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Positive response of seedlings from an old-growth grassland to soil quality improvement

Thaise de O. Bahia[1] · Hernani A. Almeida[1] · Milton Barbosa[2] · Daniel Negreiros[2,3] · Mauricio Quesada[4] · G. Wilson Fernandes[2]

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Abstract

Stress tolerance is the predominant strategy among the plant species that colonize the Brazilian rupestrian grassland (*Campo rupestre*) and is a response to edaphic conditions involving specific morphological and physiological adaptations. Motivated by the need to increase the efficiency in the production of seedlings, we experimentally evaluated whether these plant species are naturally limited in terms of optimum development and if they could have their survival and growth increased when cultivated under enhanced nutrient and water conditions and reduced iron concentration. To experimentally test this hypothesis, we cultivated seedlings of nine plant species in six distinct substrates for 150 days. The percentage of seedling survival had great variation among species (ranging from 40 to 100% survival). Eight out of the nine studied species showed higher growth and higher biomass accumulation as well as greater investment in aerial parts when grown in nursery or organic substrate (up to 10 times greater than the control treatment). This study provides evidence that these species also present reversible adjustment to cope with the poor soil conditions and are able to improve their performance in more fertile soils. Thus, seedling production for the restoration of ironstone rupestrian grasslands can be more efficient.

Keywords Biomass · *Campo rupestre* · *Canga* · Nutrient · Restoration · Survival rate

1 Introduction

Stress tolerance is a predominant strategy adopted by some plants as a response to edaphic conditions and involves specific morphological and physiological adaptations associated with low nutritional requirements and higher rates of survivorship (Bloom et al. 1985; Chapin et al. 1993; Körner 2003). Smaller, tougher, narrower, and highly sclerophyllous leaves are common in stress-tolerant species in the mountaintop rupestrian grassland (*Campo rupestre*) in southeastern Brazil (Negreiros et al. 2014a; Pierce et al. 2017). The rupestrian grasslands endure low productivity conditions caused by ironstone or quartzite soils and pronounced seasonality with a long dry period. The associated soils are usually highly acidic, shallow, sandy, with low water holding capacity, nutrient-poor, and often show high concentration of heavy metals (Vincent and Meguro 2008; Messias et al. 2013; Fernandes 2016a; Schaefer et al. 2016). The harsh conditions of the ironstone rupestrian grasslands (*canga*) limit the growth and distribution of plant species in the region and drive specific adaptations (Messias et al. 2012; Negreiros et al. 2014b; Carmo et al. 2015; Fernandes 2016b; Silveira et al. 2016). For instance, the formation of above-ground biomass of species such as *Microlicia crenulata* (DC.) Mart. and *Copaifera langsdorffii* Desf. is closely related to soil water holding capacity and granulometry (Teixeira and Lemos-Filho 2002). In the ironstone rupestrian grassland, where the soil also presents high levels of

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✉ Thaise de O. Bahia
thaiseobahia@gmail.com

1 Programa de Pós-graduação em Ecologia de Biomas Tropicais/DEBIO, ICEB/Universidade Federal de Ouro Preto, Ouro Preto, MG 35400-000, Brazil

2 Ecologia Evolutiva e Biodiversidade/DGEE, ICB/Universidade Federal de Minas Gerais, CP 486, Belo Horizonte, MG 30161-901, Brazil

3 Instituto de Ciências Biológicas e Saúde, Centro Universitário UNA, Belo Horizonte, 30180-100, MG, Brazil

4 Escuela Nacional de Estudios Superiores, Universidad Autonoma de México, Morelia, MIC, Mexico

aluminium, iron, and other heavy metals, some species are capable of absorbing cations and accumulating them in their tissues (Teixeira and Lemos-Filho 1998, 2002; Messias et al. 2013). Ribeiro et al. (2017) suggest that *Byrsonima variabilis* A. Juss. accumulates metals in its tissues as a form of defence against herbivores. These environmental filters may have been important in determining species coexistence and the composition of the local plant communities, often leading to high levels of endemism (e.g., Negreiros et al. 2014a, Carmo et al. 2015).

While the ability to survive in environments where resource and/or stress limitation occurs may be acquired through evolutionary adaptations (phylogenetic), individual ontogenetic variations (non-inherent), or reversible adjustment (acclimation; Körner 2003) also play important roles. The ability of plants to show positive and varied responses to edaphic conditions is an important adaptive factor (Gedroc et al. 1996; Pearson et al. 2003; Negreiros et al. 2009, 2014a). Some rupestrian grassland plants may modify their nutrient intake strategy via reversible adjustment. Under experimental conditions, some species inhabiting nutritionally poor environments can improve their development and growth on substrates with nutritional enrichment (Negreiros et al. 2009, 2014a). For instance, species of the genus *Baccharis* (Asteraceae) responded positively to increased resources by accumulating more biomass in their roots, stems and leaves (Negreiros et al. 2014a). However, this response to soil nutritional quality differs markedly among species (Bloom et al. 1985; Tilman 1988; Fernandes et al. 2007; Tomlinson et al. 2012; Perez-Harguindeguy et al. 2013).

The rupestrian grassland is intensively targeted for mining and road construction (Fernandes et al. 2014, 2016; Carmo et al. 2015), and due to its low resilience, the chances of spontaneous regeneration are very remote (e.g., Negreiros et al. 2009, 2011; Le Stradic et al. 2014; Fernandes et al. 2016). According to Sönter et al. (2014), in the ironstone rupestrian grassland, in addition to the allocation of compensation areas, effective conservation and revegetation activities are necessary to reduce the net loss of native vegetation. However, the restoration of degraded areas of rupestrian grassland requires special techniques, both in the preparation of the substrate and in the choice of plant species (Toy et al. 2001; Negreiros et al. 2009; Fernandes et al. 2020). Most projects aiming at environmental restoration using native species depend on the production of seedlings. Thus, in addition to using silvicultural techniques, restoration science has increasingly absorbed ecological concepts, seeking to improve seedling establishment efficiency in restored areas (Busato et al. 2012). Large-scale seedling production is the most suitable alternative needed to restore the degraded rupestrian grassland (see Negreiros et al. 2009). However, the propagation of rupestrian grassland seedlings is usually a very challenging task, and thus knowledge gap for the propagation of species is that about plant responses to edaphic factors.

Motivated by the need to increase the efficiency in the production of robust seedlings for the restoration of rupestrian grasslands and to determine the best substrate to grow these seedlings, we experimentally evaluated the performance of seedlings from nine plant species that occur widely in ironstone rupestrian grasslands in six distinct substrates. We tested the hypotheses that these plant species are naturally limited in terms of optimum development and would have their survival and growth increased when cultivated under enhanced soil conditions. Although these species are able to naturally colonize the ironstone rupestrian grasslands, tolerate, survive, and reproduce in an environment with high nutritional deficiency and high levels of heavy metals, we expected that seedlings growing in soils of higher nutrient and water status, and lower iron concentration would present higher survival rates, greater biomass and height, and more leaves. We also expected more investment in aerial parts (stem and leaf) since the plants would need to invest less in roots for mineral absorption—lower root/shoot ratio. Growing the seedlings under enhanced soils conditions would allow the production of seedlings with greater vigour and more likely to survive transplantation. The outcome of this study could have wide application in the practice of ecological restoration of rupestrian grasslands.

2 Materials and methods

Study area – The experiment was carried in a greenhouse located in the Reserva Vellozia (19° 16′ 45.7″ S, 43° 35′ 27.8″ W), Minas Gerais, Brazil. Seeds of nine plant species were collected in five different sites in Serra do Rola Moça State Park, located in the Iron Quadrangle region, in Minas Gerais, Brazil (20° 02′ 42.3″ S, 43° 59′ 89.3″ W–20° 03′ 60.3″ S, 44° 01′ 90.5″ W). The seeds were collected at different times, whenever they were available, between September 2013 and January 2015. The soil used for the experiment was sampled in the surroundings of Serra do Rola Moça State Park due to restrictions on soil extraction from the park.

Studied species – We selected plant species that were fruiting in the sampling period and/or had high importance and coverage value indexes described in the available phytosociological studies for the area (see Viana and Lombardi 2007; Jacobi et al. 2007, 2008; Messias et al. 2012). Nine species belonging to four families were studied: Asteraceae (*Baccharis dracunculifolia* DC., *Eremanthus erythropappus* (DC.) MacLeish, *Lychnophora pinaster* Mart., *Trixis vauthieri* DC.); Fabaceae (*Mimosa calodendron* Mart. ex Benth., *Copaifera langsdorffii* Desf.); Melastomataceae (*Pleroma heteromallum* D. Don (D.Don), *Pleroma candolleanum* (Mart. ex DC.) Cogn); and Lamiaceae (*Eriope macrostachya* Mart. ex Benth.). Information on aspects of occurrence, biology, and natural history of the species is presented in the Supplementary Material 1.

Experiment – To test the hypothesis, we used six different treatments named according to fertilizer dose or soil type (control, NPK/100, NPK/10, NPK, Organic, and Nursery). Firstly, all soil collected from the ironstone rupestrian grassland was manually sorted for removal of rocky fragments larger than 5 cm and completely mixed until its homogenization. The control treatment was composed only by the homogenized soil of the ironstone rupestrian grassland. For the other three treatments, the homogenized soil received increasing doses of fertilizers. The treatment called NPK consisted of the dosage 1500 g m^{-3} of NPK (4:14:8; Vitaplan®) and 2000 g m^{-3} of dolomitic limestone. This is the appropriate formulation to be applied in soil preparation for growing trees and shrubs. High phosphorus content stimulates the formation and development of roots and plant structures (Barros and Stringheta 1999). Two other treatments were composed of ironstone rupestrian grassland soil with fractions 10 and 100 times smaller than the above dosages of NPK and dolomitic limestone (called NPK/10 and NPK/100, respectively; see Negreiros et al. 2014a). The organic treatment was composed of ironstone rupestrian grassland soil with organic fertilizer (in a ratio of 2:1) produced by organic waste composting (Biodeia®). Finally, the commercial nursery substrate was composed of parts of soil, sand, and organic compound (tanned bovine manure) in the proportion 3:2:1, respectively. This treatment presents the chemical and physical characteristics of those normally used for the production of commercial seedlings (Negreiros et al. 2009). The plant responses to this type of substrate allow the evaluation of the possibility of producing such species on a large scale for use in environmental restoration programs (e.g. Gomes et al. 2018). Organic fertilization alters soil structure and water holding capacity, increases microbial activity and modifies cation and anion retention, among other benefits (Wadt 2003).

Mature seeds were collected from 10 different individuals of each studied plant species. The seeds were stored in labelled plastic bags and later screened in the laboratory. Those that had damages or signs of predation and/or disease were discarded. To obtain the seedlings, the diaspores were planted in styrofoam trays with vermiculite substrate. After 30 days of germination, the seedlings were randomly transferred to the treatments in plastic containers (10 cm in diameter and 100 cm deep, so that there was no limitation of root growth). The containers were arranged in a completely randomized design (6 treatments × 10 replicates per species), with one individual per replicate, totalling 60 individuals per species. Seedlings were cultivated for a period of 150 days. The greenhouse was covered with a shade cloth (50%) and the seedlings were irrigated by microsprinkling for 10 min twice a day, with a total of 120 ml of water per day.

Analysis of soil nutrients – The soils from each treatment were analysed to describe their chemical characteristics. To determine the pH and the content of Fe, N, P, and K, a composite sample of each treatment was submitted for analysis by the Department of Soils of the Federal University of Viçosa (following the protocol of CFSEMG 1999; Table 1). In addition, to determine the water holding capacity in the soil we subjected 10 samples (100 g) of each treatment to gravimetric test, as described below. The samples were taken to the laboratory, stored in identified paper bags, and subjected to oven drying at 70° C for 48 h. After this period, they were weighed on an analytical scale and separated into ten 100 g samples. The ten samples of each treatment were wetted to saturation and, after the complete absence of drip, the portion was reweighed to determine the water content (Novais et al. 1991).

Response variables – The percentage of plant survival in the experiment was calculated as the number of live individuals of all species at the end of the 150 days in relation to the initial total. To evaluate plant growth performance in response to the treatments, a destructive sampling of blocks of 10 seedlings of each species per treatment was carried out in three occasions, within 30, 90, and 150 days. Plant survival and growth performance were calculated as the cumulative value through the experiment period. The roots were

Table 1 Soil characterization regarding N, P, K, and Fe content (mg Kg^{-1}), pH, and H_2O—average weight of wet soil (g), with treatments (substrates) ranked in descending order for each of the soil attributes

N	P	K	Fe	H_2O	pH
Organic (4.88)	Organic (281.6)	Nursery (868.1)	NPK/100 (35.1)	Organic (143)	NPK (7.19)
Control (2.49)	Nursery (257.4)	Organic (185.3)	Organic (30.6)	Nursery (133)	NPK/10 (6.28)
NPK/100 (1.66)	NPK (30.3)	NPK/10 (151.1)	Control (28.8)	NPK/100 (129)	NPK/100 (6.21)
NPK (1.58)	NPK/10 (15.6)	NPK (106.5)	NPK/10 (27.6)	NPK/10 (129)	Organic (6.19)
NPK/10 (1.19)	Control (3.46)	NPK/100 (14.2)	NPK (26.3)	NPK (127.5)	Nursery (6.08)
Nursery (1.03)	NPK/100 (2.05)	Control (7.39)	Nursery (16.8)	Control (126)	Control (5.62)

thoroughly washed with water over a 2 mm mesh sieve, until complete removal of the adhered substrate particles. Subsequently, the total length of the seedling (from the highest tip of the root to the insertion of the last pair of leaves—mm) and the total number of leaves were recorded. To determine the production of total dry biomass, each component (root, stem, and leaves) sampled from the seedling was bagged individually, frozen and dried in an oven at 70 °C until reaching constant weight, and weighed on an analytical scale (0.001 g). With these measures, the root/shoot ratio (RSR) was calculated: RSR = RM/(LM + SM), where RM = total root dry mass; LM = total leaf dry mass; SM = total stem dry mass, as in Hunt (1982).

Statistical analyses – To test for variation in water retention capacity among treatments, we built a generalized linear model (GLM) using treatment as the predictor variable and soil wet weight (g) as the response variable. GLMs were also built to test the survival and growth hypotheses. A model was constructed to test whether the percentage of survival (response variable) varied according to the treatment and the species as well as with their interaction. For the analysis of the effect of the treatments on growth, nine models were constructed (one per species) for each of the response variables—leaf number, root/shoot ratio, total dry biomass, and overall length—with treatment as the predictive variable. All models were constructed using an appropriate error distribution for each response variable, according to the critique of the model (Crawley 2013). The mean differences in each response variable per treatment were assessed by contrast analysis (Crawley 2013).

To evaluate the effect size of the treatments on the set of variables, we performed MANOVA (Multivariate Analysis of Variance) for each of the studied species. This analysis of variance is indicated when there is more than one response variable, and a linear combination of them yields a single value of the effect of the predictor variable (Legendre and Legendre 1983). In this case, also treatment was the predictive variable, while the response variables for each of the species were leaf number, root/shoot ratio, total dry biomass, and total length.

3 Results

Analysis of soil nutrients – Overall, the organic and nursery substrates presented the highest nutrient concentration and water holding capacity ($F = 20.33$, $DF = 59$, $p < 0.05$; Table 1). However, the nursery substrate presented the lowest concentration of N. The control substrate showed the lowest values of pH, K, and water holding capacity. The NPK treatment showed a neutral pH, whereas the control was the most acidic of all. The treatment NPK/100 presented the highest amount of Fe, followed by the organic and control while the nursery presented the lowest Fe content.

Survival and growth – The percentage of plant survival at the end of the experiment (150 days) did not vary among treatments (Fig. 1a, $p = 0.55$), but varied among the plant species ($p < 0.001$; Fig. 1b). The species with the highest percentage of survival at the end of 150 days were *Copaifera langsdorffii* with 100% survival, *Trixis vauthiere* and *Pleroma heteromallum* with 93% survival each, followed by *P. candolleanum* (80%), *Eriope macrostachia* (78%), *Mimosa calodendron* (58%), *Baccharis dracunculifolia* (56%), and *Eremanthus erythropappus* (53%). All *E. erythropappus* plants in the organic treatment died. The species with the

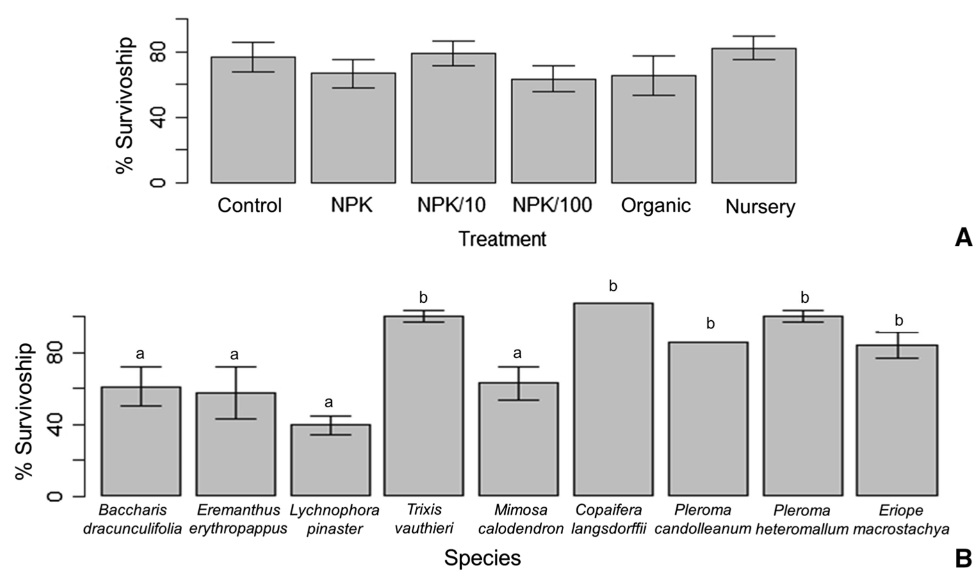

Fig. 1 **a** Mean (± SE) percent survival rate of plants of all species as a function of soil treatment after 150 days of the experiment ($p = 0.55$). **b** Mean (± SE) percent survival of the studied plant species after 150 days of the experiment ($p < 0.001$). Different letters indicate statistically different means at 5% significance

lowest survival rate was *Lychnophora pinaster*, with 36%. Survival was influenced by treatment, but with differences among species ($p < 0.001$). The survival of *E. erythropappus* and *M. calodendron* increased in the control and nursery treatments. *B. dracunculifolia* survived more in control, while *E. macrostachia* survived more in the treatment fertilized with NPK. The other species presented equal survival among the studied treatments.

In general, we found a better performance in growth and development for the substrates with greater nutrient and water availability: the nursery and organic (up to 10 times greater response magnitude than the control, Fig. 2; see also Supplementary Material 2 and 3 for detailed results). For most species (*E. erytroppappus*, *L. pinaster*, *M. calodendron*, *P. candolleanum*, and *P. heteromallum*), the highest values of leaf number, dry biomass, total length, and investment in above-ground parts (lowest root/shoot ratio) were found in the nursery substrate. The seedlings of *B. dracunculifolia* and *E. macrostachya* presented similar positive responses between the nursery and organic substrates, while *T. vauthieri* showed better performance in the organic substrate. Only the seedlings of *C. langsdorffii* showed a better overall performance in the NPK/10 treatment. Detailed results by plant species are available in Supplementary Material 2 and 3.

The performance of the evaluated species also showed improvement with soil quality enhancement when we analysed all the response variables together. The results show a positive effect of the treatments for all the species. However, each species showed a different amplitude of response. The species *P. candolleanum* (F (42) = 133.18, $R^2 = 0.898$, $p < 0.001$) and *L. pinaster* (F (16) = 3.529, $R^2 = 0.725$, $p < 0.001$) were those for which soil improvement most influenced performance and development. They were followed by *E. macrostachya* (F (41) = 7.387, $R^2 = 0.652$, $p < 0.001$), *P. heteromallum* (F (50) = 7.387, $R^2 = 0.591$, $p < 0.001$), *B. dracunculifolia* = (F (50) 4.955, $R^2 = 0.599$, $p < 0.001$), *C. langsdorffi* (F (54) = 5.634, $R^2 = 0.546$, $p < 0.001$). The species that showed the lowest effect of soil quality improvement on performance were *T. vauthieri* (F (50) = 7142, $R^2 = 0.473$, $p < 0.001$), *E. erythropappus* (F (27) = 4.763, $R^2 = 0.453$, $p < 0.001$), and *M. calodendron* (F (29) = 4746, $R^2 = 0.381$, $p < 0.001$).

4 Discussion

The studied ironstone rupestrian grassland species are tolerant to edaphic stress (Negreiros et al. 2014b; Le Stradic et al. 2015) but have demonstrated the ability to adjust to new substrate conditions. When cultivated in soil with enhanced nutrient and water availability, and reduced iron concentration, the seedlings generally presented enhanced growth (measured as biomass, height, and number of leaves), and invested more in aerial parts—corroborating the hypothesis of a positive response to nutrient increase. On the other hand, enhancing soil quality did not result in significant improvement in their survival rates. These results indicate that the studied plant species are able to tolerate, survive, and reproduce under the edaphic conditions of the ironstone rupestrian grassland. However, there is an element of reversible adjustment (acclimation; Körner 2003) involved in the performance of those plant species. Thus, although adapted to survive under the natural harsh conditions of the rupestrian grasslands (Fernandes 2016b), those plants are limited in terms of optimum development and are able to adjust and respond positively to improved soil conditions.

Although plant survival was high and varied among species, survival was not influenced by soil improvement. Figueiredo et al. (2018) also describe a high survival rate for the plant species *Periandra mediterranea* (Vell.) Taub. in post-mining substrates of ironstone soil. Some plant species grow rapidly and need more resources in certain ontogenetic stages, while others have slow growth and greater longevity (see Chapin et al. 1993; Negreiros et al. 2016). Plants growing in low resource environments have intrinsically slow growth rates and invest more in increasing their resistance (Chapin et al. 1993). These slow-growing species show physiological and morphological attributes to survive strong environmental filters such as high levels of heavy metals, soils that hamper root penetrability, poor water holding capacity, and low nutrient availability (Messias et al. 2013; Negreiros et al. 2014a). Slow-growing species may also show high amounts of defence against herbivore damage (Coley et al. 1985; Reich 2014). Negreiros et al. (2016) show that in degraded areas of quartzitic rupestrian grassland, the planted seedlings of slow-growing species had higher survival rates than the fast-growing ones. Thus, survival in adverse environments is usually prioritized in relation to growth, whereas in enriched substrates plants can allocate more resources to other functions which can compromise survival (Bloom et al. 1985).

Seedlings of eight out of the nine evaluated species, with different response amplitudes, grew and increased their biomass in response to soil quality improvement, especially in the organic and/or nursery substrate. This result shows that these substrates are the most recommendable for the production of robust seedlings of the studied species. Because the seedlings grown in these substrates will be larger (in both height and biomass) and with more roots and leaves, they will most likely be better able to survive when used in planting in environmental restoration projects. Previous studies have also shown that under mineral deficiency, plants can grow, but when optimal nutrients, water, and soil conditions are restored, as in this study, some plant species

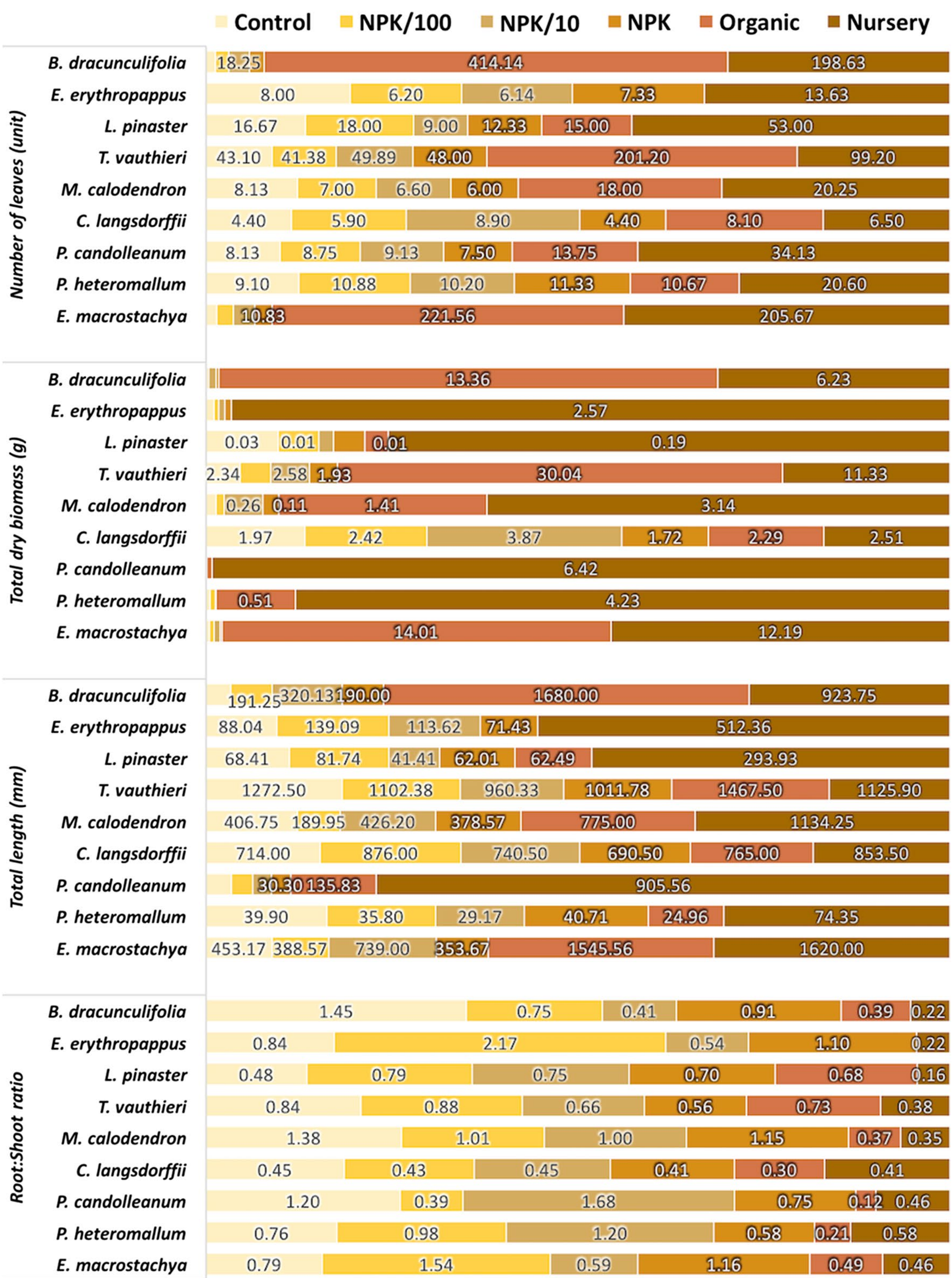

Fig. 2 Stacked bar chart of mean values (numbers) by treatment of each of the growth parameters measured for seedlings of nine plant species from an ironstone Brazilian rupestrian grassland. See Supplementary Material 2 and 3 for detailed statistical results by species. Darker colours denote soil treatments with overall higher nutrient and water status

can maximize resource uptake, favouring growth (Larcher 2000; Negreiros et al. 2009). The different amplitudes in the growth performance of the studied species can be explained, in part, by the natural occurrence of these plants in various physiognomies. Plants that colonize widely heterogeneous environments may present more variability and greater adaptability (Chapin et al. 1993). Indeed, species of restricted occurrence usually have lower phenotypic variability than species with wider distribution (Hermant et al. 2013).

The specific trade-offs between growth and survival may also explain the coexistence of species-rich communities (Kneitel and Chase 2004; Wright et al. 2010). Convergent attributes that allow successful colonization favour niche partitioning along a resource gradient and different adaptive strategies (Negreiros et al. 2016). The main plant trait spectra evident globally represent variation in plant resource economics (a trade-off between traits conferring resource acquisition and internal conservation) and in the size of plants and plant organs (Díaz et al. 2016). It shows a strong functional convergence in plant mineral nutrition strategies among severely impoverished ecosystems around the world, as demonstrated by the root specializations found in most soils with P deficiency (Oliveira et al. 2014, 2016). Here, the majority of the studied species cultivated in the organic treatment, rich in P, presented greater investment in aerial parts than in roots (lower root/shoot ratio). Fohse et al. (1991) also show that species capable of capturing more P may have a lower root/shoot ratio. Different root/shoot ratios found among distinct plants species and soil types may indicate different adaptive responses for nutrient uptake (Peret et al. 2011; Carvalho et al. 2017). Slow-growing plants also have characteristics such as the ability to accumulate reserves and efficient nutrient resorption during senescence (Chapin et al. 1993). These plants can modify morphological structures and establish symbiotic associations for improving nutrient assimilation (Figueiredo et al. 2018).

Another nutritional advantage for plants growing on substrates with high nutrient availability is the decrease in the absorption of excess heavy metals. Here, in substrate NPK/100, with higher amounts of Fe, there was no positive growth response compared to the also iron-rich control substrate. However, in the organic substrate, with more water and nutrients, the high concentration of Fe did not affect the growth performance of the plants. Foy et al. (1978) report a decrease in Pb uptake by plants grown on more nutritious soils. The opposite happened when these plants were cultivated in nutrient-poor soils; there was an increase in the concentration of heavy metals in the leaves (Foy et al. 1978). On the other hand, Ribeiro et al. (2017) suggested that metallophilic plants species, such as *Byrsonima variabilis,* that use Mn for physiological functions, may also use the available heavy metals as a defence against herbivores. This may suggest that plants that colonize environments such as the ironstone rupestrian grasslands may also succeed because they accumulate metals as a form of defence against some types of herbivores. The water holding capacity of the substrates used in the experiment was another conditioning factor for the development of these species. There was an increase in growth and biomass of plants grown in organic and nursery substrates, which presented greater water holding capacity. Assis et al. (2011) demonstrate that in Cerrado environments, hydrological factors and soil water holding capacity are the attributes that most positively influence vegetation structure and floristic composition. Water constraints may represent a strong selection pressure for the partition of plant biomass, favouring root formation (Tomlinson et al. 2012). A greater investment in roots can occur in thicker textured soils where less water retention occurs, compared to those with finer texture (Figueiredo et al. 2016). Chapin et al. (1993) show in an experiment that plants that are more sensitive to water stress may undergo a cascade of reactions, culminating in low nutrient absorption and low growth.

The plants of the nine studied species have high survival capacity, regardless of the substrate, but this capacity varied among them. Most species (except *C. langsdorffii*) responded positively to substrate enhancement, especially to substrates with good water holding capacity. These plants grew more and accumulated more biomass. This demonstrates high capacity for reversible adjustment since despite being able to survive in the depleted ironstone soil of the rupestrian grasslands, these plants can capture the resources available in more fertile soils. In addition, these responses with different amplitudes among species that colonize an environment with severe environmental filters, such as the ironstone rupestrian grasslands, can favour the colonization of multiple habitats in these environments. Thus, aiming for growth we show that robust seedling production for the restoration of ironstone rupestrian grasslands can be made more efficient for the studied species, and most likely to the majority of the rupestrian grassland species, by choosing the right substrate (nursery and organic).

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Author's contribution All authors contributed to the study conception and design. Experimental preparation and data collection were performed by Thaise de Oliveira Bahia and Hernani Alves de Almeida. Statistical analysis was performed by Thaise de Oliveira Bahia and Daniel Negreiros. The first draft of the manuscript was written by Thaise de Oliveira Bahia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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