Specialization to Extremely Low-Nutrient Soils Limits the Nutritional Adaptability of Plant Lineages

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abstract: Specialization to extreme selective situations promotes the acquisition of traits whose coadaptive integration may compromise evolutionary flexibility and adaptability. We test this idea in the context of the foliar stoichiometry of plants native to the South African Cape. Whereas foliar concentrations of nitrogen, phosphorus (P), potassium (K), calcium, magnesium, and sodium showed strong phylogenetic signal, as did the foliar ratios of these nutrients to P, the same was not true of the corresponding soil values. In addition, although foliar traits were often related to soil values, the coefficients of determination were consistently low. These results identify foliar stoichiometry as having a strong genetic component, with variation in foliar nutrient concentrations, especially [P] and [K], being identified as potentially adaptive. Comparison of stoichiometric variation across 11 similarly aged clades revealed consistently low foliar nutrient concentrations in lineages showing specialization to extremely low-nutrient fynbos heathlands. These lineages also display lower rates of evolution of these traits as well as a reduced tendency for foliar [P] to track soil [P]. Reduced evolutionary lability and adaptability in the nutritional traits of fynbos-specialist lineages may explain the floristic distinctness of the fynbos flora and implies a reduced scope for edaphically driven ecological speciation.

Keywords: adaptation, ecological specialization, foliar stoichiometry, phylogenetic niche conservatism, speciation.

Introduction

Ecological specialization may limit the evolutionary versatility of lineages, to the extent that extreme specialization is sometimes interpreted as an evolutionary dead end (Futuyma and Moreno 1988). Central to this view is the idea that specialization to a particular ecological opportunity involves the acquisition of traits whose maintenance costs and/or functionality compromise performance and competitiveness in alternative ecological settings (e.g., Anacker et al. 2011). In addition, where multiple traits are involved, coadaptation may cause traits to become genetically, developmentally, and/or functionally integrated (Caillaud and Via 2000; Hawthorne and Via 2001; Monteiro and Nogueira 2010; Crisp and Cook 2012), thereby limiting the evolutionary flexibility of the individual traits and so promoting phylogenetic conservatism in both the traits and the ecological niches they define (Crisp and Cook 2012). This lineage-level conservatism is exacerbated by reduced individual-level plasticity (Svänback and Schluter 2012), which may arise both as a consequence of the direct costs of plasticity and because selection for plasticity is relaxed under ecological specialization (Murren et al. 2015). Taken together, trait coadaptation and low plasticity are expected to limit the ecological breadth of specialist lineages, with generalists being identified as more likely progenitors of adaptive radiation (Schluter et al. 1997; Muschick et al. 2011; Hughes 2012; Hardy and Otto 2015).

Extreme edaphic environments may impose strong selection on plants, eliciting the evolution of distinctive traits or trait complexes. Plants associated with serpentine soils, for example, commonly possess distinctive morphological and physiological attributes (e.g., small, sclerophyll leaves, reduced plant stature, vacuolar storage of magnesium) that enable them to cope with the extreme physiological challenges (e.g., high concentrations of magnesium and heavy metals, low nutrient availability) imposed by these soils (Brady et al. 2005). Similarly, plants inhabiting extremely low-nutrient soils commonly possess traits (e.g., cluster roots, ericoid mycorrhizas and sclerophyllous foliage), which, while facilitating the acquisition and conservation of scarce nutrients (Lambers et al. 2006; Cramer et al. 2014), compromise plant growth responsiveness and consequent competitive ability under conditions of elevated nutrient availability (Aerts 1999; Cornelissen et al. 2001; Lynch and Ho 2005; Lambers et al. 2006). Like these other traits, variation in the stoichiometry of elemental nutrients in plant tissues may be nutritionally adaptive, with species and higher-order lineages vary-

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ing in terms of both the absolute values and evolutionary labilities of their stoichiometric attributes.

There is considerable evidence indicating that plant tissue nutrient stoichiometry has a strong genetic component. First, although the foliar stoichiometry of most plant species is somewhat plastic (e.g., Ryser and Lambers 1995; Güsewell 2004), the degree of plasticity is variable (Güsewell 2004), and many species appear to maintain their tissue stoichiometries at species- and life stage–specific values in a manner that is at least partially independent of the environment, a phenomenon referred to as stoichiometric homeostasis (Sterner and Elser 2002; Elser et al. 2010). This explains why the concentrations and ratios of elemental nutrients in plant tissues are generally less variable than their corresponding soil values (e.g., Güsewell and Koerselman 2002; Neff et al. 2006). Second, stoichiometric variation correlates with a range of other functional attributes, including leaf architecture and longevity, plant size, growth form, water/reserve storage capacity, and relative growth rate (Elser et al. 2000, 2010; Sterner and Elser 2002; Güsewell 2004; Reich and Oleksyn 2004; Wright et al. 2004; Han et al. 2005; Matzek and Vitousek 2009; Peñuelas and Sardans 2009), and seasonal fluctuations in tissue stoichiometry have been shown to be coupled to metabolic activity, with both linked to phenophase (Rivas-Ubach et al. 2012). Taken together, these observations identify plant tissue stoichiometry as being highly regulated and having a strong genetic component, a conclusion that is further supported by evidence of strong taxonomic and/or phylogenetic structuring, at least for some nutrients (Broadley et al. 2004; Kerkhoff et al. 2006; Watanabe et al. 2007; Stock and Verboom 2012; White et al. 2012). In this context, the strong environmental (e.g., temperature and soil fertility) correlates of foliar stoichiometric variation (e.g., Reich and Oleksyn 2004; Han et al. 2005) have been interpreted as adaptive (e.g., Reich and Oleksyn 2004; Elser et al. 2010).

Interestingly, while the physiological importance of absolute foliar nutrient concentrations is unquestioned (e.g., Wright et al. 2004; Elser et al. 2010), most stoichiometric research has focused on foliar nutrient ratios, partly on account of a perception that physiological processes are limited by the balance of elements present in tissues and partly because ratios control for the confounding effects of leaf architecture. Although the notion of a globally applicable optimal stoichiometric ratio for all plants, as embodied by the so-called Redfield ratio (Redfield 1958), has enjoyed some attention (e.g., Hillebrand and Sommer 1999; Knecht and Göransson 2004), it is now widely recognized that stoichiometric optima vary with metabolic requirement and consequently differ between taxa, vegetation and tissue types, and between plants of contrasting age or life stage (Elser et al. 2000, 2010; Sterner and Elser 2002; Güsewell 2004; McGroddy et al. 2004; Reich and Oleksyn 2004; Han et al. 2005; Kerkhoff et al. 2006).

The strong edaphic heterogeneity of the Cape Floristic Region (CFR) of South Africa is considered an important driver of evolution and speciation (Linder 1985, 2003; Goldblatt and Manning 2000; Verboom et al. 2004; Ellis et al. 2014), making this an ideal system for exploring the role of soil fertility in powering stoichiometric variation in plants (Stock and Verboom 2012). Whereas the coastal plains and intermontane valleys of the CFR are dominated by moderately fertile, fine-grained soils derived principally from the shale rocks of the ancient Malmesbury and Bokkeveld Groups, the mid-to-high elevations of the Cape Fold Mountains are characterized by highly leached quartzitic sands (Table Mountain Group) whose nutritional status is among the poorest recorded on Earth, particularly with regard to the availability of phosphorus (Deacon et al. 1992; Lambers et al. 2006; Bradshaw and Cowling 2014; Cramer et al. 2014). Indeed, so nutritionally impoverished are these soils that, in contrast to the low-elevation scrub flora of the CFR (renosterveld, thicket, and succulent karoo vegetation), which is fundamentally African, the heathy flora (fynbos vegetation) they support reflects the preferential recruitment of low-nutrient-adapted lineages from other continents, especially Australia, which have similarly oligotrophic soils (Bergh et al. 2014; Verboom et al. 2014). These include Proteoideae and Leucadendrinae (both Proteaceae), Schoeneae (Cyperaceae), and Restionaceae, whose members form cluster roots (Lamont 1993; Lambers et al. 2006), and Erica (Ericaceae), which forms specialized ericoid mycorrhizal associations (Read 1996). Besides these low-nutrient specialists, the CFR also hosts a suite of lineages (e.g., Asteraceae, Poaceae) that appear to lack extreme low-nutrient adaptations and associate with either more fertile environments or, more typically, a broader range of nutritional environments and their associated vegetation types.

For the purpose of evaluating the hypothesis that low-nutrient-adapted, fynbos-specialist lineages and nonspecialist lineages differ intrinsically with regard to both the concentrations and/or ratios of nutrients in their leaves and the evolutionary lability of these traits, we quantified the foliar stoichiometric attributes of a suite of Cape plant species, as well as the soils on which they grew. We predicted that nutrient levels in the leaves of fynbos-specialist lineages would be both lower and evolutionarily less labile than is the case for nonspecialist lineages. To test our hypothesis, we first assessed whether foliar stoichiometric variation is determined by phylogenetic provenance as opposed to the nutritional properties of the underlying soils. We then assessed whether and how foliar stoichiometric traits vary among 11 similarly aged, family- or order-level lineages and whether these traits and the rates at which they evolve differ in a manner that is consistent with our hypothesis. Finally, we assessed whether stoichiometric conservatism incurs a cost in terms of adaptability, with the foliar nutrient concentrations of low-

nutrient-adapted lineages reflecting a greater degree of independence of their concentrations in the soil.

Material and Methods

Species Sampling

Ninety-four accessions, representing 91 species (three species had replicate accessions) and 13 families of flowering plants, were sampled from sites distributed across the western CFR (table S1; tables S1, S2 available online). Sampling was done in such a way as to ensure the representation of both (i) low-nutrient-adapted lineages that, in the context of the CFR, associate almost exclusively with fynbos vegetation (fynbos specialists, e.g., Cyperaceae, Ericaceae, Proteaceae, Restionaceae, and Rutaceae) and (ii) lineages that associate with a broader array of vegetation types (e.g., Aizoaceae, Fabaceae, Iridaceae, Lamiales, and Poaceae). The designation of lineages as fynbos specialist versus nonspecialist was based on vegetation associations reported in Verboom et al. (2014, table 5.1), but for the purpose of corroboration, each sampled species was also scored for the major CFR vegetation types (fynbos, forest, renosterveld, succulent karoo, thicket) and geologies (quartzite, shale, granite, calcareous, acid sands) in/on which it occurs (table S2). This was based on (i) scores provided by taxonomic experts having field knowledge of the sampled lineages (see "Acknowledgments"); (ii) descriptive information in Manning and Goldblatt's (2012) conspectus of the Cape flora; and (iii) Geographical Information System overlays of species' distributions on a geographical layer depicting the major vegetation assemblages.

Within families, accessions were selected to represent as even a phylogenetic spread as possible, and all accessions were taken from natively vegetated sites, the vast majority being sampled from fynbos vegetation growing on quartzitic or granite-derived soils (table S1). For each accession, we obtained (i) a representative voucher specimen deposited in the Bolus Herbarium, University of Cape Town; (ii) 0.5–1 kg of soil sampled from the uppermost 25 cm of the soil profile immediately beneath the sample plants; and (iii) 300–500 g of fresh leaves. As far as possible, single individuals were sampled, but in some instances it was necessary to sample multiple conspecific individuals in order to secure adequate quantities of leaves. In these instances, individuals were sampled to be as close together as possible, with soil samples being taken from beneath the sampled individuals and bulked.

Phylogenetic Analysis

To enable comparative analyses in the context of a phylogenetic hypothesis with time-proportional branch lengths, alignments were generated for two plastid loci (*rbcL* and *matK*) using sequences obtained from GenBank (app. A, table A1; apps. A, B available online), which were analyzed under a lognormal relaxed Bayesian clock implemented in BEAST, version 1.8.3 (Drummond and Rambaut 2007). Since conspecific sequences were available for just 35 accessions, most were represented by congeneric sequences (app. A, table A1). This was generally unproblematic (assuming generic monophyly) since genera were represented by single species, but where multiple congeners had been sampled, existing phylogenetic studies were used to identify closely related proxies. Sequences were aligned in BioEdit, version 5.0.9 (Hall 1999), using an initial Clustal alignment step and a subsequent manual step. The resulting alignments were then combined into a BEAST input file using the program BEAUTi, version 1.8.3 (Drummond and Rambaut 2007), with separate GTR+I+G models, identified as optimal by MrModeltest, version 2.3 (Nylander 2004), specified for the two loci. In order to obtain reasonable divergence time estimates, we applied age priors to 12 internal nodes (app. A, table A2) using the posterior age estimates for these nodes produced by the comprehensive dating analysis of Magallón et al. (2015). In each instance, the age prior was set as normal, with the mean and standard deviation specified such that the mean and 95% confidence interval matched the corresponding median and 95% highest posterior density intervals, identified by Magallón et al. (2015). The tree prior was specified using a birth-death process, assuming incomplete species sampling. We ran three independent Metropolis-coupled Monte Carlo Markov chains, each of 10^7 generations, sampling every 1,000th generation. Tracer, version 1.6 (Rambaut et al. 2014), was used to check for convergence between runs and to determine the appropriate burn-ins, following which the posterior samples were combined and summarized as a maximum clade credibility (MCC) tree.

Nutrient Analyses

We focused on foliar and soil dry-mass concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and their ratios (by dry mass) to P. The choice of P as a stoichiometric denominator was guided by evidence of P limitation in many Cape plants (Lambers et al. 2006; Cramer et al. 2014). Soil samples were air-dried and sieved through a 1-mm sieve, and nutrients were analyzed by BemLab (Somerset West, South Africa). Soil pH was determined by shaking 2 g of material in 20 ml 1 M potassium chloride at 180 rpm for 60 min, centrifuging at 10,000 g for 10 min, and measuring the pH of the supernatant. Total N was determined by the combustion method using a Leco-FP528 N Analyzer (Leco, St. Joseph, MI). Following Sommers and Nelson (1972), total P was extracted via acid digestion using a 1 : 1 mixture of 1 N nitric acid and

hydrochloric acid at 80°C for 30 min, and the [P] was then determined with a Varian ICP-OES optical emission spectrometer (Varian Vista MPX, Mulgrave, Australia). Exchangeable cations were displaced from 10 g of soil sample with 25 mL of 0.2 M ammonium acetate. The samples were then filtered through Whatman No. 2 filter paper and made to 200 mL before [Ca], [K], [Mg], and [Na] were determined using ICP-OES. Leaf samples were dried in an oven at 70°C for 3 d and then milled using a steel-ball mill (MM200, Retsch, Haan, Germany) and analyzed by BemLab. Foliar [N] was determined by a Leco FP-528 N Analyzer (Leco Corporation), while [P], [K], [Ca], [Mg], and [Na] were determined by dry-ashing the ground leaves at 480°C for 8 h, dissolving the ash in 16% HCl, and then measuring the concentrations using inductively coupled plasma optical emission spectrometry.

Statistical Analysis

Statistical analyses were done in the R statistical environment, version 3.2.0 (R Development Core Team 2008), using the packages ape, version 3.3 (Paradis et al. 2004); caper, version 0.5.2 (Orme et al. 2013); geiger, version 2.06 (Harmon et al. 2008); lavaan, version 0.5-20 (Rosseel 2012); phytools, version 0.4-56 (Revell 2012); and picante, version 1.6-2 (Kembel et al. 2010). Phylogenetic comparative analyses used the BEAST MCC tree, which is available via TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S20508), with the underlying BEAST XML file available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.44g1b (Verboom et al. 2017). Trait and species ecological data are available both as supplementary tables (tables S1, S2) and from Dryad, while the analytical R code and associated input files are also available from Dryad.

For each foliar and soil nutrient concentration and P ratio, we first determined the mean, minimum and maximum values, and the fold difference (range divided by minimum) across the full species set. We then used phylogenetic generalized least squares (PGLS; Martins and Hansen 1997) regression with log-transformed data (to normalize the data and reduce the effect of outliers) to assess whether and how foliar stoichiometric attributes related to their corresponding soil values. In addition, since the foliar concentration of any individual nutrient can be influenced in a complex manner by the soil concentrations of multiple nutrients, PGLS regression was also used to assess the relationships of individual foliar nutrient concentrations to the concentrations of multiple nutrients in the soil. For this purpose, models were simplified in a stepwise manner, the predictor with the lowest significance being dropped at each step and comparative model fit being assessed using Akaike information criterion. The relative importance of soil nutrients as causal predictors of the foliar concentrations of individual nutrients was also tested using structural equation models (SEM; e.g., app. B, fig. B1), with the full suite of soil variables specified as exogenous and being allowed to covary. Parameters were estimated using maximum likelihood and the standardized coefficients reported.

Phylogenetic signal in stoichiometric traits was assessed using the tip-randomization test of Blomberg et al. (2003), with a null based on 999 replicates. To test whether and how plant lineages vary in stoichiometric attributes and the rates at which these evolve, a threshold divergence time of 80 Ma was used to define a set of 11 clades corresponding to family- or order-level taxa (fig. 1). For each clade, we determined (i) the mean tip value and (ii) the mean evolutionary rate of each foliar variable, the latter being the average evolutionary rate, expressed in normalized Felsen units (Ackerly 2009), across all divergences (i.e., contrasts) within the clade. For the purpose of identifying clade-specific mean tip values or rates that were higher or lower than expected by chance, each was ranked against a set of 999 values obtained by randomly reassigning the tips on the tree and each time recalculating the corresponding mean tip/rate value. Under a two-tailed test with $\alpha = 0.05$, rank scores <25 indicated significantly low values, while rank scores >975 indicated the opposite. For each clade, the rank scores of the mean foliar concentrations of the six nutrients studied were summed to yield a foliar nutrient index (FNI) whose values range from 6 to 6,000 (six nutrients, each with a rank score range of 1–1,000). This index has the advantages that it makes no assumptions about the relative importance of the different nutrients (all nutrients contribute equally) or the manner in which their variation is distributed.

Comparison of the mean tip and rate values of foliar stoichiometric variables allowed for a visual assessment of the hypothesis that fynbos-specialist and nonspecialist lineages differ with regard to their stoichiometric attributes and the rates at which these evolve. For the foliar attributes, we tested this idea further by comparing the fits of Brownian motion trait evolutionary models (O'Meara et al. 2006) in which the evolutionary rate was either dependent on or independent of the state of an independent binary variable distinguishing fynbos-specialist from nonspecialist lineages. For this purpose, all branches within the five fynbos-specialist crown clades were marked as fynbos specialist (state 1) and all other branches as nonspecialist (state 0).

Finally, we tested whether high- versus low-nutrient lineages (as quantified using FNI) differ with regard to the rates at which their foliar stoichiometric attributes evolve and the extent to which their foliar nutrient concentrations track the underlying soil concentrations. For this purpose, we assessed the correlations of clade-specific FNI with (i) the clade-specific mean rates of evolution in [N], [P], [K], [Ca], [Mg], and [Na] and (ii) a measure of the strength of the clade-specific associations between the foliar and soil concentrations of in-

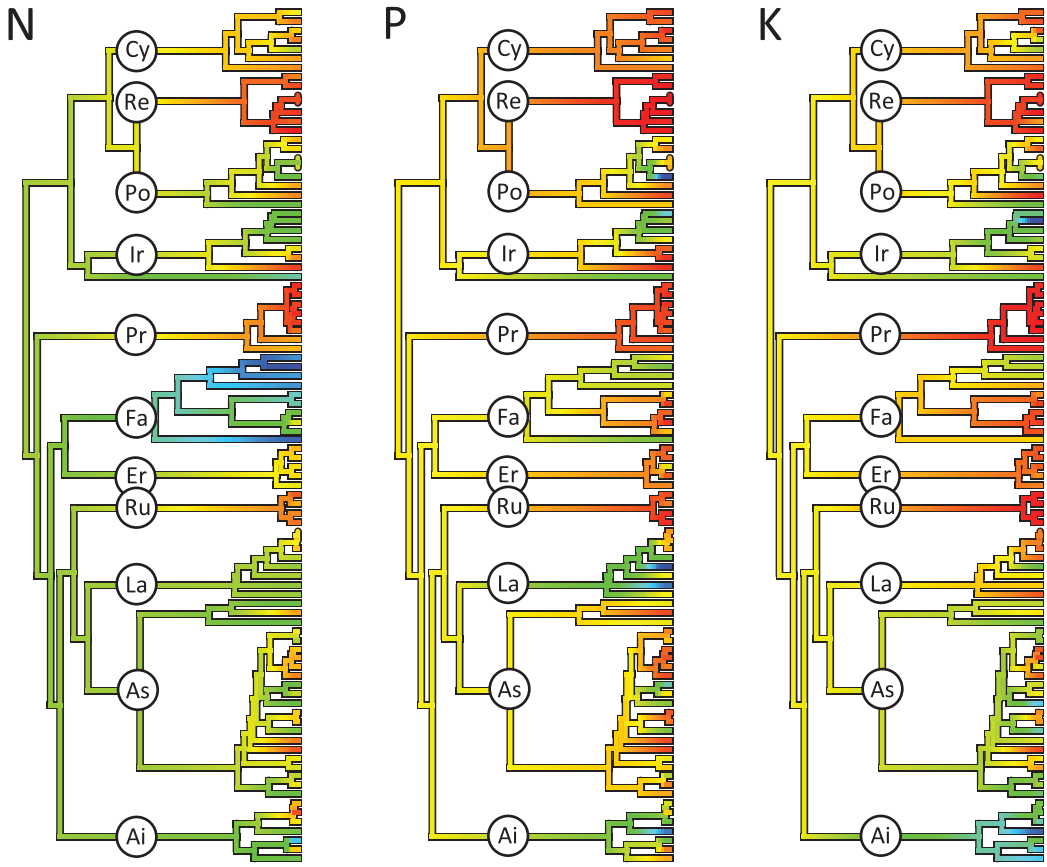

Figure 1: Variation in foliar [N], [P], and [K], determined on a dry-mass basis, across the lineages studied. Colors toward red represent low values, and those toward blue represent high values. Major lineages are indicated (circled text) as follows: Ai, Aizoaceae; As, Asterales; Cy, Cyperaceae; Er, Ericaceae; Fa, Fabaceae; Ir, Iridaceae; La, Lamiales; Po, Poaceae; Pr, Proteaceae; Re, Restionaceae; Ru, Rutaceae.

dividual nutrients. For the latter purpose, the strength of association was quantified as the mean correlation coefficient (r), based on 100 random subsamples of four species taken from the full set of species representing that clade in the study. Subsampling was necessary in order to control for differences in the number of species sampled per clade, the smallest sample size being that of Ericaceae ($n = 4$).

Results

Trait Variation

Except for [P], the foliar concentrations of all nutrients exceeded their soil concentrations by more than an order of magnitude (table 1). Also, except for [N] and [Na], foliar nutrient concentrations were less variable, in terms of fold difference, than their corresponding soil values (table 1). Of the foliar variables examined, [N] was the least variable (variation 12-fold); [K], K:P, N:P, and [P] were moderately variable (20- to 32-fold); and the concentrations of Ca, Mg, and Na and their ratios to P were the most variable (55- to 330-fold). Also, whereas foliar [K], [Ca], [Mg], and [Na] were all strongly correlated with each other across species, foliar [P] showed strong correlations only with [N] and [K], and foliar [N] was strongly linked only to [P] (fig. 2). This implies that the foliar concentrations of N and P are relatively decoupled from those of other nutrients.

Fold-difference variation in the P ratios of foliar nutrients did not differ markedly from that shown by the foliar nutrient concentrations. Whereas N:P variation was much more strongly determined by [P] (including legumes, $r^2 = 0.234$; without legumes, $r^2 = 0.388$) than by [N] (including legumes, $r^2 = 0.001$; without legumes, $r^2 = 0.045$), K:P variation was determined about equally by [K] ($r^2 = 0.141$) and [P] ($r^2 = 0.102$). Variation in the remaining P ratios, however, was consistently better explained by the concentrations of the non-P elements (Ca:P: [Ca] $r^2 = 0.368$, [P]

Table 1: Mean (min, max) and fold difference (range divided by min) of foliar and soil variables (dry mass; concentrations in g kg^{-1}) across the full data set, and corresponding mean (min, max) foliar:soil ratios

	Foliar		Soil		
Soil variable	Mean (min, max)	Fold difference	Mean (min, max)	Fold difference	Foliar:soil ratio
[N]	13 (2.9, 37)	12	1.3 (.36, 3.2)	7.9	12 (1.5, 66)
[P]	.7 (.1, 3.3)	32	.49 (.16, 8.7)	55	2.6 (.2, 11)
[K]	12 (2.1, 45)	20	.10 (<.01, .92)	234	265 (13, 1,765)
[Ca]	9.2 (.4, 58)	145	.73 (.03, 5.8)	206	25 (1.6, 164)
[Mg]	3.3 (.3, 24)	79	.16 (.01, .71)	97	44 (2.0, 676)
[Na]	3.8 (.1, 35)	330	.08 (.01, 2.8)	201	87 (1.3, 790)
N:P	24 (4.7, 123)	25	5.3 (.08, 12)	157	6.8 (.8, 77)
K:P	20 (3.6, 88)	23	.31 (.03, 1.1)	43	119 (11, 851)
Ca:P	17 (1.4, 80)	55	2.5 (.14, 17)	121	15 (.8, 254)
Mg:P	5.8 (.6, 34)	56	.59 (.01, 2.5)	195	25 (1.2, 414)
Na:P	6.5 (.2, 32)	181	.18 (.02, .84)	53	54 (1.7, 1028)

$r^2 = 0.116$; Mg:P: [Mg] $r^2 = 0.418$, [P] $r^2 = 0.066$; Na:P: [Na] $r^2 = 0.408$, [P] $r^2 = 0.042$).

Tree-Wide Phylogenetic Signal and Soil-Foliar Trait Relationships

Phylogenetic reconstructions confirmed the presence of strong phylogenetic structure in foliar nutrients, with some lineages having consistently low values and others high values (fig. 1). This pattern was, however, much less apparent in the foliar P ratios. Across the full tree, five foliar nutrient concentrations and two foliar P ratios (i.e., Ca:P and Na:P) showed significant phylogenetic signal (table 2), whereas among the soil variables, only [N] contained significant phylogenetic signal.

Despite differences in phylogenetic signal between soil and foliar variables, tree-wide PGLS regression models identified several foliar variables ([P], [K], [Na], K:P, Mg:P, and Na:P) as being significantly associated with their corresponding soil values (table 3). Since the soil-foliar associations of [Mg] and [Na] were weak or nonexistent, the strong positive associations of Mg:P and Na:P are attributable to the strong relationship of soil to foliar [P]. Thus, across all the variables studied, only foliar [P] and [K] appeared to be strongly related to their corresponding soil concentrations, and even for these two variables, soil concentrations failed to explain a large proportion of the variance in their foliar concentrations ([P], $r^2 = 0.122$; [K], $r^2 = 0.337$).

The inclusion of multiple predictors (soil nutrient concentrations) in PGLS regression models revealed that the determination of foliar nutrient concentrations is complex, with many soil nutrients potentially influencing the foliar concentrations of any individual nutrient (table 4, PGLS multiple regression). Among the soil variables considered, [P] and [K] emerged as the most consistently influential predictors (across all foliar nutrients), with [P] being the only variable to influence foliar nutrient concentrations in a consistently positive manner. SEM corroborated these results for P in identifying a consistently positive influence of soil [P] on foliar [P], [K], [Ca], [Mg], and [Na] (table 4, structural equation model).

Clade-Level Trait Variation

Comparison of the number of vegetation types occupied by species within each of the 11 clades strongly corroborated the designation of Cyperaceae, Ericaceae, Proteaceae, Restionaceae, and Rutaceae as fynbos-specialist lineages and the remainder as nonspecialist lineages (fig. 3*a*). Where species in the former clades rarely occur outside of fynbos, those in the latter commonly occupy multiple vegetation types, although there is considerable interspecific variance. This

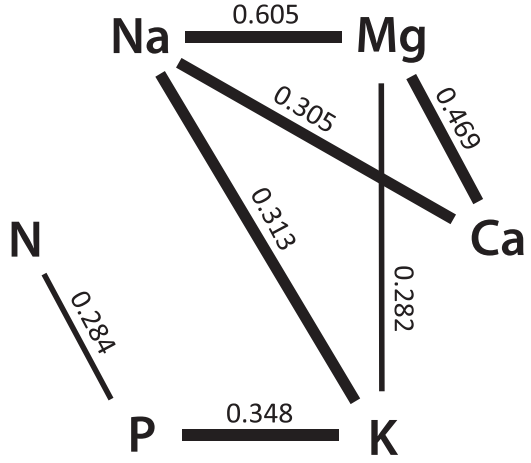

Figure 2: Cross-species correlations of nutrient foliar concentrations, the latter determined on a dry-mass basis. Lines depict correlations with $r^2 > 0.250$, all associations being positive.

Table 2: Tree-wide phylogenetic signal in foliar and soil nutrient concentrations (dry mass) and elemental ratios to P

	Leaf			Soil		
Soil variable	Obs.	Rand.	*P*	Obs.	Rand.	*P*
[N]	7.75	18.51	.049	.030	.108	.001
[P]	.027	.156	.001	.143	.491	.314
[K]	4.22	30.72	.001	.003	.004	.443
[Ca]	6.21	24.98	.005	.133	.380	.157
[Mg]	1.56	5.27	.065	.003	.006	.147
[Na]	2.03	12.1	.003	.003	.024	.132
N:P	36.6	81.0	.206	1.056	1.791	.226
K:P	60.6	70.3	.570	.026	.024	.669
Ca:P	23.2	79.9	.008	1.40	3.18	.278
Mg:P	6.31	11.6	.335	.033	.072	.099
Na:P	5.20	18.2	.006	.002	.006	.161

Note: For each variable, the observed variance of standardized contrasts ("Obs."), the mean variance of standardized contrasts based on 999 tip randomizations ("Rand."), and the associated *P* values are shown.

pattern is also apparent, albeit more weakly, when clades are compared in terms of the number of geologies with which their species associate (fig. 3*b*).

Comparison of foliar [N] and [P] and N:P across the 11 clades revealed substantial variation, with the fynbos-specialist clades consistently having low foliar [N], [P], and—with the possible exception of Cyperaceae—low foliar [K] (figs. 4, 5). With the exception of [P] in Restionaceae and [K] in Restionaceae, Ericaceae, and Proteaceae, the range of variation in foliar [N], [P], and [K] of these lineages was generally a subset of that shown by the nonspecialist lineages, which had similar minima for these variables but very different maxima (fig. 4). In contrast to the situation for foliar nutrients, the soil concentrations associated with fynbos-specialist and nonspecialist lineages did not differ dramatically (except in the case of Restionaceae) with regard to either median values or variances.

Comparison with random nulls (fig. 5*a*) confirmed the generally low-foliar-nutrient (especially [N], [P], and [K]) status of the fynbos-specialist clades. The opposite was true for Aizoaceae and Asterales, whose mean foliar [K], [Ca], [Mg], and [Na] were commonly higher than expected (fig. 5). In contrast to the concentrations, the lowest foliar P ratios were generally associated with nonspecialist clades, probably on account of their generally higher [P]. Derivation of an FNI based on the mean ranks of foliar concentrations yielded a wide range of values, indicating the existence of lineages whose foliage is generally nutrient poor and others whose foliage is generally nutrient rich. Clade-specific FNI scores displayed almost the full range of potential values (6–6,000), being highest in Aizoaceae (5,705), followed by Asterales (4,804), Iridaceae (3,348), Fabaceae (2,808), Lamiales (2,312), Poaceae (2,216), Rutaceae (1,507), Proteaceae (754), Cyperaceae (741), Ericaceae (243), and Restionaceae (89).

Foliar nutrient concentrations and possibly foliar P ratios appear to evolve at lower rates in low-FNI, fynbos-specialist lineages than is the case in high-FNI, nonspecialist lineages. This is well illustrated by reconstructions of foliar [N], [P], and [K] that identify Ericaceae, Proteaceae, and Restionaceae as being relatively invariant for these traits and Aizoaceae, Asterales, Iridaceae, Lamiales, and Poaceae as being much more labile (fig. 1). The use of normalized evolutionary rates (in Felsens), and their comparison with random nulls, broadly confirmed this pattern, with the fynbos-specialist lineages frequently showing rates of trait evolution that were lower than expected by chance (fig. 5*b*). Also supporting this pattern is the consistently better fit of trait evolutionary models that estimate separate rates of trait evolution for fynbos-

Table 3: Phylogenetically generalized least-square regression models relating foliar stoichiometric attributes to their corresponding soil variables across the full tree

Variable	β	*t*	*P*	Adj. r^2
[N]	−.042	−.259	.797	−.010
[P]	.247	3.724	<.001	.122
[K]	.188	6.951	<.001	.337
[Ca]	.063	.670	.505	−.006
[Mg]	−.106	−1.632	.106	.018
[Na]	.249	2.156	.034	.038
N:P	−.101	−1.310	.193	.008
K:P	.286	7.722	<.001	.387
Ca:P	.115	1.214	.228	.005
Mg:P	.215	3.072	.003	.083
Na:P	.312	3.064	.003	.083

Note: For each model, the slope (β), *t* statistic, *P* value, and adjusted coefficient of determination ("Adj. r^2") are shown. Concentrations were determined on a dry-mass basis, with all variables being log transformed to minimize the impact of outliers.

Table 4: Influence of soil nutrient concentrations on the foliar concentration of each nutrient studied, evaluated using phylogenetic generalized least squares (PGLS) regression and structural equation models

	Foliar variable (response)					
Soil variable (predictor)	[N]	[P]	[K]	[Ca]	[Mg]	[Na]
PGLS multiple regression:						
[N]	...	...	−.29*	...	.64*	−.48*
[P]	.30*	.47*	.15*	...	.30*	.33*
[K]	.19*	−.15*	.16*	.15*	...	−.28*
[Ca]	.07	...	...	.39*	−.22*	...
[Mg]	...	...	−.10*	−.29*	...	...
[Na]	−.51*	...	...	−.26	...	.55*
Adjusted r^2	.519	.262	.466	.154	.156	.299
Structural equation model:						
[N]	−.04	.06	.08	−.07	.15	.18*
[P]	.01	.62*	.39*	.59*	.91*	.64*
[K]	.58*	.06	.23	.25	.07	−.07
[Ca]	.40*	.10	.11	.39*	−.01	.01
[Mg]	−.30*	−.16	−.17	−.01	.03	−.09
[Na]	−.42*	−.16	−.13	−.75*	−.54*	.19
r^2	.226	.301	.231	.349	.469	.537

Note: Each column contains the parameter estimates for a separate model relating the foliar concentrations of a particular nutrient to the soil concentrations of the full set of nutrients studied (concentrations determined on a dry-mass basis). PGLS models were subjected to stepwise simplification using Akaike information criterion model comparison. In each instance, only the coefficients of variables retained in the best model are presented (otherwise marked with ellipses), with asterisks indicating significant coefficients. The coefficients of determination (r^2) associated with each model are shown.

specialist and nonfynbos lineages, with the rate estimates being consistently higher for the latter (table 5).

Clade-specific FNI was, unsurprisingly, correlated with the clade-specific mean foliar concentrations and P ratios of most nutrients (table 6). In addition, high-FNI clades were found to evolve faster with regard to foliar [P] than low-FNI clades and to show a greater tendency for foliar [P] to track soil [P] (table 6). High evolutionary lability of foliar [P] in high-FNI clades also accounts for the relationship of FNI with the rate of foliar K:P evolution, as well as the tendency for foliar K:P, Ca:P, Mg:P, and Na:P to track their corresponding soil values in high-FNI but not in low-FNI lineages.

Discussion

Two main points emerge from this study. First, we find evidence that foliar stoichiometry in plants has a strong genetic basis and conclude that stoichiometric variation has a significant adaptive component. Consistent with earlier work (e.g., Lambers et al. 2006; Cramer et al. 2014), our data identify variation in foliar [P] and [K] as being particularly significant in this regard. Second, and consistent with our primary hypothesis, we find that lineages vary considerably in their stoichiometric adaptability, with important implications for our understanding of both floristic patterning and speciation process.

The finding that most foliar stoichiometric attributes are phylogenetically structured and partly decoupled from their corresponding soil values identifies foliar stoichiometry as having a genetic component and being under some degree of homeostatic regulation (e.g., Sterner and Elser 2002; Elser et al. 2010). Both foliar nutrient concentrations and their P ratios were generally less variable than their corresponding soil values, and although foliar nutrient variation was often related to soil nutrient levels, the proportion of variance explained by these relationships was typically low (table 3). Moreover, despite a general lack of phylogenetic signal in the soil variables, foliar concentrations and P ratios generally showed strong phylogenetic signal (table 2). Although the generally weak correspondence between foliar and soil nutrient levels is partly attributable to the complex manner in which soil nutrients influence foliar nutrient concentrations, as demonstrated by our multiple PGLS regression and SEM analyses (table 4), the latter does not readily account for the strong and consistent differences in phylogenetic signal shown by foliar versus soil variables. We therefore suggest that the correlative mismatch between soil and foliar variables also reflects differences in the extent to which these variables are genetically determined. Although the observed soil-foliar mismatch is probably exacerbated by a sampling strategy that uses single individuals to represent species, this does not invalidate our analytical results

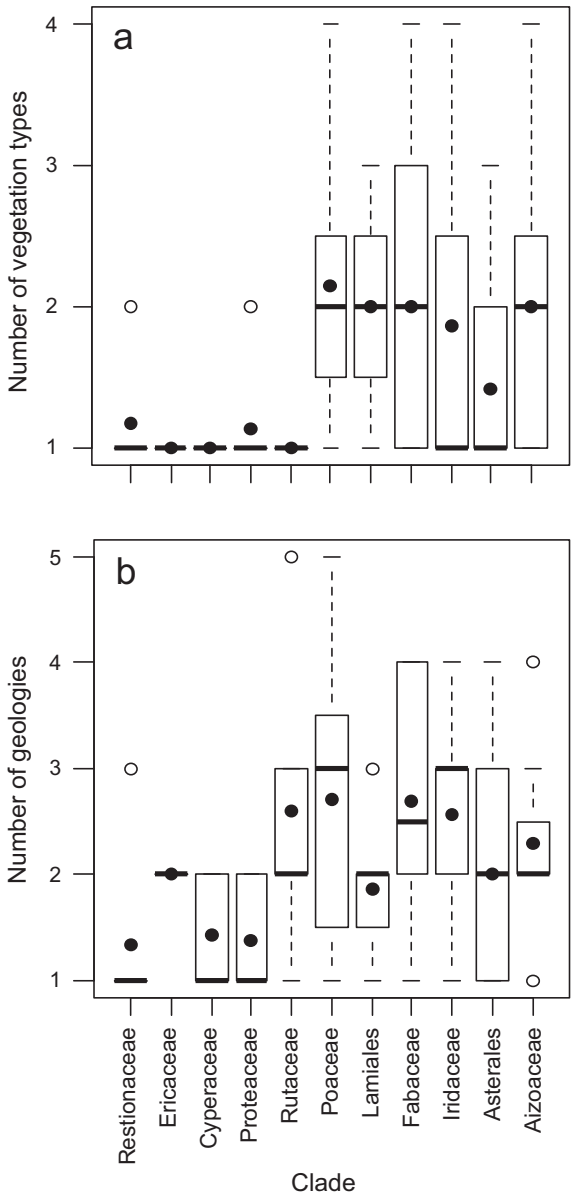

Figure 3: Box-and-whisker plots depicting the number of vegetation types (*a*) and geologies (*b*) occupied by species within each of the 11 focal clades. Heavy horizontal bars depict medians, and filled circles depict means. Boxes represent the upper and lower quartiles, whiskers the nonoutlier ranges, and open circles outliers. Clades are ordered by increasing foliar nutrient index.

since our units of analysis (tips) were individual accessions, not species, and our foliar and soil samples were appropriately matched at this level.

Our identification of foliar stoichiometry as having a strong genetic component implies that foliar stoichiometric variation, observed in both this and other studies (e.g., Reich and Oleksyn 2004; Han et al. 2005), is plausibly adaptive. In support of this idea, and consistent with earlier findings (Stock and Verboom 2012), our data clearly show that lineages that associate with fynbos soils are characterized by generally low foliar nutrient concentrations (figs. 4, 5*a*; also see FNI scores), a pattern that we interpret as indicative of physiological adaptation to conditions of chronic low nutrient availability.

While most foliar stoichiometric research has tended to focus on the ratios (e.g., N : P) of elemental nutrients rather than their absolute concentrations (e.g., Güsewell 2004; Reich and Oleksyn 2004; Kerkhoff et al. 2006; Elser et al. 2010), the low overall variability (fold difference) in [N], [P], and [K] observed here (table 1), plus the stronger phylogenetic structuring of foliar concentrations compared with that of nutrient ratios (table 2), suggests that absolute tissue nutrient concentrations are balanced at least as strongly by selection, with differences between clades probably arising as a consequence of adaptation to different selective optima. This idea is supported by the strong evolutionary foliar-soil correlations of [P] and [K] (table 3), as well as a consistent tendency for fynbos-specialist lineages to display the lowest FNI scores. While the strong patterning in foliar nutrient concentrations is partly attributable to between-clade differences in leaf architecture (e.g., sclerophylls vs. orthophylls; Wright et al. 2004), variation in the strength of correlation between individual nutrient concentrations (fig. 2), as well as variation in the strength of phylogenetic signal and/or soil-foliar relationships shown by different nutrients, indicates a residual variance that is unaccounted for by leaf architecture. That individual nutrients vary in terms of their degree of phylogenetic structuring has been noted by previous authors who attributed this variation to between-nutrient differences in the relative strength of environmental versus phylogenetic/genetic control (Watanabe et al. 2007; White et al. 2012).

Of the nutrients examined in this study, the foliar concentrations of P and K displayed the strongest phylogenetic signal (table 2), with fynbos-specialist lineages consistently having low foliar [P] and [K] (figs. 4, 5*a*). These result contrast with those of White et al. (2012), who identified shoot [Ca] and [Mg] as the strongest indicators of phylogenetic provenance, but this is likely a consequence of differences in the taxon sets and ecosystems studied. Besides showing the strongest phylogenetic structure in our study, foliar [P] and [K] (and their ratio, K : P) also showed the strongest associations with their corresponding soil levels (table 3), and soil [P] and [K] were most frequently identified as influencing the foliar concentrations of a wide range of nutrients (table 4, PGLS multiple regression). These results, paired with the moderate variability of foliar [P] and [K] (table 1), identify these elements as important agents (soil) and targets (foliar) of selection and family-level clade differentiation in the native flora of the CFR. This conclusion accords well with the acknowledged physiological importance of P and K and the observation that the availabilities of nonrenewable minerals,

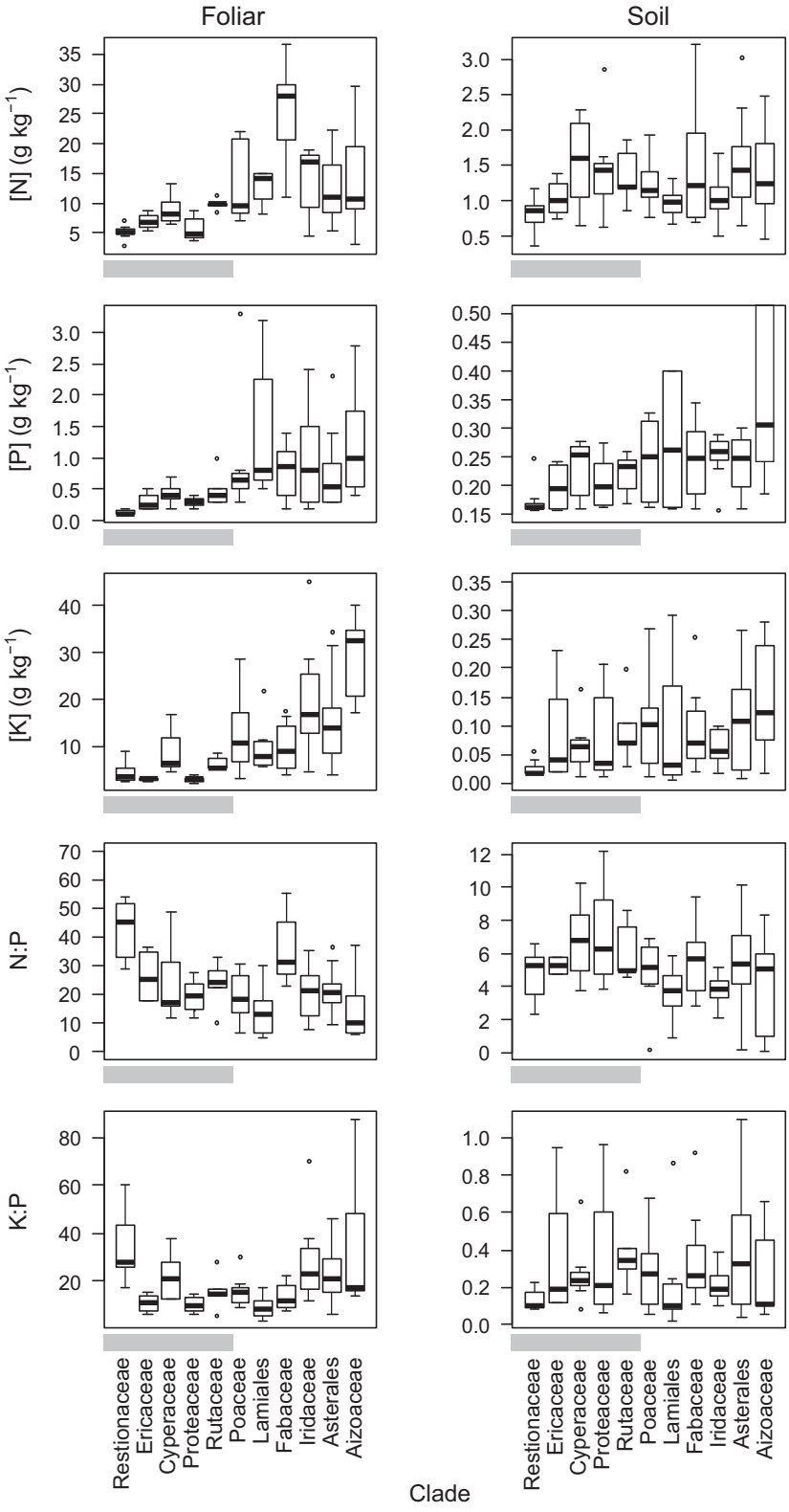

Figure 4: Box-and-whisker plots depicting clade-level variation in foliar and soil [N], [P], [K], N : P, and P : K. Clades are ordered by increasing foliar nutrient index. Horizontal gray bars below the *X*-axis of each panel distinguish fynbos-specialist lineages. To improve data visualization, outliers are omitted (foliar N : P: Fabaceae 123 g kg^{-1}; soil [P]: Poales 4.6 g kg^{-1}, Lamiales 1.2 g kg^{-1}, Iridaceae 0.54 g kg^{-1}, Asterales 5.2 and 0.85 g kg^{-1}, Aizoaceae 8.7 g kg^{-1}; soil [K]: Aizoaceae 0.92 g kg^{-1}), while the upper limits of the interquartile (2.5 g kg^{-1}) and nonoutlier (4.7 g kg^{-1}) ranges of [P] for Aizoaceae are truncated. Concentrations were determined on a dry-mass basis.

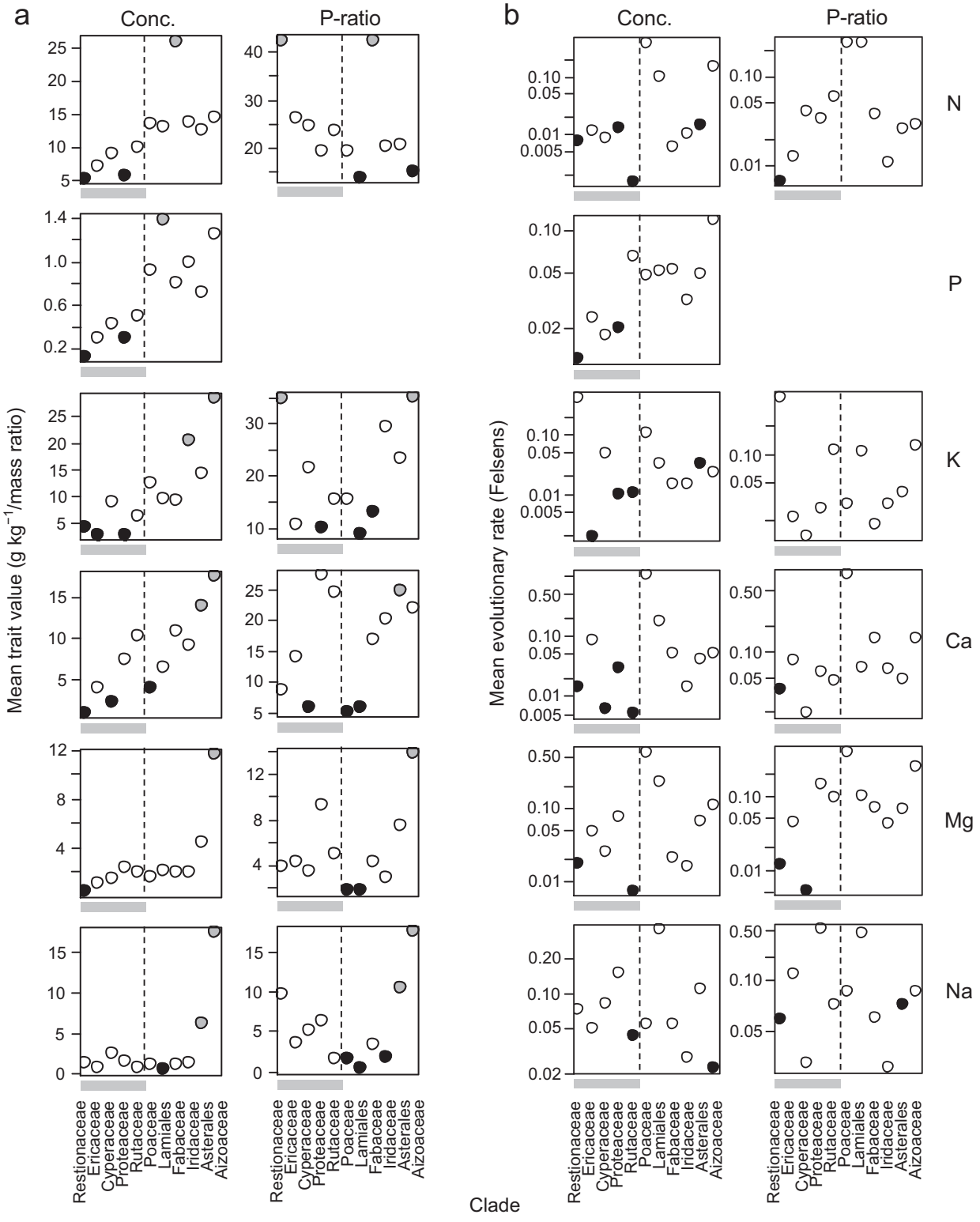

Figure 5: Mean tip values (*a*) and evolutionary rates (*b*) of foliar nutrient concentrations (dry mass) and P ratios for each of the 11 focal clades. Clades are ordered by increasing foliar nutrient index, with fynbos-specialist clades indicated with a gray horizontal bar and separated from nonspecialists by a vertical dashed line. Filled symbols indicate clade means or rates that are significantly low (black) or high (gray), as evaluated using a null produced by randomly reshuffling the tip values 999 times (two-tailed test, $\alpha = 0.05$).

Table 5: Evolutionary rates of foliar stoichiometric traits as estimated by Brownian motion models of trait evolution (O'Meara et al. 2006)

Trait	*P*	Rate (0)	Rate (1)	Ratio
[N]	<.001	10.5	.320	32.9
[P]	<.001	.04	.004	9.0
[K]	.033	4.88	2.35	2.1
[Ca]	<.001	8.26	.680	12.1
[Mg]	<.001	2.08	.126	16.5
[Na]	<.001	2.71	.195	13.9
N:P	.004	44.1	16.0	2.8
K:P	<.001	25.1	143.6	.2
Ca:P	.615	23.0	...	...
Mg:P	.081	6.25	...	...
Na:P	.058	5.15	...	...

Note: For each trait, we compared a model estimating separate rates of evolution on fynbos-specialist and nonspecialist branches (dependent model) with a model estimating a single rate on all branches (independent model). For the dependent model, "Rate (0)" is the rate estimate on nonspecialist branches, "Rate (1)" is the rate on fynbos-specialist branches, and "Ratio" is the rate ratio (rate (0)/rate (1)). Positive rate ratios indicate a lower evolutionary rate on fynbos-specialist branches. Concentrations were determined on a dry-mass basis.

such as P (Lambers et al. 2006; Cramer et al. 2014) and K (Milewski 1982), are extremely low in Cape soils on account of their derivation from minerally impoverished parent rock (Beadle 1966; Marchant and Moore 1978) and their long histories of weathering (Walker and Syers 1976; Vitousek et al. 2010; Cramer and Hoffman 2016). Unsurprisingly, the significance of P and K as drivers of plant evolution in the CFR and in the nutritionally comparable similar South Western Australian Floristic Region extends beyond tissue stoichiometry. For example, both the prevalence of cluster roots in plants from these regions (Lamont 1993; Lambers et al. 2006) and the development of seeds with exceptionally high [P] have been interpreted as low-P adaptations (Stock et al. 1990; Groom and Lamont 2010), while a general paucity of fleshy-fruited species has been attributed to K limitation (Milewski 1982). Although we identify both P and K as potentially important agents of selection in the CFR, there are indications of a preeminent role for P. Multiple PGLS regression (table 4, PGLS multiple regression) identified both soil [K] and [P] as influencing the foliar concentrations of multiple nutrients, but only in the case of [P] were the coefficients consistently positive. Moreover, SEMs identified only soil [P] as exerting a broad influence on foliar nutrient concentrations (table 4, structural equation model). Together with the observation of exceptionally low foliar:soil concentration ratios for P, relative to the other nutrients studied, these results potentially identify environmental P as being the single most important driver of nutritional evolution in Cape soils (Lambers et al. 2006; Cramer et al. 2014).

Cape soils are also characterized by generally low N availability (Stock and Allsopp 1992; Cramer et al. 2014), but the dynamic and renewable nature of soil N probably results in foliar [N] being a lesser target of selection leading to adaptive divergence than are foliar [P] and [K]. Moreover, the importance of foliar [N] as a signaling factor in plants (Wilkinson et al. 2007; Cramer et al. 2009) may impose strong constraints on its variability. Our data show that, of all the elements studied, foliar [N] was the least variable (table 1), apparently confirming its tightly regulated nature and its limited freedom to vary in response to a heterogeneous selective environment. Also supporting this interpretation are the observations that variation in foliar N:P is determined much more strongly by foliar [P] than by foliar [N], that phylogenetic signal in foliar [N] is weak (table 2, $P = .049$), and that foliar [N] is not evolutionarily associated with soil [N] (table 3). The lack of a relationship between foliar and soil [N] is also consistent with evidence (Xing et al. 2016) showing that foliar [N] variation in *Quercus wutaishanica* is more strongly influenced by soil [P] and [K] than by soil [N], a pattern that is mirrored in our data (table 4).

Interpreting variation in the remaining foliar nutrients is difficult given that, unlike N and P, whose foliar concentrations are largely decoupled from the concentrations of other nutrients, [K], [Ca], [Mg], and [Na] are tightly interrelated. Thus, while variation in the concentrations of all of these cationic elements is conceivably adaptive and under some degree of homeostatic regulation, an alternative is that the uptake and foliar concentrations of just one of these elements is regulated, with the elevated foliar concentrations of the others arising as an incidental by-product. For example, upregulation of transpiration, geared toward the increased delivery of P and K to the root-soil interface (e.g., Matimati et al. 2013), may inadvertently elevate concentrations of $Ca2+$, $Mg2+$, and $Na+$ in the rhizosphere (Cramer and Hawkins 2009), thereby facilitating the increased uptake and accumulation of these elements by the plant. Under such a scenario, the comparatively low variability of foliar [K] (table 1) identifies K as the most likely subject of stoichiometric regulation, this being further suggested by the strong correlation (fig. 2) of foliar [K] and [P], which identifies these elements as being stoichiometrically coupled. The physiological importance of tissue [K] is well established (e.g., Babita et al. 2010; Oddo et al. 2011), with its stoichiometric balance to [N] and [P] being known to influence plant growth and water limitation responsiveness (Sardans et al. 2012).

An important finding of the present study is that low-nutrient, fynbos-specialist clades (i.e., Cyperaceae, Ericaceae, Proteaceae, Restionaceae, and Rutaceae) are characterized not only by lower foliar nutrient levels (and high foliar N:P and K:P) but also a reduction in the rates at which these traits evolve (fig. 5*b*; table 5). A loss of evolutionary lability, potentially linked to adjustments in leaf architecture (Wright et al. 2004), has important ecological implications.

Table 6: Correlation of clade-specific foliar nutrient index (FNI) with clade-specific tip means, evolutionary rates, and soil-foliar correlations of foliar stoichiometric traits

	FNI vs. trait tip mean		FNI vs. trait evolutionary rate		FNI vs. trait soil-foliar correlation	
Trait	*r*	*P*	*r*	*P*	*r*	*P*
[N]	.562	.071	.225	.507	.321	.335
[P]	.736	.010	.782	.004	.759	.007
[K]	.892	<.001	−.368	.266	.499	.118
[Ca]	.883	<.001	.005	.989	.385	.243
[Mg]	.803	.003	.089	.794	.736	.010
[Na]	.752	.008	−.111	.745	.091	.789
N:P	.328	.325	−.053	.877	.563	.071
K:P	.724	.012	.622	.041	.736	.010
Ca:P	.761	.006	−.075	.825	.892	<.001
Mg:P	.650	.030	.164	.630	.884	<.001
Na:P	.466	.149	−.213	.530	.803	.003

Note: Clade-specific soil-foliar correlations were quantified as the mean of 100 correlation coefficients (*r*), each based on a subsample of four species drawn randomly from the full set of species representing each clade. Concentrations were determined on a dry-mass basis.

Since the ability to grow quickly and be competitive under high-nutrient conditions generally requires the maintenance of high foliar nutrient concentrations (Chapin 1980; Elser et al. 2000; Wright et al. 2004), the apparent inability of fynbos specialists to attain high foliar nutrient concentrations may confine them to nutritionally impoverished settings. This may account for the exceptional floristic distinctness of fynbos relative to the more eutrophically adapted African flora (renosterveld, succulent karoo, thicket vegetation) within which it is situated (Goldblatt 1978; Linder 2003; Bergh et al. 2014; Verboom et al. 2014). Although several fynbos-specialist lineages have expanded their ranges into tropical Africa (e.g., Galley et al. 2006), their consistent association there with infertile or low-temperature conditions provides further evidence of their confinement to low-productivity settings.

Evolutionary conservatism of nutritional traits in low-nutrient lineages also has consequences for speciation process. A tendency for foliar [P] to track its corresponding soil concentrations, but only in high-FNI lineages (table 6), suggests that extreme low-nutrient specialization limits the adaptability and/or plastic responsiveness of plant lineages to soil heterogeneity. Thus, although edaphically driven ecological speciation (sensu Rundle and Nosil 2005) has long been considered an important driver of Cape floristic diversity (e.g., Linder 1985; Goldblatt and Manning 2000; Ellis et al. 2014), its importance as a driver of diversification in low-nutrient-adapted, fynbos-specialist lineages may be limited. While there is plenty of evidence to show that fynbos taxa have repeatedly speciated across the quartzite-limestone (both geologies characterized by low availabilities of P) boundary to produce a floristically distinct and endemic-rich limestone form of fynbos (Cowling and Holmes 1992; Richards et al. 1995, 1997), evolutionary transitions onto the more fertile, P-rich substrata (e.g., shales) of the CFR are rare (e.g., see Hoffmann et al. 2015). Thus, as exemplified by the grass genera *Ehrharta* (Verboom et al. 2004) and *Pentameris* (Galley and Linder 2007), which have diversified onto the full range of substrata available in the CFR, the scope for edaphically motivated adaptive radiation may be greatest in lineages whose nutritional biology is relatively unspecialized. Conversely, as exemplified by Proteaceae (Latimer et al. 2009; Prunier and Holsinger 2010) and Cyperaceae (Britton et al. 2014), the strong nutritional conservatism of low-nutrient fynbos specialists may render them more susceptible to nonecological speciation (sensu Rundle and Nosil 2005) in an edaphically patchy CFR landscape (cf. Wiens 2004).

Although edaphic gradients almost certainly influence floristics and speciation process globally, the low-P soils of the CFR present a particularly extreme selective environment, akin to those presented by serpentines (Brady et al. 2005; Anacker et al. 2011). Consequently, their ecological and evolutionary impacts are likely to be unusually strong. It is well established that extremely low-P soils, such as those found in the CFR, have elicited a suite of nutritional specializations (Lamont 1982; e.g., cluster roots, ericoid mycorrhizas, parasitism, carnivory, N fixation), and we propose that adjustments in leaf physiology, associated with the maintenance of lower foliar nutrient concentrations, be added to this list. For example, Proteaceae from severely P-impoverished soils maximize photosynthetic P-use efficiency through the replacement of phospholipids with galactolipids and sulfolipids (Lambers et al. 2012). Finally, consistent with the view that extreme ecological specialization limits evolutionary versatility (Futuyma and Moreno 1988), we find evidence to suggest that this stoichiometric adaptation limits the nutritional adaptability of the lineages in which it arises, with consequences for their ecology and evolutionary biology.

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