SAMARA ARCANJO E SILVA

ALUMINUM CONCENTRATION, NUTRITIONAL STATUS AND METABOLITE PROFILE IN NATIVE CERRADO SPECIES WITH DIFFERENT RESISTANCE STRATEGIES TO THE METAL

Thesis presented to the Universidade Federal de Viçosa as part of the requirement of the Postgraduate Program in Botany for obtention of *Doctor Scientiae* degree.

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Aristéa Alves Azevedo
(Adviser)

I would like to dedicate this thesis...

To my parents, Maurício and Solange,

To my husband, my great love, Sanzio.

"*Não é sobre chegar no topo do mundo e saber que venceu,*

É sobre escalar e sentir que o caminho te fortaleceu."

(Ana Vilela)

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BIOGRAPHY

SAMARA ARCANJO E SILVA, Brazilian, married, daughter of Maurício Augusto da Silva and Solange de Fátima Arcanjo, was born in Oliveira-MG, on January 12, 1989.

In September 2007, she initiated an undergraduate program in Biological Sciences at Universidade Federal de Lavras. In March 2009, she began her scientific initiation, working for two years in the project "Evaluation of the bioindicator and phytoremediation potential of aquatic plants *Salvinia auriculata* Aulb. and *Thypha angustifolia* L. in the presence of heavy metals cadmium and lead". In the third year of her scientific initiation, she developed the monograph project "Citogenotoxicity and morphophisiological changes in *Pistia stratiotes* L. under cadmium contamination". In July 2011, she obtained her Bachelor's degree in Biological Sciences.

In August 2011, Arcanjo-Silva began her MSc studies in Botany at the Plant Biology Department (DBV/UFV), concluding them in March 2013, with the dissertation entitled "*Borreria verticillata* (Rubiaceae): nutritional characterization and morphophysiological responses to arsenic".

In April 2013, she initiated the doctorate in Botany (DBV/UFV), continuing the research line on plant tolerance to metals, and submitting her thesis on February 22nd, 2017.

INDEX

ABSTRACT

ARCANJO-SILVA, Samara, D.Sc., Universidade Federal de Viçosa, February, 2017. **Aluminum concentration, nutritional status and metabolite profile in native Cerrado species with different resistance strategies to the metal**. Adviser: Aristéa Alves Azevedo. Co-advisers: Adriano Nunes Nesi, Carlos Ernesto Gonçalves Reynaud Schaefer and Cleberson Ribeiro.

Aluminum (Al) is the most abundant metallic element in the Earth crust. Its toxicity not only depends on the total Al concentration in the soil, but also on the Al chemical form, which is highly dependent on soil pH. In acid soils (i.e., having pH lower than 5.5), Al is solubilized and toxic forms like Al^{3+} (exchangeable Al) are then released into the rhizosphere, interfering with root growth and functions and limiting crop productivity. Despite that, natural vegetation that grows on acid soils, such as the ones from the Cerrado, has developed strategies to cope with high Al^{3+} concentrations, but unfortunately, research focusing on the Al resistance mechanisms of plant species therein are still scarce. In this study, we tested the hypothesis that Al resistance strategies are constitutive features and do not depend on the concentration of the metal in soils. For that, we determined the shoot Al concentration and Al deposition sites in plants of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora*, all native Cerrado species naturally growing on acid soils with varying fertilities and metal toxicities. Nutritional and metabolic adaptations of the plants were also analyzed. Aluminum and nutrient concentrations in soil samples were determined by energy dispersive X-ray microfluorescence (µEDXRF), while in plant samples they were evaluated by both inductively coupled plasma atomic emission spectrometry and µEDXRF. Al mapping in plant samples was performed by histochemical test, X-ray probe coupled to scanning electron microscopy, and µEDXRF. Metabolic adaptations were assessed by spectrophotometric analyses and gas chromatography–mass spectrometry. *E. dysenterica* accumulated about 0.5 g Al kg^{-1} DW in the shoot. In contrast, concentrations of the metal in shoots of *Q. parviflora* and *Q. multiflora* were up to 15.0 and 20.0 g Al kg^{-1} DW, respectively, at all collection sites. *Q. parviflora* was able to hyperaccumulate Al even on a soil with negligible Al^{+3} concentration. Pectocellulosic cell walls were the preferential sites for Al deposition, but the metal was also localized in suberized cell walls and in chloroplasts. Al concentration in the species showed different correlations with soil chemical attributes. In *Q. parviflora* and *E.dysenterica,* it was positively correlated with mesotrophic soils while in *Q. multiflora* it was positively correlated with dystrophic ones.

In general, nutrient levels in *E. dysenterica* were lower and more influenced by concentration of total Al in the soil, yet no nutritional deficiency was observed. The levels of K, P, and S in *Q. multiflora* were increased in plants with highest Al accumulation. Metabolite analyses demonstrated that the levels of chlorophyll, nitrate, total amino acids, insoluble proteins, phenols, and thiobarbituric acid-reactive substances were higher in leaves of *E. dysenterica*. In contrast, *Q. parviflora* had higher non-protein thiol concentration and was more efficient in avoiding lipid peroxidation. The synthesis of compatible osmolytes and dehydroascorbate was up-regulated in both species on soils with high metal toxicity. *Q. parviflora* also showed increased levels of malate and succinate. Altogether, these findings confirm the hypothesis that neither the non-accumulator nature of *E. dysenterica* nor the Al-hyperaccumulator nature of both *Qualea* species depends on Al concentration in soils, and support the theory that species adapted to acid soils have mechanisms to cope with Al toxicity and avoid Al-induced nutritional deficiency. *Q. parviflora*, in especial, seems to have mechanisms for altering Al availability in the soil, which enables the species to hyperaccumulate Al even on a soil with negligible Al^{+3} concentration. The results on metabolic adaptations reinforce the hypothesis that phenols, thiols, and organic acids are all involved in the detoxification of Al and reactive oxygen species (ROS) in Al-hyperaccumulator species. On the other hand, the metabolic adaptations involved in ROS scavenging in *E. dysenterica*, such as phenol and dehydroascorbate production, were not sufficient to control oxidative stress in plants growing on soils with high metal toxicity.

RESUMO

ARCANJO-SILVA, Samara, D.Sc., Universidade Federal de Viçosa, Fevereiro de 2017. **Concentração de alumínio, estado nutricional e perfil metabólico em espécies nativas do Cerrado com diferentes estratégias de resistência ao metal**. Orientadora: Aristéa Alves Azevedo. Coorientadores: Adriano Nunes Nesi, Carlos Ernesto Gonçalves Reynaud Schaefer e Cleberson Ribeiro.

O alumínio (Al) é o elemento metálico mais abundante da crosta terrestre. Sua toxidez depende não somente da concentração total de Al no solo, mas também da forma química, a qual é altamente dependente do pH do solo. Em solos ácidos (com pH menor que 5,5), o Al é solubilizado e formas tóxicas como Al^{3+} (Al trocável) são liberadas na rizosfera, interferindo no crescimento e funcionamento das raízes e limitando a produtividade das culturas. Apesar disso, a vegetação natural que cresce em solos ácidos, como os do Cerrado, tem desenvolvido estratégias para lidar com altas concentrações de Al^{3+}, mas, infelizmente, pesquisas focando seus mecanismos de resistência ainda são escassas. Neste estudo nós testamos a hipótese de que as estratégias de resistência ao Al são características constitutivas e não dependem da concentração do metal nos solos. Para isso, foram determinados a concentração e os sítios de deposição de Al na parte aérea de plantas de *Eugenia dysenterica*, *Qualea parviflora* e *Q. multiflora*, espécies nativas do Cerrado crescendo naturalmente em solos ácidos com fertilidade e toxidez de metais variável. As adaptações nutricionais e metabólicas destas plantas tamém foram analizadas. As concentrações de Al e nutrientes nas amostras de solo foram determinadas por microfluorescência de raios-x por energia dispersiva (μFRXED), enquanto que nas amostras vegetais elas foram avaliadas por espectrometria de emissão atômica com plasma indutivamente acoplado e μFRXED. O mapeamento do Al nas amostras vegetais foi realizado por teste histoquímico, sonda de raios-x acoplada à microscopia eletrônica de varredura e μFRXED. As adaptações metabólicas foram avaliadas por análises espectrofotométricas e de cromatografia gasosa com espectrometria de massas. *E. dysenterica* cerca de 0,5 g Al kg^{-1} MS na parte aérea. Em contraste, a concentração do metal na parte aérea de *Q. parviflora* e *Q. multiflora* foi maior que 15,0 e 20,0 g Al kg^{-1} MS, respectivamente, em todos os sítios de coleta. *Q. parviflora* foi capaz de hiperacumular Al mesmo em solo com concentração negligenciável de Al^{+3}. As paredes pectocelulósicas foram os principais sítios de depósito de Al, mas ele também foi localizado em paredes celulares suberificadas e cloroplastos. A concentração de Al nas espécies apresentou

diferentes correlações com os atributos químicos dos solos. Em *Q. parviflora* e *E. dysenterica*, ela foi positivamente correlacionada com solos mesotróficos, e em *Q. multiflora* com solos distróficos. Em geral, os níveis de nutrientes em *E. dysenterica* foram menores e mais influenciados pela concentração de Al total no solo, contudo, não foi observada deficiência nutricional. Os níveis de K, P e S em *Q. multiflora* foram aumentados em plantas com maior acúmulo de Al. As análises metabólicas demonstraram que os níveis de clorofila, nitrato, aminoácidos totais, proteínas insolúveis, fenóis e substâncias reativas com ácido tiobarbitúrico foram maiores em folhas de *E. dysenterica*. Em contraste, *Q. parviflora* teve maior concentração de tióis não proteicos e foi mais eficiente em evitar a peroxidação lipídica. A síntese de osmólitos compatíveis e de desidroascorbato foi aumentada em ambas as espécies em solos com alta toxidez de metais. *Q. parviflora* também apresentou níveis aumentados de malato e succinato. A análise conjunta dos resultados confirma a hipótese de que a natureza não acumuladora de *E. dysenterica* e a hiperacumuladora de Al das espécies de *Qualea* não depende da concentração de Al nos solos e suporta a teoria de que espécies adaptadas a solos ácidos têm mecanismos que as permitem lidar com a toxidez do Al e evitar a deficiência nutricional induzida por este metal. *Q. parviflora*, em especial, parece ter mecanismos que alteram a disponibilidade de Al no solo, o que permite que ela hiperacumule o metal mesmo em solo com concentração negligenciável de Al^{+3}. Os resultados acerca das adaptações metabólicas reforçam a hipótese de que fenóis, tióis e ácidos orgânicos estão envolvidos na destoxificação de Al e espécies reativas de oxigênio (EROs) em espécies hiperacumuladoras de Al. Por sua vez, as adaptações metabólicas envolvidas na eliminação de EROs em *E. dysenterica*, tais como produção de fenóis e desidroascorbate, não foram suficientes para controlar o estresse oxidativo em solos com alta toxidez de metais.

INTRODUCTION

Aluminum (Al) is the most abundant metallic element and ranks third in abundance among the Earth's crust elements, after only oxygen and silicon (Sade et al. 2016). Most soil Al is fixed in minerals or bound to the surface of particles such as oxides and aluminosilicates, both of which are harmless to plants (Grevenstuk and Romano 2013). However, it has been recognized over 100 years ago that the concentrations of soluble Al increase in acid soils (Veitch 1904) and that such soluble Al is toxic to plant growth (Daikuhara 1914). When pH drops below 5.5, aluminosilicate clays and Al hydroxide minerals occurring in alkaline soils release Al-hydroxy cations and soluble mononuclear Al ($Al(H_2O)_6^{3+}$ or simply Al^{3+}) (Simões et al. 2012). The cation Al^{3+} is considered to be one of the most phytotoxic forms of Al (Sade et al. 2016) and its activity can increase up to 1000-fold for every unit decrease in pH (Kopittke and Blamey 2016).

Acid soils, which usually have high content of soluble Al, comprise approximately 3.95 billion ha of the global ice-free land or 40-50% of the world's arable land, about 60% of which is located in the tropics and subtropics (von Uexküll and Mutert 1995; Eswaran et al. 1997). In tropical South America, 85% of soils are acidic, which includes most Brazilian soils. For example, about 30 million ha of lowlands in Brazil, known locally as 'Várzeas', represent a large portion of the acid lowlands of the world that can be brought under cultivation (Fageria and Baligar 2001).

Most of the Brazilian central area is a tropical savanna, the Cerrado, which covers about 205 million ha or 23% of the country. The Cerrado is the second largest phytogeographic domain in Brazil. It also occurs in disjoint areas in the Amazon, Caatinga, and Atlantic Forest (Prance 1996; Olson et al. 2001). Most of its soils are Latosols (46%), Ultisols (15%), and Entisols (15%), usually dystrophic and acidic (pH between 4 and 5), with low natural fertility, high phosphorus (P) fixation capacity, and high saturation of Al, iron (Fe), and manganese (Mn) (Fageria and Stone 1999; Sano et al. 2008). Vegetation is formed by different physiognomies, which compose a heterogeneous landscape constituted of grassland ('campo limpo'), savannas ('campo sujo', 'campo cerrado' and 'cerrado *sensu stricto*') and forest ('cerradão') (Ribeiro and Walter 2008).

Natural acidity in tropical soils is mainly due to continuous weathering during millions of years. As rain water percolates downwards, soluble nutrients such as calcium (Ca), magnesium (Mg), and potassium (K) leach out of the top soil layers, and gradually

gets replaced by Al, Mn, and hydrogen, the elements most closely associated with soil acidity (Pattanayak and Pfukrei 2013). In addition to natural processes, agricultural practices such as removal of products from the farm, leaching of nitrogen below the plant root zone, indiscriminate use of fertilizers and build-up in organic matter contribute to soil acidification and Al solubilization (Sade et al. 2016).

Al toxicity is a major limiting factor to crop productivity in acid soils, and the root is the first organ to be affected. In the presence of Al, root elongation is rapidly inhibited by the Al binding to multiple cellular sites, including cell wall and plasma membrane, and roots become stubby, brittle, and inefficient in absorbing water and nutrients (Kochian et al. 2005; Gupta et al. 2013). Nutritional imbalance is a common symptom in plant species exposed to Al, since it also interferes with the availability and, consequently, uptake, transport and utilization of many mineral elements, especially inhibiting the Ca, Mg and K influx and increasing the N and P uptake (Sade et al. 2016). Shoot symptoms such as purpling of stems, leaves, and leaf veins; rolling of young leaves; and collapse of growing root tips or petioles are easily confused with P and Ca deficiency. Plant stunting and shortening, leaf darkening and maturation delay may also occur (Gupta et al. 2013).

Al-induced physiological changes include reduction in both stomatal opening and chlorophyll concentration, which ultimately interferes with photosynthesis and transpiration (Vitorello et al. 2005; Wang et al. 2006). Thus, Al exposure can modify plant metabolism and change the redox state of cellular components, inducing the production of reactive oxygen species (ROS) and resulting in oxidative stress (Ma et al. 2012; Ribeiro et al. 2012).

Several agronomic strategies have been proposed to manage and improve crop production in acid soils. The most adopted method is increasing soil pH by applying lime (calcium carbonate) or similar compounds, which reduces Al availability. Another strategy is the application of organic matter, which forms Al-organic acid complexes, thus reducing Al solubility (Siecińska and Nosalewicz 2017). Nevertheless, in many cases these soil improvement techniques are not practical due to their relatively high costs and to the fact that they are not efficient for alleviating subsoil acidity (Kochian et al. 2004). In that sense, the use of Al-resistant cultivars has become a promising alternative in the entire world.

Gene encoding membrane transporters and accessory transcription factors, as well as *cis*-elements that enhance gene expression, are all involved in Al resistance in many crop plants (Simões et al. 2012). Major Al resistance genes belong to the Al-activated malate

transporter (ALMT) and multidrug and toxic compound extrusion (MATE) families, being associated with malate and citrate exudation, respectively (Sasaki et al. 2004; Magalhães et al. 2007). More recently, a number of novel Al resistance genes have been identified, including genes encoding an Al^{3+}-specific Nramp transporter in the plasma membrane (Nrat1) and ABC transporters which are involved in not only Al sequestration into the vacuole (ALS1) but also transport of UDP-glucose across the plasma membrane into the cell wall (STAR1/STAR2). Such UDP-glucose transport modifies the carbohydrate composition of root cell walls and reduces Al binding and accumulation in the walls (Liu et al. 2014). The Al-induced expression of Al resistance genes is regulated by *cis*-elements and transcription factors such as STOP1 and ART1 (Simões et al. 2012).

While most of the current knowledge about Al toxicity and tolerance mechanisms is based on crop plant studies and despite the fact that an excellent progress has been made in recent years (for a review, see Kochian et al. 2015), research efforts addressing natural vegetation exposed to high Al availability are still at an exploratory phase, focusing mainly on systematics and Al accumulation patterns (Jansen et al. 2002, 2003). However, understanding the mechanisms by which plants naturally growing on acid soils adapt to high Al availability may contribute with creating efficient strategies to develop Al-resistant cultivars for use in sustainable production systems.

Despite the fact that the expression 'Al resistance' and 'Al tolerance' are often used interchangeably, in this study I use the terminology adopted by Kochian et al. (2015), according to which 'Al resistance' is used to refer to the ability of a plant to maintain reasonable growth and yield on acidic, Al-toxic soils. The mechanisms that confer this resistance are of two types: (i) Al exclusion, in which Al is prevented from entering root cells by means of physical or biochemical barriers – plants that shows these mechanisms are called Al-excluders, and they comprise the majority of plants adapted to acid soils; and (ii) Al tolerance, in which Al enters the plant and is detoxified and sequestered, thus preventing it from interacting with the sensitive components of the cell (Brunner and Sperisen 2013; Kochian et al. 2015) – which occurs in a small number of Al-accumulator plants (Jansen et al. 2003; Grevenstuk and Romano 2013).

The main strategies that contribute to Al exclusion are: Al complexation with organic acids (especially citrate, malate, and oxalate) and other compounds (e.g. mucilage and phenols) exuded by root tips in the rhizosphere, which raises the rhizosphere pH;

modification of Al^{3+}-binding sites in the walls of root cells (Brunner and Sperisen 2013; Siecińska and Nosalewicz 2017); and even exclusion of Al absorbed Al by specific Al transporters (Hartwig et al. 2007). On the other hand, Al accumulation seems to depend on the formation of less toxic organic Al complexes that prevent the contact between free Al and essential biochemical processes (Grevenstuk and Romano 2013). Organic acids and phenolic compounds are major ligands for Al detoxification, and cell walls and vacuoles are the main storage sites of the accumulated metal (Vázquez et al. 1999; Tolrà et al. 2011; Li et al. 2014). The high affinity of Al with pectocellulosic cell walls is due to the large number of free carboxyl groups in pectins and hemicelluloses (Gao et al. 2014), which can immobilize about 90% of the total Al accumulated in the cell (Chang et al. 1999).

Al-accumulator plants are often woody species from tropical regions, having evolved independently in unrelated botanical families, such as Melastomataceae, Rubiaceae, and Vochysiaceae (Jansen et al. 2002). Those species that accumulate more than 1 g Al kg^{-1} DW in leaves are considered Al-hyperaccumulators; some even seem to depend on Al for their development, and are thus called aluminophiles, such as *Vochysia thyrsoidea* (Vochysiaceae) and *Miconia albicans* (Melastomataceae), both native species from the Cerrado (Haridasan 2008). However, little is known about Al resistance in native Cerrado species. Most of the current knowledge on Al accumulation in plants is based on studies with tea (*Camellia sinensis*), buckwheat (*Fagopyrum esculentum*), and *Hydrangea macrophylla* (Kochian et al. 2015). Additionally, the Al behavior in organs of these species differs markedly from that in organs of non-accumulator species (Ma 2000).

Eugenia dysenterica DC. (Myrtaceae), popularly known as “cagaita” or “cagaiteira”, is a native fruit tree from the Cerrado and a non-Al-accumulator species. Its stem is tortuous and has a thick, fissured phellem; fruits are of the berry type, globose and yellow, and they may be consumed either fresh or processed. Leaves, fruits and bark have medicinal proprieties and the wood is utilized for small constructions and charcoal production (Silva et al. 2001). *Qualea parviflora* Mart. and *Q. multiflora* Mart. (Vochysiaceae) are two Al-hyperaccumulators popularly known as “pau-terra roxo” and “pau-terra liso”, respectively. They are native deciduous trees from the Cerrado and bear paired extrafloral nectaries on the stem, at the region near leaf insertion and at the bud base. Fruits are loculicidal capsules and bear wind-dispersed samaroid seeds (Del-Claro et al. 1996; Palermo and Miranda 2012).

E. dysenterica, *Q. parviflora*, and *Q. multifora* are widely found at the National Forest (FLONA) of Paraopeba, southeastern Brazil (Neri et al. 2012). This FLONA is a Sustainable-Use Conservation Unit that covers a 200-ha area of Cerrado (SNUC 2000) with a well-defined soil–vegetation gradient determined by soil fertility and Al concentration in the soil. The site has areas of dystrophic and mesotrophic cerradão on Red Latosol and of cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic, Yellow Latosol, and Red-Yellow Latosol (Neri et al. 2012).

Thus, this study aimed to evaluate (i) whether soil chemical features determine the concentrations of Al and nutrients in shoots of *E. dysenterica*, *Q. parviflora*, and *Q. multiflora*; (ii) whether the Al immobilization sites are related to plant capacity of Al accumulation; and (iii) how metabolic responses of plants naturally growing on acid soils can contribute to the their Al resistance. For such, shoot Al concentrations and deposition sites as well as the nutritional and metabolic responses of plants naturally growing on acid soils with different Al concentrations and availabilities were assessed.

The following chapters presented in this thesis were edited based on the format requirements of specific journals (indicated in each chapter) and adapted to the norms for thesis elaboration adopted by the Universidade Federal de Viçosa.

REFERENCES

Brunner I, Sperisen C, 2013. Aluminum exclusion and aluminum tolerance in woody plants. *Front Plant Sci* 4:1-12.

Chang YC, Yamamoto Y, Matsumoto H, 1999. Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. *Plant Cell Environ* 12:1009-1017.

Daikuhara G, 1914. *Ueber saure mineral boden.* Bulletin of the Imperial Central Agricultural Experiment Stations Japan, Tokyo, Japan. 18p.

Del-Claro K, Berto V, Réu W, 1996. Effect of herbivore deterrence by ants on the fruit set of an extrafloral nectary plant, *Qualea multiflora* (Vochysiaceae). *J Trop Ecol* 12:887-892.

Eswaran H, Reich P, Beinroth F, 1997. Global distribution of soils with acidity. In: AC Moniz (ed), *Plant–soil interactions at low pH*. Brazilian Soil Science Society, São Paulo, Brazil, pp 159–164.

Fageria NK, Stone LF, 1999. *Acidity management of cerrado and varzea soils of Brazil*. Embrapa Arroz e Feijão, Document No. 92, Santo Antônio de Goiás, Brazil.

Fageria NK, Baligar VC, 2001. Improving nutrient use efficiency of annual crops in Brazilian acid soils for sustainable crop production. *Commun Soil Sci Plant Anal* 32(7-8):1303–1319.

Gao HJ, Zhao Q, Zhang XC, Wan XC, Mao JD (2014) Localization of fluoride and aluminum in subcellular fractions of tea leaves and roots. J Agr Food Chem 62: 2313–2319.

Grevenstuk T, Romano A, 2013. Aluminium speciation and internal detoxification mechanisms in plants: where do we stand?. *Metallomics* 5:1584-1594.

Gupta N, Gaurav SS, Kumar A, 2013. Molecular basis of aluminium toxicity in plants: a review. *Am J Plant Sci* 4:21-37.

Haridasan M, 2008. Alumínio é um elemento tóxico para as plantas nativas do cerrado?. In: CHBA Prado, CA Casali (eds), *Fisiologia Vegetal: práticas em relações hídricas, fotossíntese e nutrição mineral.* Editora Manole, Barueri, Brasil, pp 1-10.

Hartwig I, Oliveira AC, Carvalho FIF, Bertan I, Silva JAG, Schmidt DAM, Valério IP, Maia LC, Fonseca DAR, Reis CES, 2007. Mecanismos associados à tolerância ao alumínio em plantas. *Semina: Ciênc Agrar* 28:219–228.

Jansen S, Broadley M, Robbrecht E, Smets E, 2002. Aluminum hyperaccumulation in angiosperms: a review of its phylogenetic significance. *Bot Rev* 68:235–269.

Jansen S, Watanabe T, Dessein S, Smets E, Robbrecht E, 2003. A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. *Ann Bot* 91:657–663.

Kochian LV, Piñeros MA, Hoekenga OA, 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu Rev Plant Biol* 55:459–493.

Kochian LV, Piñeros MA, Hoekenga OA, 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil* 274:175–195.

Kochian LV, Piñeros MA, Liu J, Magalhães JV, 2015. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annu Rev Plant Biol* 66:23.1–23.28.

Kopittke PM, Blamey FPC, 2016. Theoretical and experimental assessment of nutrient solution composition in short-term studies of aluminium rhizotoxicity. *Plant Soil* 406(1): 311-326.

Li J-Y, Liu J, Dong D, Jia X, McCouch SR, Kochian LV, 2014. Natural variation underlies alterations in Nramp aluminum transporter (NRAT1) expression and function that play key role in rice aluminum tolerance. *Proc Natl Acad Sci* 111(17):6503-6508.

Liu J, Piñeros MA, Kochian LV, 2014. The role of aluminum sensing and signaling in plant aluminum resistance. *J Integr Plant Biol* 56(3):221-230.

Ma JF, 2000. Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiol* 41:383–390.

Ma B, Gao L, Zhang H, Cui J, Shen Z, 2012. Aluminum-induced oxidative stress and changes in antioxidant defenses in the roots of rice varieties differing in Al tolerance. *Plant Cell Rep* 31:687-696.

Magalhães JV, Liu J, Guimarães CT, Lana UGP, Alves VMC, Wang YH, Schaffert RE, Hoekenga OA, Pineros MA, Shaff JE, Klein PE, Carneiro NP, Coelho CM, Trick HN, Kochian LV, 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nat Genet* 39:1156–1161.

Neri AV, Schaefer CEGR, Silva AF, Souza AL, Ferreira-Júnior WG, Meira-Neto JAA, 2012. The influence of soils on the floristic composition and community structure of an area of Brazilian Cerrado vegetation. *Edinb J Bot* 69:1-27.

Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR, 2001. Terrestrial Ecoregions of the World: A New Map of Life on Earth. *BioScience* 51:933-938.

Palermo AC, Miranda HS, 2012. Efeito do fogo na produção de frutos de *Qualea parviflora* Mart. (Vochysiaceae) em cerrado sensu stricto. *Rev. Árvore* 36(4):685-693.

Pattanayak A, Pfukrei K, 2013. Aluminium toxicity tolerance in crop plants: Present status of research. *Afr J Biotechnol* 12(24):3752-3757.

Prance GT, 1996. Islands in Amazonia. *Philos Trans R Soc Lond B Biol Sci* 351:823-833.

Ribeiro JF, Walter BMT, 2008. As principais fitofisionomias do bioma Cerrado. In: SM Sano, SP Almeida, JF Ribeiro (eds), *Cerrado: ecologia e flora*. Embrapa Cerrados, Planaltina, Brasil. pp 152-212.

Ribeiro C, Cambraia J, Peixoto PHP, Fonseca Júnior EM, 2012. Antioxidant system response induced by aluminum in two rice cultivars. *Braz J Plant Physiol* 24:107-116.

Sade H, Meriga B, Surapu V, Gadi J, Sunita MSL, Suravajhala P, Kishor PBK, 2016. Toxicity and tolerance of aluminum in plants: tailoring plants to suit to acid soils. *Biometals* 29(2):187-210.

Sano SM, Almeida SP, Ribeiro JF, 2008. *Cerrado: ecologia e flora*. Embrapa Cerrados, Planaltina, Brasil. 408p.

Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, Ryan PR, Delhaize E, Matsumoto H, 2004. A wheat gene encoding an aluminumactivated malate transporter. *Plant J* 37:645–653.

Siecińska J, Nosalewicz A, 2017. Aluminium toxicity to plants as influenced by the properties of the root growth environment affected by other co-stressors: a review. In: P de Voogt (ed), *Reviews of Environmental Contamination and Toxicology*. Springer Press, New York, USA. pp 1-26.

Silva RSM, Chaves LJ, Naves RV, 2001. Caracterização de frutos e árvores de cagaita (*Eugenia dysenterica* DC.) no sudeste do estado de Goiás, Brasil. *Rev Bras Frutic* 23(2):330-334.

Simões CC, Melo JO, Magalhães JV, Guimarães CT, 2012. Genetic and molecular mechanisms of aluminum tolerance in plants. *Genet Mol Res* 11:1949–1957.

SNUC – Sistema Nacional de Unidades de Conservação da Natureza, 2000. Lei nº 9.985, de 18 de julho de 2000. MMA/SBF.

Tolrá R, Vogel-Mikus K, Hajiboland R, Kump P, Pongrac P, Kaulich B, Gianoncelli A, Babin V, Barceló J, Regvar M, Poschenrieder C, 2011. Localization of aluminium in tea (*Camellia sinensis*) leaves using low energy X-ray fluorescence spectro-microscopy. *J Plant Res* 124:165–172.

Vázquez MD, Poschenrieder C, Corrales I, Barceló J, 1999. Change in apoplastic aluminum during the initial growth response to aluminum by roots of a tolerant maize variety. *Plant Physiol* 119:435–444.

Veitch FP, 1904. Comparison of methods for the estimation of soil acidity. *J Am Chem Soc* 26:637–662.

Vitorello VA, Capaldi FR, Stefanuto VA, 2005. Recent advances in aluminum toxicity and resistance in higher plants. *Braz J Plant Physiol* 17:129-143.

von Uexküll HR, Mutert E, 1995. Global extent, development and economic impact of acid soils. *Plant Soil* 171:1-15.

Wang J, Raman H, Zhang G, Mendham N, Zhou M, 2006. Aluminum tolerance in barley (*Hordeum vulgare* L.): physiological mechanisms, genetics and screening methods. *J Zhejiang Univ Sci B* 7:769-787.

CHAPTER 1 – Do soil chemical features determine the concentrations of aluminum and nutrients in shoots of native Cerrado plants?[(1)]

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ABSTRACT: *Aims* The ability to control aluminum (Al) and nutrient accumulation is essential for plant Al resistance on acid soils. However, little information is available regarding Cerrado species, which are naturally resistant to the metal. We studied the relationship between soil chemical attributes and the Al and nutrient levels in shoots of *Eugenia dysenterica* (non-accumulator), *Qualea parviflora*, and *Q. multiflora* (Al-hyperaccumulators) growing on different soils at the Cerrado. *Methods* Al and nutrient concentrations in plant and soil samples were determined by inductively coupled plasma atomic emission spectrometry and energy dispersive X-ray microfluorescence. *Results* Al accumulation was lower in *E. dysenterica* shoot (0.42 g kg^{-1} DW) than in *Q. parviflora* and *Q. multiflora* ones (16.84 and 23.63 g kg^{-1} DW, respectively), and few differences were observed among collection sites. Al concentration was positively correlated with mesotrophic soils in *Q. parviflora* and *E. dysenterica* and with dystrophic ones in *Q. multiflora*. *Q. multiflora* plants with higher Al accumulation also showed higher K, P, and S levels. Nutritional deficiency was not observed. *Conclusions* Al hyperaccumulation by *Qualea* spp. on soils with varying Al concentrations suggests that they can alter the availability of this metal in the soil. Data on plant nutritional status reinforce that nutrient absorption by species adapted to acid soils is not adversely affected by soil Al concentration.

Keywords: *Eugenia dysenterica*, *Qualea parviflora*, *Q. multiflora*, Al accumulation, nutritional status, soil chemical attributes

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CAPÍTULO 1 – Características químicas do solo determinam a concentração de alumínio e nutrientes na parte aérea de plantas nativas do Cerrado?

RESUMO: A habilidade de controlar o acúmulo de alumínio (Al) e nutrientes é essencial para a resistência de plantas ao Al em solos ácidos. No entanto, há pouca informação disponível sobre as espécies de Cerrado, as quais são naturalmente resistentes ao metal. Nós estudamos a relação entre os atributos químicos do solo e a concentração de Al e nutrientes na parte aérea de *Eugenia dysenterica* (não acumuladora), *Qualea parviflora* e *Q. multiflora* (hiperacumuladoras de Al) crescendo em diferentes solos no Cerrado. As concentrações de Al e nutrientes nas amostras vegetais e de solo foram determinadas por meio de espectrometria de emissão atômica com plasma indutivamente acoplado e microfluorescência de raios-x por energia dispersiva. O acúmulo de Al foi menor nas partes aéreas de *E. dysenterica* (0,42 g kg^{-1} MS) do que em *Q. parviflora* e *Q. multiflora* (16,84 e 23,63 g kg^{-1} MS, respectivamente) e poucas diferenças foram observadas entre os sítios de coleta. A concentração de Al foi positivamente correlacionada com solos mesotróficos em *Q. parviflora* e *E. dysenterica* e com solos distróficos em *Q. multiflora*. As plantas de *Q. multiflora* com maior acúmulo de Al apresentaram maiores níveis de K, P e S. Deficiências nutricionais não foram observadas. O hiperacúmulo de Al pelas *Qualea* spp. em solos com concentrações variáveis de Al sugere que elas podem alterar a disponibilidade do metal no solo. Os dados de estado nutricional reforçam que a absorção de nutrientes por espécies adaptadas a solos ácidos não é afetada adversamente pela concentração de Al no solo.

Palavras-chave: *Eugenia dysenterica*, *Qualea parviflora*, *Q. multiflora*, acúmulo de Al, estado nutricional, atributos químicos do solo

Introduction

The Cerrado is the second largest phytogeographic domain in Brazil, covering several states of its central area as well as several disjoint areas within the Amazon, Caatinga, and Atlantic Forest (Prance 1996; Olson et al. 2001). Plant features and distribution in the Cerrado are influenced by climate, soil, water availability, and fire occurrence (Ribeiro and Walter 2008). Soils therein are usually dystrophic and acidic (pH between 4 and 5), with high concentrations of exchangeable aluminum (Al^{3+}), manganese (Mn), and iron (Fe) (Kochian et al. 2004; Sano et al. 2008). Cerrado vegetation is composed of different physiognomies, which form a heterogeneous landscape constituted of grassland (campo limpo), savannas (campo sujo, campo Cerrado, and cerrado *stricto sensu*), and forest (cerradão) (Ribeiro and Walter 2008).

The National Forest of Paraopeba (FLONA of Paraopeba) at Minas Gerais state (southeastern Brazil) is a Sustainable Use Conservation Unit that covers an area of 200 ha of Cerrado (SNUC 2000). The soil–vegetation gradient therein has five classes with rather distinct characteristics, ranging from the upper landsurface closest to the calcareous Dry Forest outside the FLONA to the colluvial bottom, where the deepest Latosols occur. Soil fertility and Al concentration are two of the most important edaphic factors responsible for structural and floristic variation in the Cerrado (Neri et al. 2012).

Resistance strategies render species that grow on acid soils able to avoid Al uptake (Al-excluders) or even tolerate high internal concentration of the metal due to efficient detoxification (Al-accumulators) (Vitorello et al. 2005). Species that accumulate very high Al concentrations in leaves (more than 1 g kg^{-1} DW) are called Al-hyperaccumulators, and some of them seem even to depend on Al for their development, such as *Vochysia thyrsoidea* and *Miconia albicans*, both of which are considered aluminophile species (Haridasan 2008).

It is well known that Al-accumulator species do not occur exclusively on acid soils. *Qualea* species, for example, are indifferent to soil acidity and fertility, occurring not only in dystrophic and mesotrophic Cerradão (forest physiognomy) but also in Cerrado *sensu stricto* (savanna) at the FLONA of Paraopeba (Neri et al. 2012). Other species, however, occur exclusively on Ca-rich soils with low Al saturation, accumulating Al even under those conditions, like *Callisthene fasciculata* (Haridasan and Araújo 1988). Despite the large number of studies on Al phytotoxicity, the relationship between the soil concentration

of this metal and the amount of it that is accumulated by plants still remains unclear. Divergences on that subject are observed even in tea (*Camellia sinensis*), one of the most studied Al-accumulator species. Some authors believe that Al uptake by *C. sinensis* is affected by soil condition and Al saturation, resulting in variable Al concentrations in plants growing on different types of soils (Wong et al. 1998; Ruan et al. 2006). However, recent studies have shown that there is no significant correlation between Al concentrations in tea leaves and topsoil exchangeable Al concentration (de Silva et al. 2016).

Nutritional imbalances induced by Al exposure have been reported to several plant species. High Al^{3+} concentration promotes soil impoverishment by reducing nutrient availability and may alter the uptake, transport and utilization of most mineral elements by plants (Sade et al. 2016), as reported to calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) (Giannakoula et al. 2008), and anions such as phosphate (PO_4^{3-}) (González-Santana et al. 2012) and nitrate (NO^{3-}) (Metali et al. 2012). However, despite the negative effects of Al, species naturally growing on Cerrado soils have mechanisms that enable them to survive in those conditions, and neither Al absorption nor its translocation seem to interfere with the uptake and metabolism of nutrients like Ca, K, Mg, and P in these species (Haridasan 1982; Medeiros and Haridasan 1985). This ability to control internal nutrient levels is an important component of plant resistance to Al (Mariano and Keltjens 2005; Giannakoula et al. 2008; Serrano et al. 2011). Nevertheless, little information is available regarding the nutritional responses of Cerrado species, both Al-accumulators and non-accumulators, to acid soils.

Thus, we tested whether Al concentration in soils affects the nutrient levels in shoots of three native species from the Brazilian Cerrado, namely *Eugenia dysenterica* (non-accumulator, Myrtaceae), *Qualea parviflora*, and *Q. multiflora* (both Al-hyperaccumulators, Vochysiaceae), and whether Al accumulation in their shoots is correlated with soil chemical attributes.

Material and Methods

Study area

The study was performed in the Cerrado National Reserve at Paraopeba (FLONA of Paraopeba), Minas Gerais state, southeastern Brazil (19°16'S; 44°23'W) (SNUC 2000). The FLONA shows a well-marked soil-vegetation gradient, with phytophysiognomies

ranging from savanna (Cerrado *sensu stricto*), which occurs on dystrophic soils, to forest (Cerradão) on both mesotrophic and dystrophic soils (Neri et al. 2013). The collection sites were Dystrophic Cerradão on Red Latosol (DC-RL), Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic (C*ss*-HCD), Cerrado *sensu stricto* on Yellow Latosol (C*ss*-YL), Dense Cerrado *sensu stricto* on Red-Yellow Latosol (C*ss*-RYL), and Mesotrophic Cerradão on Red Latosol (MC-RL) (Neri et al. 2012).

Plant and soil sampling

For that purpose, we assessed the concentration and partitioning of Al and nutrients in shoots of plants collected from natural populations growing on soils with different concentrations of the metal, and performed analyses of correlation between soil total Al concentration and the Al and nutrient concentration in shoots, as well as between Al accumulation and soil chemical features. Samples of stems in secondary growth and fully expanded leaves were collected from five individuals of *Eugenia dysenterica* DC. (Myrtaceae), *Qualea parviflora* Mart., and *Q. multiflora* Mart. (Vochysiaceae) having height above 2 m, selected in plots of 20 x 100 m established by Tolentino (2011) and Neri et al. (2012) at each collection site. Samples of *E. dysenterica* were collected at all sites, while those of *Q. parviflora* were collected at MC-RL, C*ss*-RYL, C*ss*-YL, and C*ss*-HCD and those of *Q. multiflora* were collected at C*ss*-RYL, C*ss*-HCD, and DC-RL. Ten soil samples were randomly collected near the selected plants, at depths of 0.0-0.2 m.

Soil chemical analysis

Soil samples were sieved through a 200-mesh (74 μm) stainless steel sieve, and pH, concentration of Al^{3+}, acidity (H+Al), and sum of bases (SB) were determined following the method described by Embrapa (1997). Sieved samples were used to prepare pellets (0.3 g cm^{-2}) using a hydraulic press (9 t cm^{-2} during 1 min; Perkin Elmer, Waltham, MA, USA). On each pellet an area of 4 x 3 mm (1200 measurement points; 100-μm step size) was analyzed using a benchtop X-ray fluorescence spectrometer (μ-EDX-1300, Shimadzu, Kyoto, Japan) with an X-ray source of Rh tube and a Si(Li) semiconductor fluorescence detector. Quantification of chemical elements (described in Table 1) was based on the Fundamental Parameters method (Quantitative - FP), and certified samples (Soil Montana

II, NIST 2711a and BHVO-2 Basalt, USGS) were used to adjust the sensitivity coefficients of each analyzed element, as suggested by Alves et al. (2015).

Determination of Al and nutrients in plants

Stem and leaf samples were oven-dried at 70 °C, powdered in a knife mill and sieved through a 200-mesh (74 μm) stainless steel sieve. The sieved material was accurately weighed (ca. 250 mg) and digested with nitric and hydrofluoric acid in closed digestion tubes on graphite block (adapted from Paye 2014). The resulting solutions were cooled down to room temperature and their final volume was completed to 12 mL with deionized water. Al, Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP AES; Optima 8300 DV, Perkin Elmer, Shelton, CT, USA). For certification of the digestion method and of elemental quantification by ICP AES, a reference sample (bush leaves, GBW 07603) and analytical blanks were prepared in the same way as samples in each digestion batch.

Statistical analyses

Differences in chemical soil attributes and in the Al and nutrient concentrations in shoots of plants from different collection sites were tested by one- and two-way (3 species x 5 collection sites) analysis of variance (ANOVA) respectively, using software Sisvar (Ferreira 2011). Means were compared by Tukey test at the 0.05 significance level. Clustering of soil samples was evaluated by principal component analysis (PCA), and canonical correspondence analysis (CCA) was performed to estimate the correlation between soil features and Al accumulation in shoots of the species (Ter Braak 1987). Monte Carlo permutation was employed to assess the significance level of results given by the main axis of the canonical ordination (Ter Braak 1988, 1994). Software PC-ORD version 6.0 was used to perform the analyses and generate the CCA ordination axes (McCune and Mefford 2006). Pearson linear correlation was performed to assess the correlation between the concentrations of total and exchangeable Al in soils and the Al and nutrient concentrations in shoots of the species.

Results

Chemical attributes of soils

Soil chemical attributes differed among collection sites (Table 1). The MC-RL soil showed the highest values of pH (6.56), sum of bases, and base saturation, yet a negligible concentration of Al^{3+}. On the other hand, soils from the other collection sites had high values of acidity and Al saturation. Total Al concentration was highest at DC-RL, followed by C*ss*-HCD, and lowest at C*ss*-RYL ($p < 0.001$). The MC-RL soil showed an intermediate value of total Al concentration, similarly to the C*ss*-YL soil. Fe and Mn concentrations showed the same trends, of highest values in DC-RL and C*ss*-HCD soils. In contrast, Si and Zn concentrations were higher in MC-RL, C*ss*-RYL, and C*ss*-YL soils than in DC-RL and C*ss*-HCD ones. Concentration of S was higher in more closed phytophysiognomies (DC-RL, C*ss*-RYL, and MC-RL) than in open ones (C*ss*-YL and C*ss*-HCD), unlike K concentration, which was higher in the C*ss*-HCD soil than in soils from the other plant formations. The DC-RL soil showed the lowest Ca concentration ($p < 0.001$) and the MC-RL soil showed the highest P concentration ($p < 0.05$). Mg and Cu concentrations did not differ significantly among soils from the collection sites ($p = 0.89$ and 0.21, respectively).

Table 1 Chemical attributes of the surface layer (0.0-0.2 m) of Cerrado soils from the National Forest of Paraopeba, southeastern Brazil

Soil attributes	MC-RL	C*ss*-RYL	C*ss*-YL	C*ss*-HCD	DC-RL	p-value
pH (H_2O)	6.59±0.06 A	5.03±0.05 B	5.02±0.05 B	4.96±0.04 B	5.00±0.02 B	***
Al^{3+} ($cmol_c\ dm^{-3}$)	0.00±0.00 C	2.36±0.10 B	2.26±0.17 B	2.71±0.06 A	2.01±0.06 B	***
H+Al ($cmol_c\ dm^{-3}$)	3.06±0.31 D	8.79±0.21 AB	7.40±0.18 C	7.71±0.21 BC	9.78±0.15 A	***
SB ($cmol_c\ dm^{-3}$)	12.27±0.31 A	1.60±0.15 B	1.51±0.25 B	1.05±0.11 B	1.30±0.11 B	***
V (%)	80.21±1.96 A	14.93±1.07 B	16.46±2.44 B	11.74±0.95 B	11.55±0.83 B	***
m (%)	0.00±0.00 B	60.53±3.05 A	61.19±5.71 A	72.86±2.04 A	61.48±2.58 A	***
Al_2O_3 ($dag\ kg^{-1}$)	20.48±0.38 C	18.50±0.19 D	21.01±0.21 C	24.78±0.14 B	29.94±0.37 A	***
SiO_2 ($dag\ kg^{-1}$)	60.87±0.46 A	61.08±0.24 A	60.33±0.39 A	51.52±0.29 B	44.18±0.70 C	***
CaO ($dag\ kg^{-1}$)	0.34±0.05 A	0.29±0.05 A	0.19±0.01 AB	0.22±0.00 A	0.04±0.00 B	***
K_2O ($dag\ kg^{-1}$)	1.10±0.02 D	1.26±0.02 C	2.12±0.03 B	2.61±0.02 A	0.48±0.04 E	***
MgO ($dag\ kg^{-1}$)	1.52±0.04 A	1.62±0.04 A	1.48±0.12 A	1.51±0.08 A	1.54±0.04 A	ns
P_2O_5 (ppm)	684.23±122.70 A	385.34±80.85 AB	251.57±95.47 AB	270.20±75.59 AB	218.77±66.47 B	*
SO_3 ($dag\ kg^{-1}$)	0.03±0.00 ABC	0.04±0.01 AB	0.02±0.00 BC	0.01±0.00 C	0.04±0.00 A	**
Fe_2O_3 ($dag\ kg^{-1}$)	6.96±0.12 D	5.87±0.07 E	8.22±0.14 C	11.69±0.12 B	13.11±0.30 A	***
ZnO (ppm)	86.47±4.10 A	91.62±3.93 A	71.79±3.28 B	60.47±1.82 BC	54.08±2.98 C	***
MnO (ppm)	106.08±13.75 B	76.26±7.08 B	77.93±9.11 B	232.30±16.91 A	266.87±12.21 A	***
CuO (ppm)	138.20±3.61 A	132.67±3.60 A	134.14±3.68 A	137.08±3.09 A	146.55±4.08 A	ns

Means ± standard error (n=10). Different letters indicate significant difference (Tukey test). H+Al = acidity; SB = sum of bases; V = base saturation; m = Al saturation; ns: non-significant; *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; DC-RL: Dystrophic Cerradão on Red Latosol.

In the PCA on soil samples, 71.49% of the cumulative variance was explained by the first and second axes, which had eigenvalues of 6.954 and 3.055, respectively. The ordination diagram separated soils in two well-defined groups, one formed by soils samples from MC-RL, correlated with higher pH, BS, V, and concentrations of Ca, P and Si; and another formed by samples from C*ss*-HCD and DC-RL, correlated with higher acidity, Al saturation, and concentrations of total and exchangeable Al, Fe, and Mn. Soil samples from C*ss*-RYL and C*ss*-YL showed intermediate features between these two groups (Fig. 1).

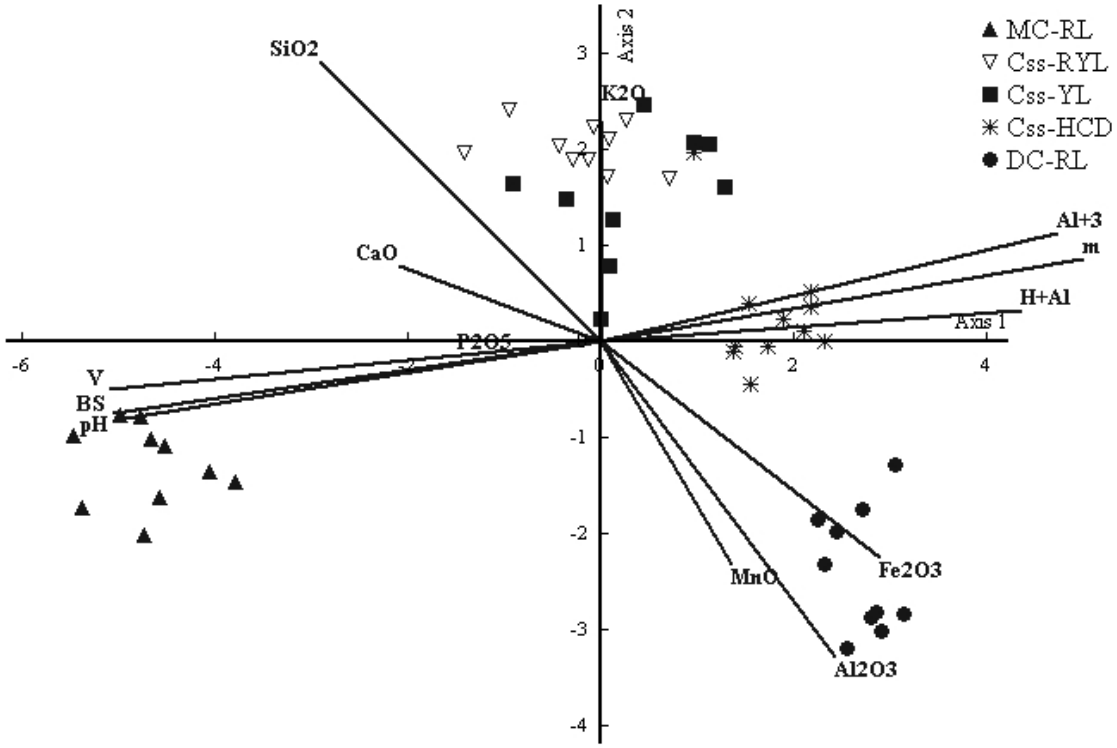

Fig. 1 Ordination diagram of Cerrado soil samples from the National Forest of Paraopeba, southeastern Brazil. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; DC-RL: Dystrophic Cerradão on Red Latosol.

Aluminum accumulation by plants

Concentration of Al in plants showed few significant differences among collection sites (Table 2). In *E. dysenterica*, mean Al concentration in the stem and leaves was 0.21 g kg^{-1} DW, averaging 0.42 g kg^{-1} DW in shoots (stem + leaves), which did not differ among

collection sites. In contrast, *Q. parviflora* accumulated on average 3.12 and 13.46 g Al kg^{-1} DW in the stem and leaves, respectively. Only stems showed difference among collection sites, with highest Al concentrations in C*ss*-HCD plants. *Q. multiflora* had 3.08 and 19.79 g Al kg^{-1} DW in its stem and leaves, respectively. The Al concentration in stems of this species did not differ among collection sites; however, in leaves it was highest in C*ss*-RYL plants. In summary, Al accumulation in shoots of the species followed the sequence *Q. multiflora* > *Q. parviflora* > *E. dysenterica* and did not differ among collection sites, except for *Q. multiflora*, which showed highest concentration at C*ss*-RYL ($p < 0.001$).

Nutrient concentration in plants

In general, the concentrations of macro and micronutrients in shoots differed among plants from different collection sites (Tables 2 and 3, respectively). *E. dysenterica* showed higher nutrient levels, especially of K, Mg, Mn, and S, at DC-RL. *Q. parviflora* plants from MC-RL showed the highest levels of K and P, while those from C*ss*-RYL showed higher Mg concentration; Mn and Zn, on the other hand, were more concentrated in plants from C*ss*-HCD. *Q. multiflora* plants from C*ss*-RYL showed the highest mean concentrations of K, P, and S, whereas those from DC-RL accumulated the highest Cu and Mn levels. Unlike Ca, concentrations of K, Mn, P, and S differed among collection sites. In the cases where nutrient accumulation differed among species in the same collection site, concentrations were overall lower in *E. dysenterica* than in both *Qualea* species, especially when nutrient levels in stems and leaves were considered separately. *E. dysenterica* showed highest concentrations only of Mn in leaves, on all soils where there was significant difference, and of P and S in shoots of MC-RL plants (Tables 2 and 3).

Table 2 Aluminum and macronutrient concentration (g kg^{-1} DW) in stems, leaves, and shoots (stem + leaves) of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora* collected on Cerrado soils with different Al concentrations in the National Forest of Paraopeba, southeastern Brazil

Species	Collection sites	Al	Ca	K	Mg	P	S
STEM							
E. dysenterica	MC-RL	0.16±0.03 Ab*	5.98±0.50 Aa	6.10±0.40 Ab	0.35±0.03 Ab	18.37±1.42 Aa	2.03±0.13 ABa
	Css-RYL	0.11±0.02 Ab	8.35±1.27 Aa	5.41±0.65 Ab	0.67±0.09 Ab	11.68±0.99 ABCb	1.99±0.18 ABa
	Css-YL	0.23±0.06 Ab	4.73±0.28 Aa	5.92±0.34 Aa	0.88±0.10 Ab	6.03±0.64 BCa	2.68±0.21 Aa
	Css-HCD	0.17±0.02 Ac	6.09±0.56 Aa	5.05±0.34 Aa	0.93±0.12 Ab	4.80±0.27 Ca	1.87±0.12 Ba
	DC-RL	0.31±0.06 Ab	4.72±0.25 Aa	8.91±3.15 Aa	1.19±0.10 Aa	13.85±5.23 ABa	2.25±0.08 ABa
Q. parviflora	MC-RL	1.91±0.09 Ca	5.13±0.27 Aa	12.53±2.26 Aa	1.37±0.08 Ba	15.91±2.30 Aa	1.59±0.10 Aa
	Css-RYL	3.48±0.42 ABa	5.92±0.77 Aa	8.26±0.86 ABb	2.36±0.25 Aa	10.66±1.56 ABb	1.41±0.07 Ab
	Css-YL	2.67±0.12 BCa	5.38±0.36 Aa	7.87±0.56 ABa	1.81±0.10 ABa	6.11±0.34 Ba	1.56±0.11 Ab
	Css-HCD	4.43±0.30 Aa	5.60±0.49 Aa	6.69±0.28 Ba	2.36±0.23 Aa	5.56±0.38 Ba	1.37±0.06 Aab
Q. multiflora	Css-RYL	3.61±0.53 Aa	7.55±0.62 Aa	14.01±2.63 Aa	2.11±0.22 Aa	24.97±2.34 Aa	1.96±0.12 Aa
	Css-HCD	2.29±0.20 Ab	5.43±0.48 Aa	6.54±0.43 Ba	2.38±0.11 Aa	4.91±0.18 Ba	1.30±0.08 Bb
	DC-RL	2.87±1.08 Aa	6.95±1.69 Aa	10.51±1.95 ABa	2.00±0.40 Aa	10.99±0.41 Ba	1.79±0.10 ABa
LEAF							
E. dysenterica	MC-RL	0.10±0.02 Ab	10.60±2.44 Aa	12.80±0.37 Aa	0.90±0.20 Ba	26.23±2.64 Aa	5.04±0.20 ABa
	Css-RYL	0.05±0.01 Ac	7.07±0.64 Aa	9.81±0.87 ABa	1.07±0.08 Bb	18.22±1.29 BCa	4.47±0.16 Bb
	Css-YL	0.21±0.02 Ab	7.65±1.70 Aa	7.67±0.62 Ba	1.08±0.14 Ba	12.72±0.52 Ca	4.44±0.15 Ba
	Css-HCD	0.19±0.02 Ac	6.75±0.57 Aa	6.80±0.32 Ba	1.50±0.26 ABa	13.71±0.15 Ca	4.35±0.17 Ba
	DC-RL	0.56±0.24 Ab	4.51±0.33 Aa	12.73±3.39 Aa	2.16±0.25 Aa	20.85±3.26 ABa	6.54±0.11 Aa
Q. parviflora	MC-RL	13.95±0.67 Aa	8.74±0.63 Aa	6.96±0.72 Ab	1.27±0.04 Aa	9.68±0.74 Ab	3.91±0.26 Aa
	Css-RYL	16.08±0.81 Ab	8.78±0.85 Aa	7.01±0.75 Aa	1.50±0.16 Aab	9.79±0.42 Ab	4.28±0.25 Ab
	Css-YL	12.96±0.44 Aa	6.60±0.25 Aa	4.90±0.31 Aa	0.97±0.04 Aa	7.47±0.21 Ab	4.86±0.27 Aa
	Css-HCD	11.91±0.50 Ab	6.68±0.29 Aa	3.28±0.03 Aa	1.15±0.07 Aa	7.52±0.17 Ab	3.43±0.09 Aa
Q. multiflora	Css-RYL	23.07±1.09 Aa	8.93±0.77 Aa	8.70±1.65 Aa	2.06±0.07 Aa	17.56±1.58 Aa	5.99±0.89 ABa
	Css-HCD	20.86±3.54 ABa	4.49±0.95 Aa	4.24±0.87 Aa	1.50±0.36 Aa	12.66±1.80 Aab	3.86±0.60 Ba
	DC-RL	17.91±1.42 Ba	5.66±0.58 Aa	7.10±0.63 Ab	1.54±0.10 Aa	17.83±1.25 Aa	6.27±0.49 Aa
SHOOT							
E. dysenterica	MC-RL	0.27±0.04 Ab	16.57±2.46 Aa	18.90±0.39 ABa	1.25±0.18 Bb	44.59±3.89 Aa	7.07±0.18 ABa
	Css-RYL	0.16±0.02 Ac	15.42±0.87 Aa	15.22±1.43 ABb	1.74±0.05 Bb	29.90±1.90 BCb	6.46±0.34 Bab
	Css-YL	0.44±0.07 Ab	12.38±1.53 Aa	13.59±0.71 ABa	1.96±0.24 Bb	18.75±0.96 Ca	7.12±0.27 ABa
	Css-HCD	0.36±0.03 Ac	12.84±0.28 Aa	11.85±0.57 Ba	2.44±0.29 ABc	18.51±0.35 Ca	6.22±0.24 Ba
	DC-RL	0.87±0.18 Ab	9.23±0.08 Aa	21.64±0.24 Aa	3.35±0.35 Aa	34.70±1.97 ABa	8.79±0.13 Aa
Q. parviflora	MC-RL	15.86±0.78 Aa	13.87±0.82 Aa	19.49±2.42 Aa	2.64±0.07 Ba	25.58±2.77 Ab	5.50±0.31 Ab
	Css-RYL	19.56±0.78 Ab	15.07±1.53 Aa	15.59±0.91 ABb	3.98±0.39 Aa	20.39±1.98 ABc	5.74±0.30 Ab
	Css-YL	15.63±0.45 Aa	11.98±0.55 Aa	12.77±0.45 ABa	2.78±0.09 Ba	13.58±0.35 Ba	6.42±0.25 Aa
	Css-HCD	16.34±0.75 Ab	12.28±0.61 Aa	9.97±0.31 Ba	3.52±0.22 ABb	13.08±0.42 Ba	4.80±0.12 Aa
Q. multiflora	Css-RYL	26.68±1.23 Aa	16.48±0.81 Aa	22.71±3.40 Aa	4.17±0.18 Aa	42.53±3.59 Aa	7.95±0.98 Aa
	Css-HCD	23.42±3.46 ABa	11.61±1.19 Aa	12.91±0.64 Ba	4.62±0.23 Aa	18.86±0.53 Ba	6.31±0.08 Ba
	DC-RL	20.78±2.00 Ba	11.75±1.75 Aa	16.23±2.10 ABa	3.54±0.29 Aa	27.13±1.24 Ba	7.89±0.34 Aa

*Means ± standard error. Means followed by the same letters do not differ statistically by Tukey test at the 5% probability level. Capital letters compare collection sites and lowercase letters compare species. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; DC-RL: Dystrophic Cerradão on Red Latosol.

Table 3 Micronutrient concentration (mg kg^{-1} DW) in stems, leaves, and shoots (stem + leaves) of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora* collected on Cerrado soils with different Al concentrations in the National Forest of Paraopeba, southeastern Brazil

Species	Collection sites	Cu	Fe	Mn	Zn
STEM					
E. dysenterica	MC-RL	11.55±1.06 Aa	67.78±11.44 Aa	57.47±13.27 Ab	45.69±3.25 Aa
	Css-RYL	13.02±0.57 Aa	129.67±35.10 Aa	56.56±8.78 Ab	42.26±4.36 Aa
	Css-YL	16.78±0.88 Aa	119.15±31.69 Aa	132.89±8.58 Ab	43.16±3.24 Aa
	Css-HCD	13.42±0.99 Aa	130.52±22.68 Aab	215.76±33.70 Ab	38.03±2.90 Ab
	DC-RL	16.64±0.32 Ab	86.14±7.53 Ab	261.67±64.81 Ab	46.50±3.34 Aa
Q. parviflora	MC-RL	10.14±0.76 Aa	40.44±6.97 Ba	1009.82±142.70 ABa	37.54±6.77 Ba
	Css-RYL	12.01±2.11 Aa	107.51±19.70 ABa	854.25±206.68 Ba	24.36±2.64 Ba
	Css-YL	11.15±1.09 Aa	73.22±10.23 ABa	1285.40±192.64 ABa	41.38±6.39 Ba
	Css-HCD	12.67±0.37 Aa	161.92±11.82 Aa	1749.27±100.25 Aa	83.20±20.20 Aa
Q. multiflora	Css-RYL	14.35±1.75 Ba	65.27±15.19 Ba	497.90±40.69 Bab	28.60±4.57 Aa
	Css-HCD	9.14±0.84 Ba	49.76±4.83 Bb	1086.52±167.57 ABa	18.94±1.57 Ab
	DC-RL	36.90±20.80 Aa	210.15±70.66 Aa	1682.98±591.43 Aa	26.46±5.78 Aa
LEAF					
E. dysenterica	MC-RL	6.43±0.24 Ba	65.58±18.46 Ab	139.61±19.56 Da	24.00±0.96 Ba
	Css-RYL	6.09±0.69 Bb	63.06±15.09 Ab	190.84±4.98 CDa	23.65±1.20 Ba
	Css-YL	6.27±0.50 Ba	83.49±15.36 Ab	311.95±15.87 Ca	22.38±1.01 Ba
	Css-HCD	4.39±0.42 Ba	102.36±7.48 Ab	534.73±37.94 Ba	22.29±0.59 Ba
	DC-RL	15.18±0.89 Aa	241.66±106.34 Ab	1121.13±10.86 Aa	33.80±4.11 Aa
Q. parviflora	MC-RL	7.88±1.00 Aa	465.55±93.83 Ba	73.70±7.68 Aa	15.26±0.42 ABb
	Css-RYL	7.20±0.47 Ab	639.95±43.48 ABa	116.92±15.04 Aa	21.09±1.64 Aa
	Css-YL	4.59±0.50 Aa	772.56±40.10 Aa	114.26±19.74 Ab	17.06±1.56 ABb
	Css-HCD	4.77±0.23 Aa	432.40±29.67 Ba	131.63±13.17 Ab	12.89±0.56 Bb
Q. multiflora	Css-RYL	10.69±2.20 Aa	789.91±103.56 Aa	135.11±10.06 Aa	19.16±1.53 Aa
	Css-HCD	4.97±0.83 Ba	550.42±105.79 Aa	152.83±30.40 Ab	17.78±2.70 Aab
	DC-RL	9.60±0.60 ABb	805.13±111.51 Aa	144.22±31.16 Ab	17.52±0.98 Ab
SHOOT					
E. dysenterica	MC-RL	17.99±1.30 Aa	132.43±22.73 Ab	197.09±31.20 Bb	69.69±3.42 Aa
	Css-RYL	19.12±1.23 Aa	192.73±33.07 Ab	247.40±7.49 Ba	65.91±5.42 Aa
	Css-YL	23.05±1.13 Aa	202.64±36.30 Ab	444.84±22.68 ABb	65.54±2.98 Aa
	Css-HCD	17.81±0.76 Aa	232.87±23.10 Ab	750.50±32.89 ABb	60.32±2.63 Aab
	DC-RL	31.82±1.22 Aa	327.80±113.87 Ab	1382.79±75.67 Aa	80.30±0.78 Aa
Q. parviflora	MC-RL	18.03±1.52 Aa	505.99±92.10 Ba	1083.53±142.98 ABa	52.80±6.96 Ba
	Css-RYL	19.04±2.07 Aa	799.61±34.77 Aa	970.64±199.41 Ba	46.36±2.80 Ba
	Css-YL	15.74±1.07 Aa	845.77±43.47 Aa	1399.66±202.04 ABa	58.45±5.89 Ba
	Css-HCD	17.43±0.35 Aa	594.31±19.37 ABa	1880.90±99.83 Aa	96.08±20.76 Aa
Q. multiflora	Css-RYL	25.04±1.29 ABa	855.19±91.00 Aa	633.01±33.69 Ba	47.76±5.98 Aa
	Css-HCD	16.77±0.57 Ba	687.13±118.17 Aa	1161.71±164.25 ABab	33.96±2.62 Ab
	DC-RL	38.72±15.72 Aa	973.06±99.51 Aa	1773.64±428.70 Aa	41.65±5.13 Ab

*Means ± standard error. Means followed by the same letters do not differ statistically by Tukey test at the 5% probability level. Capital letters compare collection sites and lowercase letters compare species. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; DC-RL: Dystrophic Cerradão on Red Latosol.

Correlation between the concentrations of total and exchangeable Al in soils and the Al and nutrient accumulation in shoots of the species – Only *E. dysenterica* showed correlation between Al concentration in the shoot and total Al concentration in the soils ($r = 0.710$, $p < 0.001$). Additionally, nutrient concentrations in that species were the most affected by soil Al concentration (Table 4). A significant positive correlation was observed between accumulated Cu, Fe, Mg, Mn, and S in shoots and total Al concentration in the soil, while Ca concentration was negatively correlated with that edaphic factor. In contrast, *Q. parviflora* showed a positive correlation with that same edaphic factor only for Mn and Zn accumulation, and a negative one for K, P, and S concentrations. *Q. multiflora*, on the other hand, showed a positive correlation with total Al concentration in the soil for Mn accumulation and a negative on for Ca and P levels (Table 4). Some differences were observed when the correlation between Al^{3+} concentrations in the soil and Al and nutrient concentrations in the plants was analyzed. The K and P concentrations in shoots of *E. dysenterica* and *Q. parviflora* were negatively correlated with Al^{+3} concentration in the soil. Additionally, *E. dysenterica* showed a positive correlation for Mg and Mn concentrations, while *Q. parviflora* showed a positive correlation for Mg and Fe levels (Table 4). In *Q. multiflora*, only Mg concentration was correlated with the Al^{+3} concentration in the soil ($r = 0.636$, $p < 0.05$).

Table 4 Pearson correlation coefficients between the concentrations of total and exchangeable Al in Cerrado soils from the National Forest of Paraopeba, southeastern Brazil, and the concentrations of Al and nutrients in shoots of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora*

Variables	Coefficient of correlation with soil total Al			Coefficient of correlation with soil Al^{+3}		
	E. dysenterica	*Q. parviflora*	*Q. multiflora*	*E. dysenterica*	*Q. parviflora*	*Q. multiflora*
Al	0.710***	-0.348	-0.457	0.135	0.211	0.224
Ca	-0.399*	-0.334	-0.547*	-0.303	-0.161	0.038
K	0.129	-0.522*	-0.404	-0.589**	-0.660**	-0.135
Mg	0.639***	-0.012	-0.372	0.464*	0.458*	0.636*
P	-0.165	-0.457*	-0.639*	-0.796***	-0.654**	-0.227
S	0.370*	-0.416*	-0.038	-0.212	-0.030	-0.358
Cu	0.476*	-0.119	0.231	0.152	-0.077	-0.371
Fe	0.420*	-0.265	0.187	0.347	0.421*	-0.465
Mn	0.941***	0.593**	0.600*	0.425*	0.342	-0.343
Zn	0.151	0.559**	-0.225	-0.218	0.276	-0.208

*p < 0.05, **p < 0.01, ***p < 0.001

The relationship between Al accumulation in plants and soil chemical attributes

Results obtained by the CCA ordination suggest that Al accumulation in plants and soil chemical features are related. The eigenvalue of only the first axis (0.411) was significant (p = 0.001), explaining 87.9% of the total variance. The high Pearson correlation coefficient (0.99), supported by the Monte Carlo permutation test, indicated that Al accumulation by the species varied significantly with the studied edaphic variables. The soil chemical variables with highest correlation on the first axis were K, Ca, pH, SB, and V (positive), and Al^{+3}, H+Al, and m (negative). The CCA ordination diagram suggests that Al concentration in shoots of *E. dysenterica* and especially *Q. parviflora* is positively correlated with the first group of variables, while in *Q. multiflora* it is more correlated with the second group (Fig. 2).

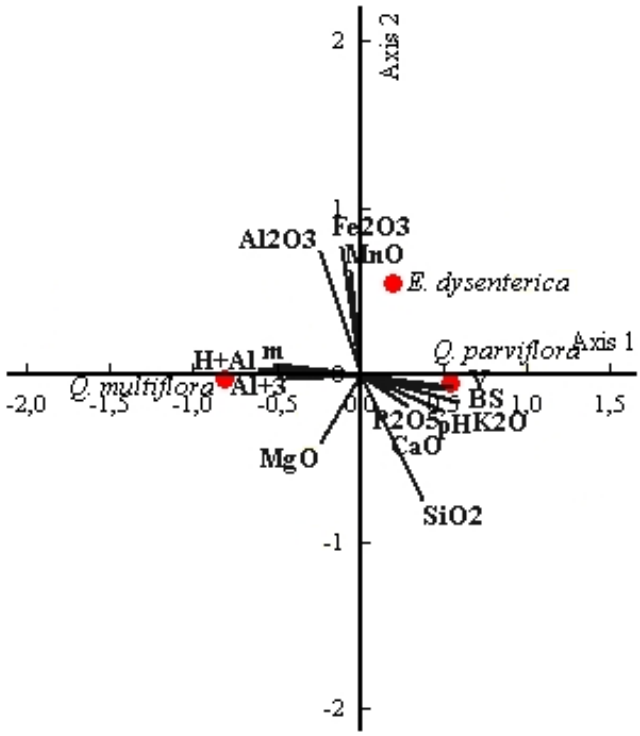

Fig. 2 Ordination diagram showing the relationship between Al accumulation in shoots of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora* and soil chemical attributes in the Cerrado from the National Forest of Paraopeba, Brazil. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; DC-RL: Dystrophic Cerradão on Red Latosol.

Discussion

Despite the high concentrations of total Al observed in soils from all collection sites, it is well known that Al availability is dependent, among other factors, on soil pH. In acid soils (having pH lower than 5.5), Al is solubilized from clay minerals, increasing Al^{3+} concentration and consequently its phytoavailability (Sade et al. 2016), as observed in our study (Table 1). Even with differences in Al availability in soils, *Q. parviflora* and *Q. multiflora* showed mean leaf concentrations higher than 10 g Al kg^{-1} DW at all sites (Table 2), confirming their hyperaccumulator nature, as described by Haridasan (1982), and evidencing that Al accumulation occurs regardless of soil fertility and Al concentration, as in *Melastoma malabathricum* (Osaki et al. 1998) and *Rudgea viburnoides* (Malta et al. 2016).

The Al hyperaccumulation by *Q. parviflora* on the MC-RL soil (with negligible Al^{3+} concentration) and the highest Al concentration in *Q. multiflora* on the C*ss*-RYL soil (with the lowest total Al concentration) (Tables 1 and 2) indicate that these species are capable to absorb Al species other than Al^{+3} or even to solubilize Al from plant-unavailable fractions (Poschenrieder et al. 2008). Exudation of organic acids, phenolic substances, and mucilage, for example, are mechanisms that increase the availability of Al and favor its uptake in Al-hyperaccumulators (Poschenrieder et al. 2015), such as *C. sinensis* and *M. malabathricum* (Watanabe et al. 2008; Hajiboland et al. 2015). However, research on the physiology of native Cerrado species in response to Al remains scarce, and more studies are necessary to evaluate the mechanisms involved in Al uptake by *Q. parviflora* and *Q. multiflora*.

Just like indicated in the literature, our results demonstrated that Al uptake responds differently to edaphic factors depending on the species (Haridasan and Araújo 1988; Serrano et al. 2011). *E. dysenterica* showed a positive correlation between Al concentration in the soil and that in the shoot, which suggests a limitation on the species mechanisms of Al exclusion, since the species is an Al-non-accumulator. In *Q. multiflora*, on the other hand, Al accumulation was correlated not only with total or available Al concentration in soils (Table 3) but also with acid and dystrophic soils (Figs. 1 and 2). Interestingly, *Q. parviflora* showed a similar response to that of *E. dysenterica*, whereby Al accumulation in its organs was positively influenced by mesotrophic soils (Fig. 1 and 2). Haridasan and Araújo (1988) also observed that *Q. grandiflora* trees growing on a Ca-rich soil have higher

Al concentration in their leaves than those that grow on a more strongly acid dystrophic latosol. This suggests that *Q. parviflora*, just as *Q. grandiflora*, may be adapted to a wide range of soil conditions, but may respond better to higher soil fertility (Haridasan and Araújo 1988).

The FLONA of Paraopeba represents not only a natural gradient of Cerrado phytophysiognomies on soils with different fertilities and Al availabilities, but also a toxicity gradient, since the concentrations of Fe and Mn increased while that of Ca decreased with increasing Al concentration in the soils (Table 1 and Fig. 1; Serrano et al. 2011). *Q. parviflora* and *Q. multiflora*, besides having shown higher Al accumulation in shoots than *E. dysenterica*, also showed higher levels of Fe and Mn, which also contributes to limiting crop yield on acid soils (Kochian et al. 2004). Moreover, foliar Mn concentration was higher in *E. dysenterica*, demonstrating that this species preferentially deposits Mn in its leaves while *Qualea* species keep the element mostly in their stems. The concentration of Mn accumulated by *E. dysenterica*, *Q. parviflora*, and *Q. multiflora* followed the concentration gradient in the soils, but it did not reach a level from which plants could be classified as Mn-hyperaccumulators, i.e. 10,000 mg kg^{-1} (van der Ent et al. 2013).

In field conditions, it is virtually impossible to ascertain direct cause-effect events. Nevertheless, we observed that *E. dysenterica*, *Q. parviflora*, and *Q. multiflora* did not reduce the uptake of nutrients known to be affected by Al toxicity, such as Ca and Mg (Kochian et al. 2005), along the soil Al gradient (Table 2). This suggests that these species, unlike most crop plants, have mechanisms that enable them to avoid the competition of Al for binding sites on root cortical cell walls and on the outer surface of the plasma membrane, which would reduce the concentration of these nutrients near the uptake sites and thus inhibit their absorption into the symplast (Marschner 2012). Maintenance of Ca and Mg levels independently of the Al concentration in leaves is a feature of plants adapted to soils with high available Al concentration (Haridasan 1987; Masunaga et al. 1998; Watanabe and Osaki 2002; Serrano et al. 2011).

Q. multiflora, besides sustaining Ca and Mg levels, was able to increase K, P, and S concentrations as it accumulated increasing Al concentrations (Table 2). Nutritional reorganization, along with avoidance of Al excess in photosynthetically active cells (see chapter 2), may contribute to the capacity of *Q. multiflora* to accumulate a higher Al

concentration than *Q. parviflora*, as our results have demonstrated. Evidence suggests that the mineral-nutrient status of plants plays a critical role in increasing plant resistance to environmental stress factors (Marschner 2012). Improvement of K-nutritional status of plants can greatly lower reactive oxygen species production by reducing activity of NAD(P)H oxidases and maintaining photosynthetic electron transport in plants under abiotic stress (Cakmak 2005). Additionally, the enhanced levels of P and S may play a role in chelating Al into insoluble Al-P complexes within the cell wall (Gaume et al. 2001; Zheng et al. 2005) and in combating oxidative stress through the antioxidant capacity of thiols (S-containing compounds) (Na and Salt 2011), respectively.

Unlike Goodland and Pollard (1978), who observed that the foliar nutrient concentrations of Al-accumulator plants from the Cerrado are generally lower than those of non-accumulator plants, we observed that *E. dysenterica* (non-accumulator) showed lower and more influenced nutrient levels than both *Qualea* species (Al-hyperaccumulators) (Tables 2-4). This data indicates that responses vary according to the species.

In summary, our results demonstrate that Al hyperaccumulation in *Q. parviflora* and *Q. multiflora* occurs on soils with varying amounts of available Al, even on those with negligible concentrations of the metal, and suggest that these species not only have mechanisms that increase the concentration of available Al in the soil but also actively absorb the metal. Additionally, Al accumulation was differently influenced by edaphic factors, whereby in *Q. multiflora* it was more correlated with dystrophic soils abundant in available Al concentrations while in *Q. parviflora* and *E. dysenterica* (the latter of which is a non-accumulator species) it was positively influenced by mesotrophic soils with lower Al availability. Data on plant nutritional status and its correlation with soil Al concentration reinforces the theory that nutrient absorption by species adapted to acid soils is not adversely affected by high concentrations of the metal in the soil. Moreover, the capacity of *Q. multiflora* to alter its K, P, and S levels in response to higher Al accumulation may contribute to the species capacity to accumulate more Al than *Q. parviflora*.

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References

Alves EEN, Costa LMC, Rocha PA, Bittencourt SFE, Faria ALL, Schaefer CEGR (2015) Utilização do μ-EDX para determinação de elementos químicos marcadores de metamorfismo em saprolitos de contato. III Simpósio Mineiro de Ciência do Solo, Viçosa-MG

Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci 168:521–530

de Silva J, Tuwei G, Zhao F-J (2016) Environmental factors influencing aluminium accumulation in tea (*Camellia sinensis* L.). Plant Soil 400:223–230.

EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária (1997) Manual de métodos de análise de solo. Centro Nacional de Pesquisa de Solos, Rio de Janeiro

Ferreira DF (2011) Sisvar: A computer statistical analysis system. Ciênc Agrotec 35:1039–1042

Gaume A, Mächler F, Frossard E (2001) Aluminum resistance in two cultivars of *Zea may* L.: root exudation of organic acids and influence of phosphorous nutrition. Plant Soil 234:73–81

Giannakoula A, Moustakas M, Mylona P, Papadakis I, Yupsanis T (2008) Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. J Plant Physiol 165:385–396

González-Santana IH, Márquez-Guzman J, Cram-Heydrich S, Cruz-Ortega R (2012) *Conostegia xalapensis* (Melastomataceae): an aluminum accumulator plant. Physiol Plant 144:134–145

Goodland R, Pollard R (1978). Aluminium and nutrient strategies of cerrado trees. II Congresso Lation-Americano de Botânica, pp 71–72

Hajiboland R, Bastani S, Bahrami-Rad S, Poschenrieder C (2015) Interactions between aluminum and boron in tea (*Camellia sinensis*) plants. Acta Physiol Plant 37:54

Haridasan M (1982) Aluminium accumulation by some cerrado native species of central Brazil. Plant Soil 65:265–273

Haridasan M (1987) Distribution and mineral nutrition of aluminium accumulating species in different plant communities of the cerrado region of central Brazil. In: San José RR, Montes R (eds) La capacidad Bioprodutiva de Sabanas. IVIC./CIET, Caracas, pp 309–348

Haridasan M (2008) Alumínio é um elemento tóxico para as plantas nativas do cerrado? In: Prado CHBA, Casali CA (eds) Fisiologia Vegetal: práticas em relações hídricas, fotossíntese e nutrição mineral. Manole, Barueri, pp 1–10

Haridasan M, Araújo GM (1988) Aluminium-accumulating species in two forest communities in the cerrado region of central Brazil. Forest Ecol Manag 24:15–26

Kochian LV, Piñeros MA, Hoekenga OA (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol 55:459–493

Kochian LV, Piñeros MA, Hoekenga OA (2005) The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274:175–195

Malta PG, Arcanjo-Silva S, Ribeiro C, Campos NV, Azevedo AA (2016) *Rudgea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplasts. Plant Soil 408:369–384

Mariano ED, Keltjens WG (2005) Long-term effects of aluminum exposure on nutrient uptake by maize genotypes differing in aluminum resistance. J Plant Nut 28:323–333

Marschner P (2012) Marschner's Mineral Nutrition of Higher Plants. Academic Press, London

Masunaga T, Kubota D, Hotta M, Wakatsuki T (1998) Mineral composition of leaves and bark in aluminum accumulators in a tropical rain forest in Indonesia. Soil Sci Plant Nutr 44:347–358

McCune B, Mefford MJ (2006) PC-ORD, version 5.0, Multivariate analysis of ecological data. MjM Software Design, Glaneden Beach. 40p

Medeiros RA, Haridasan M (1985) Seasonal variations in the foliar concentrations of nutrients in some aluminium accumulating and non-accumulating species of the cerrado region of central Brazil. Plant Soil 88:433-436

Metali F, Salim KA, Burslem DFRP (2012) Evidence of foliar aluminium accumulation in local, regional and global dataset of wild plants. New Phytol 193:637–649

Na G, Salt DE (2011) The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. Environ Exp Bot 72:18–25

Neri AV, Schaefer CEGR, Silva AF, Souza AL, Ferreira-Júnior WG, Meira-Neto JAA (2012) The influence of soils on the floristic composition and community structure of an area of Brazilian Cerrado vegetation. Edinb J Bot 69:1–27

Neri AV, Schaefer CEGR, Souza AL, Ferreira-Júnior WG, Meira-Neto JAA (2013) Pedology and plant physiognomies in the Cerrado, Brazil. An Acad Bras Cienc 85:87–102

Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR (2001) Terrestrial ecoregions of the world: A new map of life on Earth. BioScience 51:933–938

Osaki M, Watanabe T, Ishizawa T, Nilnod C, Nuyim T, Sittibush C, Tadano T (1998) Nutritional characteristics in leaves of native plants grown in acid sulfate, peat, sandy podzolic, and saline soils distributed in peninsular Thailand. Plant Soil 201:175–182

Paye HS (2014) Teor e distribuição de elementos terras raras e comparação de métodos de extração de elementos traço em solos brasileiros. Thesis, Universidade Federal de Viçosa

Poschenrieder C, Gunsé B, Corrales I, Barceló J (2008) A glance into aluminum toxicity and resistance in plants. Sci Total Environ 400:356–368

Poschenrieder C, Tolrà R, Hajiboland R, Arroyave C, Barceló J (2015) Mechanisms of hyper-resistance and hyper-tolerance to aluminum in plants. In: Panda SK, Baluška F (eds) Aluminum stress adaptation in plants. Springer International Publishing, Berlin/Heidelberg, pp 81–98

Prance GT (1996) Islands in Amazonia. Philos Trans R Soc Lond B Biol Sci 351:823–833

Ribeiro JF, Walter BMT (2008) As principais fitofisionomias do bioma Cerrado. In: Sano SM, Almeida SP, Ribeiro JF (eds) Cerrado: Ecologia e flora, Embrapa Cerrados, Brasília, pp 151–199

Ruan J, Ma L, Shi Y (2006) Aluminium in tea plantations: mobility in soils and plants, and the influence of nitrogen fertilization. Environ Geochem Health 28:519–528

Sade H, Meriga B, Surapu V, Gadi J, Sunita MSL, Suravajhala P, Kishor PBK (2016) Toxicity and tolerance of aluminum in plants: Tailoring plants to suit to acid soils. Biometals 29:187–210

Sano SM, Almeida SP, Ribeiro JF (2008) Cerrado: Ecologia e flora. Embrapa Cerrados, Brasília

Serrano HC, Pinto MJ, Martins-Loução MA, Branquinho C (2011) How does Al hyperaccumulator plant respond to a natural field gradient of soil phytoavailable Al? Sci Total Environ 409:3749–3756

SNUC – Sistema Nacional de Unidades de Conservação da Natureza (2000). *Lei nº 9.985, de 18 de julho de 2000*. MMA/SBF

TerBraak CJF (1987) The analysis of vegetation environment relationship by canonical correspondence analysis. Vegetation 69:69–77

TerBraak CJF (1988) CANOCO – A FORTRAN program for canonical community ordination by (Partial) (Detrended) (Canonical) Correspondence Analysis and redundancy analysis, version 2.1, Technical report LWA-88-2, TNO. Institute of Applied Computer Science, Wageningen

TerBraak CJF (1994) Canonical community ordination. Part I: Basic theory end linear methods. Écoscience 1:127–140

Tolentino GS (2011) Composição e partição de nicho em gradientes de solo e luz no Cerrado. Dissertation, Universidade Federal de Viçosa

van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. Plant Soil 362:319–334

Vitorello VA, Capaldi FR, Stefanuto VA (2005) Recent advances in aluminum toxicity and resistance in higher plants. Braz J Plant Physiol 17:129–143

Watanabe T, Osaki M (2002) Mechanisms of adaptation to high aluminum condition in native plant species growing in acid soils: A review. Commun Soil Sci Plant Anal 33:1247–1260

Watanabe T, Misawa S, Hiradate S, Osaki M (2008) Characterization of root mucilage from *Melastoma malabathricum*, with emphasis on its role in aluminum accumulation. New Phytol 178:581–589

Wong MH, Zhang ZQ, Wong JWC, Lan CY (1998) Trace metal contents (Al, Cu and Zn) of tea: Tea and soil from two tea plantations, and tea products from different provinces of China. Environ Geochem Health 20:87–94

Zheng SJ, Yang JL, He YF, Yu XH, Zhang L, You JF, Shen RF, Matsumoto H (2005) Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat. Plant Physiol 138:297–303

CHAPTER 2 – Are the immobilization sites of aluminum in shoots of native Cerrado species related to the species capacity to accumulate the metal? [(1)]

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ABSTRACT: The Cerrado is composed of plant species with different aluminum (Al) resistance strategies, like *Eugenia dysenterica* (non-accumulator, Myrtaceae), *Qualea parviflora*, and *Q. multiflora* (both Al-hyperaccumulators, Vochysiaceae). We evaluated whether the sites of Al immobilization in shoots of these species are related to Al concentration in the soil and to the species Al resistance strategies. Aluminum concentration in plants was determined by inductively coupled plasma atomic emission spectrometry and mapped by a histochemical test and energy dispersive X-ray microfluorescence. Relative Al abundance in tissues was measured by X-ray microanalysis coupled to scanning electron microscopy. *E. dysenterica* showed low Al concentration in the shoot (an average 0.5 g kg^{-1} DW) whereas *Q. parviflora* and *Q. multiflora* hyperaccumulated Al at all collection sites. Al was deposited preferentially in pectocelluosic walls, but also in suberized cell walls and in chloroplasts. No difference regarding sites of Al immobilization or the percentage of Al in tissues of *E. dysenterica* was observed among collection sites. The two *Qualea* species showed higher relative Al abundance in the stem medullary parenchyma and leaf hypodermis. However, the percentage of Al in palisade and spongy parenchymas was similar to the one in the hypodermis of *Q. parviflora*, but it was the lowest in *Q. multiflora*. Data demonstrates that the Al allocation pattern in tissues does not depend on the Al concentration in soils and that such pattern may be related to the species capacity to accumulate Al, thus contributing to the tolerance of *Q. multiflora* to higher internal concentrations of the metal.

Keywords: *Eugenia dysenterica*, *Qualea parviflora*, *Qualea multiflora*, acid soils, Al histolocalization, relative abundance of Al in tissues

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CAPÍTULO 2 – Os sítios de imobilização de alumínio na parte aérea de espécies nativas do Cerrado estão relacionados à sua capacidade de acumular o metal?

RESUMO: O Cerrado é composto por espécies vegetais com diferentes estratégias de resistência ao alumínio (Al), como *Eugenia dysenterica* (não acumuladora, Myrtaceae), *Qualea parviflora* e *Q. multiflora* (hiperacumuladoras de Al, Vochysiaceae). Nós avaliamos se os sítios de imobilização de Al na parte aérea destas espécies estão relacionados à concentração do metal no solo e às estratégias de resistência ao Al. A concentração de Al nas plantas foi determinada por meio de espectrometria de emissão atômica com plasma indutivamente acoplado e o mapeamento foi realizado por meio de teste histoquímico e microfluorescência de raios-x por energia dispersiva. A abundância relativa de Al nos tecidos foi mensurada por meio de microanálise de raios-x acoplada à microscopia eletrônica de varredura. *E. dysenterica* apresentou baixa concentração de Al na parte aérea (em média 0,5 g kg^{-1} MS), enquanto *Q. parviflora* e *Q. multiflora* hiperacumularam Al em todos os sítios de coleta. Paredes pectocelulósicas foram os sítios preferenciais de deposição de Al, mas ele também foi localizado em paredes suberizadas e cloroplastos. Não foi observada diferença nos sítios de imobilização e na porcentagem de Al nos tecidos de *E. dysenterica* entre os locais de coleta. As espécies de *Qualea* apresentaram maior abundância relativa de Al no parênquima medular do caule e na hipoderme da folha. Contudo, a porcentagem de Al nos parênquimas paliçádico e lacunoso foram similares àquela observada na hipoderme em *Q. parviflora* e a menor em *Q. multiflora*. Os dados demonstram que o padrão de alocação de Al nos tecidos não depende da concentração do metal no solo e que este padrão pode estar relacionado com a capacidade das espécies acumularem Al, contribuindo assim para a tolerância de *Q. multiflora* à maior concentração interna do metal.

Palavras-chave: *Eugenia dysenterica*, *Qualea parviflora*, *Qualea multiflora*, solos ácidos, histolocalização de Al, abundância relativa de Al nos tecidos

Introduction

Aluminum (Al) occurs in the lithosphere mainly as its non-phytotoxic forms, such as aluminosilicates and Al oxides (Grevenstuk and Romano 2013), and at pH values below 5.5 it is solubilized as its highly phytotoxic trivalent cation (Al^{3+}) (Hartwig et al. 2007). Acidic soils (pH < 5.5 in the surface layer) occupy about 40-50% of the world's potentially arable lands and approximately 60% are located in the tropics and subtropics, where they constrain crop production (von Uexküll and Mutert 1995). Al toxicity is the major limiting factor to plant growth and development (Poschenrieder et al. 2008), as it inhibits growth and elongation of roots, rendering them inefficient to absorb water and nutrients (Fujii et al. 2012).

Plant features and distribution in the Brazilian Cerrado are influenced by climate, soil, water availability, and fire occurrence (Ribeiro and Walter 2008). In general, soils therein are acidic (pH between 4 and 5) and dystrophic, with high exchangeable Al concentrations (Sano et al. 2008). The variation in soil characteristics in the Cerrado results in a heterogeneous landscape constituted of grassland (campo limpo), savannas (campo sujo, campo cerrado, and cerrado *stricto sensu*) and forest (cerradão) (Ribeiro and Walter 2008), all of which are composed of a wide range of species with different Al resistance strategies.

Species that are resistant to Al toxicity may have mechanisms of exclusion and/or internal tolerance that enable them to survive conditions of high concentrations of the element (Harisadan 2008a, b), being then classified as Al-excluders or Al-accumulators, respectively (Vitorello et al. 2005). Accumulation of high Al concentrations in shoots is a common trait among many plants from tropical regions. Plants that accumulate more than 1 g Al kg^{-1} DW in leaves are considered Al-hyperaccumulators. These plants have evolved independently in unrelated botanical families, especially from basal orders of Rosids and Asterids (Jansen et al. 2002; Olivares et al. 2009).

The presence of mechanisms to prevent contact between free Al and essential biochemical processes, like photosynthesis, is a prerequisite for the tolerance to high internal Al concentrations (Grevenstuk and Romano 2013). While organic acids and phenolic compounds are major ligands for Al detoxification, cell walls and vacuoles are the main storage sites of the accumulated metal (Vázquez et al. 1999; Tolrà et al. 2011; Li et al. 2014). Al has high affinity for free carboxyl groups of pectin and hemicellulose in

pectocellulosic cell walls (Gao et al. 2014) and can immobilize about 90% of the total Al accumulated in the cell (Chang et al. 1999).

Studies on Al localization have a great importance for improving our understanding of the mechanisms associated with Al toxicity and resistance (Matsumoto 2000), and they have been mostly performed with crop plants. Several techniques can be employed for that purpose, such as, for example, those associated with light microscopy, by different histochemical tests (Reyna-Llorensa et al. 2015; Malta et al. 2016); electron microscopy, laser scanning confocal microscopy (Malta et al. 2016), or X-ray microanalysis (Bressan et al. 2016; Malta et al. 2016); and X-ray spectroscopy (Campos et al. 2014). However, in native Al-resistant species the allocation pattern of the metal is a subject that remains little studied, despite the existence of many such species in the Cerrado.

Therefore, our aims were (i) to identify the sites of Al immobilization in stems and leaves of three native species to the Cerrado, all of them belonging to order Myrtales – *Eugenia dysenterica*, a non-accumulator Myrtaceae, and *Qualea parviflora* and *Q. multiflora,* two Al-hyperaccumulator Vochysiaceae –, which were collected on soils with different Al concentrations; and (ii) to determine whether these sites of immobilization are related to the Al concentration in the soil and to the Al resistance strategies of the species.

Material and Methods

Study area

The study was conducted in the Cerrado at the Paraopeba National Forest (FLONA of Paraopeba), Minas Gerais state, southeastern Brazil (19°16'S; 44°23'W) (SNUC 2000). The FLONA has a soil-vegetation gradient with distinct features: Dystrophic Cerradão on Red Latosol (DC-RL), Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic (C*ss*-HCD), Cerrado *sensu stricto* on Yellow Latosol (C*ss*-YL), Dense Cerrado *sensu stricto* on Red-Yellow Latosol (C*ss*-RYL), and Mesotrophic Cerradão on Red Latosol (MC-RL) (Neri et al. 2012), which are henceforth called collection sites. The MC-RL soil shows negligible concentration of exchangeable Al at depth 0.0-0.2 m. All collection sites show high concentrations of total Al in the soil (see chapter 1) (Table 1).

Table 1 Soil classification and concentrations of total and exchangeable Al in the surface layer (0.0-0.2 m) of Cerrado soils from the National Forest of Paraopeba, southeastern Brazil

Collection site	Phytophysiognomy	Soil	Total Al	Al^{3+}	Sampled species
MC-RL	Mesotrophic Cerradão	Red Latosol	20.48 C*	0.00 C	*Eugenia dysenterica* *Qualea parviflora*
C*ss*-RYL	Cerrado *sensu stricto*	Red Yellow Latosol	18.50 D	2.36 B	*Eugenia dysenterica* *Qualea parviflora* *Qualea multiflora*
C*ss*-YL	Cerrado *sensu stricto*	Yellow Latosol	21.01 C	2.26 B	*Eugenia dysenterica* *Qualea parviflora*
C*ss*-HCD	Cerrado *sensu stricto*	Haplic Cambisol Tb Dystrophic	24.78 B	2.71 A	*Eugenia dysenterica* *Qualea parviflora* *Qualea multiflora*
DC-RL	Dystrophic Cerradão	Red Latosol	29.94 A	2.01 B	*Eugenia dysenterica* *Qualea multiflora*

Total Al (dag kg^{-1}) and exchangeable Al (Al^{+3}; $cmol_c$ dm^{-3}). *Means followed by the same letters do not differ statistically by Tukey test at the 5% probability level (see chapter 1).

Plant sampling

Three species with wide occurrence at the FLONA of Paraopeba were selected for the study: *Eugenia dysenterica* DC. (Myrtaceae), *Qualea parviflora* Mart., and *Q. multiflora* Mart. (Vochysiaceae). Five individuals of each species, all being above 2 m high, were selected in plots of 20 x 100 m established by Tolentino (2011) and Neri et al. (2012) in each collection site. Samples of stems in secondary growth and fully expanded leaves were collected from at least two of the three species in each site, as described in Table 1.

Aluminum quantification in shoots

Stem and leaf samples were oven-dried at 70 °C, powdered in a knife mill and sieved through a 200-mesh (74 µm) stainless steel sieve. The sieved material was accurately weighed (ca. 250 mg) and digested with nitric and hydrofluoric acid in closed digestion tubes on graphite block (adapted from Paye 2014). The resulting solutions were cooled down to room temperature and their final volume was completed to 12 mL with

deionized water. Aluminum content was determined by inductively coupled plasma atomic emission spectrometry (ICP AES; Optima 8300 DV, Perkin Elmer, Shelton, CT, USA). For certification of the digestion method and of elemental quantification by ICP AES, a certificate reference sample (bush leaves, GBW 07603) and analytical blanks were prepared in the same way as samples in each digestion batch.

Microchemical Al mapping by energy dispersive X-ray microfluorescence

Pellets were prepared after applying a 9 t cm^{-2} pressure (Perkin Elmer, Waltham, MA, USA) during 1 min on 250 mg of leaf samples (particle size lower than 74 μm). The Al X-ray intensity was analyzed on an area of 40 x 30 mm (1200 measurement points; 100 μm step per point) using a benchtop spectrometer (μ-EDX-1300, Shimadzu, Kyoto, Japan) coupled with a Rh X-ray tube and a Si(Li) semiconductor detector. For microchemical Al mapping, oven-dried leaves were horizontally fixed on pure cellulose supports using adhesive tape. Areas of 40 x 30 mm (1200 measurement points; 100 μm step per point) were selected on the median portion of leaves, including the leaf margin and midrib. A calibration curve was obtained using the Al concentrations determined by ICP AES and X-ray fluorescence, after which it was used to generate quantitative maps.

Histochemical test for Al localization

Stem and leaf samples were fixed in Karnovsky solution (Karnovsky 1965), dehydrated in an ethanol series, and embedded in methacrylate resin (Historesin, Leica Instruments, Nußloch/Heidelberg, Germany). Cross sections (8 μm thick) were obtained using a rotary microtome (Spencer 820, American Optical, Buffalo, NY, USA) and subjected to a reaction with 0.5% aqueous chrome azurol S (Kukachka and Miller 1980) for 1 h, for Al detection. Positive reactions were identified by bluish staining. Photographs were taken using a light microscope (Olympus AX70TRF, Olympus Optical, Tokyo, Japan) equipped with an image capture system (Axio Vision Release 4.8.1, Carl Zeiss Vision, Jena, Germany).

Determination of relative Al abundance in plant tissues

Stem and leaf samples were fixed in Karnovsky solution (Karnovsky 1965), dehydrated in an ethanol series, and critical-point dried (CPD 030, Bal-Tec, Balzers,

Liechtenstein) using CO_2. After drying, samples were sputter-coated with carbon (Quorum Q150 T, East Grinstead, West Sussex, UK) and analyzed using an X-ray probe (X-EDS, IXRF systems, Houston, TX, USA) coupled to a scanning electron microscope (LEO 1430 VP, Zeiss, Cambridge, Cambridgeshire, UK), at the Center for Microscopy and Microanalysis (NMM) of Universidade Federal de Viçosa. The elements Al, C, Ca, Fe, Mg, N, S, and Si were selected in order to determine the relative Al abundance in plant tissues. The average percentage of Al in each tissue was calculated for evaluation of the allocation pattern of the metal in the species.

Statistical analyses

Data was subjected to analysis of variance (ANOVA) in a 3 x 5 factorial scheme (three species and five collection sites) with five replicates, using software Sisvar (Ferreira 2011). Means were compared using Tukey test at the 0.05 significance level.

Results

Aluminum concentration in shoots

The average Al concentrations in the stem and leaves of *E. dysenterica* were 0.2 and 0.3 g kg^{-1} DW, respectively, neither of which differed among collection sites (Fig. 1). *Q. parviflora* and *Q. multiflora* showed an average 3.1 g Al kg^{-1} DW in stems, and only in C*ss*-HCD did the former species accumulate significantly more Al than the latter. In leaves, the average Al concentration was 13.5 g kg^{-1} DW in *Q. parviflora* and 19.8 g kg^{-1} DW in *Q. multiflora*, both of which showed the highest mean Al accumulation at all collection sites (Fig. 1). We observed that Al concentration in leaves of *Q. parviflora* did not differ among collection sites, and that in stems such concentration showed the lowest values in plants from MC-RL. On the other hand, Al concentration in *Q. multiflora* leaves was higher in plants from C*ss*-RYL than in those from the other sites, whereas no significant difference was observed in the Al content in plant stems.

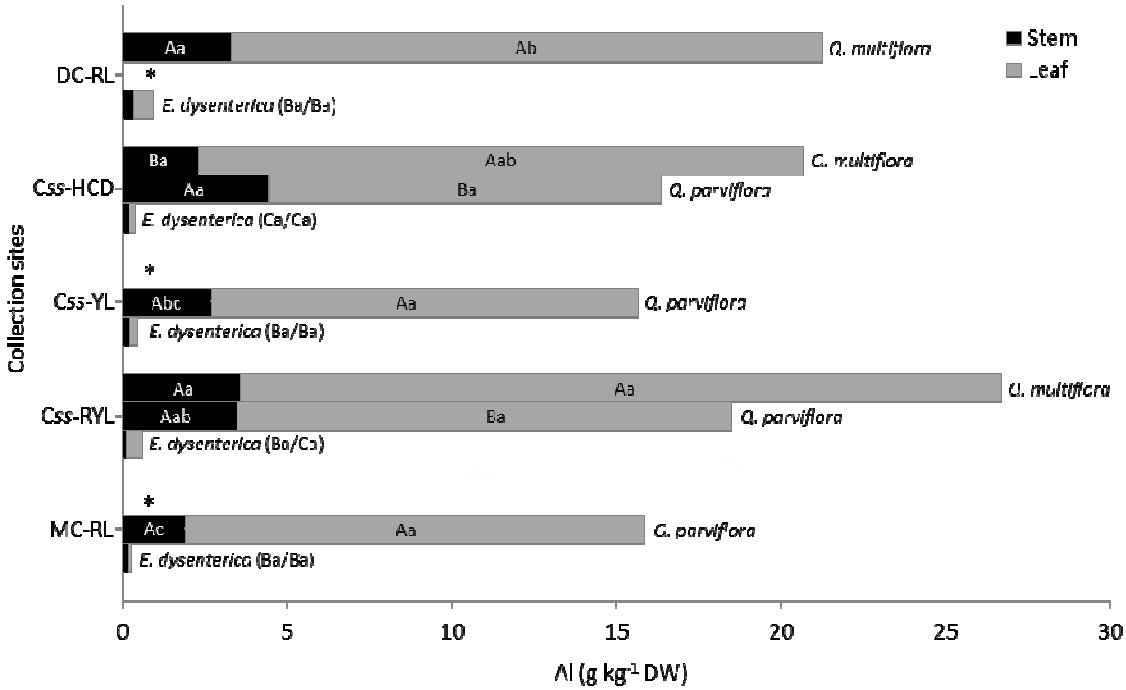


Fig. 1 Aluminum concentration assessed by ICP AES in stems and leaves of *Qualea multiflora*, *Q. parviflora*, and *Eugenia dysenterica* from the Cerrado at the National Forest of Paraopeba, southeastern Brazil. DC-RL: Dystrophic Cerradão on Red Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; MC-RL: Mesotrophic Cerradão on Red Latosol; (*) non-sampled species. Means followed by the same letters do not differ statistically by Tukey test (p = 0.05). Capital letters compare species and lowercase letters compare collection sites.

Sites of Al accumulation

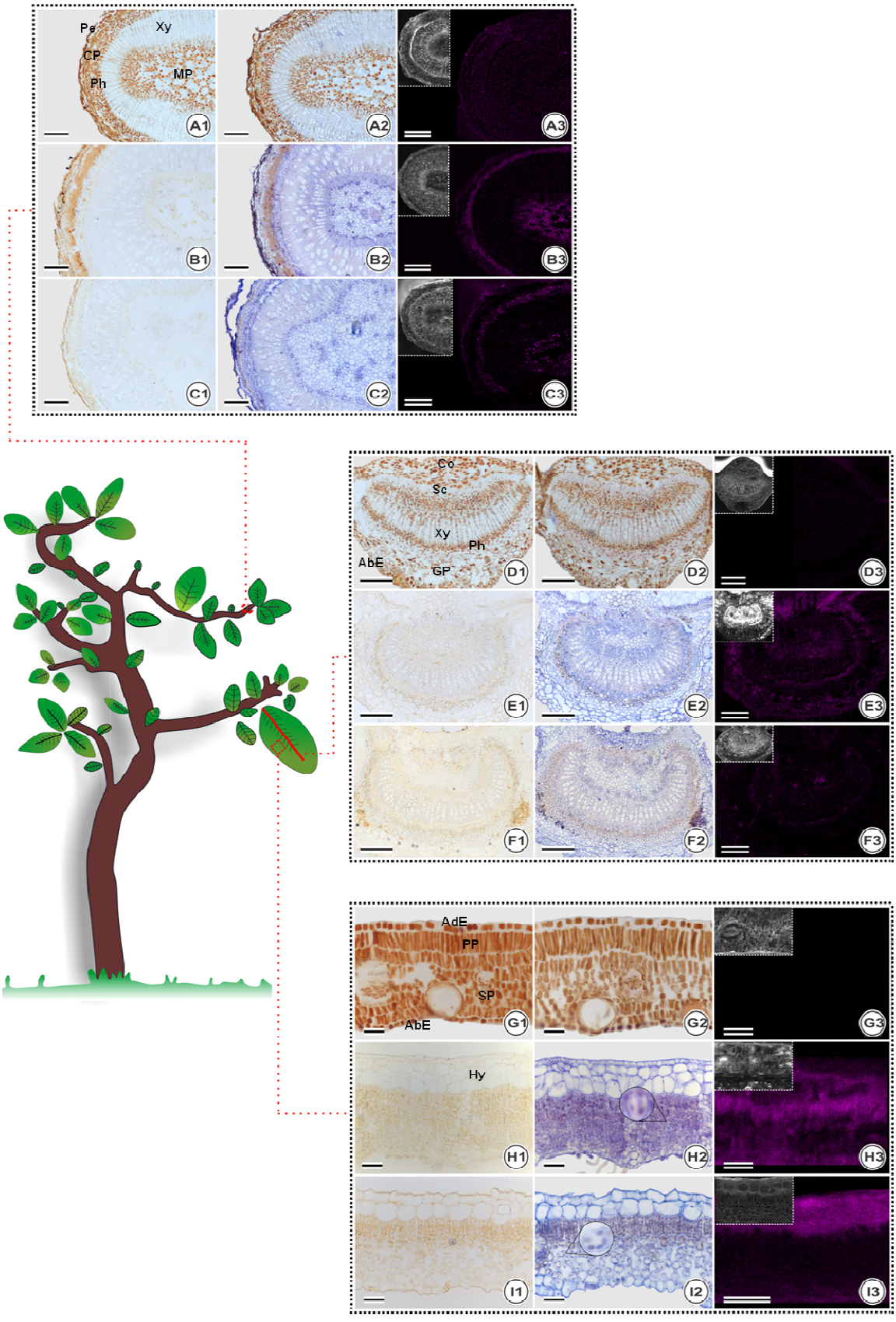
E. dysenterica showed negative results in the histochemical test for Al localization in all stem and leaf tissues (Fig. 2A, D and G). In contrast, positive reactions (bluish staining) were observed in stems and leaves of *Q. parviflora* (Fig. 2B, E and H) and *Q. multiflora* (Fig. 2C, F and I), primary cell walls being the main sites of Al accumulation in both species. In leaves, Al was accumulated in the epidermis; hypodermis; palisade and spongy parenchymas; collenchyma; xylem parenchyma; and sieve tube elements, companion cells, and parenchyma of the phloem. In stems, the metal was detected in cortical and medullary parenchymas, phloem components, vascular cambium, and xylem parenchyma. Besides pectocellulosic cell walls, the presence of Al was also observed in

chloroplasts on leaves and in suberized cell walls on stems. Lignified cell walls, including those of xylem vessel elements and sclerenchyma fibers, showed negative results for Al presence (Table 2). Al localization did not differ among collection sites.

Table 2 Sites of Al immobilization, detected by histochemical test with chrome azurol S in stems and leaves of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora* from the Cerrado at the National Forest of Paraopeba, southeastern Brazil

Tissue	Structure / Cell type	*E. dysenterica*		*Q. parviflora*		*Q. multiflora*	
		Stem	Leaf	Stem	Leaf	Stem	Leaf
Epidermis	Outer cell wall	a[a]	-	a	+	a	+
	Cell wall	a	-	a	+	a	+
	Trichome	a	a	a	+	a	+
Hypodermis	Cell wall	a	a	a	+	a	+
Phelloderm	Cell wall	-	a	-	a	-	a
Phellogen	Cell wall	-	a	-	a	-	a
Phellem	Cell wall	-	a	+	a	+	a
Parenchyma	Cell wall	-	-	+	+	+	+
	Chloroplast	a	-	a	+	a	+
Collenchyma	Cell wall	a	-	a	+	a	+
Sclerenchyma	Fiber	-	-	-	-	-	-
Phloem	Sieve tube element	-	-	+	+	+	+
	Companion cell	-	-	+	+	+	+
Vascular cambium	Parenchyma	-	-	+	+	+	+
	Cell wall	-	a	+	a	+	a
Xylem	Vessel element	-	-	-	-	-	-
	Fiber	-	-	-	-	-	-
	Parenchyma	-	-	+	+	+	+

[a] (a) absent structure or tissue; (+) positive reaction; (-) negative reaction


Pe
Xy
CP
MP
Ph
A1
A2
A3
B1
B2
B3
C1
C2
C3
Co
Sc
Xy
Ph
AbE
GP
D1
D2
D3
E1
E2
E3
F1
F2
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I2
I3

Fig. 2 Aluminum localization in the stem (A-C), midrib (D-F), and leaf blade (G-I) of *Eugenia dysenterica* (A, D, G), *Qualea parviflora* (B, E, H), and *Q. multiflora* (C, F, I) plants from the Cerrado at the National Forest of Paraopeba, southeastern Brazil. Column 1: negative control; column 2: histochemistry with chrome azurol S; column 3: Al mapping by scanning electron microscopy coupled with energy dispersive spectroscopy; detail in H2 and I2: chloroplast. *AbE* abaxial epidermis, *AdE* adaxial epidermis, *Co* collenchyma, *CP* cortical parenchyma, *GP* ground parenchyma, *Hy* hypodermis, *MP* medullary parenchyma, *Pe* periderm, *Ph* phloem, *PP* palisade parenchyma, *Sc* sclerenchyma, *SP* spongy parenchyma, *Xy* xylem. Bars: (A-F) = 200 µm, (G-I) = 50 µm.

Relative Al abundance in tissues

X-ray microanalysis coupled with scanning electron microscopy demonstrated the occurrence of low relative Al abundance, from the order of 0.2% to 1.3%, in stem and leaf tissues of *E. dysenterica* (Table 3 and Supplemental table SI). The percentage of the metal in this species was significantly lower than those observed in *Q. parviflora* (2.0% to 8.8%) and *Q. multiflora* (2.1% to 7.7%), except in sclerenchyma fibers and xylem vessel elements, both of which had relatively low mean Al values in all species (Table 3 and Supplemental table SI). A comparison of the relative Al abundance in *E. dysenterica* from distinct collection sites showed difference on neither stems nor leaf blades (Supplemental table SI). In the midrib, there was a difference only in the abaxial epidermis and sclerenchyma, both of which had the highest mean values at C*ss*-HCD and C*ss*-YL, respectively. *Q. parviflora* showed no pattern of Al accumulation related to soil, while in *Q. multiflora* the highest relative abundances of the metal were observed at C*ss*-RYL, especially in leaf tissues (Supplemental table SI).

Table 3 Comparison of mean relative Al abundances (%), determined by scanning electron microscopy coupled with an X-ray probe, in stem and leaf tissues of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora* collected in Cerrado sites at the National Forest of Paraopeba, southeastern Brazil

	Tissue	*E. dysenterica*	*Q. parviflora*	*Q. multiflora*
Stem	Cortical parenchyma	0.57 Ac*	5.03 Ba	2.11 BCb
	Medullary parenchyma	0.54 Ac	8.85 Aa	5.09 Ab
	Periderm	0.93 Ab	3.40 Ca	2.15 BCab
	Phloem	0.67 Ab	4.08 BCa	3.01 Ba
	Sclerenchyma	0.59 Aa	0.71 Da	0.75 Ca
	Xylem	0.58 Aa	0.90 Da	1.26 BCa
Midrib	Abaxial epidermis	0.98 Aa	2.06 CDa	2.12 CDa
	Adaxial epidermis	0.64 Ab	3.04 BCa	3.47 ABCa
	Collenchyma	0.57 Ab	2.88 BCa	4.10 Aba
	Ground parenchyma	0.48 Ab	4.20 ABa	5.26 Aa
	Phloem	0.60 Ab	5.31 Aa	4.21 Aba
	Sclerenchyma	0.64 Aa	0.59 Da	0.92 Da
	Xylem	0.46 Ab	1.12 Dab	2.27 BCDa
Leaf blade	Abaxial epidermis	0.68 Ab	5.70 Ba	5.99 ABa
	Adaxial epidermis	0.80 Ab	8.10 ABa	7.04 ABa
	Hypodermis	-	8.27 Aa	7.72 Aa
	Palisade parenchyma	0.66 Ac	7.21 ABa	3.19 Cb
	Spongy parenchyma	0.70 Ac	7.25 ABa	4.59 BCb

* Different letters indicate a significant difference (Tukey test, $p < 0.05$). Capital letters compare plant tissues and lowercase letters compare species.

A comparison of the Al allocation pattern in tissues revealed a difference between species (Table 3). In *E. dysenterica*, there was no difference between the Al percentage in stem and leaf tissues. In contrast, in stems of *Q. parviflora* and *Q. multiflora* the highest Al accumulation was observed in the medullary parenchyma (the innermost tissue). In leaves, *Q. parviflora* showed highest Al percentage in the midrib phloem, whereas in *Q. multiflora* this percentage was similar in the collenchyma, ground parenchyma, and phloem. The most interesting difference was observed in the leaf blade. While *Q. parviflora* accumulated higher Al percentage in the hypodermis, with similar relative abundances being observed in the palisade and spongy parenchymas, *Q. multiflora* showed the highest Al accumulation in the hypodermis, yet the palisade and spongy parenchymas had the lowest percentage of the metal (Table 3). Microchemical Al mapping along with the results of relative Al abundance revealed that in both *Qualea* species the Al accumulation occurred preferentially in leaf

blade tissues, from the midrib to leaf margin, whereas in *E. dysenterica* no significant difference was observed.

Discussion

Plants developed different strategies to cope with the high Al availability in acid soils (Poschenrieder et al. 2008). Most Al-resistant species exclude Al from both roots and shoots (García-Oliveira et al. 2014), as observed in *Eugenia dysenterica*, which has an Al concentration of about 0.8 g kg^{-1} DW in its roots (GS Tolentino, personal communication) and accumulates even lower amounts of the metal in its stems and leaves (ca. 0.2 and 0.3 g Al kg^{-1} DW, respectively) (Fig. 1). On the other hand, Al-hyperaccumulator species are capable to accumulate several thousands of mg of Al per kg dry weight in leaves (Poschenrieder et al. 2015), as shown by *Qualea parviflora* and *Q. multiflora*, both of which accumulated the metal at levels higher than 12.0 g Al kg^{-1} DW at all collection sites (Fig. 1).

Species that accumulate Al require extremely efficient mechanisms of detoxification and compartmentalization of the toxic Al^{3+} (Poschenrieder et al. 2015), one of which is Al sequestration in cell walls. Al hyperaccumulation is believed to be dependent on transpiration, which is why leaf margins and the walls of cells from upper epidermis usually have the highest Al concentrations (Shen and Ma 2001), as reported to *Camellia sinensis* (Tolrà et al. 2011; Hajiboland and Poschenrieder 2015). However, Al-accumulator species can show different mechanisms (Maejima et al. 2014), and differences may occur even between species in the same genus, as observed with *Q. parviflora* and *Q. multiflora* in our study. Despite the fact that they accumulated highest Al concentration in the leaf margins, distribution of the metal in tissues does not seem to follow the transpiration stream. Whilst in *Q. parviflora* photosynthetic and non-photosynthetic tissues showed similar Al accumulation, in *Q. multiflora* the highest Al percentages were observed in less metabolically active tissues (Tables 3 and S1), which may be a strategy to avoid contact between the metal and essential biochemical processes (Grevenstuk and Romano 2013). This difference may contribute to the capacity of *Q. multiflora* to accumulate higher Al concentration than *Q. parviflora*, as demonstrated by our results (Fig. 1).

Although the mechanisms involved in Al resistance in native accumulator plants are still poorly understood, it is believed that the metal taken up in its free form or complexed

with (in)organic ligands is transported by xylem bound to citrate (Grevenstuk and Romano 2013; Poschenrieder et al. 2015). Once in the shoot, Al can be redistributed via phloem from leaves to roots and seeds (Zeng et al. 2013), as indicated by the presence of Al in seeds of *Q. parviflora* (Arcanjo-Silva et al., unpublished data). Occasionally, Al reacts with pectins in walls of phloem cells (Bressan et al. 2016) and is accumulated in that tissue, as observed in stems and leaves of *Q. multiflora* and *Q. parviflora* (Table 2 and Fig. 3).

The sites of Al immobilization did not vary with Al concentration in soils, and sequestration of the metal in pectocellulosic cell walls can be considered an Al resistance mechanism in both Al-excluders and Al-accumulators (Malta et al. 2016). However, in excluder species the Al is retained in walls of root cells, and it therefore does not penetrate the plant (Brunner and Sperisen 2013); this seems be one of the mechanisms that avoid Al uptake in *E. dysenterica*. In contrast, Al-accumulator species such as *Q. parviflora* and *Q. multiflora* can sequestrate the metal within cell walls in the shoot, preventing it from being in direct contact with metabolically active structures and biochemical processes (Grevenstuk and Romano 2013).

Despite the preferential accumulation in less active structures, recent discoveries of Al deposition in chloroplasts of native hyperaccumulator Cerrado species (Andrade et al. 2011; Malta et al. 2016) and now of the also Cerrado-native *Q. multiflora* (Fig. 3I) and *Q. parviflora* (Fig. 3H) – conversely to what was observed by Bressan et al. (2016) – have evoked the question of whether Al has some unknown function in these organelles and in the metabolism of aluminophile plants (Haridasan 2008a). Besides the Al accumulation in chloroplasts and pectocellulosic cell walls, the occurrence of the metal in suberized walls of peridermal cells have also been observed and recently described in *Rudgea viburnoides* (Malta et al. 2016). Although Bressan et al. (2016) had reported the existence of Al-constitutive granules in parenchyma cavities on the midrib of *Q. parviflora*, no such structures were observed in our study. More research is necessary to investigate the Al-suberin interaction and to clarify the biochemical mechanisms involved in Al accumulation within chloroplasts.

Our data demonstrates that the Al allocation pattern does not depend on Al concentration in soils and that it may be related to the capacity of native Cerrado species to accumulate Al. *E. dysenterica* (an Al-non-accumulator species) showed no preferential site for Al deposition in either stem or leaf tissues. *Q. parviflora* and *Q. multiflora* (both Al-

hyperaccumulator species), on the other hand, deposited most of the Al in the innermost stem tissue; however, there was difference in their Al allocation patterns on leaves. The avoidance of Al deposition in photosynthetic tissues of *Q. multiflora* may contribute for the higher capacity of that species to accumulate the metal. The occurrence of Al in chloroplasts was once again observed and seems to be a common feature among native Al-hyperaccumulator species from the Brazilian Cerrado.

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References

Andrade L.R.M., Barros L.M.G., Echevarria G.F., Amaral L.I.V., Cotta M.G., Rossatto D.R., Haridasan M., Franco A.C. (2011) Al-hyperaccumulator Vochysiaceae from the Brazilian Cerrado store aluminum in their chloroplasts without apparent damage. *Environmental and Experimental Botany*, **70**, 37–42.

Bressan A.C.G., Coan A.I., Habermann G. (2016) X-ray spectra in SEM and staining with chrome azurol S show Al deposits in leaf tissues of Al-accumulating and non-accumulating plants from the cerrado. *Plant and Soil*, **404**, 293–306.

Brunner I., Sperisen C. (2013) Aluminum exclusion and aluminum tolerance in woody plants. *Frontiers in Plant Science*, **4**, 1–12.

Campos N.V., Pereira T.A.R., Machado M.F., Guerra M.B.B., Tolentino G.S., Araújo J.S., Rezende M.Q., Silva M.C.N.A., Schaefer C.E.G.R. (2014) Evaluation of micro-energy dispersive x-ray fluorescence and histochemical tests for aluminium detection in plants from High Altitude Rocky Complexes, Southeast Brazil. *Anais da Academia Brasileira de Ciências*, **86**, 365–376.

Chang Y.C., Yamamoto Y., Matsumoto H. (1999) Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. *Plant, Cell & Environment*, **12**, 1009–1017.

Ferreira D.F. (2011) Sisvar: A computer statistical analysis system. *Ciência e Agrotecnologia*, **35**, 1039–1042.

Fujii M., Yokosho K., Yamaji N., Saisho D., Yamane M., Takahashi H., Sato K., Nakazono M., Ma J.F. (2012) Acquisition of aluminium tolerance by modification of a single gene in barley. *Nature Communications*, **3**, 713.

Gao H.J., Zhao Q., Zhang X.C., Wan X.C., Mao J.D. (2014) Localization of fluoride and aluminum in subcellular fractions of tea leaves and roots. *Journal of Agricultural and Food Chemistry*, **62**, 2313–2319.

García-Oliveira A.L., Martins-Lopes P., Tolrà R., Poschenrieder C., Tarquis M., Guedes-Pinto H., Benito C. (2014) Molecular characterization of the citrate transporter gene TaMATE1 and expression analysis of upstream genes involved in organic acid transport under Al stress in bread wheat (*Triticum aestivum*). *Physiologia Plantarum*, **152**, 441–452.

Grevenstuk T., Romano A. (2013) Aluminium speciation and internal detoxification mechanisms in plants: where do we stand? *Metallomics*, **5**, 1584–1594.

Hajiboland R., Poschenrieder C. (2015) Localization and compartmentation of Al in the leaves and roots of tea plants. *Phyton*, **84**, 86–100.

Haridasan M. (2008a) Alumínio é um elemento tóxico para as plantas nativas do cerrado? In: Prado C.H.B.A., Casali C.A. (Eds), *Fisiologia Vegetal: Práticas em Relações Hídricas, Fotossíntese e Nutrição Mineral*. Manole, Barueri, Brazil: 1–10.

Haridasan M. (2008b). Nutritional adaptations of native plants of the cerrado biome in acid soils. *Brazilian Journal of Plant Physiology*, **20**, 183–195.

Hartwig I., Oliveira A.C., Carvalho F.I.F., Bertan I., Silva J.A.G., Schmidt D.A.M., Valério I.P., Maia L.C., Fonseca D.A.R., Reis C.E.S. (2007) Mecanismos associados à tolerância ao alumínio em plantas. *Semina: Ciências Agrárias*, **28**, 219–228.

Jansen S., Broadley M.R., Robbrecht E., Smets E. (2002) Aluminum hyperaccumulation in Angiosperms: A review of its phylogenetic significance. *The Botanical Review*, **68**, 235–269.

Karnovsky M.J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology*, **27**, 137–138.

Kukachka B.F., Miller R.B. (1980) A chemical spot-test for aluminum and its value in wood identification. *IAWA Bulletin*, **1**, 104–109.

Li J.-Y., Liu J., Dong D., Jia X., McCouch S.R., Kochian L.V. (2014) Natural variation underlies alterations in Nramp aluminum transporter (NRAT1) expression and function that play key role in rice aluminum tolerance. *Proceedings of the National Academy of Sciences USA*, **111**, 6503–6508.

Maejima E., Hiradate S., Jansen S., Osaki M., Watanabe T. (2014) Comparative analysis of aluminum accumulation in leaves of three angiosperm species. *Botany*, **92**, 327–331.

Malta P.G., Arcanjo-Silva S., Ribeiro C., Campos N.V., Azevedo A.A. (2016) *Rudgea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplasts. *Plant and Soil*, **408**, 369–384.

Matsumoto H. (2000) Cell biology of aluminum toxicity and tolerance in higher plants. *Internation Review of Cytology*, **200**, 1–46.

Neri A.V., Schaefer C.E.G.R., Silva A.F., Souza A.L., Ferreira-Júnior W.G., Meira-Neto J.A.A. (2012) The influence of soils on the floristic composition and community structure of an area of Brazilian Cerrado vegetation. *Edinburgh Journal of Botany*, **69**, 1–27.

Olivares E., Peña E., Marcano E., Mostacero J., Aguiar G., Benítez M., Rengifo E. (2009) Aluminum accumulation and its relationship with mineral plant nutrients in 12 Pteridophytes from Venezuela. *Environmental and Experimental Botany*, **65**, 132–141.

Paye H.S. (2014) Teor e distribuição de elementos terras raras e comparação de métodos de extração de elementos traço em solos brasileiros. Thesis. Universidade Federal de Viçosa, Viçosa, Brazil: 84 pp.

Poschenrieder C., Gunsé B., Corrales I., Barceló J. (2008) A glance into aluminum toxicity and resistance in plants. *Science of the Total Environment*, **400**, 356–368.

Poschenrieder C., Tolrà R., Hajiboland R., Arroyave C., Barceló J. (2015) Mechanisms of hyper-resistance and hyper-tolerance to aluminum in plants. In: Panda S.K., Baluška F. (Eds), *Aluminum Stress Adaptation in Plants*. Springer International Publishing, Switzerland: 81–98.

Reyna-Llorens I., Corrales I., Poschenrieder C., Barceló J., Cruz-Ortega R. (2015) Both aluminum and ABA induce the expression of an ABC-like transporter gene (FeALS3) in the Al-tolerant species *Fagopyrum esculentum*. *Environmental and Experimental Botany*, **111**, 74–82.

Ribeiro J.F., Walter B.M.T. (2008) As principais fitofisionomias do bioma Cerrado. In: Sano S.M., Almeida S.P., Ribeiro J.F. (Eds), *Cerrado: Ecologia e Flora*, Embrapa Cerrados, Brasília, Brazil: 152–212.

Sano S.M., Almeida S.P., Ribeiro J.F. (2008) Cerrado: Ecologia e Flora. Embrapa Cerrados, Brasília, Brazil: 408 pp.

Shen R., Ma J.F. (2001) Distribution and mobility of aluminium in an Al-accumulating plant, *Fagopyrum esculentum* moench. *Journal of Experimental Botany*, **52**, 1683–1687.

SNUC – Sistema Nacional de Unidades de Conservação da Natureza. (2000). *Lei nº 9.985, de 18 de julho de 2000*. MMA/SBF.

Tolentino G.S. (2011) Composição e partição de nicho em gradientes de solo e luz no Cerrado. Dissertation, Universidade Federal de Viçosa, Viçosa, Brazil: 80 pp.

Tolrà R., Vogel-Mikus K., Hajiboland R., Kump P., Pongrac P., Kaulich B., Gianoncelli A., Babin V., Barceló J., Regvar M., Poschenrieder C. (2011) Localization of aluminium in tea (*Camellia sinensis*) leaves using low energy X-ray fluorescence spectro-microscopy. *Journal of Plant Research*, **124**, 165–172.

Vázquez M.D., Poschenrieder C., Corrales I., Barceló J. (1999) Change in apoplastic aluminum during the initial growth response to aluminum by roots of a tolerant maize variety. *Plant Physiology*, **119**, 435–444.

Vitorello V.A., Capaldi F.R., Stefanuto V.A. (2005) Recent advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology*, **17**, 129–143.

von Uexküll H.R., Mutert E. (1995) Global extent, development and economic impact of acid soils. *Plant and Soil*, **171**, 1–15.

Zeng Q.L., Chen R.F., Zhao X.Q., Shen R.F., Noguchi A., Shinmachi F., Hasegawa I. (2013) Aluminum could be transported via phloem in *Camellia oleifera* Abel. *Tree Physiology*, **33**, 96–105.

Supplemental table SI Relative abundance (%) of Al, determined by scanning electron microscopy coupled with an X-ray probe, in different stem and leaf tissues of *Eugenia dysenterica*, *Qualea parviflora*, and *Q. multiflora* collected from five environments in Cerrado sites at the National Forest of Paraopeba, southeastern Brazil

Species	Collection site	Stem						Midrib							Leaf blade				
		CP	Pe	Ph	MP	Sc	Xy	AbE	AdE	Co	GP	Ph	Sc	Xy	AbE	AdE	Hy	PP	SP
E. dysenterica	MC-RL	0.37 Ab*	0.75 Ab	0.37 Ab	0.29 Ab	0.60 Ab	0.42 Ab	0.25 Bb	0.43 Ab	0.42 Ab	0.64 Ab	0.55 Ab	0.54 Ba	0.48 Aa	0.60 Ab	0.95 Ab	-	0.49 Ab	0.67 Ab
E. dysenterica	C*ss*-RYL	0.29 Ab	0.89 Ab	1.00 Ab	0.56 Ab	0.56 Aa	0.90 Aa	0.37 Bb	0.47 Ab	0.77 Ab	0.34 Ab	0.65 Ab	0.61ABb	0.46 Aa	0.87 Ac	0.44 Ab	-	0.65 Ac	0.58 Ab
E. dysenterica	C*ss*-YL	0.66 Ab	0.68 Ab	0.39 Aa	0.63 Ab	0.56 Aa	0.68 Aa	0.57 Ba	0.57 Ab	0.81 Ab	0.59 Ab	0.80 Ab	0.83 Aa	0.39 Ab	0.69 Ab	1.02 Ab	-	0.78 Ab	0.76 Ab
E. dysenterica	C*ss*-HCD	1.06 Ab	1.03 Ab	0.99 Ab	0.64 Ab	0.54 Aa	0.40 Ab	3.32 Aa	1.11 Ab	0.47 Ab	0.37 Ab	0.56 Ab	0.60ABab	0.56 Ab	0.59 Ac	1.01 Ac	-	0.84 Ab	0.96 Ab
E. dysenterica	DC-RL	0.47 Ab	1.29 Aa	0.62 Ab	0.58 Ab	0.72 Aa	0.52 Ab	0.39 Ba	0.61 Aa	0.37 Aa	0.44 Aa	0.46 Aa	0.63 ABa	0.41 Aa	0.62 Aa	0.57 Ab	-	0.52 Aa	0.51 Ab
Q. parviflora	MC-RL	3.14 Ca	2.71 Ba	4.27 Aa	8.46 Ba	0.93 Aa	0.98 Aa	1.86 Aa	1.83 Aa	5.34 Aa	5.24 Aa	7.52 Aa	0.52 Aa	1.05 Aa	5.18 Aa	9.72 Aa	15.11 A-	7.80 Aa	5.06 BCa
Q. parviflora	C*ss*-RYL	7.23 Aa	2.23 BCa	3.82 Aa	12.41 Aa	0.52 Ba	0.79 Aa	1.37 Aab	2.48 Aa	1.23 Bb	2.64 Ab	8.14 Aa	0.57 Ab	1.05 Aa	3.36 Ab	10.93 Aa	7.28 Ba	7.78 Aa	6.07 ABa
Q. parviflora	C*ss*-YL	5.14 Ba	4.47 Aa	1.39 Ba	6.21 Ba	0.75 ABa	0.68 Aa	1.42 Aa	1.83 Aa	2.78 Ba	4.92 Aa	4.44 Ba	0.66 Aa	1.72 Aa	4.60 Aa	6.03 Ba	5.45 B-	7.27 Aa	6.88 Aa
Q. parviflora	C*ss*-HCD	3.41 Ca	1.15 Cb	4.37 Aa	6.78 Ba	0.75 ABa	0.86 Aab	1.71 Ab	2.16 Ab	2.60 Ba	4.49 Aa	2.66Bab	0.56 Ab	0.76 Ab	3.80 Ab	5.13 Bb	5.12 Bb	5.49 Aa	4.17 Ca
Q. multiflora	C*ss*-RYL	1.25 Bb	1.15 Bab	1.79 Ab	1.60 Ab	0.76 Aa	0.58 Ba	2.45 Aa	3.53 Ba	7.13 Aa	8.89 Aa	2.63 Ab	1.09 Aa	0.90 Ba	8.99 Aa	9.33 Aa	9.85 Aa	3.78 Ab	6.18 Aa
Q. multiflora	C*ss*-HCD	1.23 Bb	3.75 Aa	1.47 Ab	2.58 Ab	-	1.28 Aa	2.25ABab	5.97 Aa	2.67 Ba	1.82 Bb	3.21 Aa	0.80 Ba	4.22 Aa	6.33 Ba	8.65 Aa	9.49 Aa	1.65 Ab	3.99 Ba
Q. multiflora	DC-RL	3.66 Aa	1.29 Ba	2.78 Aa	3.24 Aa	0.85 Aa	1.33 Aa	1.21 Ba	1.71 Ca	1.02 Ba	2.03 Ba	2.29 Aa	0.63 Ba	1.00 Ba	2.00 Ca	3.30 Ba	3.76 B-	2.31 Aa	2.31 Ca

AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; Co: Collenchyma; CP: Cortical Parenchyma; GP: Ground Parenchyma; Hy: Hypodermis; MP: Medullary parenchyma; Pe: Periderm; Ph: Phloem; PP: Palisade Parenchyma; Sc: Sclerenchyma; SP: Spongy Parenchyma; Xy: Xylem; MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-YL: Cerrado *sensu stricto* on Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic; DC-RL: Dystrophic Cerradão on Red Latosol; (-): no data obtained. *Different letters indicate a significant difference (Tukey test, $p < 0.05$). Capital letters compare environments and lowercase letters compare species.

CHAPTER 3 – Metabolic mechanisms involved in the resistance to acid soils in two native Cerrado species[(1)]

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ABSTRACT: Plants that grow on acid soils have developed mechanisms to resist adverse effects like low soil fertility and high soil acidity and metal toxicity, especially by aluminum (Al). However, the metabolic adaptations involved in the resistance of native species to such adverse conditions remain poorly understood. The metabolism of *Eugenia dysenterica* (Al-non-accumulator) and *Qualea parviflora* (Al-hyperaccumulator) plants naturally growing on acid soils with varying fertilities and metal toxicities was evaluated, and we observed that metabolic features differed between species. *E. dysenterica* showed higher levels of chlorophyll, nitrate, total amino acids, insoluble proteins, phenols, and thiobarbituric acid-reactive substances. In contrast, *Q. parviflora* had higher non-protein thiol concentration and was more efficient in avoiding lipid peroxidation. Metabolite profiling analysis demonstrated that on soils with high Al availability the synthesis of compatible osmolytes and dehydroascorbate was up-regulated in both species. *Q. parviflora* also showed increased levels of malate and succinate. These findings indicate that phenols, thiols, and organic acids play a role in the detoxification of Al and reactive oxygen species (ROS) in leaves of *Q. parviflora*. Despite the investment in production of antioxidant compounds, *E. dysenterica* was not efficient to control the occurrence of oxidative stress on acid soils with high metal toxicity. This higher susceptibility to oxidative stress may have contributed to the selection of Al exclusion as a resistance strategy in *E. dysenterica*.

Keywords: *Eugenia dysenterica*, *Qualea parviflora*, antioxidant compounds, organic acids, lipid peroxidation

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CAPÍTULO 3 – Mecanismos metabólicos envolvidos na resistência a solos ácidos em duas espécies nativas do Cerrado

RESUMO: Plantas que crescem em solos ácidos têm desenvolvido mecanismos que as permitem resistir a efeitos adversos, como baixa fertilidade e alta acidez e toxidez de metais, especialmente de alumínio (Al). Contudo, as adaptações metabólicas envolvidas na resistência de espécies nativas a tais condições adversas permanecem pobremente entendidas. O metabolismo de plantas de *Eugenia dysenterica* (não acumuladora de Al) e *Qualea parviflora* (hiperacumuladora de Al) crescendo naturalmente em solos ácidos com fertilidade e toxidez de metais variável foi avaliado e observou-se que as características metabólicas diferiram entre as espécies. *E. dysenterica* apresentou maiores níveis de clorofila, nitrato, aminoácidos totais, proteínas insolúveis, fenóis e substâncias reativas com ácido tiobarbitúrico. Em contraste, *Q. parviflora* teve maior concentração de tióis não proteicos e foi mais eficiente na evitação da peroxidação lipídica. A análise do perfil metabólico demonstrou que em solos com alta disponibilidade de Al a síntese de osmólitos compatíveis e de desidroascorbato foi aumentada em ambas as espécies. *Q. parviflora* também apresentou níveis aumentados de malato e succinato. Esses resultados indicam que fenóis, tióis e ácidos orgânicos atuam na destoxificação de Al e espécies reativas de oxigênio (EROs) em folhas de *Q. parviflora*. Apesar do investimento na produção de compostos antioxidantes, *E. dysenterica* não foi eficiente no controle do estresse oxidativo em solos com alta toxidez de metais. Esta maior susceptibilidade ao estresse oxidativo pode ter contribuído para a seleção da exclusão de Al como estratégia de resistência em *E. dysenterica*.

Palavras-chave: *Eugenia dysenterica*, *Qualea parviflora*, compostos antioxidantes, ácidos orgânicos, peroxidação lipídica

Introduction

Plant species naturally growing on acid soils developed a wide range of mechanisms to cope with adverse conditions. Adverse effects of soil acidity on plants stem mainly from the solubilization of aluminum (Al), manganese, and iron, any of which may reach toxic levels; leaching of soluble nutrients (such as calcium, magnesium, and potassium); and complexation of essential elements, especially phosphorus (Kochian et al. 2004, Pattanayak and Pfukrei 2013).

It is estimated that about 50% of the world's potentially arable lands are acidic and up to 60% of them are located in the tropics and subtropics (von Uexküll and Mutert 1995). Al toxicity is a major limiting factor to plant growth on acid soils, since when pH drops below 5.5, Al is solubilized as its trivalent cation (Al^{3+}) and becomes available for plant uptake (Serrano et al. 2011). Once absorbed, Al binds to multiple cellular sites, inhibiting root elongation, and roots then become stubby, brittle, and inefficient in absorbing water and nutrients (Gupta et al. 2013). Additionally, Al may cause reduction in stomatal opening and chlorophyll concentration, thus interfering with photosynthesis and transpiration (Vitorello et al. 2005, Wang et al. 2006), besides inducing the production of reactive oxygen species (ROS) and thereby promoting oxidative stress (Ma et al. 2012, Ribeiro et al. 2012).

The Cerrado, the second largest phytogeographic domain in Brazil (Olson et al. 2001), has soils with pH ranging between 4 and 5. This increases Al^{3+} concentration, which in turn becomes one of the main edaphic factors responsible for structural and floristic variation in the domain (Ribeiro and Walter 2008, Neri et al. 2012). Native Cerrado plants have evolved resistance mechanisms to avoid or tolerate Al^{3+} toxic effects, and according to those mechanisms such plants may be grouped in two categories: Al-excluders and Al-accumulators. Most of the plants that are adapted to counteracting Al stress prevent Al uptake through physical or biochemical barriers (Al-excluders), whereas a small number of plants absorb and store more than 1 g Al kg^{-1} DW on their aboveground tissues (Al-accumulators) (Jansen et al. 2003, Grevenstuk and Romano 2013).

Al-exclusion mechanisms may be based on Al complexation with organic acids (mainly citrate, malate, and oxalate) and other compounds (e.g. mucilage and phenols) exuded by root tips in the rhizosphere, which raises the rhizosphere pH; on the modification of Al^{3+}-binding sites in walls of root cells (Brunner and Sperisen 2013, Siecińska and

Nosalewicz 2017); or even on the exclusion of the absorbed Al by specific Al transporters (Hartwig et al. 2007). On the other hand, Al accumulation depends on Al detoxification by formation of less toxic organic Al complexes that prevent the contact between free Al and essential biochemical processes (Grevenstuk and Romano 2013).

Al detoxification strategies include sequestration in cell walls; complexation with organic and inorganic ligands in the cytoplasm; and compartmentalization in the apoplast or vacuole (Kochian et al. 2005, Grevenstuk and Romano 2013). Additionally, secondary mechanisms act to prevent/repair damage caused by the presence of free Al ions through combat of ROS by enzymatic and non-enzymatic antioxidants, such as phenolic compounds and thiols (Michalak 2006, Zagorchev et al. 2013).

Metabolite profiling is a useful and important tool to identify metabolite changes associated with plant response to stresses. However, studies with Al resistant species naturally growing on acid soils are scarce, and little is known on the physiology, biochemistry, and metabolic changes of these plants in response to soil adversity. Thereby, we assessed the metabolism of two species from the Brazilian Cerrado, *Eugenia dysenterica* (Myrtaceae, non-accumulator) and *Qualea parviflora* (Vochysiaceae, Al-hyperaccumulator), in order to elucidate the metabolic mechanisms involved in their resistance to acid soils with high Al^{3+} concentrations.

Material and Methods

Plant material

Eugenia dysenterica DC. (Myrtaceae) and *Qualea parviflora* Mart. (Vochysiaceae) are native Cerrado species naturally growing at the National Forest (FLONA) of Paraopeba, Minas Gerais state, southeastern Brazil (19°16'S; 44°23'W) (SNUC 2000). The FLONA has a well-marked soil-vegetation gradient determined by soil fertility and Al concentration (Neri et al. 2012). Samples of fully expanded leaves were collected from five individuals of each species, all being above 2 m high, selected in plots of 20 x 100 m established by Neri et al. (2012) at the Mesotrophic Cerradão on Red Latosol (MC-RL), Dense Cerrado *sensu stricto* on Red-Yellow Latosol (C*ss*-RYL), and Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic (C*ss*-HCD). Collections were performed during the autumn (May 2015), from 10:00 am to 12:00 pm. Soils in these sites have varying pH (6.59, 5.03, and 4.96, respectively), fertilities, Al^{3+} concentrations (0.00, 2.36, and 2.71 $cmol_c\ dm^{-3}$, respectively),

and total Al concentrations (20.48, 18.50, and 24.78 dag kg^{-1}, respectively) in their surface layer (0.0-0.2 m) (see chapter 1).

Biochemical analysis

Ethanolic extracts of leaf samples (50 mg) without the midrib, cooled in liquid nitrogen, were obtained according to the protocol of Kolbe et al. (2006). The soluble fraction was used for total chlorophyll, total free amino acid, and nitrate determinations, whereas the insoluble fraction was used for total insoluble protein analysis. The *a* and *b* chlorophylls, total free amino acids, and proteins were determined as detailed in Cross et al. (2006) and nitrate was determined as described by Fritz et al. (2006).

Metabolite profiling

Fully expanded leaves were collected in liquid nitrogen and 25-mg samples were homogenized using a ball mill. Extraction, derivatization, and gas chromatography–mass spectrometry (GC-MS) analysis were carried out as described by Lisec et al. (2006). Chromatograms and mass spectra were evaluated using software packages Chroma TOF 1.0 (Leco, http://www.leco.com/) and TARGET SEARCH (Cuadros-Inostroza et al. 2009). Metabolite identification was manually supervised using the mass spectral and retention index collection of the Golm Metabolome Database (Kopka et al. 2005). Peak heights of the mass fragments were normalized based on sample dry weight and on the added amount of the internal standard (ribitol). Fold change was calculated as the mean ratios of C*ss*-RYL and C*ss*-HCD samples compared with the means of MC-RL samples.

Antioxidant capacity analysis

Total thiols (TT) and non-protein thiols (NPT) were determined with 5,5´-dithiobis-(2-nitrobenzoic acid) (DTNB), following Sedlak and Lindsay (1968). Leaf samples (0.25 g) were homogenized in 0.3 mL of cold 0.1 M Tris–HCl, pH 8.0, 1 mM Na-EDTA and 1% (w/v) ascorbic acid. The homogenate was centrifuged at 10,000 *g* for 10 min at 4 °C. For TT analysis, 0.1 mL of the supernatant was mixed with the reaction buffer (0.3 mL of 0.2 mM potassium phosphate buffer, pH 8.2; 20 μL of 10 mM DTNB; and 1.58 mL of absolute methanol) and incubated for 15 min at 37 °C. NPT analysis was carried out using 1.0 mL of the supernatant mixed with 0.2 mL of 50% trichloroacetic acid (TCA) and 0.8 mL of

distilled water. After 1 h in ice bath, samples were centrifuged for 15 min at 10,000 *g*, and 0.4 mL of the supernatant was added to 0.8 mL of 0.4 M potassium phosphate buffer, pH 8.9, and 20 µL of 10 mM DTNB. Absorbances were measured at 412 nm (Multiskan GO, Thermo Scientific, Waltham, USA) and thiol concentration was estimated using the molar extinction coefficient of 14,150 mM^{-1} cm^{-1} (Riddles et al. 1979). The concentration of protein thiols (PT) was calculated by subtracting NPT from TT.

Total soluble phenol (TSP) concentration in leaves was evaluated using the Folin-Ciocalteu's reagent assay (Singleton et al. 1999). Leaf samples (0.25 g) were homogenized in 2 mL of 80% methanol and centrifuged at 12,900 *g* for 15 min at 4 ºC. The reaction mixture was made up with 7 µL of the supernatant (5-fold diluted), 93 µL of 80% methanol, and 950 µL of 10% (v/v) Folin-Ciocalteu's reagent. After 10 min, 950 µL of 7.5% (w/v) Na_2CO_3 were added to the mixture, after which the reaction was incubated at 45 ºC for 45 min. Then, a new centrifugation was performed for 3 min and the absorbance was measured at 765 nm using a microplate reader (Multiskan GO, Thermo Scientific, Waltham, USA). The results were expressed in milligrams of tannic acid equivalents per gram of fresh weight tissue (mg TA g^{-1} FW).

For phenol histolocalization, leaf samples were fixed in 10% ferrous sulphate in 4% formalin (Johansen 1940). The control test was performed by extracting phenols with methanol for 48 h before subjecting the material to the formalin solution. The material was then dehydrated in an ethanol series, embedded in methacrylate resin (Historesin, Leica), sectioned at 8 µm thick using a manual microtome (Spencer 820, American Optical, Buffalo, USA), and mounted in Permount.

Lipid peroxidation analysis

Concentration of thiobarbituric acid-reactive substances (TBARs) was used as an indicator of lipid peroxidation, being determined following Heath and Packer (1968). Leaf samples (0.20 g) were homogenized in 2.0 mL of 0.1% TCA and centrifuged at 10,000 *g* for 15 min at 4 ºC. The reaction mixture was made up with 1.0 mL of the supernatant and 1.0 mL of TBA reagent (20% w/v TCA + 0.5% w/v thiobarbituric acid), heated to 95 °C for 30 min, cooled for 15 min, and centrifuged at 10,000 *g* for 15 min. The amount of TBARs was measured by their specific absorbance at 532 nm, and the nonspecific absorbance at

600 nm was subtracted from the one at 532 nm. TBAR concentration was estimated using the molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical analyses

Data on the metabolite profiling was compared by Student's t-test at the 5% significance level. All other data was subjected to a two-way analysis of variance (ANOVA) in a 2 x 3 factorial scheme (two species and three collection sites), followed by comparison of means with Tukey test at the 5% significance level using software Sisvar (Ferreira 2011).

Results

Differences were observed in the photosynthetic pigment concentrations and nitrogen metabolism of *E. dysenterica* (non-accumulator species) and *Q. parviflora* (Al-hyperaccumulator species) (Fig. 1). *Q. parviflora* showed lower levels of chlorophyll, nitrate, and amino acids in comparison with *E. dysenterica* ($p < 0.01$), neither of which differed among collection sites. In contrast, *E. dysenterica* plants growing at C*ss*-HCD (the site with highest Al^{3+} concentration) showed lower concentrations of total chlorophyll and amino acids and higher concentrations of nitrate and protein than plants growing at the other sites. Protein concentration was lower in *Q. parviflora* than in *E. dysenterica* at C*ss*-RYL and C*ss*-HCD, and did not differ between species at MC-RL.

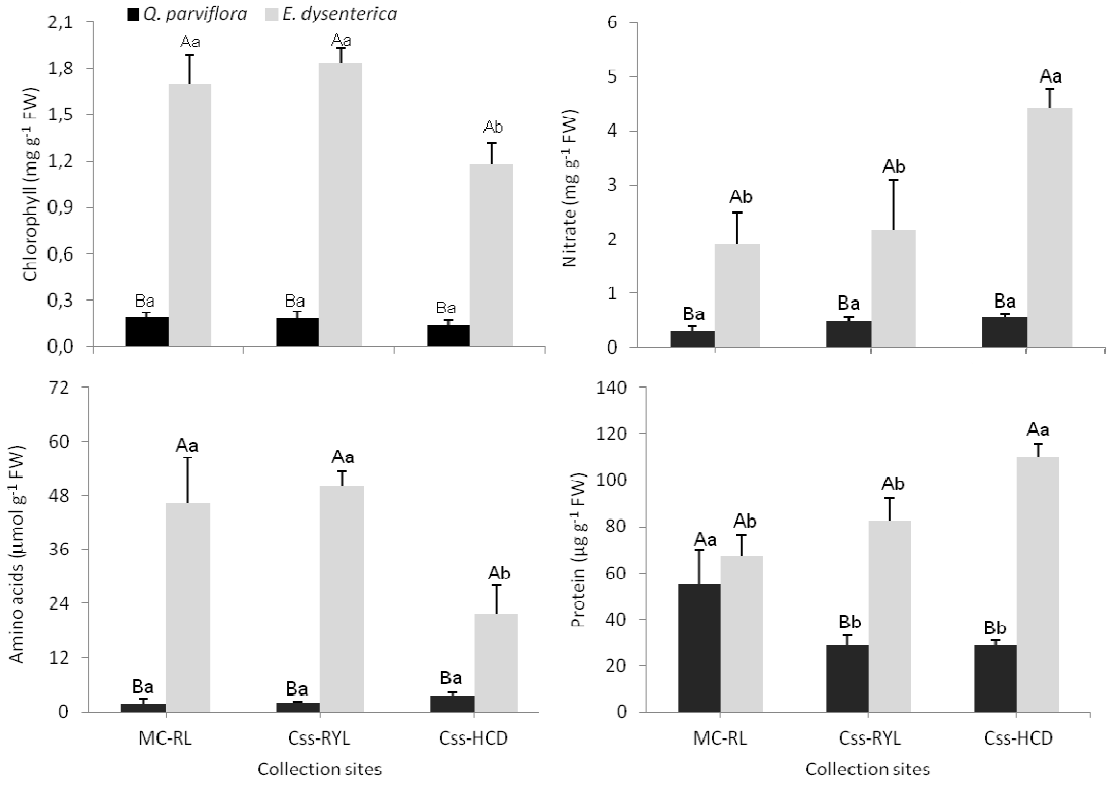

Fig. 1 Concentrations of total chlorophyll, nitrate, total free amino acids, and total insoluble proteins in leaves of *Qualea parviflora* and *Eugenia dysenterica* plants collected in the Cerrado at the National Forest of Paraopeba, southeastern Brazil. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red-Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic. Different letters indicate a significant difference (Tukey test, $p < 0.05$). Capital letters compare species and lowercase letters compare collection sites. Vertical bars represent standard deviation.

The metabolite profile of leaf samples from *Q. parviflora* and *E. dysenterica* resulted in 50 successfully annotated compounds, including 12 amino acids, seven organic acids, eight sugars, four sugar alcohols, ten fatty acids, one polyamine, and eight other metabolites (Supplemental table SI). The species showed distinct metabolisms when collected from soils with varying pH and Al availabilities; the main differences between them were mapped onto separate metabolic pathways to provide an easy overview of these distinctions (Figs. 2 and 3).

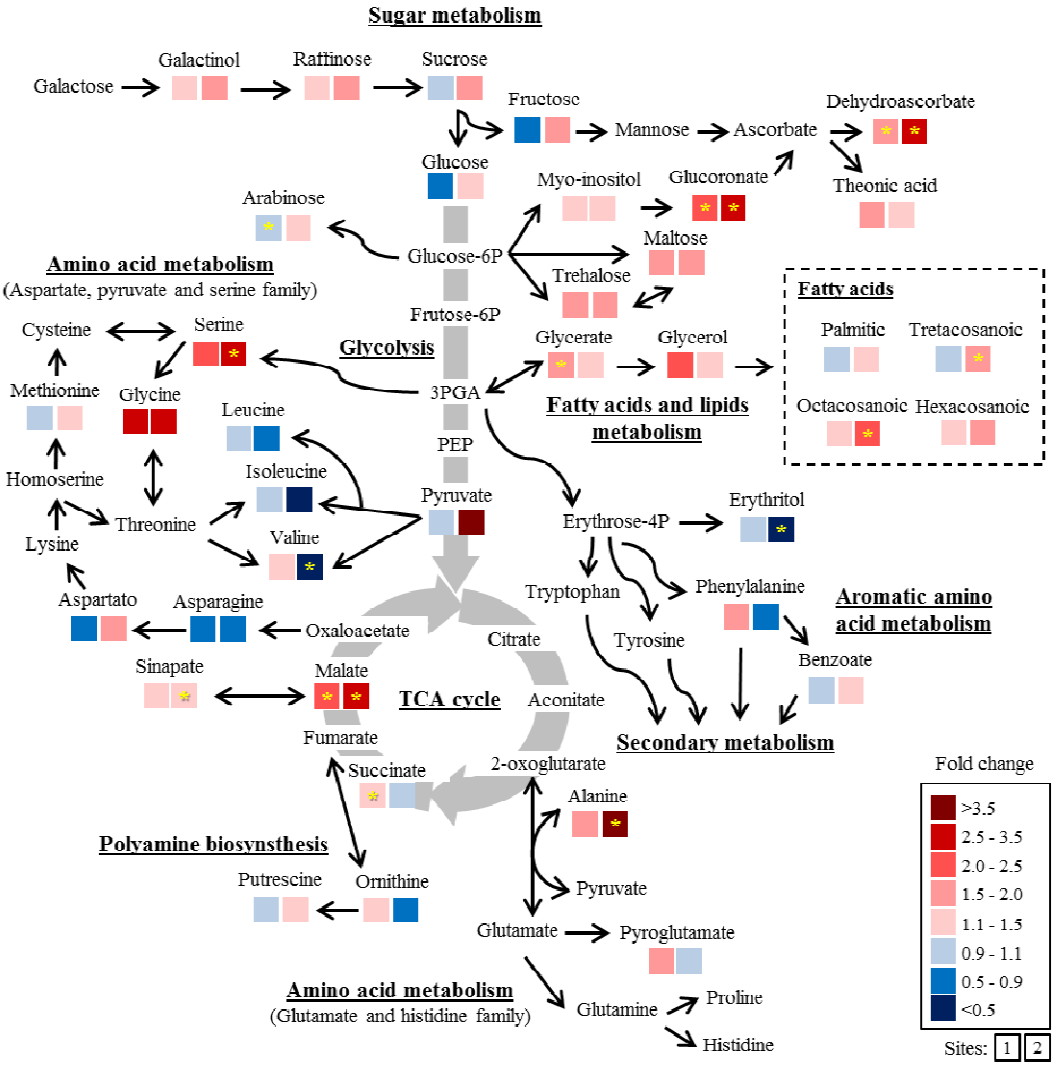

Fig. 2 Schematic summary of the major metabolic alterations in leaves of *Qualea parviflora* plants collected in the Cerrado *sensu stricto* on Red Yellow Latosol (1) and Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic (2) at the National Forest of Paraopeba, southeastern Brazil. Data is normalized with respect to the mean response calculated for plants from Mesotrophic Cerradão on Red Latosol (MC-RL). Asterisks indicate means that were considered to be significantly different from those of MC-RL plants ($p < 0.05$) by Student's t test. Legend: 3PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate; TCA cycle, tricarboxylic acid cycle.

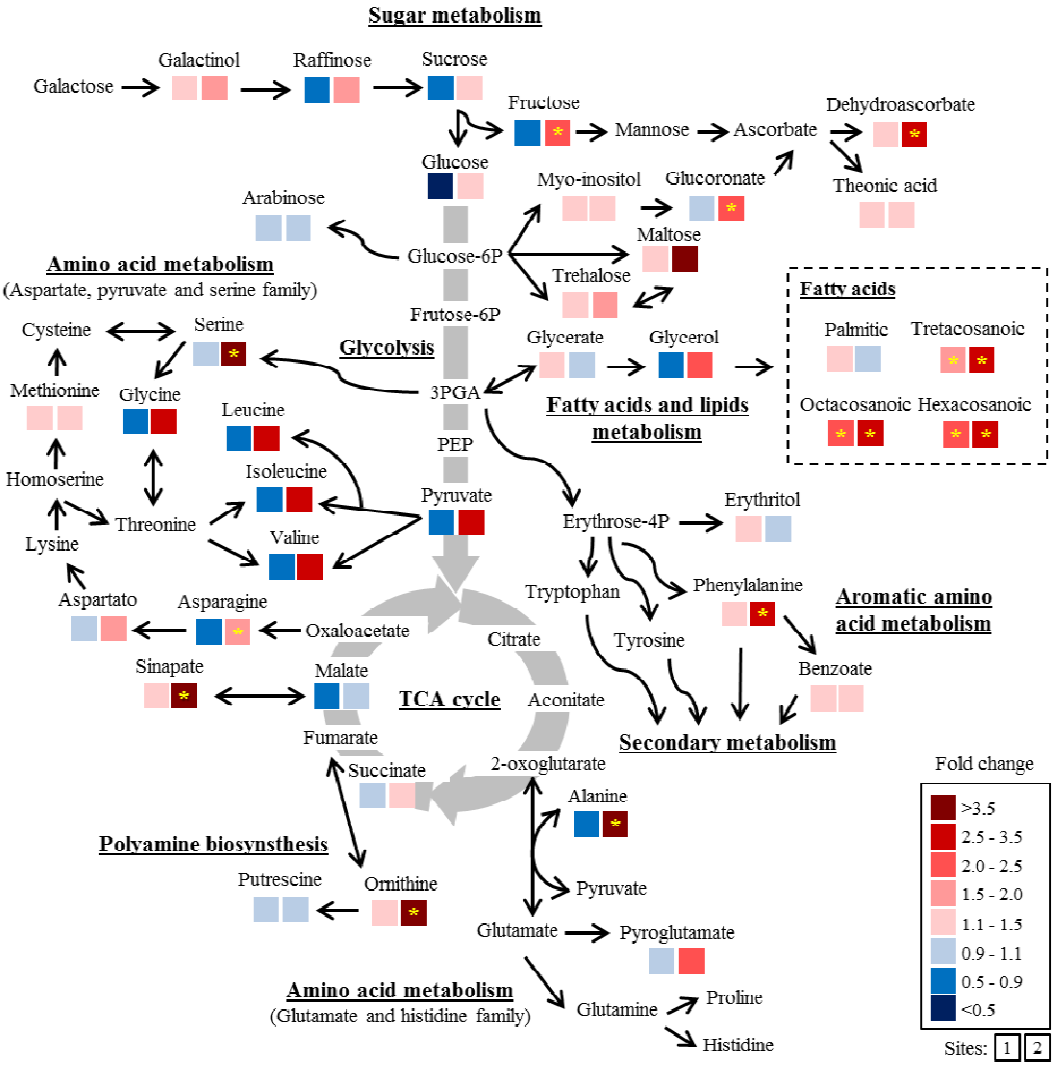

Fig. 3 Schematic summary of the major metabolic alterations in leaves of *Eugenia dysenterica* plants collected in the Cerrado *sensu stricto* on Red Yellow Latosol (1) and Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic (2) at the National Forest of Paraopeba, southeastern Brazil. Data is normalized with respect to the mean response calculated for plants from Mesotrophic Cerradão on Red Latosol (MC-RL). Asterisks indicate means that were considered to be significantly different from those of MC-RL plants ($p < 0.05$) by Student's t test. Legend: 3PGA, PEP, 3-phosphoglycerate; phosphoenolpyruvate; TCA cycle, tricarboxylic acid cycle.

Considerable differences were observed in the amino acid levels (Fig. 2). In general, the levels of phenylalanine, ornithine, and amino acids of the aspartate, pyruvate, and serine family, except serine and glycine, showed a clear decreasing trend in *Q. parviflora* plants,

especially in those from C*ss*-HCD. Significant differences were observed for valine (a reduction of about 50%), serine, and alanine (increases of about 300% and 400%, respectively). In contrast, *E. dysenterica* plants from C*ss*-HCD showed a significant increase in levels of alanine (3.25-fold), asparagine (1.78), serine (3.95), phenylalanine (3.17), and ornithine (4.01). Glycine, leucine, isoleucine, valine, and pyroglutamate also showed an increasing trend in these plants.

Similar results were observed in the species sugar levels. In general, plants collected at C*ss*-HCD showed increased contents of sugars associated with galactose metabolism. In contrast, the fructose and glucose levels in *Q. parviflora* and the raffinose, sucrose, fructose, and glucose levels in *E. dysenterica* at C*ss*-RYL showed a clear decreasing trend. The pyruvate level was similar to that of sugars in plants from C*ss*-HCD and C*ss*-RYL; however, the levels of organic acids associated with the tricarboxylic acid (TCA) cycle showed different responses between species. *Q. parviflora* showed increased levels of malate at C*ss*-RYL and C*ss*-HCD and of succinate at C*ss*-RYL. Additionally, the level of sinapate, an organic acid synthesized from malate, was also increased in C*ss*-HCD plants. As in *Q. parviflora*, the sinapate level in *E. dysenterica* was significantly higher in C*ss*-HCD plants; however, the malate level was slightly lower in comparison with MC-RL plants.

Levels of other metabolites direct or indirectly derived from glycolysis intermediates were also altered in *Q. parviflora*. Glycerate was 1.64-fold higher in C*ss*-RYL plants, glucoronate was 2.09- and 2.78-fold higher in C*ss*-RYL and C*ss*-HCD plants, respectively, and erythritol was about 50% lower in C*ss*-HCD plants. In *E.dysenterica*, there was an increase in the glucoronate level (2.09-fold) of C*ss*-HCD plants. Fatty acids increased especially in *E. dysenterica* plants from C*ss*-RYL and C*ss*-HCD. Greater increases were observed for tretracosanoic (1.83–2.94-fold), hexacosanoic (2.03–2.86) and octacosanoic (2.24–3.06) acids. The levels of tretracosanoic and octacosanoic acids were 1.78- and 2.29-fold higher, respectively, in *Q. parviflora* plants from C*ss*-HCD. Dehydroascorbate, an organic acid of particular interest, showed enhanced level in both species, especially in Css-HCD plants.

Antioxidant capacity and lipid peroxidation differed between species (Fig. 4). *Q. parviflora* had higher NPT concentration than *E. dysenterica* at all collection sites, while the opposite was observed for the TSP level. PT concentration was higher in *E. dysenterica*

than in *Q. parviflora* at Css-RYL, with no difference in the TT level. Regarding differences among collection sites, *Q. parviflora* showed higher NPT concentration at C*ss*-HCD, while no difference was observed in *E. dysenterica*. TSP concentration was highest in plants of both species at C*ss*-HCD. Neither PT nor TT concentrations differed among collection sites. Lipid peroxidation, as indicated by TBAR concentration, was higher in leaves of *E. dysenterica* plants than in those of *Q. parviflora* plants at all collection sites. TBAR levels in *E. dysenterica* were higher in plants growing at Css-HCD than in plants from the other sites. *Q. parviflora*, on the other hand, showed similar TBAR levels at all collection sites (Fig. 4).

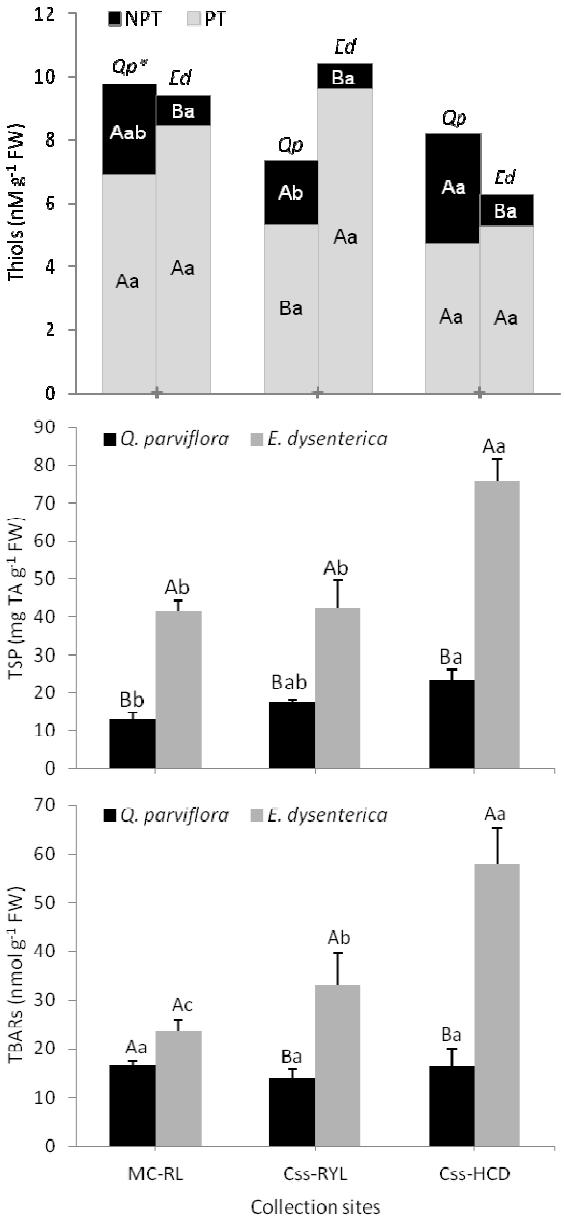

Fig. 4 Concentration of non-protein (NPT) and protein thiols (PT), total soluble phenols (TSP), and thiobarbituric acid-reactive substances (TBARs) in leaves of *Qualea parviflora* and *Eugenia dysenterica* plants collected in the Cerrado at the National Forest of Paraopeba, southeastern Brazil. MC-RL: Mesotrophic Cerradão on Red Latosol; C*ss*-RYL: Cerrado *sensu stricto* on Red Yellow Latosol; C*ss*-HCD: Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic. Different letters indicate a significant difference (Tukey test, $p < 0.05$). Capital letters compare species and lowercase letters compare collection sites. Vertical bars represent standard deviation. *(*Qp*): *Q. parviflora*, (*Ed*): *E. dysenterica*.

Histolocalization of phenolic compounds was similar in leaves of *E. dysenterica* and *Q. parviflora*, with no difference among collection sites. Positive reactions were observed in the protoplast of cells from the epidermis, hypodermis (in *Q. parviflora*), palisade and spongy parenchymas, collenchyma, and xylem and phloem parenchymas. Chloroplasts also had phenolic compounds. The outer cell wall of epidermal cells; trichomes (in *Q. parviflora*); fibers; sieve tube elements; companion cells; and vessel elements showed negative results in the test (Table 1).

Table 1 Histolocalization of phenolic compounds in leaves of *Eugenia dysenterica* and *Qualea parviflora* plants from the Cerrado at the National Forest of Paraopeba, southeastern Brazil

Tissue	Structure / Cell type	*E. dysenterica*	*Q. parviflora*
	Outer cell wall	-[a]	-
Epidermis	Protoplast	+	+
	Trichome	a	-
Hypodermis	Protoplast	a	+
Parenchyma	Protoplast	+	+
	Chloroplast	+	+
Collenchyma	Protoplast	+	+
Sclerenchyma	Fiber	-	-
	Sieve tube element	-	-
Phloem	Companion cell	-	-
	Parenchyma	+	+
	Vessel element	-	-
Xylem	Fiber	-	-
	Parenchyma	+	+

[a] (a) absent structure or tissue; (+) positive reaction; (-) negative reaction

Discussion

Plant resistance to adverse conditions of acid soils varies with the species, and Al toxicity is the main limiting factor to plant development on those soils (Nunes-Nesi et al. 2014). Besides the difference in their Al resistance strategy, *E. dysenterica* and *Q. parviflora* (non-accumulator and Al-hyperaccumulator, respectively, see chapter 1) also differed in the metabolic features of plants growing on acid soils with varying fertilities and concentrations of Al, Fe, and Mn.

Compartmentalization, complexation, and metabolic adaptation are among the major mechanisms accounting for reduced metal toxicity in plants (Cataldo and Wildung 1978). The increased nitrate concentration in *E. dysenterica* plants from C*ss*-RYL and C*ss*-HCD indicates that nitrogen uptake was up-regulated at sites with high metal toxicity. Additionally, the reduced concentration of total free amino acids in those plants may be related to their increased synthesis of proteins, both structural and enzymatic. Synthesis of compatible osmolytes, usually amino acids and sugars (Sharma and Dietz 2006, Van den Ende and Valluru 2009), and of antioxidant compounds, both enzymatic and non-enzymatic, is related to the plant capacity to control oxidative stress, and consequently to the plant resistance to abiotic stresses (Arbona et al. 2017). Despite the reduced concentration of total amino acids, *E. dysenterica* showed increased levels of asparagine, alanine, ornithine, phenylalanine, and serine. The results on metabolism of sugars and levels of amino acids in plants collected at different sites suggest that both these compound classes may be contributing to plant resistance to acid soils in the two species, as reported to *Camellia sinensis* (Xu et al. 2016).

The significant increase in malate and succinate levels in leaves of *Q. parviflora* supports the hypothesis of a role played by organic acids in Al detoxification on shoots of hyperaccumulator plants (Grevenstuk and Romano 2013). Organic acids, mainly citrate, oxalate, and malate, are the main Al-binding compounds and act in the detoxification of the metal, both internally, by formation of non-toxic Al complexes that are sequestrated in vacuoles, and externally, by exudation from the roots tips of most Al-resistant crop species and cultivars (Singh and Chauhan 2011; Nunes-Nesi et al. 2014). Thus, the reduced malate level in leaves of *E. dysenterica* may also be related to the species Al resistance strategy, by redistribution of this organic acid to the roots, which show higher Al concentration (about 0.8 g kg^{-1} DW, GS Tolentino, personal communication), and even by malate exudation, a

common feature among Al-excluder species that prevent Al uptake by chelating the metal in the rhizosphere (Brunner and Sperisen 2013).

The presence of mechanisms to prevent contact between free Al and biochemical processes is essential for tolerance to high internal Al concentrations (Grevenstuk and Romano 2013). The substantial increase in TSP concentration in *Q. parviflora* may reflect a metabolic change involved in the binding of Al in its non-toxic form (Tolrà et al. 2005), and may contribute to the species capacity to accumulate up to 15 g Al kg^{-1} DW on leaves (see chapter 1). Despite the fact that the stability constant of Al-phenol complexes is lower than the one of Al-organic acid complexes, Al-binding by phenolic compounds may be even more relevant in the less acid conditions of the apoplast or within plant cells (Tolrà et al. 2005). Thus, our data on phenolic compound histolocalization provides an important indicative that Al-phenol complexation may occur in the cytosol of *Q. parviflora*, since the localization of both Al and phenols was similar in that species, as also observed in *R. viburnoides* (Malta et al. 2016).

Enhanced production of phenols is a usual trait among plants that are subjected to different stresses. The increased level of phenylalanine, a key amino acid for the synthesis of a wide range of secondary metabolites such as phenols (Tzin and Galili 2010), in leaves of *E. dysenterica* seems to be associated with the increased concentration of these compounds, as indicated by the Folin–Ciocaulteau method. Unlike *Q. parviflora*, the increased phenol concentration in *E. dysenterica* does not seem to be related to the Al concentration in leaves, of about 0.2 g Al kg^{-1} DW (see chapter 1). The beneficial role of phenolic compounds in protecting plants is also due to scavenging of ROS generated by exposure to metals (Michalak 2006). In acid soils, not only Al but also Mn and Fe are present at excessively high levels, as observed at Css-HCD, and become potentially phytotoxic (Kochian et al. 2004). Additionally, the leaf concentration of Mn in plants collected at this site was higher than that in C*ss*-RYL and MC-RL plants (see chapter 1). Such high Mn levels may trigger an oxidative stress that can be ameliorated by the antioxidant action of phenolic compounds (Millaleo et al. 2010).

The enzymatic control of oxidative stress is another important mechanism of plant resistance to stresses, including to the one caused by Al (Inostroza-Blancheteau et al. 2012). The elevated level of dehydroascorbate, the oxidized form of ascorbate, in leaves of *E. dysenterica* and *Q. parviflora* at C*ss*-HCD suggests that the increased ascorbate peroxidase

(APX) activity is a feature of plants of these species growing on more acid soils with high metal concentration. APX catalyzes the reduction of hydrogen peroxide in water using ascorbate as the electron donor and plays an essential role in ROS scavenging (Gill and Tuteja 2010).

Despite the investment in production of antioxidant compounds, *E. dysenterica* was not able to avoid the occurrence of oxidative damage, as evidenced by the increase in lipid peroxidation and reduction in chlorophyll concentration in plants at C*ss*-HCD, the site with highest soil concentrations of Al, Fe, and Mn (see chapter 1). This higher susceptibility of *E. dysenterica* to oxidative stress may have contributed to the selection of Al exclusion as a resistance strategy in that species. On the other hand, *Q. parviflora* was able to control oxidative stress, which may have been due to the increased production of not only phenol and dehydroascorbate but also thiol. This is because glutathione (GSH) is the main NPT in the cell, participating of free radical scavenging while also contributing to the protection of membranes (Zagorchev et al. 2013). Cysteine is a key substrate for GSH biosynthesis, and although it was not identified, greater accumulation of upstream metabolites from its synthesis, such as serine and glycerate (Na and Salt 2011, Zagorchev et al. 2013), may indicate an increase in its level.

Some metabolic features seem to be more related to other environmental factors, like luminosity, than to edaphic conditions. This occurs, for example, with the levels of insoluble proteins and fatty acids on plants from C*ss*-RYL and C*ss*-HCD. Plants growing in environments with high light intensity (such as 'Cerrado *sensu stricto*') generally have more rigid leaves and thicker cuticle. The enhanced synthesis of insoluble proteins and the increased level of serine, a highly required amino acid for the production of extensins of the cell wall (Cassab 1998), in *E. dysenterica* leaves may be associated with the observed increase in leaf rigidity in plants from those sites. A similar result has been reported to *Barbacenia purpurea* plants grown under drought stress (Suguiyama et al. 2014). On the other hand, the increased levels of fatty acids, especially tetracosanoic, hexacosanoic, and octacosanoic acids, in both species may be related to an increased cuticle production (Bethea et al. 2014).

The adaptive strategies observed in *E. dysenterica* and *Q. parviflora* plants growing on acid soils with different fertilities and metal toxicities certainly contribute to their resistance to the adverse conditions of these soils, including the high Al toxicity. The more

efficient mechanisms of *Q. parviflora* for detoxification of Al (by inducing the production of organic acids and phenols) and ROS (by inducing the synthesis of phenols, dehydroascorbate, and non-protein thiols) may be related to the species capacity to hyperaccumulate Al. On the other hand, the metabolic adaptations involved in ROS scavenging, like phenol production, in *E. dysenterica* were not efficient enough to control oxidative stress, as evidenced by the increased lipid peroxidation and reduced chlorophyll concentration in plants at C*ss*-HCD.

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Author contributions

All authors participated in the conception and design of the study. S.A. and D.G. performed field collection and biochemical analysis. A.N. conducted data analysis and interpretation. A.A. discussed the results and participated in manuscript preparation. S.A. and A.N. wrote the manuscript. All authors proofread and approved the manuscript.

References

Arbona V, Manzi M, Zandalinas SI, Vives-Peris V, Pérez-Clemente RM, Gómez-Cadenas A (2017) Physiological, metabolic, and molecular responses of plants to abiotic stress. In: Sarwat M, Ahmad A, Abdin MZ (eds) Stress Signaling in Plants: Genomics and Proteomics Perspective, Vol 2. Springer-Verlag, New York, pp 1–35

Bethea FG, Park D, Mount A, Menchyk N, Liu H (2014) Effects of acute moisture stress on creeping bentgrass cuticle morphology and associated effects on foliar nitrogen uptake. HortScience 49: 1582–1587

Brunner I, Sperisen C (2013) Aluminum exclusion and aluminum tolerance in woody plants. Front Plant Sci 4: 1–12

Cassab GI (1998) Plant cell wall proteins. Annu Rev Plant Physiol Plant Mol Biol 49: 281–309

Cataldo DA, Wildung RE (1978) Soil and plant factors influencing the accumulation of heavy metals by plants. Environ Health Perspect 27: 149–159

Cross JM, von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M (2006) Variation of enzyme activities and metabolite levels in 24 *Arabidopsis* accessions growing in carbon-limited conditions. Plant Physiol 142: 1574–1588

Cuadros-Inostroza A, Caldana C, Redestig H, Kusano M, Lisec J, Peña-Cortés H, Willmitzer L, Hannah MA (2009) Target search – a Bioconductor package for the efficient preprocessing of GC-MS metabolite profiling data. Bioinformatics 10: 428.

Ferreira DF (2011) Sisvar: A computer statistical analysis system. Ciênc Agrotec 35: 1039–1042

Fritz C, Palacios-Rojas N, Feil R, Stitt M (2006) Regulation of secondary metabolism by the carbon-nitrogen status in tobacco: Nitrate inhibits large sectors of phenylpropanoid metabolism. Plant J 46: 533–548

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Bioch 48: 909–930

Grevenstuk T, Romano A (2013) Aluminium speciation and internal detoxification mechanisms in plants: Where do we stand? Metallomics 5: 1584–1594

Gupta N, Gaurav SS, Kumar A (2013) Molecular basis of aluminium toxicity in plants: A review. Am J Plant Sci 4: 21–37

Hartwig I, Oliveira AC, Carvalho FIF, Bertan I, Silva JAG, Schmidt DAM, Valério IP, Maia LC, Fonseca DAR, Reis CES (2007) Mecanismos associados à tolerância ao alumínio em plantas. Semina: Ciênc Agrar 28: 219–228

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189–198

Inostroza-Blancheteau C, Rengel Z, Alberdi M, Mora ML, Aquea F, Arce-Johnson P, Reyes-Díaz M (2012) Molecular and physiological strategies to increase aluminum resistance in plants. Mol Biol Rep 39: 2069–2079

Jansen S, Watanabe T, Dessein S, Smets E, Robbrecht E (2003) A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. Ann Bot 91: 657–663

Johansen DA (1940) Plant microtechnique. McGraw Hill Book, New York

Kochian LV, Piñeros MA, Hoekenga OA (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol 55: 459–493

Kochian LV, Piñeros MA, Hoekenga OA (2005) The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274: 175–195

Kolbe A, Oliver SN, Fernie AR, Stitt M, van Dongen JT, Geigenberger P (2006) Combined transcript and metabolite profiling of *Arabidopsis* leaves reveals fundamental effects of the thiol-disulfide status on plant metabolism. Plant Physiol 141: 412–422

Kopka J, Schauer N, Krueger S, Birkmeyer C, Usadel B, Bergmuller E, Dormann P, Weckwerth W, Gibon Y, Stitt M, Willmitzer L, Fernie AR, Steinhauser D (2005) GMD@CSB.DB: the Golm Metabolome Database. Bioinformatics 21: 1635–1638

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat Protoc 1: 387–396

Ma B, Gao L, Zhang H, Cui J, Shen Z (2012) Aluminum-induced oxidative stress and changes in antioxidant defenses in the roots of rice varieties differing in Al tolerance. Plant Cell Rep 31: 687–696

Malta PG, Arcanjo-Silva S, Ribeiro C, Campos NV, Azevedo AA (2016) *Rudgea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplasts. Plant Soil 408: 369–384

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol J Environ Stud 15: 523–530

Millaleo R, Reyes-Díaz M, Ivanov AG, Mora ML, Alberdi M (2010) Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J Soil Sci Plant Nutr 10: 476–494

Na G, Salt DE (2011) The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. Environ Exp Bot 72: 18–25

Neri AV, Schaefer CEGR, Silva AF, Souza AL, Ferreira-Júnior WG, Meira-Neto JAA (2012) The influence of soils on the floristic composition and community structure of an area of Brazilian Cerrado vegetation. Edinb J Bot 69: 1–27

Nunes-Nesi A, Santos Brito D, Inostroza-Blancheteau C, Fernie AR, Araújo WL (2014) The complex role of mitochondrial metabolism in plant aluminum resistance. Trends Plant Sci 19: 399–407

Olson DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC, D'Amico JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y, Lamoreux JF, Wettengel WW, Hedao P, Kassem KR (2001) Terrestrial ecoregions of the world: A new map of life on Earth. BioScience 51: 933–938

Pattanayak A, Pfukrei K (2013) Aluminium toxicity tolerance in crop plants: Present status of research. Afr J Biotechnol 12: 3752–3757

Ribeiro JF, Walter BMT (2008) As principais fitofisionomias do bioma Cerrado. In: Sano SM, Almeida SP, Ribeiro JF (eds) Cerrado: Ecologia e Flora, Vol 1, Embrapa Cerrados, Brasília, pp 151–199

Ribeiro C, Cambraia J, Peixoto PHP, Fonseca Júnior EM (2012) Antioxidant system response induced by aluminum in two rice cultivars. Braz J Plant Physiol 24: 107–116

Riddles PW, Blakeley RL, Zerner B (1979) Ellman's reagent: 5,5'-dithiobis(2-nitrobenzoic acid) a reexamination. Anal Biochem 94: 75–81

Sedlak L, Lindsay R (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 192–205

Serrano HC, Pinto MJ, Martins-Loução MA, Branquinho C (2011) How does Al hyperaccumulator plant respond to a natural field gradient of soil phytoavailable Al? Sci Total Environ 409: 3749–3756

Sharma S, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57: 711–726

Siecińska J, Nosalewicz A (2017) Aluminium toxicity to plants as influenced by the properties of the root growth environment affected by other co-stressors: A review. In: Voogt P de (ed) Reviews of Environmental Contamination and Toxicology. Springer Press, New York, pp 1–26

Singh D, Chauhan SK (2011) Organic acids of crop plants in aluminium detoxification. Curr Sci 100: 1509–1515

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299: 152–178

SNUC – Sistema Nacional de Unidades de Conservação da Natureza (2000). Lei nº 9.985, de 18 de julho de 2000. MMA/SBF

Suguiyama VF, Silva EA, Meirelles ST, Centeno DC, Braga MR (2014) Leaf metabolite profile of the Brazilian resurrection plant *Barbacenia purpurea* Hook. (Velloziaceae) shows two time-dependent responses during desiccation and recovering. Front Plant Sci 5: 96

Tolrà R, Poschenrieder C, Luppi B, Barceló J (2005) Aluminum-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in *Rumex acetosa* L. Environ Exp Bot 54: 231–238

Tzin, V, Galili G (2010) New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Molec Plant 3: 956–972

Van den Ende W, Valluru R (2009) Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging? J Exp Bot 60: 9–18

Vitorello VA, Capaldi FR, Stefanuto VA (2005) Recent advances in aluminum toxicity and resistance in higher plants. Braz J Plant Physiol 17: 129–143

von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. Plant Soil 171: 1–15

Wang J, Raman H, Zhang G, Mendham N, Zhou M (2006) Aluminum tolerance in barley (*Hordeum vulgare* L.): Physiological mechanisms, genetics and screening methods. J Zhejiang Univ Sci B 7: 769–787

Xu Q, Wang Y, Ding Z, Song L, Li Y, Ma D, Wang Y, Shen J, Jia S, Sun H, Zhang H (2016) Aluminum induced metabolic responses in two tea cultivars. Plant Physiol Bioch 101: 162–172

Zagorchev L, Seal CE, Kranner I, Odjakova M (2013) A central role for thiols in plant tolerance to abiotic stress. Int J Mol Sci 14: 7405–7432

Supplemental table SI Relative metabolite content in leaves of *Qualea parviflora* and *Eugenia dysenterica* plants collected in the Mesotrophic Cerradão on Red Latosol (MC-RL), Cerrado *sensu stricto* on Red-Yellow Latosol (C*ss*-RYL), and Cerrado *sensu stricto* on Haplic Cambisol Tb Dystrophic (C*ss*-HCD) at the National Forest of Paraopeba, southeastern Brazil

Metabolite	*E. dysenterica*			*Q. parviflora*		
	MC-RL	C*ss*-RYL	C*ss*-HCD	MC-RL	C*ss*-RYL	C*ss*-HCD
Amino acids						
Alanine	1.00±0.12*	0.79±0.17	**3.25±0.68**	1.00±0.21	1.86±0.70	**4.18±0.91**
Asparagine	1.00±0.19	0.73±0.09	**1.78±0.16**	1.00±0.12	0.57±0.08	0.60±0.06
Aspartate	1.00±0.17	0.95±0.09	1.51±0.36	1.00±0.09	0.81±0.22	1.55±0.46
Glycine	1.00±0.19	0.86±0.19	2.96±0.65	1.00±0.12	2.68±0.77	2.72±0.82
Isoleucine	1.00±0.18	0.87±0.21	2.89±0.78	1.00±0.13	1.06±0.23	0.46±0.11
Leucine	1.00±0.22	0.89±0.22	2.88±0.80	1.00±0.13	1.01±0.22	0.75±0.18
Methionine	1.00±0.23	1.37±0.39	1.26±0.33	1.00±0.03	0.97±0.04	1.36±0.31
Ornithine	1.00±0.20	1.26±0.06	**4.01±0.71**	1.00±0.12	1.35±0.20	0.51±0.09
Phenylalanine	1.00±0.17	1.37±0.06	**3.17±0.70**	1.00±0.13	1.64±0.31	0.89±0.21
Pyroglutamate	1.00±0.18	0.96±0.12	2.05±0.44	1.00±0.11	1.51±0.27	1.03±0.21
Serine	1.00±0.18	0.99±0.16	**3.95±0.86**	1.00±0.15	2.49±0.71	2.99±0.66
Valine	1.00±0.22	0.81±0.20	2.63±0.71	1.00±0.13	1.47±0.24	0.46±0.10
Organic acids						
Dehydroascorbate	1.00±0.13	1.39±0.08	**2.89±0.16**	1.00±0.06	**1.79±0.13**	**2.74±0.43**
Glycerate	1.00±0.32	1.36±0.50	1.03±0.22	1.00±0.15	**1.64±0.06**	1.29±0.19
Malate	1.00±0.24	0.55±0.08	0.95±0.12	1.00±0.09	**2.24±0.35**	**3.23±0.28**
Malonate	1.00±0.24	1.25±0.29	1.22±0.28	1.00±0.01	1.06±0.04	1.46±0.40
Pyruvate	1.00±0.22	0.80±0.11	2.57±0.67	1.00±0.22	0.95±0.27	3.98±1.16
Succinate	1.00±0.25	1.05±0.26	1.15±0.28	1.00±0.02	**1.28±0.05**	1.05±0.08
Threonic acid	1.00±0.26	1.25±0.38	1.50±0.28	1.00±0.11	1.69±0.26	1.33±0.17
Sugars						
Altrose	1.00±0.31	0.28±0.04	1.29±0.37	1.00±0.36	0.90±0.16	1.48±0.25
Arabinose	1.00±0.24	1.02±0.25	0.99±0.24	1.00±0.01	**0.96 ±0.01**	1.50±0.37
Fructose	1.00±0.21	0.80±0.07	**2.37±0.46**	1.00±0.41	0.69±0.17	1.57±0.27
Glucose	1.00±0.34	0.25±0.04	1.26±0.37	1.00±0.13	2.53±0.59	**3.96±0.57**
Maltose	1.00±0.06	1.26±0.25	4.97±0.63	1.00±0.12	1.57±0.28	1.98±0.58
Raffinose	1.00±0.21	0.89±0.27	1.52±0.47	1.00±0.10	1.32±0.06	1.72±0.42
Sucrose	1.00±0.25	0.70±0.19	1.11±0.29	1.00±0.04	1.00±0.06	**0.79±0.02**
Trehalose	1.00±0.21	1.23±0.22	1.83±0.42	1.00±0.16	1.67±0.38	1.71±0.40
Sugar alcohols						
Erythritol	1.00±0.27	1.39±0.19	0.93±0.22	1.00±0.15	1.06±0.28	**0.47±0.10**
Galactinol	1.00±0.24	1.43±0.30	1.82±0.43	1.00±0.12	1.26±0.08	1.60±0.47
Glycerol	1.00±0.19	0.83±0.11	2.11±0.45	1.00±0.30	2.33±0.38	1.39±0.28
myo-Inositol	1.00±0.15	1.36±0.36	1.12±0.33	1.00±0.04	1.13±0.07	1.20±0.06

Fatty acids						
Capric acid	1.00±0.30	1.25±0.28	1.31±0.16	1.00±0.08	1.19±0.10	1.71±0.25
Docosanoic acid	1.00±0.10	1.39±0.13	1.97±0.32	1.00±0.12	1.02±0.11	1.40±0.14
Eicosanoic acid	1.00±0.33	0.90±0.23	0.90±0.15	1.00±0.01	0.89±0.05	1.24±0.33
Hexacosanoic acid	1.00±0.12	**2.03±0.20**	**2.68±0.32**	1.00±0.16	1.13±0.14	1.80±0.20
Hexadecanoic acid	1.00±0.28	1.06±0.27	0.92±0.19	1.00±0.01	0.92±0.05	1.25±0.31
Octacosanoic acid	1.00±0.14	**2.24±0.22**	**3.06±0.32**	1.00±0.20	1.36±0.21	**2.29±0.21**
Octadecanoic acid	1.00±0.24	1.24±0.34	1.02±0.24	1.00±0.03	0.88±0.04	1.27±0.32
Palmitic acid	1.00±0.26	1.21±0.33	1.02±0.23	1.00±0.02	0.95±0.04	1.40±0.34
Tetracosanoic acid	1.00±0.11	**1.83±0.20**	**2.94±0.45**	1.00±0.14	1.08±0.13	**1.78±0.16**
Tetradecanoic acid	1.00±0.27	1.24±0.34	1.02±0.24	1.00±0.03	0.93±0.03	1.48±0.32
Polyamines						
Putrescine	1.00±0.26	1.07±0.28	1.00±0.26	1.00±0.01	0.93±0.05	1.24±0.35
Other metabolites						
4-hydroxy-benzoate	1.00±0.19	1.60±0.58	1.58±0.38	1.00±0.05	0.97±0.08	1.48±0.28
Benzoate	1.00±0.25	1.44±0.40	1.26±0.30	1.00±0.01	1.04±0.09	1.40±0.33
Glucuronate	1.00±0.07	0.95±0.14	**2.09±0.18**	1.00±0.09	**2.09±0.13**	**2.78±0.31**
Nicotinic acid	1.00±0.17	0.62±0.19	0.36±0.09	1.00±0.09	**0.28±0.02**	**0.22±0.05**
Phosphorate	1.00±0.30	1.54±0.22	0.26±0.05	1.00±0.06	1.17±0.10	1.82±0.47
Ribonate	1.00±0.26	1.07±0.19	1.35±0.26	1.00±0.03	1.21±0.08	**1.57±0.18**
Sinapate	1.00±0.13	1.11±0.10	**5.85±1.44**	1.00±0.06	1.16±0.11	**1.40±0.10**
Uracil	1.00±0.20	0.98±0.25	1.13±0.26	1.00±0.08	1.72±0.52	1.31±0.31

* Data was normalized with respect to the mean response calculated for plants from MC-RL. Values represent mean ± SE of five biological replicates; bold, underlined values were judged to be significantly different from those of MC-RL plants ($p < 0.05$) by Student's t-test.

FINAL CONSIDERATIONS

Eugenia dysenterica showed an average 0.21 g Al kg^{-1} DW on leaves and this concentration was not significantly altered by Al availability in soils, which confirms the non-accumulator nature of this species. The histochemical test did not detect Al in tissues of *E. dysenterica,* while the X-ray microanalysis demonstrated the occurrence of low relative Al abundances (less than 1.0%) in cell walls.

Qualea parviflora and *Q. multiflora*, on the other hand, accumulated high Al concentrations (about 13.46 and 19.79 g Al kg^{-1} DW on leaves, respectively) at all collection sites, confirming their description as Al-hyperaccumulators. Interestingly, *Q. parviflora* was able to hyperaccumulate Al even on a soil with negligible concentration of available Al, which suggests that this species has mechanisms to alter Al availability in the soil. Moreover, the concentration of accumulated Al on leaves of this species was more correlated with mesotrophic soils, which have low Al availability, as observed in *E. dysenterica* (a non-accumulator species). In *Q. multiflora*, such concentration was associated with dystrophic soils, which are abundant in available Al.

Like in other Al-hyperaccumulators, pectocellulosic cell walls were the preferential sites for Al deposition, but the metal was also localized in suberized cell walls and, once more, in chloroplasts. The presence of Al in chloroplasts, which is commonly observed in Al-hyperaccumulator species, is an intriguing fact that should inspire the performance of research regarding a possible interference of the metal with the ultrastructure and metabolism of the organelle, and even a possible role of Al in plant metabolism.

Despite the fact that nutritional deficiency is a common symptom in plants exposed to high Al concentrations, such symptom was not observed in this study. The increased levels of K, P, and S in response to higher Al accumulation, as well as the avoidance of Al deposition on photosynthetic tissues, may even be related to the capacity of *Q. multiflora* to accumulate more Al than *Q. parviflora*. This data reinforces the theory that nutrient absorption by species adapted to acid soils is not adversely affected by high Al concentrations in the soil.

Our findings on plant metabolic adaptations indicate that the resistance to adverse conditions of acid soils, including high Al concentration, in *Q. parviflora* is related to an increased synthesis of phenols, thiols, and organic acids, all of which play a role in the detoxification of Al and reactive oxygen species, thus contributing to the control of

oxidative stress. In contrast, data on lipid peroxidation demonstrates that *E. dysenterica* was not able to control this stress when growing on soils with high metal toxicity, even showing increased production of antioxidant compounds (especially phenols and dehydroascorbate).

This work is the first study using metabolite profiling analysis to elucidate the mechanisms involved in the resistance of Cerrado species naturally growing on acid soils. However, many gaps still need to be filled so that Al resistance strategies in native species can be sufficiently understood. Despite the difficulties in cultivating Cerrado species, laboratory studies conducted under controlled conditions are necessary to corroborate our results, and further research is therefore strongly encouraged.