Role of environmental filtering and functional traits for species coexistence in a harsh tropical montane ecosystem

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Environmental filtering and niche differentiation are often invoked to explain species coexistence at local scales. The ironstone *campo rupestre* of Brazil provides a biodiverse natural experiment in which edaphic gradients represent filters to test the hypothesis that plant community functional composition, despite converging on extreme stress tolerance, exhibits a co-structure with environmental parameters. At the Serra do Rola-Moça State Park, soil physico-chemical parameters were characterized alongside community-weighted mean plant functional traits and Grime's competitor, stress-tolerator and ruderal strategies for species at each sampling site. In general, species exhibited a high degree of stress tolerance (between 72.6% and 100%), while ruderalism was 0% for all species. Soil nutrients related to plant metabolism (e.g. P, Ca, Mg) were associated with the stress-tolerant strategy and with traits involved in the leaf economics and size spectra. Despite a major edaphic filter selecting stress tolerance, fine-scale microhabitat variability represented by soil parameters related to fertility (i.e. P, Ca, Mg) and water retention capacity (i.e. clay content) was associated with subtle variation in ecological strategies and functional traits of species in the ironstone *campo rupestre*.

ADDITIONAL KEYWORDS: CSR – extreme environments – functional ecology – OCBIL – plant community assembly – plant ecological strategies.

INTRODUCTION

Fundamental questions in ecology include how communities assemble, how biodiversity arises, and how species evolve alongside one another and within the local environment. Since Diamond (1975) proposed the concept of assembly mechanisms, ecologists have sought to understand the rules underlying species coexistence. Of these rules, environmental filtering, limiting similarity and niche differentiation have often been invoked to explain species coexistence at local scales (e.g. MacArthur, 1969; Westoby *et al.*, 2002; Götzenberger *et al.*, 2012; Kraft *et al.*, 2015). For example, in phosphorus-impoverished landscapes, changes in the diversity of plant nutrient-acquisition strategies are driven by low soil P concentrations (Zemunik *et al.*, 2015).

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Environmental parameters (e.g. climatic, edaphic) could act as strong filters during colonization and establishment to determine the local presence of species, which in turn drives the local prevalence of plant functional traits and ecological strategies (Pierce *et al.*, 2007; Grime & Pierce, 2012; Negreiros *et al.*, 2014). To survive in old-growth mountain top grasslands in eastern Brazil (known as *campo rupestre* in Portuguese) plants tend to exhibit morpho-physiological adaptations to improve water capture and use, as the soils are shallow and with low water retention capacity, and to withstand extreme daily temperature excursions (e.g. Jacobi *et al.*, 2007; Ferrari *et al.*, 2016; Schaefer *et al.*, 2016). Low soil nutrient availability is also an important limiting factor for plant success and distribution in this ecosystem (Benites *et al.*, 2007; Le Stradic *et al.*, 2015, Fernandes 2016a). Biotic factors such dispersal limitation are probably also involved in the assembly of *campo rupestre* communities, as the vast majority of taxa evident in this habitat exhibit no obvious mechanism for seed dispersal (Silveira *et al.*, 2013). Furthermore, other biotic factors such as seed bank dynamics (Medina & Fernandes, 2007; Silveira *et al.*, 2012; Luz *et al.*, 2018) and herbivory (e.g. Fernandes & Price, 1991) probably play important yet neglected roles in the assembly of plant communities in the *campo rupestre*. These filters should be particularly intense in the harsh ecosystems of *campo rupestre* and ultimately shape its unique, highly endemic and endangered vegetation (Fernandes & Price, 1991; Negreiros *et al.*, 2014; Fernandes, 2016a, b).

In the case of *campo rupestre* on iron substrates (a ferruginous rock matrix), plant communities are even more influenced by additional stress factors such as the acidic soils containing high contents of heavy metals (e.g. Mn, Fe, Al) (Jacobi & Carmo, 2008; Ferrari *et al.*, 2016). Metallophyte plants are common in this ecosystem (Messias *et al.*, 2013; Oliveira *et al.*, 2016, Ribeiro *et al.*, 2017). Despite the harsh abiotic conditions, this vegetation is comparatively rich in coexisting species (Jacobi & Carmo, 2012) in comparison to other landscapes with similar characteristics, such as fynbos in South Africa and kwongan in Australia, which are recognized as important centres of plant richness and endemism (Hopper, 2009; Silveira *et al.*, 2016). Therefore, the *campo rupestre* arises as part of a global biodiversity hotspot that typically characterizes an old, climatically buffered infertile landscape (abbreviated to OCBIL; Hopper *et al.*, 2016; Silveira *et al.* 2016).

Plant functional traits are important tools to unveil the dynamics and structure of ecological communities and how they respond to natural and anthropogenic disturbances (Weiher *et al.*, 1999). Functional traits are defined as any morphological, physiological or phenological feature measurable at the individual level, without reference to the environment or any other level of organization, and which impact fitness indirectly via their effects on survival, growth and reproduction (Violle *et al.*, 2007). Thus, ultimately the structure and dynamics of community and ecosystem functioning is likely to be driven by species traits (Díaz *et al.*, 2004, 2016). In this context, researchers have attempted to explain community and ecosystem functioning in many biomes worldwide in terms of plant functional traits and the suites of traits that constitute ecological 'strategies' (e.g. Chai *et al.*, 2015; de Paula *et al.*, 2015; Pierce *et al.*, 2017). Plants can acquire, process and invest resources in different ways, and the diversity of strategies has a significant impact on species composition and ecosystem functioning (Chapin *et al.*, 2000; Loreau *et al.*, 2001). This constitutes a fundamental trade-off, evident at the global scale as well as at regional and local scales, between a set of traits that permits conservation of resources and another set that allows rapid acquisition of resources. This forms a fundamental axis of plant life history known as the leaf economics spectrum (Wright *et al.*, 2004) and, more broadly, the plant economics spectrum (Reich, 2014). Another main axis of functional trait variability evident globally is related to plant size and includes co-variation in plant height, leaf area and seed mass (Díaz *et al.*, 2004, 2016; Pierce *et al.*, 2013, 2014). In summary, in a bidimensional space, plant height, leaf area and seed mass all correlate positively with each other, characterizing the size spectrum, and variation in this size-related axis is orthogonal to variation in the acquisitive–conservative axis on global and local scales (Díaz *et al.*, 2004, 2016; Cerabolini *et al.*, 2010; Pierce *et al.*, 2014, 2017).

A widely known tool to evaluate functional composition and diversity in communities considering these axis is the CSR ecological strategy scheme (C: competitiveness, S: stress tolerance, R: ruderality; Grime, 2001; Grime & Pierce, 2012), in which sets of functional traits are reported to have evolved under the operation of different levels of competition (resource pre-emption), stress (metabolic restriction) and disturbance (biomass destruction). These sets of traits can consist of easily measured 'soft' characters (e.g. Pierce *et al.*, 2017), correlated with key 'hard' functional abilities for plants (e.g. relative growth rate, leaf decomposition rate, leaf lifespan; Díaz *et al.*, 2004; Garnier *et al.*, 2017). Furthermore, these soft traits can be readily collected for large numbers of species and individuals within each species (Pérez-Harguindeguy *et al.*, 2013),

which allows replication and the comparison of results across different communities (e.g. Díaz *et al.*, 2016; Pierce *et al.*, 2017).

The CSR plant classification scheme has already enabled researchers to identify mutual and divergent effects of soil nutrients (N and P) on ecological strategies, suggesting the existence of a complex regulatory mechanism of soil fertility on trait evolution (Fujita *et al.*, 2013). This scheme provides a solid theoretical basis that allows the use of functional traits as a predictive tool, for example in restoration ecology. A meta-analysis by Pywell *et al.* (2003) using 25 experiments in grassland vegetation during restoration evaluated plant performance in relation to species functional traits and highlighted large differences in performance between species. Those species that settled and persisted in the restored plant communities exhibited traits associated with abilities such as colonization, competitiveness and vegetative regeneration, and generally possessed generalist characteristics associated with fertile habitats. This knowledge is of particular relevance for the restoration of extreme environments such as ironstone *campo rupestre*, where environmental filters are considered intense (e.g. Fernandes, 2016a, b). This has implications for applications beyond purely theoretical ecology: through discovery of the relationship between traits and the environment, the most appropriate species for the restoration of this low-resilience ecosystem can be better selected (Fernandes *et al.*, 2016a).

In the present study we evaluated the relative importance of CSR strategies involved in community assembly for different microhabitats characterized by differing soil proprieties in ironstone *campo rupestre* vegetation. In this context, we tested an initial hypothesis that a prevalence of S-selected (stress tolerance) strategies is evident in the plant community, as chronically unproductive environments are expected to favour species with traits characteristic of a conservative stress resistance syndrome (Chapin *et al.*, 1993; Negreiros *et al.*, 2014). We then tested the hypothesis that despite the operation of a strong edaphic filter selecting for stress tolerance, functional variability reflects microhabitat variability (i.e. a positive relationship between microhabitat and strategy specialization). It is expected that areas with higher organic matter content, more phosphorous, lower acidity and more exchangeable bases (i.e. relatively fertile areas) will harbour species with larger leaf area and a higher degree of competitiveness, while areas with less organic matter, phosphorous, bases and higher acidity (i.e. more infertile areas) will host species with a higher degree of stress tolerance.

MATERIAL AND METHODS

STUDY AREA

This study was conducted in the Serra do Rola-Moça State Park (20°03′07″S, 44°00′06″W) and its surroundings. This protected area is located in the so-called 'Iron Quadrangle', in the southern part of the Espinhaço Mountain Range in the transition between two biodiversity hotspots, the Cerrado and the Atlantic Forest, in Brazil (Jacobi & Carmo, 2012; see also Schaefer *et al.*, 2016). The flora and fauna of the Iron Quadrangle are influenced by these two biomes within its 7200-km² extent (Jacobi & Carmo, 2012). The region is home to several iron mines and exhibits haematitic rocky outcrops covered by *campo rupestre* vegetation, also locally known as 'canga' (Rizzini, 1979). This grassland occurs at altitudes above 900 m and is characterized by high floristic diversity, with more than 30% of species being endemic (Jacobi & Carmo, 2008), dozens of rare species (Giulietti *et al.*, 2009), and high alpha and beta species diversity (Jacobi & Carmo, 2008). The climate of the area is mesothermic with dry winters and rainy summers (Cwb); mean annual temperature is 19 °C and the annual rainfall is 1562 mm (Alvares *et al.*, 2013).

SPECIES SELECTION AND SAMPLING

We focused on herbaceous-shrub and succulent vegetation because of its importance in this ecosystem, representing more than 70% of the plant cover in the study area (Carmo & Jacobi, 2016; Carmo *et al.*, 2016). Phytosociological data were obtained in a study by S. R. Souza and M. D. M. Veloso (unpubl. data), from 52 plots (10 × 10 m) distributed in four areas, located at least 1 km apart. Each area was represented by one transect with 13 plots each one 10 m apart. The plots were aligned in a way to cover the range of soil microhabitats of each area, not necessarily in the same cardinal direction. Plots were arranged over a relatively flat area of ironstone *campo rupestre* habitat with no more than 9 m of altitudinal variation, on the mountain tops where the vegetation occurs conspicuously; they thus experience the same macroclimatic and macrotopographic conditions. We surveyed 30 plant species for the functional trait measurements, including poikilohydric sclerophyllous shrubs, rossetes and succulents. These plant species accounted for 89% of plant relative density in the plots. Vouchers of collected species are deposited at Montes Claros Herbarium (MCMG) of the Montes Claros State University (Unimontes). Six healthy adult individuals of each species were randomly sampled from the surrounding locations where the phytosociological measurements were taken.

Functional traits were collected in the middle of the rainy season (January to February 2016), the most favourable growth period in the *campo rupestre*. All measurements followed the standardized protocols described by Pérez-Harguindeguy *et al.* (2013).

TRAIT MEASUREMENTS

To quantify functional traits and CSR strategies, two leaves per individual (and whole ramets for clonal species) were collected in the field to avoid desiccation of leaf material. Collection periods were between 07:00–10:00 and 16:00–18:00 h, hence avoiding the hottest period of the day. The collected material was immediately saturated using a water spray and packed in moist paper bags which were sealed in a plastic bag and stored in a thermal box until transport to the laboratory. The plant material was kept in a refrigerator at 4 °C for a minimum of 12 h to achieve complete turgidity prior to measurements. The rehydration procedure is necessary to obtain standardized measures of leaf area (LA), avoiding leaf area shrinkage due to changes in evapotranspiration rates and dehydration during transport from field to laboratory. This allows standardized determination of specific leaf area (SLA) and leaf dry matter content (LDMC) values, despite different field conditions of each individual at the moment of sampling, as these parameters may vary substantially during the day (Pérez-Harguindeguy *et al.*, 2013).

For the calculation of CSR strategies, three key foliar traits were necessary according to the protocol proposed by Pierce *et al.* (2017): SLA, LDMC and LA. Additionally, we estimated leaf toughness (LT) which is also a trait related to the leaf economics spectrum. Measurements were taken from the two collected leaves (including the petiole) of each of the six replicate individuals of each species to generate an average value for each replicate, within 48 h of collection. Only young, fully expanded and hardened leaves without signs of herbivory, pathogens or malformation were sampled. Leaf area was obtained using a desktop scanner and the software SigmaScan Pro v.5.0 (SPSS, Inc., Chicago, IL, USA). Curved leaves were cut and positioned on the glass so that they were in complete contact with the scanner surface. Leaf water-saturated fresh weight was obtained from turgid leaves for which the surfaces were dried with soft towel paper, and the leaf dry weight was determined after 96 h of drying in an oven at 60 °C (both measurements were made using an analytical balance with a precision of 0.1 mg). For the classification of CSR strategies, values of LA, SLA and LDMC for each species were inputted into 'StrateFy' (Pierce *et al.*, 2017; available at https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1365-2435.12722) to calculate the relative proportion (%) of C-, S- and R-selection. This method was originally calibrated using data for 3068 species obtained from a global database (see Pierce *et al.*, 2017) for which multi-trait variation was then analysed by principal components analysis (PCA), trait values were regressed against PCA axes, and these regression equations were used to produce a Microsoft Excel spreadsheet capable of comparing new trait values against the global trait space. Crucially, this CSR classification method does not use each trait to directly represent the extent of C-, S- and R-selection: it is the trade-off between them, integrated and compared against trade-offs evident globally, from which CSR scores are calculated (Pierce *et al.*, 2017). As part of the development of the method, a co-inertia analysis demonstrated that trade-offs between the three leaf traits can represent variation in 14 key leaf, reproductive and whole plant functional traits, including leaf nitrogen concentration, seed mass and flowering phenology (Pierce *et al.*, 2017). We estimated LT using a digital penetrometer (Chatillon DFE-010, Largo, FL, USA) coupled to a cone-shaped tip (SPK-FMG-009A), according to Silva & Batalha (2011). To measure LT, the leaves were sustained and pressed gently under a fixed acrylic plate with three holes (3, 5 and 10 mm in diameter), each one used depending on the leaf size. Thus, in a portion of the leaf without ribs, the penetrometer was pressed against the leaf blade until it was torn. The functions related to each trait are described in Table 1.

SOIL ANALYSIS

Soil analyses were conducted in the 52 plots of 100 m². In each plot five soil samples were collected at the four vertices and at the centre of plot, at a maximum depth of 10 cm below the soil surface. The samples were air-dried and sieved to 2.0 mm prior to physicochemical analysis. The following soil attributes were analysed: Coarse sand (%); Fine sand (%); Silt (%); Clay (%); pH in water (1:2.5, v/v, soil:water suspension); Exchangeable aluminium (Al), calcium (Ca) and magnesium (Mg) extracted with 1 M KCl; and Exchangeable phosphorus (P), extracted with a double acid solution (0.025 M sulphidric acid, 0.05 M hydrochloric acid-Mehlich-1 extractor), according to the standard methods compiled by EMBRAPA (1997). The organic carbon (C) content was determined using the method of Walkley & Black (1934).

RELATIONSHIP BETWEEN ENVIRONMENTAL PARAMETERS AND SPECIES TRAITS

To test the hypothesis of a relationship between environmental parameters and species traits, we used the method developed by ter Braak *et al.* (2018) that

Table 1. CSR strategies, leaf traits surveyed, units of measurement and respective functions

Leaf trait	Abbreviation	Unit	Related functions
C-strategy	C	%	Resource pre-emption; investment of resources in further resource capture and in continued vegetative growth
S-strategy	S	%	Resource conservation; maintenance of metabolic performance in variable and limiting environments
R-strategy	R	%	Investment in propagules; regeneration of the populations in the face of repeated biomass destruction events
Leaf area	LA	mm^2	Nutrient stress and disturbances related to climatic regions
Specific leaf area	SLA	mm^2 mg^{-1}	Potential relative growth rate; leaf lifespan
Leaf dry matter content	LDMC	%	Litter decomposition; resistance to physical damage; leaf lifespan
Leaf toughness	LT	GF	Leaf lifespan; structural protection of the photosynthetic tissues; resistance to physical damage; leaf mineral nutrient contents

Function according to Lambers & Poorter (2004), Grime *et al.* (1997), Weiher *et al.* (1999), Garnier & Navas (2012), Grime & Pierce (2012), Pérez-Harguindeguy *et al.* (2013) and Pierce *et al.* (2017).

combines two simple regressions using the community-weighted mean (CWM) of species trait values and the species' niche centroid (SNC) of environmental parameters to determine the traits that are effectively filtered by environmental conditions. These regressions require three types of matrices, a community matrix (abundances of species across plots), a functional matrix (functional traits across species) and an environmental matrix (environmental variables across plots). In the present study, the community matrix included 30 species (out of 32 species identified by the phytosociological survey at the sites) by 52 sites (plots), using relative density as a measure of abundance; the functional matrix had 30 species by seven traits (i.e. C, S, R, LT, LA, LDMC and SLA); and the environmental matrix had 52 sites by 16 environmental parameters (i.e. pH, P, K, Ca, Mg, Al saturation, Base saturation, Organic carbon, Cu, Mn, Fe, Zn, Coarse sand, Fine sand, Silt and Clay). To meet normality assumptions, we log-transformed the traits LA and LT. For the environmental parameters, we log-transformed P, K, Ca, Mg, Cu, Mn, Fe and Fine sand, and used the square transformation for organic carbon content and Zn. All analyses were conducted in the R environment (R Development Core Team, 2017) using the package 'ade4' along with the functions available in ter Braak *et al.* (2018).

RESULTS

Thirty plant species belonging to 12 families were surveyed in the study area (Table 2; Supporting Information, Figure S1). The vegetation exhibited a high degree of endemism, with 43% of the species being endemic to *campo rupestre* (Table S1). Plant species showed stress tolerance values between 72.6%

and 100.0%. Twenty-six plants (87%) were classified as extreme S strategists and the remaining four (13%) were classified as S/SC (*sensu* Hodgson *et al.*, 1999) (Fig. 1). *Microlicia pseudoscoparia* (Cogn.) and *Lychnophora pinaster* Mart. were found to be the most stress-tolerant species, both exhibiting a CSR signature of 0:100:0% (C:S:R). *Trixis vauthieri* (DC.) (27:73:0%), *Vellozia compacta* (Mart. ex Schult. f.) (20:80:0%) and *Pleroma heteromallum* ((D.Don) D.Don) (20:80:0%) were notable for their relatively high values of competitiveness (i.e. high amongst local strategies, but with relatively low C-scores in absolute terms). All studied species showed values of ruderality equal to 0% (Table S1). Information regarding endemism and conservation is presented in Table S1.

The vegetation developed on a soil with high but variable acidity, with pH values ranging from 3.67 to 4.64 (Table 3). Phosphorus, heavy metals and exchangeable bases also varied greatly, more within than between sites, with some vegetation plots having up to eight times more P, 60 times more Cu and four times the base saturation than the other plots.

The analysis of trait–environment association (CWM and SNC metrics) showed that the CSR ecological strategy and three functional traits (LA, SLA and LT) were significantly related to key soil properties at the community level. Soil P was negatively related to the degree of S-selection (Fig. 2A, B) and positively related to LA (Fig. 2C, D). Soil Ca, Mg and base saturation were positively related to SLA (Fig. 2E, F; Supporting Information, Table S2) but negatively related to LT (Fig. 2G, H). Soil clay was negatively related to both LA (Fig. 3E, F) and LT (Fig. 3G, H). Manganese was negatively correlated to LT (Table S2) and aluminium saturation was negatively related to SLA but positively related to LT (Table S2).

Table 2. Functional strategies (C, S, R scores) and traits of 30 species sampled in vegetation from ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil

Family	Species	CSR category	C	S	LA	SLA	LDMC	LT
Asteraceae	*Baccharis serrulata* (Lam.) Pers.	S	15.3	84.7	341.0	7.0	36.9	104.5
	Chromolaena squalida (DC.) R.M.King & H.Rob.	S	1.9	98.1	42.9	4.4	40.4	112.2
	Lychnophora pinaster Mart.	S	0	100	15.7	2.8	44.8	330.9
	Mikania microphylla Sch. Bip.	S	3.6	96.4	63.4	6.1	38.2	126.9
	Symphyopappus brasiliensis (Gardner) R.M. King & H. Rob.	S	7.8	92.2	146.9	3.8	40.1	256.8
	Trixis vauthiere DC.	S/CS	27.1	72.9	779.9	7.0	31.9	65.1
Bromeliaceae	*Dyckia consimilis* Mez	S	3.7	96.3	135.9	1.8	19.8	297.7
Euphorbiaceae	*Microstachys corniculata* (Vahl) A. Juss. ex Griseb	S	4.2	95.8	65.0	5.8	37.0	123.6
Fabaceae	*Mimosa calodendron* Mart. ex Benth.	S	9.4	90.6	260.1	2.3	51.5	299.0
	Periandra mediterrânea (Vell.) Taub.	S	9.2	90.8	233.0	2.6	47.0	390.8
Lamiaceae	*Eriope macrostachya* Mart. ex Benth.	S	8.3	91.7	263.0	4.8	28.3	89.6
	Hyptidendron canum (Pohl ex Benth.) Harley	S	11.3	88.7	622.6	4.9	26.2	143.9
Loranthaceae	*Tripodanthus acutifolius* (Ruiz & Pav.) Tiegh.	S	14.8	85.2	557.4	2.1	49.6	260.2
Malvaceae	*Sida carpinifolia* Mill.	S/CS	15.1	84.9	348.2	5.7	36.2	82.3
Melastomataceae	*Pleroma heteromallum* (D.Don) D.Don	S/CS	20.0	80.0	1937.7	4.1	28.0	145.0
	Pleroma ferricola A.L.F.Oliveira, R.Romero & P.J.F.Guim.	S	8.0	92.0	175.7	3.6	36.6	136.7
	Microlicia pseudoscoparia Cogn.	S	0	100	2.4	3.5	9.7	70.4
Myrtaceae	*Eugenia punicifolia* (Kunth) DC.	S	6.7	93.3	156.1	3.4	49.0	251.8
Orchidaceae	*Acianthera teres* (Lindl.) Borba	S	4.5	95.5	199.3	1.0	11.9	120.1
	Cattleya caulescens (Lindl.) Van den Berg	S	6.7	93.3	328.2	1.5	15.0	279.6
	Cattleya crispate (Thunb.)Van den Berg	S	9.0	91.0	540.0	1.5	15.0	187.3
	Epidendrum secundum Jacq.	S	8.2	91.8	534.2	2.5	10.2	160.1
	Orchidaceae sp.	S	13.7	86.3	1146.7	1.5	15.2	233.7
Velloziaceae	*Barbacenia brasiliensis* Willd.	S	13.9	86.1	308.6	5.0	36.9	246.8
	Vellozia caruncularis Mart. ex Seub	S	12.8	87.2	313.0	3.7	41.2	488.4
	Vellozia compacta Mart. ex Schult. f.	S/CS	19.6	80.4	910.2	3.0	42.8	492.4
	Vellozia albiflora Pohl	S	10.4	89.6	274.2	2.9	46.8	358.9
	Vellozia tragacantha (Mart. ex Schult. f.) Mart. ex Seub	S	3.5	96.5	73.5	3.2	47.2	227.1
Verbenaceae	*Lippia grata* Schauer	S	6.7	93.3	124.4	3.6	43.0	124.9
	Stachytarpheta glabra Cham.	S	6.9	93.1	282.8	5.1	25.3	256.7

Values correspond to the mean of six replicates per species. CSR category: category classes of strategies according to Hodgson *et al.* (1999); C: competitiveness (%); S: stress tolerance (%); LA: leaf area (mm²); SLA: specific leaf area (mm² mg⁻¹); LDMC: leaf dry matter content (%); LT: leaf toughness (GF). Note that R (%) for all species was zero (not shown).

DISCUSSION

The investigation of ecological strategies revealed a vegetation characterized by highly stress-adapted species in the ironstone *campo rupestre*. This study evaluated the evolutionary strategies and functional traits of the species of ironstone *campo rupestre* vegetation and the relationship between the functional trait setting of the vegetation and soil parameters. This plant community is strongly stress-tolerant and hosts species exhibiting a strictly conservative syndrome of resource use with regard to the leaf economics spectrum (Wright *et al.*, 2004; Díaz *et al.*, 2016), such as low SLA and high LDMC. This evidence is in agreement with our first hypothesis that, as a chronically unproductive habitat, ironstone *campo rupestre* favours the coexistence of species with a conservative resource-use strategy (Chapin *et al.*, 1993; Díaz *et al.*, 2004; Wright *et al.*, 2004). When compared
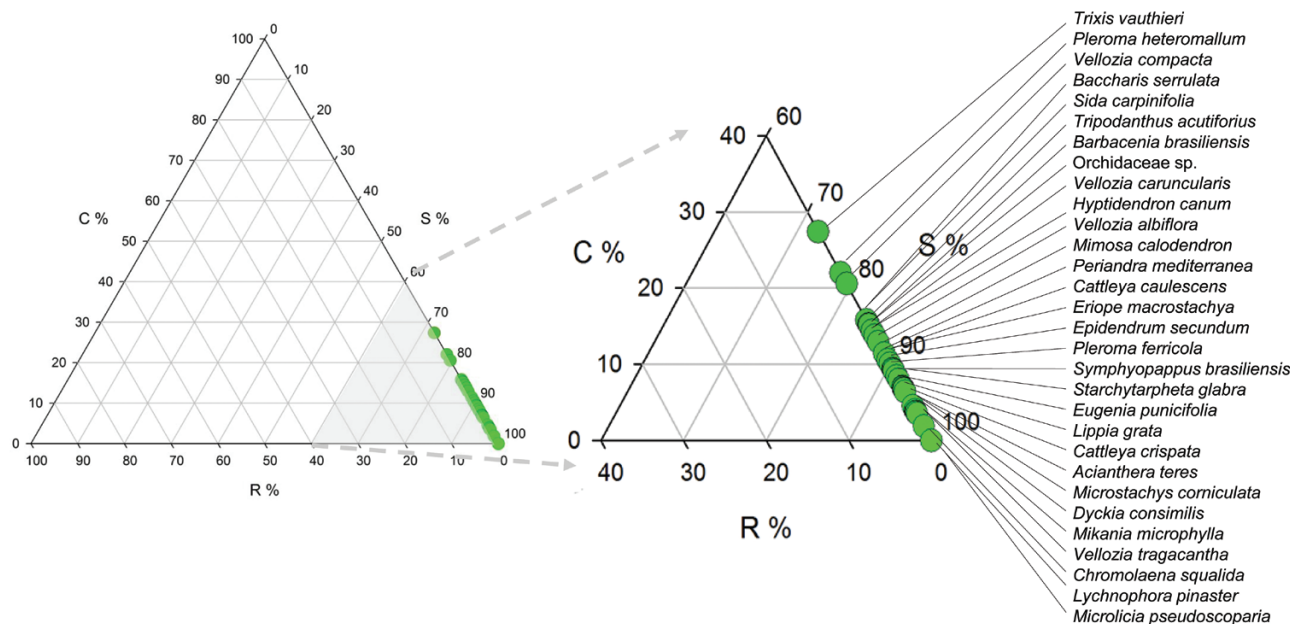
Figure 1. Relative extent (%) of C-, S- and R-selection for 30 species sampled in ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil, using the *StrateFy* analytical tool available from Pierce *et al.* (2017). C: competitiveness; S: stress tolerance; R: ruderalism. To the right is the identity of each species and further details of the proportion of C-, S- and R-selection (%).

Table 3. Physicochemical parameters of soil samples from ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil

Parameter	Values	Minimum/ maximum	CV (%)
pH (H_2O)	4.3 ± 0.03	3.7/4.6	4.73
P (mg dm^{-3})	3.75 ± 0.25	1.3/10.2	49.1
K (mg dm^{-3})	49.1 ± 12.6	31/76	18.5
Ca ($cmol_c$ dm^{-3})	1.68 ± 0.09	0.8/4.1	41.4
Mg ($cmol_c$ dm^{-3})	0.16 ± 0.01	0.1/0.4	32.7
Al saturation ($cmol_c$ dm^{-3})	29.0 ± 1.31	6.3/49.6	32.6
Base saturation (%)	12.0 ± 0.50	6.7/28.1	30.0
Organic carbon (dag kg^{-1})	8.95 ± 0.51	3.0/17.5	41.1
Cu (mg dm^{-3})	0.7 ± 0.06	0.03/1.9	61.1
Mn (mg dm^{-3})	33.2 ± 2.10	12.1/70.6	45.4
Fe (mg dm^{-3})	234.1 ± 1.72	76.8/734.3	53.1
Zn (mg dm^{-3})	8.21 ± 0.32	4.5/13.9	28.5
Coarse sand (%)	43.1 ± 1.71	9.6/65.7	28.7
Fine sand (%)	4.1 ± 0.42	0.4/16.7	73.6
Silt (%)	23.8 ± 0.97	10.7/40.1	29.3
Clay (%)	29.1 ± 0.86	15.3/41.6	21.4

Values refer to the mean of 52 plots (± SE); CV, coefficient of variation; cmolc, centimoles of charge; dag, decagram.

to similar ecosystems worldwide, such as tropical and subtropical grasslands, savannas and shrublands, the ironstone *campo rupestre* vegetation is even more stress-tolerant, in terms of its functional trait composition, with species exhibiting higher values of S than in these similar ecosystems (Pierce *et al.*, 2017), with specific examples including *M. pseudoscoparia* and *L. pinaster*.

Despite a major edaphic filter towards stress tolerance, the fine-scale microhabitat variability expressed by soil parameters related to fertility (i.e. P, Ca, Mg) and water retention capacity (i.e. clay content) reflects a subtle variation in ecological strategies and functional traits of species. Therefore, in agreement with our second hypothesis we found that relatively fertile areas harboured less stress-tolerant vegetation, whereas more infertile areas harboured more stress-tolerant vegetation, comparatively. Soil P, Ca and Mg are nutrients related to photosynthesis and ATP metabolism (Epstein *et al.*, 1972; Shen *et al.*, 2011; Schulz *et al.*, 2013). The greater concentration of P, Ca and Mg, and the greater proportion of acquisitive use of resources by plants were reflected by less stress-tolerant strategies, higher LA, higher SLA and lower LT (see Díaz *et al.*, 2016). To some extent this mirrors the situation in quartzitic *campo rupestre* and on granitic rocky outcrops (Negreiros *et al.*, 2014; de Paula *et al.*, 2015), in which edaphic environmental
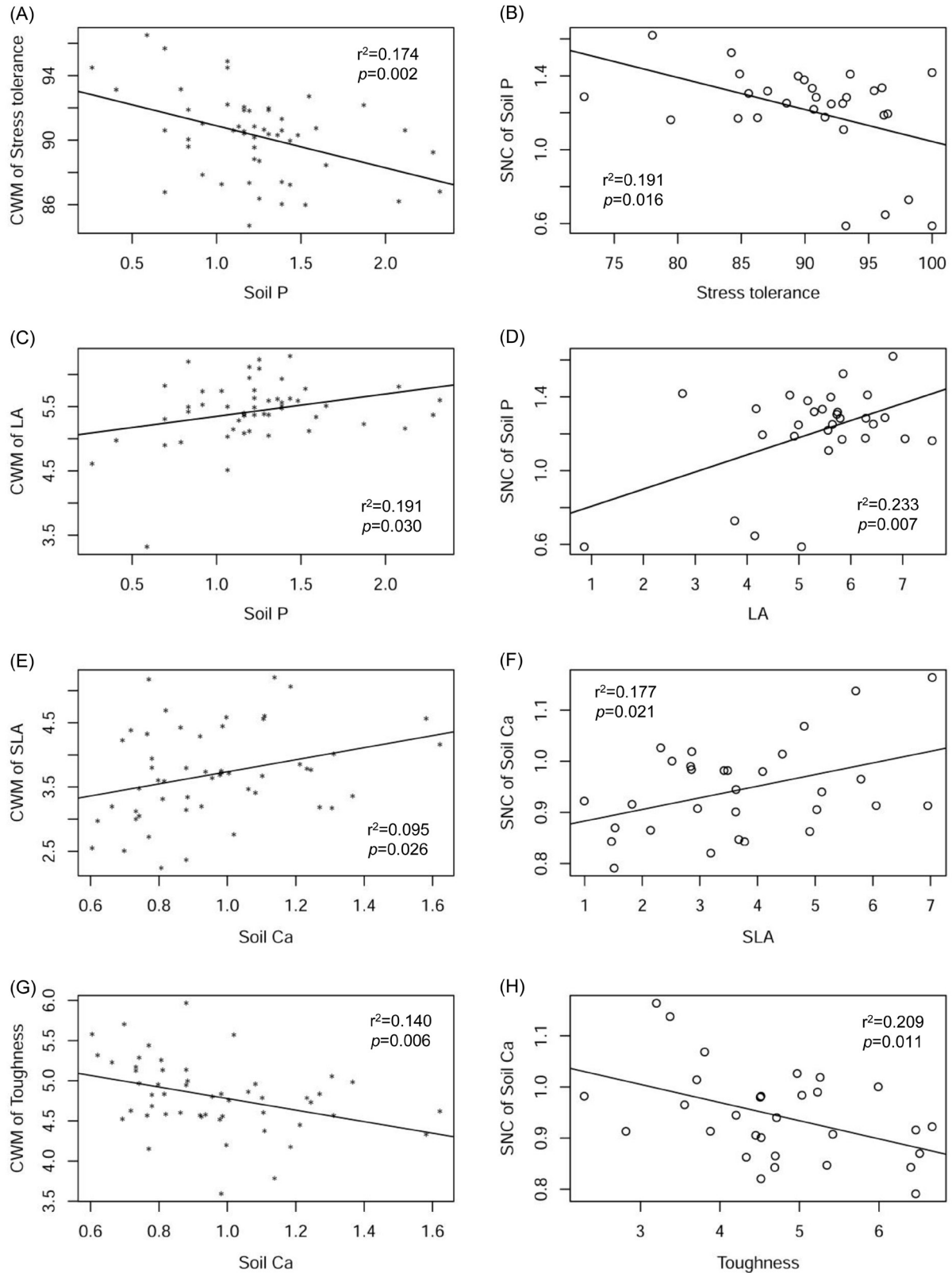
Figure 2. Analysis of trait–environment associations using community-weighted mean (CWM) and species niche centroid (SNC) metrics in ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil. Soil P content association with S strategy (A, B) and leaf area (C, D); association of soil Ca with specific leaf area (E, F) and leaf toughness (G, H). Asterisks, sites; circles, species.
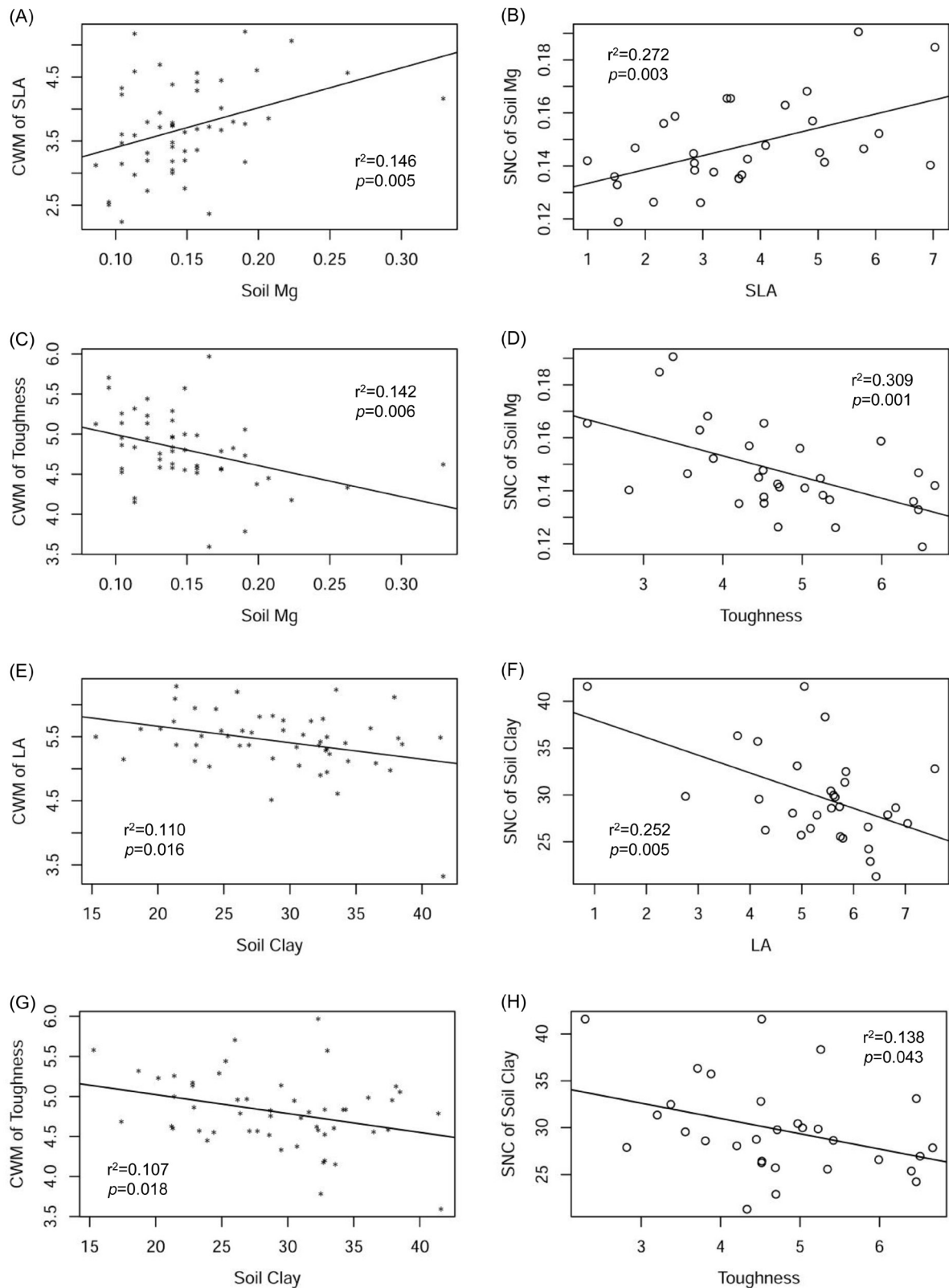
Figure 3. Analysis of trait–environment association using community-weighted mean (CWM) and species niche centroid (SNC) metrics in ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil. Soil Mg association with specific leaf area (A, B) and leaf toughness (C, D). Association of soil clay with leaf area (E, F) and leaf toughness (G, H). Asterisks, sites; circles, species.

filters lead the vegetation to a convergence of ecological strategies towards stress tolerance.

Studies comparing broad functional groups (e.g. sclerophytic shrubs, graminoids, succulents) and plant community structure and composition between different lithotypes of *campo rupestre* have also revealed a strong edaphic filter driving species assemblages and functional composition (Messias *et al.*, 2013; Carmo *et al.*, 2016; Carmo & Jacobi, 2016). However, the authors concluded that different physical soil properties among lithotypes, rather than soil fertility, explain differences in vegetation composition. Thus, it is evident that different mechanisms operate at different scales in these rocky outcrop ecosystems. As we argue here, it seems that microhabitat structure plays a meaningful role in community diversity and functional variation of ironstone *campo rupestre* species, despite the general extreme stress tolerance required for species to grow there. Carmo *et al.* (2016) analysed the surface roughness in one of the same areas of our study and found that an increase in community diversity is promoted by fine-scale surface heterogeneity, which allows a greater number of functional types to coexist. It is essential to consider that different assembly processes can operate above and below ground. Zemunik *et al.* (2015) found that in chonically impoverished soils, the functional diversity of below-ground traits related to nutrient acquisition strategies increased with increasing soil P concentration, although leaf traits converged on a high P-use efficiency. Similarly, we also found a convergence of leaf traits towards a high nutrient-use efficiency strategy (87% of species are S-strategists), but we provide a more detailed view of how changes in soil P drive subtle changes in leaf traits as well. This raises interesting questions for functional trait studies. Leaves have direct links to energy and water flux in the plant system, which metabolizes sunlight and nutrients to sustain plant life and create biomass. Therefore, the evaluation of leaf traits in the context of the leaf and plant economics spectrum (Wright *et al.*, 2004; Reich, 2014) reveal features related to nutrient acquisition at the leaf level, thus related to edaphic properties, such as the variation in stress tolerance along soil P content gradients. However, in evaluating above- and below-ground traits with an integrative perspective, there is currently no consensus about the coordination between leaf and root traits (see Weemstra *et al.*, 2016; Laliberté, 2017). Nonetheless, studies of infertile landscapes (e.g. Zemunik *et al.*, 2015; Abrahão *et al.*, 2019) show that the main environmental filters in operation are related to the edaphic factors, which may select above- and below-ground traits through different processes. Moreover, further research is needed to advance knowledge concerning the processes operating above and below

ground in the *campo rupestre*, an ecosystem already highlighted regarding the importance of below-ground processes in influencing above-ground life (Fernandes *et al.*, 2016b).

In these highly weathered substrates, the combination of phosphorus with iron and aluminium forms insoluble compounds that become unavailable to plants (e.g. Carmo & Jacobi, 2016). Additionally, the restricted nutrient availabilities of the soils result in slow organic matter decomposition that can lead to the accumulation of humic substances (Benites *et al.*, 2007). In light of OCBIL theory, ironstone *campo rupestre* species appear to be selected for the optimization of soil resource capture and use (Negreiros *et al.*, 2014; Fernandes, 2016a; Oliveira *et al.*, 2016; Silveira *et al.*, 2016).

SLA and LT are both traits related to the leaf economics spectrum (Wright *et al.*, 2004; Reich, 2014). Greater SLA values are related to lower structural reinforcement of leaves, higher growth rates and higher resource acquisition (Wright *et al.*, 2004). High LT is related to high investment in structure such as thick cell walls and small, densely organized cells. We found that both traits were related to soil fertility as expected from the leaf economics spectrum, in which increasing fertility (i.e. soil P, Ca and Mg) was associated with a relatively acquisitive resource use (i.e. higher values of SLA and lower values of LT). Leaf area reflects the size spectrum and is associated with light capture (e.g. Díaz *et al.*, 2016). Large leaves tend to lose water due to greater surface area compared to smaller leaves (Westoby *et al.*, 2002). Therefore, it is expected that an increase in LA will be accompanied either by water or nutrient availability that will allow plants to balance the transpirational and photosynthetic rates (Givnish, 1987). Our results show a functional adjustment of increased LA in microhabitats with more soil P (although leaves are not large in absolute terms), which should allow plants to optimize their metabolism and fix more carbon per water molecule loss (i.e. greater water use efficiency) by efficiently absorbing and fixing more carbon (Westoby *et al.*, 2002). Water availability is also important to carbon assimilation of *campo rupestre* plants (Lüttge *et al.*, 2007). Additionally, the concomitant relative increase in LA with increasing soil P probably underlies the relationship between increasing soil P concentration and less stress-tolerant species, according to CSR classification (Pierce *et al.*, 2017). By contrast, and in relation to soil clay gradient, higher LA and LT are associated with lower soil clay contents. Thus, in this situation greater values of LA are not related to high metabolic activity but to a relatively conservative resource use and potentially greater leaf lifespan, as clay-poor soils are usually associated with a lower

capacity for nutrient retention. Roots of Myrtaceae species are known for their capacity to create clay or silcretes (Verboom & Pate, 2013) and it is interesting to note the association of *Eugenia punicifolia* (Myrtaceae) with a plot showing the highest clay content. We only found *E. punicifolia* occurring at the highest clay contents in all our study areas, where it is present at high abundance (14 individuals).

Disturbance and biotic filters might also play a role in trait selection, but they are beyond the scope of this study. The vegetation of this ecosystem is thought to have evolved under a natural fire regime, and it is inhabited by many fire-prone (i.e. fire-resistant) species, such as species of the Velloziaceae (Silveira *et al.*, 2016). Additional field and experimental studies are needed to properly reveal the effects of fire dynamics on this vegetation. However, it is interesting to note that the CSR plant strategy related to disturbance resistance (i.e. biomass destruction) is the ruderal strategy (R), which was not identified in the studied *campo rupestre* vegetation (0% for all species evaluated here). This raises the question of whether selective pressures could be far stronger towards stress tolerance, rather than competitiveness (in terms of resource pre-emption) and disturbance resistance in this ecosystem.

It is also worth noting the capacity of CSR theory and the functional approach to reveal slightly different ecological strategies according to subtle differences in soil nutrient concentrations. The tight connection between the extent of stress tolerance and P availability observed in this study suggests that this vegetation may be very sensitive to changes in P availability, which is expected from human-induced disturbances such as fertilization of sites in direct contact with ironstone grasslands (Fernandes *et al.*, 2016a). Negreiros *et al.* (2009) showed that the increase in nutrient availability (e.g. P, K, Ca, Mg, organic matter) is not necessarily related to growth rates and biomass allocation in native species. Thus, greater nutrient availability could potentially increase the probability of invasion by exotic species in *campo rupestre* vegetation (Barbosa *et al.*, 2010; Hilário *et al.*, 2011). An increase in P availability in a P-impoverished habitat such as ironstone *campo rupestre* could have a negative impact on native vegetation (Barbosa *et al.*, 2010; Lambers *et al.*, 2013). The vegetation of *campo rupestre* evolved in an old landscape with low nutritional status (Fernandes, 2016a; Silveira *et al.*, 2016). Therefore, these plant species may have evolved metabolic strategies related to slow growth and resource conservation at the cost of capacity for rapid growth and competitive ability (Negreiros *et al.*, 2014). Thus, even under a higher concentration of soil P, shifts towards an increase in competitive ecological strategy within the native

community are not pronounced. In this scenario, the selection of competitive and acquisitive strategies with an increase in soil P availability could decrease the overall native community fitness to the detriment of the performance of invasive species (David & Menges, 2011; de Paula *et al.*, 2015). Therefore, this study provides support for the contention that an important aspect in the conservation of ironstone *campo rupestre* is to maintain its natural low soil nutrient condition, especially with regard to P content (Barbosa *et al.*, 2010; Lambers *et al.*, 2013; Fernandes *et al.*, 2016a; Knappová *et al.*, 2017).

In conclusion, this study indicates that ironstone *campo rupestre* exhibits a highly stress-tolerant herbaceous-shrub vegetation, capable of dealing with the strong environmental filters imposed by limiting edaphic conditions, but that species coexistence can nonetheless be partially explained by slight functional differences between species. Nutrients related to photosynthesis and ATP metabolism appear to be an important factor driving the functional differentiation of vegetation of ironstone *campo rupestre*. Conservation actions aiming to preserve or restore this ecosystem must take into account evident microhabitat specializations of the species: these are clear from the robust and easy-to-use CSR ecological strategy scheme. Here we helped to characterize the ecosystem of ironstone *campo rupestre* through a functional approach, identifying the CSR strategies and functional traits of the herbaceous-shrub vegetation, the predominant structural unit of this old growth grassland. We also highlight that soil characteristics and functional traits shape species distributions within the wider community despite the extremely strong habitat filtering of this metal-rich ecosystem.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. List of the 30 species and respective functional trait values sampled in the ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil.
Table S2. Linear regressions between all pairs of soil parameters (lines) and species traits (columns) from ironstone *campo rupestre* in Serra do Rola-Moça, south-eastern Brazil. For each cell are shown the r^2 and *P*-value of the regressions between: (i) species trait CWM (community-weighted mean) and soil parameter; and (ii) species traits and soil parameter SNC (species niche centroid).
Figure S1. Photographs of representative species found in the ironstone *campo rupestre* of Serra do Rola-Moça State Park, MG, Brazil.