Functional Ecology

DR GRAZIELLE SALES TEODORO (Orcid ID : 0000-0002-5528-8828)

DR HANS LAMBERS (Orcid ID : 0000-0002-4118-2272)

MS ANNA ABRAHÃO (Orcid ID : 0000-0001-9295-2292)

Article type : Research Article

Section: Plant Physiological Ecology

Editor: Dr Sally Power

Specialized roots of Velloziaceae weather quartzite rock while mobilizing phosphorus using carboxylates

Grazielle Sales Teodoro^{1,2}, Hans Lambers³, Diego L. Nascimento⁴, Patrícia de Britto Costa^{2,3}, Denisele N. A. Flores-Borges², Anna Abrahão^{2,3}, Juliana L. S. Mayer², Alexandra C. H. F. Sawaya², Francisco Sérgio Bernardes Ladeira⁴, Dalton Belchior Abdala⁵, Carlos A. Pérez⁵, Rafael S. Oliveira^{2,3}.

¹. Biology Institute, Universidade Federal do Pará, 01 Augusto Corrêa, Guamá, Belém, Brazil.

². Department of Plant Biology, Biology Institute, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz, Campinas, Brazil.

³. School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley (Perth), Australia.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2435.13324

This article is protected by copyright. All rights reserved.

⁴. Geosciences Institute, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz, Campinas, Brazil.

⁵. Brazilian Synchrotron Light Laboratory (LNLS), Brazilian Center for Research in Energy and Materials (CNPEM), Zip Code 13083-970, Campinas, São Paulo, Brazil.

Corresponding authors: Grazielle Sales Teodoro - gsales.bio@gmail.com (+55 91 99810-5826); Hans Lambers - hans.lambers@uwa.edu.au; Rafael Silva Oliveira - rafaelsooliv@gmail.com

Summary

1. *Campos rupestres* is an extremely phosphorus (P)-impoverished rocky ecosystem in Brazil. Velloziaceae is an important plant family in this environment, and some species colonize exposed quartzite rock. However, we know virtually nothing about their root development and nutrient acquisition within the rock outcrops and their possible role in rock weathering and landscape formation.
2. We tested the hypothesis that Velloziaceae dissolve P from the rock, enhancing rock-weathering. The study was carried out with two *Barbacenia* species (Velloziaceae) that colonize quartzite rocks. We assessed the root specializations and exudates, and determined the mineralogical composition of the rocks.
3. The quartzite rocks contained a low concentration of total P in a matrix composed predominantly of silica. Using transmission electron microscopy, we show root growth perpendicular to the rock-bedding planes. A micro-XRF setup at the XRF beamline of a synchrotron evidenced root-associated rock dissolution.
4. The investigated roots show novel morphological and physiological specializations, coined vellozioid roots, which are highly effective at P acquisition. These carboxylate-releasing roots function like others specialized roots in nutrient-depleted soils. The rocks represent a barrier

for most species, but due to their chemical and physical actions inside the rocks, vellozioid roots play a pivotal role in rock weathering, contributing to shaping the *campos rupestres* landscapes.

Key-words: *campos rupestres*, exudates, phosphorus, vellozioid roots, quartzite

Introduction

Plants and their associated microorganisms play a crucial role in the weathering of rock and in soil formation. However very few studies actually explored the mechanisms that are involved in these processes, and most deal with rocks that are rich in silica, which are very resistant to weathering such as quartzites (Lambers *et al.* 2009; Van Schöll *et al.* 2008; Landeweert *et al.* 2001; Taylor *et al.* 2009). Interactions between physical, chemical, and biological processes enable the weathering of bedrock and the buildup of soil, providing inorganic nutrients to terrestrial biota (Vitousek 2004; Walker & Syers 1976). Once roots have entered rock via a joint or crack, biochemical weathering is enhanced by moisture fluxes along the root, root respiration, low-molecular-weight organic acid exudation, and other rhizosphere processes (Chorover *et al.* 2007; Brantley *et al.* 2011; Burghellea *et al.* 2015; Pawlik, Phillips & Šamonil 2016). The rhizosphere processes include rhizodeposition, which comprises the release of carbon compounds from living and dying roots into the soil or rock which enhances nutrient release (Burghellea *et al.* 2015; Lambers *et al.* 2009; Hinsinger 1998). Rock weathering is an important source of phosphorus (P) in young, nutrient-rich landscapes (Walker & Syers 1976); however, little is known on this process in old and nutrient-impoverished landscapes.

OCBILs (old, climatically-buffered, infertile landscapes) are among the most nutrient-impoverished environments in the world (Hopper 2009; Lambers *et al.* 2010). OCBILs are found in several floristic provinces, for example, the fynbos in South Africa, the South West Australian Floristic Region, and the Venezuelan Pantepuis (Hopper 2009). Plant diversity in OCBILs reflects diversity in plant

Accepted Article

nutritional strategies (Oliveira *et al.* 2015; Lambers *et al.* 2010; Zemunik *et al.* 2015; Zemunik *et al.* 2018). Recently, the Brazilian *campos rupestres* have been acknowledged as an OCBIL (Silveira *et al.* 2016) as have parts of the pampas in southern South America (Sainz Rozas, Echeverria & Angelini 2012; Hopper, Silveira & Fiedler 2016). *Campos rupestres* are a grassy-shrubby mountaintop vegetation interspersed by rock outcrops in the *cerrado* domain in Brazil. OCBIL environments exhibit very low concentrations of soil total P (Benites *et al.* 2007; Hopper 2009; Silveira *et al.* 2016; Lambers *et al.* 2010; Oliveira *et al.* 2015; Benites *et al.* 2003). However, there have been very few investigations related to the concentration of P in parent material in ecosystems, especially in quartzite rocks, although this influences the P concentration in soil derived from it (Porder & Ramachandran 2013). We also know very little on and how P from the parent material enters the system via physical, chemical, and biological weathering. Rock-dwelling plants in ancient landscapes of the Brazilian *campos rupestres* offer a great opportunity to study effects of root weathering of quartzite rocks on P mining and soil formation, with concomitant effects on landscape formation.

Native species from P-impooverished-environments show several adaptive mechanisms that allow survival and growth in their natural habitats and acquisition of scarcely-available soil P resources (Lambers *et al.* 2008). These mechanisms include cluster roots and dauciform roots (Shane & Lambers 2005a) as well as sand-binding roots (Abrahão *et al.* 2019). These specialized roots exude compounds that release P that is sorbed onto soil particles or P from organic fractions, characterizing a P-mining strategy (Lambers *et al.* 2008; 2015b).

The concentration of manganese (Mn) in leaves can be used as a proxy for the release of carboxylates by roots. High Mn concentrations, relative to that of the community average, are an indication of the release of carboxylates in species in environments with a very low availability of P (Lambers *et al.* 2015a; Oliveira *et al.* 2015; Pang *et al.* 2018). In *campos rupestres*, species show great diversity of root specializations and a high diversity of nutritional strategies (Abrahão *et al.* 2014; Oliveira *et al.* 2015; Zemunik *et al.* 2018; Pereira *et al.* 2012; Abrahão *et al.* 2019). Species with dauciform or sand-binding roots present greater leaf Mn concentrations than those with arbuscular mycorrhizal

associations, indicating the importance of carboxylate-releasing P-mobilizing strategies in species with these root specializations (Oliveira *et al.* 2015).

Velloziaceae is a family comprising herbaceous to woody rosette-forming plants which has its center of diversity and endemism in Brazilian *campos rupestres* (Mello-Silva *et al.* 2011); these species comprise a third of plant cover in several *campos rupestres* communities (Zemunik *et al.* 2018).

Velloziaceae occupy different microhabitats; some species colonize exposed quartzite rock, and others occur in patches of sandy soil (Alcantara *et al.* 2015). Species growing on bare rocks have higher leaf P concentrations than those growing in soil patches (Alcantara *et al.* 2015). However, we know nothing about nutrient-acquisition strategies in rock-dwelling Velloziaceae and their importance for rock weathering. We aimed to answer the following questions. What are the P-containing minerals in quartzite rock? Do the roots of Velloziaceae penetrate the quartzite rock? Do Velloziaceae show root specializations to acquire nutrients from quartzite rocks on rock outcrops? Are those specializations functionally analogous to cluster roots or dauciform roots? How do Velloziaceae mobilize P from minerals in quartzite rocks?

We tested the hypotheses that Velloziaceae species have root specializations that are functionally similar to cluster roots and dauciform roots (Shane & Lambers 2005a), with exudates capable of mining P from the rocks. This process would allow nutrient acquisition directly from the rocks, with a major impact on rock-weathering processes, soil formation, and landscape evolution.

Methods

Study site and focal species

Our study was carried out in a *campos rupestres* area located in Serra do Cipó and Itutinga, Minas Gerais, in south-eastern Brazil. The *campos rupestres* are characterized by shallow, acid, and impermeable soils, with low water retention, interspersed with quartzitic rock outcrops. The soil P availability is among the lowest globally (Oliveira *et al.* 2015; Silveira *et al.* 2016). The rock parent

Accepted Article

material is quartzite, formed during the Precambrian and exposed to weathering since the Neoproterozoic era (700 million years of weathering) (Almeida-Abreu & Renger 2002). *Campos rupestres* plants experience strong daily thermal variation, high sunlight incidence and evapotranspiration and constant winds (Jacobi *et al.* 2007). The climate is mesothermic, Cwb (dry-winter highland climate) according to Köppen classification (Köppen 1931). The climate is typically seasonal, characterized by a wet summer from October to April, and a dry season from May to September. The annual rainfall varies from 1250 to 1550 mm. The mean annual temperature varies between 18° and 19°C.

The two Velloziaceae species, *Barbacenia tomentosa* Mart. (rock outcrops in Itutinga) and *B. macrantha* Lem. (rock outcrops in Serra do Cipó) are dominant species in the region and grow directly on bare rocks. We collected 15 individuals per species, during the rainy season, together with their natural substrate (quartzite rocks). We kept the individuals in the rock substrate collected in the field in a greenhouse at Universidade Estadual de Campinas for six months.

Data collection

Rock composition

To determine the mineralogical composition of the rocks on which the studied species occur, we collected 9 rock samples in campos rupestres at Serra do Cipó (n=4), Diamantina (n=4) and Itutinga (n=1). We ground the material collected and assessed the composition by X-ray fluorescence at the Universidade Estadual de São Paulo, in Rio Claro. In addition, we also collected samples of quartzite rocks 20 cm deep with plant roots in it (n=3) for impregnation and preparation of microscopic rock slides. These slides were analyzed under a transmission electron microscope at the Institute of Geosciences of the Universidade Estadual de Campinas. Using the images and transmission electron microscopy analyses, we determined the P-bearing minerals and investigated the relation of these two *Barbacenia* species with the rocks, as well as the role of these rock-dwelling species in the weathering

process in *campos rupestres*. The channels and tunnels in the rock slides were measured using the software Image-J in 20 rock slides.

Root description

To describe the Velloziaceae root specializations, we used scanning, light, and transmission electron microscopy (TEM). For light microscopy, we selected roots with dense root hairs and analyzed a portion of the root tip and portions in which the root hairs were concentrated. We made cross-sections of the fresh material for histochemical tests, using ruthenium red for staining polysaccharides and mucilage (Gregory & Baas 1989); Lugol's iodine solution for starch (Jensen 1962); Nile blue (Cain 1947) for neutral (stained pink) and acidic (stained blue) lipids to identify the aliphatic compounds and ferric chloride for phenolic compounds (Johansen 1940); iodinated zinc chloride (Jensen, 1962) for starch grains (stained dark blue color); phloroglucinol – HCL (Jensen, 1962) for suberin and lignin (stained pink-violet color). After the phloroglucinol test, to confirm the suberin and lignin presence, we used Sudan IV, to dye in orange the suberin and Maule reagent to dye in pink-violet color the lignin (Jensen, 1962).

Root segments were fixed in Karnovsky fixative, modified by phosphate buffer pH 7.2 for 48 h at room temperature. Those samples were dehydrated in an ascending ethanol series. After that, samples were embedded in hydroxyethyl methacrylate (Leica® historesin). The transversal and longitudinal sections (5 μm thick) were cut on a rotation microtome (Leica® RM 2145, Wetzlar, Germany). The sections were stained with toluidine blue O (Sakai 1973) and mounted in synthetic resin®. The results were documented by capturing images from the sections using an Olympus BX 51 (Tokyo, Japan) photomicroscope equipped with an Olympus DP71 camera (Tokyo, Japan).

Root sections were prepared for analysis under a TEM. For these analyses, the material was sectioned in small portions of approximately 1 mm³ and fixed in 3% (v/v) glutaraldehyde in cacodylate buffer (0.2 M pH 7.25) for 12 h. After removal of the fixative, the washing was performed in buffer and the

Accepted Article

material was fixed in a solution of 1% (w/v) osmium tetroxide (OsO₂) and kept in the dark for about 12 h. Dehydration was achieved by ascending alcohol series from 10% (v/v) until 100%. After dehydration, the material was included in hydrophilic acrylic resin LR White® "Hard Grade". Samples were included in gelatin capsules and left to polymerize at 60 °C for 12 h. The ultrafine sections were made with an ultramicrotome Leica Ultracut UCT, using a diamond razor Drukker 45, 3 mm thick. Counterstaining was performed using an aqueous solution of 2% uranyl acetate and a lead solution for 30 min each. The analyses were performed in a LEO 906 (Carl Zeiss, Germany) transmission electron microscope in Laboratory Electron Microscopy at Institute of Biology / Universidade Estadual de Campinas.

Distribution of elements in the outermost region of specialized roots

We used micro-XRF setup available at the XRF beamline of the Brazilian synchrotron light laboratory (LNLS), Campinas, São Paulo, Brazil (Pérez *et al.* 1999) to map the nutrients near a root of *B. tomentosa*. We used one sample with a dimension of 5 x 4 mm and the spatial resolution used was 20-25 µm. We mapped the following elements in the outermost region of the specialized root tissue: P, zinc (Zn) and calcium (Ca). Zinc and Ca were selected to show their spatial distributions with high resolution and to contrast with P distribution.

Root functioning: exudate release

To assess exudate release and to identify the released carboxylates, we used an ultra-high performance liquid chromatography system with a triple quadrupole mass spectrometer (UPLC-MS) and an electrospray ionization source, Acquity UPLC-TQD (Waters, Milford, MA, USA) at the Universidade Estadual de Campinas. Three 2 cm sections of fresh root tips per plant maintained in the greenhouse of *B. tomentosa* were collected and gently shaken in 0.5 ml of 0.1 % (v/v) Milli-Q water-dissolved formic acid (pH= 5.8) to avoid microbial consumption of the organic acids (Abrahão *et al.* 2014).

After 5 min, the root tips were removed, the samples were filtered, and the samples were frozen at – 20°C until analysis. Each root tip was weighed in order to express carboxylate release per unit root weight. Standards were citric, malic, oxalic, succinic, lactic, maleic, tartaric, DL-isocitric, malonic, and fumaric acid, which are all known to be released from roots and likely to be involved in P mobilization (Roelofs *et al.* 2001; Oburger *et al.* 2009).

To evaluate if the released exudates shifted during root development, we collected exudates from six individuals of *B. tomentosa* maintained in the greenhouse. From those individuals we collected roots fragments in the following stages: immature (n=3), mature (n=15), and senescent (n=4). As the investigated roots were in soil/rocks, we were not able to monitor exactly the longevity of the roots, and thus we assumed three classes 1) immature: 2-4 days; 2) mature: 6-12 days; 3) senescent: more than 15 days.

In the field, we collected young (fully mature) and senesced leaves from six individuals of *B. tomentosa* to determine the Mn concentration as a proxy for rhizosphere carboxylate concentrations. Leaves were dried until constant weight, ground, digested with nitric and perchloric acids and analyzed by atomic absorption spectrophotometry (Perkin-Elmer, MA, USA) at the Universidade Federal de Viçosa, Brazil.

Effect of carboxylates on P mobilization

We tested the effect of carboxylates on P solubilization from quartzite rocks. For this, we used the two major carboxylates released by *B. tomentosa*: malate and citrate. We ground the rock, weighed one gram and placed it in 15-ml centrifuge tubes and mixed with 5 mM KCl background solution with carboxylates added (0, 5, 10, 15 and 20 μ M). The samples were shaken for 20 min at 260 rpm and centrifuged for 20 min. The supernatant was collected with a syringe and filtered through a 45- μ m syringe-filter (Abrahão *et al.* 2014). The P was analyzed colorimetrically using the malachite green method (Motomizu, Wakimoto & Toei 1983). We used five samples of rocks (two from Itutinga outcrop and three from Serra do Cipó area) to test P solubilization.

Data analyses

To analyze whether the concentration of carboxylates changed with root development, we performed an ANOVA. To evaluate the role of exudates in P solubilization, we performed regressions between P solubilization and concentration of carboxylates. To compare the effect of malate and citrate on P solubilization we performed an ANCOVA. We performed the statistical analysis using R version 3.3.2 (R Core Team 2016).

Results

Rock mineralogical analyses

The quartzite rocks in the Espinhaço Mountain Range contained 0.14 ± 0.02 mg total P g⁻¹ in a matrix comprising predominantly silica (451 ± 8 mg Si g⁻¹) (Table 1). Some Velloziaceae occur in the bare rocks, and proliferated their roots inside them, using the rock along the bedding planes. In many cases, these plants did not use any preexisting cracks in the rocks, as evidenced by root growth perpendicular to the rock-bedding plane (Fig. 1a-c). The roots branched inside the rock (Fig. 1a-f), and formed tunnels inside the rock that varied from 0.02 ± 0.021 mm to very thick layers of roots inside the rocks (Fig. 1b-e).

The main source of P in quartzite rocks was monazite, composed of rare earth elements, mainly cerium (Ce), yttrium (Y) and gadolinium (Gd). In addition, P was associated with iron oxide (ilmenite (FeTiO₃); in this mineral the iron can be replaced by Mn and yttrium phosphate. These minerals existed as small grains in the rock matrix (Fig. 1g-h).

“Vellozioid roots” morphology and histochemistry

The root systems of *Barbacenia tomentosa* and *B. macrantha* consisted of fibrous roots with morphological specializations, which are segments with a high density of extremely long root hairs just behind the apex (Fig. 2a-f). In both species, the root hairs originated from the protoderm and the cell differentiation occurred rapidly with the elongation in the anticline direction (Fig. S1a-j). The root region covered by hairs was highly variable (from 5 mm to < 5 cm) and these hairs were up to 3 mm long (Fig. 1a-f). The P concentration in the outermost region of the root tissue was higher near the roots hairs than in portions without specializations (Fig. 2g-h). This morphological specialization in roots of Velloziaceae species has never been recorded before, and we coin the term “vellozioid roots” for this newly described structure.

Vellozioid roots contained numerous substances accumulated in both roots and root hairs (Fig. 3a-e). In the meristematic region, near the apex, we observed polysaccharides inside the root hairs and in the extracellular secretions that were stained pink with ruthenium red (Fig. 3a-b). We observed lipids inside and also on the surface of the root hairs (Fig. 3c-d). Phenols also accumulated inside the root hairs (Fig. 3e-f). Above the apex, in a region with tissue differentiation and root hairs, we observed starch grains accumulated in the root cortical cells (Fig. 3g). Lignin and suberin were present in exodermis and other cortex cells (Fig. 3h-j). Suberin was present mainly in the external periclinal wall of exodermal cells, and in thick layers of cells in the cortex region adjacent to exodermal cells (stained in dark orange) (Fig. 3j). Lignin was present in cortex cells, adjacent to cells with suberin. The lignin presence was confirmed with the Maule test (Fig. 3i). The root hairs presented invaginations along their entire length with dense cytoplasm, evidencing increased absorptive surfaces (Fig. 3k-r) and presented vesicles near the epidermis (Fig. 3l - insertion in Fig. 3k). Note the extracellular secretion on the surface of the root hair (Fig. 3n). There was probably an intense cellular communication between root hairs and subepidermal cells, as evidenced by a pit-pair in Fig. 3o. Secretions inside the root hair and the exudate are clearly heterogeneous (Fig. 3p-r). Subepidermal cells have anticlinal walls (Fig. 3r) and these cell walls had a lamellar appearance with light bands alternating with dark bands (Fig. 3s).

Carboxylate release and the functional role of vellozioid roots

Barbacenia tomentosa exuded carboxylates, mainly malate and citrate, but also succinate (Fig. 4). Isocitrate, malonate and fumarate acids were present in trace amounts only, and hence were not included in the analyses. The amount of carboxylates released was similar in immature, mature and senesced roots for citrate ($F = 2.531$; $p = 0.10$) and malate ($F = 0.136$; $p = 0.87$), while succinate was not exuded by immature roots and did not significantly differ between mature and senescent roots ($F = 0.058$; $p = 0.82$) (Fig. 4).

Furthermore, we also observed a high concentration of Mn in leaves of *B. tomentosa* ($263 \pm 53 \text{ mg kg}^{-1}$) which is an indicator of the concentration of carboxylates in the rhizosphere by roots. This high concentration was similar to that of other nonmycorrhizal species in *campos rupestres* (average 200 mg kg^{-1} (Oliveira *et al.* 2015)). The Mn concentration was greater in senesced ($346 \pm 41 \text{ mg kg}^{-1}$) leaves than in the young ones ($179 \pm 35 \text{ mg kg}^{-1}$) ($t = 2.18$; $p < 0.05$).

Phosphate solubilization from quartzite rock by carboxylates

In the treatment with malate, we observed a linear increase in soluble P concentration with increasing malate concentration. The values ranged from $5.3 \pm 1.3 \text{ mg P kg}^{-1}$ rocky substrate in the absence of carboxylates to $16.8 \pm 2.8 \text{ mg P kg}^{-1}$ rocky substrate using $20 \text{ }\mu\text{M}$ malate ($y = 0.53x + 7.33$; $R^2 = 0.81$; $p = 0.01$) (Fig. 5). Citrate also increased the soluble P concentration linearly, from $4.7 \pm 1.0 \text{ mg P kg}^{-1}$ rocky substrate in the absence of carboxylates to $10.2 \text{ mg P kg}^{-1}$ rocky substrate at $20 \text{ }\mu\text{M}$ citrate ($y = 0.31x + 4.31$; $R^2 = 0.93$; $p = 0.001$) (Fig. 5). The effect of malate on P solubilization was greater than that of citrate (*P solubilization:carboxylate type* $F = 5.29$; $p = 0.05$).

Discussion

We show a major role of two carboxylate-releasing plant species in P solubilization from quartzite rock. We provide evidence that vellozioid roots grow into the rocks, through slow chemical and physical alterations, dissolving quartzite and driving soil formation processes. The specialized biology of a plant family allows profound interactions with the rock, slowly affecting the geomorphological processes and the landscape at different scales.

The vellozioid roots, described here for the first time, are root specializations that combine anatomical and morphological modifications associated with a specialized physiology related to the release of carboxylates that mine P from quartzite rock for uptake by roots. The present results highlight a novel and significant root specialization in an iconic plant family, Velloziaceae, and we surmise that vellozioid roots are functionally similar to cluster roots and dauciform roots that mine P from extremely P-impooverished soils (Shane & Lambers 2005a). Vellozioid roots are highly efficient at P acquisition, since they can solubilize P from the rock in a matrix comprising predominantly Si (>96% of the content). This is a far more extreme situation than the strategies of plants with cluster or dauciform roots that grow in soil (Shane & Lambers 2005a) or on rock surfaces (Shi *et al.* 2019). These Velloziaceae, due to their physical and chemical actions inside the rocks, act in the process of weathering in *campos rupestres*. The present *Barbacenia* species are nonmycorrhizal (Abrahão 2018), and hence impacted the rock via root exudates, rather than exudates release by mycorrhizal hyphae (Van Schöll *et al.* 2008; Hoffland *et al.* 2004; Landeweert *et al.* 2001).

Some Velloziaceae grow directly on the rocks in *campos rupestres*, rather than in soil, and directly influence the weathering process, contributing to shape the landscape (Fig. 1). This weathering may occur initially by the exploitation of small fractures in the rocks (Frazier *et al.* 2002; Cassiani *et al.* 2009; Estrada-Medina *et al.* 2013). Alternatively, the carboxylates released by the vellozioid roots may dissolve the rock surface, and allow the roots to penetrate the rock, as evidenced by our photos. This biochemical alteration then creates a preferential water flow via channels produced by roots (Estrada-Medina *et al.* 2013; Schwinning 2013). Therefore, the water availability in these rocks

depends on fractures provided by weathering (Schwinning 2010), a process strongly enhanced by vellozioid roots. After this initial process of exploration, Velloziaceae form dense root mats, vastly increasing the space weathered in the rocks (Fig. 1). Perhaps high lignin, thick walls, mucilage may all improve the mechanical properties of these roots allowing them to grow through a hard substrate.

The quartzite rocks at Serra do Espinhaço contain a very small amount of P, 0.14 mg g^{-1} . A worldwide analysis of P concentrations in parent material shows that the mean concentration at Serra do Espinhaço is considerably less than in common silica-rich rocks, such as granite, 0.44 mg g^{-1} , and especially less than in common iron-rich rocks, *e.g.*, andesite, 1.00 mg g^{-1} (Porder & Ramachandran 2013). In quartzite rocks at Serra do Espinhaço the element with the highest concentration was silica, and its P concentration was less than that in all the rocks considered in Porder and Ramachandran (2013). This concentration of P in parent rock influences the soil P concentration (Porder *et al.* 2007; Porder & Ramachandran 2013; Augusto *et al.* 2017), which is very low in soils in *campos rupestres*, being among the most P impoverished in the world (Oliveira *et al.* 2015; Zemunik *et al.* 2018). There was no apatite in the quartzite rocks that we analyzed, but we found P in monazite, iron oxide and yttrium phosphate, in which Fe and Al hydrous oxides strongly bind the P minerals (Abel 2017), rendering them unavailable for uptake by most plants (Lambers *et al.* 2008).

Different plant P-acquisition strategies are associated with differences in soil age and soil nutrient availability (Lambers *et al.* 2015b; Lambers *et al.* 2008; Zemunik *et al.* 2015). In moderately P-impoverished soils, plants establish P-acquiring symbioses with mycorrhizal fungi, whereas in severely P-impoverished soils, the role of carboxylate-releasing root specializations becomes more important (Lambers *et al.* 2008; 2018). Mycorrhizal species coexist with non-mycorrhizal species, however, and the role of the mycorrhizal symbioses is likely to boost the defense against plant pathogens (Lambers *et al.* 2018; Albornoz *et al.* 2017). In the *campos rupestres*, different root specializations have been described, *e.g.*, sand-binding roots in *Discocactus placentiformis* (Cactaceae) (Abrahão *et al.* 2014), *Actinocephalus cabralensis* and *Paepalanthus* sp. (Eriocaulaceae) (Oliveira *et al.* 2015). In addition, there are Cyperaceae that produce dauciform roots (Oliveira *et al.* 2015). Here, we present an entirely new root specialization in Velloziaceae that colonize the quartzite

Accepted Article

rocks, vellozioid roots. Many cluster- and dauciform-root producing species occur in soil habitats in ancient landscapes impoverished in P, such as southwestern Australia (Zemunik *et al.* 2015; Lambers *et al.* 2010), the Cape region in South Africa (Lamont 2003; Lambers *et al.* 2010) and *campos rupestres* (Oliveira *et al.* 2015; Zemunik *et al.* 2018). However, this is the very first report of release of carboxylates and solubilization of P from quartzite rock mediated by plant roots. This is extremely important considering the low amount of P found in quartzite rocks, emphasizing the role of this root specialization.

The biota of the Brazilian *campos rupestres* is unique at taxonomic and evolutionary level. However, the *campos rupestres* have some analogues on other continents in relation similarity in geology and weathering patterns leading to nutrient-poor soils and others aspects (Mucina 2018). For example, in South Africa, Swaziland, Zimbabwe and Mozambique are found nutrient-poor equivalents substrates (known as Sourveld) and in some rugged and dissected Australian sandstone plateaus (revised by Mucina 2018). Therefore, we expect species with root specialization in those ecosystems, and that the importance of roots on weathering might be more important than we have assumed.

The vellozioid roots of *Barbacenia* differed morphologically and anatomically from cluster roots (comprising a cluster of rootlets along a lateral root axis) and dauciform roots (carrot-shaped roots with long hairs concentrated in a small region) (Shane *et al.* 2006a; Lambers *et al.* 2006; Güsewell 2017; Konoplenko, Güsewell & Veselkin 2017). The root specialization in *Barbacenia* species consisted of a high density of root hairs, located just below the root tip. The root hair region was quite variable in its length. Vellozioid root hairs presented a variable content (mucilage, polysaccharides, phenols, starch and others) and thick walls that may improve the mechanical properties of these roots allowing them to grow through a hard substrate. Vellozioid roots differed also from cluster roots in showing a distinct suberized exodermis, which is lacking in Proteaceae and most Fabaceae (Lambers *et al.* 2018). The absence of an exodermis may be important to rapidly release carboxylates, but renders plants likely vulnerable to pathogen attack. Since both Cyperaceae (Enstone, Peterson & Ma 2003) and Velloziaceae (present study) do have an exodermis, they may release carboxylates from their root hairs, and hence not be as susceptible to pathogen attack as Proteaceae (Lambers *et al.*

2018). Although, the root specialization in *Barbacenia* species was structurally different from that of the others described specializations, these roots were functionally analogous (Shane, Dixon & Lambers 2006b; Lambers *et al.* 2006) in terms of release of organic acids, but acquiring P from rock, rather than soil.

The numerous root hairs of vellozioid roots increased the root-rock contact area. The carboxylates are likely released from these root hairs, allowing them to mine the P that is locked up in minerals in the quartzite rock. The protons released together with carboxylates accelerate silica dissolution due to a decrease in the pH (Lucas, 2001; Thiry *et al.*, 2014). The deformation in Si grains is strong evidence that the roots dissolved the quartz (Fig. 1 d). Thus, the vellozioid roots create the microenvironment conducive to silica dissolution, contributing to the weathering in *campos rupestres*. The main organic acids released by vellozioid roots were malic and citric acid. Both, malate and citrate have affinity for iron (Fe^{3+}) and aluminum (Al^{3+}), replacing P sorbed in their oxides and hydroxides (Oburger *et al.*, 2009). Citrate is one of the major organic acids released by cluster and dauciform roots under conditions of P deficiency (Watt & Evans 1999; Shane *et al.* 2006a; Shane *et al.* 2004), but malate also appears in higher concentrations than citrate for recently-described root specialization in sandy soils in *campos rupestres* (Abrahão *et al.* 2014). The P-solubilizing relationship with carboxylate concentration highlights the importance of the exudates released by vellozioid roots. Phosphorus was efficiently solubilized, even at relatively low concentrations of the organic anions in solution. A low concentration of organic acid (5 μM) almost doubled the P availability compared with that release in the absence of carboxylates. These results indicate that the small amounts of carboxylates released by vellozioid roots were capable of efficient P solubilization from quartzite rock.

The high concentration of Mn in the leaves of *B. tomentosa* was an important indicator of the amount of carboxylates in the rhizosphere (Lambers *et al.* 2015a; Pang *et al.* 2018). Manganese solubility is likely also increased by acidification (by organic acids) (Oliveira *et al.* 2015; Pang *et al.* 2018; Lambers *et al.* 2015a). The concentration of Mn in the leaves of *B. tomentosa* was considerably greater than that of the *campos rupestres* community (Oliveira *et al.* 2015), reinforcing the important role of carboxylates in the mining strategy of these species. The Mn concentration was greatest in

older leaves, as has been found for *Hakea prostrata* (Proteaceae) (Shane & Lambers 2005b). This might reflect their age, considering many Velloziaceae are resurrection plants (Alcantara *et al.* 2015), but given that the young leaves also exhibited high Mn concentrations, this demonstrates age does not account entirely for the high Mn concentrations.

In summary, *Barbacenia* species very efficiently exploit the extremely low-P quartzite rock, using a P-mining strategy involving vellozioid roots, a novel root specialization described in this study. We show the role of Velloziaceae in weathering of quartzite rock, through chemical and physical interactions with the rock, involving exudation of carboxylates. The gradual weathering of the rock followed by the release of sand likely influences the evolution of the landscape and the diversity of substrates found in *campos rupestres*. Similar processes are likely occurring in other landscapes. For example, roots of *Pinus sylvestris* are capable of dissolving apatite, and this process may involve mycorrhizal symbionts (Calvaruso *et al.* 2013; Wallander, Wickman & Jacks 1997; van Breemen *et al.* 2000).

5. Acknowledgments

We would like to acknowledge the CAPES PVE program for a research grant to RSO (88881.068071/2014-01), scholarship to AA and PBC and a fellowship to GST and HL. This research used resources of the Brazilian Synchrotron Light Laboratory (LNLS), an open national facility operated by the Brazilian Centre for Research in Energy and Materials (CNPEM) for the Brazilian Ministry for Science, Technology, Innovations and Communications (MCTIC). We acknowledge the XRF beamline staff for the assistance during the experiments. RO received a CNPq productivity scholarship.

6. Author's contribution

G.S.T., H.L. and R.S.O. designed the study; G.S.T., D.L.N., P.B.C., D.N.A.F.B., J.M., A.A., A.C.H.F.S., C.A.P. collected the data; G.S.T. and H.L. wrote the paper with input from all authors; All authors contributed to the final version of the manuscript.

7. Data accessibility

All data is available from the Dryad Digital Repository (Teodoro et al. 2019 <https://doi.org/10.5061/dryad.6p65fh1>).

References

- Abel, S. (2017) Phosphate scouting by root tips. *Current Opinion in Plant Biology* **39**, 168-177.
- Abrahão, A. (2018) *Mineral Nutrition in Two Biodiversity Hotspots: Campos Rupestres and Kwongan*. PhD PhD, University of Western Australia, Crawley (Perth).
- Abrahão, A., Costa, P.D.B., Lambers, H., Andrade, S.a.L., Sawaya, A.C.H.F., Ryan, M.H. & Oliveira, R.S. (2019) Soil types select for plants with matching nutrient-acquisition and -use traits in hyperdiverse and severely nutrient-impoverished *campos rupestres* and *cerrado* in Central Brazil. *Journal of Ecology* **0**.
- Abrahão, A., Lambers, H., Sawaya, A.C.H.F., Mazzafera, P. & Oliveira, R.S. (2014) Convergence of a specialized root trait in plants from nutrient-impoverished soils: phosphorus-acquisition strategy in a nonmycorrhizal cactus. *Oecologia* **176**, 345-355.
- Albornoz, F.E., Burgess, T.I., Lambers, H., Etchells, H. & Laliberté, E. (2017) Native soil-borne pathogens equalise differences in competitive ability between plants of contrasting nutrient-acquisition strategies. *Journal of Ecology* **105**, 549–557.
- Alcantara, S., Mello - Silva, R., Teodoro, G.S., Drequeceler, K., Ackerly, D.D. & Oliveira, R.S. (2015) Carbon assimilation and habitat segregation in resurrection plants: a comparison between desiccation- and non-desiccation-tolerant species of Neotropical Velloziaceae (Pandanales). *Functional Ecology* **29**, 1499-1512.
- Augusto, L., Achat, D.L., Jonard, M., Vidal, D. & Ringeval, B. (2017) Soil parent material—A major driver of plant nutrient limitations in terrestrial ecosystems. *Global Change Biology* **23**, 3808-3824.

- Benites, V.D.M., Caiafa, A., Mendonça, E.D.S., Schaefer, C.E. & Ker, J.C. (2003) Solos e vegetação nos complexos rupestres de altitude da Mantiqueira e do Espinhaço. *Floresta e ambiente* **10**, 76-85.
- Benites, V.M., Schaefer, C.E.G.R., Simas, F.N.B. & Santos, H.G. (2007) Soils associated with rock outcrops in the Brazilian mountain ranges Mantiqueira and Espinhaço. *Brazilian Journal of Botany* **30**, 569-577.
- Brantley, S.L., Megonigal, J.P., Scatena, F.N., Balogh-Brunstad, Z., Barnes, R.T., Bruns, M.A., Van Cappellen, P., Dontsova, K., Hartnett, H.E., Hartshorn, A.S., Heimsath, A., Herndon, E., Jin, L., Keller, C.K., Leake, J.R., Mcdowell, W.H., Meinzer, F.C., Mozdzer, T.J., Petsch, S., Pett-Ridge, J., Pregitzer, K.S., Raymond, P.A., Riebe, C.S., Shumaker, K., Sutton-Grier, A., Walter, R. & Yoo, K. (2011) Twelve testable hypotheses on the geobiology of weathering. *Geobiology* **9**, 140-165.
- Burghilea, C., Zaharescu, D.G., Dontsova, K., Maier, R., Huxman, T. & Chorover, J. (2015) Mineral nutrient mobilization by plants from rock: influence of rock type and arbuscular mycorrhiza. *Biogeochemistry* **124**, 187-203.
- Cain, A.J. (1947) The use of Nile blue in the examination of lipoids. *Journal of Cell Science* **3**, 383-392.
- Calvaruso, C., Turpault, M.-P., Frey-Klett, P., Uroz, S., Pierret, M.-C., Tosheva, Z. & Kies, A. (2013) Increase of apatite dissolution rate by Scots pine roots associated or not with *Burkholderia glathei* PML1(12)Rp in open-system flow microcosms. *Geochimica et Cosmochimica Acta* **106**, 287-306.
- Cassiani, G., Godio, A., Stocco, S., Villa, A., Deiana, R., Frattini, P. & Rossi, M. (2009) Monitoring the hydrologic behaviour of a mountain slope via time-lapse electrical resistivity tomography. *Near Surface Geophysics* **7**, 475-486.
- Chorover, J., Kretzschmar, R., Garcia-Pichel, F. & Sparks, D.L. (2007) Soil biogeochemical processes within the critical zone. *Elements* **3**, 321-326.
- Enstone, D.E., Peterson, C.A. & Ma, F. (2003) Root endodermis and exodermis: structure, function, and responses to the environment. *Journal of Plant Growth Regulation* **21**, 335-351.
- Estrada-Medina, H., Graham, R.C., Allen, M.F., Jiménez-Osornio, J.J. & Robles-Casolco, S. (2013) The importance of limestone bedrock and dissolution karst features on tree root distribution in northern Yucatán, México. *Plant and Soil* **362**, 37-50.
- Frazier, C.S., Graham, R.C., Shouse, P.J., Yates, M.V. & Anderson, M.A. (2002) A field study of water flow and virus transport in weathered granitic bedrock. *Vadose Zone Journal* **1**, 113-124.
- Gregory, M. & Baas, P. (1989) A survey of mucilage cells in vegetative organs of the dicotyledons. *Israel Journal of Botany* **38**, 125-174.
- Güsewell, S. (2017) Regulation of dauciform root formation and root phosphatase activities of sedges (*Carex*) by nitrogen and phosphorus. *Plant and Soil* **415**, 57-72.

- Hinsinger, P. (1998) How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Advances in Agronomy* **64**, 225-265.
- Hoffland, E., Kuyper, T.W., Wallander, H., Plassard, C., Gorbushina, A.A., Haselwandter, K., Holmström, S., Landeweert, R., Lundström, U.S., Rosling, A., Sen, R., Smits, M.M., Van Hees, P.a.W. & Van Breemen, N. (2004) The role of fungi in weathering. *Frontiers in Ecology and the Environment* **2**, 258-264.
- Hopper, S.D. (2009) OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and Soil* **322**, 49-86.
- Hopper, S.D., Silveira, F.a.O. & Fiedler, P.L. (2016) Biodiversity hotspots and Ocbil theory. *Plant and Soil* **403**, 167–216.
- Jacobi, C.M., Carmo, F.F., Vincent, R.C. & Stehmann, J.R. (2007) Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem. *Biodiversity and Conservation* **16**, 2185-2200.
- Jensen, W.A. (1962) *Botanical Histochemistry: Principles and Practice*. WH FreemanWH Freeman and Co., San Francisco, CA, USA.
- Johansen, D.A. (1940) *Plant Microtechnique*. McGraw-Hill Book Company, Inc., London.
- Konoplenko, M.A., Güsewell, S. & Veselkin, D.V. (2017) Taxonomic and ecological patterns in root traits of *Carex* (Cyperaceae). *Plant and Soil* **420**, 37-48.
- Köppen, W.P. (1931) *Grundriss der Klimakunde*. Walter de Gruyter, Berlin.
- Lambers, H., Albornoz, F., Kotula, L., Laliberté, E., Ranathunge, K., Teste, F.P. & Zemunik, G. (2018) How belowground interactions contribute to the coexistence of mycorrhizal and non-mycorrhizal species in severely phosphorus-impooverished hyperdiverse ecosystems. *Plant and Soil* **424**, 11-34.
- Lambers, H., Brundrett, M.C., Raven, J.A. & Hopper, S.D. (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* **334**, 11-31.
- Lambers, H., Hayes, P.E., Laliberté, E., Oliveira, R.S. & Turner, B.L. (2015a) Leaf manganese accumulation and phosphorus-acquisition efficiency. *Trends in Plant Science* **20**, 83-90.
- Lambers, H., Martinoia, E. & Renton, M. (2015b) Plant adaptations to severely phosphorus-impooverished soils. *Current Opinion in Plant Biology* **25**, 23-31.
- Lambers, H., Mougél, C., Jaillard, B. & Hinsinger, P. (2009) Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. *Plant and Soil* **321**, 83-115.
- Lambers, H., Raven, J.A., Shaver, G.R. & Smith, S.E. (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution* **23**, 95-103.

- Accepted Article
- Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J. & Veneklaas, E.J. (2006) Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**, 693-713.
- Lamont, B.B. (2003) Structure, ecology and physiology of root clusters – a review. *Plant and Soil* **248**, 1-19.
- Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W. & Van Breemen, N. (2001) Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology & Evolution* **16**, 248-254.
- Mello-Silva, R., Santos, D.Y.a.C., Salatino, M.L.F., Motta, L.B., Cattai, M.B., Sasaki, D., Lovo, J., Pita, P.B., Rocini, C., Rodrigues, C.D.N., Zarrei, M. & Chase, M.W. (2011) Five vicariant genera from Gondwana: the Velloziaceae as shown by molecules and morphology. *Annals of Botany* **108**, 87-102.
- Motomizu, S., Wakimoto, T. & Toei, K. (1983) Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. *Analyst* **108**, 361-367.
- Mucina, L. (2018) Vegetation of Brazilian campos rupestres on siliceous substrates and their global analogues. *Flora* **238**, 11–23.
- Oburger, E., Kirk, G.J.D., Wenzel, W.W., Puschenreiter, M. & Jones, D.L. (2009) Interactive effects of organic acids in the rhizosphere. *Soil Biology and Biochemistry* **41**, 449-457.
- Oliveira, R.S., Galvão, H.C., De Campos, M.C.R., Eller, C.B., Pearse, S.J. & Lambers, H. (2015) Mineral nutrition of *campos rupestres* plant species on contrasting nutrient-impooverished soil types. *New Phytologist* **205**, 1183-1194.
- Pang, J., Ruchi, B., Zhao, H., Bansal, R., Bohuon, E., Lambers, H., Ryan, M.H., Ranathunge, K. & Siddique, K.M.H. (2018) The carboxylate-releasing phosphorus-mobilising strategy could be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. *New Phytologist* **219**, 518-529.
- Pawlik, Ł., Phillips, J.D. & Šamonil, P. (2016) Roots, rock, and regolith: Biomechanical and biochemical weathering by trees and its impact on hillslopes—A critical literature review. *Earth-Science Reviews* **159**, 142-159.
- Pereira, C.G., Almenara, D.P., Winter, C.E., Fritsch, P.W., Lambers, H. & Oliveira, R.S. (2012) Underground leaves of *Philcoxia* trap and digest nematodes. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 1154-1158.
- Pérez, C.A., Radtke, M., Sánchez, H.J., Tolentino, H., Neuenschwander, R.T., Barg, W., Rubio, M., Bueno, M.I.S., Raimundo, I.M. & Rohwedder, J.J.R. (1999) Synchrotron radiation X-ray fluorescence at the LNLS: beamline instrumentation and experiments. *X - Ray Spectrometry* **28**, 320-326.
- Porder, S., Vitousek, P.M., Chadwick, O.A., Chamberlain, C.P., Hilley, G.E. (2007) Uplift, Erosion, and Phosphorus Limitation in Terrestrial Ecosystems. *Ecosystems* **10**, 159-171, doi:10.1007/s10021-006-9011-x.

- Porder, S. & Ramachandran, S. (2013) The phosphorus concentration of common rocks—a potential driver of ecosystem P status. *Plant and Soil* **367**, 41-55.
- Roelofs, R.F.R., Rengel, Z., Cawthray, G.R., Dixon, K.W. & Lambers, H. (2001) Exudation of carboxylates in Australian Proteaceae: chemical composition. *Plant, Cell and Environment* **24**, 891-904.
- Sainz Rozas, H., Echeverria, H. & Angelini, H. (2012) Available phosphorus in agricultural soils of the Pampa and Argentina ExtraPampeana. *RIA, Revista de Investigaciones Agropecuarias* **38**, 33-39.
- Sakai, W.S. (1973) Simple method for differential staining of paraffin embedded plant material using toluidine blue O. *Stain technology* **48**, 247-249.
- Schwinning, S. (2010) The ecohydrology of roots in rocks. *Ecohydrology* **3**, 238-245.
- Schwinning, S. (2013) Do we need new rhizosphere models for rock-dominated landscapes? *Plant and Soil* **362**, 25-31.
- Shane, M.W., Cawthray, G.R., Cramer, M.D., Kuo, J. & Lambers, H. (2006a) Specialized 'dauciform' roots of Cyperaceae are structurally distinct, but functionally analogous with 'cluster' roots. *Plant, Cell and Environment* **29**, 1989-1999.
- Shane, M.W., Cramer, M.D., Funayama-Noguchi, S., Cawthray, G.R., Millar, A.H., Day, D.A. & Lambers, H. (2004) Developmental physiology of cluster-root carboxylate synthesis and exudation in harsh hakea. Expression of phosphoenolpyruvate carboxylase and the alternative oxidase. *Plant Physiology* **135**, 549-560.
- Shane, M.W., Dixon, K.W. & Lambers, H. (2006b) The occurrence of dauciform roots amongst Western Australian reeds, rushes and sedges, and the impact of phosphorus supply on dauciform-root development in *Schoenus unispiculatus* (Cyperaceae). *New Phytologist* **165**, 887-898.
- Shane, M.W. & Lambers, H. (2005a) Cluster roots: a curiosity in context. *Plant and Soil* **274**, 101-125.
- Shane, M.W. & Lambers, H. (2005b) Manganese accumulation in leaves of *Hakea prostrata* (Proteaceae) and the significance of cluster roots for micronutrient uptake as dependent on phosphorus supply. *Physiologia Plantarum* **124**, 441-450.
- Shi, J., Strack, D., Albornoz, F., Han, Z. & Lambers, H. (2019) Differences in investment and functioning of cluster roots account for different distributions between *Banksia attenuata* and *B. sessilis*, with contrasting life history. *Plant and Soil* **in press**.
- Silveira, F.O., Negreiros, D., Barbosa, N.U., Buisson, E., Carmo, F., Carstensen, D., Conceição, A., Cornelissen, T., Echternacht, L., Fernandes, G.W., Garcia, Q., Guerra, T., Jacobi, C., Lemos-Filho, J., Le Stradic, S., Morellato, L., Neves, F., Oliveira, R., Schaefer, C., Viana, P. & Lambers, H. (2016) Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant and Soil* **403**, 129-152.

- Taylor, L.L., Leake, J.R., Quirk, J., Hardy, K., Banwart, S.A. & Beerling, D.J. (2009) Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology* **7**, 171-191.
- Van Breemen, N., Finlay, R., Lundström, U., Jongmans, A.G., Giesler, R. & Olsson, M. (2000) Mycorrhizal weathering: a true case of mineral plant nutrition? *Biogeochemistry* **49**, 53-67.
- Van Schöll, L., Kuyper, T.W., Smits, M.M., Landeweert, R., Hoffland, E. & Van Breemen, N. (2008) Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant and Soil* **303**, 35-47.
- Vitousek, P.M. (2004) *Nutrient Cycling and Limitation: Hawaii as a Model System*. Princeton University Press, Princeton.
- Walker, T.W. & Syers, J.K. (1976) The fate of phosphorus during pedogenesis. *Geoderma* **15**, 1-9.
- Wallander, H., Wickman, T. & Jacks, G. (1997) Apatite as a P source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Plant and Soil* **196**, 123-131.
- Watt, M. & Evans, J.R. (1999) Linking development and determinacy with organic acid efflux from proteoid roots of white lupin grown with low phosphorus and ambient or elevated atmospheric CO₂ concentration. *Plant Physiology* **120**, 705-716.
- Zemunik, G., Lambers, H., Turner, B.L., Laliberté, E. & Oliveira, R.S. (2018) High abundance of non-mycorrhizal plant species in severely phosphorus-impooverished Brazilian campos rupestres. *Plant and Soil* **424**, 255-271.
- Zemunik, G., Turner, B.L., Lambers, H. & Laliberté, E. (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants* **1**, 10.1038/nplants.2015.1050.

Table 1. Mineral element concentration (mg g⁻¹) in quartzite rock in *campos rupestres*. The rock samples were collected in Itutinga, Diamantina and Serra do Cipó National Park (n=9), Minas Gerais, Brazil. The values shown are averages and standard deviation (n=9).

Mineral	Si	Ti	Al	Fe	Mn	Mg	Ca	Na	K	P
Element	451 +	0.6 +	7 +	6 +	0.09 +	0.5 +	0.3 +	0.2 +	4.6 +	0.14 +
	8	0.4	4	3	0.05	0.4	0.15	0.10	3.5	0.02

Figure 1. a) *Barbacenia tomentosa* growing in rock outcrops in Itutinga, Minas Gerais, Brazil. The individuals used in greenhouse were collected at this site; **b)** roots of *B. tomentosa* on quartzite rocks; **c)** roots of a Velloziaceae species creating layers in quartzite rock in *campos rupestres* at Serra do Cipó, Minas Gerais, Brazil. Individuals of *B. macrantha* were collected at Serra do Cipó. **d, e, f)** rock slices; the fissures in the rocks represent the tunnels and channels resulting from root activities (some of them are evidenced by the arrows); the weathering process can be viewed from d (how the process starts) until c (thicker layer of roots); the roots can be viewed in the rock slides; **g)** transmission electron microscopic image showing the mineral composition in the quartzite matrix. **h)** The points 1, 2, 3 and 4 had their mineralogical composition analysed. The points 1 and 4 contained only silicon (Si) and oxygen (O). The points 2 and 3 contained phosphorus (P), O, potassium (K), titanium (Ti), yttrium (Y), gadolinium, (Gd), dysprosium (D), sodium (Na); aluminium (Al); manganese (Mn), iron (Fe), potassium (K), and chlorine (Cl).

Figure 2. *Barbacenia tomentosa* and *B. macrantha* roots collected on their natural substrate of quartzite rock; **a)** Roots of *B. tomentosa* showing dense root hairs; **b, c, d)** Vellozioid roots of *B. tomentosa*; **e)** Scanning electron micrograph of the root hairs of *B. tomentosa*; **f)** Scanning electron micrograph of a vellozioid root of *B. macrantha*; **g)** Vellozioid root of *B. tomentosa*; **h)** Distribution of phosphorus (red) in the outermost region of root shown in g.

Figure 3. Histochemical (a-i) and transmission electron microscopic images (j-r) showing dense substances in the epidermal and subepidermal cells, as well as in the root hairs. **a)** Test with ruthenium red, showing the presence of polysaccharides and mucilage in the epidermis and root hairs; **b)** Detail of polysaccharides and mucilage inside the root hairs; **c)** Lipids in the epidermis stained blue; **d)** Presence of lipids inside the root hairs; **e)** Phenols in the wall and in the tips of the root hairs (stained with ferric chloride); **f)** Detail of the bases of the root hairs showing the presence of phenols; **g)** Starch grains accumulated in root cortical cells (stained in dark blue); **h)** Lignin and suberin stained with phloroglucinol – HCL (pink); **i)** Lignin stained in red stained with Maule; **j)** Suberin stained in orange; **k-s)** Transmission electron microscopy of vellozioid roots; **k)** Invaginations of root hairs, increasing their surface area with dense content inside them; **l)** Detail of the vesicles in the root wall; **m)** Detail of the dense content inside the root hairs; **n)** Invaginations of the root hairs with dense substances; **o)** Connection between two root hairs; **p)** Detail of root hair content; **q)** Root hairs releasing their content ; **r)** Thick cell wall in root hair.

Figure 4. Carboxylate concentrations during vellozioid root development in *Barbacenia tomentosa*: immature (2-4 days), mature (6-12 days) and senescent (more than 15 days). There were no significant differences in carboxylate concentrations among the developmental stages.

Figure 5. Solubilized phosphorus (P) from quartzite rock collected in *campos rupestres* as affected by the addition of carboxylates (citrate – black circle – and malate – red square); n=5. Bars represent standard errors.

Figure S1: Light microscopy images of roots of *Barbacenia tomentosa*.