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ORIGINAL ARTICLE

Low fertilization optimizes the water use efficiency of an Amazonian *canga* grass for mineland rehabilitation

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ABSTRACT

The peculiar characteristics of mining waste substrates represent a significant challenge for environmental rehabilitation. Here, we evaluated the revegetation potential of *Paspalum cinerascens* on substrates from mining areas of Serra dos Carajás, a region harboring a large mine complex in the eastern Brazilian Amazon. *Paspalum cinerascens* is a native grass widely distributed in the *canga* ecosystem, a vegetation type covering iron ore reserves. Seeds of *P. cinerascens* harvested in *canga* were germinated in sterilized quartzite sand and the seedlings grown in controlled conditions for 90 days. The seedlings were then cultivated in *canga* topsoil (control, without fertilization) and mining waste substrate with half and complete fertilization currently applied at the beginning of mineland rehabilitation in Serra dos Carajás. Regardless of fertilization, plants grown in the mining waste substrate did not differ in carbon assimilation, tillering rate and root biomass, despite higher leaf nutrient content and lower root: shoot ratio when compared to plants in *canga* topsoil. Compared to the control, complete fertilization led to significantly taller plants, higher shoot biomass and reduced water use efficiency. Half fertilization led to higher phosphorus and water use efficiency and stomatal density. Our results confirmed that *P. cinerascens* has adaptive traits to grow and thrive in the harsh environmental conditions of post iron ore mining, and can be used in rehabilitation processes. Moreover, half fertilization led to plants with optimized water loss in exchange for carbon without significant costs to plant growth, an interesting trait for rehabilitation in areas experiencing water restrictions.

KEYWORDS: rock outcrops; land degradation; water restrictions, Poaceae, Carajás

Baixa fertilização otimiza a eficiência do uso da água de uma gramínea nativa da canga amazônica para reabilitação de minas

RESUMO

As características peculiares dos substratos de remanescentes da mineração (estéril de mina) representam um desafio para a recuperação ambiental. Neste estudo avaliamos o potencial de uso de *Paspalum cinerascens* para revegetação de estéril de mina na Serra dos Carajás, Pará (Brasil). *Paspalum cinerascens* é uma gramínea nativa amplamente distribuída nas cangas, vegetação típica dos campos rupestres que cobrem reservas de minério de ferro. As plantas de *P. cinerascens* foram cultivadas em *topsoil* de canga (controle) e em estéril de mina com meio e completo regime de fertilização atualmente empregado para revegetação das áreas mineradas na Serra dos Carajás. Foram utilizadas sementes coletadas nas cangas e as plantas foram cultivadas em condições controladas por 90 dias. Independentemente da fertilização, plantas cultivadas em estéril de mina não apresentaram diferenças significativas na assimilação de carbono, perfilhamento ou biomassa radicular, apesar de valores mais elevados de nutrientes foliares e menor razão raiz: parte aérea quando comparadas às plantas em *topsoil* de canga. A fertilização completa resultou em plantas mais altas, maior biomassa aérea e menor eficiência no uso da água. Metade da fertilização aumentou a densidade estomática, a eficiência de uso da água e de fósforo. Esses resultados confirmaram que *P. cinerascens* possui características adaptativas para crescer e prosperar em condições ambientais adversas remanescentes da mineração, sendo indicada para uso em processos de recuperação de áreas degradadas na Serra dos Carajás. Além disso, o uso de metade do regime de fertilização otimiza o uso da água pelas plantas sem perdas significativas de crescimento, uma característica desejável para recuperação de áreas com restrições hídricas.

PALAVRAS-CHAVE: afloramentos rochosos; degradação do solo; restrições hídricas, Poaceae, Carajás

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INTRODUCTION

Active land rehabilitation aims to accelerate the recovery of environmental function and services lost or negatively impacted by anthropogenic activities (SER 2004). Severe ecosystem degradation arising from human activities such as open pit mining may impose a series of challenges to rehabilitation. During the process of iron ore extraction, open pit mines dig huge craters, and a large part of the raw materials extracted (mine waste substrates) accumulate in enormous piles (Chen *et al*. 2018) and require rapid soil cover to reduce the risk of damages associated with erosion (sediment runoff, water contamination, etc.) (Gastauer *et al*. 2018). Nonetheless, such substrates are commonly acidic, poor in organic matter, nutrients and soil organisms, and show low water retention capacity (Guedes *et al*. 2020; Silva *et al*. 2018), which impair plant growth and make it difficult to revegetate their surface. Therefore, to improve the chemical limitations of these soils, liming and fertilization are part of the amendments applied during the beginning of the rehabilitation processes to promote plant growth (Grant *et al*. 2007; Carvalho *et al*. 2018).

In addition to the benefits of rapid soil cover (Pietrzykowski 2019) fast-growing plant species are advantageous for improving the low carbon content belowground (usually close to zero at severely disturbed sites or in recently built up soils, such as minelands after topsoil removal) (Guedes *et al*. 2021) and promoting the return of some ecosystem services, such as soil nutrient cycling (Cristescu *et al*. 2013; Ranjan *et al*. 2015). In the beginning of the rehabilitation processes, seeds of nonnative annual herbs are conventionally used, mostly because of their feasibility to be applied in broad areas (Silva et al. 2018). While less information exists on native species, they may be better adapted to unique and severe edaphic and climatic conditions, such as post mining substrates (Oliveira de Araujo *et al*. 2020; Caldeira *et al*. 2021), and can contribute to restoring local network interactions, thereby reducing the risk of biological invasions and the emergence of undesired new ecosystems (Gastauer *et al*. 2020*a*). Efforts have been made to select promising local native species to restore mining areas in many countries such as Brazil (Silva *et al*. 2018; Zappi *et al*. 2018; Caldeira *et al*. 2021), Russia (Treschevskaya *et al*. 2019) and South Africa (Titshall *et al*. 2013), among others. The successful use of native species was reported in the reclamation of coal mining in the North American Appalachians (Fields-Johnson *et al*. 2012) and for the restoration of ecosystem functions of bauxite mines in Western Australia (Grant *et al*. 2007).

Grass species are known for their rapid growth, high efficiency in water use and carbon fixation, allowing them to create a homogenous ground cover, reducing erosion processes (Siqueira-Silva *et al*. 2019; Oliveira de Araujo *et al*. 2020). In a recent work with *Paspalum cinerascens* (Döll) A.G.Burm. & C.N.Bastos, Caldeira *et al*. (2021) highlighted the potential of this species to colonize mining waste substrates. *Paspalum cinerascens* is a native perennial grass of the *canga* ecosystems of Serra dos Carajás, in the eastern Brazilian Amazon (Viana *et al*. 2018). Covering large iron ore reserves in the mountain outcrops of the Amazon rainforest, the *canga* vegetation is well adapted to the adverse local environmental conditions, marked by a thin and metal rich soil, high radiation, wind, temperature and severe seasonal drought period (Skirycz *et al*. 2014). As a perennial grass widely distributed in the *canga* of Serra dos Carajás (Viana *et al*. 2018), *P. cinerascens* was listed as one of the most promising species for mineland reclamation in this region (Zappi *et al*. 2018), due to its elevated tolerance to iron concentrations in the substrate (Caldeira *et al*. 2021), as has also been reported for other species of *Paspalum* (Figueiredo *et al*. 2018; Oliveira de Araujo *et al*. 2020).

In order to further assess the potential of *P. cinerascens* for revegetation purposes in Serra dos Carajás, in this study we evaluated the growth of this grass in the substrate remaining from iron ore extraction in different nutritional requirement scenarios. As the species shows high carbon assimilation in *canga* environments (Caldeira *et al*. 2021), we expect that *P. cinerascens* seedlings can grow and thrive in the mining waste substrate with little fertilization requirements.

MATERIAL AND METHODS

Plant material and germination

Seeds of *P. cinerascens* were collected at the end of the rainy season (May 2019) from the grassland of the *canga* plateau N1 (6° 2’32.30”S, 50°16’19.76”W) of Serra dos Carajás (Pará state, Brazil) (Viana *et al*. 2018). In the Plant Growth Laboratory of Instituto Tecnológico Vale (Belém, Brazil), the seeds were sown over sterilized quartzite sand in plastic boxes (Gerbox, 11 cm x 11 cm x 4 cm) and kept in a growth chamber (SCG 120, Weiss Technik, Loughborough, UK) with a 12:12 h photoperiod, 80 μmol m^{-2} s^{-1} photosynthetic photon flux density, 28:22 °C day:night temperature regime and 60% air humidity. Water loss by evaporation was replaced daily. Seedlings were collected after the emission of primary roots and leaves and transferred to pots of 1 dm^3 containing the substrates.

Plant growth conditions

Substrate collection and analysis - Seedling growth was compared between two substrates, the *canga* topsoil collected in the *canga* plateau N1 and the mining waste substrate collected from a representative location of the N4-N5 mines of Carajás Mineral Province (6° 4’35.37”S, 50° 9’31.17”W) of Serra dos Carajás. After collection, both substrates were air-dried and sieved to remove particles larger than 1 cm. The physical and chemical properties of the substrates were determined after homogenization and sieving through a 4 mm mesh (Supplementary Material, Table S1). The chemical characterization was carried out following Teixeira *et al.* (2017). The pH was determined by a pH electrode in a 1:2.5 soil-to-water ratio, and the organic carbon was determined using the potassium dichromate ($K_2Cr_2O_7$) method. The available phosphorus (P), potassium (K), boron (B), zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) contents were determined using the Mehlich-1 method (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4), where P was determined by colorimetry, K by flame photometry and the other elements by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Exchangeable Ca, Mg and Al were measured using atomic absorption spectrophotometry on 1 M KCl extracts with the addition of lanthanum oxide. The soil texture was determined as described by Kettler *et al.* (2001).

Experimental design - Because of its low nutrient content, and to simulate the field revegetation practices currently used in the Carajás Mineral Province, the mining waste substrate was used with two fertilization treatments: i) the complete fertilization protocol [100 mg dm^{-3} N (CH_4N_2O), 200 mg dm^{-3} P ($Na_2PO_4H_2O$), 100 mg dm^{-3} K (KCl), 60 mg dm^{-3} S, 0.5 mg dm^{-3} B (H_3Bo_3), 5 mg dm^{-3} Zn ($ZnSO_4.7H_2O$), 1.5 mg dm^{-3} Cu ($CuSO_4.7H_2O$), and 0.15 mg dm^{-3} Mo ($(NH_4)_6Mo7O_{24}.4H_2O$)]; and ii) half the fertilization protocol (half of all nutrient doses). As a native species of *canga* ecosystem, we hypothesized that *P. cinerascens* may require low nutrient input, which can also further reduce the potential recruitment of invasive species. Except for N, all nutrients were applied before seedling transfer to the substrate. N fertilization was carried out in three applications at regular intervals of 30 days (0, 30 and 60 days). The *canga* topsoil was used as the control without any fertilization. We used eight repetitions for each treatment and the control, each repetition consisting of one pot of 1.2 dm^3 containing a single plant. Plants were cultivated for 90 days.

Environmental conditions - The plants were cultivated in growth shelters with temperature and relative air humidity monitored every 15 minutes with a thermocouple connected to a datalogger (RHT10, Extech Instruments, Boston, USA). The daily air temperature varied between 25 and 37 °C, and the vapor pressure deficit varied between 0.4 and 2.5 kPa (Supplementary Material, Figure S1). The water availability was held at 70% of the soil water retention capacity by replacing water loss from evapotranspiration with distilled water after daily monitoring of the pot weight.

Plant measurements

Tillering rate, biomass and nutrient partitioning - The tillering rate was determined as the number of tillers produced by the plants after 90 days of cultivation. Then, the shoot and root biomass were harvested separately, washed and oven-dried at 62 °C to a constant weight. The materials were then ground in a Wiley mill (Retsch, Düsseldorf, Germany) to determine the concentration of major nutrients and micronutrients. For nutrient determination, samples of 250 mg of plant tissue were mixed with 2 mL of 65% HNO_3 (MERCK®, Darmstadt, Germany), 2 mL of H_2O_2 and 5 mL of Milli-Q water (Master System MS 2000, Gehaka, São Paulo- Brazil) and then digested in a microwave oven (Mars Xpress, CEM Corporation, Matthews, NC, USA) at 80 ºC for 3 min, 150 ºC for 5 min and 180 ºC for 10 min. The digested extracts were filtered using slow-filtering blue-band filter paper. The filtered extract was transferred to 50-mL Falcon tubes, and the final volume was adjusted with Milli-Q water. Macro and micronutrient levels were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES).

Nutrient use efficiency (NUE) was determined based on the dry mass production and nutrient content. The NUE was estimated as follows (Siddiqi and Glass 1981):

$$NUE = {m_d^2}/{m_n}$$

where, is the shoot dry mass (mg) and is the accumulated nutrient mass in the shoots.

Gas exchange - The gas exchanges were measured two days before plant harvesting in the fully developed leaves of the main stem of each plant with a gas analyzer (LI-6400XTR, LICOR, Lincoln, NE, USA). The measurements were carried out in the morning (between 9:00 and 11:30), and the environmental conditions inside the cuvette were adjusted to a PPFD of 1000 µmol m^{-2} s^{-1}, block temperature 28 °C, vapor pressure deficit 1.8 kPa and air CO_2 concentration of 400 ppm. Carbon assimilation rate (A), stomatal conductance (gs), transpiration (E) and internal carbon (Ci) were estimated. We also calculated the intrinsic water-use efficiency (iWUE) as the ratio of A: gs, the carboxylation efficiency as A: Ci and the ratio of intercellular to ambient CO_2 (Ca) as Ci Ca^{-1}.

Leaf morphological traits – Five leaf samples were collected from the middle region of the leaf lamina of fully expanded leaves. All samples were fixed in FAA 70 (formaldehyde, acetic acid, 70% ethanol, 1:1:18 v/v) for 24 hours and preserved in 70% ethanol. Histological free-hand cross-sections of the leaf lamina were stained with toluidine blue (O'Brien *et al.* 1964) and mounted in 50% glycerin. The epidermal printing method (Segatto *et al.* 2004) was used for stomatal characterization. The slides were observed and photomicrographed under an optical microscope (Zeiss Scope A.1) coupled to an AxioCam TCc 5 digital camera. The images were analyzed using Moticplus 2.0 software, previously calibrated with a micrometric slide provided by the manufacturer. We measured the epidermis thickness on the adaxial and abaxial sides, mesophyll, bulliform cells, guard cells, midrib and metaxylem. The stomatal density of the area of the abaxial epidermis was calculated. Nail polish imprints were taken from the abaxial surface of mature leaves. Stomatal densities were determined by light microscopy from leaf imprints. Four square areas were analyzed in each leaf to obtain the stomatal density.

Data analysis

Generalized linear models (GLMs) with Gaussian and Poisson errors were built to evaluate the influence of different substrates on the physiological, anatomical and morphological response of *P. cinerascens*. Contrast analysis was performed as a post hoc test for all GLM analyses. All analyses were performed using the R platform (R Core Team 2018) and the *coms* package was used for contrast analyses with the function RT4Bio.

RESULTS

Plant growth and gas exchange

The plants grown in the mining waste substrate did not differ significantly from the control in tillering rate and root biomass (Figure 1). Plant height, leaf number and shoot biomass were significantly higher in plants that received complete fertilization compared to the other treatment and the control. The root:shoot ratio was significantly lower in the mining waste substrate, regardless of the fertilizations applied. The carbon assimilation rate, the ratio of intercellular to ambient CO_2 (Ci Ca^{-1}) ratio and carboxylation efficiency did not differ significantly among substrates (Figure 2). Stomatal conductance and transpiration rate were significantly higher in plants growing in mining waste substrate, regardless of fertilization, while intrinsic water use efficiency was significantly higher in plants receiving half fertilization and in the control.

Leaf nutrient content

Four out of eight nutrients with significant variation (N, K, Mg, S) were higher in the treatments than in the control, while P only was significantly higher in mine waste receiving complete fertilization (Table 1). Ca, Mn and Fe were significantly higher in the control, where both Mn and Fe reached considerably high values. B, Cu, and Zn did not differ significantly among the substrates. NUE was significantly higher for P in mining waste substrate with half fertilization, while plants grown on mine waste with complete fertilization had greater efficiency in the use of Ca and Mn (Table 2).

Leaf morphological traits

Most of the leaf anatomical traits did not differ significantly among treatments (Table 3). Midrib thickness and stomatal

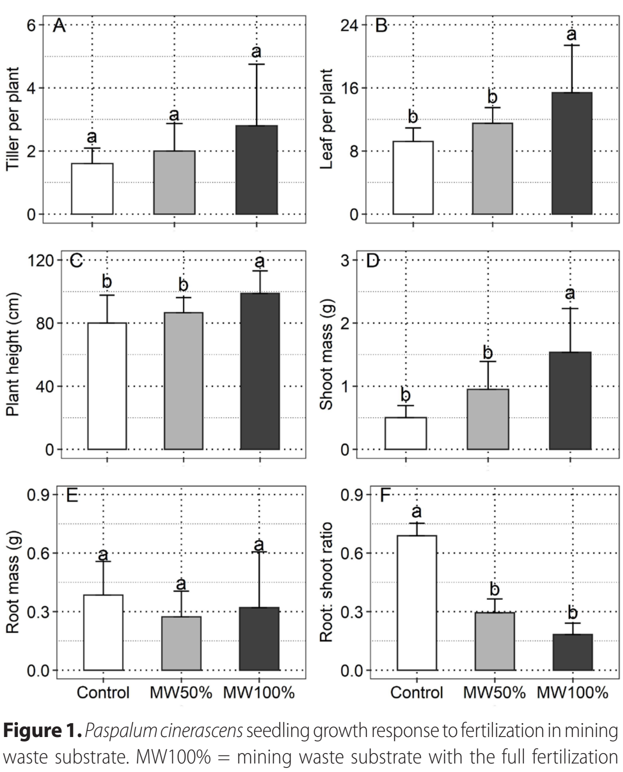

Figure 1. *Paspalum cinerascens* seedling growth response to fertilization in mining waste substrate. MW100% = mining waste substrate with the full fertilization regime used on site for revegetation; MW50% = mining waste substrate with 50% standard fertilization regime. Columns represent the mean of eight repetitions and the error bars the 95% confidence intervals. Bars carrying the same letters indicate no significant pairwise difference between the treatments and control according to a contrast test.

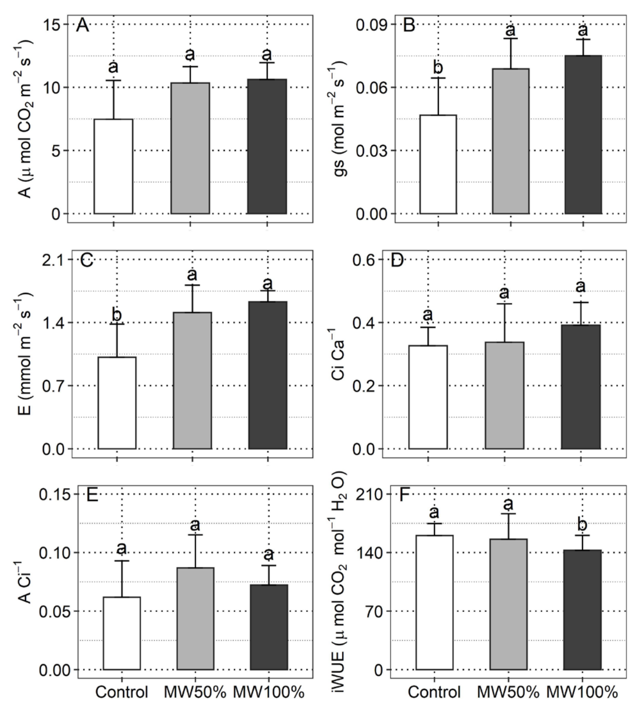

Figure 2. Gas exchange parameters of *Paspalum cinerascens* seedlings cultivated in mining waste substrates. MW100% = mining waste substrate with the full fertilization regime used on site for revegetation; MW50% = mining waste substrate with 50% standard fertilization regime. A – carbon assimilation rate (A); B – stomatal conductance (gs); C – transpiration rate (E); D – ratio of intercellular to ambient CO_2 (Ci Ca^{-1}); E – carboxylation efficiency (A Ci^{-1}); F – intrinsic water use efficiency (iWUE). Columns represent the mean of eight repetitions and the error bars the 95% confidence intervals. Bars carrying the same letters indicate no significant pairwise differences between the treatments and control according to a contrast test.

Table 1. Nutrient contents in the leaves of *Paspalum cinerascens* grown on *canga* topsoil (control) and mining waste substrate receiving the complete (MW100%) or half (MW50%) of the standard revegetation fertilization. Values are the mean of eight replicates ± standard deviation. Different letters within columns indicate significant pairwise differences according to a contrast test.

Substrate	N $g\ kg^{-1}$	P $g\ kg^{-1}$	K $g\ kg^{-1}$	Ca $g\ kg^{-1}$	Mg $g\ kg^{-1}$	S $g\ kg^{-1}$	B $mg\ kg^{-1}$	Cu $mg\ kg^{-1}$	Fe $mg\ kg^{-1}$	Mn $mg\ kg^{-1}$	Zn $mg\ kg^{-1}$
Control	4.91 ± 4.1b	0.36 ± 0.0b	7.6 ± 1.6b	3.3 ± 0.3a	3.0 ± 0.9b	1.6 ± 0.3b	23.5 ± 1.7a	4.2 ± 0.64a	1441 ± 378a	1053.7 ± 206a	39.2 ± 9.3a
MW50%	11.01 ± 1.8a	0.37 ± 0.1b	20.1 ± 1.4a	2.2 ± 0.3b	5.5 ± 1.0a	4.4 ± 0.8a	18.3 ± 7.6a	5.7 ± 0.95a	1382 ± 686b	194.1 ± 11.2b	35.1 ± 1.9a
MW100%	13.02 ± 1.0a	0.89 ± 0.0a	22.1 ± 2.6a	2.3 ± 0.8b	5.3 ± 1.6a	4.7 ± 1.5a	20.7 ± 6.0a	5.2 ± 1.83a	799 ± 294c	186.5 ± 17.9b	37.5 ± 4.8a

N – nitrogen, P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, S – sulfur, B – boron, Cu – copper, Fe – iron, Mn – manganese, Zn – zinc

Table 2. The nutrients use efficiency of *Paspalum cinerascens* grown on *canga* topsoil (control) and mining waste substrate receiving the complete (MW100%) or half (MW50%) of the standard revegetation fertilization. Values are the mean of eight replicates ± standard deviation. Different letters within columns indicate significant pairwise differences according to a contrast test.

Substrate	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
						$mg^2\ g^{-1}$					
canga	0.19±0.05a	3.20±0.40b	0.16±0.03a	0.30±0.01b	0.41±0.11a	0.75±0.17a	0.0±0.00a	0.28±0.03a	0.001±0.00a	0.001±0.00c	0.03±0.00a
MW50%	0.26±0.06a	8.37±2.60a	0.14±0.05a	1.40±0.70b	0.56±0.30a	0.70±0.38a	0.19±0.14a	0.52±0.18a	0.002±0.00a	0.014±0.00b	0.08±0.02a
MW100%	0.31±0.21a	4.42±2.60b	0.19±0.12a	2.30±2.20a	0.93±0.86a	1.08±1.03a	0.22±0.20a	0.94±0.77a	0.005±0.00a	0.021±0.13a	0.11±0.07a

N – nitrogen, P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, S – sulfur, B – boron, Cu – copper, Fe – iron, Mn – manganese, Zn – zinc

Table 3. The leaf anatomical traits of *Paspalum cinerascens* grown on *canga* topsoil (control) and mining waste substrate receiving the complete (MW100%) or half (MW50%) of the standard revegetation fertilization. Values are the mean of eight replicates ± standard deviation. Different letters within columns indicate significant pairwise differences according to a contrast test.

Substrate	AdaxEpi (µm)	AbaxEpi (µm)	BC (µm)	Mesophyll (µm)	Midrib (µm)	Metaxylem (µm)	PDS (µm)	EDS (µm)	SD (stomata per mm^2)
canga	11.3 ± 1.3 a	11.0 ± 1.5 a	60.0 ± 15.0 a	43.0 ± 9.0 a	488 ± 93 b	28.1 ± 5.0 a	27.3 ± 7.0 a	14.2 ± 4.0 a	238 ± 110 c
MW50%	10.0 ± 1.3 a	11.4 ± 1.3 a	63.6 ± 10.0 a	51.1 ± 8.8 a	580 ± 62 a	26.4 ± 6.7 a	23.3 ± 4.1 a	15.4 ± 2.5 a	523 ± 180 a
MW100%	9.7 ± 1.5 a	10.4 ± 1.7 a	59.5 ± 4.6 a	45.5 ± 2.8 a	596 ± 30 a	24.3 ± 2.4 a	29.8 ± 8.5 a	17.2 ± 2.3 a	421 ± 158 b

AdaxEpi: adaxial epidermis; AbaxEpi: abaxial epidermis; BC: bulliform cell; EDS: equatorial diameter of the stomata; PDS: polar diameter of the stomata; SD: stomatal density.

density were significantly higher in plants grown in mining waste substrate compared to the control, and stomatal density was significantly higher in plants grown in mining waste with half fertilization compared to complete fertilization. Stomatal density of plants in *canga* topsoil was 46% of that in mining waste with half fertilization, and 57% of that in mining waste with complete fertilization. Despite the differences in stomatal density, stomatal size (polar and equatorial) did not differ significantly among the substrates.

DISCUSSION

Paspalum cinerascens growth performance in mining waste substrate was successful, presenting similar results to the control treatment, which corresponds to its native growth substrate. It was able to develop tillers and root biomass similar to that in *canga* topsoil and it improved the shoot biomass in the mining waste. The maintenance of tillering capacity and the number of leaves is an important feature for protecting the soil against erosion and even increasing the soil biomass, helping to simulate natural successional trajectories (Gastauer *et al*. 2020*b*). Shoot biomass gain among plants grown in mining waste increased with fertilization, reinforcing its potential as a species with high carbon fixation capacity (Caldeira *et al*. 2021) which is a desired characteristic to start land rehabilitation (Gastauer *et al*. 2020*b*). The observed lack of difference in the carbon assimilation rate among treatments indicated that *P. cinerascens* might be able to grow and thrive under harsh conditions. Furthermore, leaf tissue thickness generally did not differ among the substrates, corroborating its carbon assimilation capacity, as mesophyll leaf thickness is directly linked to the total soluble protein and chlorophyll contents and, consequently, to the carbon assimilation rate (Herrera *et al*. 2009). Although changes in stomatal density affect photosynthesis and transpiration performance (Sun *et al*. 2014; Cai *et al*. 2017) the higher values of stomatal conductance, transpiration and stomatal density found in plants grown on mining waste did not negatively affect the rate of carbon assimilation. In fact, this high carbon assimilation capacity may occur due to the stomatal dumbbell shape of grass species, which show rapid stomatal aperture response with small changes in turgor (Chen *et al*. 2017).

The fast establishment of vegetation cover, as indicated by shoot traits (biomass, leaf number and plant height), is important for soil protection against erosion and contributes to improving the organic matter and nutrient cycling, and, consequently, the overall gain in the chemical quality of the substrate, favoring effective revegetation (Poorter *et al.* 2012; Silva *et al.* 2018). *Mimosa acutistipula* var. *ferrea* Barneby, *Parkia platycephala* Benth, *Stryphnodendron pulcherrimum* (Willd.) Hochr. and *Chloroleucon acacioides* (Ducke) Barneby & J. W. Grimes (Fabaceae) are some of the native *canga* species that also showed benefits similar to *P. cinerascens*, displaying rapid growth and soil cover when cultivated in mining waste substrate (Silva *et al.* 2018). On the other hand, the higher investment in the root system observed in individuals grown in *canga* topsoil (which was also observed by Caldeira *et al.* 2021) may be linked to nutrient restrictions due to the lack of fertilization. As *canga* topsoil shows lower nutrient availability than mining waste, more energy allocation to the roots is required to collect nutrients and sustain plant growth (Postma *et al.* 2014). This may explain the different trends observed in plants grown under fertilized conditions. On the other hand, the reduced investment in the root system may compromise water acquisition, especially in scenarios of water restriction during the dry season in Carajás Mineral Province (Skirycz *et al.* 2014). Plants tend to proportionally allocate more resources to the most limiting resource uptake, to achieve a functional balance (Poorter *et al.* 2012). As *P. cinerascens* is well adapted to the water restrictions of *canga* environments (Viana *et al.* 2018), it is expected that, growing on mining waste substrate, the species will fine-tune between root and shoot growth when experiencing water shortage. Thus, conditions allowing more efficient water use, such as those observed when only half fertilization was applied, may be advantageous to perennial species such as *P. cinerascens,* by preserving the soil water for a longer period.

The higher leaf contents of N, P, K, Mg and S promoted by substrate fertilization led to an increase in the shoot biomass, due to the importance of these elements in photosynthetic metabolism. Leaf N determines photosynthetic processes, including light absorption and carbon reaction enzymes (Hikosaka 2004). The addition of N may also have influenced the greater thickness of the midrib of plants grown in mining waste, as also observed in *Arabidopsis thaliana* (Cai *et al.* 2017). Phosphorus is one of the main limiting elements for plant growth and development, as it plays a vital role in morphogenesis and physiological processes (Yan *et al.* 2018). When available in lower quantities, P can restrict the regeneration of ribulose-1,5-bisphosphate (RuBP), which in turn may impair the optimal photosynthesis gain with N availability (Reich *et al.* 2009). Also, P is a key element for determining root growth (Broschat and Klock-Moore 2000). In environments with P deficiency, plants can show pronounced changes in root morphology and growth to improve P acquisition (Bello 2021). In cases of P availability, despite an overall stimulus to root growth, it may also be accompanied by a decrease in the root:shoot ratio because of a relatively higher increment in the shoot component (Kim *et al.* 2008). However, *canga* vegetation is adapted to shallow soils with low nutrient availability (Skirycz *et al.* 2014) and may show lower nutrient demand. Despite the increase in nutrient supply by fertilization, very little gain in nutrient use efficiency was observed. Furthermore, carbon assimilation rates varied minimally with nutrient availability. It is possible that this species behaves as a resource-conserving species, having little response to changes in soil nutrient availability in the short term. This pattern was observed in two species of Fabaceae native to *canga* environments (*Dioclеia apurensis* and *Bauhinia longipedicellata)* (Ramos *et al.* 2020). Plants adapted to soils with low nutrient availability have characteristics to address these environmental restrictions (Silveira *et al.* 2016). This neutral behavior illustrates adaptive fitness responses and the use of resource-conserving traits, which corroborates with previous studies showing that native species from an environment with low water availability may not respond to changes in nutrient availability (Davidson *et al.* 2011; Heberling and Fridley 2015; Knauf *et al.* 2021). Some observed changes, such as the higher root:shoot ratio and similar nutrient use efficiency values in plants, regardless of fertilization, lead to a new homeostatic state, aiming to compensate for the negative effects of a possible water deficit on photosynthesis (Flexas *et al.* 2006). However, few anatomical adjustments were observed for *P. cinerascens*. This demonstrates an adaptation of this species to the site, not needing to carry out a structural rearrangement in its leaves to succeed in establishing growth on mining waste. This adaptation of a native species to mining waste is important to combat invasive species, in addition to reestablishing ecological interactions to provide an effective restoration of the ecosystem.

The plants grown in mining waste substrate showed higher values of stomatal conductance and transpiration rate, which can also benefit the absorption of nutrients from the soil. In addition to this benefit, the higher water loss requires an increment in water capture by the root system, which is higher in plants receiving complete fertilization, because of its more developed shoot part. To meet this demand without significant alterations of the root biomass, it is expected that the plant responds in other aspects of root plasticity such as a higher investment in fine roots and/or adjustments in aquaporin expression, which are proteins that modulate water absorption and transport throughout the plant body (Kaldenhoff *et al.* 1998; Caldeira *et al.* 2014; Maurel and Nacry 2020). However, such adjustments may also allow a decrease in iWUE of plants growing with complete fertilization, despite not having been harmful to biomass accumulation. In the long term, this lower iWUE can harm the establishment and persistence of *P. cinerascens* in areas requiring rehabilitation. Otherwise, the higher iWUE of *P. cinerascens* grown with half fertilization was equivalent to plants in *canga* topsoil. Under this condition, *P.*

cinerascens was able to efficiently manage water in exchange for carbon, representing an important adaptive characteristic to sustain vegetative growth and save water without substantial costs for the carbon assimilation rate (Lawson and Blatt 2014). In addition, the increased iWUE did not occur at the expense of biomass reduction compared to plants grown in *canga* topsoil.

Despite the importance of adding nutrients to degraded areas, the fertilization level is a crucial point in practice to start rehabilitation (Silva *et al*. 2018; Caldeira *et al*. 2021). Knowing the optimal amount of fertilization can prevent waste, the recruitment of weeds and environmental pollution, such as the eutrophication of water resources (Kanter *et al*. 2020; Wang *et al*. 2020). Excess nutrients can promote the recruitment of invasive ruderal species that are superior competitors under greater availability of nutrients in soil (Ribeiro *et al*. 2017). Although plants grown in mining waste with the standard fertilization protocol for revegetation in the Carajás Mineral Province had the highest shoot biomass, they had the lowest iWUE value. In contrast, plants cultivated in mining waste with half fertilization had the same values as plants grown in native *canga* soil, and iWUE was not affected.

CONCLUSIONS

Plants of *Paspalum cinerascens* were able to grow and thrive in mining waste substrates receiving either half or the complete fertilization protocol applied for initial rehabilitation processes in Carajás Mineral Province. Despite the gain in shoot biomass and leaf number by using complete fertilization, the plants also showed decreased water use efficiency. On the other hand, when they received half of the fertilization protocol, the plants showed intrinsic water use efficiency similar to plants grown in *canga* topsoil, emphasizing the benefits of a reduced application of nutrients in mining waste substrates for effective mineland rehabilitation. The species showed few anatomical leaf changes in the different treatments, efficiently used nutrients and saved water, showing high potential to protect the soil during both the dry and rainy seasons. Thus, we suggest that half of fertilization currently applied would be enough to optimize the growth and persistence of this species in revegetation protocols in Carajás.

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SUPPLEMENTARY MATERIAL (only available in the electronic version)

Boanares *et al*. Low fertilization optimizes the water use efficiency of an Amazonian canga grass for mineland rehabilitation

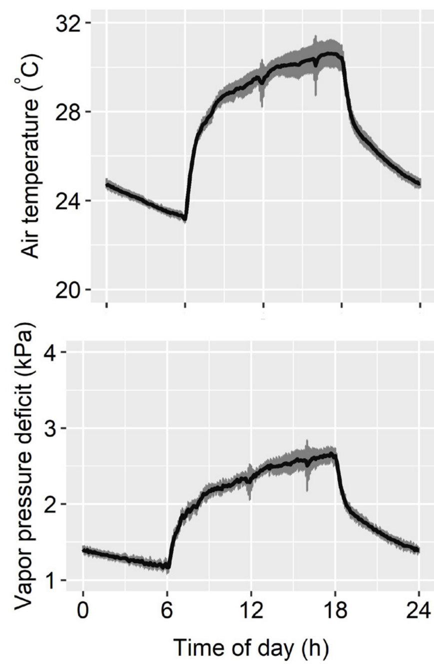

Figure S1. Mean circadian cycle of air temperature and vapor pressure deficit during the 90 days of the growth experiment of *Paspalum cinerascens* in *canga* topsoil and mining waste substrate. Error bars represent the standard deviation.

Table S1. Nutritional and physical characterization of *canga* topsoil and mining waste substrate used in the growth experiment of *Paspalum cinerascens*.

Parameter	Measure unit	*Canga*	Mining waste
pH		5.10	5.10
OC	%	2.60	0.40
N	%	0.00	0.00
P	mg dm^{-3}	10.30	14.80
K	mg dm^{-3}	52.90	8.10
S	mg dm^{-3}	28.90	92.50
B	mg dm^{-3}	0.06	0.20
Zn	mg dm^{-3}	1.50	1.70
Fe	mg dm^{-3}	185.00	76.20
Mn	mg dm^{-3}	127.00	5.50
Cu	mg dm^{-3}	0.10	0.40
Ca	cmolc dm^{-3}	1.00	0.20
Mg	cmolc dm^{-3}	0.60	0.10
Al	cmolc dm^{-3}	0.10	0.01
Sand	%	39.00	39.30
Clay	%	31.00	16.70
Silt	%	30.00	44.00