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PLANT GROWTH AND METABOLISM OF EXOTIC AND NATIVE *CROTALARIA* SPECIES TO MINELAND REHABILITATION IN THE AMAZON

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Dissertation submitted to Federal Rural University of Amazônia, as part of the requirements for obtaining the *Magister Scientiae degree in Agronomy*.

Advisor: Prof. PhD. Silvio Junio Ramos

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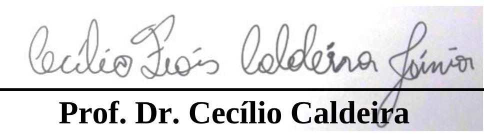

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Resumo

Apesar de seus enormes benefícios, a atividade mineradora é responsável por intensas alterações na vegetação e nas propriedades do solo. Assim, após a exploração, é necessário reabilitar as áreas mineradas criando melhores condições para o estabelecimento de espécies vegetais, principalmente nativas, o que ainda é um desafio. Este trabalho teve como objetivo avaliar a fertilização mineral e orgânica sobre o crescimento, compostos de carbono e metabolismo do nitrogênio (N) de duas espécies de Crotalaria (Crotalaria spectabilis (espécie exótica)) e Crotalaria maypurensis (espécie nativa da Província Mineral de Carajás – PMC) cultivadas em uma pilha de estéril de uma área de mineração de ferro da PMC. Foram testadas sete adubações (I = sem aplicação de fertilizantes; II = NPK; III = NPK + micronutrientes; IV = NPK + micronutrientes + composto orgânico; V = PK; VI = PK + micronutrientes; VII = PK + micronutrientes + composto orgânico). A adubação contribuiu para o maior crescimento de ambas as espécies de Crotalaria e os tratamentos a base de NPK e micronutrientes apresentaram os melhores resultados (até 257%) enquanto a adubação orgânica não promoveu diferenças satisfatórias. A crotalária exótica apresentou maior número de nódulos, massa seca dos nódulos, teores de clorofila a e b e apresentou amônio livre como forma predominante de N, refletindo maiores incrementos na produção de biomassa em relação à espécie nativa. Apesar de apresentar menor crescimento, o uso de espécies nativas na reabilitação de áreas de mineração deve ser considerado, principalmente por ser uma espécie que apresenta bom desenvolvimento e atende a legislação vigente, sendo uma oportunidade para restaurar a biodiversidade local.

Palavras-chave: Fixação biológica de Nitrogênio, área de mineração, metabolismo do nitrogênio, nutrição vegetal.

Abstract

Despite its enormous benefits, mining activity is responsible for intense changes on vegetation and soil properties. Thus, after exploration, it is necessary to rehabilitate the mined areas creating better conditions to the establishment of plant species, especially native ones, which is still a challenge. This study aimed to evaluate mineral and organic fertilization on growth, carbon compounds and nitrogen (N) metabolism of two *Crotalaria* species [*Crotalaria spectabilis* (exotic species) and *Crotalaria maypurensis* (native species from Carajás Mineral Province (CMP) cultivated in a waste pile from an iron mining area in CMP. Seven fertilizations were tested (i = without fertilizers application; ii = NPK; iii = NPK + micronutrients; iv = NPK + micronutrients + organic compost; v = PK; vi = PK + micronutrients; vii = PK + micronutrients + organic compost). Fertilization contributed for the greater growth of both *Crotalaria* species and treatments based on NPK and micronutrients presented the best results (up to 257%) while organic fertilization did not promote satisfactory differences. Exotic *Crotalaria* presented greater number of nodules, nodule dry mass, chlorophyll *a* and *b* contents and presented free ammonium as the predominant form of N reflecting greater increments in biomass production compared to native species. Although presenting lower growth, the use of native species in the rehabilitation of mining areas should be considered, mainly because it is a species that presents good development and meets the current legislation, being an opportunity to restore local biodiversity.

Keywords biological nitrogen fixation; mining area; nitrogen metabolism; plant nutrition

SUMÁRIO

1. CONTEXTUALIZATION

Mining in the Carajás Mineral Province (CMP) began in the 1980, with excavation being carried out using an open-air bench, forming the mine pits and also the waste piles. (LAMEGO, 2012). It is an activity of great importance and contribution to the Brazilian economy. (IBRAM, 2020), however, it causes changes in the local biodiversity, with changes in the landscape, due to the removal of vegetation, requiring the mitigation of negative impacts and the rehabilitation of mined areas.

Brazilian environmental legislation determines that mining companies submit repair/damage requests to areas affected by the activity, with the aim of reducing negative impacts on local biodiversity, especially in conservation units, such as the Carajás National Forest (VIANA et al., 2016). Where the Mineral Complex of Carajás has been installed for the extraction of iron, copper and manganese.

Thus, in the environmental constraints of this complex, there is a recommendation for the use of native species for the revegetation of areas impacted by mining, combined with the use of non-aggressive and easy-to-control exotic/commercial species (ICMBIO, 2014).

Some studies in recovered mined areas point out that the recovery of these areas with native species has facilitated the process of ecological succession (JESUS et al., 2016; SILVA et al., 2018). In addition, the use of species from the Fabaceae family, as a result of green manuring, has contributed to improving the stability of these soils, ensuring greater biological activity throughout the process and maintenance of fertility, due to the enrichment with organic matter and biological fixation of nitrogen (HERNANI; PADOVAN, 2014).

Organic matter, incorporated by green manure, reflects positively on the physical, chemical and biological attributes of the soil, since it guarantees living cover provided by the plant's canopy; mulch resulting from shoots; in addition to the action of microorganisms in their root systems (SOUZA et al., 2014). The use of legumes as green manure has been observed in studies, which demonstrate that they have promoted an increase in the organic matter content and, consequently, an increase in the cation exchange capacity, as well as the levels of N, by BNF (HERNANI; PADOVAN, 2014).

BNF, provided by the symbiosis between rhizobium and legumes, contribute to the economic and environmental viability of the systems, as it reduces the need to apply nitrogen fertilizers to widely cultivated species (FARIAS et al., 2016), as well as being important for soil stabilization and improvement of ecological services.

Thus, the use of legumes, especially native species, in programs for the Recovery of Degraded Areas (RAD), plays an important role as it contributes to the preservation and maintenance of the system. However, despite the importance and need to use native species in the recovery of these areas, the selection of promising species for revegetation requires a better knowledge of their performance in these new environments formed by mining, being essential a greater understanding of growth and nutritional requirement, the in order to maximize its use in recovery activities at CMP (CARVALHO et al., 2017).

This study aimed to evaluate mineral and organic fertilization on growth, carbon compounds and nitrogen (N) metabolism of two Crotalaria species [Crotalaria spectabilis (exotic species) and Crotalaria maypurensis (native species from Carajás Mineral Province (CMP) cultivated in a waste pile from an iron mining area in CMP. This last species is not yet used in revegetation activities in the PMC, but it has potential for use in revegetation due to the high seed production and rapid biomass production. (ZAPPI et al., 2018).

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ARTICLE

Plant growth and metabolism of exotic and native *Crotalaria* species to mineland rehabilitation in the Amazon

Abstract

Despite its enormous benefits, mining activity is responsible for intense changes on vegetation and soil properties. Thus, after exploration, it is necessary to rehabilitate the mined areas creating better conditions to the establishment of plant species, especially native ones, which is still a challenge. This study aimed to evaluate mineral and organic fertilization on growth, carbon compounds and nitrogen (N) metabolism of two *Crotalaria* species [*Crotalaria spectabilis* (exotic species) and *Crotalaria maypurensis* (native species from Carajás Mineral Province (CMP)] cultivated in a waste pile from an iron mining area in CMP. Seven fertilizations were tested (i = without fertilizers application; ii = NPK; iii = NPK + micronutrients; iv = NPK + micronutrients + organic compost; v = PK; vi = PK + micronutrients; vii = PK + micronutrients + organic compost). Fertilization contributed for the greater growth of both *Crotalaria* species and treatments based on NPK and micronutrients presented the best results (up to 257%) while organic fertilization did not promote satisfactory differences. Exotic *Crotalaria* presented greater number of nodules, nodule dry mass, chlorophyll *a* and *b* contents and presented free ammonium as the predominant form of N reflecting greater increments in biomass production compared to native species. Although presenting lower growth, the use of native species in the rehabilitation of mining areas should be considered, mainly because it is a species that presents good development and meets the current legislation, being an opportunity to restore local biodiversity.

Keywords biological nitrogen fixation; mining area; nitrogen metabolism; plant nutrition

Introduction

Ferruginous cangas are ecosystems with vegetation typical of rupestrian fields, which is directly associated with the rocky substrate (Viana et al. 2016). These areas are home to species adapted to extreme conditions, such as acidic soils, low nutrient contents, low water availability, and high temperatures (Mota et al. 2018). The Amazon cangas can be found in the National Forest of Carajás and Campos Ferruginosos National Park. These conservation units are located in Carajás Mineral Province (CMP), Easter Amazon. The CMP is one of the most important mineral provinces in the world, covering the largest iron (Fe) reserves, with potential for exploration and export of bauxite, manganese, gold, copper, nickel and kaolin (Teixeira et al. 2021). Despite the enormous mineralogical potential of the CMP, it is necessary to consider that the mining activity is also responsible for the intense change in land cover and use (Sonter et al. 2014).

Revegetation is the most applied technique to start mineland rehabilitation, however, its main challenge is in the establishment of species capable to dealing with post-mining environments (Ramos et al. 2019). Due to mineral extraction activity and the removal of organic matter and minerals, minelands are extremely depleted in nutrients and with severe changes in the physical, biological and chemical properties (Gastauer et al. 2019a; Pourret et al. 2016; Ramos et al. 2022). Then, during the selection of the species, the rapid growth and accumulation of biomass must be considered, as they contributed to the improvement of soil organic matter levels, soil protection and nutrient cycling, which facilitate the establishment of new individuals (Pratiwi et al. 2021; Silva et al. 2018).

The use of a wide variety of native species are recommended by legal norms in Brazil for rehabilitation purposes because they compose the local community being adapted to the characteristics of the region, and contributing to the return of ecosystem services (Gastauer et al. 2019b, 2021; Ramos et al. 2019). According to Gastauer et al. (2019a) functional characteristics of native species such as nutrient accumulation and their adaptations to the environment to be recovered place the species among those with the highest chance of success for the rehabilitation of mined areas. On the other hand, exotic species can negatively impact the diversity of the communities and threaten the success of rehabilitation (Gastauer et al. 2021).

Among the species used in the initial mineland rehabilitation and to improve the physical, chemical, and biological properties of the soil, those belonging to the Fabaceae family stand out, as they can help in the structuring and stabilization of soil aggregates, ensuring a higher content of organic matter, as well as improving biological activity (Costa et al. 2021; Pratiwi et al. 2021). The genus *Crotalaria* (subfamily Papilionoideae, tribe Crotalarieae) is one of the five largest in the Fabaceae family (Lewis et al. 2005; Mattos et al. 2018). *Crotalaria* species has the potential to associate with nitrogen (N) fixing bacteria and these interactions can improve the soil N availability (Berriel et al. 2020) and plant growth in mining areas.

Crotalaria spectabilis is an exotic species originated from tropical Asia that is eased to develop in poor soils and has been studied/applied for revegetation of mined areas (Melo et al. 2014; Zanchi et al. 2021) and green fertilization purposes (Teodoro et al. 2011) worldwide. On the other hand, *Crotalaria maypurensis* Kunth, is the only native *Crotalaria* species from canga environments in Serra dos Carajás (eastern Brazilian Amazon) and has the potential to be used in programs for the rehabilitation of degraded areas. It is a species that has rapid growth and easy propagation, as it produces a large number of seeds with good germination (Mattos et al. 2018). In addition, it guarantees the incorporation of a large amount of biomass.

The use of the native species *Crotalaria maypurensis* may represent environmental and economic gains in the process of environmental rehabilitation. However, studies are needed on the performance of this species in mining-affected areas for a better understanding of its growth and nutritional requirements (Carvalho et al. 2017). In addition, it is important to understand the effect of N metabolism on plant growth (Carlisle et al. 2012). Thus, the objective of the present study was to evaluate the mineral and organic fertilization on the growth, nodulation, and the production of N compounds in *Crotalaria maypurensis* (native species of the ferruginous cangas from Carajás) cultivated in mining waste and compared with the *Crotalaria spectabilis* (exotic species) (that currently being used in planting cocktails for mineland rehabilitation in CMP). Then, we intended to evaluate the potential use of these both *Crotalaria* species for rehabilitation of Fe mining waste learning about their metabolism (i.e. evaluation of N inorganic forms) and how it impacts on growth.

Material and methods

Substrate characterization and fertilization treatments

The substrate used for plant cultivation was a post mining substrate - raw materials brought from the C horizon after Fe ore extraction in CMP. The substrate has very low levels of organic matter (0.5%) and nitrogen (0.05%) (Table 1). Thus, to test nitrogen and organic fertilization jointly to basic fertilization normally applied in the field (PK and micronutrients), seven substrate fertilization treatments were tested: T0 - without addition of fertilizers; T1 - NPK; T2 - NPK + micronutrients; T3 - NPK + micronutrients + organic compost; T4 - PK; T5 - PK + micronutrients; T6 - PK + micronutrients + organic compost). The commercial organic compost (composting of plant parts from different species with a sandy loam soil - ratios kept confidential) is routinely applied in revegetation activities in CMP (Guedes et al. 2021) (Table 1). It was applied at 5% rate (5/95 compost/soil mixture). Regarding the plant nutrients, when applied (present or omitted depending on treatment), the amounts were: 75 mg of N; 185 mg P, 75 mg K; 0.5 mg of B; 1.5 mg of Cu; 0.15 mg of Mo; 5 mg of Zn per dm^3 of mining waste soil via $Ca(NO_3)_2$; $CaH_4P_2O_8$; KCl; H_3BO_3; $CuSO_4.5H_2O$; Na_2MoO_4, and $ZnSO_4$. The chemical characterization of both waste soil and organic compost followed methodology described in Teixeira et al. (2017).

Table 1. Chemical and physical properties of the iron mining waste soil and organic compost used in this research.

Analysis	Mining waste	Organic compost
pH (H_2O)	6.40	6.90
P (mg dm^{-3})	22.5	0.80
N (%)	0.05	0.91
K (mg dm^{-3})	57.2	606
S (mg dm^{-3})	30.0	672
Ca ($cmol_c$ dm^{-3})	2.30	16.9
Mg ($cmol_c$ dm^{-3})	3.30	4.21
B (mg dm^{-3})	0.53	3.73
Cu (mg dm^{-3})	0.70	2.40
Fe (mg dm^{-3})	11.1	138
Mn (mg dm^{-3})	13.5	33.7
Zn (mg dm^{-3})	8.60	3.70
OM (%)	0.50	9.58
H+Al ($cmol_c$ dm^{-3})	1.80	1.20
SB ($cmol_c$ dm^{-3})	5.80	25.0
CEC ($cmol_c$ dm^{-3})	51.5	26.2
Sand (g kg^{-1})	605	600
Clay (g kg^{-1})	270	350
Silt (g kg^{-1})	125	50.0

OM = Organic matter; SB = Base saturation; CEC = Cation exchange capacity at pH 7.0.

Plant species and seed treatment

Two species of the genus *Crotalaria* (Fabaceae family) were used, being the *Crotalaria spectabilis* Roth., commercial/exotic species, and the *Crotalaria maypurensis* Kunth., species native from the areas of Brazilian Amazon ferruginous cangas. A detailed morphological description of *Crotalaria maypurensis* can be found in Mattos et al. (2018). The species were selected due to their presence near mining areas (*Crotalaria maypurensis*) and the use in rehabilitation projects in CMP (*Crotalaria spectabilis)*.

The seeds of the native species were collected in the canga areas, close to the iron ore mines of Carajás, at the end of the fruit-filling period and before the opening of the beans to maintain the maximum germinative potential. Conversely, the seeds of the commercial species were acquired in specialized trade and are widely marked in Brazil for environmental recovery purposes. Before sowing, seeds of the two *Crotalaria* species were submitted to dormancy breaking with manual scarification for a homogeneous germination of the two species.

Experimental design and growing conditions

The study was conducted in a greenhouse with average temperature of 28 °C. The experimental design was completely randomized with four replicates, using the two species combined with seven substrate fertilization treatments described above. Five seeds of the *Crotalaria* species were sown in a pot containing 2.5 dm^3 of mining waste soil (species grown separately in each pot). A solution containing the aforementioned micronutrients was applied at sowing, while concentrations of total macronutrients were subdivided with applications at sowing and 36 days after sowing. Both solutions were applied directly on soil surface. After 20 days of emergence, thinning was performed, leaving only one plant per pot. The plants were cultivated for 90 days, and during this period, they were irrigated daily with deionized water, keeping the humidity at 70% of field capacity.

Growth parameters and nutrient quantification

After 90 days of cultivation, the plants were evaluated for height using a graduated tape, considering the apical meristem as the standard. Subsequently, shoot dry matter (SDM) and root dry matter (RDM) were measured. Then the plants were washed with distilled water and dried in an oven at 65 °C until constant weight. Thus, leaves were collected from the whole plant, ground, and the N content was determined according to Malavolta et al. (1997).

Nodule measurements

The seeds were not inoculated with Rhizobium, however, nodule number per plant (NN), nodule dry matter (NDM), and nodulation efficiency (NE) were determined because both species have the potential to perform N biological fixation. The NE is the ratio between total-N content in shoot dry matter (g) and NDM (g) (Melo and Zilli 2009). The N accumulation in shoot (NAS) was calculated by the ratio between shoot dry matter (g) and total-N content (Claessen 1997).

Nitrogen and carbon compounds

Nitrogen and carbon compounds in leaves and nodules were extracted by the method described by Bieleski and Turner (1966). The material of both species was sampled at the time of harvest, in the morning. For each gram of leaf dry material, 10 mL of MCW solution (60% methanol, 25% chloroform, 15% deionized H_2O) was added to the sample and 5 mL of MCW solution for nodule sample. Then, 1 mL chloroform + 1.5 mL H_2O was added for each 4 mL supernatant. After phase separation, the water-soluble phase was used for analysis of the following N [nitrate (NT) (Cataldo et al. 1975), free ammonium (FA) (McCullough 1967), allantoin (AL) and allantoic acid (AA) (Van Der Drift, 1970), total soluble amino acids (TSA) (Yemm and Cocking 1955)] and carbon [sucrose (SU) and total soluble carbohydrates (TSC) (Bieleski and Turner 1966)] compounds.

Photosynthetic pigments

For the quantification of pigments (chlorophyll *a* and *b*), full developed leaves were sampled from each plant in the moment of harvest and stored in a freezer at -80°C. Then, 0.2 g of leaf tissue was macerated in liquid N, and transferred to a Falcon tube, which was completed to 10 mL with 90% acetone (v/v). The extracts were stored for 24 hours, in the dark, before being submitted to centrifugation (300 rpm for 20 min.). Then the supernatant was diluted in the proportion of 1 mL of sample to 4 mL of acetone. The samples were transferred to quartz cuvettes and the absorbance was measured in a spectrophotometer (ICP-AES Spectro Genesis, Kleve, Germany). The concentrations of chlorophyll *a* and *b* (μg mL^{-1}) were determined using formulas proposed by Jeffrey and Humphrey (1975).

Statistical analysis

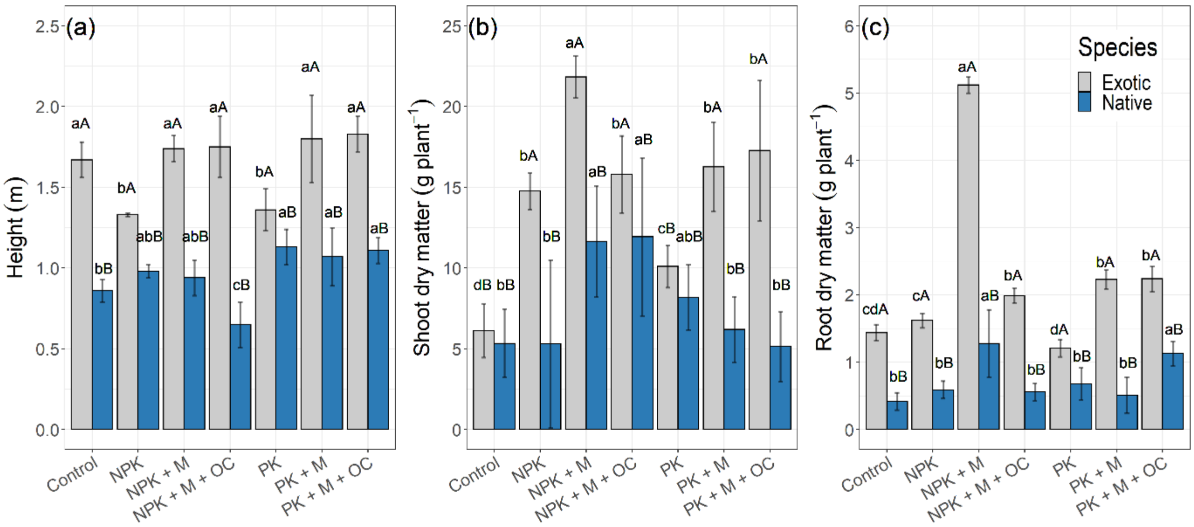
The data were submitted to analysis of variance after checked for normality using Shapiro-Wilk test. Means of treatments were compared by Duncan's test at 5% probability level. All statistical analyses were performed in R environment version 3.6.1 (R Core Team 2019).

Results

Growth parameters

Height of the exotic *Crotalaria* species was higher compared with the native one in all treatments tested (Figure 1a). The application of fertilizers in the exotic species did not provide height gains compared with control (no fertilization). Conversely, for the native species, the treatments PK, PK + micronutrients, and PK + micronutrients + organic compost increased plant height (up to 31.4%).

Fig. 1. Height (a), shoot dry matter production (b) and root dry matter production (c) in two *Crotalaria* species cultivated with organic and mineral fertilization. Columns with different lowercase letters between fertilization under equal species and capital letters between species [*C. spectabilis* (exotic species) and *C. maypurensis* (native species)] under equal treatment indicate significant difference from the Duncan test (*P*<0.05). Vertical bars represent standard



deviation (n=4). Control = without addition of fertilizers; OC = organic compost.

The exotic species produced more biomass than native one with increments up to 300% in root dry biomass (NPK + micronutrients) and 236% in shoot dry biomass (PK + micronutrients + organic compost) (Figure 1b, c). Both *Crotalaria* species showed the highest plant biomass production (shoot and root) with the application of the treatment NPK + micronutrients - up to 257% increase compared with control.

Application of organic compost increased plant biomass compared with control, however, no effect or decrease of biomass was observed when compared with NPK + micronutrients treatment.

Nodule measurements

In this study, plant species were not inoculated with Rhizobium, however nodulation was found for both species. Nodule number was higher in the commercial species, especially with the application of the treatment NPK + micronutrients + organic compost (Figure 2a). For the native species, the treatment with PK + micronutrients + organic compost, promoted greater nodulation. The absence of N fertilization allowed greater nodule dry matter in both species, except for the treatment NPK + micronutrients (Figure 2b).

In general, native species presented higher nodulation efficiency compared to commercial species, mainly through treatments NPK, NPK + micronutrients, and PK (Figure 2c). For the commercial species, the highest nodulation efficiency was observed in control treatment (no fertilization) and it did not differ from native species. On the other hand, in general, the native *Crotalaria* accumulated less N in the shoot than the commercial one, which was responsive to treatments with and without N, especially with PK + micronutrients (149%) and PK + micronutrients + organic compost (161%) (Figure 2d).

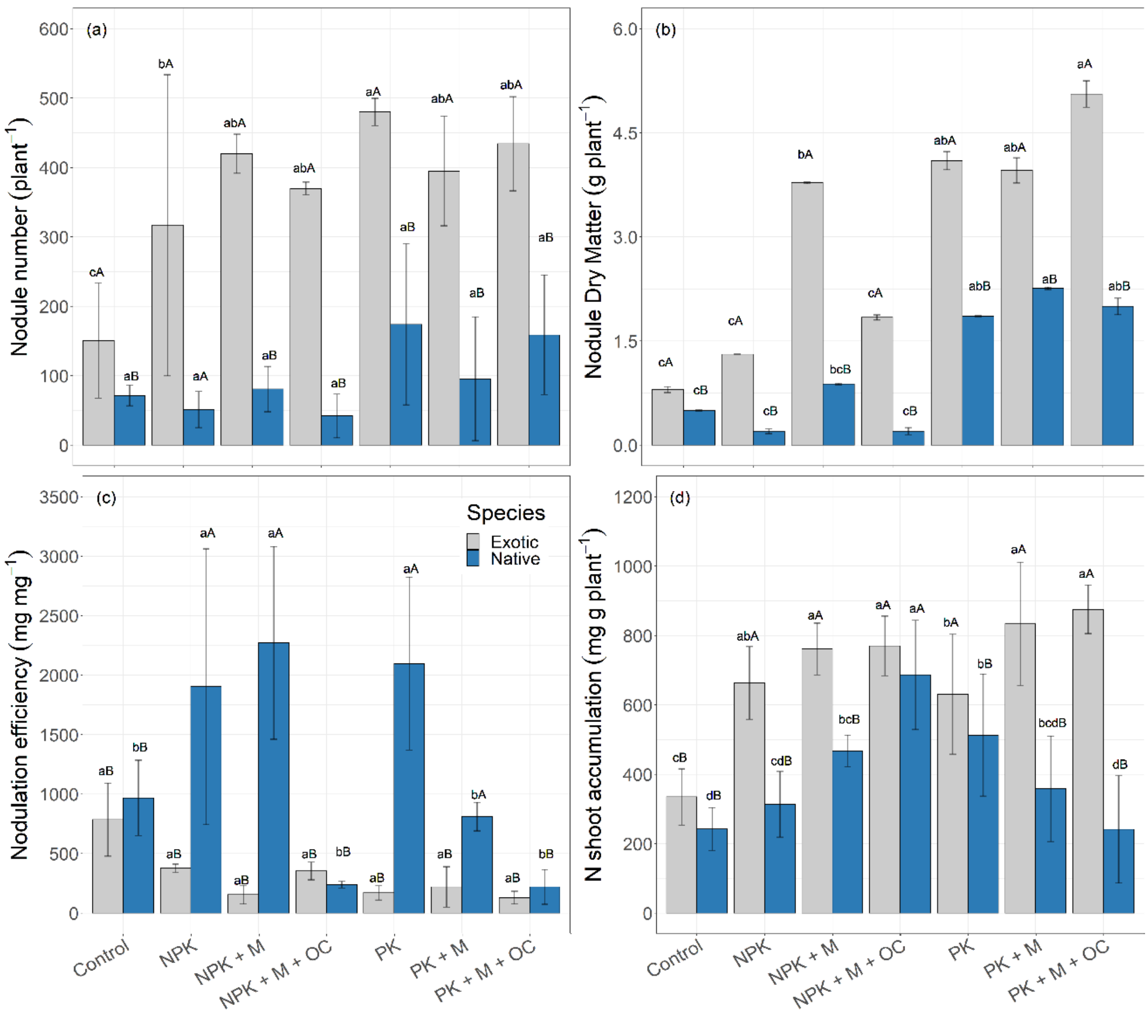


Fig. 2. Nodule number (a), nodule dry matter production (b), nodulation efficiency (c) and N shoot accumulation (d) in two *Crotalaria* species cultivated with organic and mineral fertilization. Columns with different lowercase letters between fertilization under equal species and capital letters between species [*C. spectabilis* (exotic species) and *C. maypurensis* (native species)] under equal treatment indicate significant difference from the Duncan test (P<0.05). Vertical bars represent standard deviation (n=4). Control = without addition of fertilizers; OC = organic compost.

Photosynthetic pigments

For concentrations of chlorophyll *a* and *b*, there was a response only as a function of the species. It was observed that the exotic species (*C. spectabilis*) had higher chlorophyll concentration than native species (*C. maypurensis*) for all treatments studied (Figure 3).

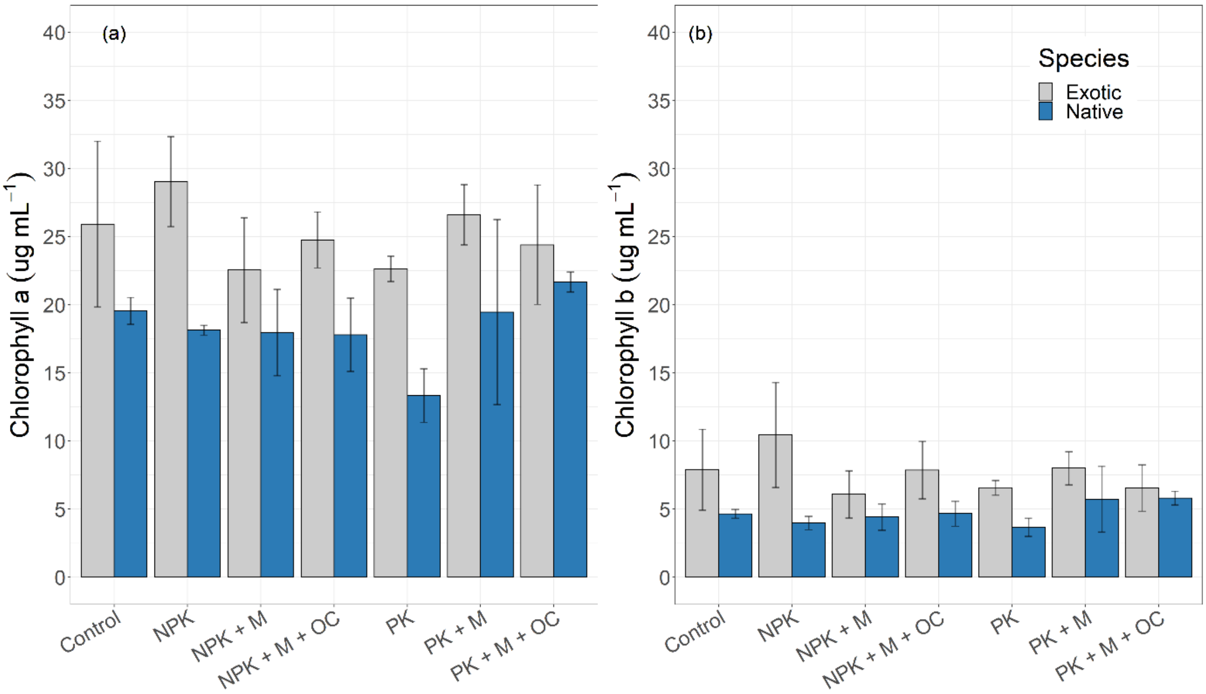


Fig. 3. Concentrations of chlorophyll *a* (a) and *b* (b) in leaves of two *Crotalaria* species, *C. spectabilis* (exotic species) and *C. maypurensis* (native species) cultivated under different treatments. Columns with different lowercase letters between fertilization under equal species and capital letters between species [*C. spectabilis* (exotic species) and *C. maypurensis* (native species)] under equal treatment indicate significant difference from the Duncan test ($P<0.05$). Vertical bars represent standard deviation (n=4). Control = without addition of fertilizers; OC = organic compost.

Nitrogen and carbon compounds

The nitrate concentration found in the leaves was higher than that observed in the nodules for both species (Table 2 and 3). The native *Crotalaria* species presented the highest nitrate concentration in the treatments PK + micronutrients + organic compost (leaves) and PK (nodules). On the other hand, for the exotic species no effect of fertilization was observed. The native species showed higher free ammonium concentration in the leaves in the NPK-based treatments, while exotic species had low performance. Contrarily, in the nodules, the opposite effect was observed (Table 3).

Ureides (allantoin and allantoic acid) were found in higher concentration in the nodules compared with the leaves regardless of the species tested (Table 2 and Table 3). Native species obtained the highest values of allantoin concentration in the leaves in the treatments without fertilization (control), NPK + micronutrients, NPK + micronutrients + organic compost, and PK + micronutrients + organic compost. The treatment NPK promoted a higher concentration for the exotic species. In the nodules, the exotic species had the highest allantoin concentration with the application of NPK and PK, however, the native species appears with the highest averages among species in the treatments based on NPK + micronutrients + organic compost, PK + micronutrients and PK + micronutrients + organic compost (Table 3).

In general, the native species obtained higher values of allantoic acid in the leaves and nodules than exotic *Crotalaria* species, with the highest averages identified in the leaves and nodules with the application of NPK + micronutrients (8% higher than control) and PK (15.3% higher than control), respectively (Table 2). The highest allantoic acid average in nodules for exotic species was observed in the NPK treatment, with 6.3% increments compared with control (Table 3).

Table 2. Leaf nitrogen compounds in two *Crotalaria* species cultivated with organic and mineral fertilization.

Species	Treatments	Nitrate (µmol g DM^{-1})	Free ammonium (µmol g DM^{-1})	Allantoin (µmol g DM^{-1})	Allantoic acid (µmol g DM^{-1})	Total soluble amino acids (µmol g DM^{-1})
C. spectabilis (exotic species)	Control	24.9 ± 0.87a*	473 ± 8.12b*	659 ± 7b*	66.5 ± 1.24c*	98.1 ± 2.65a*
	NPK	21.1 ± 0.49a*	63.1 ± 11.5g*	722 ± 20a*	68.5 ± 1.25bc^{ns}	81.1 ± 4.14b^{ns}
	NPK + micronutrients	33.9 ± 1.00a*	124 ± 3.67f*	589 ± 5c*	66.4 ± 1.34c*	77.1 ± 4.34bc^{ns}
	NPK + micronutrients + organic compost	20.5 ± 0.92a*	181 ± 3.16e*	588 ± 11c*	66.5 ± 0.44c*	75.8 ± 2.51c*
	PK	20.9 ± 0.63a*	576 ± 11.8a*	544 ± 13d^{ns}	65.9 ± 1.92c*	66.3 ± 2.30d*
	PK + micronutrients	20.1 ± 0.36a*	439 ± 9.22c*	594 ± 7c^{ns}	72.0 ± 2.80a^{ns}	70.2 ± 1.33d*
	PK + micronutrients + organic compost	23.7 ± 0.52a*	422 ± 4.68d*	605 ± 9c*	71.1 ± 0.73ab^{ns}	60.9 ± 1.62e*
C. maypurensis (native species)	Control	258 ± 33.9b	192 ± 15.9d	708 ± 20a	69.6 ± 3.27b	105.8 ± 3.80a
	NPK	229 ± 4.20c	175 ± 2.11e	589 ± 13d	71.2 ± 2.62b	78.0 ± 1.28c
	NPK + micronutrients	224 ± 3.35c	304 ± 5.12b	674 ± 12b	75.3 ± 2.31a	80.5 ± 1.21c

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NPK + micronutrients + organic compost	251 ± 8.56b	273 ± 7.03c	641 ± 14c	70.8 ± 1.41b	86.9 ± 2.41b
PK	253 ± 5.06b	349 ± 7.37a	544 ± 9e	69.9 ± 1.86b	61.1 ± 4.24d
PK + micronutrients	265 ± 14.9b	301 ± 9.14c	606 ± 23d	71.4 ± 2.17b	60.2 ± 6.11d
PK + micronutrients + organic compost	289 ± 6.07a	170 ± 3.53d	607 ± 14d	71.7 ± 0.61b	49.7 ± 4.37d

Means followed by different lowercase letters between fertilization (organic and mineral under equal species) and * between species [C. spectabilis (exotic species) and C. maypurensis (native species)] under equal treatment indicate significant difference from the Duncan test ($P<0.05$). Means ± SD, n = 4. ns = not statistically significant.

Table 3. Nodule nitrogen compounds in two *Crotalaria* species cultivated with organic and mineral fertilization.

Species	Treatment	Nitrate (μmol g DM^{-1})	Free ammonium (μmol g DM^{-1})	Allantoin (μmol g DM^{-1})	Allantoic acid (μmol g DM^{-1})	Total soluble amino acids (μmol g DM^{-1})
C. spectabilis (exotic species)	Control	18.0 ± 1.00a*	274 ± 12.2f*	1597 ± 69b^{ns}	155 ± 6.52bc*	198 ± 12.85c*
	NPK	18.9 ± 0.66a*	718 ± 10.1a*	2120 ± 50a*	165 ± 4.60a*	281 ± 8.28a*
	NPK + micronutrients	14.5 ± 0.61a*	365 ± 3.98d*	1384 ± 40d	138 ± 3.23d*	222 ± 5.16b*
	NPK + micronutrients + organic compost	14.9 ± 1.07a*	258 ± 10.6f*	1499 ± 30c*	156 ± 4.54bc^{ns}	184 ± 8.19d*
	PK	16.0 ± 0.47a*	492 ± 11.9b*	1641 ± 43b*	139 ± 0.98d*	143 ± 6.52e*
	PK + micronutrients	13.6 ± 0.37a*	389 ± 15.3c*	1465 ± 30cd*	148 ± 5.93c*	137 ± 10.4e*
	PK + micronutrients + organic compost	13.1 ± 0.33a*	3056 ± 23.6e*	1427 ± 60cd*	158 ± 11.4ab^{ns}	136 ± 8.54e*
C. maypurensis (native species)	Control	161 ± 5.36c	196 ± 12.2e	1532 ± 59c	175 ± 5.05b	212 ± 12.56b
	NPK	146.3 ± 2.76d	289 ± 6.40d	1376 ± 29d	125 ± 3.30d	204 ± 7.75b
	NPK + micronutrients	204 ± 8.97b	310 ± 10.5c	1388 ± 42d	174 ± 2.87b	207 ± 5.24b
	NPK + micronutrients + organic compost	163 ± 11.57c	117 ± 3.20f	1895 ± 86a	137 ± 5.36b	215 ± 12.1ab
	PK	217 ± 11.59a	414 ± 12.9b	1483 ± 54c	202 ± 10.1a	207 ± 13.3b

PK + micronutrients	204 ± 3.45b	423 ± 18.9ab	1544 ± 73c	172 ± 4.69bc	228 ± 9.11a
PK + micronutrients + organic compost	169 ± 10.12c	435 ± 11.2a	1761 ± 51b	165 ± 5.87c	200 ± 8.60b

Means followed by different lowercase letters between fertilization (organic and mineral under equal species) and * between species [*C. spectabilis* (exotic species) and *C. maypurensis* (native species)] under equal treatment indicate significant difference from the Duncan test ($P<0.05$). Means ± SD, n = 4. ns = not statistically significant.

The concentration of total soluble amino acids found in leaves was lower than that observed in nodules, for both species (Table 2 and Table 3). It is noted that the native and exotic species presented the highest content in the leaves in the control treatment (Table 2). In the nodules, in general, the native species had higher averages compared to exotic *Crotalaria* species. The highest increments with fertilization were found with the NPK (42%) and PK + micronutrients (7.4%) application for exotic and native species respectively.

The sucrose concentration in the leaves was higher in the native species for all treatments (Figure 4). The highest averages were found with PK + micronutrients + organic compost application for native species and PK and PK + micronutrients for exotic species, with an increase of 24.5% and 46% compared with control, respectively. In the nodules, the highest sucrose concentrations were identified with PK application for both species.

In general, total soluble carbohydrates were higher in the nodules, when compared to the leaves (Figure 4). In the leaves, native species also presented the highest averages and the PK + micronutrients + organic compost was the best treatment. For nodules, the exotic species obtained the highest average in the treatment with PK + micronutrients and native species with control.

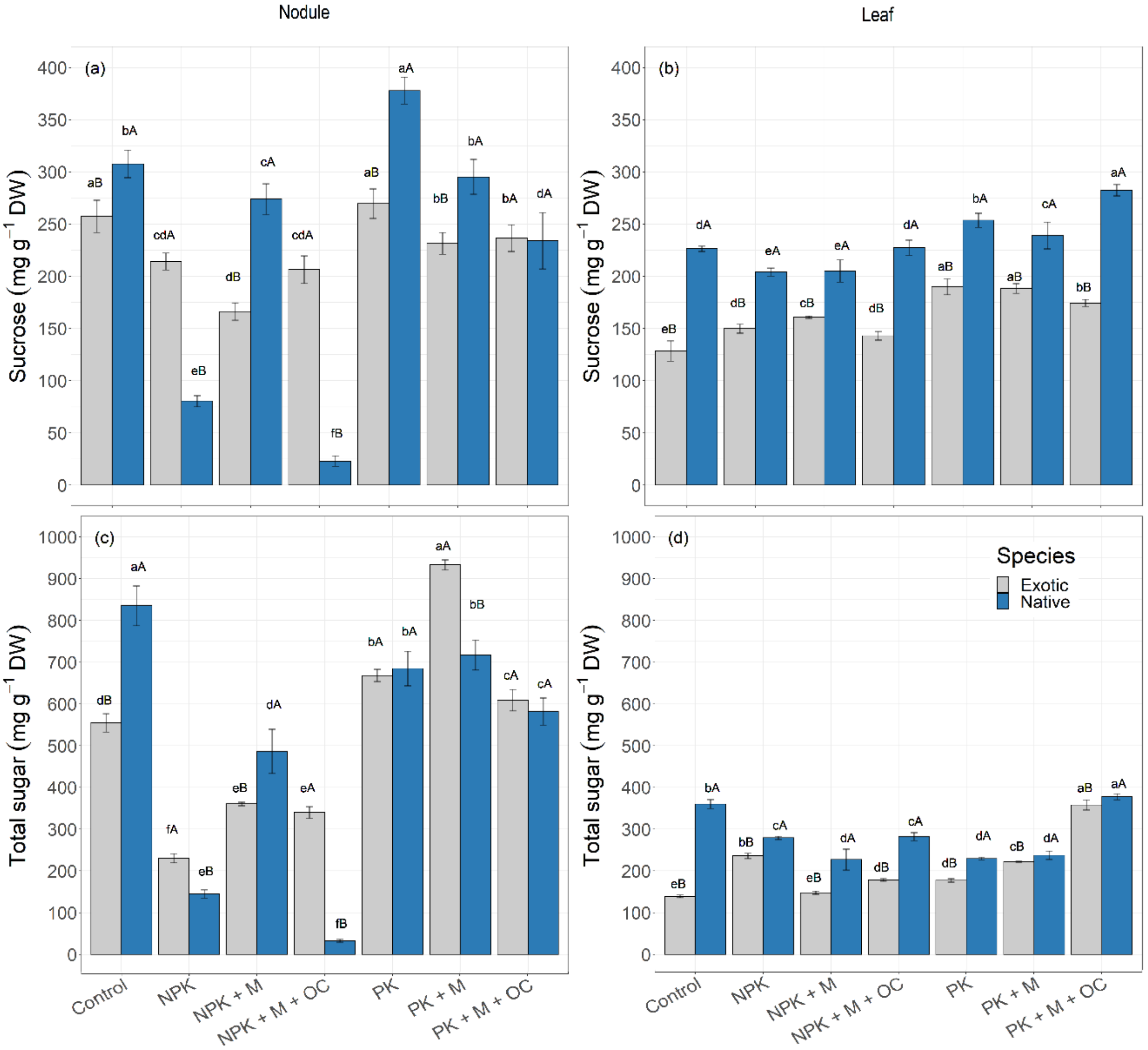

Fig. 4. Concentrations of nodule sucrose (a), leaf sucrose (b), nodule total sugars (c) and leaf total sugars (d) in two *Crotalaria* species cultivated under different mineral and organic fertilization treatments. Columns with different lowercase letters between fertilization under equal species and capital letters between species [*C. spectabilis* (exotic species) and *C. maypurensis* (native species)] under equal treatment indicate significant difference from the Duncan test (P<0.05). Vertical bars represent standard deviation (n=4). Control = without addition of fertilizers; OC = organic compost.

Discussion

Growth and compounds production in *Crotalaria* species

The height of the exotic *Crotalaria* species was superior to the native *Crotalaria* species in all treatments, however, significant gains in height were observed for the native species when submitted to treatments without N addition. In another study, no effect of nitrogen fertilization on the production of shoot was observed for the legumes *Canavalia ensiformis* (L.) DC., *Cajanus cajanvar* var. Flavus DC., Mucuna deeringiana (Bort) Merr and *Crotalaria juncea* L. (Mangaravite et al. 2014). This is especially relevant for rehabilitation of mined areas, where N is usually a limiting factor (Ramos et al. 2022).

The absence of N fertilization also allowed greater nodule dry matter production in native species. In another study with legumes, Ramires et al. (2018) reported the greater nodule formation and nodule dry matter production in beans in the absence of N fertilization, with values 78.7% higher than those obtained with N application. Also, soybean nodulation may be inhibitied with nitrate supply (Yamashita et al. 2019). This can be explained by the N interference in the synthesis of flavonoids, which are metabolites with important functions in molecular communication between the plant and N-fixing bacteria (Liu and Murray 2016). In the pre-infection stage, the plant exudes different substances through the roots, such as carbohydrates, amino acids, and phenolic compounds (flavonoids) creating a chemical gradient in the rhizosphere and resulting in the attraction of the bacteria to the root surface (Mandal et al. 2010). Flavonoid levels decrease with N application, promoting less chemotaxis and, consequently, less bacterial infection in the roots, that is, decrease or inhibition of the nodulation.

In general, the N absence increased ureides concentration. These composts are the main forms of N transport into plant from the N biological fixation, being transferred from the nodules to the shoot through the xylem (Carter and Tegeder 2016) - which explains the greater concentration in the nodules compared to leaves, regardless of the species studied. When N biological fixation occurs, a more intense synthesis of ureides is observed, which can be considered the best way to evaluate the nodulation efficiency in legumes that transport these compounds (Camargos and Sodek 2010; Izaguirre-Mayoral et al. 2018). Data obtained in this study confirm the relationship between these variables, with higher nodulation efficiency and allantoin values being observed in the treatment without fertilization for the native species.

Native *Crotalaria* species showed increased biomass with the treatments applied, which highlights the important role of fertilization in the growth of these species to start revegetation activities of environments depleted in nutrients such as mined areas. These results corroborate with previous studies carried out by Silva et al. (2018) when they show an increase in initial growth of four native Fabaceae species from cangas of the CMP when such plants were cultivated in fertilized substrates. They also concluded that fertilizers should be applied whenever possible to achieve successful revegetation.

Although organic fertilization increased plant biomass compared to control, its application, in the tested conditions, could be supressed as it reduced or do not have effect in total biomass production compared to treatment with NPK and micronutrients application for both species. On the other hand, addition of micronutrients had positive effects on plant growth, demonstrating that mineral fertilization had a higher impact on the studied *Crotalaria* species. Vidal et al. (2020) also found that organic amendments, produced through composting without clay mineral addition, had no or negative effects on *Lolium perenne* and *Phaseolus vulgaris* growth. These authors associated the results with the maturity stage of the compost.

Scoring species

The exotic *Crotalaria* species had better dry mass production when compared to the native species. It is important to point out that our results demonstrated that *C. maypurensis* and *C. spectabilis* have different metabolism in the presence of N inorganic forms (nitrate and free ammonium). The assimilation of NO_3^- has a higher energy demand compared to the assimilation of NH_4^+, which needs to be previously reduced to ammonium during the formation of N compounds (Guo et al. 2007). The nitrate concentration was higher in the native species (its main form of N uptake) and, since its assimilation demands greater energy compared to the assimilation of free ammonium, the native species had lower growth and biomass production (Carlisle et al. 2012).

In addition, the higher concentrations of chlorophyll *a* and *b* observed for exotic species may be involved in the increased biomass produced, since they are the point of entry of energy in the plant allowing greater production of photoassimilates (Oliveira et al. 2015). The greater nodulation presented by the exotic species may have positively influenced the increase of N accumulation in the leaves and, consequently, the chlorophyll levels. This relationship between the variables have been observed in cowpea plants (Pereira et al. 2018). Greater N availability triggers increase of chlorophyll concentrations in leaves because the N is essential during the chlorophyll biosynthesis (Bassi et al. 2018).

Regarding native *Crotalaria*, this species presented higher nodulation efficiency than the commercial species, yet presenting lower nodule number. Although these variables are linked, it is noteworthy that this efficiency is possibly conditioned by the presence of active nodules, which is an indication of favourable conditions to the occurrence of N biological fixation. Despite not knowing the N biological amount provided from the N biological fixation, we can assume that the differences in N accumulation come from the activity of the nodules, considering that plants obtained similar growth receiving or not N supply.

Native *Crotalaria* species had the highest total soluble amino acids values in the leaves. These compounds are related to an additional amount of N for nitrogen-fixing bacteria that could be metabolized in the root system and transported to the shoot in the form of amino acids (Moreira et al. 2014). Thus, total soluble amino acids may have a direct influence on N biological fixation, modifying nitrogenase enzyme activity (Reis and Döbereiner 1998) and justifying the growth of native species (shoot and root dry mass).

The native *Crotalaria* species has potential for soil rehabilitation in the mined areas of the CMP, as it can contribute to an increase in the soil N supply. In this context, the N biological fixation favours the reduction of the levels of fertilizers applied, the cultivation costs and environmental impacts, supporting the rehabilitation of degraded areas, especially those where the loss of soil organic matter was intense. The advantages presented by the native species may be related to the better adaptation to areas of canga, which are characterized by a strong incidence of solar radiation, shallow soils with a high concentration of metals, in addition to high temperatures and strong water seasonality, marked by severe droughts (Mattos et al. 2018; Silva et al. 2018). These conditions point to adaptations of the species *C. maypurensis*, which may contribute to the growth in limiting areas, such as in substrates after mining.

In sum, it should be highlighted the greatest contribution to N input without the need to add external N source, the overall robustness and the contribution to the protection of endangered canga flora with the native species. On the other hand, exotic species has benefits such as increased growth and overall performance. Thus, based on experimental conditions, both *Crotalaria* species seems to be adequate for rehabilitation of mined substrates in areas of Brazilian Amazon ferruginous cangas. However, considering competitive exclusion, risk for hybridization, among other issues, further studies should be carried out to analyze competition of both species with a risk assessment before introduction of both species and monitoring after seeding.

In addition, mining sites may differ in terms of substrate depth, soil physical attributes, content of potentially toxic elements, rainfall and temperature regime, and all these conditions should be tested to better understand their adaptability to mining soil. Also, further studies should assess changes in critical substrate attributes, possibly over long periods of time or with repeated *Crotalaria* species applications.

Conclusions

This research revealed that mineral fertilization contributes for the greater growth of *Crotalaria* species in Technosol of Carajás. The treatments based on NPK and micronutrients presented the best results for both *Crotalaria* species, showing the need for fertilization with all nutrients in mining waste soil aiming to promote higher plant growth. In general, organic fertilization did not promote satisfactory differences.

Exotic *Crotalaria* species presented higher N accumulation and chlorophyll contents in its tissues, reflecting greater increments in biomass production compared to native species. Contrarily, native *Crotalaria* species presented nitrate as the predominant form of N in their leaf tissues, which may be one of the reasons to the lower growth of this species, since more energy is spent to metabolize the nitrate in plant tissues.

Considering the higher nodulation efficiency and considerable biomass production, the native species can be promising in projects for the rehabilitation of mined areas, with special evidence for the ecosystems found in the CMP mainly by the fast growth and persistence in the aforementioned areas, which contributes to the protection of endangered canga flora and is also favorable to meet legal requirements. Finally, planting both species together, may link the benefits of rapid growth and high biomass protection of *C. spectabilis* with the biodiversity protection issues with *C. maypurensis*. However, further studies should be carried out to analyze competition of both species and to better understand their adaptability to mining environmental conditions.

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