Vellozioid roots allow for habitat specialization among rock- and soil-dwelling Velloziaceae in campos rupestres

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Abstract

1. Plant growth on harsh substrates (habitat specialization) requires specific traits to cope with stressful conditions.

2. We tested whether traits related to nutrient acquisition (root colonization by fungal symbionts, and plant morphological and physiological specializations), and nutrient use (leaf nitrogen (N) and phosphorus (P) concentrations and N- and P-remobilization efficiency), were related to habitat specialization for 27 species of Velloziaceae growing either in soil or on rocks in extremely P-impoverished campos rupestres habitats. If habitat specialization were to drive trait sorting, then we expect traits to differ between those substrates.

3. Both soil and rock-dwelling species presented a very low proportion of root length colonized by arbuscular mycorrhizal and dark-septate fungi. However, rhizosheaths were only observed in soil-dwelling species, and vellozioid roots, a specialization that allows for mining P and dissolving quartzite rock, were mostly found in rock-dwelling species. We did not observe differences in nutrient-use traits between rock- and soil-dwelling species.

4. Root specializations are strongly correlated with microhabitats, and the presence of vellozioid roots seems to mediate bare rock specialization. There is an overall P limitation of plant productivity both on rock and in soil of campos rupestres, which does not drive the sorting of traits related to above-ground nutrient use and symbiotic P acquisition. Therefore, nutrient impoverishment is indeed a very strong environmental filter in campos rupestres as a whole, but habitat specialization plays an important role in the spatial distribution of Velloziaceae between contrasting substrates.
1 | INTRODUCTION

Plants with specializations that allow them to grow in specific soils contribute greatly to global plant diversity; however, the processes driving these habitat specializations are poorly understood (Cacho & Strauss, 2014). The ability of a species to acquire resources under stressful conditions incurs costs and reduces a species’ competitiveness in non-extreme soils (Cacho & Strauss, 2014). Therefore, a trade-off between stress resistance and competitiveness might explain endemism through habitat specialization and the generation of biodiversity in habitats with variable substrates (i.e., geodiverse habitats) (Anderson et al., 2015; Hjort, Gordon, Gray, & Hunter, 2015; Rajakaruna, 2017). In harsh geodiverse environments as in the rocky mountain top, seasonally dry grasslands in Brazil, the campos rupestres (Figure 1a,b) (Silveira et al., 2016), species with different plant traits occupy different substrates (Alcantara et al., 2015; Zappi, Moro, Meagher, & Nic Lughadha, 2017). For example, species with different below-ground traits for nutrient acquisition such as specialized roots or mycorrhizal associations occur on contrasting substrates (Abrahão, Costa, Lambers, et al., 2019).

In severely phosphorus (P)-impoverished soils of campos rupestres formed from extremely low P parent material such as quartzite (Porder & Ramachandran, 2013), the costs of P acquisition are very high (Raven, Lambers, Smith, Shaver, & Westoby, 2018). This might lead to a trade-off in plant investment among different P-acquisition strategies (Abrahão, Costa, Lambers, et al., 2019; Ryan et al., 2012). At relatively higher soluble inorganic soil P concentrations, mycorrhizas represent an effective strategy for P uptake, because thin mycorrhizal hyphae incur smaller carbon (C)-construction costs than root morphological and physiological specializations (e.g., the release of carboxylates that mobilize insoluble soil P) (Raven et al., 2018). Surveys of arbuscular mycorrhizal (AM) fungal spores in soil have revealed a high diversity in campos rupestres (de Carvalho et al., 2012; Coutinho, Fernandes, Barbosa, Valério, & Goto, 2015). However, the proportion of the root length colonized by these fungi is very low (Abrahão, Costa, Lambers, et al., 2019; Oliveira et al., 2015; Zemunik, Lambers, Turner, Laliberté, & Oliveira, 2018). When nutrients, especially nitrogen (N), are available in organic forms (Benites et al., 2005; Benites, Schaefer, Simas, & Santos, 2007), dark-septate fungi, poorly known fungal symbionts with strongly melanized hyphae that colonize roots of plants, can have positive effects on plant growth and nutrient uptake (Berthelot, Chalot, Leyval, & Blaudez, 2019; Newsham, 2011). Dark-septate fungi are important root endophytes in campos rupestres plant species (Abrahão, Costa, Lambers, et al., 2019; Oliveira et al., 2015; Zemunik et al., 2018), but we do not know how strong their association is with species growing on different substrates.

When P is severely limiting, as in campos rupestres, root specializations are more efficient than mycorrhizas (Raven et al., 2018). Dauciform roots, sand-binding roots forming rhizosheaths (Abrahão, Costa, Lambers, et al., 2019; Abrahão, Lambers, Sawaya, Mazzafera, & Oliveira, 2014; Oliveira et al., 2015) and the recently described vellozioid roots (Teodoro et al., 2019) are prevalent in campos rupestres. Rhizosheaths are soil sheaths strongly bound to the roots, associated with long root hairs that increase root-soil contact and mucilage release (Brown, George, Neugebauer, & White, 2017; North & Nobel, 1997; Pang, Ryan, Siddique, & Simpson, 2017), which enhances the uptake of poorly mobile P (Brown et al., 2017; Kidd et al., 2018; McCully, 1999; Zhang et al., 2018). Vellozioid roots (Figure 1c) were described in rock-dwelling Velloziaceae, which is an ubiquitous plant family in campos rupestres; they are capable of dissolving the quartzite rocks, releasing strongly bound P through carboxylate release and contributing to biological weathering of rocks (Porder, 2019; Teodoro et al., 2019). These vellozioid roots are therefore expected to be more effective in rocks than in soils. Interestingly, soil-dwelling Velloziaceae form rhizosheaths (Abrahão, Costa, Lambers, et al., 2019), but we do not know whether they also are formed by rock-dwelling species. Because Velloziaceae grow both in soil (Figure 1d) and on rocks without any soil (Figure 1e) (Alcantara et al., 2015), they provide an excellent model for testing habitat specialization in campos rupestres. If the vellozioid roots were more carbon-costly than rhizosheaths, they would only be an advantage for rock-dwelling species, where investment in them would be rewarded through the acquisition of monazite-bound P (Teodoro et al., 2019). We expect that due to the costs of habitat specialization of the vellozioid roots, species with these roots might be less abundant in soils.

In the campos rupestres, the total N concentration ([N]) in rock parent material is extremely low, and N slowly accumulates in soil, mainly due to biological N₂ fixation (Vitousek, Menge, Reed, & Cleveland, 2013; Vitousek, Porder, Houlton, & Chadwick, 2010). Because of these N inputs in campos rupestres over time, N is less limiting than P for plant growth (Oliveira et al., 2015). In campos rupestres, N₂ fixation is performed by free-living cyanobacteria (Alves, Silva, Oliveira, & Medeiros, 2014; Elbert et al., 2012; Jacobi, do Carmo, Vincent, & Stehmann, 2007) and by the N₂-fixing symbionts of legumes (dos Reis Jr et al., 2010). Furthermore, termites, that build mounds and tunnels around plant roots and stems (Figure 1f,g), may contribute to nutrient input through N cycling and N₂ fixation by bacteria in their hindgut (French, Turner, & Bradbury, 1976; Nishi, Vasconcellos-Neto, & Romero, 2013; Schaefer, Marins, Corrêa, & Nunes, 2016). Due to the low pH and water saturation in summer, organic matter mineralization is low (Benites et al., 2007) and N is likely to be mainly available in organic forms. Strategies to acquire
FIGURE 1  Landscape heterogeneity in campos rupestres creates geodiverse habitats where rock outcrops are interspersed within a soil matrix with (a) or without (b) a gravel cover. (a) Campos rupestres at Serra do Cipó, Santana do Riacho, Minas Gerais, Brazil. (b) Campos rupestres with white sands and the Pedra Grande rock outcrop at the back at Serra do Cabral, Joaquim Felício, Minas Gerais, Brazil. (c) Vellozioid roots of Barbacenia tomentosa collected in the field. (d) Soil-dwelling Vellozia stenocarpa presents deciduous senesced leaves (arrow), where leaf sheaths remain attached to the caudex, while leaf blades are shed; Serra do Cabral. (e) Rock-dwelling Barbacenia filamentifera with marcescent leaves (arrow) grows without any soil; Guinda-Sopa road. (f) Termite mounds are a common feature in campos rupestres and greatly contribute to nutrient cycling; Barbacenia cf. riedeliana grows next to a termite mound, likely benefiting from additional nutrients brought in by the termites (arrow); Fazenda Galheiros, Diamantina municipality, Minas Gerais, Brazil. (g) Termite tunnel next to Barbacenia cf. riedeliana roots (arrow); Fazenda Galheiros. (h) Non-desiccation-tolerant Barbacenia flava (red arrow) with marcescent leaves and desiccation-tolerant Vellozia nivea in a desiccated state (blue arrows) in the dry season at Serra do Cipó.
organic N such as associations with dark-septate fungi may be favoured in soil-dwelling plants (Berthelot et al., 2019).

Once nutrients have been taken up, they are allocated to specific plant functions, including leaf production (Aerts & Chapin, 1999). Thus, leaf nutrient concentrations and stoichiometry are a proxy for nutrient availability and plant functioning (Lambers & Poorter, 1992; Reich, 2014; Wright et al., 2004). Leaf N: P ratios of plants from campos rupestres are high (Abrahão, Costa, Lambers, et al., 2019; Oliveira et al., 2015), whereas leaf N and P concentrations are very low, indicating strong P limitation (Elser, Fagan, Kerkhoff, Swenson, & Enquist, 2010). Community-weighted manganese concentrations [Mn] in mature leaves can be used as a proxy for nutrient-acquisition strategies (Lambers, Hayes, Laliberté, Oliveira, & Turner, 2015), because carboxylates release both soil P and soil-bound Mn (Lambers, Chapin, & Pons, 2008; Lambers et al., 2015). As a result, carboxylate-releasing plants accumulate Mn in leaves (Gardner & Boundy, 1983; Lambers et al., 2015; Pang et al., 2018). Conversely, AM fungi intercept Mn, decreasing leaf [Mn] in host plants (Kothari, Marschner, & Römheld, 1991). Therefore, carboxylate-releasing species are expected to present greater leaf [Mn] than mycorrhizal species (Lambers et al., 2015). In summary, leaf N: P ratios can indicate N or P limitation for plant productivity, and leaf [Mn] can be used as an above-ground proxy for below-ground plant functions.

Nutrient remobilization is an important plant strategy on nutrient-impoverished soils (Hayes, Turner, Lambers, & Laliberté, 2014; Kobe, Lepczyk, & Iyer, 2005; Vergutz, Manzoni, Porporato, Novais, & Jackson, 2012). For example, in Velloziaceae, the P-remobilization efficiency can reach 92%, but we do not know how nutrient remobilization varies among species growing on different substrates (Abrahão, Costa, Lambers, et al., 2019; Alcantara et al., 2015).

We tested the following hypotheses: (a) soil-dwelling and rock-dwelling species present a similarly low proportion of root length colonized by AM fungi; (b) soil-dwelling Velloziaceae, which are exposed to a greater availability of organic N present more colonization by dark-septate fungi; (c) soil-dwelling and rock-dwelling species show differences in root specialization type, with rhizosheath formation in soil-dwelling species and vellozioid roots in rock-dwelling species; (d) rock-dwelling species have similar leaf [P] and lower leaf [N], and consequently lower leaf N: P ratios, than soil-dwelling species; (e) rock-dwelling species remobilize P equally efficiently and N more efficiently than soil-dwelling species, due to putatively low N availability in rock. In summary, we expect nutrient-acquisition and nutrient-use traits to allow for habitat specialization in Velloziaceae, contributing to niche differentiation and the generation and maintenance of biodiversity in campos rupestres (Augusto, Fanin, & Bakker, 2019).

2 | MATERIALS AND METHODS

2.1 | Study sites

The campos rupestres are a vegetation complex mainly located within the Cerrado phytogeographic region, but also within the Caatinga and Amazonian phytogeographic regions (Alves et al., 2014). Although the studied areas are located within the Cerrado, the very distinct rock parent material provided opportunities for a very distinct flora (Colli-Silva, Vasconcelos, & Pirani, 2019; Silveira et al., 2016). Campos rupestres are known for their high plant species diversity (Alves & Kolbe, 1994; Le Stradic, Buisson, & Fernandes, 2015), which is strongly related to substrate heterogeneity (Lima et al., 2018; Negreiros, Le Stradic, Fernandes, & Rennó, 2014; Teodoro et al., 2019). The rock parent material is extremely nutrient poor, leading to nutrient-impoverished soils, with acidic pH, high aluminium (Al) concentrations and low magnesium (Mg) and calcium (Ca) concentrations (Benites et al., 2007). The soil patches can vary from thin soil layers over gravel to deep sandy depositions in valleys and depressions (Figure 1a–e) (Benites et al., 2007; Conceição & Pirani, 2016). During the dry winter, plant growth is slow, and the vegetation is prone to fire (Oliveira et al., 2016). The Espinhaço Range, where our sites were located, extends 1,200 km in the south–north direction in eastern Brazil (Figure 2) and harbours most of the campos rupestres (Silveira et al., 2016). Due to the mining potential of the campos rupestres, these systems are under severe threat and understanding how these plants function before their habitat is destroyed is essential if we are to restore plant diversity and ecosystem functions after mining (Fernandes, Barbosa, Negreiros, & Paglia, 2014; Fernandes, Toma, Angrisano, & Overbeck, 2016).

The regional climate at the study site is classified as Cwb, according to Köppen’s system (Köppen, 1900; Köppen, 2003; Kottek, Grieser, Beck, Rudolf, & Rubel, 2006), with marked seasonality, a dry cool winter and a rainy warm summer. Mean minimum monthly temperatures, calculated from data recorded from 1961 to 2016 by a meteorological station at Conceição do Mato Dentro (19°1′12″S; 43°25′48″W) in the southern Espinhaço Range, range from 10°C in July to 19°C in January, and maximum monthly temperatures range from 25°C in July to 30°C in February (http://www.inmet.gov.br/portal/). Mean annual precipitation is 1,313 mm (http://www.inmet.gov.br/portal/).

2.2 | Species choice

The monocot family Velloziaceae comprises 304 species in five genera (Flora do Brasil 2020, 2019; World Checklist of Selected Plant Families, 2018). The vast majority are concentrated in Brazil (222 species in two genera: 197 occur in the Cerrado region, of which 193 are restricted to campos rupestres: of the remaining, 46 species occur in the Caatinga, 24 in the Atlantic Forest and five in Amazonia (Flora do Brasil 2020, 2019). The two Neotropical lineages of Velloziaceae show independent shifts to faster diversification, concentrated, respectively, within the genus Vellozia during the Oligocene/Miocene transition (23.1 Mya) and within the genus Barbacenia during the middle/late Miocene (13.4 Mya) (Alcantara, Mello-Silva, & Ree, 2018).

Velloziaceae form rosettes with a caudex surrounded by aerial roots that grow to reach the substrate (Ayensu, 1973). These aerial roots are covered by persistent leaf sheaths that help retain moisture and allow for direct shoot-water uptake (Ayensu,
1973; Oliveira, Dawson, & Burgess, 2005; Smith, 1962). Some Velloziaceae have persistent senesced leaves (marcescent leaves, Figure 1c), and some have deciduous senesced leaves (Figure 1d). Additionally, some Velloziaceae are desiccation tolerant (DT, Figure 1h); that is, they have the ability to equilibrate leaf water potential with that of dry air (Porembski & Barthlott, 2000). Desiccated leaves fully rehydrate when water is supplied, and recover to exhibit a normal metabolism (Alcantara et al., 2015; Gaff, 1987). Non-desiccation-tolerant species (non-DT, Figure 1h) maintain photosynthetic activity during the dry season (Alcantara et al., 2015). Non-DT Velloziaceae present lower leaf [P] and [N] than DT species (Alcantara et al., 2015). The interplay with water- and carbon-acquisition strategies might influence nutrient use in different substrates, so we explored whether deciduousness or desiccation tolerance would influence N and P use.

We collected 27 species of Velloziaceae from 30 populations located at five sites along 300 km of the Espinhaço Range in the state of Minas Gerais (Table 1, Figure 2). The first site was a private reserve at Serra do Cipó, in the southern Espinhaço Range. The second site was Fazenda Galheiros, next to the BR 259 road between Curvelo and Gouveia. The third site was in rock outcrops next to the road between Guinda and Sopa in the Diamantina Municipality. The fourth site was next to the MG 220 road to Conselheiro Mata, and the fifth was in the Serra do Cabral State Park. The species were classified as DT or non-DT (Table S1) according to literature records (Alcantara et al., 2015; Gaff, 1987; de Mello-Silva, 1994) and personal observations. Six species with uncertain DT classification were removed from the analyses. When we were not able to identify the species in the field, individuals with reproductive structures were collected and vouchers were incorporated in the Herbarium of the
TABLE 1 Coordinates of the campos rupestres collection sites along the southern Espinhaço Range in Minas Gerais, Brazil

| Site number | Place               | Location          | Latitude  | Longitude |
|-------------|---------------------|-------------------|-----------|-----------|
| 1           | Vellozia Reserve    | Serra do Cipó     | 19°16'56"S | 43°35'38"W|
| 2.1         | Fazenda Galheiros   | Diamantina Plateau| 18°36'01.9"S | 43°52'55.5"W|
| 2.2         | Fazenda Galheiros   | Diamantina Plateau| 18°35'13.9"S | 43°53'42.9"W|
| 3.1         | Guinda-Sopa road    | Diamantina Plateau| 18°12'53.2"S | 43°42'11.4"W|
| 3.2         | Guinda-Sopa road    | Diamantina Plateau| 18°49.5"S   | 43°42'50.6"W|
| 3.3         | Guinda-Sopa road    | Diamantina Plateau| 18°6'32.1"S | 43°44'5.5"W|
| 4           | Conselheiro Mata    | Diamantina Plateau| 18°18'31.7"S | 43°54'18.0"W|
| 5           | Serra do Cabral State Park | Serra do Cabral | 17°42'28"S | 44°11'35"W|

2.3 | Soil collection and analyses

Soil and rock samples were collected in May 2015, at the end of the rainy season. At each site, we collected a compound sample (500 g) of soil between 0 and 10 cm depth with a 5-cm-diameter soil corer. We also collected soil from a termite mound to compare it with the bulk soil. Soil P, potassium (K), Ca, and Mg were extracted by ion exchange resin (van Raij, de Andrade, Cantarella, & Quaggio, 2001). Phosphorus was determined colorimetrically, and K, Ca and Mg by atomic absorption spectrophotometry (AAS) (PerkinElmer 3110) (van Raij et al., 2001). Boron (B) was extracted in hot water and determined colorimetrically (van Raij et al., 2001). Copper (Cu), iron (Fe), zinc (Zn) and Mn were extracted with diethylene-triamine-pentaacetic acid (DTPA) and determined by AAS (PerkinElmer 3110) (van Raij et al., 2001). Soil pH was analysed after extraction with 0.01 M CaCl₂ (van Raij et al., 2001). Soil organic matter was determined by dichromate oxidation according to van Raij et al. (2001) and soil texture by the Bouyoucos procedure (Gee & Or, 2002). The soil samples collected in May 2015 were analysed at Laboratório de Análises de Solos at the Luiz de Queiroz College of Agriculture, Brazil.

2.4 | Rock collections and analyses

Approximately 500 g of rock samples per site was collected with hammer and chisel and was crushed and pulverized at the Department of Petrology and Metallogeny of the State University of São Paulo (UNESP, in Rio Claro). Additionally, we collected samples from termite mounds and tunnels to assess their contribution to the nutrition of Velloziaceae at site 2.2 (see Table 1 for site locations). The chemical analyses were conducted by X-ray fluorescence spectrometry (Gomes, Formoso, & Trescases, 1984) in a Philips PW 2400 fluorescence spectrometer equipped with a Philips PW 2510 sample holder (XRF, Philips, model PW 2400) at the State University of São Paulo (UNESP, in Rio Claro). Because the soil and rock nutrient analyses were performed at different laboratories, the results are presented in different units (mg/dm³ for soil analyses or mg/kg for rock analyses). Since we did not calculate soil density, we cannot make conversions. However, previous studies have shown that the soil density in campos rupestres is around 1 kg/dm³ (Benites, Caiafa, Mendonça, Schaefer, & Ker, 2003); therefore, we can compare the values.

2.5 | Root collection and assessment of rhizosheaths, vellozioid roots and fungal colonization

We collected at least 30 cm of fresh roots from four individuals for each of the 30 species in May 2015, at the end of the growing (rainy) season. Vellozia nivea, Barbacenia flava and B. rubrovirens were collected from two sites, whereas all other species were collected from a single site. The samples were immediately stored in 50% (v/v) ethanol. Prior to mycorrhizal assessment, all the roots were examined for the presence of long root hairs that formed rhizosheaths or vellozioid roots (Brown et al., 2017; Pang et al., 2017; Teodoro et al., 2019) with a stereomicroscope (Leica D80) and photographed with a Leica DFC295 3.0 MP digital camera with focus stacking using Leica Application Suite 3.8.0 (Leica). The presence of rhizosheaths was recorded, if the roots still had soil attached after shaking the roots in the vials and gently scrubbing with a paint brush (Buckley, 1982). The presence of vellozioid roots was recorded if we observed what appeared to be sequential root hair zones and elongation zones along the same root axis (Figure 1c), but not necessarily with soil attached (Teodoro et al., 2019).

For assessment of colonization by mycorrhizal fungi and dark-septate endophytes, root clearing and staining were performed as in Abrahão, Costa, Lambers, et al. (2019). The stained roots were stored in acidified glycerol until assessment of mycorrhizal colonization with the slide method where 30 1-cm root segments (Giovanetti & Mosse, 1980) were examined at 200× magnification with a compound microscope (Olympus BX51) mounted with a Leica DFC 295 digital camera (Leica). For each segment, we observed 10 visual fields under the compound microscope and recorded how many fields presented AM fungal structures. The proportion of the root length colonized was calculated as the proportion of visual fields...
observed with fungal structures, divided by the total number of visual fields observed. Roots were recorded as colonized by AM fungi if Arum- or Paris-type structures (Dickson, 2004) such as arbuscules, vesicles or hyphal coils were observed. We used the same method to record dark-septate fungi and recorded the presence of sclerotia and melanized septate hyphae. We did not observe ectomycorrhizas or ericoid mycorrhizas or fine root endophytes.

### 2.6 | Leaf collection and analyses

We collected mature and senesced leaves from the same individuals from which we collected roots. Mature leaves were collected from the second and third rows of leaves, counting outwards from the centre of the rosette. Marcescent senesced leaves are retained around the caudex and were collected from the first row of dried leaves. Since the sampling was done during the rainy season, the senesced leaves could not be confounded with desiccated leaves. Senesced leaves from deciduous species were collected from the ground or rock surface. Leaves were dried at 60°C for five days and ground in liquid N with a Geno/Grinder 2010 (SPEX SamplePrep) using stainless steel bearings for 6 min at 950 rpm. A subsample of mature and senesced leaves was digested in concentrated HNO₃: HClO₄ (4:1) (Malavolta, Vitti, & De Oliveira, 1997) and analysed colorimetrically for P with the vanadate–molybdate reagent. A subsampled as 100% × (1 − [Nutrient]senesced/[Nutrient]mature) (Nardoto, & N-remobilization efficiency from senesced leaves was calculated (Bradstreet 1965) and N determined by AAS, as above. Phosphorus- and N-remobilization efficiency from senesced leaves was calculated as 100% × (1 − [Nutrient]senesced/[Nutrient]mature) (Nardoto, Bustamante, Pinto, & Klink, 2006).

### 2.7 | Statistical analyses

All statistical analyses involved model selection using corrected Akaike information criterion (AICc) (Zuur, Ieno, Walker, Saveliev, & Smith, 2009) in the R environment (R Development Core Team, 2017). Linear mixed models were used with species as a random factor using the nlme package (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2019). To compare plant traits between substrates, a model with intercept only (null model) was compared with a model with substrate type as an independent variable. To check for correlations between plant traits and soil or rock chemical properties, a model with intercept only (null model) was compared with a model with the substrate chemical property as an independent variable. Model assumptions were checked graphically (Zuur et al., 2009), and if they were not met, variance was modelled using generalized least-squares models (gls) with the nlme package (Pinheiro et al., 2019). If the models presented residual heteroscedasticity or non-normality, plant attributes were modelled using generalized linear mixed models (glmm) with gamma distribution with the glmmTMB R packages (Brooks et al., 2017). Because mycorrhizal and dark-septate colonization data were expressed as a percentage, they were modelled using glmm with negative binomial distributions with the glmmTMB package (Skaug, Fournier, Bolker, Magnusson, & Nielsen, 2016) adding 10⁻¹¹ to all the values, because zeros cannot be included in the model. The presence or absence of rhizosheaths or vellozioid roots in each species was modelled with generalized linear models (glm) with a binomial distribution using glm. If the differences in AICc between models were less than two units, the most parsimonious model was chosen (Arnold, 2010). If any term was in the selected model, Tukey post hoc comparisons were performed with the lsmeans package (Lenth, 2016).

### 3 | RESULTS

#### 3.1 | Substrate nutrient analyses

All soils were nutrient poor and very acidic: pH analysed in CaCl₂ varied between 3.2 and 5.3. Resin [P] of the soil samples varied between 1 and 3 mg P/dm³ soil, while total soil [N] varied from 60 to 2,541 mg N/kg soil. Maximum resin-extracted [K] was very low (<20 mg/dm³) and below detection limit (<7.8 mg/dm³) in half of the samples. Resin-extracted [Ca] and [Mg] were below detection limit in most samples (<12 and 20 mg/dm³ soil for Ca and Mg, respectively), only reaching 80 and 36 mg/dm³ (for Ca and Mg, respectively) in one sample. The total amount of organic matter ranged from 3 to 58 g/dm³ soil. Available soil [Mn] was also below the detection limit in several samples (<0.5 mg Mn/dm³ soil), and maximum DTPA-extractable [Mn] was 3.6 mg Mn/dm³ soil. Available Fe varied considerably, from 3 to 193 mg Fe/dm³ soil.

The rock samples were all quartzite, so mainly composed of Si (439–460 g Si/kg rock), with Fe (6–23 g Fe/kg rock) and Al (1.6–13.7 g Al/kg rock) the second and third most abundant elements. Total [Ca] varied between 143 and 643 mg Ca/kg rock and total [Mg] from 60 to 1,146 mg Mg/kg rock. Total [K] varied from 332 to 9,048 mg K/kg rock. Available soil [Mn] was below the detection limit in several samples (<0.5 mg Mn/dm³ soil), and maximum DTPA-extractable [Mn] was 3.6 mg Mn/dm³ soil. Available Fe varied considerably, from 3 to 193 mg Fe/dm³ soil.

Termite mounds presented much greater total [Ca] (1,500 mg Ca/kg) and total [Mg] (603 mg Mg/kg). Total [K] in termite mounds was 7,609 mg K/kg soil. Maximum resin-extracted [K] was very low (<20 mg/dm³) and below detection limit (<7.8 mg/dm³) in half of the samples. Resin-extracted [Ca] and [Mg] were below detection limit in most samples (<12 and 20 mg/dm³ soil for Ca and Mg, respectively), only reaching 80 and 36 mg/dm³ (for Ca and Mg, respectively) in one sample. The total amount of organic matter ranged from 3 to 58 g/dm³ soil. Available soil [Mn] was also below the detection limit in several samples (<0.5 mg Mn/dm³ soil), and maximum DTPA-extractable [Mn] was 3.6 mg Mn/dm³ soil. Available Fe varied considerably, from 3 to 193 mg Fe/dm³ soil.

#### 3.2 | Nutrient-acquisition strategies and fungal symbionts

Thirteen out of the 29 species assessed for fungal colonization presented typical AM structures such as arbuscules, coils, vesicles and hyphae (Figure 3a,b). The average proportion of the root length colonized by AM fungi in colonized species was very low (10%) and did not differ with substrate (Tables S2 and S3, Figure 4a).

Dark-septate fungi were not observed in any roots of species growing on rocks, but 10 of the 19 soil-dwelling species were colonized, with an average of 23% of the root length colonized (Tables S2 and S3).
and S3, Figure 3c-f). Only eight out the 29 species were colonized by both AM and dark-septate fungi (Table S2). We did observe intermingling of the two types of fungi (Figure 3b).

We observed rhizosheaths in 12 of the 20 soil-dwelling species (Table S2, Figure 4b). We observed vellozioid roots mainly in rock-dwelling species: eight of the nine rock-dwelling species had vellozioid roots, while only four of the 20 soil-dwelling species had vellozioid roots (Table S2, Figure 4c).

### 3.3 Desiccation tolerance and leaf deciduousness

Fifteen populations were classified as DT and eleven as non-DT; five species with unknown classification or with insufficient material for senesced-leaf nutrient analyses were removed from the analyses (Table S1). Only seven species shed their senesced leaves, the remainder presenting senesced-leaf blades attached to the caudex (marcescent leaves, Table S1).
3.4 | Leaf nutrient analyses

Average mature leaf [P] varied sixfold, between 0.25 and 1.55 mg P/g leaf dry weight (Table S1, Figure 5a). Mature leaf [P], on average, did not differ between rock-dwelling and soil-dwelling species (Table S3, Figure 5a), DT or non-DT, or deciduous or marcescent leaves (Table S3, Figure S1a). Senesced leaf P varied 21-fold, between 0.03 and 0.65 mg P/g (Table S1, Figure 5a). Phosphorus-remobilization efficiency varied significantly, from 3% to 95%, but was not related to substrate (Tables S1 and S3, Figure 5b), desiccation tolerance or senesced-leaf deciduousness (Table S3, Figure S1b). Mature leaf [P] was not related to resin [P] in soil-dwelling species (Table S3, Figure 5c) or total [P] in rock-dwelling species (Table S3, Figure 5d).

Mature leaf [N] varied fivefold, between 4.2 and 23 mg N/g (Table S1, Figure 6a). Senesced leaf [N] also varied fivefold between 3 and 16 mg N/g (Table S1, Figure 6a). Mature and senesced leaf [N] did not differ with substrate (Table S3, Figure 6a), desiccation tolerance, or senesced-leaf deciduousness (Table S3, Figure S1c). Nitrogen-remobilization efficiency including all individuals varied significantly from −72% (i.e., N accumulation in senesced leaves) to 70% and was not related to the substrate (Tables S1 and S3, Figure 6b), desiccation tolerance or senesced-leaf deciduousness (Table S3, Figure S1d). Mature leaf [N] was not related to total soil [N] in soil-dwelling species (Table S3, Figure 6c); total [N] was not measured in rock samples. Mature leaf N: P ratios varied four-fold, between 11 and 47 (Table S1). Mature leaf N: P ratios did not differ with substrate (Table S3, Figure 7).

Mature leaf [Mn] varied from 43 to 2014 mg Mn/kg, while senesced leaf [Mn] varied from 28 to 2,211 mg Mn/kg; neither depended on substrate (Table S2, Table S3, Figure 8a). Mature leaf [Mn] increased with increasing available soil [Mn] in soil-dwelling species (Table S3, Figure 8b), but was similar among species growing on the rocks (Table S3, Figure 8c).

4 | DISCUSSION

This study aimed to determine which plant traits were associated with habitat specialization and allowed the establishment of Velloziaceae on bare rocks or in sandy soils. We found that vellozioid roots were prevalent in rock-dwelling species and rhizosheaths in soil-dwelling species, indicating that habitat specialization is associated with root morphological and physiological differentiation. The ability of the Velloziaceae to grow on bare rocks accelerates soil formation, releases rock-derived nutrients to the ecosystem and allows, in the long term, the establishment of other species (Porder, 2019). We only observed dark-septate fungi in roots of soil-dwelling species, probably related to the greater amount of organic N in soil. We observed similar leaf [P] and [N] in rock-dwelling and soil-dwelling Velloziaceae. Thus, both soil and rock-dwelling species likely encountered extremely low levels of available P and, consequently, presented similarly low proportions of their root length colonized by AM fungi, as found before at very low P availability (Oliveira et al., 2015; Zemunik et al., 2018). Contrary...
to our expectations, rock-dwelling and soil-dwelling Velloziaceae presented similar leaf [N] and N-remobilization efficiency, possibly due to N inputs from free-living cyanobacteria or termites in rocks (Alves et al., 2014; Schaefer et al., 2016). Leaf desiccation tolerance and leaf deciduousness did not affect leaf [P], [N], or P- and N-remobilization efficiencies. Our data suggest that both substrates offer highly P- and moderately N-limiting conditions for growth which then leads to similar nutrient-use strategies. The type of root specialization seems to be important for habitat specialization and plant establishment on bare rocks. Therefore, nutrient impoverishment is a very strong environmental filter in campos rupestres, but the trade-offs in the costs of obtaining P from the rocks are associated with root morphophysiological edaphic specializations in Velloziaceae.

4.1 | Nutrient-acquisition strategies

In the quartzite rocks in the campos rupestres, P is strongly bound in monazite (Teodoro et al., 2019); therefore, although total [P] was higher in rocks, available [P] was likely similarly low in soil and rock. Arbuscular mycorrhizal fungi are ineffective (Parfitt, 1979) and very costly (Raven et al., 2018) when available [P] is very low. This would explain why the proportion of the root length colonized was equally low for both substrates. However, even a low level of colonization is likely important to enhance defence against pathogens (Lambers et al., 2018). Our study adds to the framework of the importance of non-mycorrhizal P-acquisition strategies when P is severely limiting. Historically, most studies have been carried out in temperate N-limited systems (Lambers, Raven, Shaver, & Smith, 2008) that do not reach the levels of P impoverishment of ancient tropical soils, especially not the levels in the extremely P-poor soils of campos rupestres. We highlight the importance of non-mycorrhizal strategies for P mining in extremely P-poor habitats.
We only observed dark-septate fungi in roots of soil-dwelling Velloziaceae. Dark-septate fungi present a greater positive effect on plant biomass when organic N is the major source on N for plants (Newsham, 2011). It is possible that the accumulation of organic N in soils, due to unfavourable conditions for mineralization (Benites et al., 2007), favoured dark-septate fungi in soil-dwelling species. The availability of viable spores in the quartzite rock may restrict the growth of dark-septate fungi in rock-dwelling Velloziaceae. However, due to the great variation in the proportion of root length colonized by dark-septate fungi, there were no significant differences between substrates.

We expected rhizosheaths in soil-dwelling species and vellozioid roots in rock-dwelling species as alternative strategies for P acquisition, to be more prominent than mycorrhizas. Indeed, we did observe rhizosheaths in 60% of the soil-dwelling species, and in none of the rock-dwelling species, and vellozioid roots in 90% of the rock-dwelling species and in only 20% of the soil-dwelling species. Vellozioid roots appear essential to grow on rocks due to their capacity to penetrate the rock matrix by dissolving the quartzite by roots through dissolution of the quartzite by roots between the rock bedding planes which leads to an increased moisture-holding capacity. However, several species of Brazilian Velloziaceae also occur in granite (i.e. Atlantic rainforest inselbergs close to the coastal line of Brazil) (Porembski, Martinelli, Ohlemüller, & Barthlott, 1998; Barreto et al., 2013; Salgado et al., 2008; Wray & Sauro, 2017). The success of vellozioid roots in these rocks is related to the increase in rock porosity through dissolution of the quartzite by roots between the rock bedding planes which leads to an increased moisture-holding capacity. However, several species of Brazilian Velloziaceae also occur in granite (i.e. Atlantic rainforest inselbergs close to the coastal line of Brazil) (Porembski, Martinelli, Ohlemüller, & Barthlott, 1998); there are also some Andean and African species. The challenges posed by different lithologies depend on their chemical and physical characteristics. Therefore, it is possible that the roots of Velloziaceae present different morphologies and functions in species growing on different lithologies. Further studies are needed to understand how these species grow on rocks posing
different mechanical resistance due to the different amounts of easily alterable minerals (Barreto et al., 2013).

4.2 | Nutrient use strategies

We expected rock-dwelling species to exhibit similar leaf [P], lower leaf [N] and lower leaf N: P than soil-dwelling species. However, leaf [P], [N] and N: P ratios did not depend on substrate. The leaf [P] in this study varied threefold among rock-dwelling species and among soil-dwelling species. This suggests that there is a great variation in the within-substrate availability of both P and N in campos rupestres rocks and soils, or that the Velloziaceae species differ greatly in nutrient acquisition, perhaps related to desiccation tolerance and senesced-leaf deciduousness. Further detailed studies can assess how habitat specialization, leaf deciduousness and desiccation tolerance interact and influence nutrient acquisition and nutrient use in Velloziaceae.

Leaf [N] of soil- and rock-dwelling Velloziaceae was slightly lower (10 to 16 mg N/g dw) than the global average of terrestrial native plants (18 mg N/g), but leaf [P] was only half (0.5 to 0.7 mg N/g) of the global average (1.43 mg P/g) (Vergutz et al., 2012). This is reflected in most species showing N: P ratios above 20, which indicates a strong P, rather than N, limitation of plant growth (Elser et al., 2010; Güsewell, 2004). Campos rupestres substrates have been exposed to weathering for a very long time (Barreto et al., 2013), leading to strong P losses from quartzite that contained very little P to start with (Porder & Ramachandran, 2013). Despite the strong P impoverishment in campos rupestres soils, P addition would probably not stimulate faster growth, due to the inherently extremely slow growth of the Velloziaceae, but would rather allow the establishment of fast-growing species which could competitively exclude the Velloziaceae.

Nutrient remobilization is expected to be greater when nutrients are limiting for growth (Hayes et al., 2014; Kobe et al., 2005; Richardson, Peltzer, Allen, McGlone, & Parfitt, 2004; Vergutz et al., 2012). We observed high P (up to 85%) and modest mean N-remobilization efficiencies per species (up to 55%) in both rock- and soil-dwelling species. This also suggests that, on average, P is more strongly limiting for growth in species growing on both substrates. There was a very large variation in both P- and N-remobilization efficiencies, independent of substrate. This very large variation is possibly due to the variation in N availability among both rocks and soils. Nutrient remobilization from marcescent leaves may well continue for longer than in deciduous species, and aerial roots can also have access to leachate from these decomposing marcescent leaves. We expected deciduousness and desiccation tolerance to influence P and N remobilization; however, we did not observe greater remobilization in marcescent or DT species. Hence, we did not find habitat specialization in above-ground traits related to P- and N-remobilization efficiencies in Velloziaceae.

We observed very high leaf [Mn] (>500 mg Mn/kg) in six rock- and soil-dwelling species (Table S1). Since crop plants require, on average, 50 mg Mn/kg (Epstein & Bloom, 2005), and soil and rock [Mn] were low, the very high leaf [Mn] in some Velloziaceae is likely due to the release of carboxylates into the substrate (Lambers et al., 2015), as demonstrated for Barbacenia tomentosa and B. macrantha (Teodoro et al., 2019). This process plays an important role in rock weathering (Benites et al., 2007; Teodoro et al., 2019), in quartzite dissolution (arenization) and the formation of soils (Drever, 1994; Sauro, 2014), creating new niches and allowing the establishment of other species (Conceição & Pirani, 2016; Porder, 2019).

5 | CONCLUSIONS

We observed similar below- and above-ground traits related to nutrient acquisition and nutrient use in soil- and rock-dwelling Velloziaceae. The strongly bound P in rocks likely poses similar challenges for plants to those growing on soils, with very little total P and a very low P availability. As such, species growing on either substrate present similar nutrient-acquisition strategies, relying very little on AM fungi for P acquisition and predominantly on non-mycorrhizal carboxylate-releasing strategies. Interestingly, soil- and rock-dwelling species exhibited different root specialisations, showing their role in habitat specialization. We conclude that habitat specialization is mediated by vellozioid roots that facilitate plant growth on the rocks, accelerating soil formation and influencing landscape geomorphology and future plant establishment.

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AUTHORS’ CONTRIBUTIONS

A.A., P.d.B.C., G.S.T., H.L., D.L.N., M.H.R. and R.S.O. conceived the ideas and designed methodology; A.A., G.S.T., P.d.B.C. and S.A.L.A.
collected the data; A.A. analysed the data and led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

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Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.vnwxk6dn9 (Abrahão, Costa, Teodoro, et al., 2019).

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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