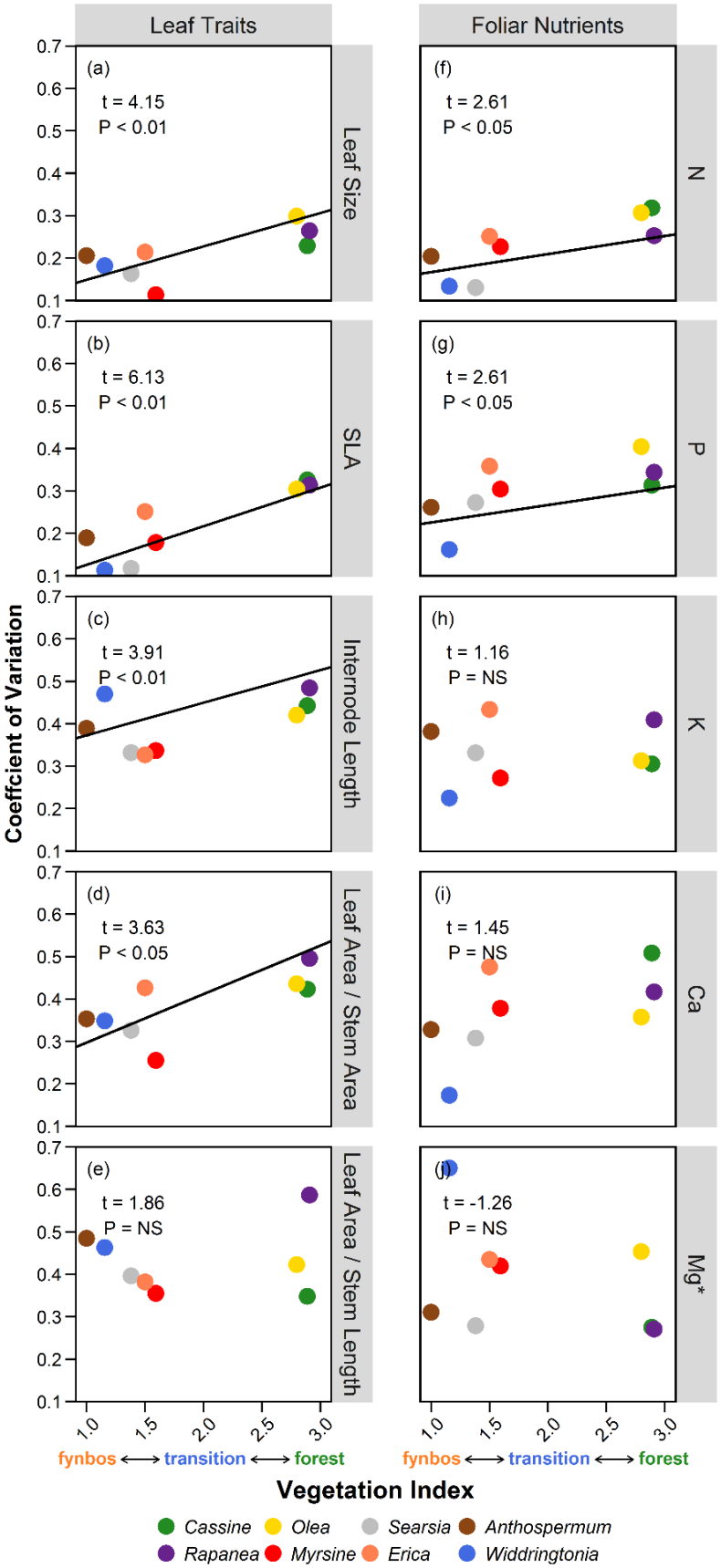

Figure 4.3 Variation in species leaf traits and foliar nutrition (per area) in relation to their relative abundance across the forest–fynbos habitat gradient (Vegetation Index). Circles represent species coefficient of variation (CV). Regression lines and corresponding statistics were derived from PGLS, using 'nlme' 3.1 (Pinheiro *et al*., 2017) in R. For Mg* (j) values were divided by 3 for scaling purposes.

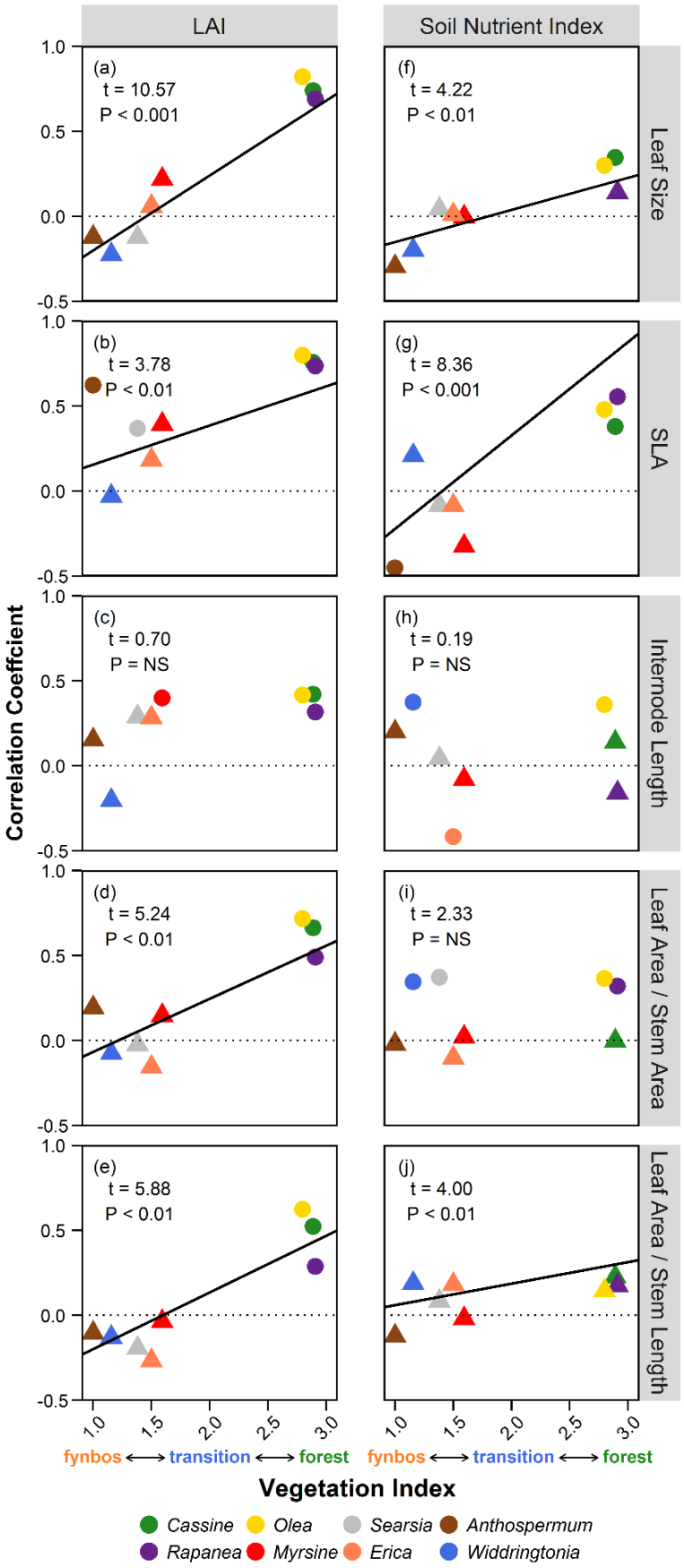

Figure 4.4 The predictive response of species leaf traits to LAI (a – e) or Soil Nutrient Index (f – j) in relation to their relative abundance across the forest–fynbos habitat gradient (Vegetation Index). Circles (P < 0.05) and triangles (P = not significant) indicate the significance of trait–environment linear regressions. Regression lines and corresponding statistics were derived from PGLS, using 'nlme' 3.1 (Pinheiro *et al*., 2017) in R.

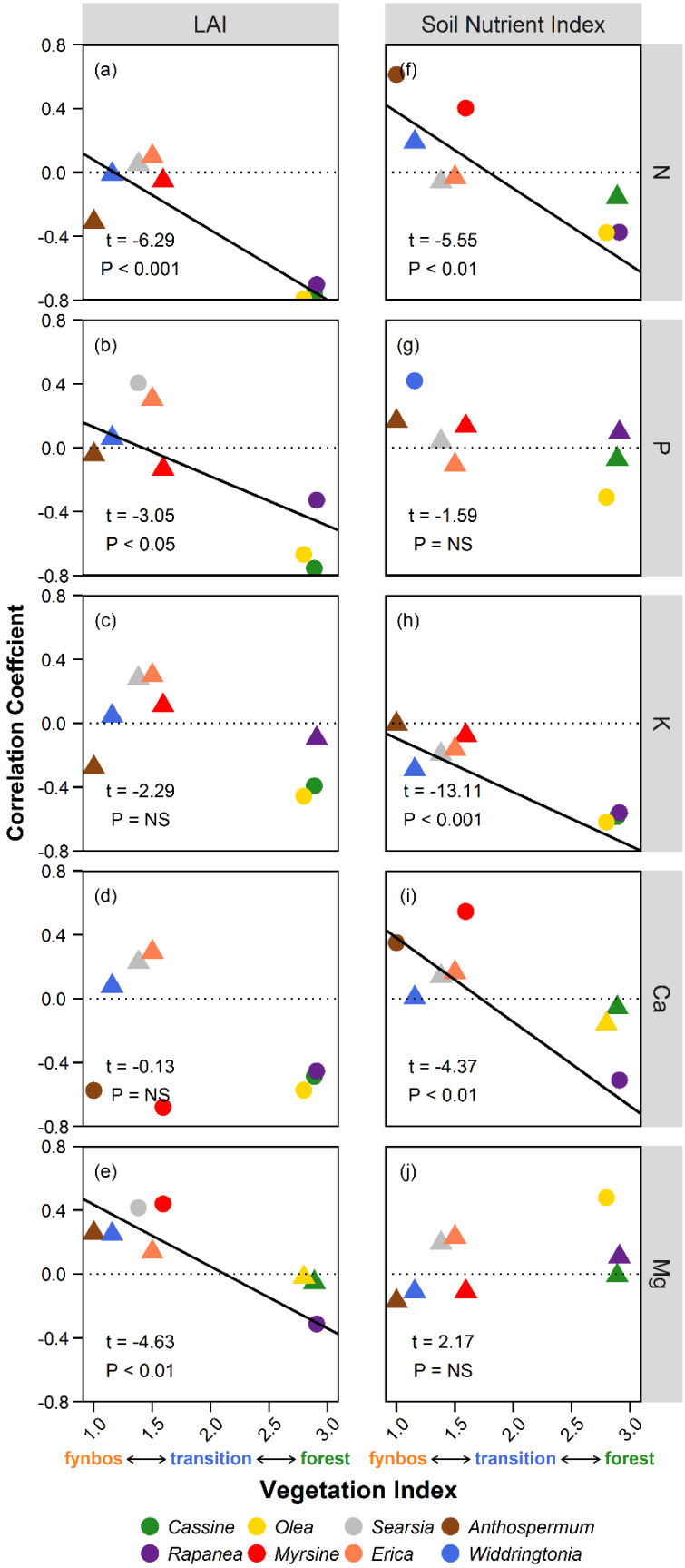

Figure 4.5 The predictive response of species foliar nutrition (expressed per area) to LAI (a – e) or Soil Nutrient Index (f – j) in relation to their relative abundance across the forest–fynbos habitat gradient (Vegetation Index). Circles (P < 0.05) and triangles (P = not significant) indicate the significance of trait–environment linear regressions. Regression lines were derived from PGLS, using 'nlme' 3.1 (Pinheiro *et al*., 2017) in R.

In contrast to the general pattern observed in the structural leaf traits, the response of foliar nutrition per area, specifically [N], [P] and [Mg] to LAI was negatively correlated with Vegetation Index (Figs. 4.5a, b & e, see Fig. S4.3 for foliar nutrition per mass). Driving this pattern were the forest species whose foliar nutrition (per area) tended to decline with LAI. This is highlighted by *O. capensis* whose leaf [N] decreased by 41% with increasing LAI (low LAI: mean ± 95% confidence interval = 2.2 ± 0.3 g m^{-2} versus high LAI: 1.3 ± 0.2 g m^{-2}). With the exception of [Mg], the foliar nutrient concentration of fynbos and transition species were not responsive to changes in LAI (Fig. 4.5e). Unlike forest species, [Mg] in the leaves of fynbos and transition species increased with LAI, the response being significant in *Myrsine africana* and *S. lucida*. Similarly, foliar [N] and [Ca] in fynbos and transition species' leaves increased with SNI, whereas their concentrations in forest species were negatively correlated with the soil index (Figs. 4.5f & i). This relationship was most consistently demonstrated by *Rapanea melanophloeos*, whose foliar [N], [K] and [Ca] were significantly higher on soils with a low versus high SNI (Figs. 4.5f, h & i, Table S4.1).

Discussion

Consistent with previous studies investigating phenotypic plasticity across resource gradients (e.g. Goulart *et al*., 2001; Gratani *et al*., 2006; Portsmuth & Niinemets, 2007), my analyses show that species that experience variable light availability during the course of their development (Fig. 4.1a), express a range of phenotypes (Figs. 4.4 & 4.5, Tables S4.1 & 4.2). Besides the variable selective regime associated with closed environments, the relatively fertile soils of environments experiencing such a dynamic light regime (Figs. 4.1b & S2.1; Chapter 3), may contribute to the realisation of aboveground phenotypic plasticity through the alleviation of belowground constraints (Lortie & Aarssen, 1996; van Kleunen & Fischer, 2005; Valladares *et al*., 2007). By contrast, species inhabiting oligotrophic soils (Figs. 4.1b & S4.1) that require specialization to conservative growth forms for persistence (Figs. 4.2a & b; Valladares *et al*., 2000, 2007), showed little phenotypic variation with resource supply (Figs. 4.4 & 4.5). Moreover, these differences in phenotypic plasticity, may account for the observed asymmetry in the colonization of open-canopy vegetation by closed-canopy trees during the long-term absence of fire (e.g. Brown & Podger, 1982; Manders & Richardson, 1992; McCoy *et al*., 1999; Hoffmann *et al*., 2009).

While the source of trait variation across environmental gradients could be due to variation in genotype (i.e. ecotypes), the very small distances associated with the gradients make this unlikely. The strong and clustered phenotypic response of forest leaf traits to LAI (Figs. 4.4 & 4.5) thus implies that the forest light environment provides a strong selective pressure for the expression of plasticity. This is

perhaps not surprising as the changing light conditions through closed canopies from the shaded understorey (Fig. 4.1a; mean LAI = 3.2 m^2 m^{-2}) to the unshaded upper storey. This LAI change is relatively small when compared to other temperate forests (e.g. 5.7 m^2 m^{-2}, Asner et al., 2003), but likely selects for opposing plant trait values. The larger and thinner leaves, coupled with greater proportional display of leaf area (leaf area/stem length) at high relative to low LAI (Figs. 4.4a & d, Table S4.1) is expected to enhance light interception in the low, often mottled light conditions of forest understoreys (Chapter 3; Givnish, 1988; Valladares & Niinemets, 2008). Furthermore, the negative relationship between foliar [N] and [P] per area, and LAI (Figs. 4.5a & b, Table S4.2) may support the contrasting rates of photosynthesis (high versus low) commonly observed between sun and shade leaves (Givnish, 1988; Kitajima, 1994; Valladares & Niinemets, 2008). The significant correlations of leaf structural and nutritional traits with SNI (Figs. 4.4 & 4.5), however, suggest that nutrient availability may be critical to the realisation of plasticity (Lortie & Aarssen, 1996; van Kleuen & Fischer, 2005; Portsmuth & Niinemets, 2007; Valladares *et al*., 2007). The higher nutrient status of forest soils (Figs. 4.1b & S4.1) reduce the demand for investment in belowground structures and allow trees to grow taller and produce nutritionally expensive larger and thinner leaves, which enhance their ability to capture light (Bloom *et al*., 1985; Lusk & Contreras, 1999; Westoby *et al*., 2002; Cramer, 2012) relative to open habitat species. Selective pressures associated with emergent light gradients and favourable soil conditions are thus both critical to the expression of phenotypic plasticity in closed-canopies such as forest.

In contrast to closed canopies, specialization to relatively nutrient poor soils (Fig. S4.1; Cramer *et al*., 2014; Verboom *et al*., 2017) may have contributed to the generally weak phenotypic responsiveness of open-canopy fynbos species to both LAI and SNI variation (Figs. 4.4 & 4.5). It could be argued that the environmental gradient sampled across fynbos–transition habitats was not large enough to trigger a phenotypic response (Fig. 4.1 & S4.1). Supporting this, the exposure to a strong light gradient (i.e. 24-100% irradiance) in a greenhouse resulted in significant plasticity in gaseous exchange properties (see Figs. 3.6 & 3.7), suggesting that the lack of availability of variation in the field may partially result in the underestimation of species plasticity. The possession of conservative traits (i.e. small, sclerophyllous leaves, Figs. 4.2a & b) by fynbos species in a nutritionally constrained environment, however, is commonly associated with reduced morphological plasticity so as to prevent the production of phenotypes that are unsustainable under predominantly unfavourable conditions (Chapin, 1980; Valladares *et al*., 2000; 2007). Similarly, the occurrence of specialized nutrient acquisition strategies, such as mycorrhizae and cluster roots, among fynbos species (Lamont, 1982; Allsopp & Stock, 1993) possibly contributes to their lack of plasticity in leaf nutritional traits (e.g. foliar [P] per area and mass; Figs. 4.5g & S4.2) even when nutrient availability is variable (e.g. P-

Olsen, Fig. S4.1). In general, plants that experience limited nutrient supply also tend to allocate more biomass belowground to aid acquisition (Bloom *et al*., 1985; Aerts & Chapin, 2000; Lambers *et al*., 2008). This bias in allocation could limit the response of fynbos species to changes in aboveground conditions such as reduced light availability as plants adapted to one limiting factor can rarely cope with simultaneous constraints (Valladares *et al*., 2007). Consequently, it is the specialization to oligotrophic soils, requiring conservative resource use coupled with an enhanced capacity for nutrient acquisition, which underpins a lack of plasticity in relatively oligotrophic open canopies such as fynbos.

Despite the generally limited phenotypic responsiveness of fynbos and transition species, a few species, namely *Anthospermum aethiopicum*, *Myrsine africana* and *Searsia lucida*, showed responsiveness in some traits to changes in LAI and SNI (Figs. 4.4 & 4.5, Tables S4.1 & 4.2) implying that not all species are specialized to the high light and low nutrient conditions in fynbos. Although common in fynbos, these three species also occur in more than four biomes in South Africa (Mucina & Rutherford, 2006), including Albany Thicket, in which light gradients are common (Holmes & Cowling, 1993). Moreover, within fynbos, their distribution includes the occupation of granite-, shale- and sandstone-derived substrates (Mucina & Rutherford, 2006) that vary in nutrient availability (Cramer *et al*., 2014). Thus, the plasticity I observed in these species may be consequence of their wide distribution and the environmental heterogeneity it includes.

While the occurrence of fynbos vegetation on multiple substrates (Bergh *et al*., 2014) indicates that some species may experience spatial variation in nutrient availability, species may also be subject to temporal fluctuations in nutrient availability as evidenced by the temporary flush in soil P in the early stages of post-fire succession (Brown & Mitchel, 1986). The changes in floristic composition through succession (Kruger, 1979; Hoffmann *et al*., 1987), however, imply that few species experience fire-driven nutrient heterogeneity except for long-lived resprouters. The production of variable leaf forms by some resprouters, similar to that observed between juvenile and adult foliage in some conifers (e.g. Hill & Broadribb, 1999; Greenwood *et al*., 2009), suggests that they are plastic to post-fire nutrient fluctuations. For instance, in Restionaceae common to Australian and South African sclerophyllous shrublands, adult resprouters tend to have tall, sclerophyllous culms but immediately post-fire they produce clusters of short culms (Pate & Delfs, 1999; Haaksma & Linder, 2000), possibly to exploit the increased nutrient availability. The high levels of endemism and beta diversity in fynbos (Manning & Goldblatt, 2012; Bergh *et al*., 2014), nonetheless, suggest that the probability of phenotypic plasticity in the vegetation type is low, with species more commonly habitat specialists.

While phenotypic plasticity has been postulated to enable the evolutionary shift of lineages between biomes (Donoghue & Edwards, 2014), it may also be critical to understanding asymmetrical colonization across open- and closed-canopy biomes (Brown & Podger, 1982; Manders & Richardson, 1992; McCoy *et al*., 1999; Hoffmann *et al*., 2009). Phenotypic plasticity in forest species may facilitate their colonization of neighbouring open-canopy vegetation as their ability to reduce leaf size and increase sclerophylly will aid their tolerance of relatively hotter (Little *et al*., 2012; Ibanez *et al*., 2013), moisture stressed (Manders, 1990a) and nutrient poor soils (Figs. 4.1 & S4.1) encountered in open habitats. Underlying the functional significance of this plasticity are evolutionary patterns identified by Onstein *et al*. (2014), where the shift of three forest families into fynbos was associated with a reduction in leaf size and SLA. By contrast, specialization to a hot, dry and nutritionally constrained environment coupled with the conservative leaf traits and specialized root adaptations such conditions require, may contribute to the inability of open-canopy species to colonize closed-canopy vegetation. For instance, while, sclerophyllous leaves on shrubs inhabiting fynbos or the *Eucalyptus* species that dominate Australian savannas (O'Grady *et al*. 2000; Prior *et al*., 2003) may promote nutrient conservation, their failure to respond to changing light conditions (Fig. 4.4) will limit their capacity to capture light in the understorey of forests. In a similar manner, investment in belowground structures such as cluster roots or N_2-fixing nodules (e.g. leguminous trees common to savannas, Mott *et al*., 1985; Scholes & Archer, 1997; Oliveira-Filho & Ratter, 2002) for nutrient acquisition and lignotubers/rhizomes for post-fire resprouting (Bond & Midgley, 2001) that open habitats require, will likely restrict aboveground growth. In savannas, however, lignotubers on their own might not prevent the development of trees; rather the combination of lignotubers and thick bark to enhance fire survival will slow tree growth and thus their ability to compete with forest trees for light (Hoffmann & Franco, 2003; Hoffmann *et al*., 2012; Ondei *et al*., 2016). Thus, I propose that it is differences in plasticity coupled with a trade-off in above versus belowground allocation that act together to maintain open- versus closed canopy biome boundaries.

Chapter 5

General Discussion and Synthesis

The role of stochastic versus deterministic processes in influencing the coexistence of biomes such forest and fynbos

How do two biomes, such as forest and fynbos, which contrast dramatically in physiognomy (i.e. trees versus shrubs) and floristic composition (Chapter 2) coexist stably in the same landscape without one state dominating the other? At a species-level, two contrasting theories have emerged to explain patterns of species distribution, diversity and coexistence. These are classical niche theory, which emphasizes the importance of deterministic processes (Hutchinson, 1959; MacArthur & Levins, 1967; Vandermeer, 1972; Tilman, 1982) and the more recent neutral theory, which emphasizes stochastic processes (Bell, 2001; Hubbell, 2001). A species niche refers to the ecological conditions that a species requires for growth and reproduction. Consequently, coexistence enabled by niche differentiation requires either that species occupy different habitats or they exploit different resource pools in the same habitat, as no two species can compete equally for a resource. By contrast, neutral theory assumes that species are ecologically equivalent, where differences in resource requirement and competitive ability between species are absent with the result that coexistence is random. Both theories have been explored with respect to species coexistence within fynbos (e.g. Richards *et al*., 1997; Latimer *et al*., 2005; Thuiller *et al*., 2006; Araya *et al*., 2011); however, there has been no discussion of their roles in influencing the coexistence of forest and fynbos biomes. In this synthesis I have focussed on forest–fynbos boundaries in the Cape Floristic Region (CFR) as an example of open- versus closed-canopy boundaries that exist elsewhere in South Africa (Chapter 2) and globally (e.g. Enright *et al*., 2001; Hoffmann *et al*., 2009; Staver *et al*., 2011; Wood & Bowman, 2012).

Until the late Miocene (5.3 – 11.6 Ma) the area now incorporating the CFR was largely covered in mesic forest, with fynbos restricted to mountain tops, according to paleo records (Coetzee, 1983; Scott 1995; Dupont *et al*., 2013; Roberts *et al*., 2017). From the late Miocene with the onset of increasing summer aridity particularly in the western CFR, however, it has been hypothesized that forest distribution contracted and became restricted to mesic habitats (Verboom *et al*., 2014; Linder & Verboom, 2015). This apparent historical sensitivity of forest species to moisture deficits is consistent with the contemporary pattern of forest distribution coinciding with greater precipitation in the driest quarter relative to fynbos (Chapter 2), which experiences summer drought (Bradshaw & Cowling, 2014). While seasonal moisture availability may influence the broad distribution of forest and fynbos, both biomes frequently co-occur in areas that receive some of the highest rainfall in the region (e.g.

Orangekloof MAP = 1227 mm; Luger & Moll, 1993), implying a limited role for climate in influencing forest–fynbos coexistence. Nevertheless, forest distribution is still associated with moisture availability given that their soils tend to have a higher moisture content than adjacent fynbos (Manders, 1990a; see Fig. 5.1), probably because forest patches frequently occur on streambanks, rocky screes and in ravines (Campbell & Moll, 1977; McKenzie *et al*., 1977; Manders, 1990a) that act as drainage lines and so accumulate moisture (Western *et al*., 1999). In addition to moisture availability, these topographical features serve as fire refugia for fire-sensitive forest species as they act as barriers to fire, which commonly occurs in fynbos (Geldenhuys, 1994; Watson and Cameron, 2001). The association between forest and fynbos distribution and the imposed heterogeneity in fire and moisture availability suggests that each species in each biome have different ecological requirements and that their coexistence is possibly influenced by niche differentiation.

The existence of distinct fire and soil moisture niches may not purely be a result of heterogeneity in climate and topography, but also emerge from a difference in vegetation structure between forest and fynbos (see Fig. 5.1). Forest trees are tall and form closed canopies whereas low stature fynbos shrubs have open canopies, with the consequence that light availability in forest understoreys is significantly lower and more dynamic relative to fynbos (Chapter 3). Low light availability will lead to a reduction in temperature and evaporative demand in forest, thus contributing to the maintenance of higher soil moisture availability (Manders & Richardson, 1992). Structural divergence may also contribute to the contrasting incidence of fire in forest and fynbos. The pronounced separation of tree canopies from their litter layer coupled with lower foliar concentrations of secondary compounds and higher foliar moisture content compared to fynbos species limits the intrusion of fires into forest (van Wilgen *et al*., 1990). Moreover, a lack of fire in forest allows for leaf litter to accumulate that would otherwise be burnt, which when combined with leaves that are not as sclerophyllous and have higher nutrient concentrations compared to fynbos species (Chapters 3 & 4), potentially results in increased nutrient cycling (Hobbie, 2015). This difference in nutrient cycling between forest and fynbos is almost certainly the source of the relatively higher nutrient availability in forest soils (Chapter 4; Manders, 1990a; Cramer, 2010; Coetsee *et al*., 2015; Cowling & Potts, 2015; see Fig. 5.1) given that both biomes frequently inhabit the same geological formation (i.e. sandstone, see Cramer *et al*. in Appendix S5). The ability of forest and fynbos vegetation to modify their own resource and disturbance regimes (i.e. niche construction *sensu* Odling-Smee *et al*., 1996), appears to signal a limited role for neutral processes in explaining the distribution of forest and fynbos taxa.

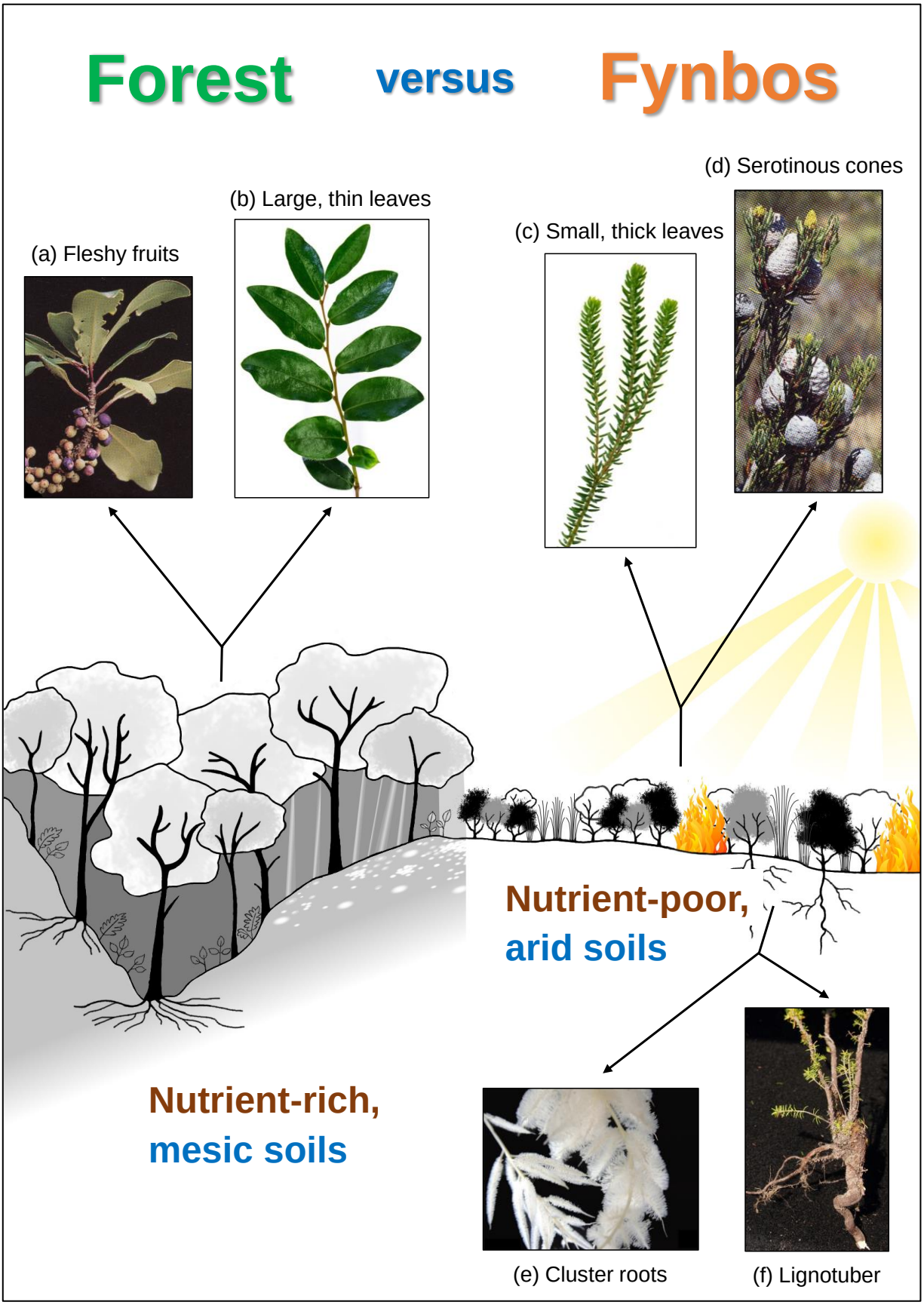

Figure 5.1 A cross-sectional view of a forest–fynbos boundary showing the turnover in environmental regimes and vegetation. Highlighted are the differences light availability and patchiness, soil nutrient and moisture availability, vegetation structure (trees versus shrubs) and some adaptive traits, including: (a) *Rapanea melanophloeos* fruits (van Wyk & van Wyk, 1997); (b) *Dispyros whyteana* branch (Photo: KF Packer); (d) *Phylica ericoides* branch (Photo: KF Packer); (d) *Leucadendron* sp. nov. cones (Rebelo, 2001); (e) *Leucadendron* var. chameleon cluster roots (Lambers & Shane, 2007); (f) *Erica coccinea* lignotuber (Segarra-Moragues & Ojeda, 2010).

The steep turnover in abiotic conditions between forest and fynbos is paired with an equally dramatic switch in adaptive traits (see Fig. 5.1), which possibly reinforces niche differentiation between the observed biomes. For instance, where many forest species rely on of fleshy fruits for reproduction (Manders, 1990b; Cowling *et al*., 1997), fynbos species largely depend on serotinous cones, post-fire flowering and smoke germinated seeds to ensure persistence (Brown, 1993; Lamont & Downes, 2011; Kraaij & van Wilgen, 2014). Given that fire may potentially stimulate reproduction in fynbos species (but see Bradshaw *et al*., 2011), indicates that the distribution of fynbos is likely limited to fire-prone environments. Similarly, the adaptive traits that fynbos species produce in order to persist in the relatively resource constrained environments they inhabit may restrict their distribution. Light-saturated, nutrient-poor and seasonally dry conditions in fynbos environments has led to the selection of small, sclerophyllous and erect leaves which reduce light interception (Falster & Westoby, 2003), promote heat shedding (Yates *et al*., 2010), and nutrient conservation (Cramer *et al*., 2014). Furthermore, many fynbos species, such as those in Proteaceae, invest in cluster roots (Lamont, 1982) and deep root systems (Higgins *et al*., 1987; Manders and Smith, 1992) to enhance the acquisition of nutrients and water. While these leaf and root traits aid the tolerance of constrained resource conditions, they are not suitable in environments where light is limiting and probably contribute to their exclusion from forest (Chapters 3 & 4).

Unlike fynbos, forest understorey environments select for increased investment in aboveground traits, including elongated stems and large, thin and flat leaves with photosynthetic systems that maximize carbon gain under low, dynamic light conditions (Chapters 3 & 4, Givnish, 1988; Valladares & Niinemets, 2008). Although these traits enable shade tolerance, they may limit forest species to nutrient-rich and moist soils, which alleviate trees from belowground constraints and promote aboveground growth. The importance of favourable belowground conditions to forest persistence is highlighted by the inability of climax species to grow and survive under fynbos conditions (i.e. low nutrient soils, summer drought), possibly as a result of their small root systems (Manders & Smith, 1992) and low plasticity in xylem vessel anatomy (February & Manders, 1999). Therefore, as with fynbos species, traits that facilitate species persistence in forest may compromise their ability to compete in fynbos habitats, providing support for the role of niche differentiation in driving forest–fynbos coexistence.

Although niche differentiation may explain the distribution of fynbos and its exclusion from forest understoreys, it does not fully explain the distribution of forest species, especially when considering the colonization of fynbos vegetation by forest species during the long-term absence (up to 50 years) of fire (McKenize *et al*., 1977; Manders & Richardson, 1992). The ability of forest species to establish

in an environment in which resource availability contrasts strongly with their native habitat, implies that environmental filtering is of little relevance to species distribution, thus providing support for the role of stochastic processes in influencing forest–fynbos coexistence. Successful recruitment and establishment in fynbos, however, is limited to a handful of pioneer forest species (Chapter 4; Manders & Richardson, 1992). The ability of these species to tolerate constrained resource supply in fynbos is probably enabled by their plasticity in leaf traits (e.g. leaf size, specific leaf area; Chapter 4) and/or the development of deep root systems (e.g. *Kiggelaria africana*; Manders & Smith, 1992), which increase resource acquisition and conservation. In comparison to this neutrality exhibited by pioneer forest species, the lack of fynbos or climax forest species establishment in opposing habitats (Manders & Richardson, 1992) implies that their distribution is more dependent on imposed and emergent environmental heterogeneities. Hence, the role of deterministic versus stochastic processes in influencing the distribution of species may vary between biomes and species.

Contrary to Manders and Richardson (1992) findings, which indicate that high soil nutrient availability is important to the establishment of climax forest species, Bond (2010), in a study comparing plant and soil nutrient stocks between forest and fynbos environments, proposed that fynbos soils indeed have sufficient nutrient availability to support the development of forest trees. The success of invasive alien tree species across the fynbos biome (Wilson *et al*., 2014) appears to provide support for this proposal and questions the role of niche differentiation in influencing species coexistence. Closer inspection of the alien trees present in fynbos, however, reveals that many of them are native (e.g. *Acacia saligna*, Western Australia; *Pinus halepnsis*, Mediterranean Basin) to environments that are very similar to fynbos in terms of climate, soil nutrition and the incidence of fire (Cramer *et al*., 2014). These species also possess sclerophyllous and/or phyllodinous leaves, specialized root adaptations (e.g. N_2-fixation, *Acacia* spp.; ectomycorrhizae, *Pinus* spp.), serotinous cones and heat stimulated seed germination (Jeffery *et al*., 1988; Morris *et al*., 2011), all traits that are advantageous in nutrient-poor and fire-prone environments. While these traits are similar to those exhibited by fynbos species, they actually provide alien invasive species with a competitive advantage. For example, *Acacia* species are able to rapidly develop deep root systems to enhance water access (Witkowski, 1991) and significantly increase soil N (Yelenik *et al*., 2004), which together competitively exclude fynbos species (Morris *et al*., 2011). A difference in competitive ability is counter to the principles of neutral theory, implying that stochastic processes are unlikely to explain the coexistence of alien trees and fynbos.

The steep turnover in adaptive traits and environmental conditions, which appear to emerge largely from the different floristic components, provides strong support for the role of deterministic processes in influencing the coexistence of forest and fynbos biomes. The ability of pioneer forest species to

establish in fynbos, however, implies that neutral processes may also influence their spatial distribution and coexistence with fynbos. Evidence for the role of multiple processes influencing species coexistence has led several authors to state that it is not necessarily about one process versus another but rather that both deterministic and stochastic processes may influence coexistence to varying degrees (Adler *et al*., 2007; Vergnon *et al*., 2009; Chase & Myers, 2011). Specifically, from the findings I presented here, the coexistence of forest and fynbos biomes appears to be explained by niche differentiation to a large degree, with neutral process perhaps only applying where pioneer forest and fynbos species coexist. Although the present work focuses on the forest–fynbos boundary, the ubiquity in environmental turnover and asymmetrical colonization across open- and closed-canopy boundaries elsewhere (e.g. forest–maquis in New Caledonia, Enright *et al*., 2001; forest–moorland in Tasmania, Wood & Bowman, 2012; forest–grassland in South Africa, Gray & Bond, 2015; forest–savanna in Brazil, Hoffmann *et al*., 2009 and Australia, Tng *et al*., 2013), suggests that it may have broader applications.

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Supporting Information

Appendix S2

Table S2.1 List of genera shared between neighbouring biomes. Genera listed are only for the nine most sampled boundaries.

Biome Boundary					
Forest – Grassland	Forest – Fynbos	Forest – Savanna		Fynbos – Albany Thicket	
Acacia	Aloe	Acacia	Hugonia	Acalypha	Gasteria
Apodytes	Asparagus	Albizia	Hypoestes	Adromischus	Gazania
Asplenium	Blechnum	Alchornea	Justicia	Aloe	Gnidia
Combretum	Brabejum	Aneilema	Lagynias	Anthospermum	Grewia
Crocosmia	Cassine	Balanites	Lannea	Argyrolobium	Gymnosporia
Cyperus	Cunonia	Baphia	Manilkara	Aristida	Haworthia
Diospyros	Curtisia	Bauhinia	Maytenus	Aspalathus	Helichrysum
Dombeya	Cynanchum	Brachylaena	Metarungia	Asparagus	Hermannia
Encephalartos	Diospyros	Canthium	Ochna	Asplenium	Heteropogon
Englerophytum	Ehrharta	Carissa	Olea	Athanasia	Hibiscus
Faurea	Euclea	Combretum	Panicum	Azima	Indigofera
Gymnosporia	Gerbera	Commiphora	Pavetta	Barleria	Lampranthus
Pavetta	Grewia	Coptosperma	Philenoptera	Berkheya	Lotononis
Plectranthus	Heeria	Crassula	Plectranthus	Bobartia	Lyperia
Pteris	Ischyrolepis	Croton	Prionostemma	Brachiaria	Merxmuellera
Rapanea	Laurophyllus	Dalbergia	Pteleopsis	Bulbine	Oedera
Rhus	Maytenus	Dialium	Putterlickia	Capparis	Otholobium
Streptocarpus	Metrosideros	Diospyros	Rhoicissus	Centella	Oxalis
Syzygium	Olea	Dombeya	Rhus	Ceropegia	Pelargonium
Thunbergia	Oxalis	Encephalartos	Sclerochiton	Cheilanthes	Pentaschistis
	Podocarpus	Englerophytum	Stangeria	Chrysanthemoides	Polygala
	Pterocelastrus	Eragrostis	Streptocarpus	Chrysocoma	Pterocelastrus
	Rapanea	Faurea	Strychnos	Commelina	Pteronia
	Rhus	Grewia	Syzygium	Cotyledon	Rhoicissus
	Rumohra	Gymnosporia	Tarchonanthus	Crassula	Rhus
	Schoenoxiphium	Heteropyxis	Thunbergia	Cymbopogon	Sansevieria
			Trichilia	Cynodon	Sceletium
			Xeroderris	Cyrtanthus	Schotia
				Digitaria	Sebaea
				Diospyros	Selago
				Dodonaea	Senecio
				Drimia	Spiloxene
				Ehrharta	Sporobolus
				Elytropappus	Stapelia
				Eragrostis	Stipa
				Eriocephalus	Stoebe
				Eriospermum	Sutera
				Euclea	Tephrosia
				Euphorbia	Themeda
				Euryops	Thesium
				Eustachys	Trachypogon
				Felicia	Tristachya
				Ficinia	Zygophyllum

Table S2.1 cont.

Biome Boundary				
Fynbos – Succulent Karoo			Fynbos – Strandveld	
Adromischus	Gasteria	Quaqua	Afrolimon	Passerina
Afrolimon	Gazania	Rhus	Aspalathus	Pelargonium
Albuca	Geissorhiza	Romulea	Asparagus	Phylica
Aloe	Gladiolus	Ruschia	Babiana	Polygala
Amphiglossa	Gnidia	Salvia	Carpobrotus	Psoralea
Amphithalea	Haemanthus	Sceletium	Cassine	Pterocelastrus
Antimima	Haworthia	Selago	Chironia	Putterlickia
Arctotis	Helichrysum	Senecio	Chrysanthemoides	Rhus
Aspalathus	Heliophila	Sparaxis	Cineraria	Romulea
Asparagus	Hemimeris	Stachys	Cladoraphis	Ruschia
Asplenium	Hermannia	Stapelia	Clutia	Salvia
Babiana	Indigofera	Steirodiscus	Crassula	Senecio
Ballota	Ischyrolepis	Stipagrostis	Cullumia	Tetragonia
Berkheya	Ixia	Strumaria	Cynanchum	Thamnochortus
Bulbinella	Justicia	Struthiola	Cynodon	Thesium
Cephalophyllum	Lachenalia	Sutera	Diospyros	Trachyandra
Chlorophytum	Lampranthus	Tetragonia	Ehrharta	Ursinia
Chrysanthemoides	Lapeirousia	Thamnochortus	Eriocephalus	Wahlenbergia
Chrysocoma	Lebeckia	Thesium	Euclea	Willdenowia
Cladoraphis	Limeum	Trichogyne	Euphorbia	Zygophyllum
Clutia	Lobostemon	Tylecodon	Felicia	
Conophytum	Lotononis	Ursinia	Ficinia	
Cotula	Manulea	Wahlenbergia	Freesia	
Crassula	Maytenus	Wiborgia	Geissorhiza	
Cyphia	Microloma	Willdenowia	Gladiolus	
Delosperma	Montinia	Zaluzianskya	Helichrysum	
Didelta	Moraea	Zygophyllum	Hermannia	
Digitaria	Nenax		Indigofera	
Diosma	Nylandtia		Ischyrolepis	
Diospyros	Oedera		Knowltonia	
Dodonaea	Olea		Lachenalia	
Drimia	Oncosiphon		Lampranthus	
Drosanthemum	Ornithogalum		Leysera	
Ehrharta	Osteospermum		Maytenus	
Elytropappus	Othonna		Metalasia	
Eragrostis	Oxalis		Morella	
Eriocephalus	Pelargonium		Myrsine	
Eriospermum	Pentaschistis		Nemesia	
Euclea	Pentzia		Olea	
Euphorbia	Phyllobolus		Osteospermum	
Euryops	Phymaspermum		Osyris	
Felicia	Polygala		Otholobium	
Ficinia	Pteronia		Othonna	

Table S2.1 cont.

Biome Boundary					
Fynbos – Renosterveld				Savanna – Grassland	
Acmadenia	Disa	Leucadendron	Sparaxis	Acacia	Dicoma
Acrodon	Dodonaea	Lichtensteinia	Sporobolus	Acalypha	Dierama
Adromischus	Drosanthemum	Liparia	Stachys	Alepidea	Digitaria
Agathosma	Ehrharta	Lobostemon	Stoebe	Alloteropsis	Diheteropogon
Albuca	Elegia	Lotononis	Strumaria	Aloe	Diospyros
Aloe	Elytropappus	Manulea	Sutera	Ancylobotrys	Dombeya
Amphithalea	Eragrostis	Marasmodes	Tephrosia	Andropogon	Ehretia
Anisodontea	Erepsia	Maytenus	Tetragonia	Anthephora	Elephantorrhiza
Annesorhiza	Erica	Merxmuellera	Thamnochortus	Anthospermum	Elionurus
Anthospermum	Eriocephalus	Metalasia	Themeda	Aptosimum	Encephalartos
Antimima	Eriospermum	Micranthus	Thesium	Argyrolobium	Englerophytum
Arctopus	Euchaetis	Microloma	Tribolium	Aristida	Enneapogon
Arctotis	Euclea	Mohria	Tristachya	Asparagus	Eragrostis
Argyrolobium	Euphorbia	Montinia	Tritoniopsis	Aster	Eriosema
Aristea	Euryops	Moraea	Tylecodon	Barleria	Eriospermum
Aristida	Eustachys	Muraltia	Ursinia	Becium	Euclea
Aspalathus	Felicia	Myrsine	Wahlenbergia	Berkheya	Eulalia
Asparagus	Ficinia	Oedera	Watsonia	Bewsia	Euphorbia
Athanasia	Freesia	Oftia	Wiborgia	Blepharis	Euryops
Babiana	Freylinia	Olea	Willdenowia	Bothriochloa	Faurea
Barleria	Galenia	Ornithogalum	Zygophyllum	Brachiaria	Felicia
Berkheya	Gazania	Otholobium		Brachylaena	Ficus
Bobartia	Geissorhiza	Oxalis		Brachystelma	Frithia
Brachiaria	Gladiolus	Passerina		Bulbostylis	Gazania
Bulbinella	Gnidia	Pelargonium		Calpurnia	Gerbera
Centella	Gymnosporia	Pentaschistis		Celtis	Gladiolus
Chrysanthemoides	Haworthia	Peucedanum		Centella	Gnidia
Chrysocoma	Helichrysum	Phylica		Chaetacanthus	Grewia
Cliffortia	Heliophila	Plecostachys		Chamaecrista	Gymnosporia
Clutia	Hermannia	Printzia		Cheilanthes	Haemanthus
Corymbium	Hesperantha	Protea		Chrysocoma	Helichrysum
Cotula	Hibiscus	Pseudoselago		Clerodendrum	Hemizygia
Crassula	Hordeum	Psoralea		Coddia	Hermannia
Cullumia	Hyparrhenia	Pteronia		Combretum	Heteromorpha
Cymbopappus	Hypodiscus	Putterlickia		Commelina	Heteropogon
Cymbopogon	Indigofera	Restio		Crassula	Hibiscus
Cynanchum	Ischyrolepis	Rhus		Crotalaria	Hippobromus
Cynodon	Ixia	Romulea		Cussonia	Huernia
Cyrtanthus	Knowltonia	Ruschia		Cymbopogon	Hyparrhenia
Delosperma	Lachenalia	Salvia		Cynodon	Hyperacanthus
Didelta	Lampranthus	Selago		Cyrtanthus	Hypoxis
Diosma	Lapeirousia	Senecio		Dichrostachys	Indigofera
Diospyros	Lebeckia	Serruria		Dicliptera	Inezia

Table S2.1 cont.

Biome Boundary				
Savanna – Grassland		Renosterveld – Succulent Karoo		
Ipomoea	Ruellia	Acacia	Euphorbia	Olea
Justicia	Schizachyrium	Adromischus	Euryops	Ornithogalum
Kleinia	Schoenoxiphium	Aloe	Felicia	Osteospermum
Kohautia	Seemannaralia	Amphiglossa	Foveolina	Otholobium
Kyllinga	Selago	Amphithalea	Galenia	Othonna
Kyphocarpa	Senecio	Androcymbium	Galium	Oxalis
Lannea	Setaria	Anthospermum	Gazania	Pelargonium
Ledebouria	Solanum	Antimima	Geissorhiza	Pentaschistis
Lippia	Sporobolus	Antithrixia	Gethyllis	Pentzia
Lopholaena	Stachys	Arctotheca	Gladiolus	Pharnaceum
Lotononis	Syncolostemon	Argyrolobium	Glottiphyllum	Phylica
Loudetia	Tarchonanthus	Aristida	Gnidia	Plantago
Lycium	Tephrosia	Aspalathus	Gorteria	Polygala
Lydenburgia	Teucrium	Asparagus	Helichrysum	Psilocaulon
Melhania	Themeda	Athanasia	Heliophila	Pteronia
Melinis	Thunbergia	Babiana	Hemimeris	Quaqua
Melolobium	Trachypogon	Ballota	Hermannia	Rhus
Microchloa	Tragus	Berkheya	Hesperantha	Rhynchopsidium
Monocymbium	Triaspis	Bulbinella	Ixia	Romulea
Moraea	Tricalysia	Carissa	Karroochloa	Rosenia
Nidorella	Tridentea	Carruanthus	Lachenalia	Ruschia
Orbea	Tristachya	Cheiridopsis	Lampranthus	Schismus
Osteospermum	Urochloa	Chrysocoma	Lapeirousia	Selago
Ozoroa	Vangueria	Clutia	Lasiospermum	Senecio
Pachycarpus	Vernonia	Cotula	Lepidium	Stachys
Panicum	Vitex	Crassula	Lessertia	Stipa
Parinari	Wahlenbergia	Cyanella	Leysera	Strumaria
Paspalum	Watsonia	Cynodon	Limeum	Sutera
Pavetta	Xerophyta	Delosperma	Lotononis	Syringodea
Pavonia	Ziziphus	Diascia	Lycium	Tetragonia
Pearsonia		Didelta	Macledium	Thesium
Pellaea		Dimorphotheca	Manulea	Trachyandra
Pentanisia		Diospyros	Maytenus	Tribolium
Pentzia		Disa	Merxmuellera	Trichodiadema
Plectranthus		Dodonaea	Microloma	Tripteris
Pogonarthria		Drimia	Montinia	Tritonia
Pollichia		Drosanthemum	Moraea	Tylecodon
Protea		Ehrharta	Muraltia	Ursinia
Pterocarpus		Elytropappus	Nemesia	Viscum
Rabdosiella		Eragrostis	Nenax	Wahlenbergia
Rhoicissus		Eriocephalus	Nylandtia	Wiborgia
Rhus		Eriospermum	Oedera	Zaluzianskya
Rhynchosia		Euclea	Oftia	Zygophyllum

Table S2.2 List of families shared between neighbouring biomes. Families listed are only for the nine most sampled boundaries.

Biome Boundary					
Forest – Grassland	Forest – Fynbos	Forest – Savanna		Fynbos – Albany Thicket	
Acanthaceae	Amaryllidaceae	Acanthaceae	Poaceae	Acanthaceae	Rubiaceae
Amaryllidaceae	Anacardiaceae	Amaryllidaceae	Polygalaceae	Aizoaceae	Rutaceae
Anacardiaceae	Apocynaceae	Anacardiaceae	Proteaceae	Amaryllidaceae	Salvadoraceae
Apocynaceae	Asparagaceae	Annonaceae	Pteridaceae	Anacardiaceae	Santalaceae
Araliaceae	Asphodelaceae	Apocynaceae	Rhamnaceae	Apiaceae	Sapindaceae
Aspleniaceae	Asteraceae	Araliaceae	Rubiaceae	Apocynaceae	Scrophulariaceae
Asteraceae	Blechnaceae	Arecaceae	Rutaceae	Asparagaceae	Thymelaeaceae
Capparaceae	Celastraceae	Asteraceae	Salicaceae	Asphodelaceae	Vitaceae
Celastraceae	Cornaceae	Bignoniaceae	Sapindaceae	Aspleniaceae	Zygophyllaceae
Combretaceae	Cunoniaceae	Burseraceae	Sapotaceae	Asteraceae	
Cyperaceae	Cyperaceae	Capparaceae	Scrophulariaceae	Brassicaceae	
Ebenaceae	Dryopteridaceae	Celastraceae	Thymelaeaceae	Campanulaceae	
Fabaceae	Ebenaceae	Combretaceae	Vitaceae	Capparaceae	
Gentianaceae	Euphorbiaceae	Commelinaceae	Zamiaceae	Celastraceae	
Gesneriaceae	Fabaceae	Crassulaceae	Zygophyllaceae	Commelinaceae	
Icacinaceae	Iridaceae	Cucurbitaceae		Crassulaceae	
Iridaceae	Lauraceae	Cyperaceae		Cyperaceae	
Lamiaceae	Malvaceae	Ebenaceae		Ebenaceae	
Malvaceae	Myrtaceae	Erythroxylaceae		Euphorbiaceae	
Myrtaceae	Oleaceae	Euphorbiaceae		Fabaceae	
Oleaceae	Orchidaceae	Fabaceae		Gentianaceae	
Orchidaceae	Oxalidaceae	Gesneriaceae		Geraniaceae	
Poaceae	Penaeaceae	Iridaceae		Hypoxidaceae	
Primulaceae	Poaceae	Lamiaceae		Iridaceae	
Proteaceae	Podocarpaceae	Linaceae		Lamiaceae	
Pteridaceae	Primulaceae	Loganiaceae		Malvaceae	
Rhamnaceae	Proteaceae	Malvaceae		Orchidaceae	
Rubiaceae	Restionaceae	Meliaceae		Oxalidaceae	
Salicaceae	Rhamnaceae	Myrtaceae		Poaceae	
Sapotaceae	Rubiaceae	Ochnaceae		Polygalaceae	
Scrophulariaceae	Rutaceae	Oleaceae		Pteridaceae	
Thymelaeaceae	Sapindaceae	Orchidaceae		Ranunculaceae	
Zamiaceae	Scrophulariaceae	Phyllanthaceae		Rhamnaceae	

Table S2.2 cont.

Biome Boundary					
Fynbos – Succulent Karoo		Fynbos – Strandveld		Fynbos – Renosterveld	
Acanthaceae	Rubiaceae	Aizoaceae	Thymelaeaceae	Acanthaceae	Pteridaceae
Aizoaceae	Rutaceae	Amaryllidaceae	Zygophyllaceae	Aizoaceae	Ranunculaceae
Amaryllidaceae	Santalaceae	Anacardiaceae		Amaryllidaceae	Restionaceae
Anacardiaceae	Sapindaceae	Apocynaceae		Anacardiaceae	Rhamnaceae
Apiaceae	Scrophulariaceae	Asparagaceae		Anemiaceae	Rosaceae
Apocynaceae	Solanaceae	Asphodelaceae		Apiaceae	Rubiaceae
Asparagaceae	Thymelaeaceae	Asteraceae		Apocynaceae	Rutaceae
Asphodelaceae	Zygophyllaceae	Campanulaceae		Asparagaceae	Santalaceae
Aspleniaceae		Celastraceae		Asphodelaceae	Sapindaceae
Asteraceae		Crassulaceae		Asteraceae	Scrophulariaceae
Boraginaceae		Cyperaceae		Boraginaceae	Thymelaeaceae
Brassicaceae		Ebenaceae		Brassicaceae	Zygophyllaceae
Campanulaceae		Euphorbiaceae		Campanulaceae	
Celastraceae		Fabaceae		Celastraceae	
Colchicaceae		Gentianaceae		Crassulaceae	
Crassulaceae		Geraniaceae		Cyperaceae	
Cyperaceae		Iridaceae		Ebenaceae	
Ebenaceae		Lamiaceae		Ericaceae	
Euphorbiaceae		Malvaceae		Euphorbiaceae	
Fabaceae		Molluginaceae		Fabaceae	
Geraniaceae		Myricaceae		Geraniaceae	
Iridaceae		Oleaceae		Iridaceae	
Lamiaceae		Plumbaginaceae		Lamiaceae	
Malvaceae		Poaceae		Malvaceae	
Molluginaceae		Polygalaceae		Molluginaceae	
Montiniaceae		Primulaceae		Montiniaceae	
Oleaceae		Ranunculaceae		Oleaceae	
Oxalidaceae		Restionaceae		Orchidaceae	
Plumbaginaceae		Rhamnaceae		Oxalidaceae	
Poaceae		Rubiaceae		Poaceae	
Polygalaceae		Rutaceae		Polygalaceae	
Polygonaceae		Santalaceae		Primulaceae	
Restionaceae		Scrophulariaceae		Proteaceae	

Table S2.2 cont.

Biome Boundary			
Savanna – Grassland		Renosterveld – Succulent Karoo	
Acanthaceae	Oleaceae	Aizoaceae	Santalaceae
Aizoaceae	Orchidaceae	Amaranthaceae	Sapindaceae
Amaranthaceae	Poaceae	Amaryllidaceae	Scrophulariaceae
Amaryllidaceae	Proteaceae	Anacardiaceae	Solanaceae
Anacardiaceae	Pteridaceae	Apiaceae	Tecophilaeaceae
Apiaceae	Rhamnaceae	Apocynaceae	Thymelaeaceae
Apocynaceae	Rosaceae	Asparagaceae	Zygophyllaceae
Araliaceae	Rubiaceae	Asphodelaceae	
Asparagaceae	Sapindaceae	Asteraceae	
Asphodelaceae	Sapotaceae	Brassicaceae	
Asteraceae	Scrophulariaceae	Campanulaceae	
Boraginaceae	Solanaceae	Celastraceae	
Campanulaceae	Thymelaeaceae	Colchicaceae	
Cannabaceae	Velloziaceae	Crassulaceae	
Capparaceae	Verbenaceae	Ebenaceae	
Caryophyllaceae	Vitaceae	Euphorbiaceae	
Celastraceae	Zamiaceae	Fabaceae	
Chrysobalanaceae		Geraniaceae	
Combretaceae		Hypoxidaceae	
Commelinaceae		Iridaceae	
Convolvulaceae		Lamiaceae	
Crassulaceae		Malvaceae	
Cyperaceae		Molluginaceae	
Ebenaceae		Montiniaceae	
Euphorbiaceae		Oleaceae	
Fabaceae		Orchidaceae	
Hypoxidaceae		Oxalidaceae	
Iridaceae		Plantaginaceae	
Lamiaceae		Poaceae	
Malpighiaceae		Polygalaceae	
Malvaceae		Polygonaceae	
Moraceae		Rhamnaceae	
Myrtaceae		Rubiaceae	

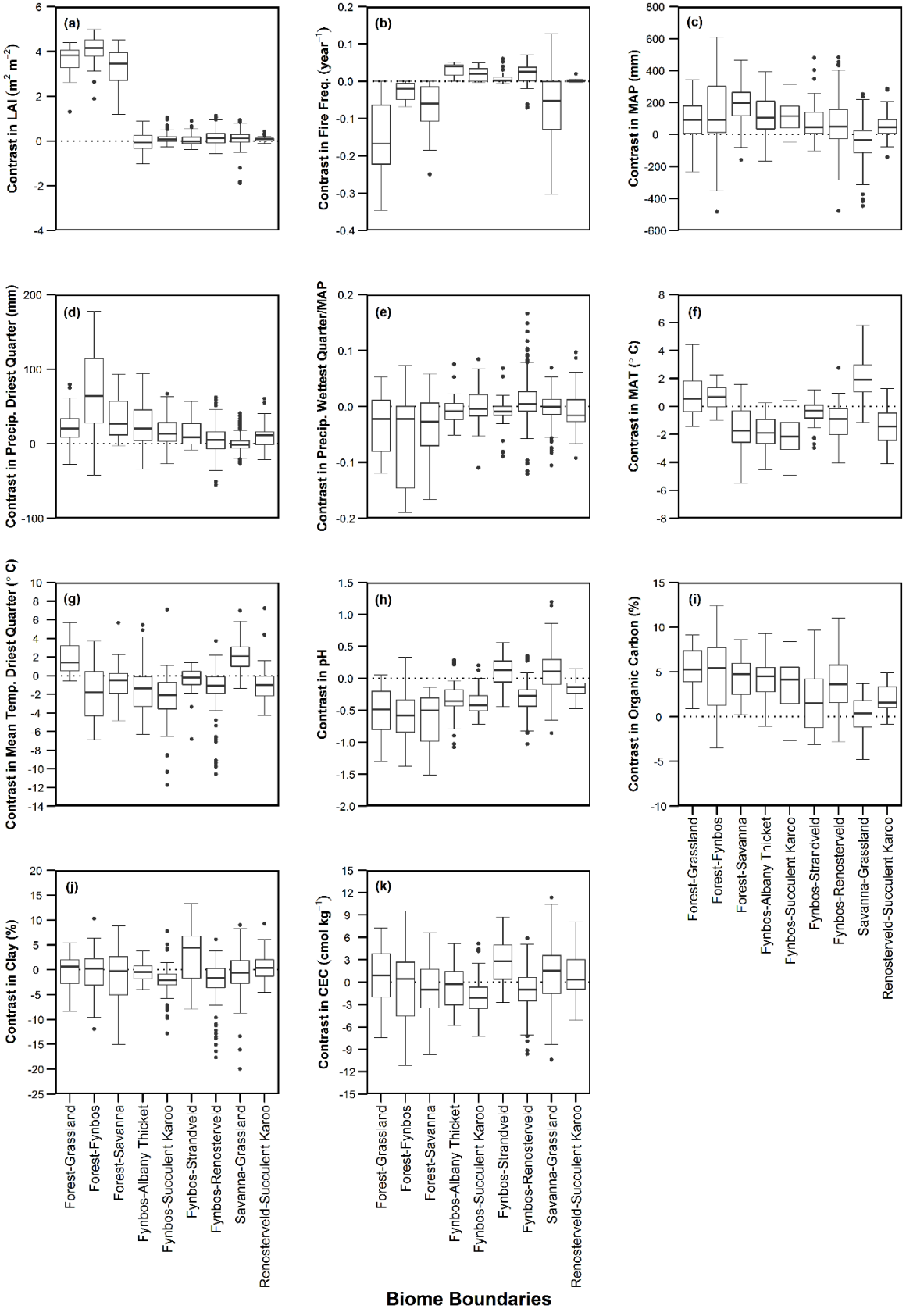

Figure S2.1 Variation in environmental turnover across the nine most sampled boundaries. Values are differences between biomes with direction of turnover provided by subtracting the second biome from the first (e.g. Forest – Grassland). Box corresponds to interquartile range (IQR: 25th and 75th percentiles). Whiskers extend from the box to the lowest value within 1.5*IQR, with points representing outliers. Broken line indicates zero for reference. Freq. = frequency, Precip. = precipitation and Temp. = temperature.

Appendix S3

Experimental growth media

For experiment one, plants were grown in a granite-derived soil (Table S3.1). Before potting, the soil was air-dried and passed through a 10 mm sieve to remove large stones and pieces of organic matter. I then added a cocktail of fertilizer at a rate of 2 and 5g L^{-1} to the surface layers of fynbos and forest species potting bags, respectively, to balance the nutrient availability. The cocktail consisted of 17% Multicote 8 (42-0-0 N:P:K, Haifa Group, South Africa) and 83% Multicote 8 (15-3-12, N:P:K + Mg + Micronutrients). Multicote is a slow release granular fertilizer encapsulated in a multilayer polymeric coating. In experiment two, I added the same cocktail of Multicote, however, at rate of 3g L^{-1} for all species, to a sand medium (Table S3.1). Furthermore, I supplemented the calcium content by adding 0.6g L^{-1} of $CaSO_2$. Both nutrient supplements were mechanically mixed into the sand.

The soil used in experiment one was analysed at BemLab (Somerset West, South Africa). I air-dried the soil and passed it through a 1 mm mesh sieve before analysis. Soil pH was determined by shaking 2 g of soil in 20 ml of 1M KCl at 180 rpm for 60 min, centrifuging 10,000 g for 10 min, and measuring the pH of the supernatant. Total N was measured by the combustion method using a Leco-FP528 N analyzer (Leco, St. Joseph, MI). Total P was extracted by via acid digestion using a 1:1 mixture of 1 N HNO_3 and HCl at 80°C for 30 min (Sommers & Nelson, 1972) with P concentration determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Varian Vista MPX, Mulgrave, Australia). Plant available P was determined by extracting 6.6 g of soil in Bray II solution (Bray & Kurtz, 1945) before filtering and analysing using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Varian Vista MPX). Exchangeable cations were displaced from 10 g of soil with 25 ml of 0.2 M ammonium acetate. The sample was then filtered through Whatman No. 2 filter paper and made to 200 ml before concentrations of Ca, K, Mg and Na were quantified using ICP-AES.

The sand in experiment two was analysed at the Institute for Plant Production (Department of Agriculture, Western Cape, South Africa) for the following properties: pH, P-citric, total K and exchangeable cations. Soil pH was determined following the same method as for the soil above. P-citric was extracted from the sand in 1% (w/v) citric acid, following the protocols of the Soil Science Society of South Africa. Exchangeable cations (Na, K, Ca, Mg) were extracted with ammonium acetate and EDTA at pH 4.65 and their concentrations determined using a Thermo ICP iCAP 6000 Series Spectrometer (Thermofisher Scientific, Surrey, UK). Total N and C were analysed using mass a spectrometrometer at the Department of Archeometry (University of Cape Town). Exactly 190.21 mg of sand was placed in a 9 x 5 mm tin capsule (Säntis Analytical, Teufen, Switzerland). The sample was

then combusted in a Flash 2000 organic elemental analyzer and the gasses passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all from Thermo Scientific, Bremen, Germany). Results were calibrated using two in-house and one IAEA standard.

Table S3.1 Nutritional properties of the growth mediums used during the two pot experiments.

Experiment	Medium	pH	N	C	P	Bray II P	Citric P	K	Na	K	Ca	Mg
			mg g^{-1}			mg kg^{-1}			cmol(+) kg^{-1}			
1	Soil	6.7	0.15	3.9	191	89	-	69	0.1	0.2	6.4	0.24
2	Sand	6.0	0.1	1.0	-	-	18	10	-	-	0.6	0.08

Table S3.2 Biomass and leaf properties (mean ± 95% CI) of all species sampled. Where 95% CI is missing, only one replicate was sampled.

Species	Irradiance (%)	Plant Dry Weight (g)	Shoot:Root	LAR ($m^2\ kg^{-1}$)	Leaf Size (mm^2)	SLA ($m^2\ kg^{-1}$)	LA / SA ($m^2\ m^{-2}$)	LA / SL ($m^2\ m^{-1}$)
forest	24	10.4 ± 4.0	2.3 ± 0.4	2.3 ± 0.6	1578 ± 612	6.3 ± 1	100.5 ± 40.4	0.042 ± 0.006
C. foveolatus	54	12.9 ± 5.8	2.5 ± 0.9	2.7 ± 1.0	1201 ± 513	7.3 ± 1.3	130.6 ± 80.9	0.047 ± 0.007
	100	12.0 ± 2.4	3.3 ± 0.4	3.1 ± 0.6	1456 ± 872	7.5 ± 0.6	139.6 ± 57.7	0.051 ± 0.009
	24	29.6 ± 8.4	8.7 ± 4.1	4.5 ± 1.5	850 ± 444	9.3 ± 1.4	290.2 ± 156	0.031 ± 0.010
D. whyteana	54	41.2 ± 12.1	8.0 ± 4.9	3.3 ± 0.9	621 ± 201	8.2 ± 1.2	209 ± 157.6	0.032 ± 0.011
	100	68.7 ± 44.5	5.1 ± 3.5	2.9 ± 1.0	802 ± 390	6.9 ± 0.7	149.1 ± 94.5	0.031 ± 0.017
	24	136.9 ± 45	3.3 ± 0.8	4.0 ± 1.5	1943 ± 388	13.2 ± 4.1	311.1 ± 100.1	0.067 ± 0.022
K. africana	54	130.5 ± 60.2	3.2 ± 1.2	4.3 ± 1.0	1785 ± 803	14.8 ± 2.3	266.4 ± 59.1	0.055 ± 0.015
	100	147.8 ± 54.2	3.0 ± 0.6	3.4 ± 1.2	1938 ± 1370	12.4 ± 4.9	237.6 ± 73.2	0.068 ± 0.024
	24	160.8 ± 31.0	2.6 ± 0.8	3.1 ± 1.0	898 ± 190	8.7 ± 1.5	333.3 ± 204.4	0.040 ± 0.017
O. europaea	54	151.9 ± 31.2	2.6 ± 0.5	2.6 ± 0.7	985 ± 715	8.1 ± 1.6	315.9 ± 113.3	0.044 ± 0.028
	100	206.9 ± 31.2	2.2 ± 0.5	2.2 ± 0.3	683 ± 491	6.8 ± 0.7	348.6 ± 339.6	0.055 ± 0.06
	24	76.2 ± 24.4	2.6 ± 0.3	4.6 ± 1.5	6404 ± 2124	11 ± 3.3	220.3 ± 69.3	0.184 ± 0.06
R. melanophloeos	54	81.8 ± 42.1	2.9 ± 0.8	4.2 ± 1.4	5802 ± 2824	10.4 ± 2.1	157.4 ± 71.2	0.110 ± 0.050
	100	85.3 ± 47.4	2.6 ± 0.3	4.3 ± 0.8	4880 ± 1321	10.4 ± 0.7	169.7 ± 110.6	0.108 ± 0.067
fynbos	24	30.5 ± 15.2	5.9 ± 1.6	3.4 ± 0.0	6 ± 4	8.3 ± 0.5	139.4 ± 27.5	0.008 ± 0.002
E. versicolor	54	48.0 ± 16.2	5.8 ± 0.9	4.3 ± 0.9	5 ± 2	8.7 ± 2.6	257.5 ± 120.2	0.008 ± 0.002
	100	46.0 ± 4.4	5.6 ± 1.9	3.4 ± 1.0	6 ± 2	7.0 ± 1.2	223.7 ± 242.3	0.01 ± 0.002
	24	21.0 ± 44.0	3.5 ± 4.2	1.6 ± 0.3	6 ± 2	4.3 ± 1.0	92.2 ± 23.3	0.004 ± 0.002
P. ericoides	54	23.4	9.6	2.5	4 ± 2	5.3	82.4	0.004
	100	27.2 ± 34.2	4.9 ± 4.3	2.6 ± 0.6	5 ± 1	5.4 ± 1.4	99.4 ± 59.6	0.005 ± 0.002
	24	116.8 ± 33.7	6.5 ± 1.2	3.2 ± 0.6	1020 ± 788	9.3 ± 1.2	171.8 ± 38.8	0.029 ± 0.013
S. lucida	54	156.4 ± 26.9	5.7 ± 0.8	3.3 ± 0.2	860 ± 436	8.3 ± 0.3	216.3 ± 85.5	0.028 ± 0.006
	100	177.1 ± 16.2	6.5 ± 1.3	3.1 ± 0.1	1054 ± 655	7.7 ± 0.3	201.5 ± 36.8	0.026 ± 0.004

Table S3.3 Light response curve properties (mean ± 95% CI) of all species sampled.

Species	Irradiance (%)	A_{max} ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	R_d ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Quantum Yield (ϕ)	LCP ($\mu mol\ m^{-2}\ s^{-1}$)
forest	24	4.9 ± 2.1	0.17 ± 0.11	0.05 ± 0.01	3.6 ± 1.5
D. whyteana	54	6.6 ± 6.1	0.38 ± 0.05	0.05 ± 0.01	7.7 ± 0.4
	100	7.3 ± 3.5	0.32 ± 0.20	0.05 ± 0.01	6.9 ± 2.4
	24	6.4 ± 3.3	0.30 ± 0.14	0.03 ± 0.01	9.3 ± 3.3
K. africana	54	7.7 ± 3.7	0.25 ± 0.17	0.04 ± 0.02	5.9 ± 1.7
	100	7.6 ± 6.2	0.49 ± 0.43	0.05 ± 0.02	10.4 ± 4.0
	24	8.9 ± 2.6	0.17 ± 0.23	0.04 ± 0.02	3.7 ± 2.8
O. capensis	54	9.5 ± 1.3	0.25 ± 0.21	0.05 ± 0.01	5.3 ± 2.1
	100	9.9 ± 1.4	0.23 ± 0.09	0.04 ± 0.01	5.9 ± 1.7
	24	9.9 ± 5.2	0.34 ± 0.18	0.05 ± 0.01	7.1 ± 1.9
O. europaea	54	9.5 ± 8.6	0.49 ± 0.40	0.04 ± 0.03	15.7 ± 9.3
	100	10.9 ± 5.9	0.64 ± 0.28	0.05 ± 0.01	12.7 ± 2.1
	24	4.3 ± 1.1	0.24 ± 0.29	0.03 ± 0.02	7.4 ± 3.8
R. melanophloeos	54	4.1 ± 3.0	0.60 ± 0.51	0.03 ± 0.01	17.6 ± 7.6
	100	7.3 ± 1.8	0.85 ± 0.47	0.05 ± 0.01	19.8 ± 7.6
fynbos	24	16.9 ± 4.3	0.00 ± 0.00	0.06 ± 0.03	2.0 ± 0.9
B. lanuginosa	54	20.5 ± 5.7	0.00 ± 0.00	0.07 ± 0.04	4.6 ± 0.8
	100	21.8 ± 4.4	0.70 ± 0.69	0.07 ± 0.02	6.2 ± 2.7
	24	8.8 ± 2.0	0.09 ± 0.16	0.03 ± 0.02	0.0± 0.0
E. versicolor	54	11.2 ± 4.0	0.11 ± 0.15	0.04 ± 0.01	0.2 ± 0.3
	100	17.2 ± 6.3	0.45 ± 0.29	0.04 ± 0.0	9.1 ± 4.2
	24	12.9 ± 2.5	0.47 ± 0.27	0.04 ± 0.02	5.1 ± 5.0
P. ericoides	54	13.2 ± 9.6	0.24 ± 0.47	0.03 ± 0.01	4.2 ± 1.7
	100	21.1 ± 5.2	0.78 ± 0.24	0.05 ± 0.01	10.7 ± 3.5
	24	7.7 ± 1.8	0.05 ± 0.05	0.04 ± 0.02	11.1 ± 0.6
S. lucida	54	8.4 ± 2.9	0.23 ± 0.27	0.05 ± 0.01	4.7 ± 6.9
	100	12.2 ± 4.1	0.37 ± 0.38	0.05 ± 0.02	15.0 ± 2.8

Table S3.4 Steady-state gaseous exchange properties, fluorescence and root respiration (mean ± 95% CI) of all species sampled. Where 95% CI is missing, only one replicate was sampled.

Species	Irradiance (%)	Assimilation ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Dark Respiration ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	ΦPSII	Root Respiration ($nmol\ CO_2\ g^{-1}\ root\ DW\ s^{-1}$)
forest	24	1.9 ± 0.7	0.68 ± 0.33	0.62 ± 0.04	13.3 ± 13.5
C. foveolatus	54	3.0 ± 2.3	0.65 ± 0.33	0.31 ± 0.04	9.8 ± 7.8
	100	10.2 ± 2.1	0.86 ± 0.50	0.21 ± 0.02	11.4 ± 3.2
	24	2.7 ± 1.8	0.8 ± 0.34	0.58 ± 0.09	18.5 ± 12.4
D. whyteana	54	5.2 ± 3.1	0.73 ± 0.42	0.37 ± 0.04	9.7 ± 5.8
	100	8.3 ± 5.1	1.02 ± 0.61	0.20 ± 0.05	8.6 ± 10.9
	24	1.0 ± 2.3	0.36 ± 0.12	0.34 ± 0.18	1.9 ± 0.7
K. africana	54	1.6 ± 1.1	0.25 ± 0.12	0.14 ± 0.03	3.1 ± 3.4
	100	3.1 ± 2.1	0.58 ± 0.36	0.08 ± 0.02	2.6 ± 0.6
	24	2.6 ± 1.9	0.27 ± 0.25	0.61 ± 0.11	1.6 ± 1.0
O. europaea	54	5.5 ± 1.0	0.47 ± 0.14	0.40 ± 0.10	1.9 ± 1.1
	100	5.2 ± 3.9	0.52 ± 0.10	0.18 ± 0.05	1.8 ± 0.9
	24	2.2 ± 0.5	0.67 ± 0.54	0.58 ± 0.04	2.2 ± 0.9
R. melanophloeos	54	1.6 ± 0.9	0.62 ± 0.24	0.30 ± 0.06	2.9 ± 1.0
	100	4.8 ± 4.1	0.85 ± 0.34	0.15 ± 0.06	2.5 ± 1.5
fynbos	24	0.5 ± 1.8	1.22 ± 1.38	0.56 ± 0.02	7.4 ± 1.5
E. versicolor	54	0.7 ± 2.1	0.66 ± 0.33	0.34 ± 0.13	4.9 ± 3.3
	100	1.8 ± 1.6	0.72 ± 0.25	0.21 ± 0.05	4.0 ± 1.6
	24	1.0 ± 1.1	0.68 ± 0.49	0.56 ± 0.13	19.6 ± 8.1
P. ericoides	54	0.5 ± 2.1	0.72 ± 0.72	0.21 ± 0.26	12.6
	100	8.2 ± 8.6	0.87 ± 0.68	0.24 ± 0.13	9.0 ± 5.2
	24	1.4 ± 0.7	0.34 ± 0.29	0.44 ± 0.14	3.1 ± 1.5
S. lucida	54	1.0 ± 0.3	0.39 ± 0.10	0.21 ± 0.07	2.3 ± 0.6
	100	2.6 ± 1.7	0.38 ± 0.19	0.14 ± 0.03	2.1 ± 0.7

Table S3.5 Induction and lightfleck sequence properties (mean ± 95% CI) of all species sampled.

Species	Irradiance	$T_{50\%A}$	$T_{90\%A}$	Induction State 30 s	Carbon Gain (mmol m^{-2})		Light-Use Efficiency (%)	
	(%)	(s)		(%)	5 s	30 s	5 s	30 s
forest	24	662 ± 46	1484 ± 912	42 ± 25	0.25 ± 0.12	0.36 ± 0.13	101 ± 51	104 ± 41
D. whyteana	54	652 ± 24	1292 ± 571	38 ± 17	0.3 ± 0.19	0.4 ± 0.23	91 ± 41	103 ± 35
	100	663 ± 24	1271 ± 389	42 ± 7	0.37 ± 0.07	0.41 ± 0.03	107 ± 21	103 ± 16
	24	1107 ± 512	1978 ± 259	12 ± 9	0.11 ± 0.06	0.24 ± 0.15	39 ± 33	78 ± 28
K. africana	54	1193 ± 376	1901 ± 311	10 ± 20	0.17 ± 0.19	0.29 ± 0.20	45 ± 39	70 ± 33
	100	1065 ± 463	1749 ± 524	20 ± 33	0.16 ± 0.08	0.33 ± 0.09	59 ± 71	102 ± 60
	24	808 ± 313	1446 ± 533	28 ± 18	0.30 ± 0.17	0.39 ± 0.13	69 ± 27	82 ± 13
O. capensis	54	761 ± 145	1504 ± 438	26 ± 10	0.34 ± 0.12	0.41 ± 0.14	75 ± 26	84 ± 19
	100	861 ± 200	1395 ± 811	20 ± 18	0.2 ± 0.06	0.33 ± 0.21	44 ± 13	67 ± 40
	24	816 ± 266	1795 ± 92	17 ± 13	0.33 ± 0.20	0.44 ± 0.16	73 ± 33	91 ± 20
O. europaea	54	933 ± 544	1694 ± 724	18 ± 26	0.26 ± 0.27	0.42 ± 0.27	59 ± 59	93 ± 56
	100	759 ± 276	1329 ± 784	35 ± 22	0.45 ± 0.29	0.57 ± 0.38	92 ± 51	121 ± 64
	24	1372 ± 414	1950 ± 458	8 ± 11	0.04 ± 0.05	0.09 ± 0.08	20 ± 22	40 ± 36
R. melanophloeos	54	1458 ± 252	2114 ± 124	5 ± 26	0.03 ± 0.02	0.08 ± 0.08	20 ± 21	75 ± 100
	100	983 ± 632	1613 ± 909	31 ± 27	0.18 ± 0.18	0.24 ± 0.16	63 ± 68	99 ± 51
fynbos	24	808 ± 135	1732 ± 325	19 ± 2	0.55 ± 0.01	-0.13 ± 0.44	128 ± 8	-22 ± 93
B. lanuginosa	54	942 ± 312	1789 ± 307	18 ± 9	0.57 ± 0.25	0.14 ± 1.14	138 ± 143	38 ± 204
	100	1078 ± 397	1946 ± 196	18 ± 10	0.45 ± 0.19	0.24 ± 0.20	71 ± 27	44 ± 48
	24	1160 ± 268	1778 ± 229	11 ± 6	0.13 ± 0.12	-0.09 ± 0.3	23 ± 23	-14 ± 50
E. versicolor	54	1228 ± 142	1759 ± 249	3 ± 5	0.09 ± 0.09	-0.06 ± 0.23	22 ± 23	-11 ± 48
	100	1152 ± 283	1739 ± 374	8 ± 8	0.16 ± 0.15	0.03 ± 0.03	23 ± 23	5 ± 4
	24	1129 ± 119	1760 ± 485	7 ± 2	0.12 ± 0.10	-0.01 ± 0.91	36 ± 32	-9 ± 241
P. ericoides	54	1042 ± 171	1595 ± 192	9 ± 3	0.14 ± 0.32	-0.23 ± 0.21	31 ± 70	-43 ± 33
	100	773 ± 221	1522 ± 258	28 ± 11	0.47 ± 0.33	0.24 ± 0.17	88 ± 65	43 ± 27
	24	1104 ± 182	1777 ± 191	13 ± 6	0.19 ± 0.15	0.32 ± 0.19	23 ± 17	34 ± 18
S. lucida	54	1085 ± 113	1714 ± 104	10 ± 7	0.16 ± 0.08	0.35 ± 0.19	17 ± 9	35 ± 18
	100	1106 ± 277	1717 ± 278	9 ± 12	0.20 ± 0.21	0.41 ± 0.26	23 ± 26	47 ± 31

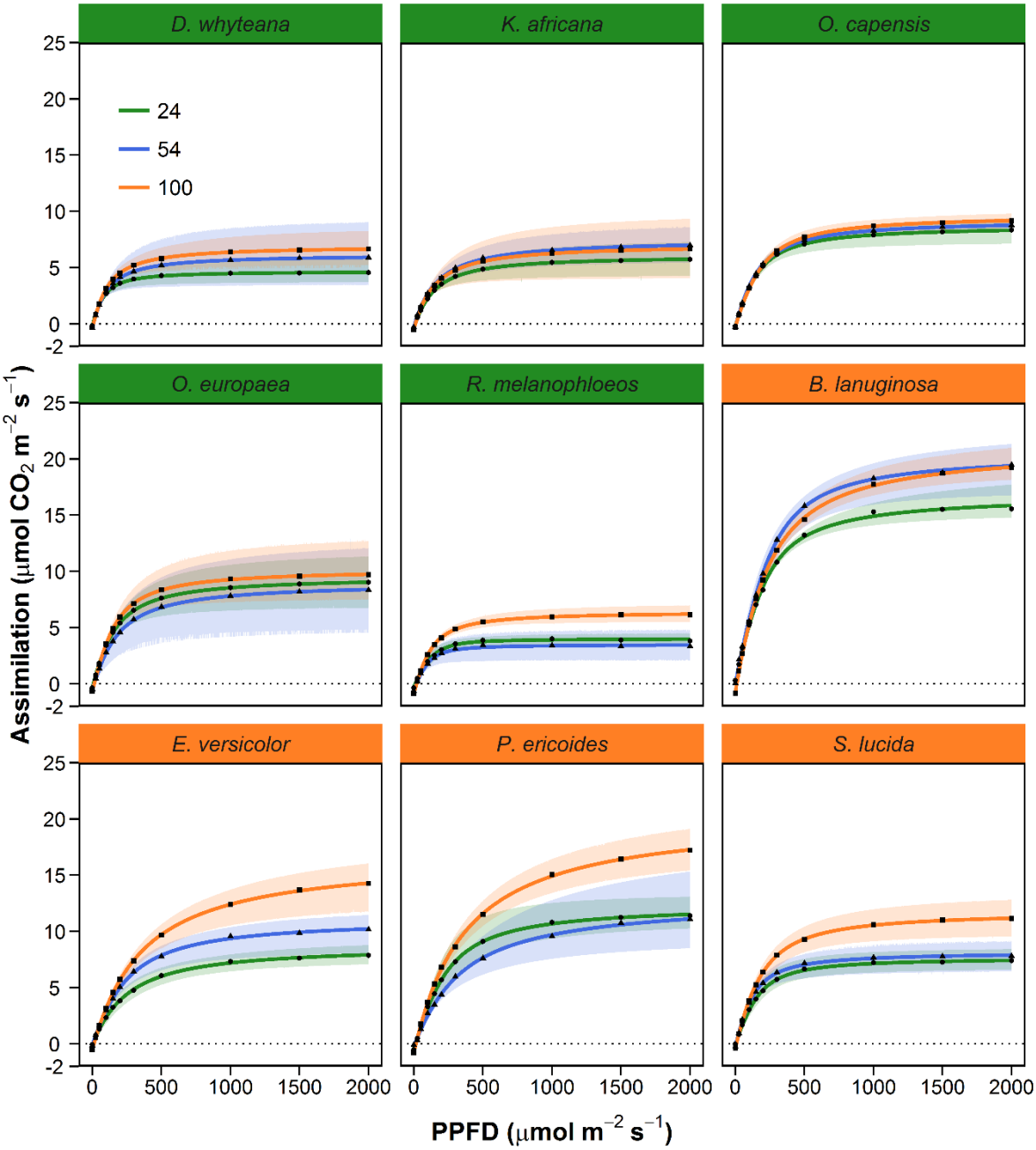

Figure S3.1 Steady-state photosynthetic response of forest (green) and fynbos (orange) species to varying light intensities. Points represent the mean of replicates of each species grown under 24% (circle), 54% (triangle) and 100% (square) irradiance levels. Lines represent mean curves, fitted using a non-rectangular hyperbola model that was optimized using differential evolution in R. Ribbons represent the boostrapped 95% confidence intervals for each light treatment curve.

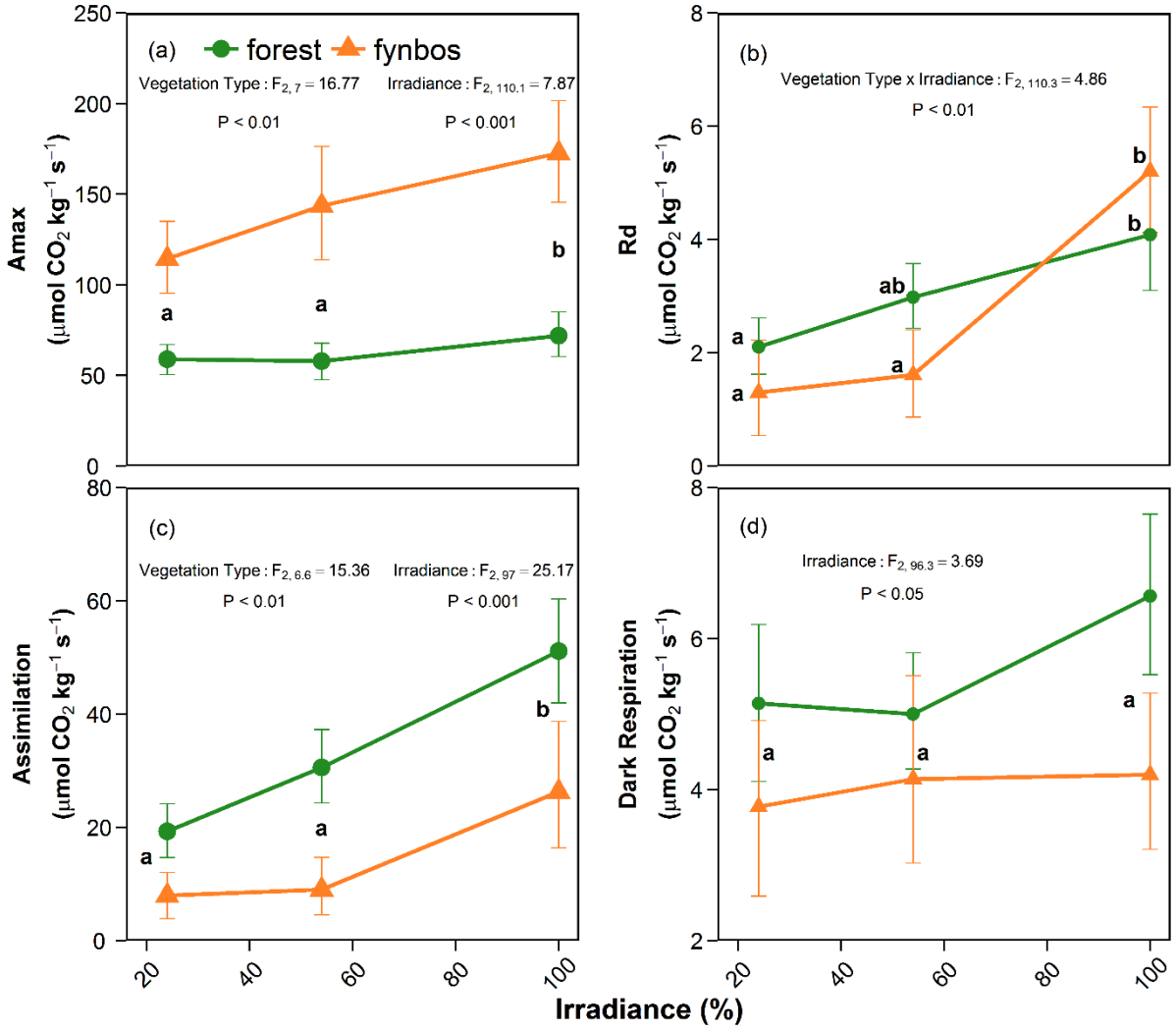

Figure S3.2 Gaseous exchange properties expressed per mass of forest and fynbos species derived from light response curves (a – b) and steady-state rates recorded at 120, 400 and 1000 µmol m^{-2} s^{-1} in the 24%, 54% and 100% irradiance treatments, respectively (c – d). Points and error bars represent the mean ± 95 % confidence interval of replicates grown under each light treatment. Letters denote significant differences between vegetation types and/or light treatments (P < 0.05) from a linear mixed effects model (fixed factor: vegetation type x irradiance, random factor: species). Where significant main effects or interactions occurred, F-stat and P-values are reported.

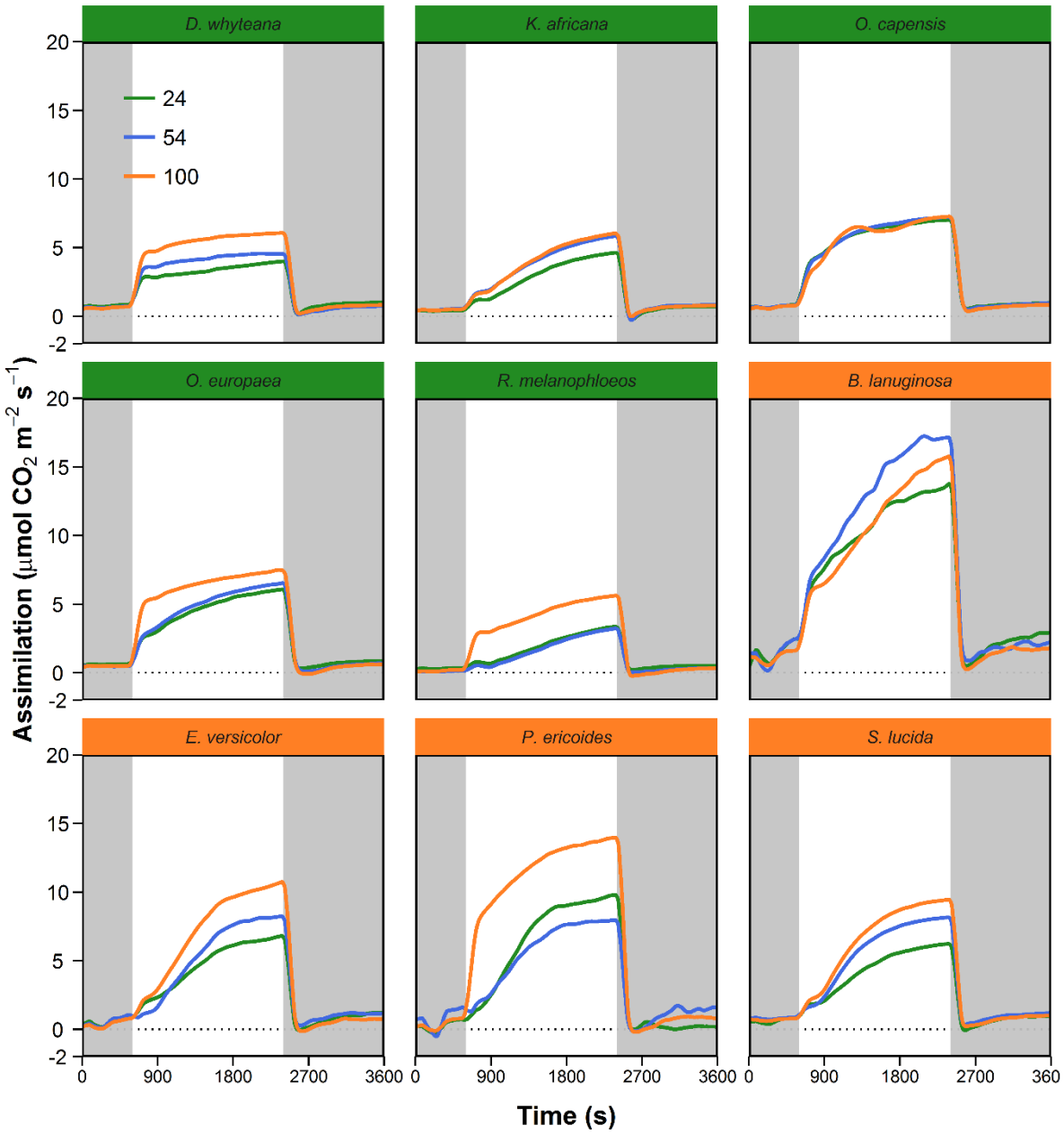

Figure S3.3 The photosynthetic induction gain and loss of forest (green) and fynbos (orange) species to rapid changes in light intensity. Plants were illuminated at low (grey fill: 20 µmol m^{-2} s^{-1}) for 10 minutes followed by high (white fill: 1000 µmol m^{-2} s^{-1}) for 30 minutes and then back to low intensity for 20 minutes. Lines represent the means of replicates of each species grown under 24%, 54% and 100% irradiance.

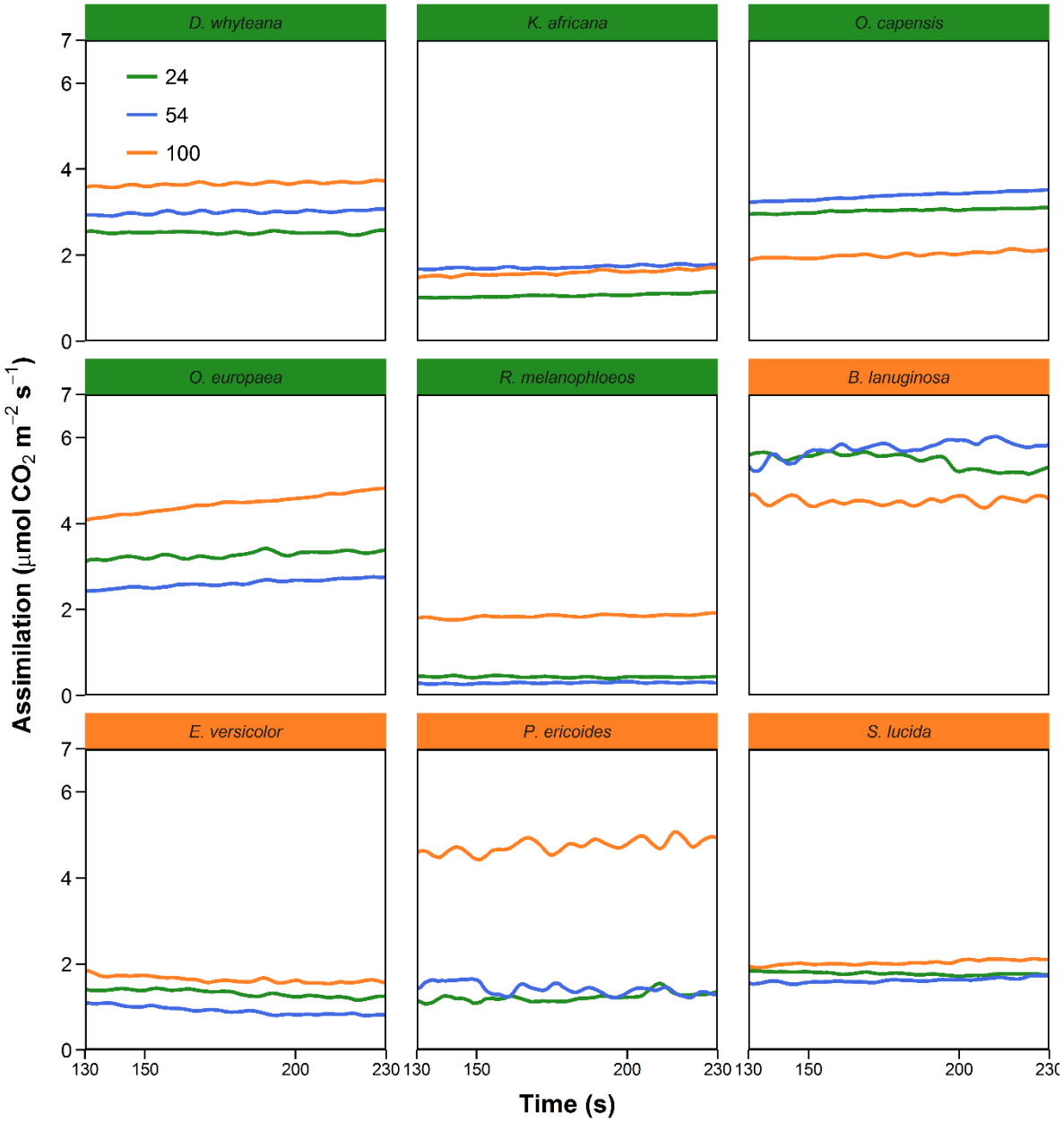

Figure S3.4 Temporal changes in forest and fynbos species photosynthetic rates during a sequence of lightflecks where 5 s high intensity flecks (1000 µmol m^{-2} s^{-1}) were separated by 5 s of low intensity light (20 µmol m^{-2} s^{-1}). Lines represent the means of replicates of each species grown under 24%, 54% and 100% irradiance covering the last 100 s of the sequence.

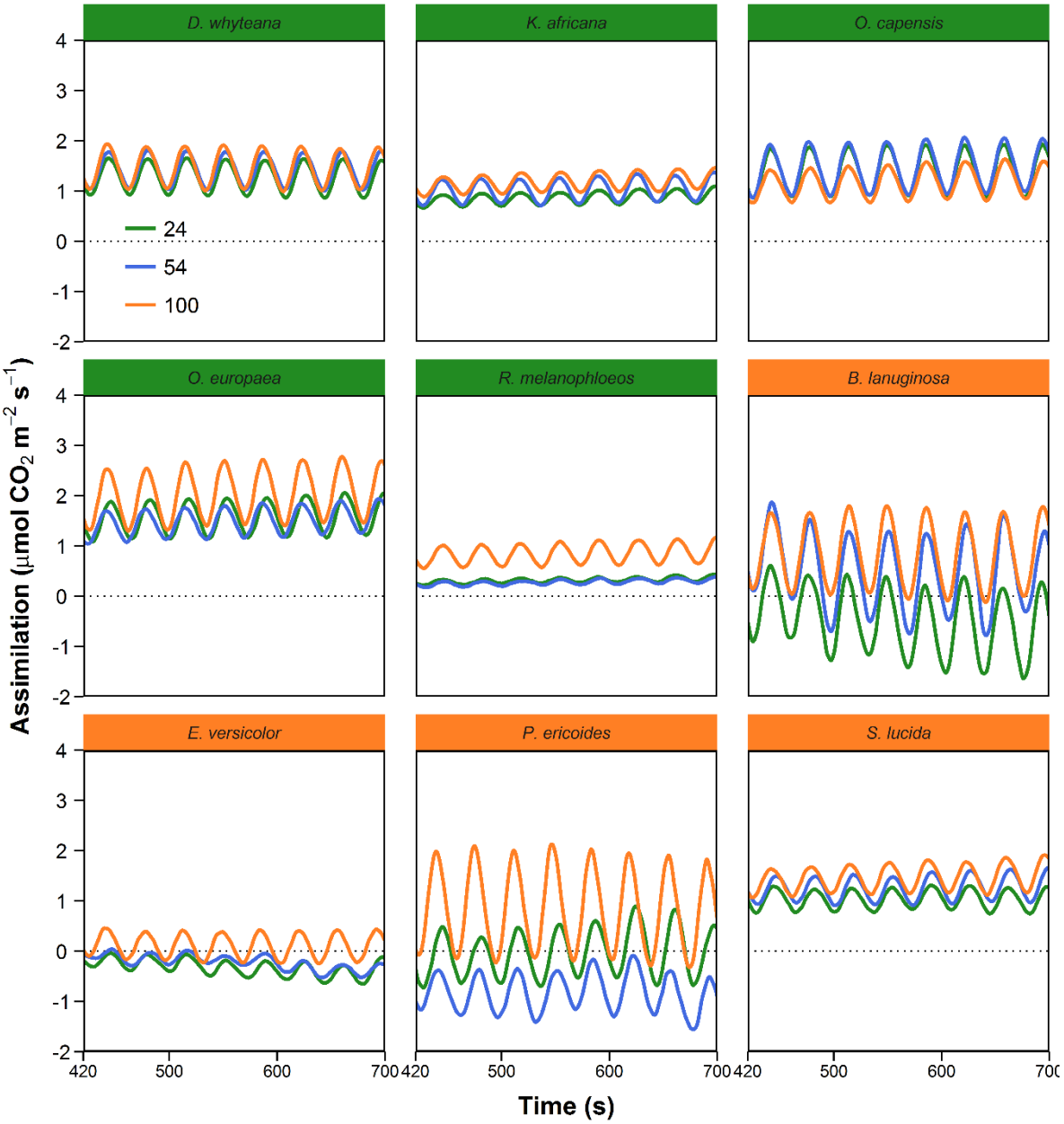

Figure S3.5 Temporal changes in forest and fynbos species photosynthetic rates during a sequence of lightflecks where 5 s high intensity flecks (1000 µmol m^{-2} s^{-1}) were separated by 30 s of low intensity light (20 µmol m^{-2} s^{-1}). Lines represent the means of replicates of each species grown under 24%, 54% and 100% irradiance covering the last 280 s of the sequence.

Appendix S4

Table S4.1 Species relative slope values (log) derived from significant structural trait–environment linear regressions. Missing value indicates a non-significant regression.

Environmental Property	Species	Leaf Size (mm)	SLA (m^2 kg^{-1})	Internode Length (m)	Leaf Area / Stem Area (m^2 m^{-2})	Leaf Area / Stem Length (m^2 m^{-1})
LAI	*Cassine*	0.26	0.35	0.007	0.43	0.01
	Rapanea	0.27	0.30	0.001	0.37	0.05
	Olea	0.38	0.31	0.008	0.45	0.02
	Myrsine	-	-	0.003	-	-
	Searsia	-	0.26	-	-	-
	Erica	-	-	-	-	-
	Anthospermum	-	0.73	-	-	-
	Widdringtonia	-	-	-	-	-
Soil Nutrient Index	*Cassine*	0.03	0.04	-	-	-
	Rapanea	-	0.06	-	0.07	-
	Olea	0.03	0.05	0.002	0.06	-
	Myrsine	-	-	-	-	-
	Searsia	-	-	-	0.28	-
	Erica	-	-	-0.001	-	-
	Anthospermum	-	-0.15	-	-	-
	Widdringtonia	-	-	0.002	0.26	-

Table S4.2 Species relative slope values (log g m^{-2}) derived from significant foliar nutrient–environment linear regressions. Missing value indicates a non-significant regression.

Environmental Property	Species	N	P	K	Ca	Mg
LAI	*Cassine*	-0.40	-0.40	-0.21	-0.33	-
	Rapanea	-0.30	-0.17	-	-0.29	-0.79
	Olea	-0.41	-0.41	-0.25	-0.33	-
	Myrsine	-	-	-	-1.64	6.17
	Searsia	-	0.96	-	-	5.07
	Erica	-	-	-	-	-
	Anthospermum	-	-	-	-1.34	-
	Widdringtonia	-	-	-	-	-
Soil Nutrient Index	*Cassine*	-	-	-0.08	-	-
	Rapanea	-0.04	-	-0.10	-0.09	-
	Olea	-0.05	-0.05	-0.08	-	0.41
	Myrsine	0.13	-	-	0.31	-
	Searsia	-	-	-	-	-
	Erica	-	-	-	-	-
	Anthospermum	0.25	-	-	0.27	-
	Widdringtonia	-	0.13	-	-	-

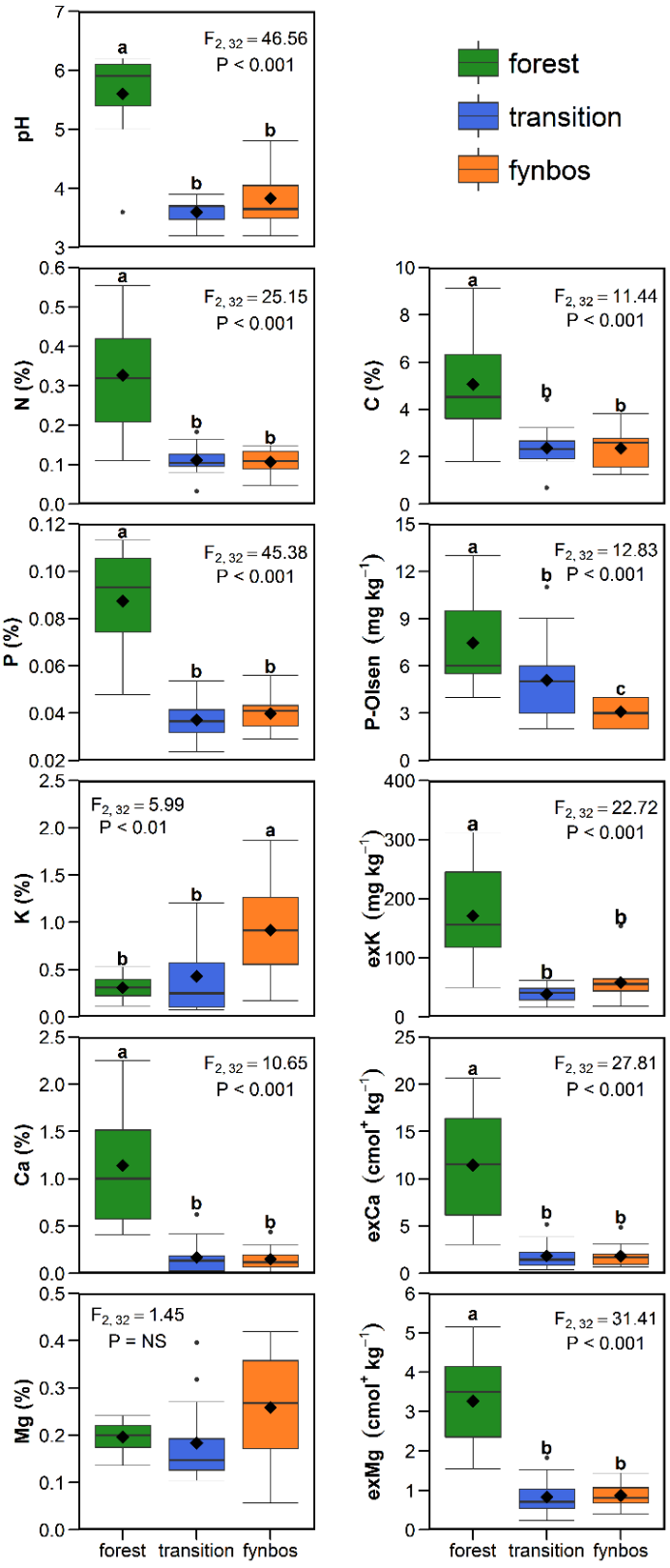

Figure S4.1 Variation in soil nutrient properties between neighbouring vegetation types. Box corresponds to interquartile range (IQR: 25th and 75th percentiles). Whiskers extend from the box to the lowest value within 1.5*IQR, with circles representing outliers. Diamonds represent means with letters indicating significant differences between vegetation types determined from a one-way ANOVA.

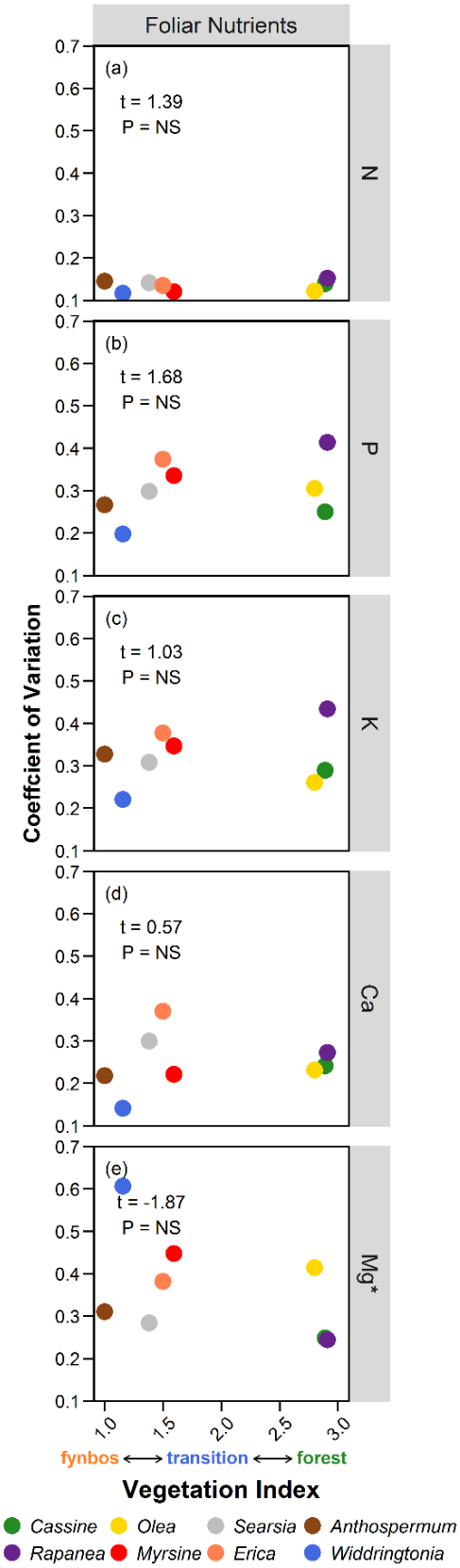

Figure S4.2 Variation in species foliar nutrition (per mass) in relation to their relative abundance across the forest–fynbos habitat gradient (Vegetation Index). Circles represent species coefficient of variation (CV). Statistic were derived from PGLS, using 'nlme' 3.1 (Pinheiro *et al*., 2017) in R. For Mg* (j) values were divided by 3 for scaling purposes.

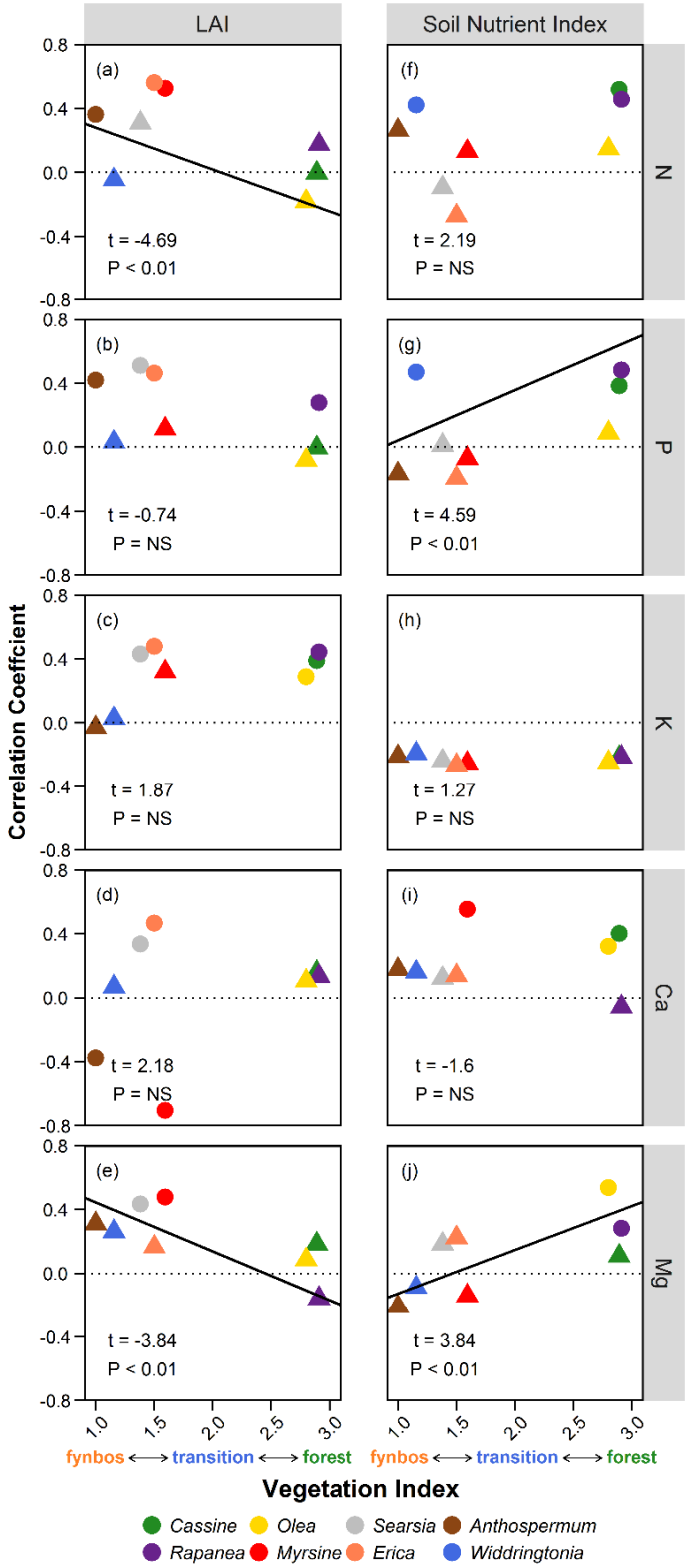

Figure S4.3 The predictive response of species foliar nutrition (expressed per mass) to LAI (a – e) or Soil Nutrient Index (f – j) in relation to their relative abundance across the forest–fynbos habitat gradient (Vegetation Index). Circles (P < 0.05) and triangles (P = not significant) indicate the significance of trait–environment linear regressions. Regression lines and corresponding statistics were derived from PGLS, using 'nlme' 3.1 (Pinheiro *et al.*, 2017) in R.

Appendix S5

Are forest-shrubland mosaics of the Cape Floristic Region an example of alternate stable states?

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Abstract

The idea of alternate stable states (ASS) has been used to explain the juxtaposition of distinct vegetation types within the same climate regime. ASS may explain the co-existence of relatively inflammable closed-canopy Afrotemperate Forest patches ("Forest") within fire-prone open-canopy Fynbos in the Cape Floristic Region (CFR) on sandstone-derived soils. We evaluated the hypothesis that although fire and local topography and hydrology likely determined the paleogeographic boundaries of Forest, present-day boundaries are additionally imposed by emergent edaphic properties and disturbance histories. We studied vegetation and edaphic properties of Forest-Transition-Fynbos vegetation at two sites within the CFR on sandstone-derived soils and tracked historical change using aerial photography. Whereas Forest and Fynbos have changed little in extent or density since 1945, Transition vegetation increased into areas formerly occupied by Fynbos. Forest soils were ubiquitously more nutrient-rich than Fynbos soils, with Transition soils being intermediate. These edaphic differences are not due to geological differences, but instead appear to have emerged as a consequence of different nutrient cycling within the different ecosystems. Soil nutrients are now so different that a switch from Fynbos to Forest is unlikely, in the short term. Floristically, Transitional vegetation is more similar to Fynbos than Forest and may be less resilient to changes in exogenous drivers (e.g., fire). Our findings are consistent with the idea that geologically Forest and Fynbos are largely fire-derived long-term ASS, with the stability of each state reinforced by marked soil nutrient differences. In contrast, the intermediate Transitional vegetation that might switch states is unlikely to be stable.

Keywords: Alternate stable states, ecosystem resilience, edaphic properties.

Introduction

In theory, alternative stable states can develop despite sharing the same environment (Lewontin, 1969). In practice, however, there is considerable debate as to whether natural ecosystems display alternative states that remain stable over ecologically-relevant timescales, and whether shifts between states are caused by changes in exogenous disturbance agents (e.g., fire; Wilson & Agnew 1992) or by changes in endogenous ecosystem interactions (e.g. loss of predators, Beisner *et al*., 2003; increase in elephants, Pellegrini *et al*., 2017). The coexistence of contrasting vegetation types within the same climate envelope has commonly been interpreted as a case of such alternative stable states (ASS; Warman & Moles, 2009; Staver *et al*., 2011; Hirota *et al*., 2011; Dantas *et al*., 2013; Pausas, 2015). An alternative view is that physical factors other than climate (Veenendaal *et al*., 2015), such as edaphic limitations, local hydrology and land-use history (Sankaran *et al*. ,2008, Staver *et al*., 2011) account for vegetation patterns. Since stabilising feedbacks alter soil and other environmental properties (e.g. Jobbágy & Jackson 2000, 2004), it has been difficult to attribute vegetation mosaics to, for example, different soils determining different vegetation or to the emergence of ASS where different vegetation creates different soil properties. Soil chemistry is, however, more likely to change in response to different vegetation than soil physical properties (e.g. soil texture), providing a potential method for unscrambling the different processes. Claims for edaphic control of vegetation mosaics, versus the mosaics controlling contrasting edaphic properties therefore require precise edaphic and historical information for differentiating the alternatives (Veenendaal *et al*., 2015; Lloyd & Veenendaal, 2016).

Changes in the prevalence of fire have been suggested to account for the switch between fire-prone open-canopy and relatively poorly flammable closed-canopy vegetation (e.g. Manders, 1990; Manders & Richardson; 1992, Manders *et al*., 1992; Staver *et al*., 2011; Wood & Bowman, 2012; Coetsee *et al*., 2014). Boundaries between these vegetation types may change over time, suggesting that the internal stabilising feedbacks of each stable state can be over-ridden. Such changes may occur over long time periods (decades to centuries). For example, forest islands in sclerophyllous Tasmanian shrublands were explained through the "ecological drift" model of Canadell *et al*. (1996) that was suggested to lead to alternate states over a period of several centuries (i.e. *ca.* 500 years). This conceptual model allows deviations from average fire frequencies to overcome resilience thresholds and cause state transitions (Wood & Bowman, 2012). An alternate explanation for the Tasmanian forest-shrubland mosaic was proposed by Mount (1979) who interpreted the patterns as being the stable product of differential fire susceptibility defined by site characteristics (e.g. topography) and consequently resulting in different fuel loads. Essentially, these two models incorporating fire can be

viewed as an emergent vegetation patterning model in which alternate states can exist (i.e. Jackson, 1968) versus an environmentally imposed vegetation patterning (i.e. Mount, 1979). Wood & Bowman (2012) found localised vegetation transitions in southwest Tasmania close to forest boundaries with strong fire-vegetation-soil feedbacks, and conlcuded in favour of an emergent vegetation pattern. The slow rates of forest expansion (0.8% over 22 years), however, led the authors to argue that imposed patterns could also coexist within their study area. In this ecosystem both emergent and imposed processes were subsequently concluded to operate over short- (decadal to centenial) and longer-term (centenial to millenial) time scales, respectively (Thomas *et al*., 2010).

The partial dichotomy between emergent and imposed vegetation patterning is highly relevent to the Forest-Fynbos boundary in the Cape Floristic Region (CFR) of South Africa. Patches of Afrotemperate Forest occur in a matrix of lower stature, fire-prone, open-canopy, sclerophyllous shrub vegetation known as Fynbos. Forest-Fynbos boundaries can be sharp (< 10 m), although the vegetation may also grade between Forest and Fynbos through a transitional vegetation that is a mixture of both (McKenzie *et al*., 1977). Fire frequency and intensity are thought to be critical to determining regional forest boundaries (Phillips, 1931; McKenzie *et al*., 1977; Manders & Richardson 1992). Forest-Fynbos boundaries often occur along drainage lines, wind-shadows, slope-breaks and rock screes, all of which represent an imposed limit to fire frequency and/or intensity (van Wilgen *et al*. 1990, Geldenhuys 1994). Furthermore, Forest and Fynbos species have different flammability traits, with shrubby Fynbos being more susceptible to fire than tall Forests (van Wilgen *et al*., 1990), although fire does on occasion transgress the forest boundary (Kraaij *et al*., 2013) during hot, dry and windy conditions (Bradshaw & Cowling, 2014). These differences in flammability suggest that the two vegetation types may also contribute to the position of their boundary resulting in emergent spatial patterning of vegetation with fire adaptation in Fynbos preventing the expansion of fire-sensitive Forest species (van Daalen, 1981; Manders, 1990). Consistent with this, the spatial transitions between these vegetation types have consequently been considered to represent the coexistence of ASS (Coetsee *et al*., 2015).

Humans may alter the fire regime or harvest vegetation and consequently alter the position of vegetation boundaries, potentially resulting in an ASS switch. For example, Pillans (1926) suggested that frequent burning, along with farming and timber harvesting contributed to the decline of Forest cover on the Cape Peninsula between 1700-1900. In contrast, fire protection due to urbanization may have contributed to the expansion of Forests into Fynbos (Poulsen & Hoffman, 2015), despite the higher frequency of fires over the last four decades (Forsyth & van Wilgen, 2008). Likewise, the replacement of rain forest by moorland in Tasmania has been attributed to anthropogenic fires during the Late Glacial period (Fletcher & Thomas, 2010), resulting in contemporary forest expansion being

recolonization, rather then *de novo* expansion. An alternative or possibly complementary driver of the contemporary increase in closed canopy cover might be elevated CO_2 (e.g. Bond & Midgley, 2012) or other aspects of climate- or environmental-change, such as nutrient deposition, which might also contribute to ASS switches.

In addition to a high degree of taxonomic (Power *et al*., 2017) and structural turnover (Forsyth & van Wilgen, 2008), these boundaries are, however, commonly associated with strong nutritional dissimilarities (Manders, 1990; Cramer, 2010; Cowling & Potts, 2015) For example, Forest soils have higher C, N, Ca and K than Fynbos soils, even when occupying the same geology, implying that Forests increase the fertility of soils while frequent fires in Fynbos promote mineralization and loss of nutrients (Coetsee *et al*., 2015). Although this nutritional pattern was consistent across the majority of sites, these authors concluded that there was scant evidence for edaphic conditions contributing to ecological discontinuities across Forest-Fynbos boundaries. Instead they favoured a fire-dependent model for vegetation discontinuities and concluded that these vegetation types represented ASS that could be altered by anthropogenically induced changes in fire regime (Coetsee *et al*., 2014). This putative vegetation change in response to fire regime does not, however, consider how edaphic constraints, including those created by the ASS, might contribute to the stability of the characteristics of the vegetation. If soils do have a role in dictating the structure and flammability of the vegetation, then the perturbation of vegetation by an altered fire regime would have to be sustained for long enough to override the inherent resilience of the ecosystem, including the soil properties, in order for the ecosystem to switch to an ASS.

In the debate as to whether ASS exist between Forest and Fynbos, we therefore need to consider time and the degree to which the sites are fire-prone as a contibuting factors. Emergent boundaries have undoubtedly been reinforced over the long-term through persistent occupancy by a particular vegetation that influences the soil and fire prevalence. We hypothesised that Forest-Fynbos boundaries exhibit a degree of resilience to perturbation that is contingent on the contemporary edaphic circumstances. Switches between alternate stable states are consequently less likely in areas where edaphic properties preclude one or the other of the alternate states. We predict that a consequence of edaphic discontinuities between Forest and Fynbos is that (1) Forests are constrained to more fertile soils and do not readily invade soils where Fynbos exists in a stable state, demonstrating that these are very stable alternate states. 2) In contrast, the transitional vegetation with intermediate susceptibility to fire, occurring on soils that are also intermediate between Forest and Fynbos, is most likely to be sensitive to changes in disturbance. This transitional vegetation may tend towards canopy closure in the absence of disturbance and to a more open-canopy with disturbance, exhibiting the ability to switch

to alternate states depending on disturbance regimes, but these switches are unlikely to be stable. To evaluate these predictions, we explored the vegetation and edaphic properties, and fire history of Forest-Transition-Fynbos in the CFR at two distinct sites.

Materials and Methods

Study sites

The study was conducted at two sites situated in Table Mountain National Park, Blinkwater ravine and Orange Kloof. Blinkwater (33.960003° S, 18.394640° E) is on the western slopes of Table Mountain, above a pipeline built in the mid-1880's. The construction of the pipeline was photographically documented, providing a record of the vegetation at the time (Poulsen & Hoffman, 2015) and was also subject to aerial photographic survey from 1945 onwards (Fig. 1). In the middle part of the ravine, where water flows during the winter months the vegetation is classified as Southern Afrotemperate Forest ("Forest"; Mucina & Rutherford ,2006) and contains trees up to 8 m in height. Adjacent to the ravine the vegetation is dominated by ericoid and proteoid shrubs up to 2.5 m in height, in addition to graminoids. The upper slopes are classified as Peninsula Sandstone Fynbos but this grades into Peninsula Granite Fynbos (collectively "Fynbos") downslope (Mucina & Rutherford, 2006). A transitional vegetation type ("Transition") dominated by thicket and Forest precursor shrub and tree species such as *Phylica buxifolia* and *Cassine peragua* up to 4 m in height occurs between Forest and Fynbos. The boundary between Transition and Fynbos on the southern side is coincident with a line of boulders running downslope (Fig. 1). Soils at the study site are skeletal overlying shallow Table Mountain Sandstone rock, although granitic outcrops and relatively isolated boulders also occur downslope. There are also shale bands of limited extent, exposed in the cliff faces approximately 500 m upslope from the study site. In each of the vegetation types (i.e. Fynbos, Transition and Forest) we selected three, 10 x 5 m plots upslope from the pipeline. Three soil samples were collected in each plot (Fig. 1).

Orange Kloof (-33.994899° S, 18.394476° E) is a 285 ha valley that has been actively managed for over 50 years with aerial photographs of the area available from 1955 onwards (Fig. 1). Management practices have included the active prevention of tree harvesting and fires within close proximity to Forest patches, with no fire occurring since 1972 (McKenzie *et al*., 1977). The vegetation contains typical elements of Peninsula Sandstone Fynbos on west facing valley slopes at higher elevations while the lower valley and large sections of the east facing slope are dominated by indigenous Southern Afrotemperate Forests, interspersed with Peninsula Granite Fynbos (Mucina & Rutherford, 2006). The lower area is made up of Basement Granite frequently overlaid by Table Mountain Shale and Table

Mountain Sandstone (McKenzie *et al*., 1977). Sandstone-derived soils are generally shallow in depth (< 0.5 m) while granitic soils are deeper (< 2 m) with relatively higher total N and P (McKenzie *et al*., 1977). The area designated Peninsula Granite Fynbos is today largely dominated by what we designate here as Transition vegetation, but was termed "scrub" by (McKenzie *et al*., 1977). Five sites across Orange Kloof were selected for sampling. At four sites, three 10 x 5 m plots covering each of the vegetation types (Fynbos, Transition and Forest) were selected. Within a 50 m radius of each plot three surface soil samples were collected. Each of the plots covering the three vegetation types were selected in close proximity to each other ensuring consistency in geology (largely sandstone). In the lower reaches of the valley where granitic soils are dominant, one comparison site was established, but from which Fynbos was missing (Fig. 1, site E).

Vegetation assessment

Within each plot the identity and percentage abundance of all plant species were recorded. Repeat digitised aerial photographs covering Blinkwater (1945–2015) and Orange Kloof (1955–2016) sites were used to quantify change in woody cover over time. Prior to analysis, images were georeferenced using the coordinates of continuously identifiable control points (e.g. rocks, roads) obtained from Google Earth. Misalignments between images and control coordinates were corrected using thin plate spline transformation and cubic spline resampling, implemented in QGIS (Quantum GIS Development Team, 2014). To quantify the extent of woody cover for each year, a 25 x 25 m grid with 5 m^2 cells was centred over each plot. Vegetation of each cell was then manually classified as either 'open-canopy' or 'closed-canopy' based on the presence of woody vegetation occupying ≥ 50% of the cell following the approach of Wood and Bowman (2012). Vegetation change across Forest, Transition and Fynbos sites was then calculated as the percentage of woody cover that changed over the sample period.

Normalised difference vegetation indices (NDVI) at 10 m resolution were obtained through Google Earth Engine (https://developers.google.com/earth-engine) from the Sentinel-2 MultiSpectral Instrument for the period Jun 2005 – Jun 2016. The process flow comprised import of the satellite image collection, application of a cloud mask (based on a Google Earth Engine cross-sensor algorithm which masks pixels with reflectance values outside of set thresholds for the visible and infrared bands), calculation of the NDVI over a time series of satellite images, and calculating the upper quartile of time series NDVI values to avoid problems with cloud cover induced at low values of NDVI. NDVI was calculated using $\mathrm{NDVI} = \rho_{\mathrm{NIR}} - \rho_{\mathrm{Red}}/\rho_{\mathrm{NIR}} + \rho_{\mathrm{Red}}$ (Tucker 1979) in which ρ_{NIR} was the "B8" band (842 nm) and ρ_{Red} the "B4" band (665 nm) of the multispectral images.

Soil and rock sampling and preparation

Surface leaf litter was cleared away and soils were collected using a 0.07 m diameter auger to a depth of 0.3 m. Soils were air dried for 1 week, weighed, and then passed through a 1 mm sieve. Sieved samples were then split by repeatedly quartering the soil and taking opposite quarters for each successive sub-sample to provide samples of appropriate volume (Gerlach *et al*., 2002) for physical and chemical analyses. Additional samples were collected for charcoal analysis at depths of 0-10 cm, 10-20 cm and 20-30 cm and air-dried for 48 h prior to analysis. Chemical characteristics of the soils were assessed using measures of extractable elements to assess plant available nutrients and characteristics of the intact soil total elemental composition from which organic material had been removed via dry-ashing (see below).

Samples of granite and sandstone rocks occurring in or near Fynbos, Transition and Forest plots at Blinkwater were removed from boulders to provide a reference of geological parent material. The rock surface was cleaned with a steel brush followed by washing with a domestic power washer to remove lichens and other deposits before being crushed.

Soil particle size analysis

Soils samples were heated to 450°C for 12 h in a furnace to remove organic matter (dry-ashed) prior to particle size distributions being measured using a Malvern Master-Sizer 2000. Each sample was subjected to 300 s ultrasonic dispersal to ensure complete disaggregation of particles. The proportion of the soil particles in each size class was recorded and plotted. These size classes were then summed into categories representing clay, silt, very fine sand, fine sand, medium sand, coarse sand and very coarse sand, according to the Wentworth grain size chart (William *et al*., 2006).

Soil and rock total elemental analysis

Sieved and dry-ashed soil, and crushed rock samples were milled in a mortar and pestle to a fine powder. The powder was placed in sample cups with a polypropylene bottom and assessed in a Spectro Xepos X-Ray Fluorescence (XRF) analyzer (Spectro, Amatek materials analysis division, Kleve, Germany). Measurements were conducted in a helium atmosphere using a silicon drift detector. The instrument was calibrated by using a certified standard GBW07312 (National Research Center for CRMs, Beijing, China), for which elemental concentrations were obtained from NOAA Technical memorandum NOS ORCA 68 (1992). Based on this data a weathering ratio was calculated as ([Ca] + [Mg] + [K])/[Zr] following the example of Chittleborough (1991), but substituting K for Na.

Available soil nutrient analysis

Mass spectrometer analysis for soil C, N, $\delta^{13}C$ and $\delta^{15}N$ was conducted in the Department of Archeometry (University of Cape Town). Approximately 40 mg of sieved soil that was not dry-ashed was weighed into tin capsules (Elemental Microanalysis Ltd, Devon, U.K.) and combusted in a Thermo Flash EA 1112 series elemental analyzer from where the gasses were fed into a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy). Two in-house standards and one IAEA standard were used to calibrate the results.

The Institute for Plant Production (Department Agriculture: Western Cape, South Africa) conducted the following soil nutrient analyses on sieved soil that was not dry-ashed: pH, electrical conductivity, K, Mn, Na, Cu, Zn, Ca, Mg and two measures of plant available P: P-Citric (extracted in 1% (w/v) citric acid) and P-Olsen (Olsen, 1954) following protocols of Soil science society of South Africa (1990). Soil pH was determined by shaking 2 g soil in 20 ml 1 M KCl at 180 rpm (Hermle Z420, Gosheim, Germany) for 60 min, centrifuging at 10 000 g for 10 min and measuring the supernatant pH. Exchangeable cations were extracted with NH_4-acetate and EDTA at pH 4.65, and their concentrations determined using a Thermo ICP iCAP 6000 Series Spectrometer (Thermofisher Scientific, Surrey, UK).

Charcoal analysis

Samples of 100 g of air-dried soil were stirred into 10% (w/v) KOH and left overnight before sieving through 1.4 mm mesh and washing with water. The sieved material was suspended in water in a petri dish and the charcoal fragments extracted by hand under a dissecting microscope. The fragments were counted and weighed after drying at 70°C for 48 h.

Statistical analysis

Chao estimates of extrapolated species richness from species accumulation curves (Chao, 1987; Chiu *et al.*, 2014) were determined using the *specpool* function of the 'vegan' library (Oksanen *et al.*, 2016) in R (R development Core Team, 2014). To compare turnover in species richness between vegetation types (i.e. Fynbos, Transition and Forest), Sørensen's dissimilarity coefficient (i.e. the number of species shared between two vegetation zones divided by the total number of species present in the two vegetation zone) was calculated using the function *shared* from the 'rich' library (Rossi, 2011) in R. If the number of species shared between two sites is denoted as a and the numbers of unique species (not shared) as b and c, then Sørensen's coefficient = $(b + c) / (2a + b + c)$ (Oksanen *et al.*, 2016).

Analysis of similarity (ANOSIM) in the library 'vegan' library provided a test of whether there were significant differences between Forest, Fynbos and Transition vegetation types.

To examine the separation or overlap of the three vegetation types (Forest, Transition, Fynbos) with respect to soil nutrition, a linear discriminant function analysis was conducted using the *lda* function in the 'MASS' library (Venables & Ripley, 2002) in R. We selected soil variables (pH, Ca, Mg, Olsen P, Na, K, Cu, Zn, Mn, total N, organic matter, clay and $\delta^{15}N$) that represent important plant and ecosystem characteristics. A one-way ANOVA followed by a post-hoc Tukey HSD test using the 'agricolae' library (Mendiburu, 2016) in R, was used to check for significant ($P < 0.05$) differences in soil nutrients, rates of change in woody cover and NDVI between Forest, Transition and Fynbos across both study sites.

Results

Current vegetation characteristics

Forest and Fynbos vegetation types are floristically distinct, as is evident from the fact that there were no shared species between them at Blinkwater and only 1% at Orange Kloof (Table 1). We identified Transition vegetation as being a combination of elements of both Fynbos and Forest and located sample sites in areas in which change in vegetation has occurred over the last 6 decades (Fig. 1). At Blinkwater, of a total of 30 species in Transition vegetation, 9 were common with Fynbos and 6 with Forest. At Orange Kloof, of a total of 53 species in Transition, 25 were common with Fynbos and 6 with Forest. Thus at both sites Transition shared elements of both Fynbos and Forest, but according to Sørensen's dissimilarity coefficients (Table 1) was floristically more similar to Fynbos.

Although vegetation types at Blinkwater were relatively homogeneous between sites, there was significant turnover in species between the three vegetation types (Fig. 2a). Forest and Transition at Orange Kloof had similar Sørensen's coefficients to those at Blinkwater, however, Fynbos exhibited greater variance between sites at Orange Kloof than at Blinkwater (Table 1). The greater variance of Fynbos at Orange Kloof is probably the consequence of a broader geographical sampling (Fig. 1). Nevertheless, the differences between vegetation types at Orange Kloof were large and significant (Fig. 2a). Blinkwater had fewer species than Orange Kloof for all three vegetation types, consistent with the greater range of sites sampled at Orange Kloof. Furthermore, at both Blinkwater and Orange Kloof, the species pools of each vegetation type diminished in the following sequence Transition > Fynbos > Forest, consistent with Transition vegetation containing species from both Fynbos and

Forest. Overall, the annual upper quartile of NDVI values at Blinkwater was lower than at Orange Kloof (P = 0.017, Fig. 3). Moreover, NDVI was significantly higher in Forest than in Fynbos, with Transition being intermediate at both sites.

Table 1 Comparison of the species richness of Fynbos (Ff), Transition (Tr) and Forest (Fo) communities at the Orange Kloof and Blinkwater study sites. On the diagonal (grey background) is the observed richness for each community, above the diagonal is the richness common to pairs of communities (shared richness) and below the diagonal is the total richness for pooled pairs of communities. In total, Blinkwater had 67 species and Orange Kloof had 94 in the plots sampled. The percentage of species at each site within a vegetation type is shown. Sørensen's dissimilarity coefficient is an index of turnover in species richness between two vegetation types.

		Species number			Species (%)			Sørensen	
Site	Vegetation	Ff	Tr	Fo	Ff	Tr	Fo	Ff	Tr
Orange Kloof	Ff	47	25	1	50	27	1		
	Tr	75	53	6	80	56	6	0.50	
	Fo	66	67	20	70	71	21	0.97	0.84
Blinkwater	Ff	27	9	0	40	13	0		
	Tr	48	30	6	72	45	9	0.68	
	Fo	39	36	12	58	54	18	1	0.71

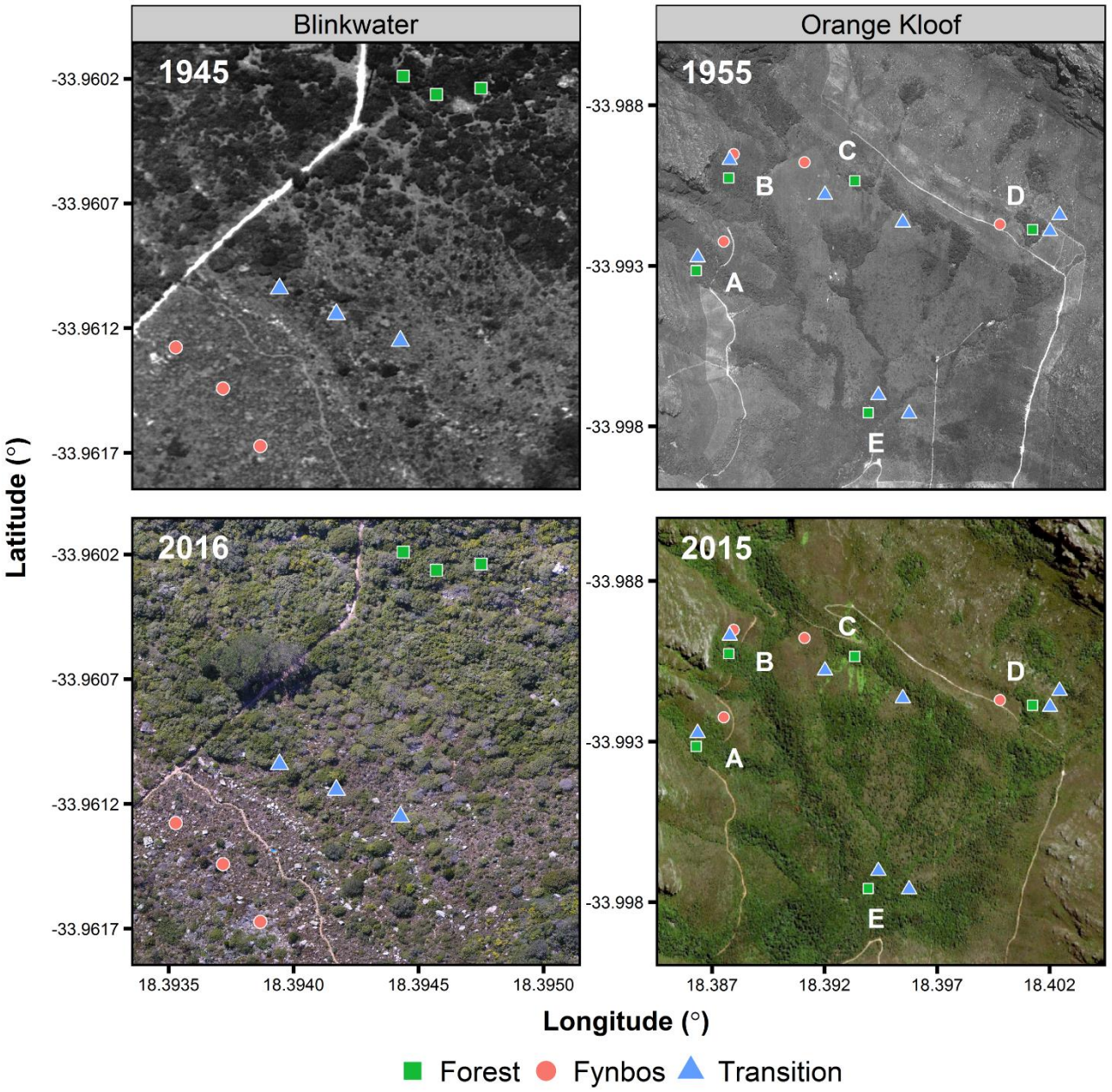

Figure 1 Historical aerial photographs of the Blinkwater and Orange Kloof sites compared to contemporary images. Within each site the sampling locations are marked for each of the vegetation types (Fynbos, Transition, Forest). Sampling at Orange Kloof was in a larger geographic area and occurred in 5 different sites. Site E lacked Fynbos vegetation and was on granite-derived soils (as opposed to sandstone-derived soils elsewhere) and was thus excluded from statistical analyses although the data was retained for presentation. At Blinkwater a walking path running southeast lies along a rock scree that defines the boundary between Transition and Fynbos vegetation. The large tree along the northwest path ("Pipe track") at Blinkwater is an alien *Pinus* sp.

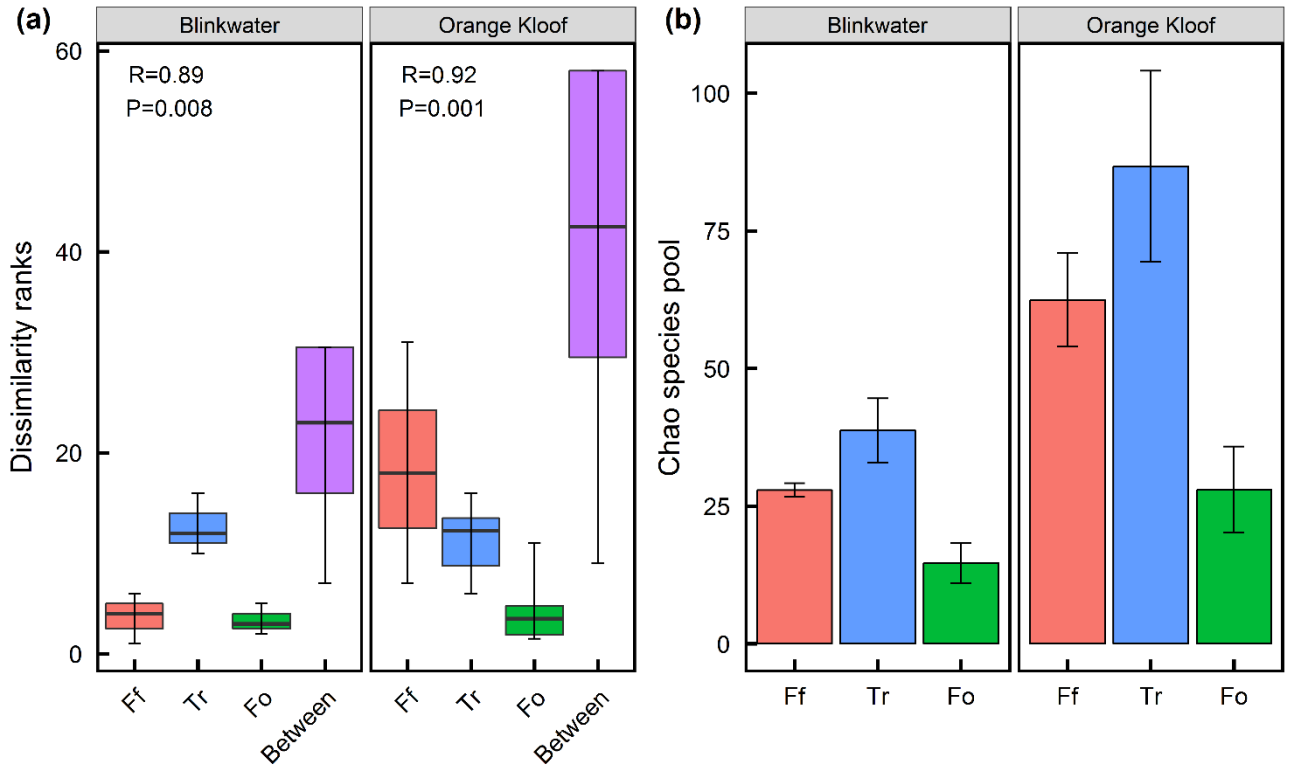

Figure 2 Analysis of (a) species (dis)similarities and (b) the Chao estimates of species pool size. Dissimilarities were calculated using analysis of similarities (ANOSIM) showing the compositional differences within vegetation types (Fynbos = Ff, Transition = Tr, Forest = Fo) compared with the dissimilarity between vegetation types ("Between"). The statistical significance of the R-value of the ANOSIM test is shown. Chao species pools represent the estimates of extrapolated species richness from species accumulation curves (Chao, 1987, Chiu *et al*., 2014).

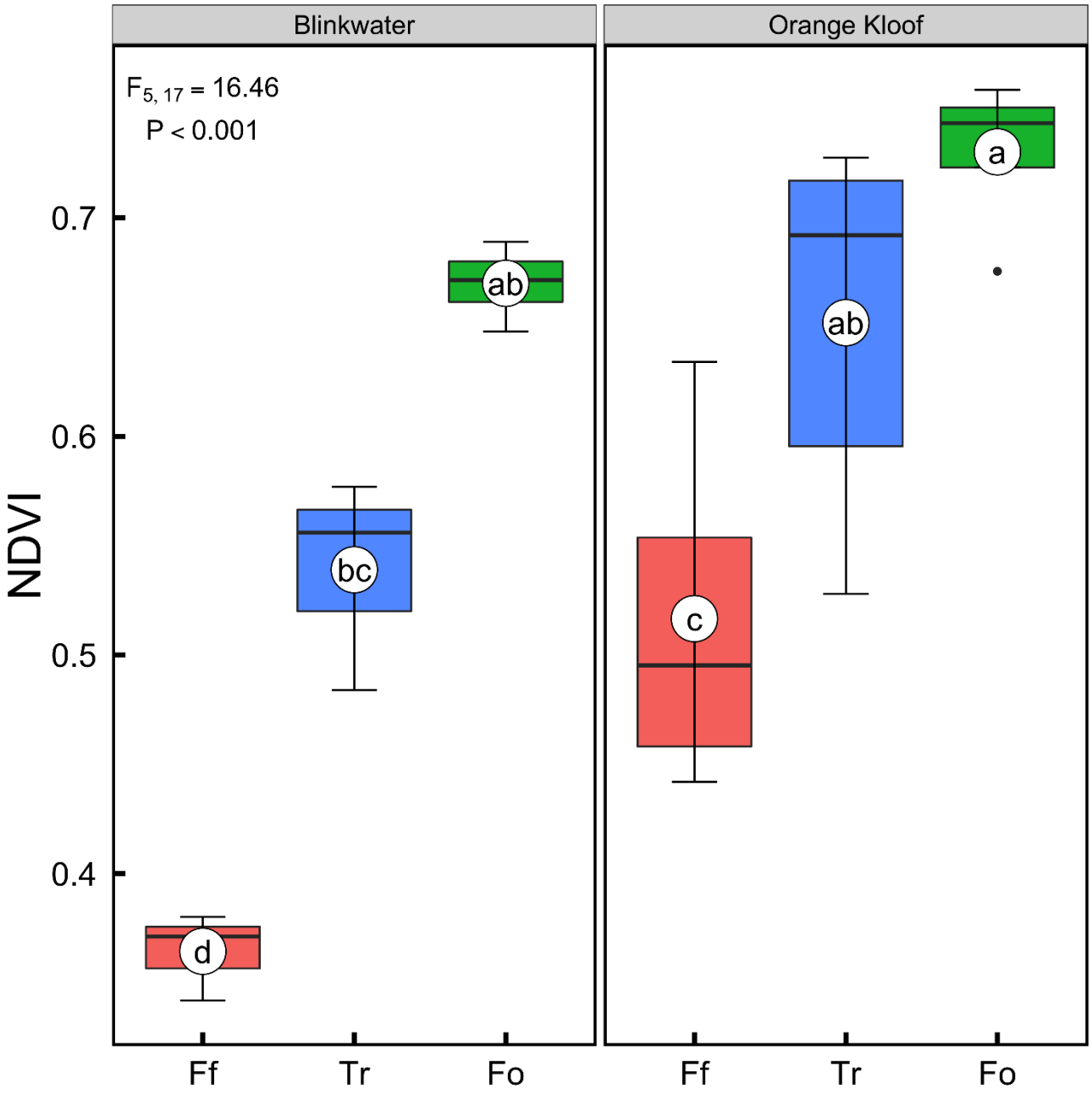

Figure 3 Variation in the upper-quartile of NDVI values between vegetation types (Fynbos = Ff, Transition = Tr, Forest = Fo) at each study site. The boxes and horizontal lines represent the first and third quartiles and the medians, respectively. The whisker represents 1.5 x the interquartile range and outliers above/below are shown as points. Circles represent the mean with letters indicating the significant interaction between vegetation types and sites from a one-way ANOVA, for which the F value and P values is given.

Vegetation change

Consistent with the definition of the vegetation types, woody cover was high for Forest at both Orange Kloof and Blinkwater (Fig. 4a). Woody cover of Forest increased over the duration of the observations at Blinkwater by 0.25% per annum, but remained completely closed at all Orange Kloof sites (Fig. 4b). Although woody cover of Fynbos and Transition is distinct at Blinkwater today, this was not the case

in the preceding decades over which woody cover of both Fynbos and Transition increased, as shown by the repeat photographs (Fig. 1, 4). At Orange Kloof, Fynbos woody cover has been lower than that of Transition from the 1960's onwards, with only small annual increases in Fynbos (0.08% per annum) while Transition increased at a faster rate (0.55% per annum, Fig. 4b), which is similar to the rate of change reported by Luger and Moll (1993) of 0.41% per annum (applying a linear regression to their data) for Orange Kloof.

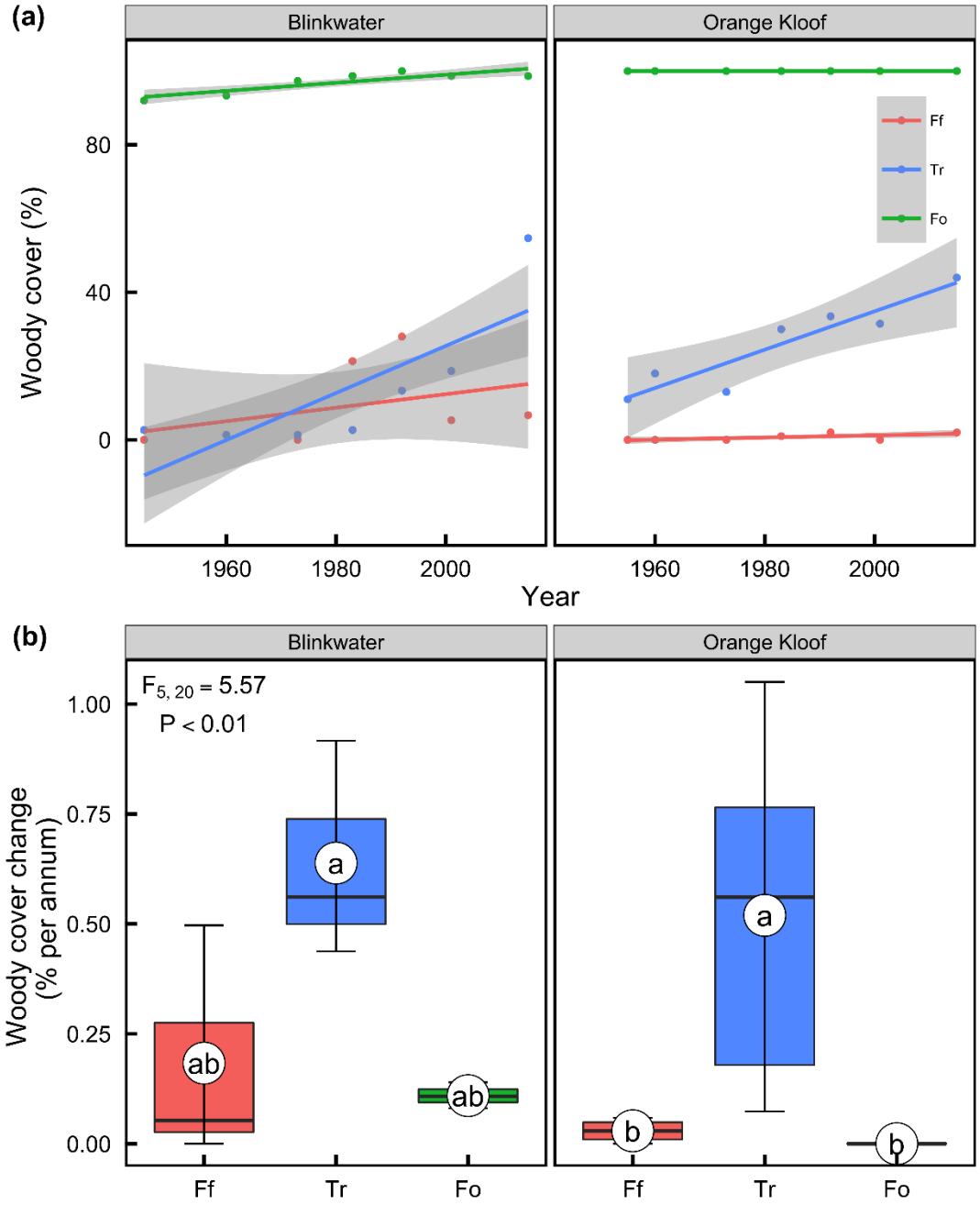

Figure 4 Differences in the change in woody cover with time between vegetation type (Fynbos = Ff, Transition = Tr, Forest = Fo) at both study sites. Lines represent linear fits and the grey bands are the 95% confidence intervals. Linear fits (a) for each individual plot were used to calculate the (b) rates of wood change for both sites and all vegetation types. Other details as in Fig. 3.

Soil texture

Overall, soil texture was remarkably similar between vegetation types on sandstone-derived soils (Fig. 5). The only significant ($P < 0.05$) differences at Blinkwater involved very coarse sand, which was lower in the Fynbos than in either Transition or Forest soils (Supplementary material Appendix 1, Table S1). The texture of soils at Orange Kloof was similar to Blinkwater, but with more very fine sand at Orange Kloof in Transition, less coarse sand in Fynbos and less very coarse sand in all vegetation types. We reported site E at Orange Kloof (Fig. 1) separately from the others because this site had no Fynbos vegetation in the vicinity. Occurring on granite-derived soils, the site was also distinct in texture, having a greater concentration of finer material and correspondingly less coarse particles (Fig. 5).

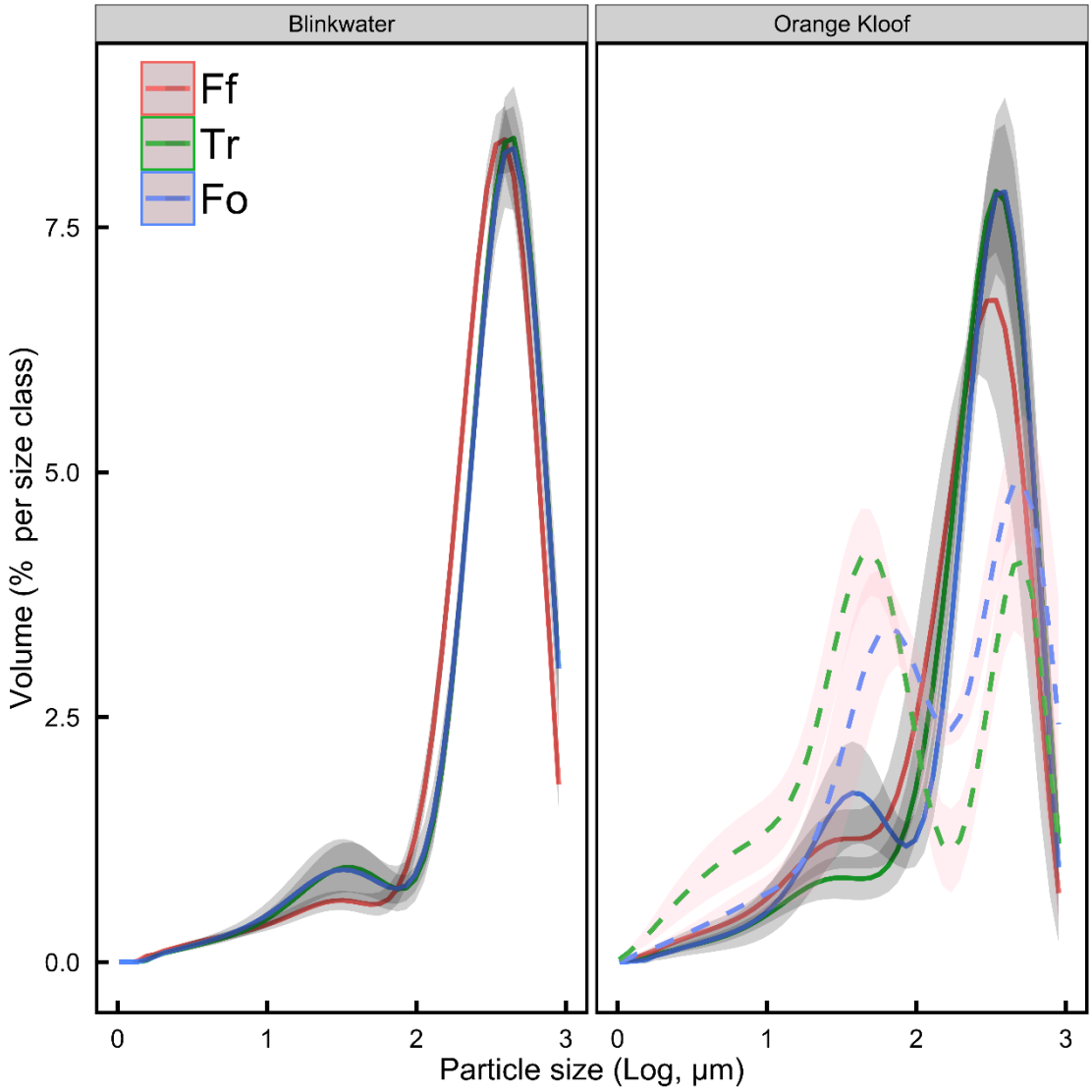

Figure 5 Comparison of the proportion of the soil volume (%) comprising different particle sizes (log scale) between vegetation type (Fynbos = Ff, Transition = Tr, Forest = Fo) at both study sites. Grey ribbons represent the 95% confidence bands. The broken lines with pink ribbons are for the Orange Kloof granitic site (Fig. 1) that was excluded from inclusion in statistical analyses.

Soil chemical properties

Many plant available nutrient concentrations were considerably higher in granite than in sandstone rocks (Supplementary material Appendix 1, Table S2). As a consequence, we restricted sampling to sandstone-derived soils, apart from site E at Orange Kloof (Fig. 1). Fynbos soils had significantly lower total concentrations of C, P, Ca and Sr than Forest at both sites (Fig. 6). Only at Orange Kloof were K, Fe and Al higher in Fynbos than in Forest. Generally, Transition soils were intermediate between Fynbos and Forest, except for Orange Kloof, where concentrations of C, P, Ca and Sr in Transition soils were more similar to the Fynbos than Forest soils. Of the properties affecting nutrient availability, pH, total N, P-citric, K, Ca, Mg, and organic matter were all higher in Forest than Fynbos soils at both sites (Fig. 7). Although Transition soils were intermediate between Fynbos and Forest at Blinkwater, Transition soils from Orange Kloof were generally statistically indistinguishable from those of Fynbos (Fig. 7).

To show the relative availability of P-citric and extractable K we expressed them as a proportion of their totals (Fig. 8). For P a greater proportion of the P was available in Forest soils than in Fynbos. A similar pattern occurred for K at the Orange Kloof. N:P ratios were indistinguishable across vegetation types. Differences between ratios of P and K to Zr and the weathering ratio (Fig. 8) generally showed higher values in Forest than in Fynbos, indicating greater loss of P and K and greater weathering of Fynbos than of Forest soils.

Charcoal

The weights and counts of charcoal in the soils were relatively high in all vegetation types at both sites (Fig. 9). This indicates that fires have occurred ubiquitously through these two areas. The lack of significant differences between vegetation types probably reflects the high variability of the data due to quantification of charcoal being done by individually picking out fragments. Despite the variability, Orange Kloof had significantly greater concentrations of charcoal in Fynbos and Forest than did Blinkwater.

Prediction of vegetation based on soil data

Linear discriminant analysis (LDA) was able to clearly differentiate between the three vegetation types at Blinkwater, based entirely on soil data (Fig. 10). At Orange Kloof, Fynbos and Transition showed considerable overlap, but Forest was still clearly differentiated. LDA, however, failed to separate these vegetation types when based on soil textural data (data not shown).

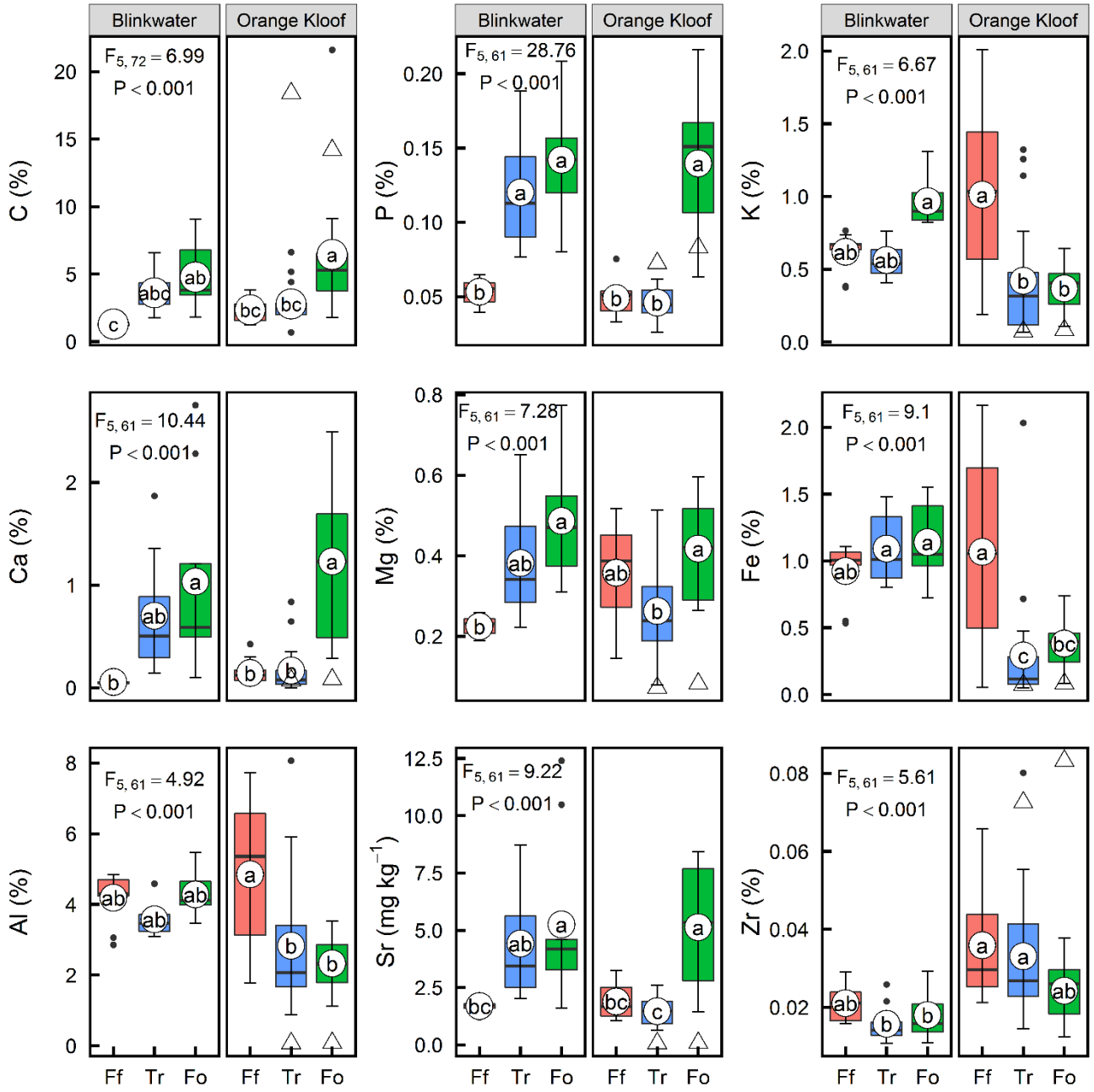

Figure 6 Variation in soil total elemental concentrations between vegetation types (Fynbos = Ff, Transition = Tr, Forest = Fo) at both study sites. The site on granitic-soil at Orange Kloof is represented by Δ. Other details as in Fig. 3.

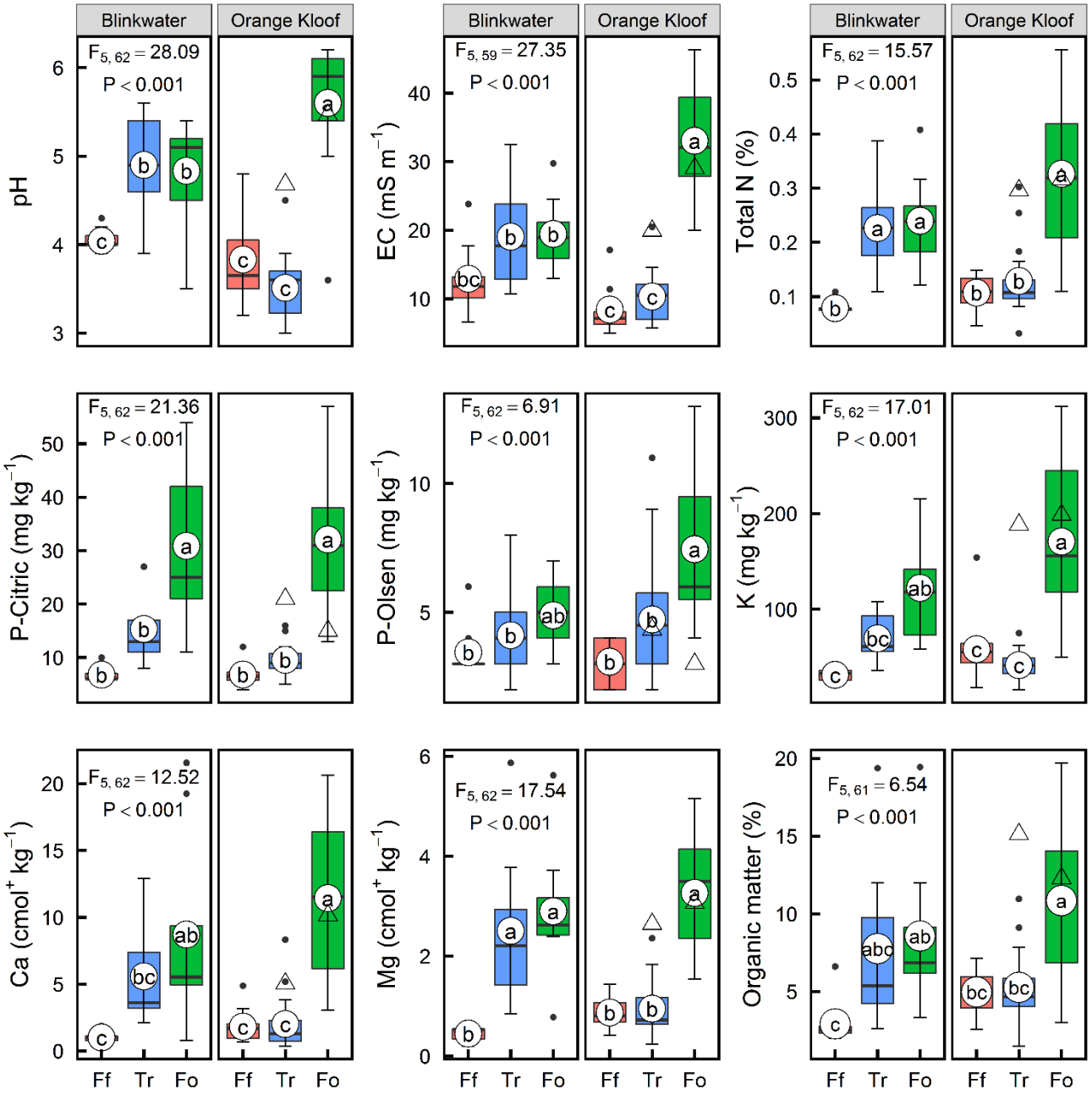

Figure 7 Variation in soil chemical characteristics determined using standard soil analysis protocols to assess plant–available nutrients between vegetation types (Fynbos = Ff, Transition = Tr, Forest = Fo) at both study sites. The measured characteristics include electrical conductivity (EC) and organic matter determined from weight loss on ignition. Other details as in Fig. 3.

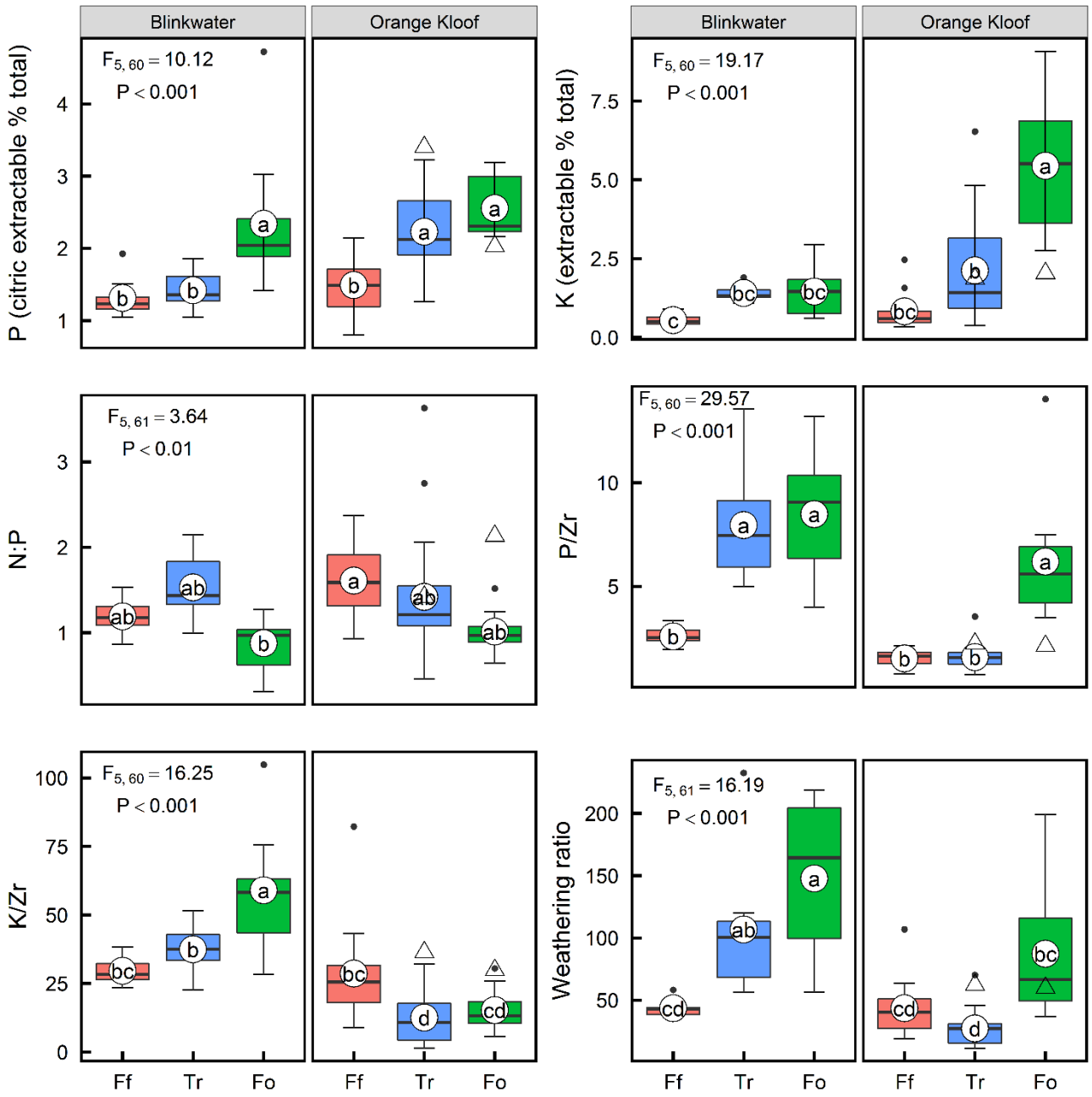

Figure 8 Variation in ratios of soil chemical characteristics between vegetation types (Fynbos = Ff, Transition = Tr, Forest = Fo) at both study sites. Citric acid extractable P and extractable K are expressed relative to total P and K determined using XRF analysis. Total N is expressed relative to total P (N:P). P:Zr, K:Zr and weathering ratios are all based on XRF elemental analyses. Other details as in Fig. 3.

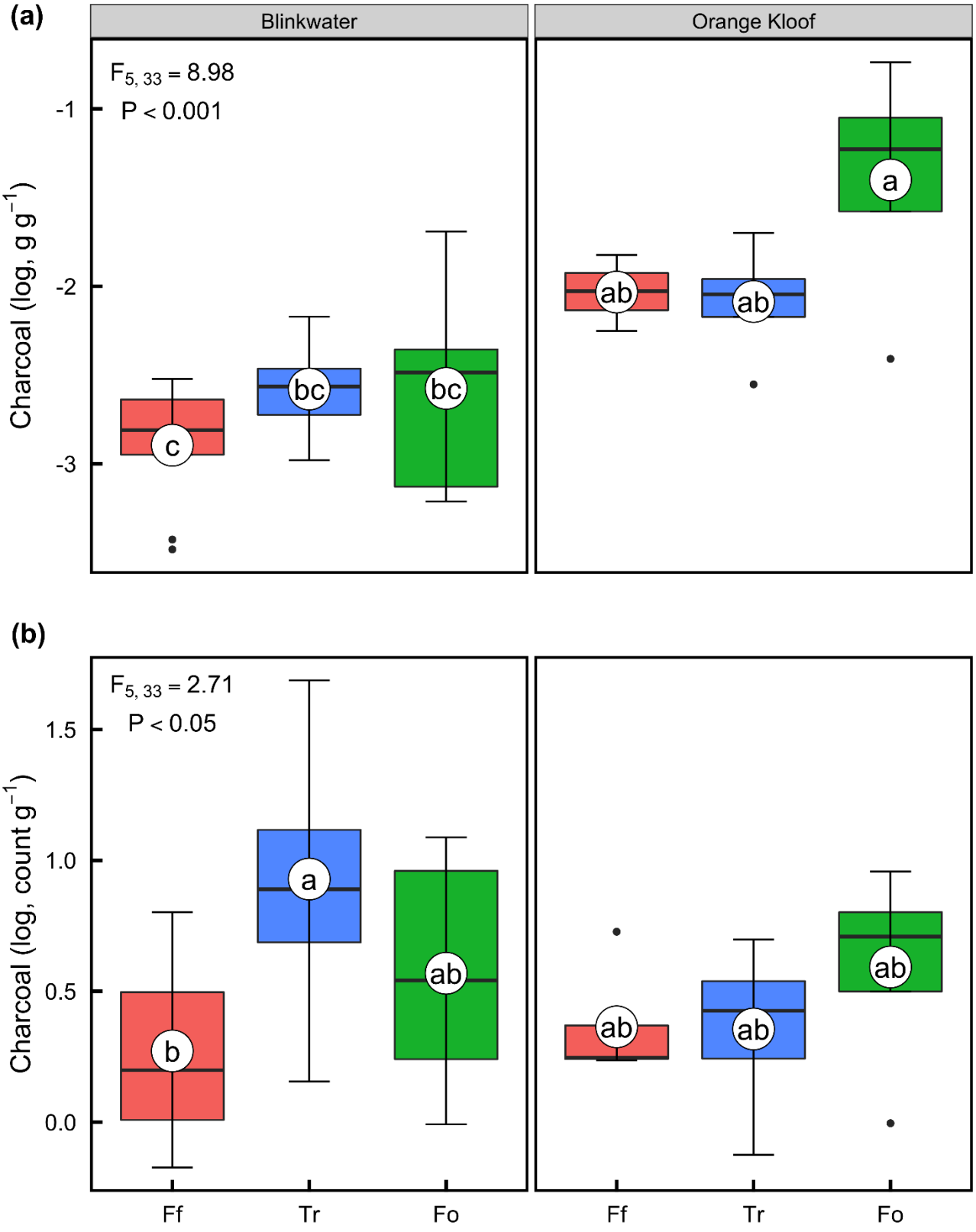

Figure 9 Variation in the weight and number of fragments of charcoal (logged) extracted per weight of soil between vegetation types (Fynbos = Ff, Transition = Tr, Forest = Fo) at both study sites. Other details as in Fig. 3.

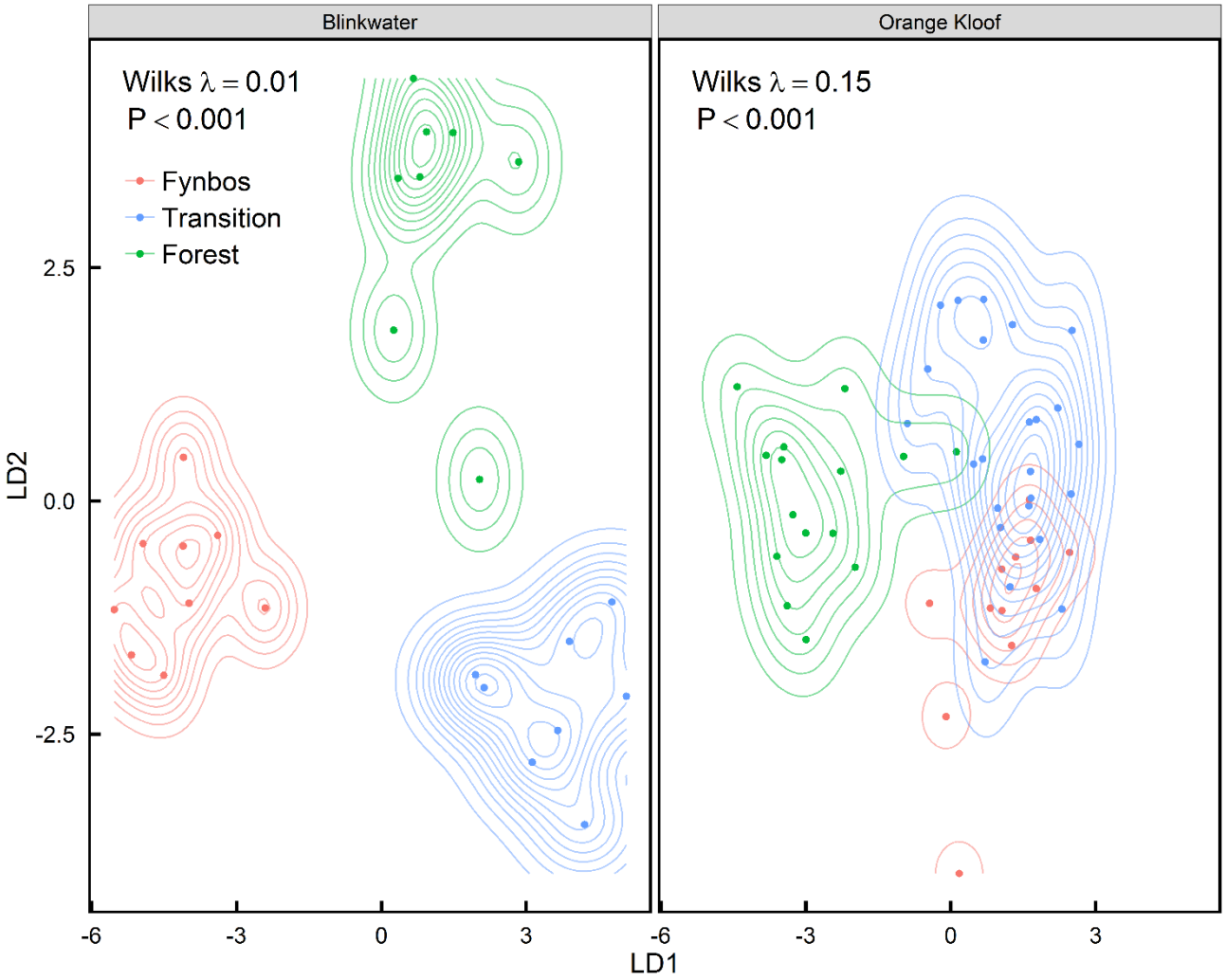

Figure 10 Linear discriminant analysis of the vegetation types at both sites using soil variables. For Blinkwater and Orange Kloof LD1 explained 86% and 69%, respectively, of the between-vegetation type explained variance. Results of MANOVA using Wilk's λ are shown. Soil properties determined using standard soil analysis protocols that were not collinear (R < 0.7) and were included in the LDA were pH, Ca, Mg, Olsen P, Na, K, Cu, Zn, Mn, total N, organic matter, clay and $\delta^{15}N$.

Discussion

We found no evidence for switches between Forest and Fynbos as ASS over the decades for which aerial photographs are available. This is contrary to the interpretation of some authors (e.g. Phillips, 1931; Coetsee *et al.*, 2014) that Forest colonises adjacent Fynbos if fire is excluded, and that Forest- and Fynbos-soils do not exclude the alternate vegetation. Indeed, van Daalen (1981) found no evidence that Forest species regenerate in Fynbos. Furthermore, Masson and Moll (1987) clearly separated Fynbos, Transition and Forest based on floristic composition, and concluded that the boundaries were strongly associated with edaphic properties (texture, pH and P) and moisture. The increased woody cover in Transition vegetation over time (Fig. 4) could be interpreted as providing support for the

capacity of this Transition zone to exist as ASS. This interpretation is consistent with that of Wood and Bowman (2012) for Tasmanian moorlands with forest patches. The fact that canopy closure is incomplete and that soil and vegetation properties are intermediate suggests, however, that this Transition vegetation may rather be an unstable seral stage (e.g. Phillips, 1931).

The existence of charcoal in all vegetation types at Blinkwater and Orange Kloof indicates exposure to fire. While fire return intervals in Fynbos are variable (7–55 years, but mostly 10–20 years) but relatively regular, fires do also occasionally transgress into Forest under high fire danger conditions (Kraaij *et al*., 2014) leaving a charcoal record. Furthermore, clearing and burning of forest vegetation is known to have taken place in the 19th century (Poulsen & Hoffman, 2015). Infrequent intense fires and land-use history (e.g. Luger & Moll, 1993; Poulsen & Hoffman, 2015) and the edaphic circumstances may suggest that the documented changes in Transition vegetation are at least partially the consequence of reestablishment of vegetation, rather than *de novo* canopy closure, as is the case for rain forests in Tasmanian moorlands (Fletcher & Thomas, 2010). It is possible that this Transition vegetation is in a constant state of flux between more open- and closed-states, depending on prevailing disturbance patterns.

For Forest and Fynbos to switch states, the climate and edaphic properties should be capable of supporting either vegetation type. For example, Wood and Bowman (2012) found that Tasmanian forest and non-forest patches had distinct P concentrations, but there was also considerable overlap between edaphic properties. In our study, Forest and Fynbos plots within a site were no more than 200 m apart and thus the climate is similar, although there may have been differences in local hydrology. It is possible that Forest soils are deeper than the Fynbos soils, but the sandstone derived soils at Orange Kloof are all generally relatively shallow (< 0.5 m deep; McKenzie *et al*, 1977; Masson & Moll, 1987; Campbell & Moll, 1977). Since there was also very little difference in soil texture between the Forest and Fynbos plots, this suggests that Forest and Fynbos ecosystems represent long-term ASS on the same sandstone-derived geology. Concentrations of the relatively immobile element Zr (Shahid *et al*., 2013) also did not differ significantly between Forest and Fynbos (Fig. 6), although, the relatively lower K:Zr and P:Zr ratios likely indicates greater losses of K and P from Fynbos than from Forest soils (Fig. 8). Moreover, the higher weathering ratio of Forest soils than Fynbos soils indicates that mobile elements have been leached (e.g. Cramer & Hoffman, 2015) out of the Fynbos soils or lost through repeated pyro-mineralisation (Stock & Lewis, 1986). The nutritional strategies of Fynbos that allow them to access scarce and sparingly available nutrients (reviewed by Cramer *et al*., 2014) may also exacerbate nutrient losses through leaching and fire. Thus, differences in topography and

pedogenic pathways likely account for the strong nutritional differences between Forest and Fynbos soils, despite the shared geology.

The Forest-Fynbos nutritional differences (Figs. 7 and 8) were particularly pronounced for pH, total N, total P, K, Ca and Mg. Since the sandstone-geology is shared, these nutritional differences have emerged as a consequence of divergent nutrient cycling in Forest and Fynbos (e.g. Hobbie, 2015). In the case of Forest, a lack of frequent fires and the accumulation of organic carbon are likely to drive nutrient accumulation, whereas the lower stature open-canopy and fire-prone Fynbos results in nutrient depleted soils (e.g. Neary *et al*., 1999). These emergent differences in edaphic characteristics between Forest and Fynbos represents a degree of resilience to change that supports the idea that these are ASS, since such large nutrient differences on geologically identical substrates, we speculate, may only emerge over time scales of multiple decades to centuries. Therefore, from an edaphic perspective, intact Forest and Fynbos vegetation types are particularly stable alternative biome states. The Transitional vegetation sites are, however, in areas that are nutritionally intermediate between Forest and Fynbos at Blinkwater, and more similar to Fynbos at Orange Kloof. We suggest that Transition vegetation is successional to forest (e.g. Phillips, 1931), existing in areas that are intermediate in fire-susceptibility and/or the capacity to generate closed-canopy forest due to insufficient access to water and nutrients, and may therefore exist as a relatively open- or closed-canopy forms. Their trajectory of change is unlikely to produce forests without sustained anthropogenic modification of the fire regime.

Despite many consistent Forest-Fynbos differences in edaphic properties, there is also a degree of variability in those. These site-differences are apparent from the differences in NDVI between the Blinkwater and Orange Kloof sites, which is not surprising considering that Blinkwater receives only about 60% the annual precipitation (i.e. 714 ± 23 mm, mean ± SE, data from South African Weather Service between 1960–2015) of Orange Kloof (1227 mm), with the latter being additionally augmented by an estimated 500 mm fog precipitation (Luger & Moll, 1993). This might additionally be related to different histories or to the proximity to the coast and aspect. For example, Blinkwater ravine is a north-west facing slope 1.6 km from the coast, whereas Orange Kloof is a minimum of 3.5 km from the coast in a protected valley. These differences are likely to have contributed to higher soil pH, EC and Olsen P for Forest sites at Orange Kloof than at Blinkwater. Averaging between sites is likely to obscure specific Fynbos-Forest-soil differences, several of which may act in concert to determine the vegetation state. Despite these site-specific differences there were also extremely consistent differences in some soil nutrients between the sites. There has been considerable focus on scarce soil P as an indicator of Fynbos vegetation (reviewed in Cramer *et al*., 2014) and that was indeed consistently different between Forest and Fynbos soils at the two sites. However, there were also

differences in pH, EC, N, K, Ca and Mg. The turnover between Forest and Fynbos vegetation cannot therefore be associated with any one of these edaphic characteristics in isolation.

Transition vegetation has been considered an example of expanding Forest (McKenzie *et al*., 1977). In our analysis, the Transition vegetation shared more floristic similarities with Fynbos than with Forest, particularly at Orange Kloof, possibly indicating that the Transition vegetation is recovering from disturbance and is transitional in both space and time. While the changes in Transition woody cover over the past decades have been linear, at *ca*. 40% it is still far from the closed canopy exhibited by Forest (*ca*. 100%). Since we chose study plots on the basis of the earliest available aerial photographs, this is not attributable to misclassification of former Transition vegetation as Forest vegetation. The relatively open canopy of Transition vegetation is also consistent with the floristic composition. We thus suggest that this Transition vegetation zone is a relatively unstable intermediate zone, in which regime shifts could occur. The existence of Transition vegetation zones may be attributable to the degree of protection from fire. For example, the rocky scree southwest of the Transition vegetation at Blinkwater (Fig. 1) is likely to reduce the fire incidence driven by prevailing south-westerly winds. Over prolonged time periods less frequent exposure to fire than Fynbos coupled with smaller nutrient feedbacks (e.g. due to leaf litter and soil volume explored) than in Forest may explain the intermediate nutrient status of these Transition zones.

This raises the question as to whether, in the absence of disturbance, Transition vegetation will continue to increase in density towards a Forest, or whether it will persist as transitional vegetation between Forest and Fynbos. There is little information on the rate at which vegetation can alter soil chemical properties in the Fynbos region. Colonising Forest trees can be observed on aerial photographs, but soil modification beneath their canopies has not been compared to uninvaded Fynbos. Trajectories of change to Fynbos with increased fire frequency or harvesting of Forests or replacement by Forests of Fynbos as a result of extended fire protection requires further study to establish the rates of change.

Conclusion

Considering the disparate nutritional characteristics of Forest and Fynbos, we suggest these are generally unlikely to switch to an alternate state, unless perturbations persist for periods of multiple decades to centuries and are capable of altering soil properties. In this system, large differences in soil chemistry are commonly caused by the vegetation, and are not intrinsic (i.e. geological). Rates of vegetation change are, however, far slower than recorded for tropical grassy ecosystems which can switch to closed forest where fires are suppressed for 30 to 40 years (Bond *et al*., 2005; Durigan

& Ratter, 2016). These relatively stable Forest-Fynbos boundaries might be the consequence of extreme depletion of soil nutrients by Fynbos and intense canopy fires. Transitional areas between Forest and Fynbos with intermediate vegetation and edaphic characteristics, because of either different geologies or resulting from partial protection from fire, may more rapidly switch states, but are unlikely to be stable. Establishing that ecosystems are indeed ASS is tricky, however, considering the longevity of woody species, slow changes to edaphic properties and the interplay between emergent and imposed determinants.

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Supporting Information

Appendix S1

Table S1 Comparison of the mean ± SE of texture components of the soils for the Blinkwater and Orange Kloof sites in Fynbos (Ff), Transition (Tr) and Forest (Fo) vegetation types. Different letters indicate significant differences between the logit transform of the texture class determined using a two-way ANOVA with the sites and vegetation types as factors and post-hoc Tukey tests ($P < 0.05$). Each texture class was analysed separately.

	Blinkwater			Orange Kloof		
Texture	Ff	Tr	Fo	Ff	Tr	Fo
Clay	0.97±0.09a	0.75±0.06a	0.79±0.11a	1.42±0.21a	1.7±0.36a	1±0.24a
Silt	9.3±0.7a	12.4±1.3a	12.6±1.8a	17.6±2a	20.4±3.2a	20.5±2.9a
Very fine sand	5.4±0.5ab	4.2±0.3b	4.3±0.6b	10.6±1.9a	9.1±1a	8.4±1.1ab
Fine sand	21±0.6a	14.7±0.7a	14.8±0.6a	24±1.8a	18.4±1.8a	16.8±1.2a
Medium sand	39.8±0.7a	37.6±0.7a	37.2±1a	32.4±2.6a	32±2.3a	33.7±2.4a
Coarse sand	23.1±0.8a	28.4±0.9a	28.1±1.6a	13.9±2.8b	17.9±1.6ab	18.8±1.9ab
Very coarse sand	0.56±0.12b	1.96±0.32a	2.12±0.4a	0.1±0.09b	0.38±0.12b	0.7±0.37b

Table S2 Comparison of the oxide composition (mg kg^{-1}) of granite and sandstone rock samples collected at Blinkwater ravine (mean ± SE, n = 3). The ratio of granite: sandstone is shown with the P values derived from Student's t tests.

Oxide/ Element	Granite	Sandstone	Granite/ Sandstone	P value
Al	27001 ± 7783	13947 ± 2874	1.9	0.136
P	4189 ± 2589	3980 ± 166	1.1	0.927
K	26265 ± 205	1830 ± 327	14.4	**0.000**
Ca	5780 ± 980	469 ± 58	12.3	**0.001**
Ti	1834 ± 68	453 ± 47	4	**0.000**
V	73 ± 8	15 ± 2	4.7	**0.000**
Cr	18 ± 5	13 ± 0	1.4	0.224
Mn	351 ± 34	90 ± 38	3.9	**0.004**
Fe	26192 ± 3521	12657 ± 4292	2.1	0.069
Ni	11 ± 1	4 ± 0	2.8	**0.001**
Cu	12 ± 1	4 ± 1	2.6	**0.004**
Zn	58 ± 4	24 ± 5	2.4	**0.004**
Se	0.067 ± 0.011	0.054 ± 0.002	1.2	0.254
Br	0.28 ± 0.04	0.12 ± 0	2.2	**0.005**
Rb	149.87 ± 19.25	3.12 ± 0.64	48	**0.000**
Sr	100 ± 19	15 ± 5	6.9	**0.004**
Y	23.49 ± 3.34	3.09 ± 1.37	7.6	**0.001**
Zr	145 ± 10	48 ± 5	3	**0.000**
Nb	6 ± 1	4 ± 0	1.5	0.076
Hg	206 ± 16	155 ± 4	1.3	**0.017**
Tl	2.69 ± 0.13	1.42 ± 0.12	1.9	**0.001**
Pb	47.44 ± 6.34	7.16 ± 1.44	6.6	**0.001**
Bi	2.07 ± 0.16	1.13 ± 0.05	1.8	**0.001**