



## More is not always better: responses of the endemic plant *Vellozia nanuzae* to additional nutrients

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### ABSTRACT

Soil nutrients are one of the main drivers of plant species composition and distribution, mainly due to the role they play in plant survival and reproductive success. However, the nutritional requirements of plants inhabiting their native ecosystems are still poorly known. This is the case for most species of *campo rupestre* vegetation, which are found on infertile soils of quartzitic and ferruginous origin. The present study evaluated the effects of macronutrients and substrates on survival and growth of the micro-endemic *Vellozia nanuzae* (Velloziaceae). Plant mortality was about 95 % higher in soil enriched with macronutrients and almost 100 % in soils with added manure in the first 30 days. Individual plants from treatments with added nutrients had lower growth (53 % less) compared to individuals on natural substrates. In conclusion, *Vellozia nanuzae* survived and developed better on soil of its original habitat even though it is acidic and poor in nutrients. Our results show that more nutrients are not always better for the survival and development of native species that inhabit harsh ecosystems.

**Keywords:** *campo rupestre*, plant nutrition, rupestrian grasslands, soil nutrients, Velloziaceae

## Introduction

Plant survival and development are closely related to soil nutrient availability (Chapin III 1980; Epstein & Bloom 2004). Many nutrients, such as N, P, K, Ca, Mg, S, B, Cu, Cl, Fe, Mn, Mo and Zn, are essential to tissue formation and hence plant metabolism and development (Uchida 2000; Razaq *et al.* 2017). On the other hand, when in excess in natural ecosystems, these nutrients can be harmful and lead to plant mortality (Uchida 2000; Echart & Cavalli-Molina 2001; Negreiros *et al.* 2014). Anthropogenic activities (Melnikova & Sasai 2020) and global climate change (Hunt *et al.* 2020) are important sources of increased soil fertilization (Miller & Miller 1998; Matias *et al.* 2011; Xu *et al.* 2019), a global phenomenon that may affect

native species inhabiting low fertile habitats (Fernandes 2016a). Other soil parameters may interact with nutrients further changing their availability in the soil. For instance, increased acidity can reduce the availability of N, P, K, Ca, Mg, and S, and thus soil fertility (*e.g.*, Arens 1958; Goodland & Ferri 1979; Schaefer *et al.* 2016). The physical attributes of soil, such as texture and granulometry, also play important roles in plant development and success. For instance, sandy soils contain a large amount of coarse particles and have lower cation exchange capacity, which result in better soil aeration but reduced water retention and nutrient concentrations (Rawls *et al.* 1991; Klein & Klein 2015). Altogether, soil attributes represent important filters that ultimately determine plant success and hence plant community assembly (see Wijesinghe *et al.* 2005; Negreiros *et al.* 2014).

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Fertilization techniques used to reduce acidity, such as liming, and alterations to soil substrate have been widely used to achieve increased vigor and development of plants. Although widely used in agriculture and silviculture, such techniques are not always successful for every ecosystem, as many plant species do not respond to them in the same way. In fact, many plant species develop better on soils that are considered nutritionally poor, such as in many natural grasslands and savannic formations (e.g., Haridasan 1988; 2000; Negreiros *et al.* 2009). It is surprising that many ecosystems that are considered nutrient-poor, acidic and with high aluminum concentrations, such as *campo rupestre* (rupestrian grassland), possess high plant diversity and endemism (Conceição *et al.* 2016; Fernandes 2016a). *Campo rupestre* is an ancient grassy ecosystem found in areas above 900 m where rocky outcrops prevail (e.g., Fernandes 2016a; Silveira *et al.* 2016). As these species grow on nutrient-impoverished soils and under harsh environmental conditions, the vegetation is predominantly sclerophyllous and herbaceous with scattered shrubs and trees (Negreiros *et al.* 2014; for details see Fernandes 2016a).

There are several reasons to better understand the nutritional responses of plants of the *campo rupestre* ecosystem. First, knowledge of plant growth and development is of major importance for the restoration of this ecosystem, which has experienced high rates of habitat conversion and impacts from climate change (e.g., Fernandes *et al.* 2014; 2018; Fernandes 2016b; Pena *et al.* 2017). The restoration of *campo rupestre* is not an easy task due to the high climatic seasonality with a long period of water shortage and high soil temperatures, among other factors (see review in Fernandes *et al.* (2016). Another challenge is achieving restoration by seeding because the low viability of the seeds of many species requires that an extremely high quantity be used (Stradic *et al.* 2014; Dayrell *et al.* 2016; but see Negreiros *et al.* 2016; Gomes *et al.* 2015; 2018). Second, habitat conversion results in drastic changes to soil structure and quality (Morgan & Connolly 2013; Fernandes *et al.* 2016). For instance, the conversion of natural soils to cultivation can result in compaction, erosion and leaching of nutrients as well as excessive fertilization, contamination by pesticides and acidification or alkalization (Hillel 2007). Moreover, another common land use in *campo rupestre* is mining, the activities of which cause deep and irreversible disruption to soil structure with the removal of the thin surface soil layer (topsoil) (e.g., Fernandes *et al.* 2014), along with its seed and root bank, further impacting plant colonization and seed germination (Fernandes *et al.* 2016). An opposite effect is that of road paving, which leads to the deposition of calcium, mostly along road margins (Barbosa *et al.* 2010). Calcium deposition leads to decreased aluminum toxicity, which produces habitat conditions that enable invasion by exotic ruderal species (Barbosas *et al.* 2010; Hilário *et al.* 2011). Third, the management and conservation of endemic plant

species require special understanding and attention given the increasing threats to *campo rupestre* (e.g., Fernandes *et al.* 2018), a first step of which is understanding plant responses to nutrient availability.

The herbaceous species *Vellozia nanuzae* (Velloziaceae) is a micro-endemic of the *campo rupestre* ecosystem in Serra do Cipó, Minas Gerais, Brazil. The species is only found among a few rocky crevices (Mello-Silva 2015) in a limited geographic range. Given its restricted distribution, there is an urgent need to gain more knowledge about the physiology and propagation of the species so that proactive conservation strategies and adequate management can be deployed (Fernandes *et al.* 2007). Moreover, this species is also known to have a high potential for bioprospecting (Barbosa & Fernandes 2008). The leaves of *V. nanuzae* contain secondary compounds that have potential for cancer treatment, such as nanuzone and 11 $\beta$ -hydroxy-nanuzone (Pinto *et al.* 1988). The species also has potential for cosmetic use due to the resin produced by all of its organs (Maurya & Yadav 2005).

The aim of the present work was to compare the survival and growth of *V. nanuzae* seedlings in different substrates. To do so, soil from the natural habitat of the species was used with varying the levels of nutrient addition and proportions of other substrates. Two hypotheses were tested. Hypothesis I: Seedlings of *V. nanuzae* exposed to nutrient addition will exhibit lower survival and higher growth rates than seedlings exposed to soils with lower nutrient availability. The rationale behind this hypothesis is that species that are tolerant to nutritionally poor soils will survive better in soils of lower nutritional quality. Some plant species that live in nutritionally poor habitats have traits that retain and conserve nutrients, and thus ensure increased survival under such conditions (e.g., Coley *et al.* 1985; Grime & Campbell 1991; Arendt 1997; Waremboourg & Estelrich 2001). In addition, Negreiros *et al.* (2014) found that some *campo rupestre* species have lower survival in soils with added nutrients, further providing support for this hypothesis. Nutritional increments generally stimulate increased plant growth; however, for plant species naturally inhabiting low fertility soils the response is less pronounced, if at all, because of their tolerance strategies, such as a stress resistance syndrome that confers slow growth (Chapin III *et al.* 1993; Aerts & Chapin 2000). Hypothesis II: seedlings of *V. nanuzae* grown in naturally occurring soil of *campo rupestre* will have a higher proportion of roots compared to aerial biomass. It is expected that plants tolerant of nutrient-poor soils will allocate more to roots due to limited nutrients (Chapin III 1980; Bloom *et al.* 1985; Wilson 1988; Mooney & Winner 1991; Moreira & Klink 2000; Hoffmann & Franco 2003). A common strategy for plant species of the *campo rupestre* ecosystem is strong investment in their underground structures due to strong pressures on aerial parts, such as fire and herbivory (see reviews in Fernandes 2016a; Pausas *et al.* 2018). Furthermore, roots are responsible for water absorption during the dry period,





hence supporting the contention of high investment in the root system (Brum *et al.* 2017).

## Materials and methods

### Study area

The experiment was carried out in a greenhouse at Vellozia Reserve (19°17'46" S, 43°35'28" W; 1,200 m), Serra do Cipó, Minas Gerais, Brazil, between December 2016 and June 2017. The local climate is classified as mesothermic (Cwb), according to the classification of Köppen, which is characterized by dry winters and wet summers. The average annual rainfall is 1,500 mm and the average temperature varies between 17.4 and 19.8 °C (see Madeira & Fernandes 1999).

### Experimental design

Seeds of *V. nanuzae* L.B.Sm. & Ayensu were obtained through manual collection of ripe fruits in September 2016. The seeds for the experiment were sorted from those having signs of predation, pathogens or malformations. The seeds were then seeded in six 128-cell styrofoam trays (3 cm X 3 cm X 5.1 cm) with four or five seeds per cell. The substrate used for seed germination and seedling production was composed of equal parts of washed sand, vermiculite and vegetable soil (modified from Macedo *et al.* 1993).

After 120 days, 600 individuals of 0.5 to 1 cm in height (average height = 0.75 cm) were collected and transferred to plastic containers (8.5 cm in diameter and 18 cm in depth) of six treatments (n= 100 individuals per treatment): control (soil from the natural habitat of *V. nanuzae*); three treatments with the addition of different concentrations of nitrogen (N), phosphorus (P), and potassium (K) (NPK/100: 15 g/m<sup>3</sup> of NPK + 20 g/m<sup>3</sup> in dolomitic limestone; NPK/10: 150 g/m<sup>3</sup> of NPK+ 200 g/m<sup>3</sup> in dolomitic limestone; and NPK: 1500 g/m<sup>3</sup> of NPK + 2000 g/m<sup>3</sup> in dolomitic limestone); and two treatments with substrates containing different proportions of control soil (C), sand (S) and manure (M) (3:2:1 and 2:0:1, respectively) (see Tab. 1 for details). We used the manure marketed as Terral Esterco®. About 1.5 kg of soil was added to each container. The NPK used was 10-10-10 (10% N, 10% P<sub>2</sub>O<sub>5</sub>, 10% K<sub>2</sub>O), Vitaplan® brand. The amount of formulation of these macronutrients was based on the manufacturer's (Vitaplan) recommendations. The amount de NPK used for NPK/100 (15 g/m<sup>3</sup>) and NPK/10 (150 g/m<sup>3</sup> of NPK) was, respectively, 100 and 10 less than those prepared for NPK (1500 g/m<sup>3</sup>). The *campo rupestre* soil used for the experiment was collected at points near where the species occurs in Vellozia Reserva. The soil was collected to a depth of 10–20 cm and within a 20 cm diameter around each point. After drying, the collected soils were sieved with a 5 mm mesh, to remove large fragments of gravel, and homogenized.

**Table 1.** Treatments with different concentration of nitrogen (N), phosphorus (P), and potassium (K) and proportions of substrates — native soil (control - C), sand (S) and manure (M) — used to test survival and growth of *Vellozia nanuzae* (Velloziaceae).

Treatments	Substrate	Liming	Fertilization
Control (C)	<i>Campo Rupestre</i>	No	No
NPK/100	(C)	Yes = 20 g/m <sup>3</sup> in dolomitic limestone *	15 g/m <sup>3</sup> of NPK
NPK/10	(C)	Yes = 200 g/m <sup>3</sup> in dolomitic limestone.	150 g/m <sup>3</sup> of NPK
NPK	(C)	Yes = 2000 g/m <sup>3</sup> in dolomitic limestone.	1500 g/m <sup>3</sup> of NPK
C+S+M	(C) + sand + manure (3:2:1).	No	No
C+M	(C) and manure (2:1).	No	No

\*Dolomitic limestone (PRNT 80 %, CaO 37 %, MgO 14 %, humidity 5 %).

Three soil samples from each treatment, chosen at random, were homogenized and sent for chemical and physical analyses prior to the experiment. The analyses were performed by Departamento de Solo, Universidade de Viçosa, Brazil. The method used was according to Donagema *et al.* (2011). The following parameters were measured for each sample: pH; organic matter, N, P, K, Ca<sup>2+</sup> and Mg<sup>2+</sup> content; microelements S and B; potential acidity (H + Al); sum of bases (SB); cation exchange capacity at pH 7.0 (T); effective cation exchange capacity (t); base saturation index (V %); and remaining phosphorus (P-Rem). The results for each treatment are presented in Table 2.

The containers containing the substrates and seedlings were arranged in a completely randomized design of six treatments × 100 replicates for 600 individual seedlings. The seedlings were grown in a greenhouse covered with shading material (50 %) and irrigated by micro-sprinklers for one minute twice a day. The containers possessed holes allowing free drainage of water. Manual weeding was performed weekly to eliminate invasive plants. Individuals of each treatment were evaluated once a month for five months (150 days) after transplantation.

### Assessment of survival and growth

To evaluate plant survival according to treatment, the numbers of surviving and dead individuals were quantified after 30, 60, 90, 120 and 150 days. To analyze Hypothesis I, in relation to survival, a regression analysis was performed following a Weibull survival distribution with censored data (Pinder III *et al.* 1978; Crawley 2012), where the response variable was time to death of each plant and the explanatory variable was the nutrient addition treatment, with five levels: Control (C), NPK/100, NPK/10, NPK, C+S+M, and C+M. Individuals that remained alive until the end



**Table 2.** Chemical and physical properties of the soil of the six treatments used to test survival and growth of *Vellozia nanuzae*. Three treatments had the addition of different concentrations of nitrogen (N), phosphorus (P), and potassium (K): 1) NPK/100 (15 g/m<sup>3</sup> of NPK + 20 g/m<sup>3</sup> in dolomitic limestone); 2) NPK/10 (150 g/m<sup>3</sup> of NPK+ 200 g/m<sup>3</sup> in dolomitic limestone); and 3) NPK (1500 g/m<sup>3</sup> of NPK + 2000 g/m<sup>3</sup> in dolomitic limestone). Three treatments had different proportions of substrates — native soil (control - C), sand (S) and manure (M): 1) C; 2) C+S+M; 3) 2C:1M.

Parameters	Control	NPK/100	NPK/10	NPK	C+S+M	C+M
pH (H <sub>2</sub> O)	5.17	6.38	5.94	6.72	7.26	7.22
N (dag/kg)	0.08	0.05	0.05	0.07	0.1	0.29
P (mg/dm <sup>3</sup> )	0.3	0.4	1.6	29.3	72.4	142
K (mg/dm <sup>3</sup> )	11	35	68	367	536	1.17
Ca <sup>2+</sup> (c mol <sub>c</sub> /dm <sup>3</sup> )	0.56	2.32	2.78	12.6	5.06	7.39
Mg <sup>2+</sup> (c mol <sub>c</sub> /dm <sup>3</sup> )	0.04	0.18	0.21	0.54	1.19	2.16
H + Al (c mol <sub>c</sub> /dm <sup>3</sup> )	1.7	2	1.7	1.7	0.8	1.7
SB (c mol <sub>c</sub> /dm <sup>3</sup> )	0.63	2.59	3.16	14.1	7.62	12.5
t (c mol <sub>c</sub> /dm <sup>3</sup> )	0.63	2.59	3.16	14.1	7.62	12.5
T (c mol <sub>c</sub> /dm <sup>3</sup> )	2.33	4.59	4.86	15.8	8.42	14.2
V %	27	56.4	65	89.3	90.5	88.1
P-Rem (mg/L)	25.7	23	27	26.1	43	43.9
Coarse sand (kg/kg)	0.19	0.21	0.19	0.19	0.50	0.19
Fine sand (kg/kg)	0.33	0.28	0.30	0.31	0.19	0.29
Silt (kg/kg)	0.35	0.35	0.36	0.36	0.20	0.35
Clay (kg/kg)	0.12	0.14	0.13	0.13	0.09	0.15

Footnote: pH in water, KCl and CaCl 1:2.5 ratio; N extracted by sulphurous digestion, Kjeldhal distillation; P and K extracted by Extractor Mehlich 1; Ca<sup>2+</sup> and Mg<sup>2+</sup>, extractor 1 mol / L KCl; H + Al, extractor 0.5 mol / L calcium acetate pH 7.0.

of the experiment (150 days) were recorded and used in the analysis as censored data to inform the final model. The trendlines were not fitted to the dataset in the chart; we preferred to use the raw data, showing how mortality happened over time. To analyze the growth performance in each treatment was evaluated over time by recording rosette diameter (cm) and the total number of leaves every 30 days. Rosette diameter was measured using a digital caliper (accuracy of 0.01 mm) while the number of leaves was counted with the aid of a manual counter. The relative growth rate (RGR) of seedling diameter was calculated following Hunt 1982:  $RGR = (\ln D_{final} - \ln D_{initial}) / (t_{final} - t_{initial})$  where, D = diameter; and t = time. In order to determine relative growth rate (RGR) of seedling diameter, a linear model was constructed for each plant, and the slope used as a proxy for growth rate in each treatment. In this way, a single value was generated for each replicate (plant), which was used as the response variable in a Generalized Linear Model (GLM)-ANOVA with treatment as the explanatory variable. Models were then subjected to contrast analysis, with levels of treatment without significant differences (P > 0.05) being lumped. Only treatments where survival rates were higher than 50% were compared in the RGR analysis to achieve a minimum balanced model design.

To analyze Hypothesis II, production of dry biomass (roots and shoots) was recorded for each individual survivor through destructive collection after the last measurement of the experiment. The roots were carefully washed with water on a 2 mm mesh sieve to remove particles of substrate adhered to them. The production of dry biomass was determined by bagging each component collected from the plants. The bagged components were then frozen and taken

to the laboratory for oven drying at 70 °C until reaching constant weight (weighed with an analytical balance at 0.001 g precision), according to Chiariello *et al.* (1989). To evaluate biomass partitioning between plant organs (root and shoot), the following parameters were calculated for each plant using root and shoot biomass values, as carried out by Sainju *et al.* 2017: Root/ Shoot ratio (RSR) = MR/ MC; where: MR = root dry mass and MC = shoot dry mass. Data were analyzed by GLM-ANOVA with treatment being the explanatory variable. Models were submitted to contrast analysis, with levels of treatment without significant differences (P > 0.05) being grouped. Here we only compared the RSR of treatments where survival rates were higher than 50%.

All statistical analyses were performed using R Statistical Software (R Development Core Team 2014).

## Results

### Soil properties

All of the properties related to the chemistry and texture of soil varied among treatments (Tab. 2). The *campo rupestre* soil (control) was the most acidic and nutritionally poor. We noted that the addition of NPK and manure led to an increase in pH and macro and micronutrients. The pH was about 29% and 39% higher for the control (pH = 5.17) than for the NPK (pH=6.72) and 2C:1M (pH= 7.22) treatments, respectively. The average amount of nitrogen for the control was 0.08 dag/kg and was similar to the three treatments of NPK (NPK/100, NPK/10, NPK). However, nitrogen content was 3.6 times higher for 2C:1M (0.29 dag/kg) than for the



control (0.08 dag/kg). Mean phosphorus content was 97-fold higher for the NPK treatment (29.3 mg/dm<sup>3</sup>) and about 473 times higher (142 mg/dm<sup>3</sup>) for the 2C:1M treatment than for the control (0.3 mg/dm<sup>3</sup>). The average potassium content was higher for treatments C+S+M (536 mg/dm<sup>3</sup>) and NPK (367 mg/dm<sup>3</sup>) than for the control (11 mg/dm<sup>3</sup>). Mean magnesium content was 0.04 cmolc/dm<sup>3</sup> for the control and 14 % higher for the NPK treatment and 54 % for the 2C:1M treatment. Calcium level was highest for the NPK treatment (12.6 cmolc/dm<sup>3</sup>) and lowest for the control (0.56 cmolc/dm<sup>3</sup>). The highest values for cation exchange capacity (14.1 cmol/dm<sup>3</sup>) were for the NPK treatment. Coarse sand (0.19-0.21 kg/kg), fine sand (0.28-0.33kg/kg), silt (0.35-0.36 kg/kg), and clay (0.12-0.15 kg/kg) were similar among control, NPK/100, NPK/10, NPK, and 2C:1M treatments. The treatment C+S+M had the highest values for coarse sand content (0.5 kg/kg) and the lowest values for fine sand (0.19 kg/kg), silt (0.20 kg/kg), and clay (0.09 kg/kg).

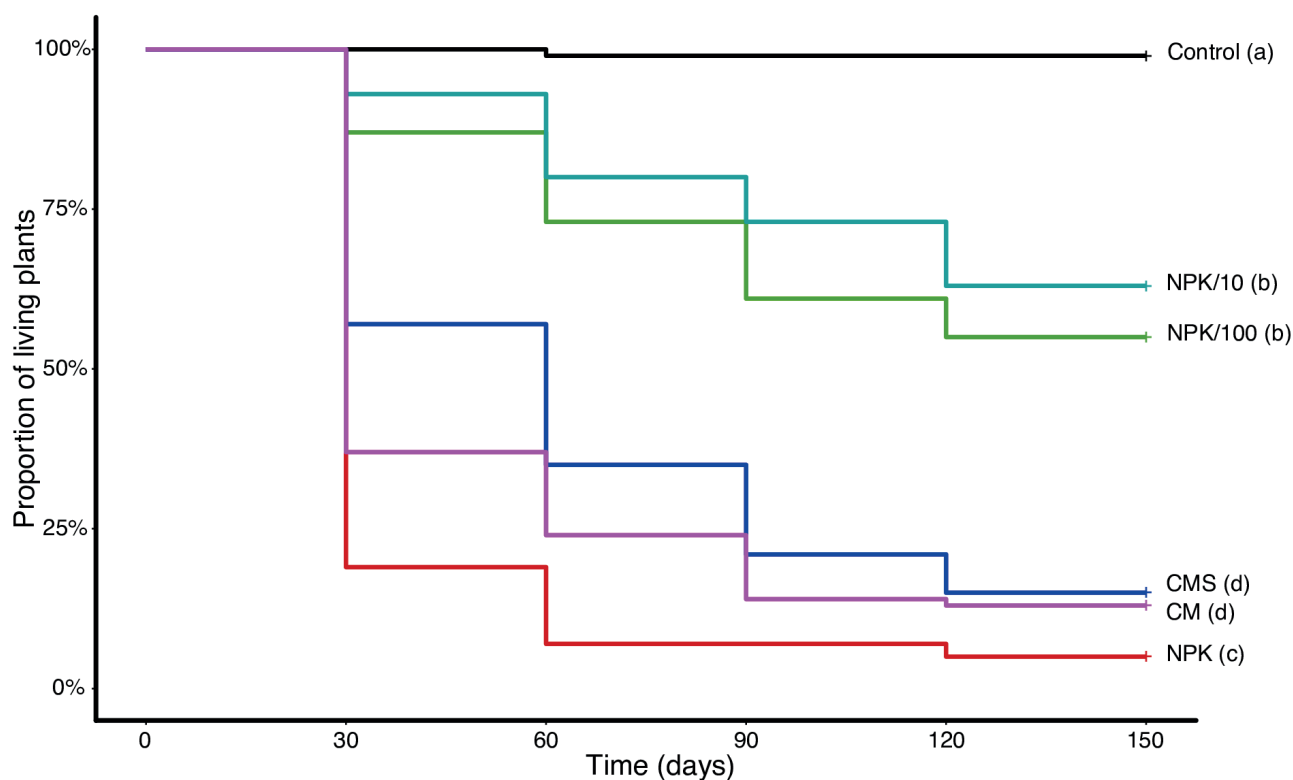
### Seedling survival experiment

The survival of *V. nanuzae* seedlings was significantly lower in all treatments relative to the control treatment (native soil;  $\chi^2 = 235.41$ , DF=5,  $P < 0.001$ ), while the Tukey post-hoc analysis revealed that only manure ( $P = 0.43$ )

and high levels of NPK addition ( $P = 0.72$ ) did not differ in their influence on plant survival (Fig. 1). In fact, while only one individual in the control treatment died during the experiment, there was an average of 41 % mortality for individuals receiving treatments of low levels of NPK addition (*i.e.*, NPK/100 and NPK/10); 85 % mortality for manure treatments, and 95 % mortality for the NPK treatment. Due to the extremely high mortality rates in CM, CMS and NPK treatments, they were not used in further growth analysis, since survival is the most limiting variable for a plant and such unbalanced design could bias the analysis.

### Seedling growth performance

Regarding plant growth, *V. nanuzae* individuals growing in the control treatment exhibited significantly greater increases in diameter than individuals grown in all other treatments within the 150 days of observation ( $F_{2,213} = 16.56$ ,  $P < 0.001$ , Figs. 2, 3A). Control plants (native soil) exhibited a two-times greater increase in diameter than plants grown in NPK enriched soils (NPK/100 and NPK/10). We noticed that the individuals of the CM treatment languished after 60 days with decreasing diameter.



**Figure 1.** Proportion of surviving plants of *V. nanuzae* under treatments of different concentrations of macronutrients and substrate composition over time (days). Three treatments had the addition of different concentrations of nitrogen (N), phosphorus (P), and potassium (K): 1) NPK/100 (15 g/m<sup>3</sup> of NPK + 20 g/m<sup>3</sup> in dolomitic limestone); 2) NPK/10 (150 g/m<sup>3</sup> of NPK+ 200 g/m<sup>3</sup> in dolomitic limestone); 3) NPK (1500 g/m<sup>3</sup> of NPK + 2000 g/m<sup>3</sup> in dolomitic limestone). Three treatments had different proportions of substrates (soil native (control - C), sand (S) and manure (M)): 1) C; 2) 2C:1M; 3) 3C:2S:1M.

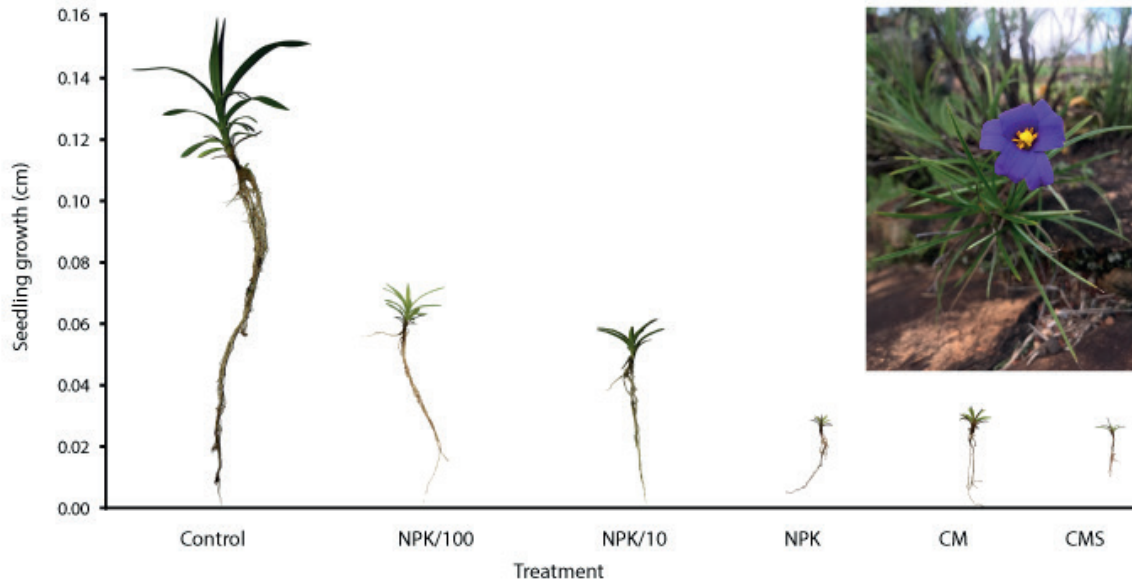


The number of leaves per plant did not differ significantly among treatments ( $F_{2,213}=0.9, P=0.40$ ).

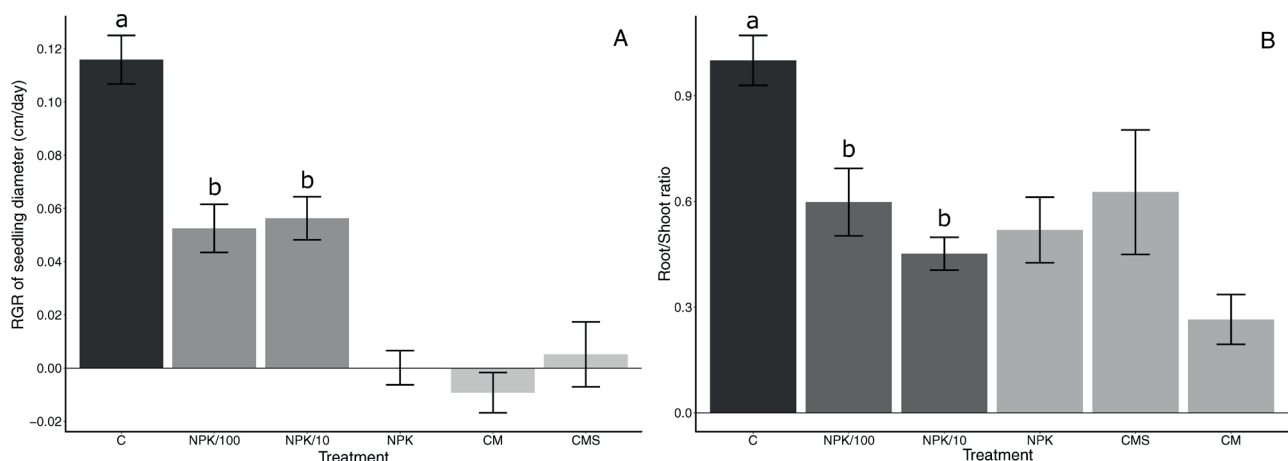
The root/shoot biomass ratio was also significantly higher for individuals of *V. nanuzae* grown in the control treatment ( $F_{2,168}=37.19, P<0.001$ , Fig. 3B) compared to individuals grown in nutritionally enriched soils and soils with different substrates.

## Discussion

Seedlings of *V. nanuzae* grown in soil from their natural habitat had significantly higher survival and growth rates than seedlings grown in soils enriched with nutrients. These results corroborate Hypothesis I — native plants that naturally inhabit nutrient poor soils will have lower survival



**Figure 2.** *Vellozia nanuzae* L.B.Sm. & Ayensu (Velloziaceae), an endemic species of the *campo rupestre* ecosystem. Individuals of *V. nanuzae* that remained alive until the end of the experiment (150 days) selected randomly under different concentrations of macronutrients and substrate composition. Three treatments had the addition of different concentrations of nitrogen (N), phosphorus (P), and potassium (K): 1) NPK/100 (15 g/m<sup>3</sup> of NPK + 20 g/m<sup>3</sup> in dolomitic limestone); 2) NPK/10 (150 g/m<sup>3</sup> of NPK+ 200 g/m<sup>3</sup> in dolomitic limestone); 3) NPK (1500 g/m<sup>3</sup> of NPK + 2000 g/m<sup>3</sup> in dolomitic limestone). Three treatments had different proportions of substrates (soil native (control - C), sand (S) and manure (M)): 1) C; 2) 2C:1M; 3) 3C:2S:1M.



**Figure 3. A.** Average ( $\pm$  standard error) relative growth rate (RGR) of seedling diameter (cm/day) of *V. nanuzae* under treatments of different concentrations of macronutrients and substrate composition. **B.** Average ( $\pm$  standard error) root-to-shoot biomass ratios of individuals of *V. nanuzae* under different concentrations of macronutrients and substrate composition. Three treatments had the addition of different concentration of nitrogen (N), phosphorus (P), and potassium (K): 1) NPK/100 (15 g/m<sup>3</sup> of NPK + 20 g/m<sup>3</sup> in dolomitic limestone); 2) NPK/10 (150 g/m<sup>3</sup> of NPK+ 200 g/m<sup>3</sup> in dolomitic limestone); 3) NPK (1500 g/m<sup>3</sup> of NPK + 2000 g/m<sup>3</sup> in dolomitic limestone). Three treatments had different proportions of substrates (soil native (control - C), sand (S) and manure (M)): 1) C; 2) 2C:1M; 3) 3C:2S:1M.



rates when cultivated in nutrient enriched soils than when grown in soil with low nutritional availability. *Campo rupestre* soil is nutritionally poor (see review by Schaefer *et al.* 2016). Even so, the growth and survival of *V. nanuzae* plants in this natural substrate were greater, indicating that the species is highly tolerant to their restrictive habitat. In fact, individuals that developed in nutritionally enriched substrates exhibited a drastic reduction in survival, reinforcing the hypothesis of tolerance to the nutrient-poor environment (see Tab. 2) of the *campo rupestre* ecosystem. It is also important to highlight changes in soil Ca<sup>2+</sup> content and pH under the different treatments (Tab. 2). High acidity did not seem to be a limiting factor of seedling development and survival for *V. nanuzae*. The direct negative effects of high pH or a high Ca concentration in the soil, and inability to use the phosphorus pool of the soil, also suggest that this species presents a calcifuge behavior (Tyler & Olsson 1993; Lee 1999). In this case, calcium acts as secondary messenger, which requires the maintenance of a low concentration of this nutrient (Lee 1999). However, more biochemical and physiological experiments are required to better elucidate this behavior. In another nutritional experiment, Negreiros *et al.* (2014) reported that *Baccharis dracunculifolia*, while showing increased growth in soils of higher nutritional quality, had lower survival rates. Although the tolerance of some plant species to nutritionally poor soils has already been demonstrated for some harsh ecosystems (e.g., Haridasan 1988; 2000), the mechanisms are mostly unknown and hence in need of further investigation.

The results of the present study are valuable for the conservation and management of species of the *campo rupestre* ecosystem, considering that innumerable activities have a direct influence on the nutritional quality of its soils. For example, Barbosa *et al.* (2010) demonstrated that the paving of roads in the *campo rupestre* ecosystem increased the calcium content and decreased the aluminum content of the soil along road margins. They also showed that this habitat disturbance resulted in a completely different plant community, often with a high number of ruderal and invasive species. The same has been observed as the result of the installation of pasture systems (e.g., Vendramini *et al.* 2007; Schaefer *et al.* 2016). Therefore, environmental restoration projects designed for the *campo rupestre* ecosystem need to take into consideration the nutritional requirements of plants and their temporal and functional dynamics (Fernandes *et al.* 2016).

In addition to increased survival rates, plants of *V. nanuzae* grown in soil from the *campo rupestre* ecosystem (i.e., control plants) also had higher average growth rates than those grown in enriched soils, hence providing support for Hypothesis I. These results indicate that nutritional enrichment of nutritionally poor environments can result in toxic conditions for native species, and thus trigger adverse effects at the ecosystem level (Fernandes & Price 1991). Although other species of the *campo rupestre* ecosystem

had an opposite response with higher growth rates with nutritional enrichment (*Calliandra fasciculata*, Negreiros *et al.* 2009; *Collaea cipoensis*, Negreiros *et al.* 2009; *Baccharis dracunculifolia*, Negreiros *et al.* 2014), these species are widespread (*C. fasciculata*: Barneby 1998), autochorous (*C. cipoensis*: Gélvez-Zúñiga *et al.* 2018), or pioneer (*B. dracunculifolia*: Müller *et al.* 2007) species. Clearly, the number of species studied so far does not allow us yet to propose a general trend for the response of *campo rupestre* species. Future habitat-wide studies that focus on plant responses to nutrition will aid in the construction of a more robust hypothesis for *campo rupestre* species. Regardless, we argue that disruption of soil stability and quality lead to strong changes in soil nutritional conditions that harm the survival and development of *campo rupestre* plant species. Soil conservation measures for areas close to populations of *V. nanuzae* could represent an important strategy for ensuring its survival and minimizing the chances for extinction given its rarity in the landscape.

The number of leaves has been shown to be a conservative trait (see Cui *et al.* 2018). The control plants and those subjected to different nutritional treatments had similar numbers of leaves, despite the physicochemical differences among the soils. This plant trait was maintained in soils with nutritional enrichment, while relative growth rates and biomass were reduced.

Plants of *V. nanuzae* grown under conditions of low soil nutrients and high toxicity had greater root development than those grown with enriched soil, further supporting Hypothesis II. This hypothesis predicted that seedlings of *V. nanuzae* grown in naturally occurring *campo rupestre* soil will have a higher proportion of roots compared to aerial biomass. Soil acidity and high aluminum concentration often result in reduced root biomass (Echart & Cavalli-Molina 2001; Caires *et al.* 2008); this was not, however, observed in *V. nanuzae*. The higher proportion of biomass in the roots of *V. nanuzae* can be interpreted as a response or tolerance to low nutrient availability (Lilienfein *et al.* 2001), water stress (Hoffmann & Franco 2003) and/or frequent fire (Castro & Kauffman 1998), all of which are important environmental filters in the *campo rupestre* ecosystem (Negreiros *et al.* 2014; see reviews in Fernandes 2016a). The greater root development of *V. nanuzae* would favor the nutrient uptake and assimilation necessary for growth in depleted soils (Berbara *et al.* 2006; Oliveira *et al.* 2016). Moreover, in nutrient-impooverished soils of *campo rupestre*, belowground specializations (rhizosheaths, mycorrhizas, and dark septate fungi) can be relevant traits for plant survival (Coutinho *et al.* 2015; Oliveira *et al.* 2015; Abrahão *et al.* 2019). Abrahão *et al.* (2019) verified that among 27 species of Velloziaceae, most (16 species) possessed rhizosheaths with long root hairs in soils poorest in P, which allows habitat specialization among rock-dwelling and soil-dwelling and favors a greater uptake of soil resources. Relationships between plants and soil are also not unidirectional. Increased nutrients can stimulate or



favor the antagonistic soil biota and suppress protagonists (Zandt *et al.* 2019). Although recent advances have been made in understanding plant-microorganism associations in *campo rupestre* (review in Oki *et al.* 2016), future studies that elucidate the relationship between nutrition and microorganism diversity and its effects on growth and development of *V. nanuzae* are needed. In conclusion, *Vellozia nanuzae* is tolerant to nutrient poor soil and develops better in soil from its original habitat, which is characterized as acidic and nutrient poor. Our results show that more nutrients are not always better for survival and development of native species that inhabit harsh ecosystems. Furthermore, the findings presented here reinforce the importance of conserving soil in order to conserve the species. There are hundreds of other species of the megadiverse *campo rupestre* ecosystem (see Fernandes 2016c) that may possess similar or even stronger or more finely tuned tolerance to soil poverty and toxicity, and hence are equally under threat from habitat disturbance and climate change. Given the present lack of governance and plans to lead effective conservation and monitoring of the *campo rupestre* ecosystem, and the lack of investment in Brazilian biodiversity and conservation (Fernandes *et al.* 2018), extinctions of plant species, such as *V. nanuzae*, are imminent.

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