UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Biologia Vegetal

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FROM THE FIELD TO THE RECOVERY OF DEGRADED AREAS: an ecophysiological approach in the selection of native plant species from Banded Iron Formations (BIFs)

Belo Horizonte
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FROM THE FIELD TO THE RECOVERY OF DEGRADED AREAS: an ecophysiological approach in the selection of native plant species from Banded Iron Formations (BIFs)

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Doutora em Biologia Vegetal.

Orientador: Prof. Dr. Eduardo Gusmão Pereira

Coorientador: Profa. Dra. Queila Souza Garcia

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CAMILLA OLIVEIRA RIOS

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Às 8 horas do dia 16 de dezembro de 2022, reuniu-se a Comissão Examinadora de Tese indicada pelo Colegiado do Programa de Pós-Graduação em Biologia Vegetal para julgar, em exame final, o trabalho intitulado **"From the field to the recovery of degraded areas: an ecophysiological approach in the selection of native plant species from banded iron formations (BIFs)"** , requisito final para obtenção do grau de Doutor em Biologia Vegetal, área de concentração **Fisiologia Vegetal e Ecologia.** Abrindo a sessão, o Presidente da Comissão, professor Dr. Eduardo Gusmão Pereira, após dar conhecimento aos presentes do teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores:

Comissão Examinadora - Resultado

Dr. Eduardo Gusmão Pereira (UFV) - aprovado

Dr. Vitor Nascimento (UFLA) - aprovado

Dr. Dener Márcio da Silva Oliveira (UFV) - aprovado

Dra. Anna Abrahão (Universidade Federal do Ceará) - aprovado

Dr. Marcel Giovanni Costa França (UFMG) - aprovado

Após defesa da aluna, a comissão reuniu-se, sem a presença da candidata e do público, para julgamento e expedição do resultado final.

Pelas indicações, a candidata foi considerada aprovada.

O resultado final foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou-se a ata presente, que será assinada por todos os membros participantes da Comissão Examinadora. Belo Horizonte, 16 de dezembro de 2022.

Assinaturas dos membros da comissão examinadora:

Documento assinado eletronicamente por **Eduardo Gusmão Pereira, Usuário Externo**, em 16/12/2022, às 11:57, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Documento assinado eletronicamente por **Marcel Giovanni Costa França, Professor do Magistério Superior**, em 20/12/2022, às 10:38, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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RESUMO

É importante que a recuperação de áreas impactadas por atividades antropogênicas e mudanças climáticas seja realizada com espécies vegetais nativas e que o ecossistema de referência, bem como seus aspectos abióticos, sejam considerados. O objetivo do trabalho foi compreender como as relações ecológicas e fotossintéticas da vegetação de canga sob condições de campo e casa de vegetação podem contribuir para a seleção de espécies a serem utilizadas na recuperação de áreas degradadas. Foram selecionadas espécies vegetais nativas, pertencentes a canga couraçada a canga nodular. As análises foram realizadas durante a estação seca e chuvosa. As espécies apresentaram diferentes respostas em relação as estações climáticas às quais são expostas ao longo do ano e ao microambiente ao qual se encontram. Apesar da fraca correlação entre a estratégia ecológica de tolerância ao estresse e variáveis fotossintéticas, as espécies vegetais nativas da canga apresentaram respostas adaptativas resilientes que diferiram do ecossistema e variaram de acordo com a heterogeneidade dos microhabitats. A baixa fertilidade e chuvas reduzidas durante a estação seca levou a manutenção ou incrementos nos parâmetros-chave F_v/F_m, índices de clorofila, ϕ_{PSII} e ϕ_{NPQ}. Dentre as espécies estudadas, *J. monticola*, *L. pinaster*, *B. reticularia*, *P. mediterranea* e *M. splendens* são possíveis espécies facilitadoras, sendo fundamentais para a resposta de cada microambiente. Quando desenvolveram no rejeito de mineração de ferro, as estratégias de resistência apresentadas pelas espécies, *J. caroba*, *M. splendens* e *P. mediterranea*, foram comprovadas pelas respostas fotoquímicas e de crescimento. Os parâmetros fisiológicos analisados destacaram a diversidade de estratégias adaptativas encontradas em espécies vegetais de afloramentos ferruginosos, mostrando seu potencial para recuperação de áreas degradadas, pois além de beneficiarem outras espécies, conseguem também sobreviverem em condições adversas.

Palavras-chave: Formações Ferríferas Bandadas, campo rupestre, mudanças climáticas, dinâmica de comunidades, biomas gramíneos tropicais, características fisiológicas, mineração

ABSTRACT

It is important that the recovery of areas impacted by anthropogenic activities and climate change be carried out with native plant species and that the reference ecosystem, as well as its abiotic aspects, be considered. The objective of this study was to understand how the ecological and photosynthetic relationships of canga vegetation under field and greenhouse conditions can contribute to the selection of species to be used in the recovery of degraded areas. Native plant species were selected, belonging to the *couraçada canga* and the *nodular canga*. The analyses were carried out during the dry and rainy seasons. The species showed different responses in relation to the climatic seasons to which they are exposed throughout the year and to the microenvironment to which they are found. Despite the weak correlation between the ecological strategy of stress tolerance and photosynthetic variables, native canga plant species showed resilient adaptive responses that differed from the ecosystem and varied according to the heterogeneity of the microhabitats. Low fertility and reduced rainfall during the dry season led to maintenance or increases in key parameters F_v/F_m, chlorophyll indices, ϕ_{PSII} and ϕ_{NPQ}. Among the studied species, *J. monticola*, *L. pinaster*, *B. reticularia*, *P. mediterranea* and *M. splendens* are possible facilitator species, being fundamental for the response of each microenvironment. When they developed in the iron mining tailings, the resistance strategies presented by the species, *J. caroba*, *M. splendens* e *P. mediterranea*, were better explained by the photochemical and growth responses. The analyzed physiological parameters highlighted the diversity of adaptive strategies found in plant species of ferruginous outcrops, showing their potential for the recovery of degraded areas, since in addition to benefiting other species, they can also survive in adverse conditions.

Keywords: Banded Iron Formations, *campo rupestre*, climate change, community dynamics, tropical grassy biomes, physiological characteristics, mining

SUMÁRIO

INTRODUÇÃO GERAL

A dinâmica das comunidades vegetais é formada a partir da exposição a filtros bióticos e abióticos. Esses são capazes de determinar quais espécies e características irão predominar em um habitat específico, e juntos compor um ecossistema (Lavorel & Garnier, 2002). Entretanto, mudanças ambientais afetam diretamente os indivíduos e os serviços ecossistêmicos, trazendo severos prejuízos a natureza (Suding *et al.*, 2008; Bu *et al.*, 2019). Uma forma de preservar as espécies nativas e garantir sua sobrevivência sob eventos climáticos futuros seria através do estudo das características de cada espécie quando expostas a diferentes condições, sejam elas características funcionais, nutricionais, hidráulicas, fotoquímicas, antioxidantes e/ou partição de carboidratos. Com estes resultados aumentamos a capacidade preditiva de interpretar como as mudanças globais influenciarão os ecossistemas (Suding *et al.*, 2008; Kearney *et al.*, 2021) e caracterizar possíveis espécies a serem utilizadas em projetos de recuperação de áreas degradadas.

Dentre as características adaptativas apresentadas pelos vegetais, as estratégias fisiológicas desempenhadas em habitats sob potenciais estressores ambientais contribuem para uma melhor performance de desenvolvimento. Quando expostas a ambientes com alta incidência luminosa, em níveis acima da capacidade de utilização pela fotossíntese, e déficit hídrico sazonal, as plantas podem apresentar danos ao aparato fotossintético (Lemos Filho, 2000; Souza et al., 2009). Entretanto, a energia resultante do processo de absorção luminosa, uma vez absorvidas pelo complexo antena das clorofilas, pode ser dissipada por três vias distintas: através da transferência de elétrons na fotossíntese que está relacionada ao rendimento quântico efetivo do FSII (ϕ_{PSII}); por meio da dissipação de forma não fotoquímica regulada (ϕ_{NPQ}), capaz de proteger o aparato fotossintético contra superexcitação e subsequente dano ao centro de reação do FSII (Baker, 2008; Pereira *et al.*, 2018); e ainda por meio de incrementos no rendimento quântico de dissipação de energia de forma não regulada no FSII (ϕ_{NO}) que podem levar a formação de espécies reativas de oxigênio (EROS) e comprometer o estabelecimento vegetal (Pereira *et al.*, 2018).

Formações ferríferas conhecidas mundialmente por banded iron formations (BIFs) estão entre os ecossistemas que apresentam características severas as espécies vegetais. Apesar do isolamento edáfico, do relevo topográfico e das restrições físicas do solo, as áreas de BIFs comportam altos níveis de biodiversidade florística (Markey & Dillon, 2008; Gibson *et al.*, 2010). Os campos rupestres ferruginosos, situados sobre áreas de BIFs no Brasil, também conhecidos como *cangas*, são ecossistemas extremamente heterogêneos com grande diversidade de ambientes microedáficos e com vegetação excepcionalmente rica e altamente endêmica (Fernandes et al., 2018; Silveira et al., 2016), classificadas funcionalmente como estresse tolerante (Negreiros *et al.*, 2014; Dayrell *et al.*, 2018). A vegetação associada se encontra sob constante estresse ambiental devido a presença de solos altamente intemperizados e pobres em nutrientes, alta irradiância e marcante sazonalidade climática (Giulietti *et al.*, 1987; Rapini *et al.*, 2008; Fernandes, 2016; Oliveira *et al.*, 2016; Abrahão *et al.*, 2019). É ainda um habitat com diferentes disponibilidades de recursos e grande variação nas condições bióticas e abióticas (Denney *et al.*, 2020), podendo ser diferenciado em microhabitats incluindo a canga couraçada e nodular.

Apesar da importância dos serviços ecossistêmicos prorcionados pelas cangas e de sua grande biodiversidade, esses ambientes são extremamente degradados pela atividade mineradora no Brasil, pois são responsáveis por fornecer grande porção do minério de ferro mundial (Ramanaidou & Wells, 2014). A atividade mineradora resulta em alterações nas condições físico-quimica do solo, aumento na concentração de metais e formação de lagoas de decantação de rejeito e pilhas de estéreis. Sobreviver a essas condições antrópicas requer estratégias morfofisiológicas específicas que garantem a aclimatação vegetal (Ginocchio *et al.*, 2017). Desta forma, é importante que trabalhos que visem a recuperação destas áreas degradadas levem em consideração o ambiente a ser recuperado, bem como seus potenciais estressores e os fatores que limitam o estabelecimento e crescimento vegetal. Outro fator determinante para o sucesso da restauração consiste em compreender as características e as tolerâncias das espécies-alvo. É preciso que as espécies selecionadas sejam capazes de germinar e se desenvolver nestes ambientes, bem como que as condições do local atendam aos requisitos da espécie e não exceda os limites de tolerância (Valliere *et al.*, 2021). Por isso, é de suma importância que a recuperação de áreas degradadas leve em consideração o ecossistema de referência, os aspectos bióticos e abióticos limitantes e as espécies a serem introduzidas, diminuindo assim o risco de invasão biológica e aumentando a chance de sobrevivência da espécie (Kienberg *et al.*, 2014; Aslan, 2019; Gastauer *et al.*, 2019).

Assim como a extração de minério de ferro, várias outras atividades antrópicas vêm trazendo sérios prejuízos ao meio ambiente. Na tentativa de restaurar os serviços ecossistêmicos e conter o rápido declínio da biodiversidade, foi criado pelas Nações Unidas a "Década da Restauração dos Ecossistemas", estabelecida entre os anos de 2021 a 2030 (UNEP e FAO 2020). Além do reflorestamento, a década almeja também a recuperação de formações campestres, como pradarias e savanas, desta forma, a reabilitação de áreas minerárias promoverá o aumento da biodiversidade primária e dos serviços ecossistêmicos, e irá reduzir os impactos ambientais decorrentes da atividade.

Neste contexto, esta tese tem como objetivos gerais compreender a relação entre as estratégias ecológicas e os ajustes fotossintéticos, caracterizar a interação entre as respostas funcionais e mecanísticas de espécies vegetais nativas de afloramentos ferruginosos e determinar quais parâmetros são eficazes na seleção de espécies com maior chance de sucesso em trabalhos de recuperação de áreas degradadas e em diferentes condições ambientais.

CAPÍTULO 1: Can ecological strategies be explained by photochemical efficiency in ironstone outcrops vegetation?

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ABSTRACT

Aims Vegetation in ironstone outcrops is under potential environmental stressors. The aim of this work was to understand the relationships between the ecological strategies of species from an ironstone outcrops in Brazil and their photosynthetic adjustments, and to contribute for the selection of species for the recovery of mined environments.

Methods A total of 22 species belonging to 12 families were selected over *canga* vegetation. The physiological characteristics, chlorophyll *a* fluorescence and chlorophyll indices, were evaluated during the rainy and dry seasons. Analysis of competitor, stress-tolerator, ruderal (CSR) ecological strategies was performed during the rainy season for each species using functional leaf characteristics.

Results The species presented the following ecological strategies: tolerance to stress (74.76%), competition (22.31%) and ruderality (2.93%). Despite a weak correlation between the ecological stress-tolerant strategy and photosynthetic variables, the low soil fertility and reduced rainfall during the dry season led to photochemical adjustments. The species were divided into five distinct groups based on the ability to use and dissipate excess light energy. Some species showed maintenance or increments in photochemical efficiency parameters, chlorophyll indices, and the capacity to avoid damage to the photosynthetic complex through a photoprotective process during the dry season.

Conclusions The photochemical responses observed highlighted the diversity of adaptive strategies found in plant species from ironstone outcrops. The different photosynthetic adjustments are related to the competition capacity and stress tolerance mechanisms. Thus, the analyzed physiological parameters can be used for selecting plant species for the recovery of environments impacted by mining companies.

Keywords: *Campo rupestre*, CSR strategies; photosynthesis, physiological plasticity, tropical grassy biomes

1. INTRODUCTION

Plant communities with high biodiversity in nutrient-impoverished and stress-prone habitats are formed by environmental filtering events – the continuous dispersion of plants and their specialization to local environmental conditions (Laliberté *et al.*, 2014; Zappi *et al.*, 2017). In these environments, plasticity in photosynthetic traits becomes essential to the physiological mechanisms of plants to thrive in the different environmental conditions to which they are exposed (Turcotte & Levine, 2016; Fenollosa & Munné-Bosch, 2018). The microenvironmental variation in a given ecosystem has the potential to buffer the responses of native populations against environmental changes and thus allow them to persist locally (Denney *et al.*, 2020). In this way, microhabitat variations can protect local populations from the effects caused by climate change (Silva *et al.*, 2013), and may also favor the evolution of adaptive strategies (Denney *et al.*, 2020).

The *campo rupestre* ecosystem of Brazil possesses a great diversity of micro-edaphic environments with a mosaic of associated vegetation. The flora is exceptionally rich and highly endemic, and although *campo rupestre* occupies an area corresponding to less than 1% of the Brazilian land surface, it houses about 15% of the country’s vascular plant diversity (Silveira *et al.*, 2016). *Campo rupestre* is mainly composed of an herbaceous layer, with small sclerophyllous shrubs and sub-shrubs and evergreen plants. The predominance of ancient strains with reduced dispersal capacity, high endemism, high vulnerability to soil removal, and biological specialization for the acquisition of resources allows the *campo rupestre* to be considered alongside *fynbos* (Cape Floristic Region) and *kwongkan* (southwest Australia), as a biota representative of OCBIL theory, since it is old, climatically buffered and infertile landscapes (Silveira *et al.*, 2016; Hopper *et al.*, 2021).

The vegetation associated with *campo rupestre* is under constant selective pressure due to potential environmental stresses due to infertile soils, seasonal drought, and exposure to excessive irradiance and strong winds (De Carvalho *et al.*, 2012; Messias *et al.*, 2013). The *Quadrilátero Ferrífero* (QF) of the state of Minas Gerais hosts formations of ferruginous *campo rupestre* on itabirite, known as *canga*. *Canga* differs from quartzitic *campo rupestre* by having a higher proportion of rocks, less fine soil content, relatively greater fertility and a higher level of trace metals, especially Fe (Messias *et al.*, 2013; Schaefer *et al.*, 2016a; Gomes *et al.*, 2021b). Despite its high biodiversity and ecological importance, human actions, such as urbanization and iron mining activities, are responsible for the degradation of *canga*. These impacts favor erosion and hinder natural regeneration, since *campo rupestre* ecosystems are unable to recover from severe anthropogenic disturbances (Le Stradic *et al.*, 2018; Buisson *et al.*, 2019), in addition to leaving the environment more susceptible to colonization by invasive species (Barbosa *et al.*, 2010; Negreiros *et al.*, 2011; Tameirão, LB *et al.*, 2021).

As observed for the *campo rupestre* vegetation (Negreiros *et al.*, 2014; Dayrell *et al.*, 2018), native *canga* plant species possess a series of traits that allow them to be functionally classified as stress tolerant, among the ecological strategies known as CSR (C: competitive, S: stress-tolerant and R: ruderal) (Grime & Pierce, 2012; Tameirão, LB *et al.*, 2021). We consider stress as an environmental factor that negatively reduces some physiological processes below the maximum rate that the plant could sustain (Lambers & Oliveira, 2019). Therefore, plants make up for the damaging effects of stress through mechanisms that occur at different times scales depending on the nature of the stress and the physiological processes that have been affected. Plant success in stressful environments is related to some degree of resistance to stress, which in turn differ widely between species and range from stress avoidance to stress tolerance mechanisms (Lambers & Oliveira, 2019). However, in this article, we are considering the stress tolerance strategy as a broader ecological perspective, which involves conserving resources and maintaining metabolic performance in harsh environments (Grime *et al.*, 1997; Perez-Harguindeguy *et al.*, 2013; Pierce *et al.*, 2017).

Canga vegetation presents great biodiversity due mainly to edaphic and topographic variation at fine-scale as well as the high surface heterogeneity to which they are exposed and microclimate characteristics (Carmo *et al.*, 2016; Do Carmo & Jacobi, 2016). However, the *canga* species have more restricted geographical distributions, less competitiveness and greater resistance to stress, as well as small-size individuals with smaller and narrower leaves and greater sclerophylly (Negreiros *et al.*, 2014).

Knowing the different photosynthetic strategies that plants employ under continuous environmental stressors, mainly those related to energy use which contributes to improved physiological performance, can provide markers for the selection of promising plant species for the recovery of environments impacted by iron mining. Excess light at levels above the utilization capacity for photosynthesis (Lemos Filho, 2000; Pereira *et al.*, 2018), in combination with high seasonal rainfall, low relative air humidity and low soil moisture in the winter, can cause damage to the photosynthetic complex. Once the light is absorbed by chlorophyll in the antenna complex, it can be dissipated by three distinct and competing processes. The use of light energy for electron transfer in photosynthesis is related to the effective quantum yield of PSII (ϕ_{PSII}). The excess energy absorbed can be dissipated in a non-photochemically regulated manner (ϕ_{NPQ}), protecting the photosynthetic apparatus against overexcitation and subsequent damage to the PSII reaction center (Baker, 2008; Pereira *et al.*, 2018), which is associated with the xanthophyll cycle and heat dissipation (Baker, 2008; Baraldi *et al.*, 2008). However, increases in the quantum yield of non-regulated energy dissipation in PSII (ϕ_{NO}) occur when plants do not display efficient mechanisms for dissipating excess energy in a regulated way, which can lead to the formation of reactive oxygen species (ROS) due to excess incident energy and compromise their establishment in environments with intense stressors (Pereira *et al.*, 2018).

Since it is an environment with shallow and nutrient-poor soil, high irradiance and climatic seasonality, we hypothesize that *canga* vegetation will predominantly possess a higher proportion of species with ecological strategies close to stress tolerance, and that these will be associated with greater energy dissipation through regulated non-photochemical processes in PSII and consequent reduction of the effective quantum yield of PSII during the dry season. In this study, we aimed to understand the relationships between ecological strategies present in *canga* species and photosynthetic adjustments in response to climatic seasonality, in order to understand which parameters are useful in the selection of species for the recovery of mined environments. Considering the Decade on Ecosystem Restoration established by the UN in an attempt to restore ecosystem services and contain the rapid decline in biodiversity, this work brings contributions that aim to favor the physiological selection of species to be used in the recovery of environments impacted by iron ore mining since the selection of suitable plant species is essential for successful restoration programs, especially in natural grassland habitats (Dudley *et al.*, 2020a), such as *cangas*.

2. MATERIAL AND METHODS

2.1 Study area

The investigated area is located in the extreme north of Serra da Moeda, known locally as Serra da Calçada (20°05’55,2”S, 43°58’ 58,2”W), in the municipality of Brumadinho, Minas Gerais, Brazil (Fig.1). Located in the southern portion of Serra do Espinhaço and west of the *Quadrilátero Ferrífero*, Serra da Moeda has been degraded by iron ore mining. Among the vegetation types found in the region are gallery forest, forest islands, open savanna, *campo rupestre* on quartzite and *campo rupestre* on itabirite or *canga* (Giulietti *et al.*, 1987; Zappi *et al.*, 2017), with the study area being of the latter. In the *canga* area were established 10 plots of 10 m × 10 m distributed along a 200 m transect (Caminha-Paiva *et al.*, 2022). The plots were distributed on *nodular* and *couraçada canga*.

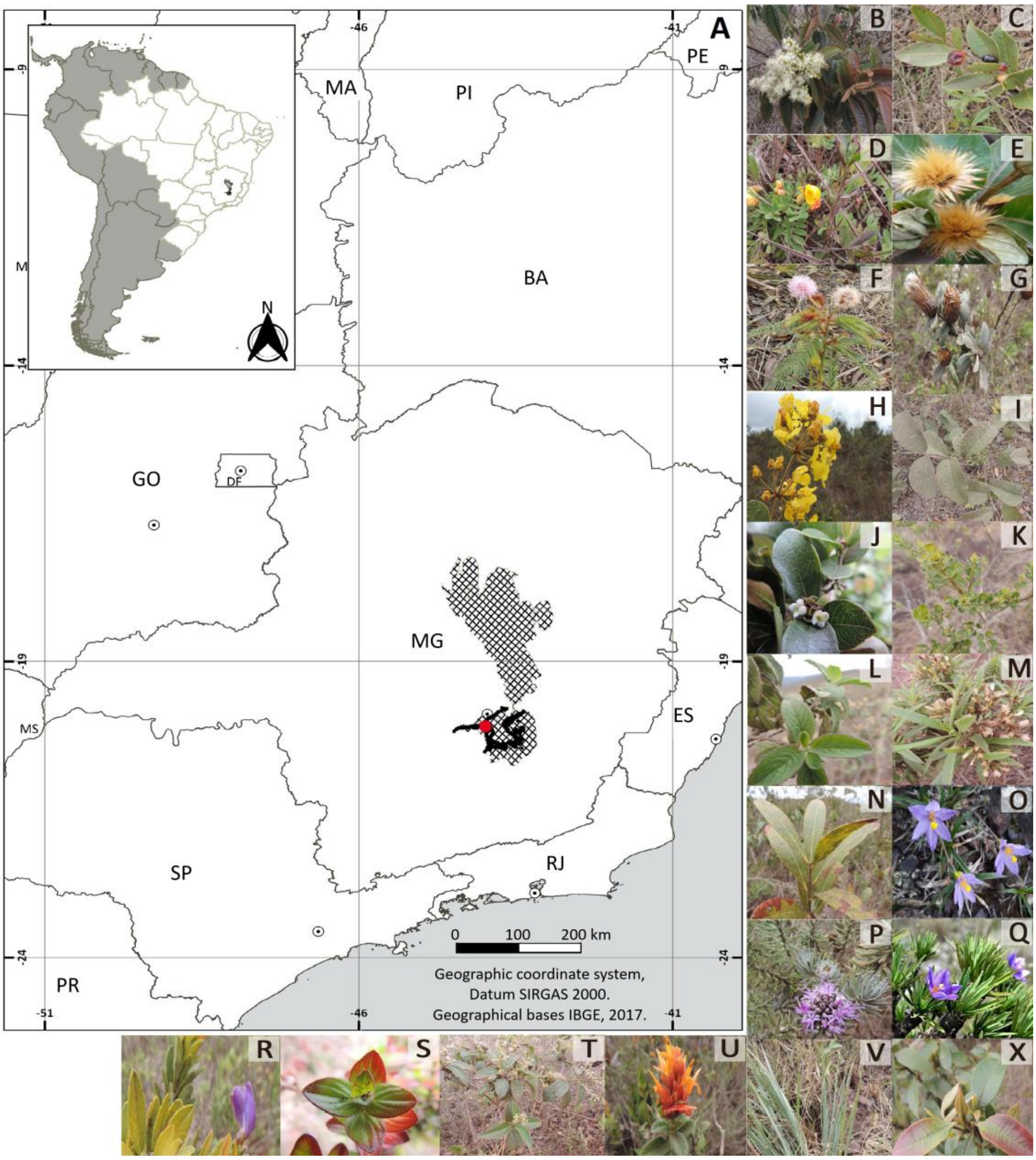


Fig. 1 Study area in *canga* (A). Dashed area indicates the Serra do Espinhaço Biosphere Reserve, black area represents the *Quadrilátero Ferrífero* and the red dot indicates the study area in Serra da Calçada, Brumadinho, MG. Panels on the right and below show the 22 studied species. *Miconia trianae* (B), *Eugenia bimarginata* (C), *Chamaecrista mucronata* (D), *Hololepis pedunculata* (E), *Mimosa pogocephala* (F), *Dasyphyllum velutinum* (G), *Peixotoa tomentosa* (H), *Banisteriopsis malifolia* (I), *Cordiera concolor* (J), *Baccharis reticularia* (K), *Eriope macrostachya* (L), *Trixis vauthieri* (M), *Kielmeyera coriacea* (N), *Vellozia caruncularis* (O), *Lychnophora pinaster* (P), *Vellozia compacta* (Q), *Periandra mediterranea* (R), *Diplusodon microphyllus* (S), *Leandra aurea* (T), *Justicia monticola* (U), *Lagenocarpus cf. rigidus* (V) and *Myrcia splendens* (X). State abbreviations: MG Minas Gerais, SP São Paulo, PR Paraná, RJ Rio de Janeiro, ES Espírito Santo, MS Mato Grosso do Sul, GO Goiás, BA Bahia, PI Piauí, MA Maranhão and PE Pernambuco.

2.2 Climate

The local climate is Aw (tropical with a dry winter) according to the classification of

Köppen and Geiger, with an average temperature of 20.7°C, a low annual thermal amplitude, dry winters (May to October) and rainy summers (November to April), and an average annual rainfall of 2041.6 mm (Fig.2).

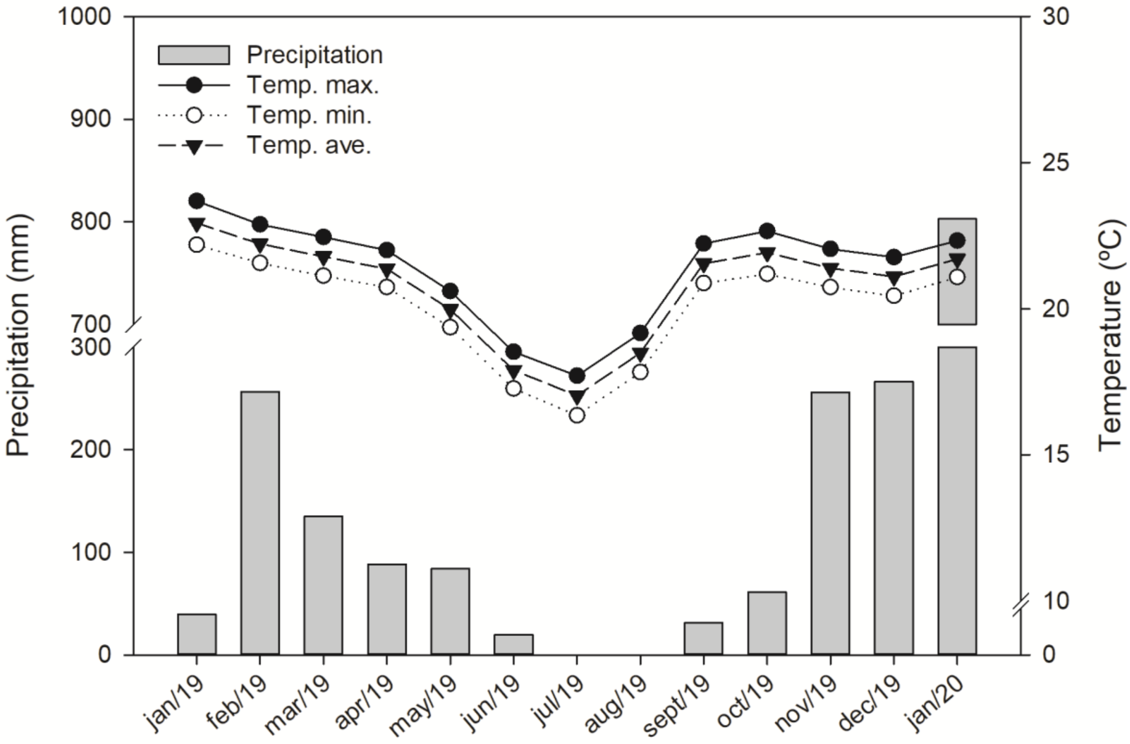


Fig. 2 Precipitation and temperature during the study period, including the rainy and dry seasons. Information extracted from the database of the National Institute of Meteorology (INMET) for the automatic station A555, Ibirité (Rola Moça), located 8.0 km from the study area.

The average temperature of soil and air during the rainy season were 19.9ºC and 22.9ºC, and during the dry season they were 22.4ºC and 17ºC, respectively. The analyses were performed with four type K thermocouple coupled to a model 1384 dataloger, Omega, Taiwan. Two being arranged under the ground and the other two in the air. Measurements were performed at 1-hour intervals for 5 days.

The maximum photosynthetically photon flux density (PPFD) in the rainy and dry season was 2792 µmol m^{-2} s^{-1} and 2016,05 µmol m^{-2} s^{-1}, respectively (both measured around noon with open skies, without clouds using a HD 2102.2 radiometer, Delta OHM, Italy). Measurements were performed at 1-hour intervals for 5 days.

2.3 Soil characterization

In the 10 plots established along the transect, the volumetric water content of the soil (measured with a TDR 100 probe, Fieldscout, USA) was evaluated. During the rainy and dry periods was 13.4% and 6.5%, respectively. The portable probe used for TDR measurement consisted of a pair of parallel tapered stainless steel rods, 3.3 cm apart. Standard mode was selected for measurements. The soil was drilled with the rods and they became a parallel pair of a transmission line. The length of the rods used for the analyses were 7.6 cm. Measurements were performed at 1-hour intervals for 5 days.

In the study area, the 10 plots established along the transect are located on ferriferous substrates with a clay-sandy texture, and high levels of silt and sand. It is also markedly stony, poor in nutrients, and with low cation exchange capacity. As a consequence of the advanced degree of weathering, the pH values are slightly acidic. The substrate is also poor in Ca, K and Mg and has high Fe contents. Five subsamples of bulk soil were collected in each plot (4 corners and 1 centre point) at 0–20-cm depth, and mixed thoroughly to produce a single composite homogeneous representative sample for each plot. A subset of each composite sample (ca. 500 g) was used for physical-chemical analysis: pH; available P, K, Mn, Fe, Zn, S, and Cu; exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+}; soil organic carbon (SOC); granulometry; and particle density (Tab. 1). Soil analysis followed the recommendations of (Teixeira *et al.*, 2017). P and K were analyzed with the Mehlich-1 extraction method; Ca^{2+}, Mg^{2+}, and Al^{3+} with 1 mol L^{-1} KCl extraction; and SOC following the Walkley-Black method.

Table 1. Physico-chemical characteristics of soils of the *canga* in the study area.

Variables	Canga
P (mg dm^{-3})	0.92±0.08
K (mg dm^{-3})	30.9±2.18

Ca^{2+} (cmol$_c$ dm^{-3})	0.89±0.08
Mg^{2+} (cmol$_c$ dm^{-3})	0.20±0.02
Fe (mg dm^{-3})	172.48±8.18
Mn (mg dm^{-3})	27.24±3.41
Zn (mg dm^{-3})	3.15±0.29
Cu (mg dm^{-3})	1.13±0.08
S (mg dm^{-3})	5.51±0.42
Al^{3+} (cmol$_c$ dm^{-3})	0.63±0.09
H + Al (cmol$_c$ dm^{-3})	9.24±0.50
SB (cmol$_c$ dm^{-3})	1.17±0.10
ECEC$_t$ (cmol$_c$ dm^{-3})	1.81±0.13
CEC$_T$ (cmol$_c$ dm^{-3})	10.41±0.52
V (%)	11.47±0.96
m (%)	34.37±4.22
res-P (mg L^{-1})	34.34±1.37
OM (dag kg^{-1})	4.32±0.38
pH H_2O	4.56±0.13
Coarse sand (kg kg^{-1})	0.55±0.02
Fine sand (kg kg^{-1})	0.12±0.02
Silt (kg kg^{-1})	0.11±0.01
Clay (kg kg^{-1})	0.22±0.01
Textural classification	Sandy clay loam

Soil analyses were performed with composite samples from the study area (n = 10). Legend: H + Al. potential acidity; SB sum of exchangeable bases; ECECt effective cation exchange capacity; CECT cation exchange capacity at pH 7; V base saturation index; m aluminum saturation index; res-P residual phosphorus; SOC soil organic carbon. Mean ± standard error

2.4 Plant species

Based on the structure and composition of plant species and studies carried out in the same site (Gomes *et al.*, 2021b; Caminha-Paiva *et al.*, 2022), 22 species with C3 photosynthetic metabolism were selected belonging to the following families (Fig. 1): Asteraceae (5 species), Fabaceae (3), Velloziaceae (2), Melastomataceae (2), Myrtaceae (2), Malpighiaceae (2), Acanthaceae (Krause & Weis), Calophyllaceae (Krause & Weis), Lythraceae (Krause & Weis), Cyperaceae (Krause & Weis), Lamiaceae (Krause & Weis), and Rubiaceae (Krause & Weis). Representative branches of each species were collected, herborized and identified to species (Fig. 1). A total of 10 individuals (replications) per species studied were selected throughout the plots. Although the plots were adjusted along a transect, the distribution of individuals within the plot is heterogeneous, so the number of individuals in each plot was not standardized. However, plants were selected based on a minimum distance of 2 meters between individuals. Plant samples were identified based on botanical literature, consultation with experts, and by comparison with samples in the BHCB herbarium at the Federal University of Minas Gerais.

2.5 Analysis of photosynthetic traits

Physiological traits were assessed for 10 individuals (replicates) per species during the rainy season (January 2019) and the dry season (July 2019), the same individuals were used in both seasons. Chlorophyll *a* fluorescence analyses were performed with the Mini-PAM fluorometer (Heinz Walz, Effeltrich, Germany). Initial fluorescence (F_0) and maximum quantum yield of photosystem II (PSII) (F_v/F_m) were determined after at least 30 minutes of acclimatization of leaves in the dark (Cailly, 1996). The leaves were then exposed to incident radiation at the leaf level (546 to 1,801 µmol m^{-2} s^{-1}) and a pulse of saturating light was applied to determine the following parameters: F = fluorescence in *steady state* before the pulse of saturating light; F_m’ = maximum fluorescence of the illuminated plant tissue; $\phi_{PSII} = (F_m'-F)/F_m'$ = effective quantum yield of PSII; $\phi_{NPQ} = F/F_m' - F/F_m$ = quantum yield of regulated non-photochemical energy dissipation of PSII(Cailly, 1996); $\phi_{NO} = F/F_m$ = quantum yield of unregulated non-photochemical energy dissipation of PSII (Cailly, 1996). and NPQ = Fm-Fm’/Fm’ = non-photochemical quenching (Bilger & Björkman, 1990). Apparent electron transport rate (Manzoni *et al.*) was calculated as ETR = 0.5 × lA × ϕ_{PSII} × PPFD, where 0.5 is the assumed proportion of how much is absorbed/used by PSII reaction centers (Melis *et al.*,

1987) and lA is the leaf absorbance set at 0.84.

Chlorophyll indices were measured in the same leaves using a portable ClorofiLOG meter (CFL1030, Falker, Brazil). In addition to the total chlorophyll index, chlorophyll *a*, chlorophyll *b*, and the chlorophyll *a*/*b* ratio were also evaluated. The measurements were taken three times in the middle portion of a fully expanded leaf and then averaged as a replicates.

2.6 Analysis of CSR ecological strategies

The analysis of CSR ecological strategies was carried out with 10 mature and apparently healthy individuals (repetitions) per species in January during the rainy season of 2020. The CSR values were calculated for each species (Pierce *et al.*, 2017) using three functional characteristics of the leaves: leaf area (LA, mm^2), leaf dry weight (LDW, mg) and leaf fresh weight (LFW, mg). The measurement of these characteristics followed the protocol proposed by Perez-Harguindeguy *et al.* (2016). Whole shoots were collected in the field while avoiding the stronger sunlight period (11: 00-15: 00). The shoots were then packaged in paper bags, saturated with water using a spray bottle, sealed in plastic bags and stored in an insulated box to be transported to the laboratory, 76 km away. In the laboratory, the collected shoots were kept in a refrigerator at 4°C for at least 12 hours and until complete turgidity. Measurements were performed on three leaves (including the petiole) per individual within 24h after collection in the field. Only fully expanded leaves were used, with no signs of pathogens, herbivory, or malformation (Cornelissen *et al.*, 2003). Leaf area was obtained using a portable scanner and Image J Software. Curved leaves were cut into smaller fragments and positioned so as the entire leaf surface could be scanned. Fresh leaf weight was obtained from turgid leaves previously dried with paper towels, while dry leaf weight was determined with an analytical scale (accurate to 0.01 mg) after 96 hours of oven drying at 60ºC. Although the CSR calculation considers only three characteristics of the leaf, it represents a series of ecological strategies and plant functioning (Pierce *et al.*, 2017; Dayrell *et al.*, 2018). Values of leaf area (LA), specific leaf area (SLA), and leaf dry matter content (LDMC) for each species were entered into 'StrateFy' to calculate the proportion (%) of C-, S-, and R-selection for CSR strategies (Pierce et al. 2017).

2.7 Statistical analysis

The R program, version 3.0.0 was used to calculate mean and standard deviation values of the following parameters: maximum quantum efficiency of PSII (F_v/F_m), initial fluorescence (F_0), total chlorophyll index, effective quantum yield of PSII (ϕ_{PSII}), quantum yield of regulated dissipation of non-photochemical energy from PSII (ϕ_{NPQ}), quantum yield of unregulated dissipation of non-photochemical energy from PSII (ϕ_{NO}) and ecological CSR strategies. The Shapiro-Wilk test was applied to check the occurrence of normal distribution in each data set. For normally-distributed data sets, the Bartlett test was performed to verify variance homogeneity. Data sets significantly deviating from normality were log-transformed.

Analysis of variance (one-way ANOVA) and a-posteriori Duncan's tests were applied to compare seasons means. A repeated-measures ANOVA and a-posteriori Duncan's tests were also used for each species separately.

Correlation analysis was performed using the corrplot, Hmisc and Performance Analytics statistical packages. Correlations with "x" are non-significant interactions with p>0.005.

3. RESULTS

Higher values for the maximum quantum efficiency of PSII (F_v/F_m) were observed during the rainy season, with values close to 0.8 for most species (Fig. 3A). The species: *L. aurea, B. malifolia, E. bimarginata, E. macrostachya, J. monticola, K. coriacea, M. splendens, P mediterranea, D. microphyllus,* and *H. pedunculata* had lower F_v/F_m during the dry period. Only *M. pogocephala* had a significantly higher F_v/F_m during the dry season (Fig. 3B).

Overall, initial fluorescence was higher during the rainy season (Fig. 3C). However, most species did not show significant differences between rainy and dry seasons, except for *M. pogocephala*, *V. compacta*, *E. macrostachya*, and *D. velutinum* (Fig. 3D).

Higher levels of total chlorophyll were observed during the rainy season (Fig. 3E). However, only *B. reticularia*, *L. aurea*, *C. mucronata*, *K. coriacea*, *P. mediterranea*, and *D. microphyllus* showed significant differences between seasons. Comparison among species showed *E. bimarginata* to have significantly higher levels of total chlorophyll in both seasons than all other species (Fig. 3F).

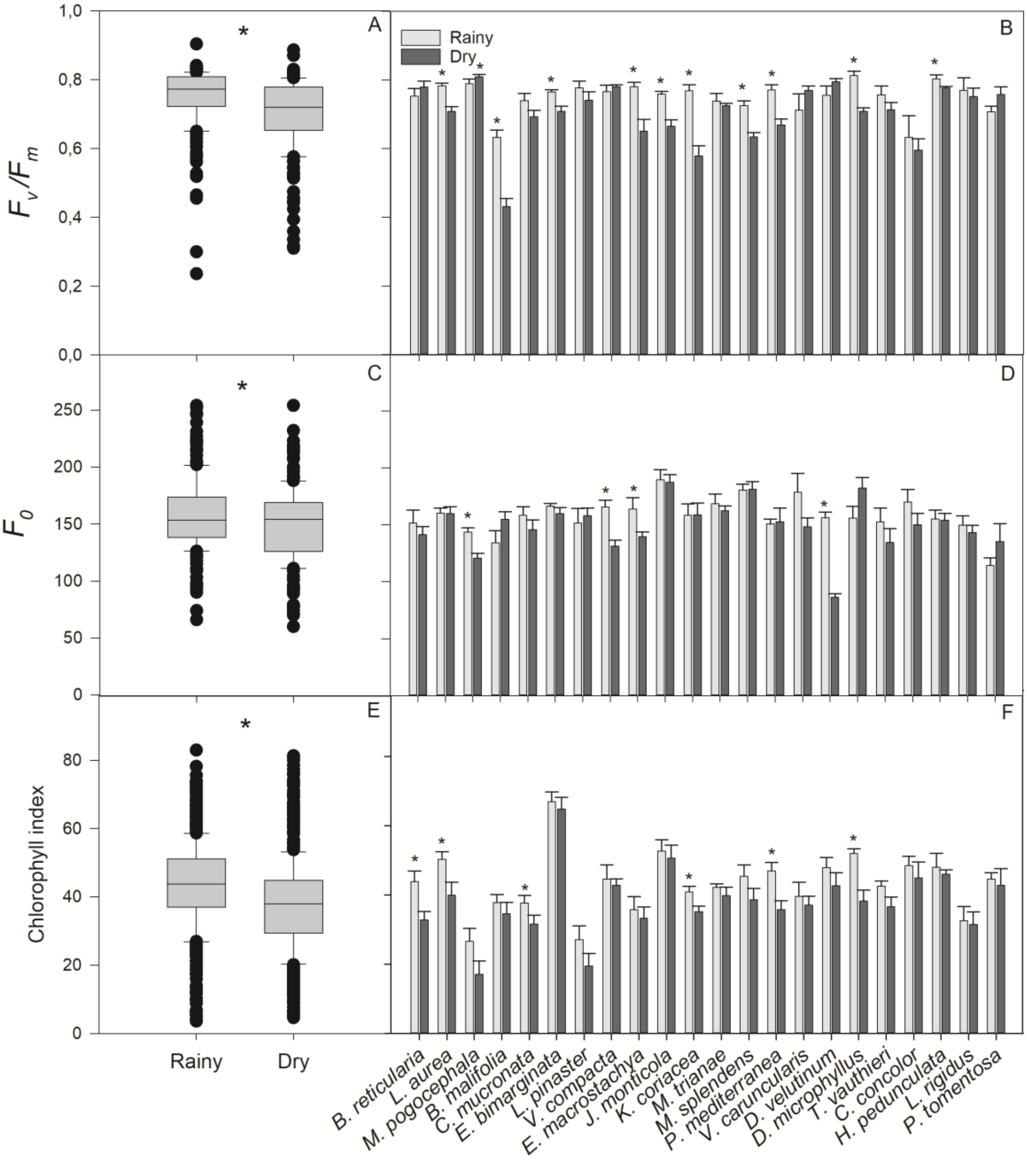


Fig. 3 Maximum quantum efficiency of PSII (F_v/F_m, A and B), initial fluorescence (F_0, C and D) and total chlorophyll index (E and F) for 22 *canga* plant species in the rainy and dry seasons. Boxplots compare seasons regardless of species. Asterisks (*) indicate a significant difference between seasons according to Duncan's test at 5% probability. The bars for each species represent the average ± standard error of ten repetitions.

The *canga* species showed higher PSII effective quantum yield (ϕ_{PSII}) during the rainy season (Fig. 4A), as observed for *B. malifolia*, *C. mucronata*, *E. bimarginata*, *V. compacta*, *E. macrostachya*, *J. monticola*, *K. coriacea*, *M. trianae*, *M. splendens*, *P. mediterranea*, and *V.*

caruncularis. However, the opposite was observed for *B. reticularia*, *L. aurea*, and *M. pogocephala* (Fig. 4B) with significantly higher ϕ_{PSII} in the dry season. Overall, the quantum yield of regulated non-photochemical energy dissipation of PSII (ϕ_{NPQ}) did not differ significantly between seasons (Fig. 4C). However, it was higher for the species *B. reticularia*, *L. aurea*, *M. pogocephala*, *D. velutinum*, *D. microphyllus*, and *T. vauthieri* during the rainy season and during the drought for the species *E. macrostachya*, *J. monticola*, *K. coriacea*, *Miconia trianae*, *M. splendens*, *P. mediterranea*, and *V. caruncularis* (Fig. 4D). The dry season resulted in an increase in quantum yield of unregulated dissipation of non-photochemical energy from PSII (ϕ_{NO}) (Fig. 4E), as observed for *C. mucronata*, *E. bimarginata*, *L. pinaster*, *D. velutinum*, *D. microphyllus*, and *T. vauthieri*. Only *M. pogocephala* had significantly higher values of ϕ_{NO} during the rainy season (Fig. 4F). The strategy of dissipating excess energy adopted by each species during the dry and rainy season and the adjustments in photochemical efficiency were used to separate the species in five different groups.

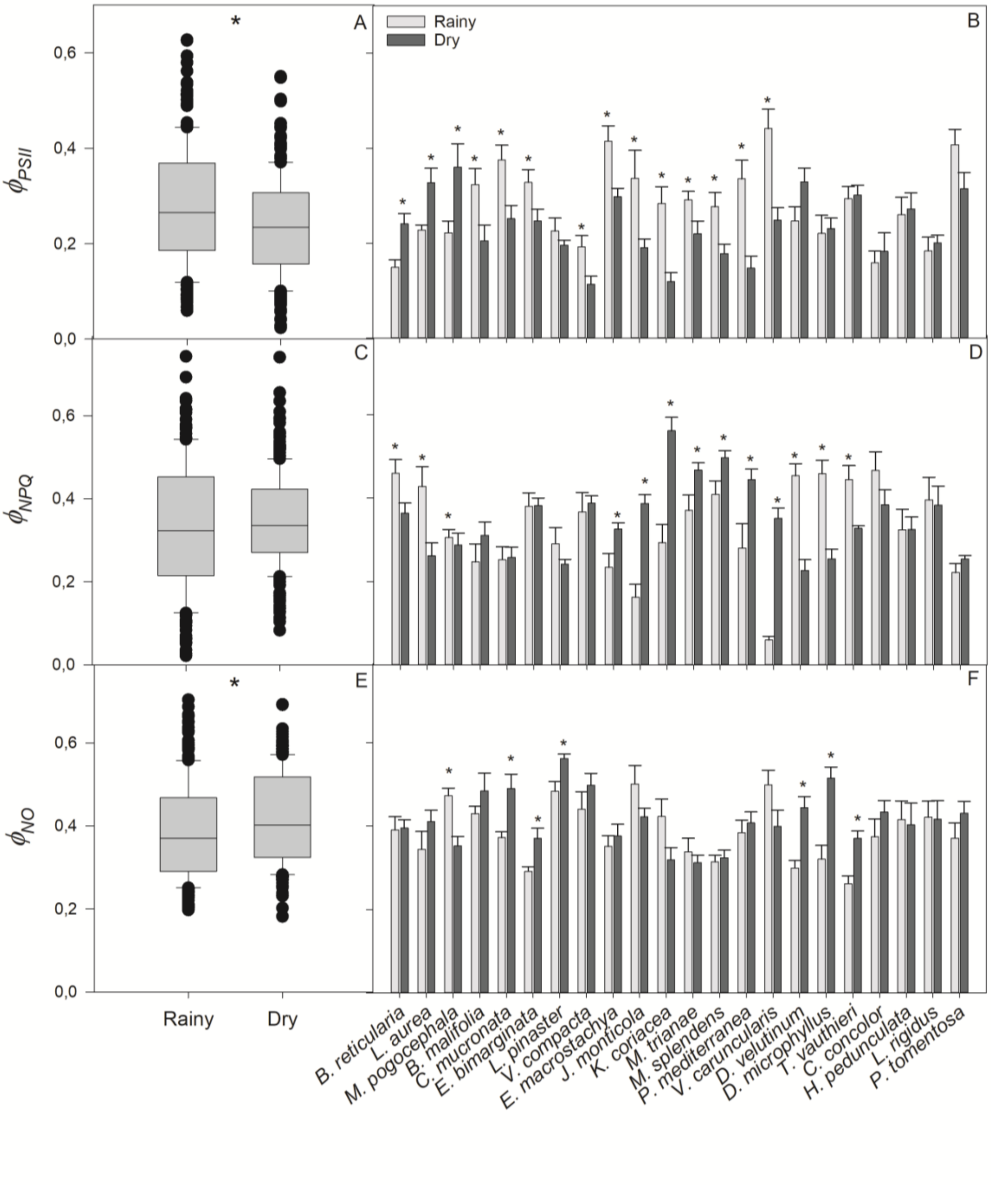


Fig. 4 Effective quantum yield of PSII (ϕ_{PSII}, A and B), quantum yield of regulated dissipation of non-photochemical energy from PSII (ϕ_{NPQ}, C and D) and and quantum yield of unregulated dissipation of non-photochemical energy from PSII (ϕ_{NO}, E and F) for 22 *canga* plant species in the rainy and dry seasons. Boxplots compare seasons regardless of species. Asterisks (*) indicate a significant difference between seasons according to Duncan's test at 5% probability. The bars for each species represent the average ± standard error of ten repetitions.

The five distinct groups were set according to the ability of the studied species to use and dissipate excess light energy, highlighted by the variables ϕ_{PSII} and ϕ_{NPQ}, respectively, in the rainy season in relation to the dry season: Group 1 - characterized by increases in ϕ_{PSII} and decreases in ϕ_{NPQ}; includes the species *B. reticularia*, *Leandra aurea*, and *M. pogocephala*; Group 2 - characterized by decreases in ϕ_{PSII} and maintenance of ϕ_{NPQ} during the seasons; includes the species *B. malifolia*, *C. mucronata*, *E. bimarginata*, *L. pinaster*, and *V. compacta*; Group 3 - characterized by decreases in ϕ_{PSII} and increases in ϕ_{NPQ} in the dry season; includes the species *E. macrostachya*, *J. monticola*, *K. coriacea*, *M. trianae*, *M.. splendens*, *P. mediterranea*, and *V. caruncularis*; Group 4 - characterized by no significant difference for ϕ_{PSII} and decreases in ϕ_{NPQ} in the dry season; includes the species *D. velutinum*, *D. microphyllus*, and *T. vauthieri*; and Group 5 - characterized by no differences in any of the variables of the use of light energy between dry and wet seasons; includers the species *C. concolor*, *H. pedunculata*, *L. rigidus*, and *P. tomentosa* (Fig. 5, Tab. S1). The ϕ_{NO} /ϕ_{NPQ} ratio was different between seasons only for groups 3 and 4. For group 3, increases in ϕ_{NPQ} values were observed, and for group 4, increases in ϕ_{NO} and reduction in ϕ_{NPQ} during the dry season. The reduction in ϕ_{NPQ} values and the increase in ϕ_{NO} during the drought for groups 1 and 2, respectively, did not lead to a difference between the ϕ_{NO}/ϕ_{NPQ} ratio (Tab. S1).

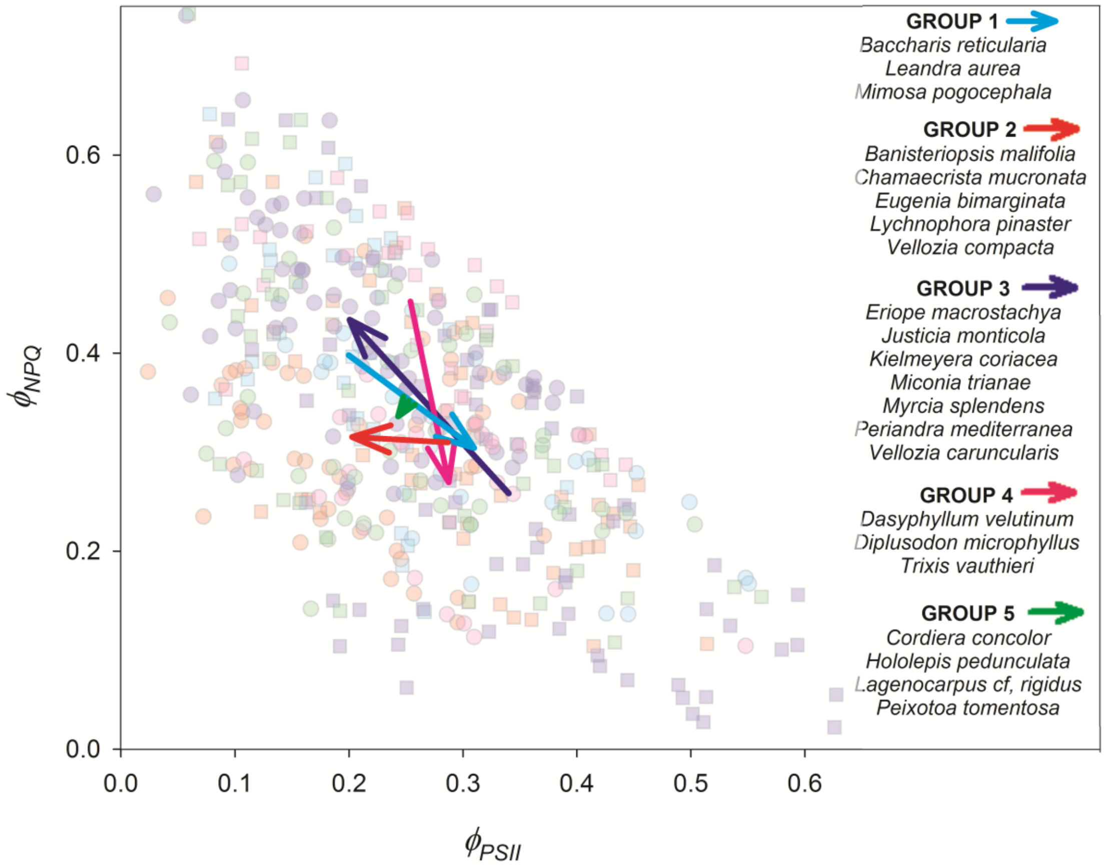
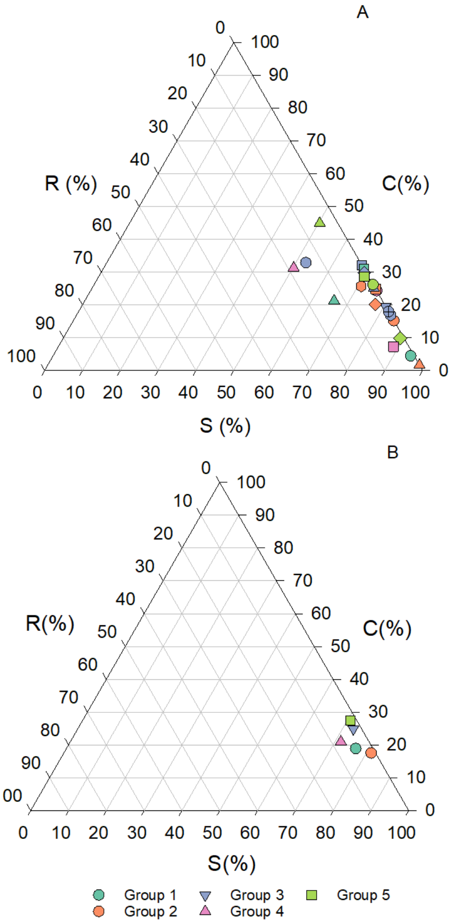


Fig. 5 Grouping of the 22 native species of *canga* into five different groups (see text), according to effective quantum yield of PSII (ϕ_{PSII}) and quantum yield of regulated non-photochemical energy dissipation of PSII (ϕ_{NPQ}) during the rainy season and the dry season. Statistical significances according to Duncan's test at 5% probability are presented in Table S1.

The studied native plant species of *canga* presented ecological strategies characteristic of stress tolerance (74.76%), competition (22.31%) and ruderal (2.93%) (Fig.6A). Among the 5 groups selected according to the ϕ_{PSII} and the ϕ_{NPQ} (Fig.5), group 2 is composed of the species with the highest average percentage of stress tolerance (Fig. 6). The species *L. pinaster* had the highest proportion of adaptive stress-tolerant strategy (98.35%) and *T. vauthieri* the lowest values (50.22%). Group 5 species are the most competitive, with an average percentage of 27.40%, with *H. pedunculata* standing out with 44.91% competition capacity. All the analyzed species showed a very low percentage of ruderality, with the species of Group 4 being the most ruderal with an average of 7.51%, reaching up to 18.49%

for *T. vauthieri*.



Species	C(%)	S(%)	R(%)
● *Baccharis reticularia*	4.46	94.62	0.92
■ *Leandra aurea*	30.92	69.08	0.00
▲ *Mimosa pogocephala*	21.24	65.96	12.80
● *Banisteriopsis malifolia*	27.71	70.90	3.39
● *Chamaecrista micronata*	15.20	84.80	0.00
■ *Eugenia bimarginata*	24.86	75.14	0.00
▲ *Lychnophora pinaster*	1.65	98.35	0.00
◆ *Vellozia compacta*	20.18	77.41	2.41
● *Eriope macrostachya*	32.88	52.67	14.45
■ *Justicia monticola*	32.11	67.89	0.00
▲ *Kielmeyera coriacea*	25.11	74.53	0.36
◆ *Miconia trianae*	29.80	69.66	0.55
● *Myrcia splenden*	16.87	83.13	0.00
▼ *Periandra mediterranea*	19.58	80.42	0.00
● *Vellozia caruncularis*	17.95	82.05	0.00
● *Dasyphyllum velutinum*	24.29	75.71	0.00
■ *Diplusodon microphyllus*	7.27	88.68	4.05
▲ *Trixis vauthieri*	31.28	50.23	18.49
◆ *Cordiera concolor*	9.84	89.15	1.01
▲ *Hololepis penduculata*	44.91	50.32	4.78
● *Lagenocarpus cf, rigidus*	26.20	73.80	0.00
■ *Peixotoa tomentosa*	28.68	70.23	1.09

Fig. 6 Ecological strategies, C = competition, S = stress tolerance, and R = ruderal, for each of the studied species (A) and for each of the analyzed groups (B). The table on the right shows the percentage of CSR for each species. See Fig. 5 for descriptions of the groups Fig. 5.

The correlation analysis carried out among the CSR analysis, chlorophyll indices and chlorophyll fluorescence demonstrated that there was no relationship between the stress tolerance ecological strategy and photochemical variables. However, a positive correlation was observed between the competition strategy and chlorophyll indices (*a*, *b* and total) in dry and rainy seasons (Fig. S1). There was also a negative correlation between competition and ϕ_{NO} in the dry season and a positive correlation with ϕ_{PSII} in the rainy season.

4. DISCUSSION

Tolerance to stress and different photochemical adjustments are strategies that contribute to the dynamics of *canga* vegetation and guarantee their survival in this highly diversified ecosystem. The ecological stress tolerance strategy presented by plants might comprise a variety of photosynthetic strategies to cope with low nutrient availability and rainfall seasonality in *canga*. Different environmental stressors can be considered strong predictors of ecological stress tolerance strategies in *campo rupestres*, particularly soil infertility (Messias *et al.*, 2013; Schaefer *et al.*, 2016a), climatic seasonality with prolonged droughts, and intense exposure to solar radiation (Negreiros *et al.*, 2009; De Carvalho *et al.*, 2012). As these are extremely old ecosystems, their development is accompanied by the depletion of soil nutrients (Wright *et al.*, 2004; Turner & Condron, 2013). Therefore, the low availability of P (Lambers *et al.*, 2011; Abrahão *et al.*, 2019) and also the acidic pH and high concentrations of Fe in *canga* influence the different physiological strategies that guarantee their survival during the dry season. We showed that low soil fertility and reduced rainfall during the dry season led to photochemical adjustments that mainly concerning the plasticity in the patterns of excess light energy dissipation, reducing possible oxidative damage (Kaiser *et al.*, 2019) and ensuring the occupation of a wide variety of microhabitats.

Environments with severe stressors, such as the *campo rupestre*, are composed of long-lived and slow-growing species (Dayrell *et al.*, 2018), resulting in few morphological adjustments throughout the period of a year. In addition, the low availability of nutrients in the soil also results in the selection of species with slow growth (Manzoni *et al.*, 2017), long organ life-span (Reich, 2014), and high efficiency in the use and remobilization of nutrients (Veneklaas *et al.*, 2012; Hayes *et al.*, 2014). This interacting with high solar irradiance tends to favor plant species with thicker leaves, longer leaf life-spans and lower photosynthetic and respiratory rates (Wright *et al.*, 2004). *Canga* species, despite having less photosynthetic capacity when compared to species with short leaf life-spans and lower leaf mass per area, have physiological adjustments that guarantee plasticity and survival in the face of climatic seasonality. Phenotypic plasticity was also observed in different populations of *Myrcia amazonica* found in *campo rupestre* and gallery forest during the dry and rainy season, reflecting adaptations to different microclimates (da Silva Moraes *et al.*, 2017).

Our results provide evidence of the existence of a spectrum of photochemical characteristics in native species of *canga* adapted to local environmental conditions. These photochemical adjustments may be involved in the coexistence of the high number of species, since these traits can impact both niche and fitness differences of plants in this environment (Turcotte and Levine 2016). *Campo rupestre* occurs in regions of seasonally dry climate, with two to five months of drastic reductions in rain (Alves *et al.*, 2014), which, consequently, implies a reduction in F_v/F_m (Fig. 3A). In addition to the severe edaphoclimatic conditions, low surface water retention capacity, high intensity of solar radiation, and low concentration of nutrients can be limiting factors for the growth, establishment, and survival of plants in *campo rupestre* (Jacobi *et al.*, 2008; Skirycz *et al.*, 2014; Nunes *et al.*, 2015; Vasconcelos *et al.*, 2016). Once exposed to adverse conditions, such as prolonged drought, the overproduction of reactive oxygen species can cause damage to the photosynthetic machinery of plants (Sharma *et al.*, 2012). In this way, photochemical adjustments guarantee the dissipation of excess energy safely, allowing the species to survive during stress and resume growth under favorable conditions (Rios et al. 2021). The dry season also resulted in a reduction in the values of F_v/F_m for species of Melastomataceae in xeric and mesic microhabitats of *campo rupestre* (Castro *et al.*, 2018). Despite this downward trend in F_v/F_m values during the driest months, not all species responded in this way, showing that some are able to maintain F_v/F_m in both seasons (Fig. 3B).

Changes in PSII non-photochemical energy dissipation provide dynamic mechanisms capable of reducing the risks of photoinhibition (Kaiser *et al.*, 2019). The decrease in F_v/F_m values during the drought period was not the result of possible photoinhibitory damage, but rather adjustments in the light energy dissipation process, especially for Group 3 species, in which greater regulated non-photochemical dissipation was observed (Tab. S1). Adjustments in the electron flow partition between assimilative and non-assimilative processes contribute to the suppression of photoinhibition (Franco *et al.*, 2007). The high irradiance associated with less water availability during the dry period promoted photooxidation of photosynthetic pigments, with a consequent reduction in F_v/F_m and an increase in ϕ_{NO} but not enough to decrease ϕ_{PSII} in the species *D. microphyllus*. The same was observed for *E. bimarginata*, but there was no reduction in the chlorophyll index and ϕ_{PSII} was lower during drought, which may have resulted in photoinhibitory damage in both species. (Fig. 4F). However, they may have an efficient mechanism for eliminating ROS so as not to cause severe damage, such as enzymatic mechanisms that minimize the phytotoxic effects of ROS (Schützendübel & Polle, 2002). The high irradiance for *campo rupestre* also promoted a reduction in the photosynthetic pigments of *Myrcia amazonica* but it was not enough to reduce the assimilation of CO_2 (da Silva Moraes *et al.*, 2017).

A high prevalence of stress tolerance syndromes were found in the studied native species of *canga* (Fig. 6), as observed by Negreiros *et al.* (2014) for herbaceous species of *campo rupestre*. The different photochemical strategies observed among the species is one of the key points for the great diversity of *cangas* since they guarantee survival in the face of environmental stresses, preventing oxidative damage that can otherwise leave plants to death ((Siqueira-Silva *et al.*, 2019; Rios *et al.*, 2021). This diversity of photochemical strategies explains the non-correlation between the ecological stress-tolerant strategy and each of the analyzed physiological variables (Fig. S1). During periods of drought, reductions in the hydraulic conductivity of plants are common, which in turn limits the flow of water, resulting in loss of stomatal cell turgor and consequent reduction in stomatal conductivity that will influence gas exchange (Sperry, 2000; Vieira *et al.*, 2017). In view of stomatal closure and high irradiance, variations in the dissipation of excess light energy are observed. There was a greater dissipation of energy in a regulated manner, as observed for species belonging to Group 3. Group 2, on the other hand, contains species with the highest percentage of tolerance to stress, however there was an increase in ϕ_{NO} during the dry season, which did not cause damage to the photosynthetic apparatus as no differences were observed between seasons for the F_v/F_m parameters and chlorophyll indexes (Tab. S1).

The ecological strategy of competition observed in some extent among the studied species results from an ability to invest in growth and a rapid acquisition of resources (Pierce *et al.*, 2017). The positive correlation between the competition strategy and the photosynthetic parameters (chlorophyll and ϕ_{PSII}) is also related to the greater leaf area that guarantees a greater surface of light capture. Those functional and photosynthetic traits directly influences the competition strategy. However, the low competitive percentage presented by the studied species in *canga* ecosystem is due to variation in functional traits, which reduce hierarchical characteristics and consequently improve interactions, since phenotypic plasticity reduces the intensity of competition and promotes coexistence (Navarro-Cano *et al.*, 2019).

Higher levels of total chlorophyll ensure greater light absorption (Pereira et al., 2018), which in turn is associated with greater CO_2 assimilation and greater ϕ_{PSII} conferring biomass gain and competitive improvement (Carmona *et al.*, 2019). The lack of variation in the total chlorophyll index between seasons for all species belonging to the five groups, as well as the increase or maintenance of ϕ_{PSII} during the rainy season, except for Group 1, explain the positive correlation with the ecological strategy of competition. On the other hand, the highest values of ϕ_{NO} for species in groups 2 and 4 during the dry period are negatively correlated with the competition strategy, since increases in ϕ_{NO} may be related to impaired light utilization and dissipation in the dry season, and may lead to oxidative damage and consequent decrease in the capacity to compete for resources. Among the groups analyzed here, species belonging to groups 5 and 3 were shown to have a higher percentage of competitive strategy. The greater ability to use light energy in the photosynthetic process by the species of these groups is confirmed by the maintenance of chlorophyll indexes during the climatic conditions of the two seasons and by increments or maintenance of ϕ_{PSII} during the rainy season and ϕ_{NPQ} during drought (Tabela S1). The use of energy in thermal dissipation (ϕ_{NPQ}) provides a balance between the absorption and use of light, thereby minimizing the chances of photoxidative damage (Demmig-Adams & Adams, 2006).

With the loss of areas of *cangas* increasing, efforts to restore this ecosystem to preserve biodiversity and ecosystem services are of paramount importance. (Resende *et al.*, 2013; Parr *et al.*, 2014; Bond, 2016). The restoration of these environments impacted by mining activities should be based on the reintroduction of target plant species that are extinct locally or that provide an increase in the abundance of desirable species whose populations have suffered disturbances (Buisson *et al.*, 2019). The efficient selection of species is based on plant characteristics capable of maximizing the establishment of a highly diverse plant community where ecological interactions are also restored (Forup *et al.*, 2008).

The photochemical responses in the use of light energy are related with different adaptive strategies of plant species from *canga* and with climatic seasonality. Most of the studied species were able to dissipate excess energy so as not to experience damage to the photochemical complex through regulated dissipation of non-photochemical energy and through adjustments in the use of light energy for electron transfer in the photochemical process of photosynthesis. In severe environments with multiple stressors, such as the *cangas*, the maintenance or increments in the key parameters F_v/F_m, chlorophyll indices, ϕ_{PSII} and ϕ_{NPQ} during the dry season demonstrates the ability of the strategies of competition and tolerance to stress presented by these species. These physiological mechanisms demonstrate the ability of these species to dissipate excess energy in order to avoid oxidative damage, and are, therefore, promising to be used in projects for the recovery of degraded areas. Because these proved to be resistant even when exposed to severe environmental conditions.

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7. AUTHOR CONTRIBUTIONS

EGP: Funding acquisition and resources, project administration, supervision, conceptualization, formal analysis, writing-reviewing, and editing. COR: conceptualization, investigation, formal analysis, writing-original draft. PAP: investigation, writing-reviewing and editing. MZ and MB: formal analysis, writing-reviewing, and editing. All authors approved the manuscript.

8. COMPETING INTERESTS

The authors declare that they have no conflict of interest. All authors approved the manuscript.

9. DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPLEMENTARY MATERIAL

Plant and soil

Can ecological strategies be explained by photochemical efficiency in ironstone outcrops vegetation?

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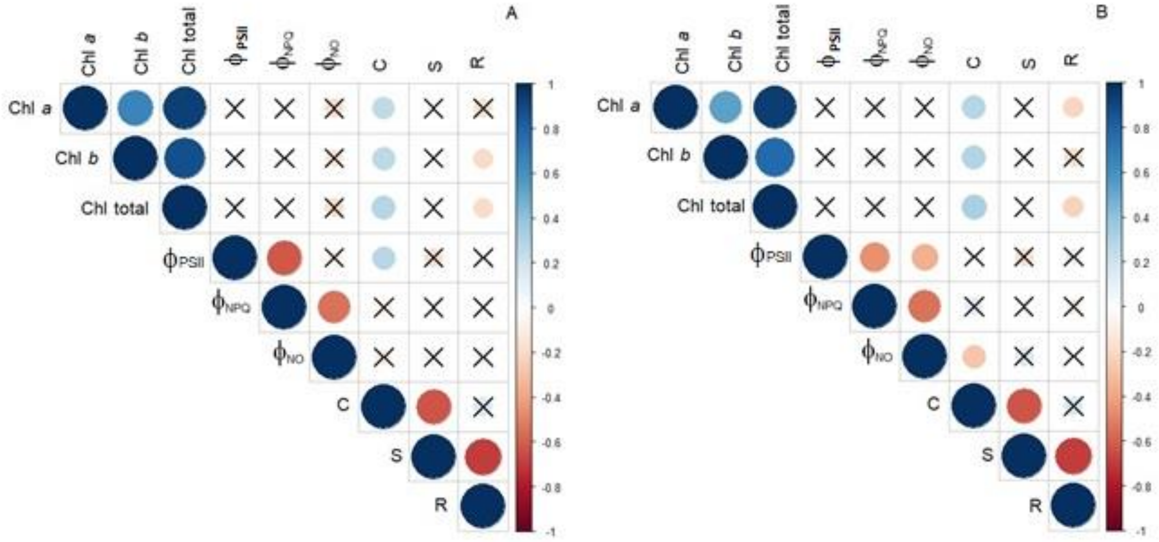

Figure S1. Correlation matrix between ecological strategies (C = competition; S = stress tolerance; R = ruderal) and photosynthetic variables (Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*; Chl total = total chlorophyll; ϕ_{PSII} = effective quantum yield of PSII; ϕ_{NPQ} = quantum yield of regulated dissipation of non-photochemical energy from PSII; and ϕ_{NO} = quantum yield of unregulated dissipation of non-photochemical energy from PSII) during the rainy season (A) and the dry season (B). Blue circles represent positive correlations and red circles negative correlations, while the greater size of the circle and the greater intensity of the color represent stronger correlations. X represents non-significant interactions (>5% probability).

Table S1. Averages (±standard error) for physiological analyses of total chlorophyll (Chl), F_0, F_v/F_m, ϕ_{PSII}, ϕ_{NPQ} and ϕ_{NO} for the different groups during the rainy and dry season.

Variables	**Group 1**		**Group 2**		**Group 3**		**Group 4**		**Group 5**	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
Chl	40.3±2.23Aa	30.0±2.14Aa	42.8±2.10Aa	38.7±2.38Aa	43.4±0.66Aa	38.6±0.69Aa	47.5±0.86Aa	39.2±0.58Aa	43.4±1.17Aa	41.3±1.06Aa
F_0	151.9±1.52Aa	140.5±3.59Aa	155.2±1.85Aa	149.9±1.68Aa	170±1.63Aa	161.4±2.07Aa	154.9±0.35Aa	134.3±8.73Aa	147.2±3.73Aa	145.6±1.29Aa
F_v/F_m	0.775±0.00Aa	0.765±0.01Aa	0.736±0.01Aa	0.671±0.02Aa	0.751±0.00Aa	0.670±0.01Aa	0.775±0.01Aa	0.738±0.01Aa	0.729±0.01Aa	0.721±0.01Aa
ϕ_{PSII}	0.200±0.01Bb	0.310±0.01Aa	0.289±0.01Aab	0.203±0.01Ba	0.340±0.01Aa	0.200±0.01Ba	0.254±0.01Aab	0.287±0.01Aa	0.253±0.02Aab	0.243±0.01Aa
ϕ_{NPQ}	0.398±0,01Aab	0.304±0.01Bab	0.307±0.01Ab	0.316±0.01Aab	0.258±0.01Bb	0.434±0.01Aa	0.452±0.00Aa	0.269±0.01Bb	0.352±0.02Aab	0.336±0.01Aab
ϕ_{NO}	0.403±0.01Aa	0.386±0.01Aa	0.404±0.01Ba	0.481±0.01Aa	0.402±0.01Aa	0.366±0.01Aa	0.294±0.01Ba	0.443±0.01Aa	0.396±0.00Aa	0.421±0.00Aa
ϕ_{NO}/ϕ_{NPQ}	1.25±0.16Ab	1.44±0.13Ab	1.67±0.15Ab	1.75±0.13Aab	3.10±0.42Aa	0.93±0.05Bc	0.69±0.05Bb	1.93±0.21Aa	1.53±0.17Ab	1.47±0.13Ab

Different capital letters indicate a significant difference between seasons within the same group. Different lowercase letters indicate a significant difference among groups within the same season. Averages were compared by the Duncan test at 5% probability.

Table S2. Averages (±standard error) for physiological analyses of NPQ, ETR and PPFD (μmol m^{-2} s^{-1}) for the different species during the rainy and dry season.

	NPQ		**ETR**		**PPFD**	
Species	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**	**Dry**
B. reticularia	1,32±0,2	0,96±0,10	113,25±11,55	130,47±16,97	1815,9±37,85*	1256,0±71,11
L. aurea	1,51±0,25*	0,68±0,10	126,84±10,75	167,76±21,1	1365,4±136,62	1247,0±125,16
M. pogocephala	0,66±0,05	0,81±0,05*	112,31±11,59	135,8±13,12	1317,5±166,27	984,4±85,27
B. malifolia	0,61±0,12	0,74±0,13	191,03±23,82*	92,89±14,21	1438,1±122,16	1142,5±66,22
C. mucronata	0,69±0,1	0,57±0,08	204,92±21,28*	126,69±11,14	1351,7±133,77	1252,0±67,43
E. bimarginata	1,34±0,14	1,09±0,11	141,51±21,6	141,4±13,31	1071,2±189,65	1405,8±84,92
L. pinaster	0,64±0,12	0,43±0,02	87,14±18,74	101,9±10,25	978,3±160,53	1223,2±73,23
V. compacta	1,04±0,26	0,83±0,10	73,77±10,34	58,55±9,74	934,6±153,75	1191,3±65,36
E. macrostachya	0,72±0,12	0,93±0,11	203,91±24,3	161,55±13,03	1237,7±180,5	1296,9±76,13
J. monticola	0,33±0,06	0,95±0,08*	84,29±12,13	95,64±6,72	836,5±159,71	1226,2±42,49
K. coriacea	0,84±0,21	2,00±0,31*	136,95±11,23*	64,34±11,83	1220,7±98,79	1223,0±108,36
M. trianae	1,29±0,24	1,54±0,11	147,13±17,7	121,44±14,43	1231,3±132,88	1354,8±79,73
M. splendens	1,35±0,15	1,57±0,09	111,76±17,97	92,73±10,51	1071,7±176,81	1275,7±93,78
P. mediterranea	0,89±0,28	1,15±0,12	180,8±21,96*	69,57±9,14	1378,6±157,81	1174,0±85,6
V. caruncularis	0,12±0,01	0,99±0,14*	176,97±31,78	111,6±12,27	1093,3±209,34	1065,3±46,33
D. velutinum	1,59±0,17*	0,54±0,08	139,4±24,65	162,02±13,54	1254,7±191,36	1228,1±124,01
D. microphyllus	1,58±0,2*	0,52±0,07	111,95±19,79	148,08±14,21	1409,4±185,43	1532,9±37,51
T. vauthieri	1,83±0,24*	0,9±0,04	137,49±29,58	180,4±13,79	114,6±214,86	1441,9±94,79
C. concolor	1,51±0,3	0,93±0,13	89,15±19,29	92,02±18,26	1360,4±177,82	1252,7±60,95
H. pedunculata	0,96±0,22	0,98±0,17	141,92±20,20	155,47±24,43	1335,0±186,59	1338,6±103,67
L. rigidus	1,18±0,3	1,12±0,21	73,17±12,05	104,12±9,53	1041,1±170,57	1254,7±81,86
P. tomentosa	0,65±0,09	0,61±0,04	206,67±35,09	174,12±20,48	1192,8±172,99	1333,0±85,12

* indicate a significant difference between seasons within the same species. Averages were compared by the Duncan test at 5% probability.

CAPÍTULO 2: Beyond functional traits: how does microenvironment heterogeneity shape the mechanistic responses of plant species in a tropical ironstone outcrop?

Manuscrito preparado de acordo com as normas para submissão ao periódico New Phytologist

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ABSTRACT

- Understanding how climate change influences response and effect traits helps us interpret and predict future environmental scenarios in hyper-diverse nutrient impoverished ecosystems. Our objective is to understand the interaction between the functional and mechanistic responses of plant species native to different microenvironments of banded iron formations (BIFs) under seasonal adjustments.
- Native plant species from two distinct microenvironments of BIFs were selected. One microenvironment is characterized by a large proportion of exposed rock and the other formed by fragmented ironstone. The analyses were carried out during the dry and rainy seasons.
- The lower precipitation resulted in higher concentration of total carbohydrates and higher accumulation of starch in the shoot. The environmental heterogeneity reflected in a great diversity of physiological strategies developed by native plant species. Mechanistic responses and functional adjustments of native plant species highlighted the differences in microhabitats from the whole ecosystem.
- The abiotic characteristics of each microenvironment were determinant for the diversity of functional and mechanistic responses of the analyzed plant species, which varied according to the heterogeneity of habitats. Some key species are most relevant to the ecosystem response and showed the resilient of BIFs to climatic seasonality.

Keywords: Banded Iron Formations, *canga*, community dynamics, microhabitats, nurse species, response-effect framework.

1. INTRODUCTION

Environmental changes affect the functioning of ecosystems through different responses of individuals belonging to plant populations (Suding *et al.*, 2008; Bu *et al.*, 2019). Understanding how plant community functional traits are affected by abiotic factors increases the predictive ability to interpret how global changes will influence ecosystems (Suding *et al.*, 2008; Kearney *et al.*, 2021). However, little is known about how the dynamics of mechanistic responses in plants influence the functional adjustments of the ecosystem in a systemic way.

The ecosystem is affected by changes in community dynamics, these are formed from abiotic and biotic filters. Filters determine which species and traits are able to resist each specific habitat (Lavorel & Garnier, 2002). Trait-based studies have sought simultaneously explaining the responses of individual plants to biotic and abiotic agents, as well as their effect on the ecosystem (Lavorel & Garnier, 2002). Species characteristics provide direct information about the strategies they use to grow and reproduce. Traits are the measurable biological characteristics such as morphology, physiology and phenology that shape their performance in the ecosystem (Violle *et al.*, 2007; Cadotte *et al.*, 2011; Gallagher *et al.*, 2021). As such, understanding the systemic functioning of a community involves the integration of response and effect traits. The first is determined by the community's response to a given change and the second corresponds to how the changed community will affect ecosystem services (Suding *et al.*, 2008). However, plant responses to environmental factors can be analyzed using traits that are more practical and easy to measure, but which are not necessarily related to a specific functional mechanism; or through more complex traits, less accessible but with an impact directly related to plant functionality (Hodgson *et al.*, 1999) specially in hyper-diverse ecosystems.

Most trait-based studies are focused on leaf morphological characteristics, specific leaf area (SLA), or its reciprocal, leaf mass per area (LMA), as well as leaf nutrient concentration, height and leaf morphology (Westerband *et al.*, 2021). The LMA is an important leaf functional trait (Hikosaka, 2004) commonly associated with photosynthetic traits. Species with a high LMA have expensive and long-lived leaves, with thicker or denser leaf tissues, with a low concentration of nutrients and a low photosynthetic and respiration rate (Wright *et al.*, 2004). Plant species that have coriaceous leaves and a high LMA are commonly found in arid and semi-arid regions (Niinemets, 2001) and in ecosystems with nutrient-poor soils, as observed by Mariano *et al.* (2021) when comparing the hyper-diverse *campo rupestre* with other Brazilian biomes. All these parameters are important and express considerable variations in the plant community. However, in addition to morphofunctional traits, other factors, such as photochemistry, antioxidants and carbohydrate partition, are also important to be measured. They expand knowledge about the ecosystem and are related plant responses to environmental variations. When compared to works involving leaf characteristics, fewer studies report hydraulic, root or reproductive characteristics. This scarcity of data undermines the understanding of covariance at the species level that directly influences ecosystem processes (Westerband *et al.*, 2021). However, it is not yet known how morphofunctional traits relate to others, especially in hyper-diverse environments. The microenvironmental heterogeneity present in iron rich rocky outcrops plays an important role in determining local diversity as it favors the coexistence of plant species and contributes to the high species richness (Badia *et al.*, 2021).

Iron formations known worldwide as banded iron formations (BIFs) consist of finely interstratified chemical sediments rich in iron oxides, carbonates and/or silicates that are precursor deposits of the largest sources of iron ore in the world. (Pat Shanks, 2014). BIFs are currently responsible for supplying 50% of the world's iron ore (Ramanaidou & Wells, 2014). They harbor high levels of floristic biodiversity determined by edaphic isolations, topographic relief and physical restrictions of the extremely shallow soil (Markey & Dillon, 2008; Gibson *et al.*, 2010). BIFs are functionally different from the surrounding landscape, harboring significantly different proportions of species with different calcification and nutrient acquisition strategies (Gibson *et al.*, 2010; Cross & Lambers, 2021).

The ferruginous *campo rupestre*, known as *canga*, is mainly composed of detrital fragments of hematite from weathered BIF, cemented together by goethite (Levett *et al.*, 2021). They are extremely heterogeneous ecosystems with a great diversity of microedaphic environments and exceptionally rich and highly endemic vegetation (Fernandes et al., 2018; Silveira et al., 2016). The flora is under constant selective pressure from highly weathered and nutrient-poor soils, high solar radiation, with a high rate of evapotranspiration and marked seasonality of rainfall (Giulietti *et al.*, 1987; Rapini *et al.*, 2008; Fernandes, 2016; Oliveira *et al.*, 2016; Abrahão *et al.*, 2019). In this way, macro and microenvironmental variations expose the native population to a mosaic of resource availability, different abiotic conditions and a variety of biotic interactions (Denney *et al.*, 2020). *Cangas* can be differentiated into *couraçada canga* and *nodular canga*. Habitats that are found on *couraçada canga* have a large proportion of exposed rock resulting in warmer soils and less physical space for plant establishment (Jacobi & Carmo, 2011; Carmo & Jacobi, 2016; Schaefer *et al.*, 2016b). The *nodular canga* is characterized by fragmented iron rocks, which allows the development of a greater number of species (Carmo & Jacobi, 2016).

The ability to adapt to these strong environmental filters as well as to small specific variations of its microhabitat provided the formation of vegetation with different adaptive strategies specialized to these conditions and determined the patterns of biodiversity found in this ecosystem (Zappi *et al.*, 2019; Fernandes *et al.*, 2020). Unfortunately, biodiversity and ecosystem services provided by *cangas* have been increasingly threatened, mainly due to the construction of highways and mining activity (Barbosa et al. 2010; Fernandes et al. 2016b; Fernandes e Ribeiro 2017; Pena et al. 2017). The floristic composition of this ecosystem is closely associated with the topography and substrate, being vulnerable to the impacts of mineral exploitation (Markey & Dillon, 2008). A great potential gain is achieved with the integration of species traits within the scope of conservation science. Once studied, species traits can be used in practice in conservation projects, such as reserve planning, threat mitigation and ecosystem restoration (Gallagher *et al.*, 2021). Traits may be able to accurately predict species responses when exposed to environmental changes and extinction risks (O'Grady *et al.*, 2004; Chichorro *et al.*, 2019; Carmona *et al.*, 2020).

In this work, the *canga* was used as a model of BIF ecosystem to understand how seasonal adjustments in the mechanistic and functional attributes of response and effect of the native plant community interact and guarantee the resilience of plants in these environments. We expect that the resilient responses of *canga* ecosystem to seasonal environmental characteristics vary according to the level of microenvironmental heterogeneity. Representative species from *couraçada canga* are expected to present more efficient mechanisms capable of minimizing the damage caused by seasonal water scarcity and nutritional deficit, through the reduction of water potential and consequent accumulation of non-structural carbohydrates and proline. When subjected to stresses that cause dehydration, cells perform osmotic adjustments by increasing solute concentration, ensuring balance in the cytosol (Kishor *et al.*, 2005). In species from the *nodular canga*, we expect a smaller reduction in the maximum quantum efficiency of PSII, an increase in the levels of antioxidant enzymes and in the absorption of essential nutrients. We expect that the *canga* vegetation, adapted to different microhabitats, will present different strategies depending on the peculiar characteristics of the soil in each microenvironment. Our objective is to understand the interaction between the functional and mechanistic responses of plant species from banded iron formations (BIFs) that shapes the ecosystem’s microenvironmental heterogeneity under seasonal adjustments.

2. MATERIALS AND METHODS

2.1 Study area

The study area is located at the northern end of Serra da Moeda, locally known as Serra da Calçada (20°05’55.2”S e 43°58’ 58.2”W), municipality of Brumadinho, Minas Gerais. As it is located inside the Quadrilátero Ferrífero in Minas Gerais, Serra da Calçada is surrounded by mining companies and areas impacted by the extraction of iron ore. Among the different phytophysiognomies found in the area, the analyses were carried out with native plant species of *couraçada* and *nodular canga*.

2.2 Plant material

Initially, field surveys were carried out in order to select representative plots of the local vegetation for the study. Ten plots of 10 m^2 were established, 10 m apart from each other (Gomes *et al.*, 2021a; Rios *et al.*, 2022). The plots were demarcated along the *couraçada* and *nodular canga* vegetation. The species were selected based on the work developed by Rios et al. (2022), in which 22 species of *canga* were separated into 5 distinct groups according to their ability to use and dissipate excess light energy.

Group 1 - characterized by increases in effective quantum yield of PSII (ϕ_{PSII}) and decreases in quantum yield of regulated dissipation of non-photochemical energy from PSII (ϕ_{NPQ}); Group 2 - characterized by decreases in ϕ_{PSII} and maintenance of ϕ_{NPQ} during the seasons; Group 3 - characterized by decreases in ϕ_{PSII} and increases in ϕ_{NPQ} in the dry season; Group 4 - characterized by no significant difference for ϕ_{PSII} and decreases in ϕ_{NPQ} in the dry season; and Group 5 - characterized by no differences in any of the variables of the use of light energy between dry and wet seasons. From these groups, ten representative species of the *canga* vegetation, at a functional level and in response to the environment, were selected. The selected individuals were demarcated and georeferenced, using the same individuals during the dry and rainy seasons (Fig. 1).

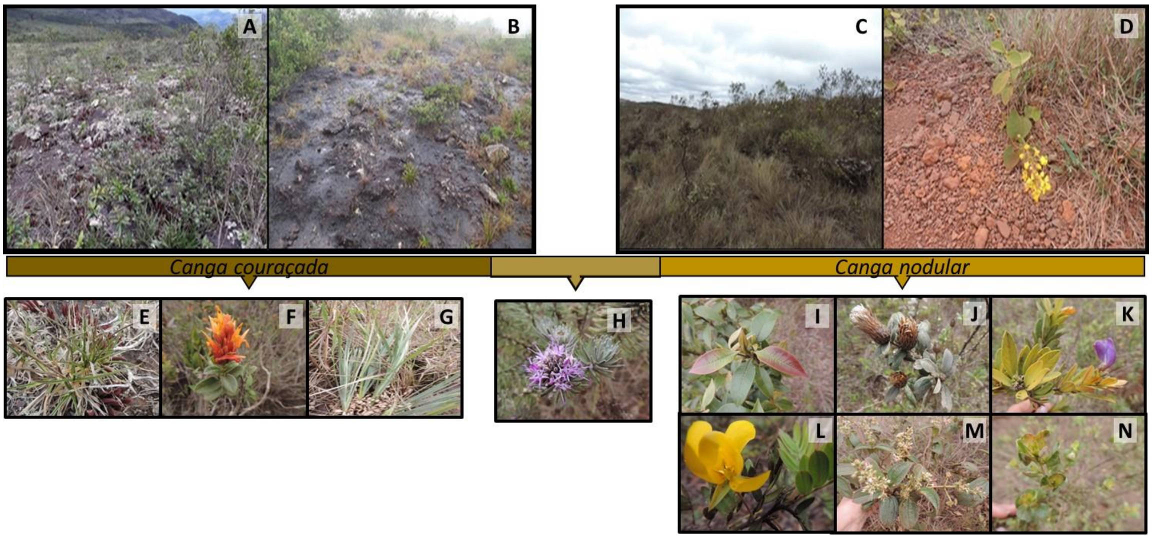

Figure 1. General aspects of the ferruginous *campo rupestre*: *canga couraçada* (A-B) and *nodular canga* (C-D) and its species studied. *Vellozia caruncularis* Mart. ex Seub.(E), *Justicia monticola* (Nees) Profice (F) and *Lagenocarpus rigidus* (Kunth) Nees (G) located in the *canga couraçada* microhabitat*; Lychnophora pinaster* Mart. (H), common in both microhabitats; and *Myrcia splendens* (Sw.) DC. (I), *Dasyphyllum velutinum* (Baker) Cabrera (J), *Periandra mediterranea* (Vell.) Taub. (K), *Chamaecrista mucronata* (Spreng.) H.S.Irwin & Barneby (L), *Leandra aurea* (Cham.) Cogn.(M) and *Baccharis reticularia* DC(N) located in the *nodular canga* microhabitat.

2.3 Climate

The local climate has low annual thermal amplitude, with rainy summers and dry winters, being classified as Aw (tropical, with dry winter), according to Köppen and Geiger (Climate-data.org, 2020). The *Serra da Calçada* presents an average annual temperature of 20.7°C and rainfall of 2041.6 mm (INMET, 2020).

The volumetric soil water content (measured with a TDR 100 probe, Fieldscout, USA) during the rainy and dry seasons was 13.4% and 6.5%, respectively. The portable probe used for TDR measurement consisted of a pair of parallel conical stainless steel rods, 3.3 cm apart. Default mode has been selected for measurements. The ground was drilled with the rods and they became a parallel transmission line pair. The length of the rods used for the analysis was 7.6 cm. Measurements were performed at 1 hour intervals for 5 days.

The average soil and air temperatures during the rainy season were 30.7ºC and 29.1ºC for *nodular canga* and 20.5ºC and 22.1ºC for *couraçada canga*, respectively. In the dry period, the average temperature of the soil was 23.9ºC and the air temperature was 17.0ºC for *nodular canga* and 26.6ºC and 18.3ºC for *couraçada canga*, respectively. The analyses were performed at each day of measurements with four type K thermocouples coupled to a model 1384 data logger, Omega, Taiwan. Two being arranged under the ground and the other two in the air. Measurements were performed at 1 hour intervals for 5 days.

The maximum photosynthetically active photon flux density (PPFD) during the rainy season in the *nodular canga* was 1420 μmol m^{-2} s^{-1} and in the *couraçada canga* 1067 μmol m^{-2} s^{-1}. In the dry season, it was 1029 μmol m^{-2} s^{-1} for *nodular canga* and 1062 μmol m^{-2} s^{-1} for *couraçada canga* (both measured with open sky, without clouds using an HD 2102.2 radiometer, Delta OHM, Italy). Measurements were taken at 1 hour intervals for 5 days in both seasons and habitat.

2.4 Soil

A subset of each sample (ca 500g) was analyzed for substrate chemical characteristics, P, K, Ca, Mg, Fe, Mn, Al, pH, H, potential acidity, sum of exchangeable bases, effective cation exchange capacity, cation exchange capacity at pH 7, base saturation index, aluminum saturation index and soil organic matter, where protocols were followed according to Da Silva *et al.* (1998).

The water content of soil samples in the dry and rainy seasons was determined on a gravimetric basis, calculated as the difference between wet weight (WW) and dry weight (DW) using the following equation: $SWC (\%) = 100 \times [(WW / DW) / DW]$.

2.5 Evaluation of water status traits

Relative leaf water content (RWC) was measured at approximately noon on the first fully expanded leaf. Three 1 cm diameter disks were removed from each leaf sample, and the fresh weight (FW) was measured immediately. Turgid weight (TP) was determined after rehydration of leaf discs immersed in distilled water, and kept at 4ºC for 24 hours. The dry weight (DW) was determined by placing the leaf discs in an oven at 65ºC to reach a constant weight. The RWC was calculated as the difference between the different weights of the leaves, using the following equation: $RWC (\%) = 100 \times [(FW - DW) / (TW - DW)]$ according to (Barrs & Weatherley, 1962).

From the analysis of the functional traits, the leaf water content was calculated $(LWC) = ((LFW-LDW)/LFW)*100$.

The leaf water potential of 10 individuals of each species was determined during predawn, using a pressure chamber model 1505D-EXP (PMS Instrument Company, USA) during the dry and rainy seasons.

The analyses of the proline concentration of the leaves were carried out in both seasons of the year. Free proline was extracted by the sulfosalicylic acid method and determined in a spectrophotometer at 520 nm, according to Bates *et al.* (1973).

2.6 Assessment of nutrient concentration in the root and shoot of plants

During the dry and rainy seasons, in the field, roots and aerial parts were collected.

These were taken to the laboratory where they were washed with running water and deionized water. The roots were also washed with a DCB solution (sodium dithionite, sodium citrate and sodium bicarbonate), which ensures the removal of iron and other metals attached to the external portion of the root (Pereira *et al.*, 2014). Then, the material was dried at 75°C for 72 hours. Subsequently, nitrogen (N) concentrations were determined using the Kjeldahl method by atomic absorption spectrophotometry (Tedesco *et al.*, 1995; de Oliveira, 1997). The analyses of P, Fe and Mn of the plant parts were performed according to Malavolta *et al.* (1997) in atomic absorption spectrophotometer. The N:P ratio was also calculated.

2.7 Evaluation of morphofunctional traits in canga ecossystem

Analyses were performed with mature and apparently healthy individuals, 10 replicates per species during the rainy and dry seasons. Whole plant samples were collected in the field, avoiding the period of the strongest solar radiation (11:00-15:00). They were then placed in paper bags, saturated with water through a spray bottle, sealed in plastic bags and stored in an insulating box to be transported to the laboratory, 76 km away. In the laboratory, the collected samples were kept in a refrigerator at 4°C for at least 12 hours until complete turgor. Measurements were performed on three leaves (including the petiole) per individual within 24 hours after field collection. Only fully expanded leaves were used, with no signs of pathogens, herbivory or malformation (Cornelissen *et al.*, 2003). Leaf area was obtained using a handheld scanner and Image J software. Curved leaves were cut into smaller pieces and positioned so that the entire leaf surface could be scanned. The weight of the dried leaves was determined with an analytical balance (accurate to 0.01 mg) after 96 hours of drying in an oven at 60ºC. With the analysis of leaf area (LA), leaf fresh weight (LFW) and leaf dry weight (Blanchar *et al.*) the other morphofunctional traits were calculated. Leaf succulence index (LSI) = (LFW-LDW)/(LA/10), leaf mass per area (LMA) = LDW/(LA/1000), leaf dry matter content (LDMC) = LDW/LFW*100 and specific leaf area (SLA) = LA/LDW.

2.8 Evaluation of carbohydrate partitioning traits

Carbohydrates were determined in fully expanded leaves and roots, both collected during the dry and rainy seasons. Samples of 0.5g of the dried and ground plant material were homogenized in a mortar with 5 ml of 80% ethyl alcohol, at boiling point, to carry out the extraction, this process was carried out twice.

The pellet was used for the determination of starch, through extraction with sulfuric acid and perchloric acid and determination in a spectrophotometer at 630 nm second Hodge and Hofreiter (1962). And the supernatant of the extraction was used for the quantification of total soluble sugars with phenol sulfuric acid in a spectrophotometer at 490nm according to Dubois *et al.* (1956).

2.9 Evaluation of pigment index and photochemical traits

Photosynthetic analyses were carried out during the dry and rainy seasons, always on the same individuals previously identified.

The chlorophyll content index was measured with the ClorofiLOG portable meter (CFL1030, Falker, Brazil). Measurements were taken three times on the median part of the fully expanded leaf, and the mean of the three measurements was calculated as a replicate.

It was also evaluated with the aid of DUALEX Scientific (FORCE-A, Orsay, France), the NBI® (Nitrogen Balance Index), which is the Chlorophyll / Flavonoids ratio (related to the allocation of Nitrogen / Carbon) that can be used as a proxy for the nitrogen status of plants (Casa *et al.*, 2015; Agati *et al.*, 2016). The NBI was not calculated for the species *B. reticularia*, *L. pinaster* e *V. caruncularis* due to the leaf area of these species being smaller than necessary for reading.

Chlorophyll *a* fluorescence was measured on the same leaves used for chlorophyll measurements, using a Mini-PAM fluorometer (Heinz Walz, Effeltrich, Germany). The maximum quantum yield of photosystem II (PSII) (F_v/F_m) was determined in dark-acclimated leaves for at least 30 minutes (Genty *et al.*, 1989). Then, rapid light response curves were obtained by using the Mini-PAM fluorometer to determine the instantaneous capacity of photosynthetic response to excessive solar radiation. At first, the leaves were dark-acclimated for 30 min to determine F_0 and F_m. Posteriorly, the leaves were exposed to the light conditions where plants grown for at least 10 min and the light response curve was started. The leaves were exposed to increasing actinic light intensities (0–2000 μmol m^{-2} s^{-1}) during nine 10-s intervals, and the steady-state fluorescence (F_s) and maximum light-adapted fluorescence (F_m') were measured. At each light intensity, we measured the effective quantum yield of PSII (ϕ_{PSII}); apparent electron transport rates through PSII (Manzoni *et al.*); photochemical (qP) and non-photochemical quenching (NPQ). The ETR_{max} e NPQ_{max} variables were determined at a maximum photosynthetic photon flux density (PPFD) of 2000 μmol m^{-2} s^{-1}.

2.10 Evaluation of antioxidant traits

The extent of lipid peroxidation was measured through malonaldehyde (MDA) concentrations determined with samples of fully expanded leaves and roots, according to Hodges *et al.* (1999). A portion of the same samples was used for determinations of H_2O_2 contents according to Velikova *et al.* (2000). The activities of superoxide dismutase (SOD;

EC 1.15.1.1) (Beauchamp & Fridovich, 1971), catalase (CAT; EC 1.11.1.6) (Havir & McHale, 1987), and ascorbate peroxidase (APX; EC 1.11.1.11) (Nakano & Asada, 1981) were also evaluated in leaf samples. Total protein was determined in crude extracts, according to Bradford (1976), using bovine serum albumin (BSA) as a standard. The analyses were carried out in both seasons of the year.

2.11 Experimental design and statistical analysis

Initially, the responses of the species at the ecosystem level were analyzed, considering the seasons throughout the year, dry and rainy season. A simple ANOVA was performed, and the ExpDes.pt package was used. Next, the different microenvironments present in the canga were considered. A 2x2 factorial scheme was adopted, with two seasons (dry and rainy) and two microhabitats (*couraçada canga* and *nodular canga*). A factorial analysis was performed, and the Car and ExpDes.pt packages were used. The R program, version 3.6.2 was used to calculate mean and standard deviation values. The Shapiro-Wilk test was applied to check the occurrence of normal distribution in each data set. For normally-distributed data sets, the Bartlett test was performed to verify variance homogeneity. Data sets significantly deviating from normality were log-transformed. Analysis of variance (one-way ANOVA) and a-posteriori Tukey’s tests were applied to compare seasons means. A repeated-measures ANOVA and a-posteriori Tukey’s tests were also used to compare microhabitats and seasons.

3. RESULTS

3.1 Seasonal water deficit causes an increase in oxidative damage and a reduction in the absorption of nutrients in canga species at ecosystem level

In a broader aspect, the native plant species of the analyzed BIF ecosystems showed different responses to the climatic seasonality to which they are exposed throughout the year. Climatic seasonality resulted in higher soil moisture during the rainy season as well as higher water potential, relative water content in leaves and also a rise in the proline content. The concentration of P in the roots and Mn in the shoot and the N:P ratios in *canga* species were the same in both seasons, but the accumulation of N, Fe and Mn in the roots and P and N in the shoot was higher in *canga* vegetation during the rainy season. The higher concentration of N in plant tissues resulted in a higher ratio of chlorophyll/flavonoids as observed by the NBI index. Only the accumulation of Fe in the shoot of *canga* species was greater during the dry season (Fig. 2 and Table S1). Regarding the morphofunctinal traits, a higher LMA and LSI were observed during the dry season in native BIF vegetation, which resulted in higher SLA observed in the rainy season. However, the climatic seasonality did not affect the morphofunctional traits LA, LFW, LDW and LDMC.

During the period of soil water stress, the native species from the areas of BIFs presented an adaptive strategy, increasing the concentration of total carbohydrates and starch in the shoot. In the roots, starch remained unchanged and total carbohydrates were higher during the rainy season. The water deficit resulted in lower levels of total chlorophyll which consequently resulted in a lower maximum quantum efficiency of PSII and lower electron transport rate. During the dry period, higher quenching of energy dissipation in a regulated manner was also observed, and higher production of H_2O_2 in the shoot and higher lipid peroxidation in the roots. However, the oxidative damage resulting from the dry period did not provide an increase in the activity of antioxidative enzymes (Table S1 and Fig. 2).

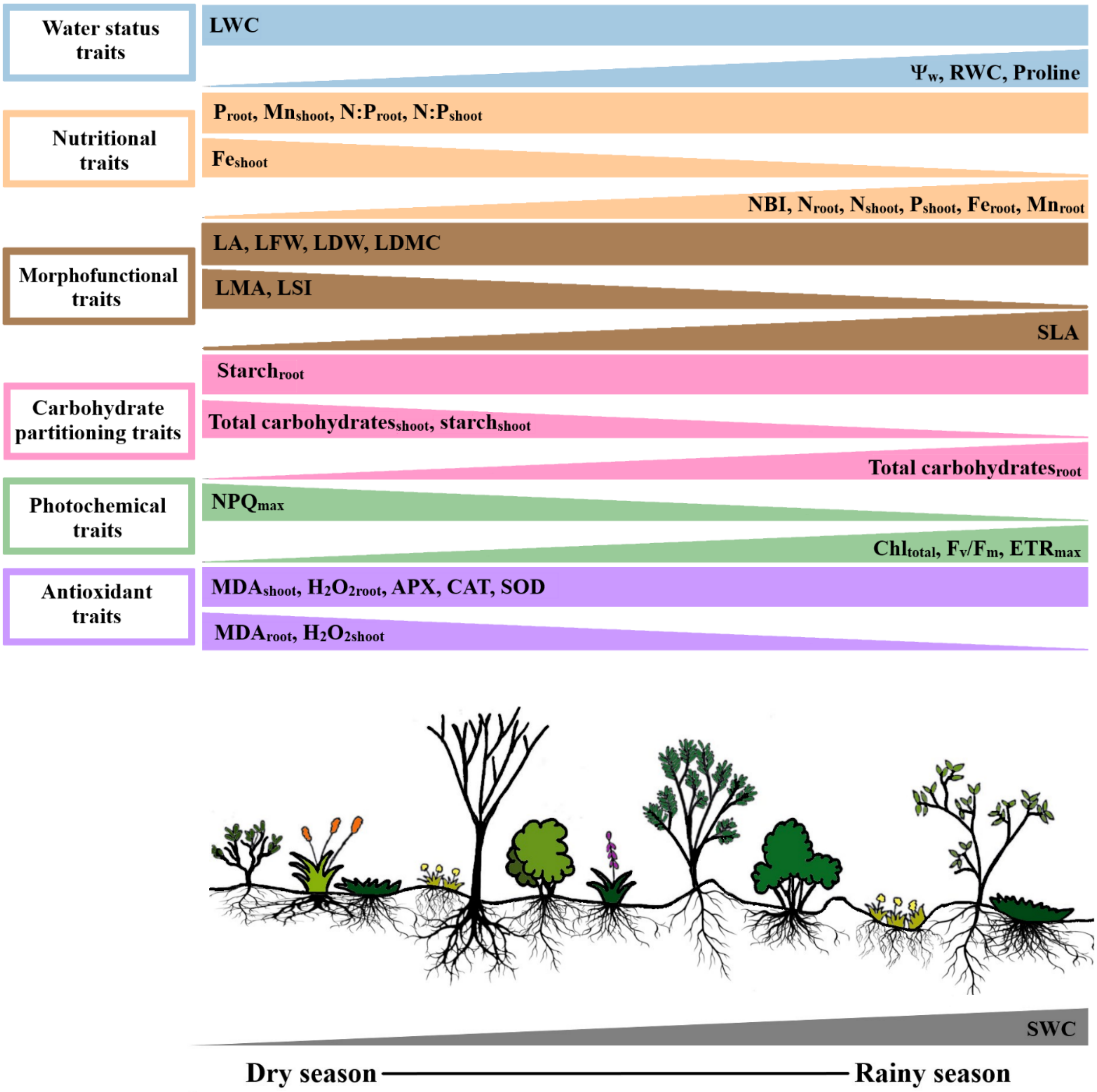

Figure 2. Water status traits (leaf water content – LWC, water potential - Ψw, relative water content in the leaf – RWC), nutritional traits (nitrogen – N, phosphorus - P, iron - Fe, manganese – Mn present in the roots and aerial, NBI - Nitrogen Balance Index), morphofunctional traits (leaf area - LA, leaf fresh weight – LFW, leaf dry weight – LDW, leaf mass per area – LMA, leaf succulence index – LSI, leaf dry matter content – LDMC and specific leaf area – SLA), carbohydrate partitioning traits, photochemical traits (maximum quantum efficiency of the PSII - Fv/Fm, maximum non-photochemical extinction NPQmax, total chlorophyll – Chl total, apparent rate of maximum electron transport - ETRmax) and antioxidant traits (malonaldehyde concentration – MDA and hydrogen peroxide - H2O2 in roots and shoots, activity of the enzymes superoxide dismutase – SOD, catalase – CAT and ascorbate peroxidase – APX) of native species of the *canga* ecosystem exposed to natural climatic seasonal conditions.

3.2 Species adapted to different microhabitats presented different responses to seasonal water deficit

The *canga* areas are extremely heterogeneous ecosystems with a great diversity of microedaphic environments that are decisive for the ecophysiological responses of plant species. In this work we consider two distinct microenvironments, the *nodular canga* and the *couraçada canga* that presented a reduction in SWC in the dry season (Fig.3a). In both microhabitats, plant species also showed more negative water potential in the dry season (Fig. 3b), indicating that even with the lowest water potential observed for the *couraçada canga* plants in the dry season (Fig. 3b and Fig S1a), this did not result in a very severe water deficit for the plants. Only for the *couraçada canga* plants a lower RWC was observed during the dry season (Fig. 3c). Plants in the *nodular canga* microenvironment showed differences in proline levels between seasons (Fig. 3d). *L. pinaster*, a species present in both microhabitats, but predominates in the *couraçada canga*, was the only species that presented a water deficit resistance strategy based on increases in proline values during the dry season (Fig. S1b and also see Fig. 6c). During the rainy season, *B. reticularia*, *L. aurea*, *M. splendens* and *V. caruncularis* also showed an increase in proline accumulation (Fig. S1b).

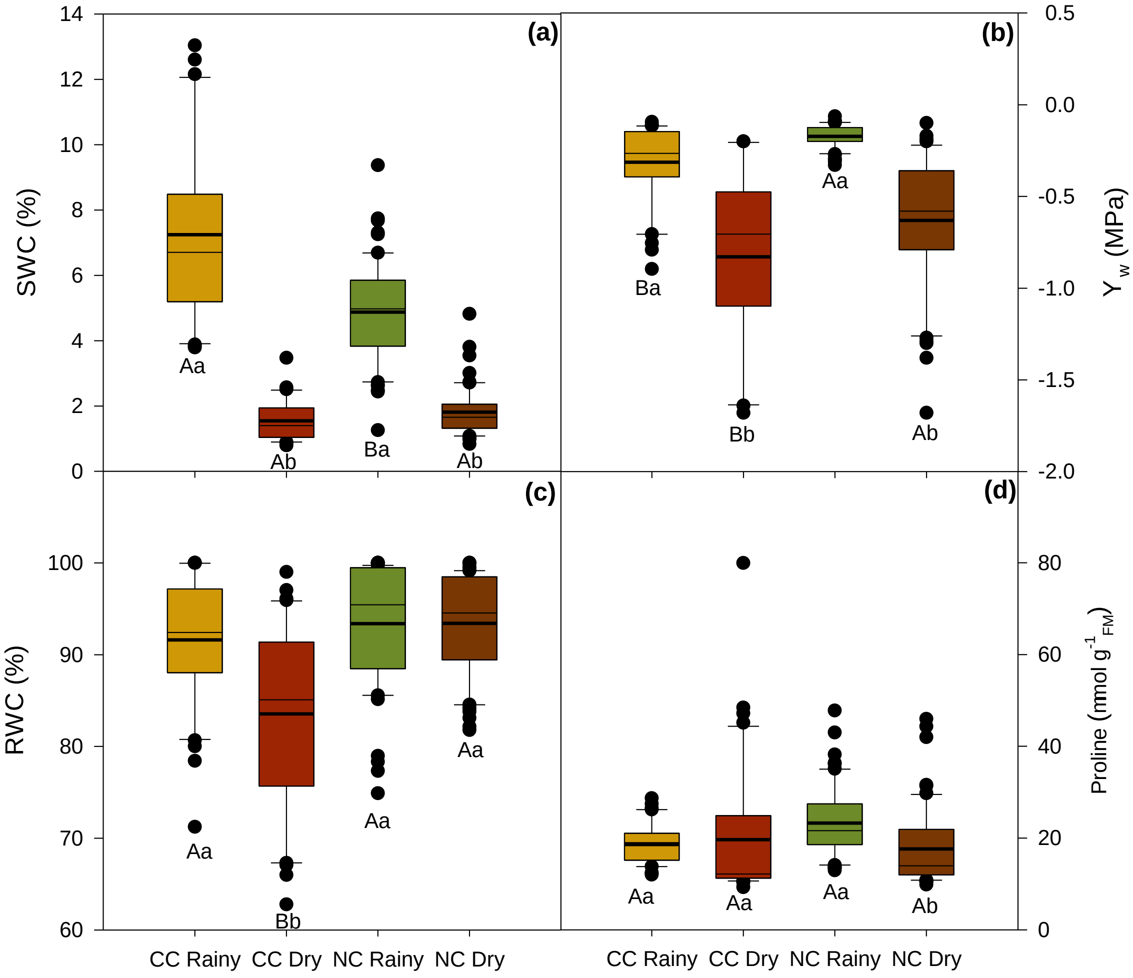

Figure 3: Soil water content – SWC (a), water potential - Ψ_w (b), relative water content in the leaf - RWC (c) and proline concentration (d) in native plants of the *couraçada* (CC) and *nodular canga* (NC) microhabitats exposed to the rainy and dry season. Values in boxplots are means (bold line), median (iner line) and the points represent each outliers of the data range. Means with different capital letters represent difference between areas (*couraçada* and *nodular*) and small letters difference between seasons (rainy and dry) according to Tukey's test at 5% probability.

The patterns visualized in the field and the distinct physical characteristics of the substrate demonstrate that the analyzed species are found in different microhabitats dispersed along the outcrop of ferruginous rock. In addition to the distinct physical characteristics of the substrate, the *nodular canga* habitats also present higher potential acidity, cation exchange capacity and concentration of organic matter. Because it is an old and highly weathered ecosystem, the concentration of P present in all analyzed niches was extremely low (Table 1). Among the native species of each of the microhabitats *J. monticola* and *M. splendens* were the most determinant species for the soil chemical characteristics of the *couraçada* and *nodular canga*, respectively (Table S2).

Table 1. Chemical characteristics of the soil from *couraçada* and *nodular canga*.

Soil traits	***Couraçada***	***Nodular***
P (mg/dm^3)	4.04±0.47	4.79±0.23
K (mg/dm^3)	58.22±2.93	63.30±1.52
Ca (cmolc/dm^3)	2.41±0.17	2.73±0.17
Mg (cmolc/dm^3)	0.58±0.05	0.58±0.04
Fe (mg/dm^3)	183.28±11.44	202.98±6.80
Mn (mg/dm^3)	85.36±9.34	80.13±6.54
Al (cmolc/dm^3)	0.21±0.02	0.86±0.55
pH H_2O	4.90±0.06	4.87±0.03
pH $CaCl_2$	4.01±0.05	4.00±0.03
H (cmolc/dm^3)	9.04±0.57	10.76±0.39*
H+Al (cmolc/dm^3)	9.25±0.59	11.06±0.40*
SB (cmolc/dm^3)	3.13±0.22	3.48±0.20
t (cmolc/dm^3)	3.34±0.21	3.78±0.19
T (cmolc/dm^3)	12.39±0.48	14.54±0.41*
V (%)	27.62±2.43	24.28±1.29
m (%)	8.00±1.13	9.86±0.95
OM (dag/Kg)	7.51±0.29	8.91±0.27*

Soil analyses were performed with composite samples from the whole study area (n = 100). Subtitle: H + Al= potential acidity; SB= sum of exchangeable bases; t= Effective cation exchange capacity; T= Cation exchange capacity at pH 7; V= Base saturation index; m= aluminum saturation index; OM= soil organic matter. Mean ± standard error. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

The nutritional traits for both microenvironments showed distinct responses to the ecosystem as there was no difference between the dry and rainy seasons when analyzing the concentration of N and Mn present in the roots (Table 2). Despite no significant difference in root P concentration (Fig. 4a), the species found in the *couraçada canga* accumulated higher concentrations of P in the shoot and Fe in the roots and shoots (Fig.4b and 4c). On the other hand, the N:P ratio in the aerial part was lower in *couraçada canga* plants only when associated with water deficit. There was also a reduction in the *nodular canga* for the rainy season. (Table 2). However, the highest concentration of Fe present in the plant tissues of the *couraçada canga* species, as well as the concentration of P in the shoot, was mainly due to the responses of the species *J. monticola* and *V. caruncularis* (Fig. S2d-f and see Fig. 5a and 6d). The species *J. monticola* showed accumulation of N, Mn and P in the roots and P, Fe and N in the shoot above the ecosystem average for both seasons. The same occurred for the concentration of Fe and Mn present in the shoot of the species *L. pinaster* (Fig S2f-h and see Fig. 6c). In the *nodular canga*, all species presented Fe concentration in the roots below the ecosystem average. On the other hand, *M. splendens* and *L. aurea* showed a high concentration of Mn in the roots (Fig. S2g and see Fig. 5d-e). The same occurred for the accumulation of N and consequently the N:P ratio in the roots of *P. mediterranea* (Fig. S2a-i and Fig. 5f).

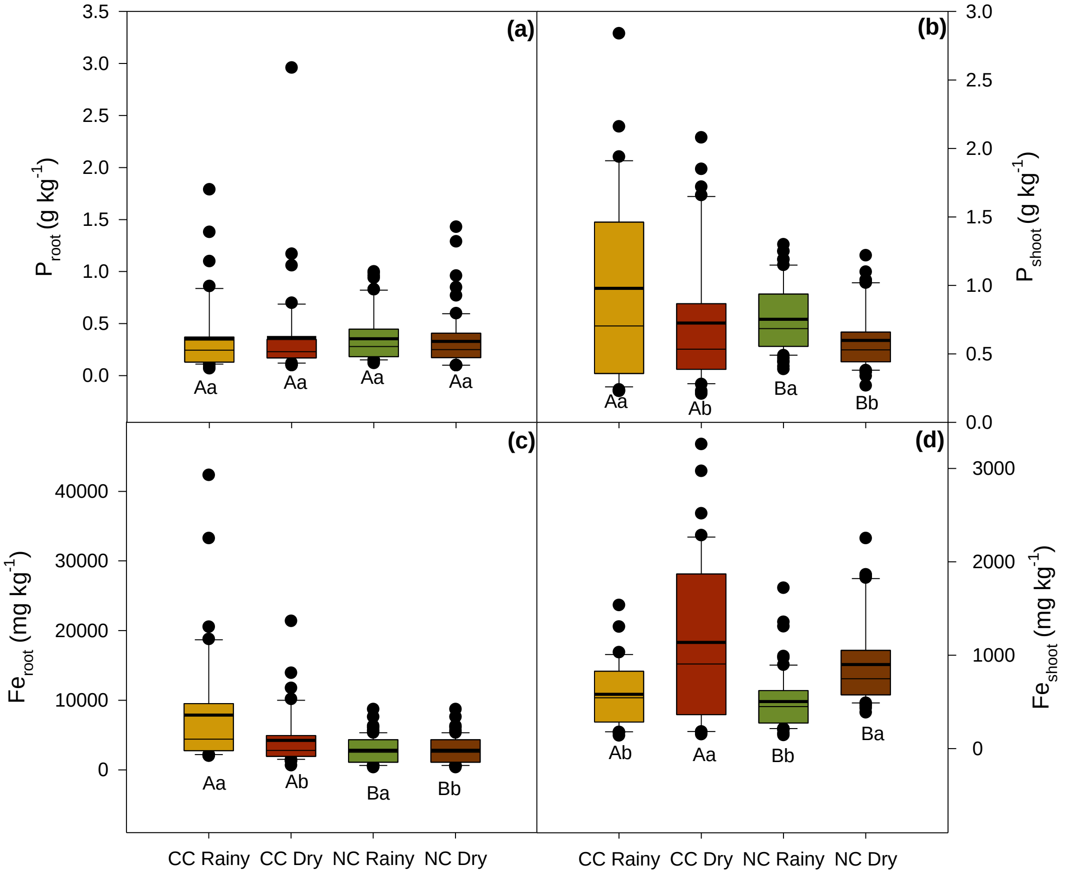

Figure 4. Phosphorus (a and b) and iron (c and d) present in roots (a and c) and shoots (b and d) in native plants of the *couraçada* (CC) and *nodular canga* (NC) microhabitats exposed to the rainy and dry season. Values in boxplots are means (bold line), median (iner line) and the points represent each outliers of the data range. Means with different capital letters represent difference between areas (*couraçada* and *nodular*) and small letters difference between seasons (rainy and dry) according to Tukey's test at 5% probability. Statistical analyses were performed separately for root and shoot.

Regarding the morphofunctional traits, LA, LFW and LDW there was no difference between microhabitats and climatic seasonality (Table 2). However, the highest values of LA, LFW and LDW were observed for *L. rigidus* and *L. aurea* and the lowest for *B. reticularia* and *L. pinaster*. The difference between seasons was observed only for the LA of *L. aurea* and *J. monticola* and LDW of *L. aurea* (Fig. S3a-c and Fig. 5d, Fig. 6a). Among the calculated parameters, only LMA, LSI and SLA showed differences between habitats and seasons. LMA and LSI were higher for the species growing in the *couraçada canga* and during the dry season. Increases in SLA values were observed for the *nodular canga* species during the rainy season (Table 2), mainly for the species *B. reticularia* and *D. velutinum* (Fig.S4d). Although there is no difference between the microhabitats.

The concentration of total carbohydrates and starch present in the roots and shoots reflects the ecosystem responses. Only the concentration of total carbohydrates present in the shoot was different, in which the plant species in the *couraçada canga* showed no difference between the seasons and in the *nodular canga* a lower concentration was observed during the dry season (Table 2). Regardless of the niche in which they are found, all species showed increases in the concentration of total carbohydrates in the root during the rainy season (Fig. S5a). This higher concentration of total carbohydrates resulted in a greater accumulation of starch in the shoot during the period of water deficit. However, the starch concentration in the roots showed no difference between the seasons, but a smaller increase was observed in the *couraçada canga* plants (Table 2). Among the *couraçada canga* species, only *J. monticola* did not present increases in the starch concentration in the shoot during the dry season, whereas in the *nodular canga B. reticularia*, *L. aurea*, *L. pinaster* and *P. mediterranea* were determinant for this increase in starch on shoots during water shortage (Fig. S5d). The accumulation of starch in the roots of the *nodular canga* plants was higher mainly due to the increments of *D. velutinum,* its concentration was 5 times higher than the ecosystem average during the rainy season (Fig. S5c and Fig. 5c). *M. splendens* and *L. rigidus* also showed increases in the concentration of carbohydrates in the shoot (Fig. S5b). The same occurred for the concentration of starch present in the aerial part of *C. mucronata* and *J. monticola* (Fig. 5b and Fig. 6a).

The season with the least precipitation resulted in a lower photosynthetic efficiency in *nodular canga*, and lower electron transport rate and total chlorophyll content in both microhabitats (Table 2). The reduction of F_v/F_m values during the dry period was determined in the *nodular canga* by the species *B. reticularia*, *P. mediterranea* and *M. splendens* and in the *couraçada canga* by *J. monticola* and *L. rigidus* (Fig.S6a). This reduction in F_v/F_m values for the species *B. reticularia* and *P. mediterranea* resulted in a lower maximum electron transport rate during the drought (Fig.S6c). Consequently, an increase in energy dissipation in the unregulated form was observed for plants exposed to *couraçada canga* during drought (Table 2). The environment of the *couraçada canga* caused its plant species to have lower

ETR_{max} values (Table 2). However, *B. reticularia, C. mucronata* and *P. mediterranea* were determinant for the higher rate of electron transport in the *nodular canga* (Fig. S6c). As well as the ecosystem, plants from *couraçada canga* in the rainy season had lower NPQ_{max} (Table 2). *L. rigidus* was the only species that showed higher energy dissipation in an unregulated way during the dry season (Fig. 6b and S6). The decline in total chlorophyll levels during the dry season was observed for all species except for *L. pinaster*, with no difference between seasons (Fig. S6b). Despite the decrease in the total chlorophyll content, the NBI values were lower for *nodular canga* species and remained constant between seasons, that is, the increase in flavonoids was proportional to the decrease in chlorophyll during the dry season (Table 2).

During the dry season, a higher concentration of H_2O_2 was produced in the shoot for both microhabitats (Table 2). Only *L. rigidus* showed no increase (Fig. S7d). The water deficit also resulted in higher levels of MDA in the roots of *the couraçada canga* species (Table 2). In relation to the microhabitats, during the drought, the *nodular canga* species have lower production of MDA in the roots (Table 2). *L. rigidus* and *J. monticola* showed increments above the ecosystem average for MDA concentration present in roots during drought (Fig. 6a-b). Native species from both microenvironments showed similar enzymatic responses throughout the year (Fig. S8). However, species adapted to *nodular canga* showed higher activity of SOD and CAT enzymes. In the *couraçada canga*, the enzyme APX showed greater activity (Table 2). During the dry season *P. mediterranea* increased the activity of the enzyme superoxide dismutase. *P. mediterranea* together with *M. splendens* were determinant for the higher SOD activity of the *nodular canga* (Fig. 5e-f and Fig.S8a). The activity of the catalase enzyme did not differ between the seasons for any of the species analyzed, however, *B. reticularia* stood out from the others because it showed greater activity of this enzyme when compared to the other species (Fig. 5a). The climatic seasonality also did not change the activity of the APX enzyme, but all the analyzed species showed high activity of this enzyme regardless of the season. *L. aurea*, *L. rigidus* and *L. pinaster* had the highest APX values (Fig. 5d, Fig. 6b-c).

Table 2. Nutritional, morphofunctional, carbohydrate partitioning, photochemical and antioxidant traits in native plants of *couraçada* and *nodular canga* microhabitats during the rainy and dry seasons.

	Analyzed variables	***Couraçada***		***Nodular***	
		Dry	**Rainy**	**Dry**	**Rainy**
Nutritional traits	N_{root}	8.31±0.98Aa	10.18±1.14Aa	8.267±0.36Aa	9.96±0.59Aa
	N_{shoot}	15.09±1.31Ab	19.67±1.54Aa	17.518±0.38Ab	19.70±0.81Aa
	Mn_{root}	150.58±44.32Aa	209.89±34.96Aa	187.22±25.07Aa	269.45±34.90Aa
	Mn_{shoot}	417.66±62.51Aa	574.77±78.21Aa	571.55±45.76Aa	509.03±39.89Aa
	$N:P_{root}$	28.70±1.81Aa	35.45±2.90Aa	34.88±2.55Aa	33.48±1.95Aa
	$N:P_{shoot}$	23.52±1.15Ba	25.00±1.49Aa	31.35±0.96Aa	27.56±1.25Ab
Morphofunctional traits	**LA**	756.30±109.82Aa	839.31± 117.43Aa	722.65± 90.73Aa	731.77± 70.65Aa
	LFW	461.64±69.54Aa	449.98±65.60Aa	400.81±53.43Aa	348.44±40.98Aa
	LDW	206.11±36.76Aa	196.14±33.49Aa	170.65±23.33Aa	144.44±16.34Aa
	LMA	252.02±10.32Aa	228.40 ±11.63Ab	231.04± 12.38Ba	183.59 ±8.84Bb
	LSI	3.81±0.16Aa	3.20±0.09Ab	3.21±0.18Ba	2.61±0.11Bb
	LDMC	40.11±1.45Aa	41.10±1.58Aa	42.46±1.01Aa	41.40±0.96Aa
	SLA	4.26±0.19Bb	4.91±0.28Ba	4.99±0.26Ab	6.02±0.22Aa
Carbohydrate partitioning traits	$Carb._{root}$	60.53±8.82Ab	226.37±16.35Aa	74.98±5.18Ab	188.91±17.98Ba
	$Carb._{shoot}$	182.30± 13.56Ba	177.61± 13.95Aa	273.05 ±15.11Ab	188.40± 17.40Aa
	$Starch_{root}$	221.23 ±20.07Ba	105.81± 9.12Ba	375.70±30.84Aa	353.02± 27.20Aa
	$Starch_{shoot}$	197.59 ±11.64Aa	76.34 ±9.44Ab	139.45 ±10.07Ba	68.14 ±8.33Ab
Photochemical traits	F_v/F_m	0.76±0.01Ab	0.77±0.008Ab	0.75±0.007Ab	0.77±0.008Aa
	F_0	137.67±9.39Ab	175.03±12.55Aa	145.70±9.89Ab	176.64±11.05Aa
	ETR_{max}	91.64±16.50Bb	117.81±21.94Ba	115.98±20.04Ab	145.75±24.03Aa
	NPQ_{max}	1.16±0.31Aa	0.50±0.13Bb	0.77±0.21Aa	0.65±0.20Aa
	Chl. *a*	24.30±1.45Bb	28.32±1.59Ba	26.34±0.68Ab	29.57±0.59Aa
	Chl. *b*	10.39±1.33Ab	14.22±1.38Aa	10.58±0.84Ab	15.07±0.78Aa
	Chl. total	36.69±2.45Ab	42.54±2.66Aa	36.92±1.34Ab	44.64±1.26Aa
	Chl. *a/b*	4.72±0.93Aa	2.57±0.25Ab	3.67±0.52Aa	2.33±0.16Ab
	NBI	26.92±0.86Aa	28.58±0.70Aa	20.64±0.62Ba	20.79±0.59Ba
Antioxidant traits	MDA_{root}	137.01±16.44Aa	54.49±9.95Ab	86.39±8.81Ba	66.68±8.02Aa
	MDA_{shoot}	104.25±12.20Aa	87.98±10.63Aa	92.59±9.42Aa	89.82±12.52Aa
	H_2O_{2root}	110.80±15.70Aa	108.71±8.51Aa	96.93±15.63Aa	111.46±7.31Aa

H_2O_{2shoot}	293.15±20.35Aa	180.76±20.36Ab	280.88±16.77Aa	174.68±12.85Ab
SOD	4.68±0.36Ba	5.52±0.58Ba	8.18±0.57Aa	8.03±0.48Aa
CAT	0.04±4.62e-3Ba	0.03±5.38e-3Ba	0.05±7.80e-3Aa	0.04±7.63e-3Aa
APX	7.38±0.67Aa	6.42±0.70Aa	5.08±0.47Ba	4.18±0.45Ba

The measurement units of each analyzed variable are: N (g/kg), Mn (mg/kg), leaf area - LA (mm^2), leaf fresh weight – LFW (mg), leaf dry weight – LDW (mg), leaf mass per area – LMA (g m^{-2}), leaf succulence index – LSI (g water dm^{-2}), leaf dry matter content – LDMC (% of fresh weight), specific leaf area – SLA (mm^2 mg^{-1}), total carbohydrates (mg g^{-1}), starch (mg g^{-1}), malonaldehyde concentration – MDA (nmol g^{-1} FM), hydrogen peroxide - H_2O_2 (mg g^{-1} FM), activity of the enzymes superoxide dismutase – SOD (U $min^{-1}mg^{-1}_{prot}$), catalase – CAT (μmol H_2O_2 min^{-1} mg^{-1} $_{prot}$) and ascorbate peroxidase – APX (μmol ASA min^{-1} mg^{-1} $_{prot}$). The values represent the means ± standard error. Means with different capital letters represent difference between areas (*couraçada* and *nodular*) and small letters difference between seasons (rainy and dry) according to Tukey's test at 5% probability.

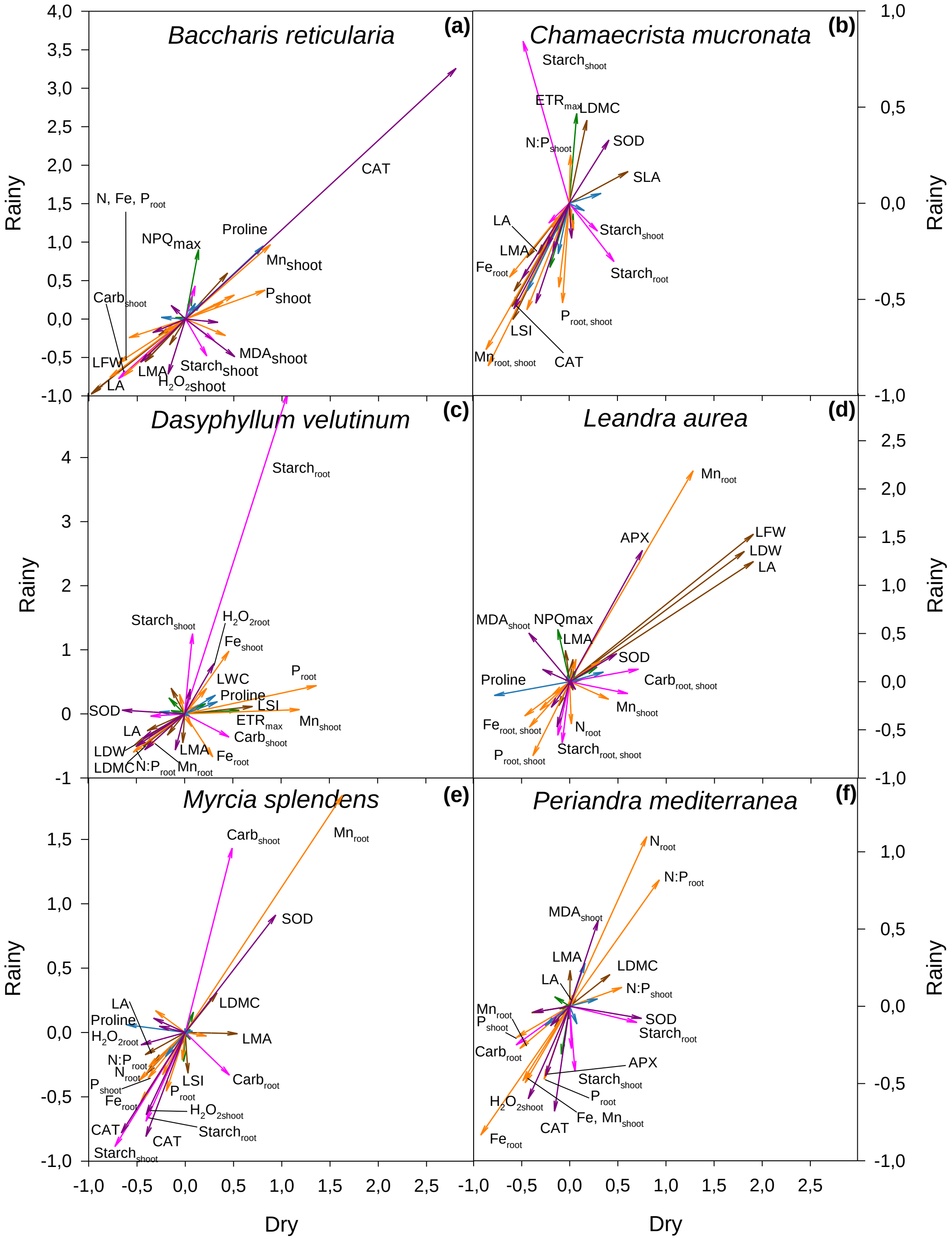

Figure 5. Morphophysiological responses of native plants of the nodular canga. The start point of each vector represents the ecosystem average and the end point the average of each trait evaluated during the rainy and dry seasons. The colors represent blue- Water status traits (LWC, Ψ_w, RWC and Proline), orange- Nutritional traits (P, Mn, Fe, N, N:P in roots and shoots and NBI), brown- Functional traits (LA, LMA, LFW, LDW, LDMC, LSI and SLA), pink- Carbohydrates partition traits (Starch and Total carbohydrates in roots and shoots), green- Photochemical traits (NPQ_{max}, ETR_{max}, Chlorophyll total and F_v/F_m) and purple- Antioxidant traits (MDA and H_2O_2 in the root and shoot, CAT, SOD and APX).

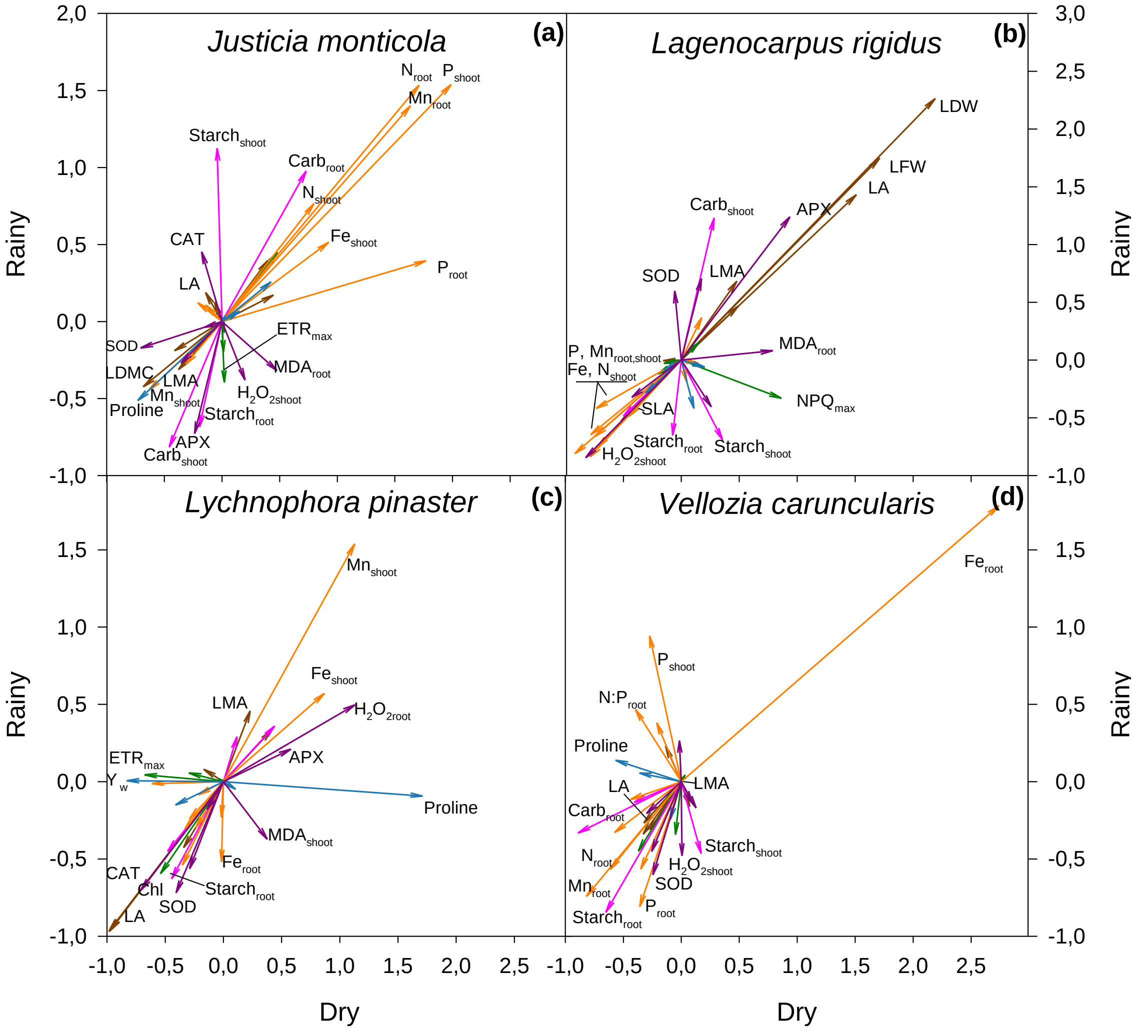

Figure 6. Morphophysiological responses of native plants of the *couraçada canga*. The start point of each vector represents the ecosystem average and the end point the average of each trait evaluated during the rainy and dry seasons. The colors represent blue- Water status traits (LWC, Ψ_w, RWC and Proline), orange- Nutritional traits (P, Mn, Fe, N, N:P in roots and shoots and NBI), brown - Morphofunctional traits (LA, LMA, LFW, LDW, LDMC, LSI and SLA), pink- Carbohydrates partition traits (Starch and Total carbohydrates in roots and shoots), green- Photochemical traits (NPQ_{max}, ETR_{max}, Chlorophyll total and F_v/F_m) and purple- Antioxidant traits (MDA e H_2O_2, in the root and shoot, CAT, SOD e APX).

4. DISCUSSION

The environmental heterogeneity of *canga* ecosystems, present on BIFs, resulted in mechanistic responses and functional adjustments of native plant species different from the ecosystem average. The *couraçada* and *nodular canga* are environments with different physical characteristics, temperature and soil water retention capacity. These different characteristics reflect a great diversity of physiological strategies developed by native plant species. Climatic seasonality, dry and rainy season, affected the ecosystem directly through changing temporarily the abiotic factors and indirectly through effects on physiology, morphology and behavior of plant species. However, among all the analyzed species, some proved to be decisive for the responses found in the different habitats of *canga* ecosystem.

Ecosystem functioning is determined by community dynamics (Bu et al., 2019). In this way, changes at the community level will likely affect the ecosystem, that is, the community's response to environmental changes results in different effects that will lead to changes in ecosystem processes (Suding et al., 2008). Corroborating the characteristics of perennial plants, the accumulation of starch in the shoot was one of the responses used by the species studied during the dry season, which contributed to the increases in the values of LMA and LSI. Under these conditions, perennial plants allocate the fixed carbon for long-term storage (non-structural carbohydrates), which can be used during drought conditions or for later regrowth (Chapin III et al., 1990; Sala et al., 2010). These trends can be interpreted as a resource conservation strategy (Diaz et al., 2004) adopted by the species when exposed to water deficit and consequently lower nutrient absorption, low SWC and RWC. Plant species adapted to resource-poor habitats generally have low photosynthetic and respiratory rates, low leaf nutrient content, high LMA, and long leaf life span (Mason & Donovan, 2015). This spectrum represents a continuum of resource-conserving strategies that produce high-investment, low-productivity leaves that persist much longer (Wright et al., 2001; Mason & Donovan, 2015). The high LMA values of the *campos rupestres* species, associated with the high contents of leaf dry matter, low P concentration in plant tissues and high N:P ratios (Güsewell, 2004) characterize the species as tolerant to stress according to the triangular CSR scheme (Grime, 1977; Negreiros *et al.*, 2014; Dayrell *et al.*, 2018; Rios *et al.*, 2022). However, these sort of environmental changes and morphofunctional responses have also been implicated in the reduction of total chlorophyll, F_v/F_m and ETR and increases in NPQ, H_2O_2 and MDA.

Regarding water absorption by plant cells, this only occurs when the water potential of the cytosol and vacuole is slightly lower than that of the surrounding environment. When subjected to stresses that cause dehydration, cells perform osmotic adjustments by increasing solute concentration. However, balance in the cytosol is maintained by compatible solutes such as proline and glycine-betaine (Kishor *et al.*, 2005). Although significant, the difference in water potential of plants between the dry and rainy seasons was very small and could be not sufficient for the accumulation of proline in response to water deficit. In addition to osmotic function, proline also plays multiple roles in stress acclimation, recovery, and signaling

(Szabados & Savouré, 2010). After periods of stress, such as the dry season, proline pools are responsible for providing a reducing potential for mitochondria through the oxidation of this amino acid, providing electrons for respiration and contributing to the supply of energy in the resumption of growth, during of the rainy season (Hare & Cress, 1997; Kishor *et al.*, 2005).

Although native *canga* species are predominantly stress tolerant, the diversity of the plant community as well as the functional variations of the species is directly related to the structure of the microhabitat in which it is found (Tameirão, LBS *et al.*, 2021; Rios *et al.*, 2022). In addition to the strong seasonality, rainy summers and dry winters, the *canga* environments also present shallow soils with rocky matrices with a small portion of organic matter and low water retention capacity (Fernandes *et al.*, 2014; Negreiros *et al.*, 2014). However, the higher indices of cation exchange capacity and organic matter present in the *nodular canga* generated some benefits for the species of this habitat when compared to the *couraçada canga*. Unlike the whole ecosystem responses and the *couraçada canga*, the plant species of the *nodular canga* were able to maintain the RWC, NPQ_{max} and levels of MDA in roots during the dry period. Despite the increase in H_2O_2 in the shoot, during the dry season, the absence of increases in the values of NPQ_{max} and MDA demonstrates the stress tolerance strategy developed by these species. The species showed mechanisms capable of ROS scavenging and inhibiting possible oxidative damage (Mhamdi & Van Breusegem, 2018). The higher activity of SOD and CAT enzymes of the species from the *nodular canga* microhabitat was effective in ROS control and fundamental for these results.

The highest soil moisture contents observed during the rainy season, with higher values in the *canga couraçada* demonstrates that the fragmented rocks of the *nodular canga* allow greater water drainage (Carmo & Jacobi, 2016). In addition to the low percentage of water in the soil, the *canga* is also an ecosystem that is extremely poor in nutrients. The studied substrates showed very low availability with values close to the most P-depleted soils in the world (Lambers *et al.*, 2011; Abrahão *et al.*, 2019). This low concentration of P occurs because this nutrient is originally found in the primary material and is then lost during soil development (Wright *et al.*, 2004; Turner & Condron, 2013). The concentration in plant tissues of N and P below the minimum necessary to maintain the cells results in tissue senescence (Batten & Wardlaw, 1987), whereas the excessive increase leads to toxic effects. In order to contain this shortage of P, native species of *campo rupestres* have presented root specializations such as rhizosheaths (Abrahão *et al.*, 2019). The low concentration of nutrients present in the soil, especially P and N, results in a reduction of those nutrients in plant tissues.

P-deficient plants increase the rate of P uptake and reduce the rate of N uptake, while N-deficient plants do the opposite (Aerts & Chapin III, 1999). Thus, N:P ratios above 20 suggest a greater P limitation than N limitation for plant growth (Oliveira *et al.*, 2015). The increase in the N:P ratio may have contributed to the increases in leaf dry matter (Atkinson, 1973) in plants from *canga* environments, probably reflecting the accumulation of assimilated products such as starch or secondary compounds (Vance *et al.*, 2003). In perennial plants, P deficiency (high N:P ratio) accelerates leaf senescence more than N deficiency (Güsewell, 2004). However, despite the low concentration of P and N, a study developed by Abrahão *et al.* (2019) showed that species from *campos rupestres* show high photosynthetic efficiency in the use of these nutrients as well as remobilization during leaf senescence. The low concentration of P present in the leaves, associated with the moderate concentration of N and the high LMA, reinforces the conservative strategy of nutrient use adopted by the species (Aerts & Chapin, 1999; Bustamante *et al.*, 2012). Regarding Mn concentration, despite the low availability in the soil, the concentration in roots and shoots was quite high (> 50 mg/kg, see (Epstein & Bloom, 2005)). The extreme conditions presented by *canga* substrates, acidic pH, low P concentration and high Fe concentrations, reflect the great diversity of plant nutritional strategies as well as the diversity present in *cangas* (Zemunik *et al.*, 2015). The higher SWC as well as the higher absorption of Fe and P by the native species of the *couraçada canga* corroborated for adaptive strategies aimed at thicker leaves and longer shelf life, when compared to species of the *nodular canga* (Wright *et al.*, 2004).

The two distinct *canga* phytophysiognomies defined by Rizzini (1997) are of common geological origin, but throughout their development they began to show differences in soil properties, concentration of chemical elements and proportion of exposed rock (Vincent & Meguro, 2008). The results obtained highlighted a great floristic heterogeneity associated with a variety of microenvironments dispersed over BIF formations. The abiotic conditions to which the vegetation is exposed, despite being severe, provided the conditions for the establishment of a global biodiversity hotspot with a vegetation rich in species and with a high degree of endemism when compared to landscapes with similar characteristics (Hopper, 2009; Silveira *et al.*, 2016). However, ecosystem functioning is related to the disproportionate contribution of each species, with their particular functional characteristics and different levels of correlation with the environment, as showed in this work.

Trait measurements reveal the contribution of each species to ecosystem functioning (Cadotte *et al.*, 2011). Among the species that contribute the most to the ecosystem, facilitator species benefit from closely associated neighbors (Padilla & Pugnaire, 2006), favor diversity and community dynamics, and are essential for the survival and growth of some plants (Pugnaire *et al.*, 1996; Kikvidze *et al.*, 2005). The proper selection of nurse species to be used in projects for the recovery of degraded areas is of paramount importance. Among the studied species *J. monticola* and *L. pinaster* are possible nurse species of the *canga couraçada*. Both species were fundamental to the response of this microenvironment, since they resulted in increases in the values of P and Fe in the shoot and soil moisture, as well as in the reduction of water potential and relative water content in the leaf. In the *nodular canga*, *B. reticularia*, *P. mediterranea* and *M. splendens* were the main species responsible for the characterization of the soil, for the lowest LMA contents and for the highest water potential, electron transport rate, non-photochemical energy dissipation and antioxidant enzyme activity. However, measuring and understanding each of the mechanistic traits, as well as selecting species capable of altering the conditions of their microenvironment in order to provide more suitable habitats for other species, is fundamental for making conservation and restoration decisions.

When exposed to climatic seasonality, native plant species of *canga* showed resilient adaptive responses that differed from the ecosystem and varied according to the heterogeneity of microhabitats. The particular characteristics of the soil of each microenvironment were determinant in the functional and mechanistic responses of the species, which presented responses with different levels of correlation with the environment.

5. DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. All authors approved the manuscript.

8. AUTHOR CONTRIBUTIONS

EGP: Funding acquisition and resources, project administration, supervision, conceptualization, design of the research, formal analysis, writing-reviewing, and editing. COR: design of the research, performance of the research, data analysis, collection, interpretation, conceptualization, investigation, formal analysis, writing-original draft. PAP: performance of the research, investigation, data collection, writing-reviewing and editing. All authors approved the manuscript.

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SUPPLEMENTARY MATERIAL

New Phytologist

Beyond functional traits: how does microenvironment heterogeneity shape the mechanistic responses of plant species in a tropical ironstone outcrop?

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Table S1. Characterization of native plant species in the BIF ecosystem

	Analyzed variables	Dry	Rainy
Water status traits	**Ψ_W (Le Stradic *et al.*)**	-0.73±0.41	-0.24±0.02*
	LWC (% of fresh weight)	58.47±0.84	58.71±0.85
	RWC (%)	88.48±1.12	92.50±0.96*
	SWC (%)	1.68±0.10	6.06±0.31*
	Proline (μmol g^{-1}_{FM})	18.64±1.71	21.01±0.85*
Nutritional traits	**N_{root} (g kg^{-1})**	8.29±0.67	10.07±0.87*
	N_{shoot} (g kg^{-1})	16.31±0.85	19.68±1.18*
	P_{root} (g kg^{-1})	0.35±0.05	0.35±0.04
	P_{shoot} (g kg^{-1})	0.66±0.05	0.87±0.07*
	Fe_{root} (mg kg^{-1})	2899.16±405.68	5347.30±813.44*
	Fe_{shoot} (mg kg^{-1})	1018.72±95.42*	543.41±47.21
	Mn_{root} (mg kg^{-1})	168.90±34.69	239.67±34.93*
	Mn_{shoot} (mg kg^{-1})	494.61±54.13	541.90±59.05
	$N:P_{root}$	32,41± 1,72	34,27± 1,64
	$N:P_{shoot}$	28,22± 0,83	26,64± 0,96
	NBI	23.78±0.74	24.69±0.64*
Morphofunctional traits	**LA (mm^2)**	739.47±10.02	785.54±9.40
	LMA (g m^{-2})	241.54±1.13*	206.00±1.02
	LFW (mg)	425.14±42.33	389.06±36.10
	LDW (mg)	184.83±20.26	165.13±16.69
	LSI (g water dm^{-2})	3.45±0.13*	2.84±0.08
	LDMC (% of fresh weight)	41.52±0.84	41.28±0.85
	SLA (mm^2 mg^{-1})	4.70±0.18	5.57±0.18*
Carbohydrate partition traits	**Total carbohydrates $_{root}$ (mg g^{-1})**	67.76±7.00	207.64±17.16*
	Total carbohydrates $_{shoot}$ (mg g^{-1})	227.68±14.34*	183.01±15.68
	Starch $_{root}$ (mg g^{-1})	298.47±25.46	229.42±18.16
	Starch $_{shoot}$ (mg g^{-1})	168.53±10.86*	72.24±8.89
Photochemical	**Chl total**	35.81±1.90	43.59±1.97*

traits	**F_v/F_m**	0.75±0.01	0.77±0.01*
	ETR $_{max}$	170.43±8.92	206.49±11.32*
	NPQ $_{max}$	1.66±0.14*	1.22±0.10
Antioxidant traits	**MDA $_{root}$ (nmol g^{-1} $_{FM}$)**	111.70±12.62*	60.58±8.99
	MDA $_{shoot}$ (nmol g^{-1} $_{FM}$)	98.42±10.81	88.60±11.58
	H_2O_2 $_{root}$ (mg g^{-1} $_{FM}$)	103.86±15.66	110.09±7.91
	H_2O_2 $_{shoot}$ (mg g^{-1} $_{FM}$)	287.01±18.56*	177.72±16.61
	SOD (U $min^{-1}mg^{-1}_{prot}$)	6.43±0.47	6.77±0.53
	CAT (µmol H_2O_2 min^{-1} mg^{-1} $_{prot}$)	0.05±0.01	0.04±0.01
	APX (µmol ASA min^{-1} mg^{-1} $_{prot}$)	6.23±0.57	5.30±0.57

Mean ± standard error. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

Table S2. Chemical characteristics of the ryzhospheric soil close to each of the analyzed species.

Soil traits	***L. aurea***	***L. rigidus***	***J. monticola***	***P. mediterranea***	***M. splendens***	***C. mucronata***	***D. velutinum***	***V. caruncularis***	***B. reticularia***	***L. pinaster***
P (mg dm^{-3})	4.75±0.54	3.29±0.28	6.03±1.46	5.33±0.49	5.24±0.68	4.87±0.59	4.02±0.51	2.92±0.15	4.66±0.54	2.98±0.20
K (mg dm^{-3})	69.25±3.83	55.66±0.50	67.50±7.12	68.50±3.91	62.97±3.19	58.54±3.11	61.82±4.09	53.42±5.71	59.77±3.72	52.62±5,29
Ca (cmolc dm^{-3})	3.25±1.03	1.86±0.59	3.42±1.08	2.28±0.72	3.00±0.95	2.70±0.85	2.26±0.72	2.58±0.82	2.80±0.88	1.59±0.50
Mg (cmolc dm^{-3})	0.70±0.09ab	0.33±0.03b	0.93±0.12a	0.55±0.06ab	0.54±0.12ab	0.61±0.08ab	0.55±0.08ab	0.54±0.03ab	0.54±0.09ab	0.36±0.04b
Fe (mg dm^{-3})	201.25±17.47a	234.05±13.29a	100.97±11.29b	206.58±9.39a	212.75±23.85a	203.20±16.74a	203.71±13.81a	215.26±20.07a	191.09±17.75a	224.22±12.74
Mn (mg dm^{-3})	96.44±13.91ab	53.42±8.19b	155.81±13.46a	70.1±11.69b	87.58±23.59ab	72.35±9.84b	62.17±8.36b	59.63±4.74b	90.16±22.40ab	43.74±8.11b
Al (cmolc dm^{-3})	0.19±0.02	0.48±0.02	0.08±0.01	4.41±3.65	0.42±0.07	0.29±0.05	0.27±0.06	0.17±0.03	0.32±0.05	0.23±0.03
pH H_2O	4.91±0.08bc	4.62±0.13c	5.29±0.06a	4.90±0.07bc	4.76±0.09bc	5.04±0.06ab	4.81±0.07bc	4.79±0.05bc	4.81±0.08bc	4.70±0.04bc
pH $CaCl_2$	4.01±0.09b	3.87±0.05b	4.41±0.08a	4.05±0.05ab	3.90±0.09b	4.12±0.07ab	3.94±0.07b	3.88±0.03b	4.00±0.06b	3.78±0.05b
H (cmolc dm^{-3})	9.75±0.58ab	11.56±0.61ab	5.15±0.41c	9.2±0.51bc	13.83±1.10a	10.90±1.03ab	9.40±0.86b	9.83±0.86ab	11.16±0.71ab	11.28±0.94ab
H+Al (cmolc dm^{-3})	9.94±0.59b	12.04±0.61ab	5.23±0.42c	9.52±0.53b	14.25±1.44a	11.2±1.06ab	9.67±0.87b	10±0.89b	11.48±0.73ab	11.51±0.95ab
SB (cmolc dm^{-3})	4.12±0.20ab	2.33±0.21ab	4.52±0.45a	3.00±0.35ab	3.72±0.76ab	3.46±0.39ab	2.97±0.30ab	3.25±0.11ab	3.489±0.56ab	2.09±0.17b
t (cmolc dm^{-3})	4.31±0.49ab	2.81±0.19ab	4.59±0.44a	3.33±0.31ab	4.14±0.72ab	3.76±0.37ab	3.25±0.26ab	3.42±0.09ab	3.80±0.52ab	2.32±0.16b
T (cmolc dm^{-3})	14.06±0.67ab	14.37±0.71ab	9.75±0.58c	12.53±0.58bc	17.97±1.02a	14.67±1.18ab	12.64±0.83bc	13.25±0.84bc	14.97±0.65ab	13.60±0.98bc
V (%)	29.14±3.35b	16.20±1.21b	46.03±3.20a	23.91±2.54b	20.76±4.09b	24.13±2.45b	24.36±2.97b	25.44±1.97b	23.32±3.42b	16.03±1.84b
m (%)	5.53±1.34bc	17.96±1.91a	1.87±0.34c	11.85±3.01abc	13.13±2.47ab	8.83±1.78abc	10.01±2.95abc	4.97±0.85bc	10.22±1.96abc	10.67±1.85ab
MO (dag Kg^{-1})	8.83±0.76	8.88±0.25	6.48±0.59	9.45±0.52	9.26±0.78	8.35±0.61	8.01±0.57	7.54±0.22	9.66±0.72	7.84±0.65

Soil analyses were performed with composite samples from the study area (n = 100). Caption: H + Al potential acidity; SB sum of exchangeable bases; t Effective cation exchange capacity; T Cation exchange capacity at pH 7; V Base saturation index; m aluminum saturation index; MO soil organic matter. The values represent the means ± standard error. Means with different letters represent difference between species, according to Tukey's test at 5% probability.

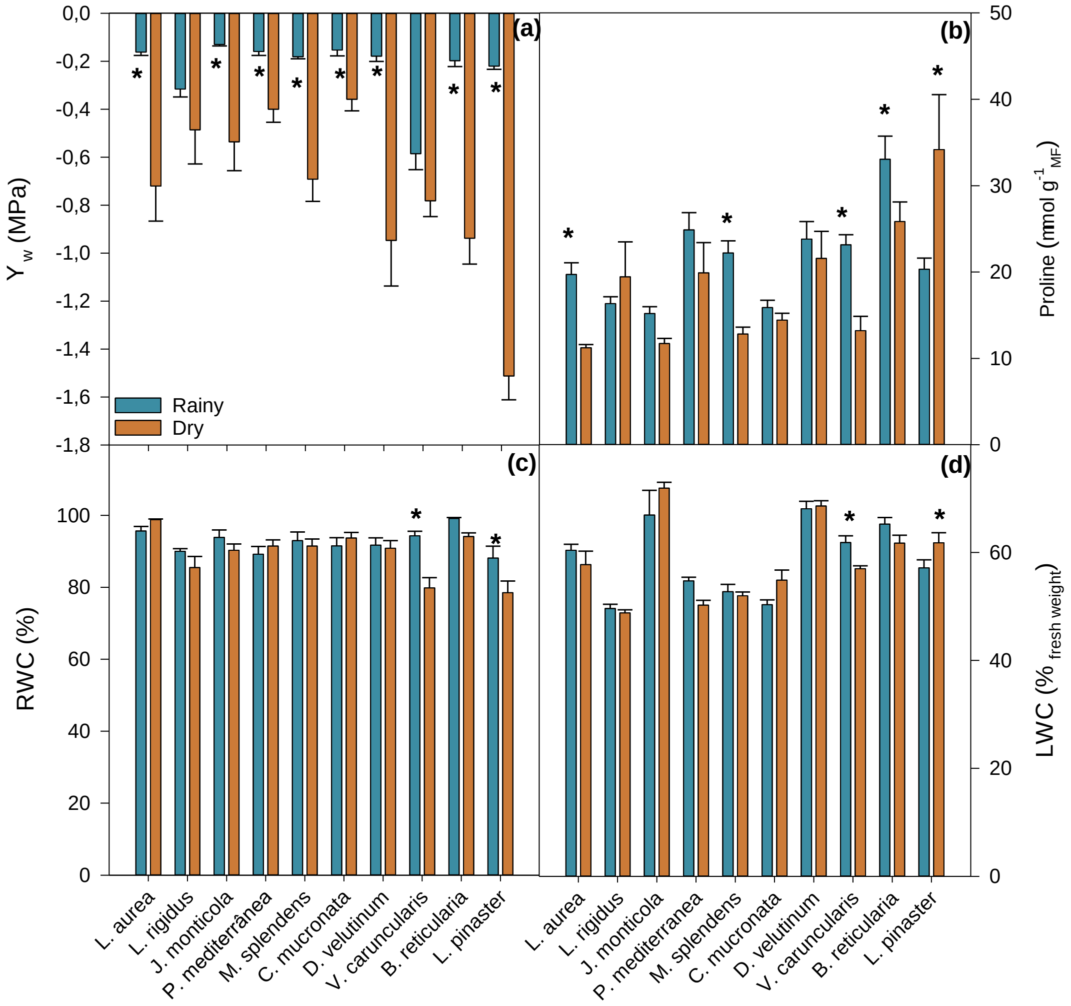

Figure S1: Water potential - Ψ_w (a), proline concentration (b), relative water content in the leaf - RWC (c), leaf water content – LWC (d) in native plants of *campo rupestre*, *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* e *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

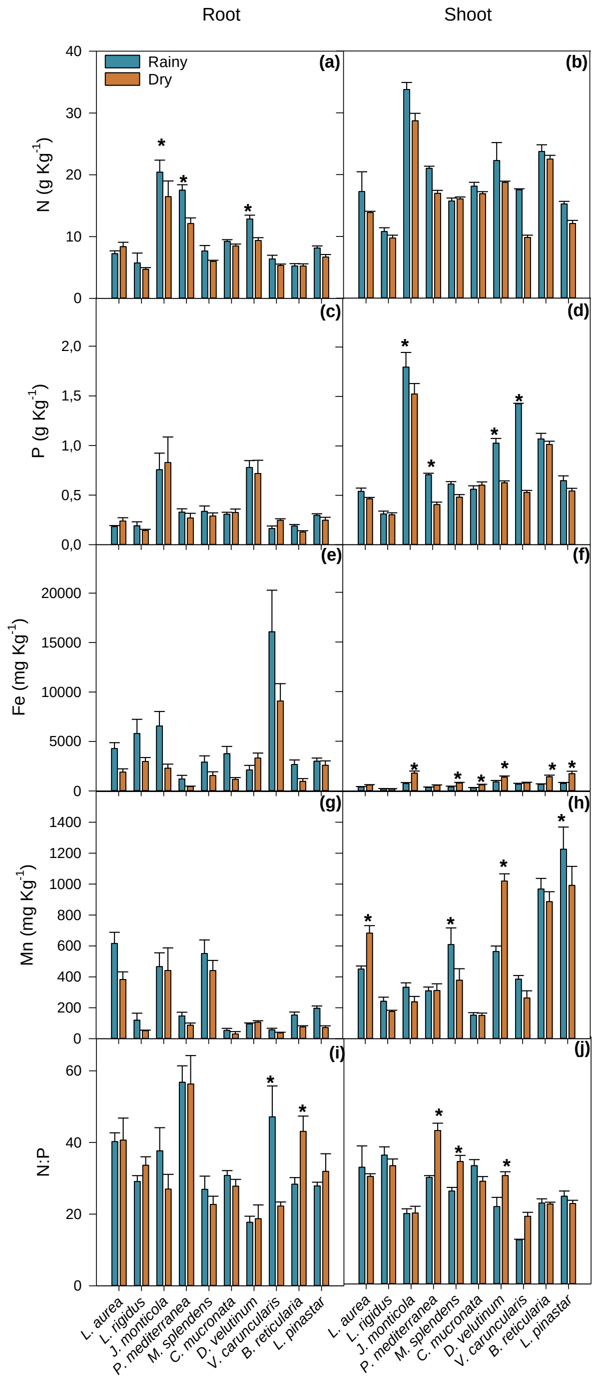

Figure S2. Nitrogen (a and b), phosphorus (c and d), iron (e and f), manganese (g and h) and N:P ratio (i and j) present in the roots (a, c, e, g and i) and aerial part (b, d, f, h and j) in native plants from *campo rupestre*, *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* e *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability. Statistical analyses were performed separately for root and shoot.

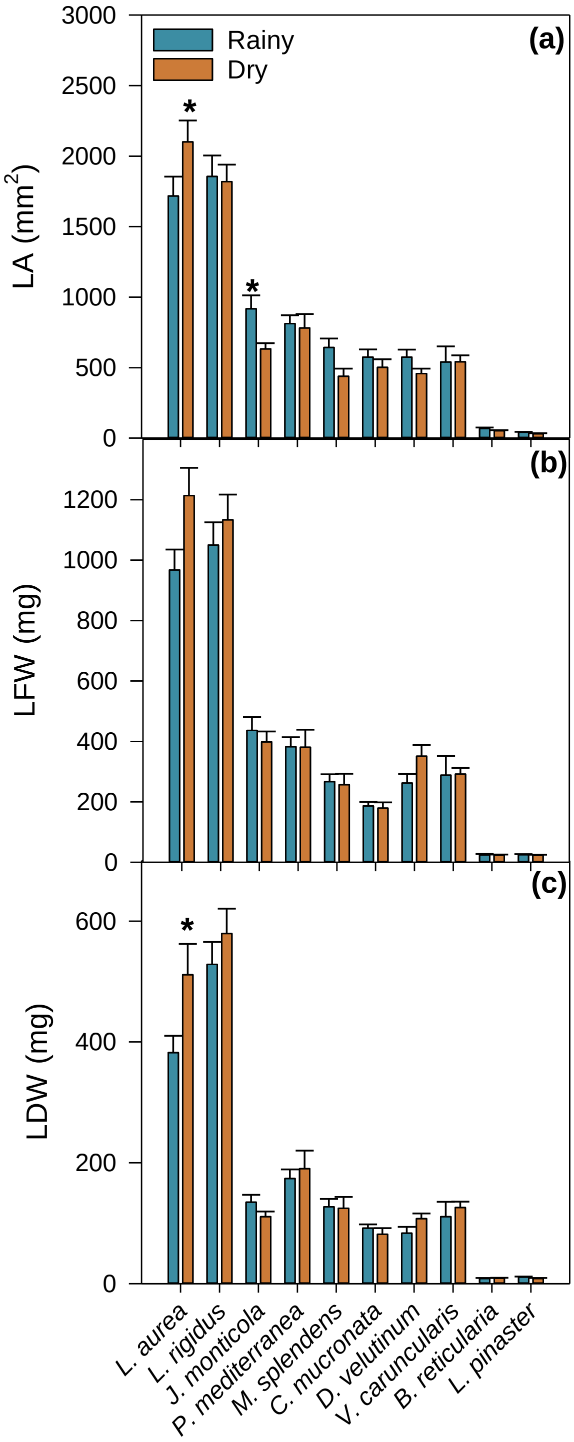

Figure S3. Leaf area - LA (a), leaf fresh weight – LFW (b) and leaf dry weight - LDW (c) in native plants from *campo rupestre*, *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* e *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

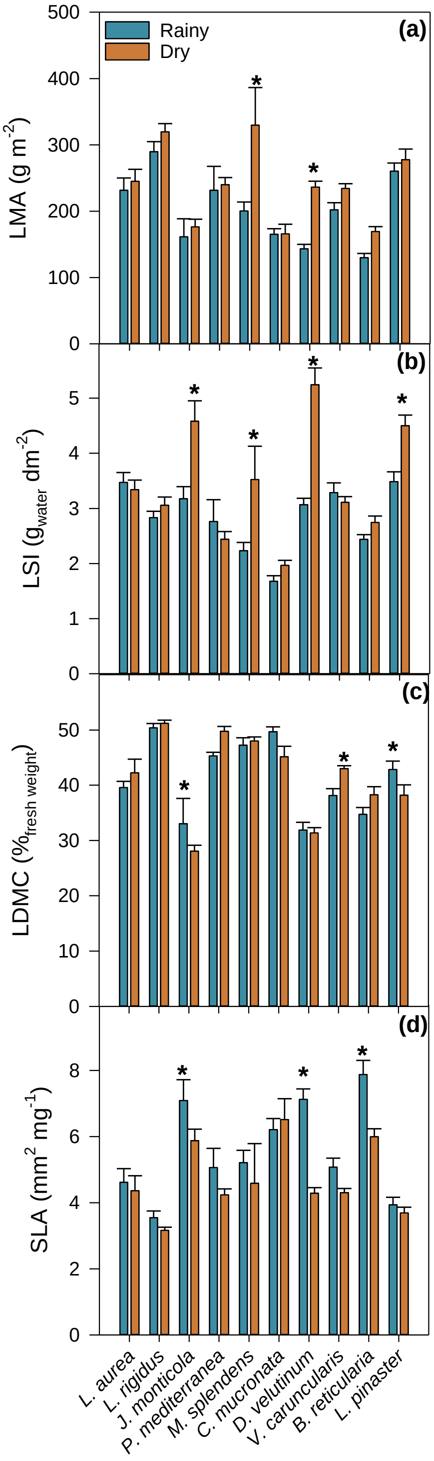

Figure S4. Leaf mass per area – LMA (a), leaf succulence index – LSI (b), leaf dry matter content – LDMC (c) and specific leaf area – SLA (d) in native plants from *campo rupestre*, *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* e *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

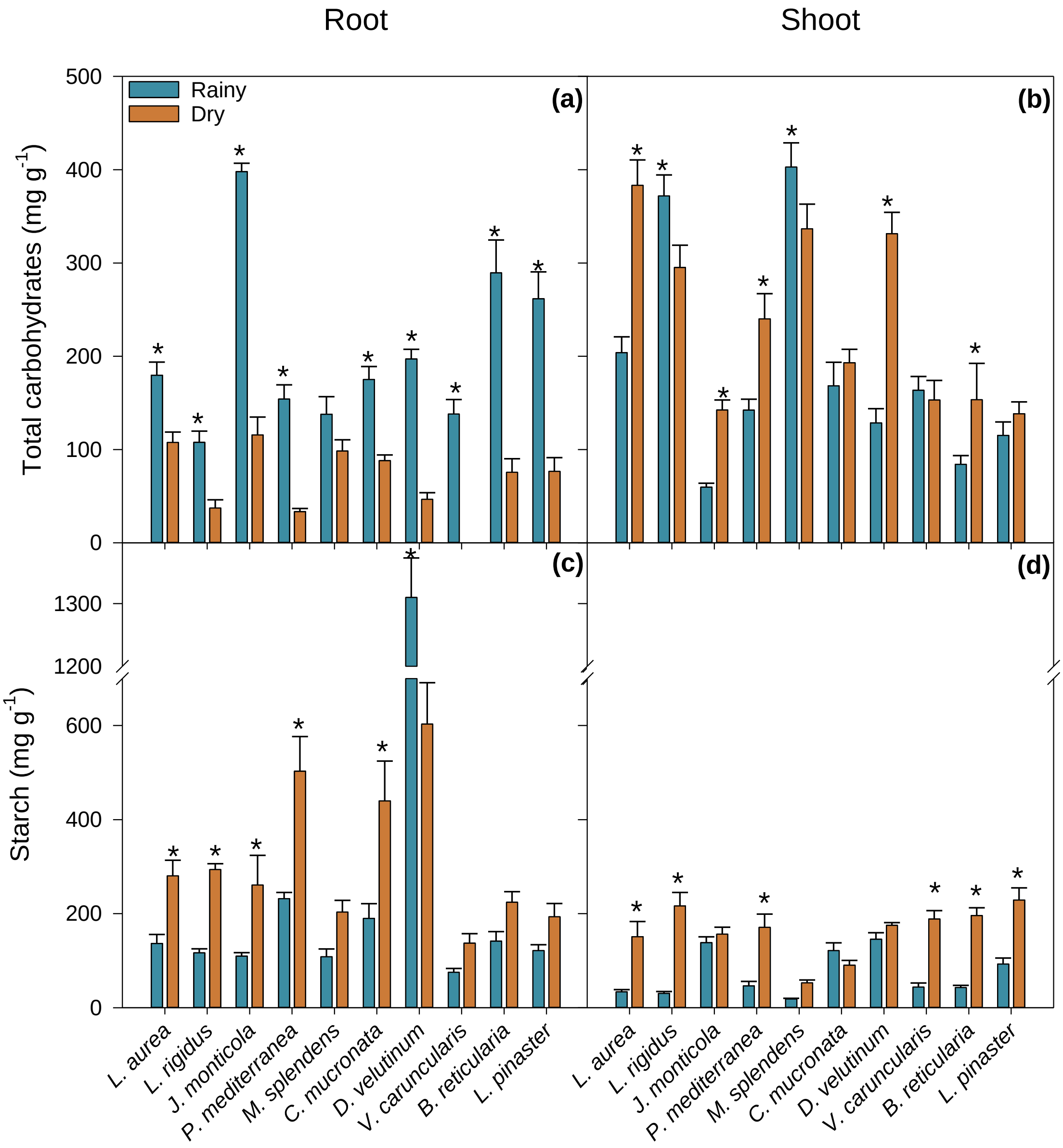

Figure S5. Total carbohydrates (a and b) and starch (c and d) present in roots (a and c) and shoots (b and d) in native plants from *campo rupestre B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* e *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability. Statistical analyses were performed separately for root and shoot.

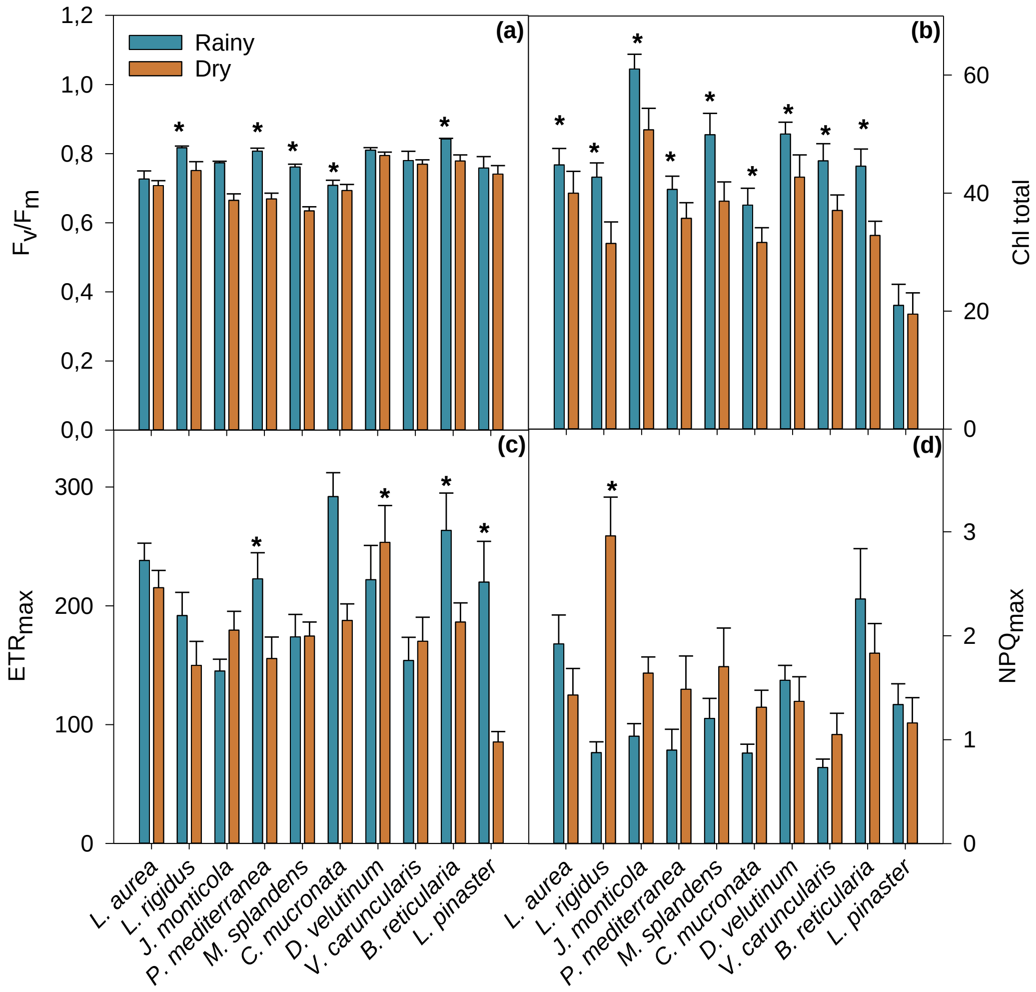

Figure S6: Maximum quantum efficiency of PSII (a), total chlorophyll content (b), maximum electron transport rate (c) and maximum non-photochemical extinction (d) in native plants from campo rupestre, *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* e *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

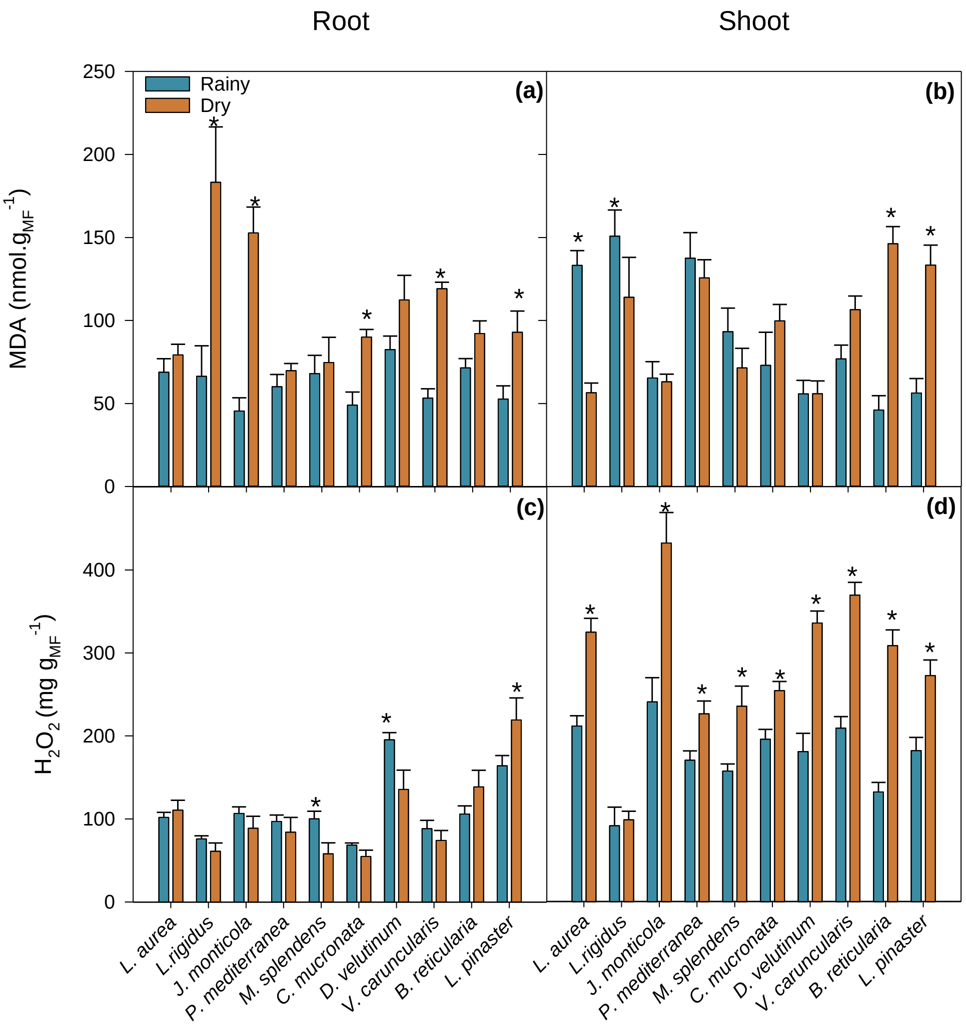

Figure S7: Malonaldehyde concentration – MDA (a and b) and hydrogen peroxide -H_2O_2 (c and d) in roots (a and c) and shoots (b and d) of *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* and *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability. Statistical analyses were performed separately for root and shoot.

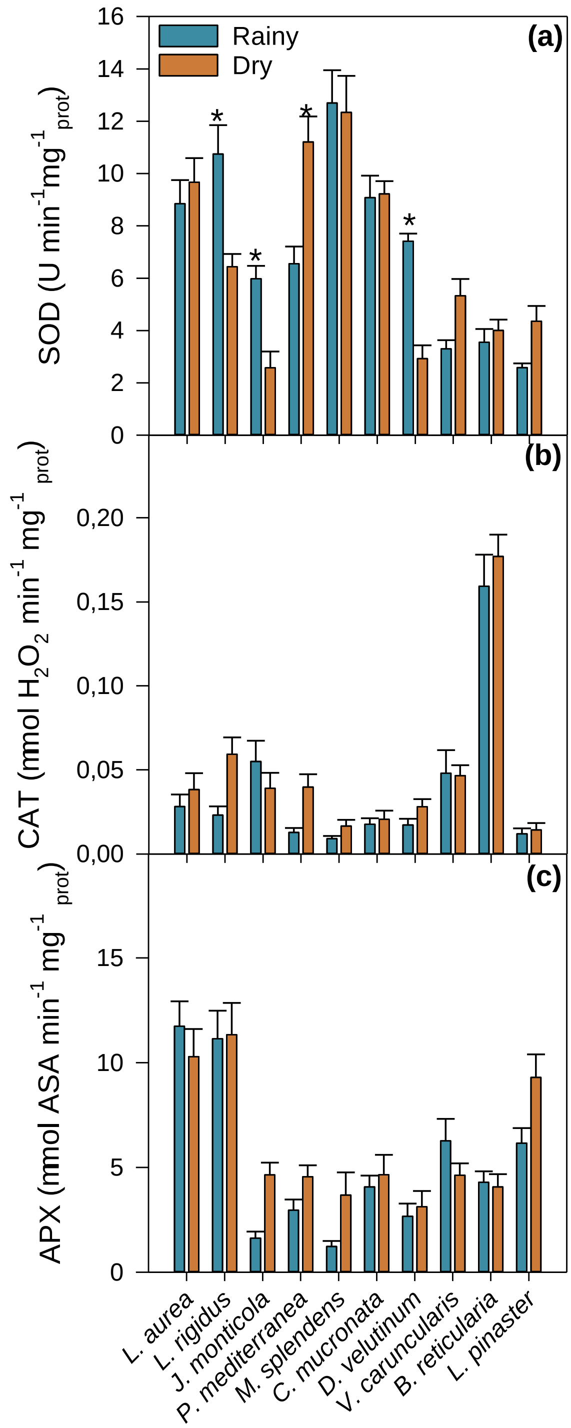

Figure S8: Activity of the enzymes superoxide dismutase – SOD (a), catalase – CAT (b) and ascorbate peroxidase – APX (c) in leaves of *B. reticularia*, *C. mucronata*, *D. velutinum*, *J. monticola*, *L. aurea*, *L. pinaster*, *L. rigidus*, *M. splendens*, *P. mediterranea* and *V. caruncularis* exposed to rainy and dry seasons. The bars represent the means ± standard error of ten repetitions. Means followed by an asterisk showed differences according to Tukey's test at 5% probability.

CAPÍTULO 3

Photochemical rather than morphofunctional attributes are decisive in the responses of native plant species from ferruginous outcrops when grown in iron mining substrates

Manuscrito preparado de acordo com as normas para submissão ao periódico Functional Plant Biology

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ABSTRACT

Functional diversity of native plant species from reference ecosystems is important for the recovery of degraded areas. The objective of this work was to evaluate if the relationship between morphofunctional and photosynthetic attributes of native canga species represents its functional group differences when grown in iron mining tailings substrate. The experiment was carried out with species belonging to different functional groups: a widespread semideciduous tree-shrub, *Myrcia splendens* (Sw.) DC; an endemic deciduous shrub, *Jacaranda caroba* (Vell.) DC; and a nitrogen-fixing herbaceous species, *Periandra mediterranea* (Vell.) Taub. The species were grown in two conditions, a reference soil and iron ore tailing. All species showed germination percentages above 73%. Despite belonging to different functional groups, when grown in tailings, the morphofunctional attributes were similar between species. *M. splendens* was the species most affected by the conditions imposed by the iron ore mining tailings, with reduction in the light-use efficiency and electron transport. *P. mediterranea*, had satisfactory growth and maintenance of photosynthetic attributes. *J. caroba* sown in the tailings increased effective quantum yield of PSII. The photochemical and growth assessments were able to better explain the adaptive strategies developed by the species, guaranteeing the success of revegetation.

Keywords: functional groups, chlorophyll *a* fluorescence, *canga*, nitrogen-fixing, widespread semideciduous, deciduous shrub, ecological restoration.

1. INTRODUCTION

The advance of environmental degradation caused by human activities has brought irreparable losses to biodiversity (Weisse & Goldman, 2019). In an attempt to restore ecosystem services and amend the rapid decline of biodiversity, great efforts have been made (UNCCD, 2017). However, often these efforts are concentrated in forested areas, neglecting habitats such as grasslands and savannas (Stanturf *et al.*, 2019; Dudley *et al.*, 2020b; Holl & Brancalion, 2020). In order to encourage and support this cause, the United Nations created the "Decade of Ecosystem Restoration", established between the years 2021 to 2030 (UNEP e FAO 2020). Among the ecosystems to be restored, the rehabilitation of mining areas contributes to promoting the increase of primary biodiversity and ecosystem services, reducing the environmental impacts resulting from the activity (Giljum *et al.*, 2022).

The recovery of areas degraded by metal extraction activities is a challenging task, as they are environments with severe stress-prone characteristics, such as high solar radiation, low organic matter content, low nutrient content, high soil compaction and seasonal droughts (Rios *et al.*, 2021; Cruz *et al.*, 2022; Santos *et al.*, 2022; Silva *et al.*, 2022; Rios *et al.*, 2023). Surviving these conditions requires specific morphophysiological strategies that ensure the plants acclimatize to the physicochemical factors of the soil (Ginocchio *et al.*, 2017). Therefore, it is extremely important that the rehabilitation of these areas takes into account the reference ecosystem, the limiting abiotic aspects of these environments and that it is carried out with native species, thus reducing the risk of biological invasion and increasing the chance of survival of the species (Kienberg *et al.*, 2014; Aslan, 2019; Gastauer *et al.*, 2019).

Landscapes degraded by iron ore mining bring with them serious environmental problems, such as threats to biodiversity and loss of ecosystem services (Meira *et al.*, 2016; de Castro Pena *et al.*, 2017; Fernandes & Ribeiro, 2017; Giljum *et al.*, 2022). The areas explored for the extraction of iron ore are, in most cases, natural environments of ferruginous *campo rupestre*, also known as *canga*. *Cangas* are environments originated from banded iron formations composed of rocky outcrops vegetation with common presence of metallophytes or at least metal-tolerant species (Le Stradic *et al.*, 2014) forming a mosaic with herbs and shrubs over nutrient-poor itabirite (Gagen *et al.*, 2019; Miola *et al.*, 2021). It is an ecosystem composed of native plant species with a high degree of endemism and considerable functional diversity. The selection of species belonging to different functional groups of the reference ecosystem guarantees greater success in the recovery of areas impacted by mining (Miller *et* *al.*, 2012). The characteristics of the seeds are also important to be considered in works aiming the recovery of degraded areas, since the lack of knowledge about seeds of native species generates a challenge for their use (Maciel *et al.*, 2022). Once the main seed traits are known, it is also important to study the development of the species to be used. With the purpose of predicting the performance of the plant when exposed to the area to be recovered, functional characteristics are often used (Balazs *et al.*, 2020). The evaluations of functional differences, based on tools proposed by functional ecology, allow the identification of species to be used in projects for the initial rehabilitation of waste dumps (Gastauer *et al.*, 2020). Some authors still defend the selection of plant species with widely different functional characteristics, minimizing competition and maximizing facilitation (Navarro-Cano *et al.*, 2019).

However, studies that are based only on the morphofunctional characteristics of plants consider intraspecific variability insignificant when compared to interspecific variability (Albert *et al.*, 2010). On the other hand, within a single plant species there is a wide variation of characteristics between individuals (Albert *et al.*, 2010; Poorter *et al.*, 2018). In this way, changes in the approach, based on the mechanistic responses of individuals, are capable of altering the interpretation of plant characteristics. Thus, the physiological mechanisms of plants allow us to understand how plants establish and persist in highly disturbed environments and can be used in projects for the recovery of degraded areas (Valliere *et al.*, 2021). Plant species adapted to severe environmental conditions present a diversity of adaptive strategies, for example, increases in photochemical efficiency parameters, increase in chlorophyll levels and development of photoprotective processes (Rios *et al.*, 2022), besides a higher concentration of proline and reduction of oxidative damage, through lower content of maloaldehyde and hydrogen peroxide (Rios *et al.*, 2023). Among the possible methodological tools to be used, the plant ecophysiology techniques can assist in site preparation and selection of plant material, by providing information on performance, and finally, improving the ability to predict restoration results (Valliere *et al.*, 2021). Chlorophyll *a* fluorescence is a practical, fast and non-destructive method, with great potential for selecting plant species with efficient resource use strategies (Guimarães *et al.*, 2022; Rios *et al.*, 2022). This assessment provides an overview of how the plant is distributing light energy throughout its photochemical process.

In previous study carried out in *canga*, Rios *et al.* (2022), carried out in *canga*, identified and selected some species under field conditions, with the purpose of using them in rehabilitation projects in areas impacted by mining companies. These selected species showed greater photoprotection capacity, accumulation of non-structural carbohydrates, increased levels of antioxidant enzymes and leaves with high diversity of morphofunticonal traits such as leaf mass per area (LMA), leaf area (LA), leaf fresh weight (LFW), leaf dry weight (Blanchar *et al.*), leaf succulence index (LSI) and leaf dry matter content (LDMC). However, more knowledge is needed about the responses of native *canga* species to tailings conditions in order to understand whether the possible tolerance mechanisms of the species are related to the cross-talk between morphofunctional and physiological characteristics when subjected to areas impacted by mining. In this context, the hypothesis of the work is that native *canga* species belonging to three distinct functional groups, will present different adaptive characteristics when grown in iron ore tailings. Among them, we expect that the nitrogen-fixing herbaceous species *Periandra mediterranea* presents satisfactory responses since the symbiosis promotes N absorption and favors its acclimation in depleted soils, through the accumulation of dry matter, the increase of chlorophyll and consequently higher net assimilation rate and photosynthetic efficiency. For the widespread semideciduous tree-shrub species *Myrcia splendens*, we hypothesize that it presents rapid leaf development and mechanisms that minimize oxidative damage through energy dissipation in a regulated manner. Unlike the others, we expect that the deciduous shrub species *Jacaranda caroba* of endemic occurrence will present less satisfactory responses, such as a decrease in its development, through a reduction in the functional characteristics of growth and in the photosynthetic analysis, due to its limited range. The objective of this work was to evaluate if the relationship between morphofunctional and photosynthetic attributes of native *canga* species represents its functional group difference when grown in iron mining tailings substrate.

2. MATERIAL AND METHODS

2.1. Studied species

The plant species *Jacaranda caroba* (Vell.) DC, *Myrcia splendens* (Sw.) DC and *Periandra mediterranea* (Vell.) Taub. were selected because they are native species with wide distribution over *canga* areas and representative of three important functional groups - endemic, widespread and N fixer, respectively. The selection of native species abundant in environments with edaphic conditions similar to the areas to be revegetated is of paramount importance (Madejón *et al.*, 2003; De Carvalho *et al.*, 2012).

J. caroba is a species belonging to the family Bignoniaceae, ranges from shrubs to small trees, reaching an average height of 0.5 to 2.5 meters, with phytogeographic domains between the Cerrado and the Brazilian Atlantic Forest (Pereira & Mansano, 2008). It has bipinnate compound leaves with coriaceous or subcoriaceous leaflets that vary from elliptical to rhomboid and opposite phyllotaxis, its flowers vary in color from magenta to purple and elliptical, dry and dehiscent capsular fruits, exposing the winged membranous seeds (Gentry, 1992; Lohmann & Pirani, 1996).

M. splendens is a semideciduous tree species belonging to the Myrtaceae family, occurring from Mexico to southern Brazil (Oliveira-Filho & Fontes, 2000; Morais & Lombardi, 2006). They can reach 18 m in height and 30 cm in diameter, have red or green leaves, white flowers and reddish fruits with fleshy pulp (Carvalho, 2008).

P. mediterranea is a shrub legume native to all regions of Brazil and parts of Bolivia, with great abundance in *campo rupestre* and the ability to establish associations with nitrogen-fixing bacteria and mycorrhizal fungi (Menéndez *et al.*, 2016). Has the potential for revegetation of degraded areas (Figueiredo *et al.*, 2018).

2.2. Experimental conditions

The experiment was carried out at the Universidade Federal de Viçosa, Campus Florestal, Brazil (19º 53' 20.23” S e 44º 25' 56.38” O). The local climate according to Köppen is classified as tropical, with two well-defined seasons, rainy summers and dry winters, and an average annual rainfall of 1500 mm. The plants were cultivated inside the greenhouse, with relative humidity, temperature and maximum irradiance of 52%, 21ºC and 606.9 μmol m^{-2} s^{-1}, respectively during the experiment.

2.3. Seed collection and biometrics

Initially, seeds of all species were collected in a *canga* area located in Serra da Calçada, Brumadinho, Minas Gerais, Brazil (20°05’55,2”S, 43°58’ 58,2”W). Biometric evaluations of the seeds were carried out using 100 healthy seeds collected from georeferenced matrices. The weighing was measured on a scale with a precision of 0.001 g, where the fresh mass was obtained and the length, width and thickness measurements were performed with a digital caliper of 0.01 mm (Table 1).

Table 1. Biometry of *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) seeds

Species	Fresh biomass (g)	Length (mm)	Width (mm)	Thickness (mm)
Jacaranda caroba	0.01±0.00c	3.79±0.03c	4.03±0.03b	0.69±0.01c
Myrcia splendens	0.09±0.00a	6.94±0.09a	4.54±0.06a	3.44±0.06a
Periandra mediterranea	0.04±0.00b	6.51±0.02b	4.00±0.02b	2.59±0.01b

Analysis of mean fresh mass, length, width and thickness ± standard error (n=100 seeds of each species). Letters mean statistical difference between species, according to Tukey's test at 5% probability

2.4. Germination experiment

All seeds were previously washed with 1.5% sodium hypochlorite for 5 minutes, then washed with deionized water. The germination experiment was conducted in Petri dishes. Two treatments were adopted for the germination tests, the control treatment – double layer of germination paper - and the mining tailings treatment - 30g of iron mining tailings. . The tailings used during the experiment were collected at the Pico Mine belonging to the Vargem Grande complex of mining VALE (20°12'48.65"S, 43°52'6.51"W). The seeds of *M. splendens* were removed from the fruit and disposed for each treatment in 13 Petri dishes with 30 seeds each. A photoperiod of 12 hours and alternating temperatures between 20 and 30ºC were used in the germination chamber. For *J. caroba*, four Petri dishes with 40 seeds were used for each treatment, which were placed in a photoperiod of 12 hours and 25ºC in the germination chamber. For *P. mediterranea*, the seeds were scarified in the portion opposite the embryo in order to overcome the physical dormancy of the seed, then they were distributed in four Petri dishes for each treatment with 30 seeds each. A photoperiod of 12 hours and 25ºC was used for this species in the germination chamber. For the three species, each replication corresponds to the total number of seeds in each Petri dish. To reduce the loss of water by evaporation, the Petri dishes were kept closed and opened only for daily irrigation. The position of the plates inside the germination chamber was randomly changed every day. The plates were inspected daily, and the germination criterion used was that of Labouriau (1983), in which the seed is considered to have germinated when the primary root protrudes. The germinated seeds were counted daily and the experiment was ended when there was no germination for ten consecutive days. For each treatment and species, the germination percentage and speed index were calculated. The percentage was calculated by the ratio between the number of germinated seeds and the total number of seeds, and the germination speed index (GSI) was calculated according to (Maguire, 1962).

2.5. Transplantation and seedling development

The seedlings from the germination tests were initially transplanted into tubes containing vermiculite (control treatment) and iron ore tailings. After acclimatization and satisfactory root growth, 48 plants of each species were transferred to plastic pots (2L) containing two different substrates: a very clayey reference soil (unmined substrate), from areas adjacent to an iron mine; and an iron ore tailings (mining substrate) originating from the Pico Mine, belonging to VALE S.A (Table 2). Eight pots were used for each treatment, each containing 3 individuals.

Table 2. Physicochemical classification of the reference soil (control) and iron ore tailings

Soil traits	Reference soil	Iron ore tailing
P (mg dm^{-3})	1.0	8.33
K (mg dm^{-3})	44	0
Ca^{2+} (cmol$_c$ dm^{-3})	0.29	0.31
Mg^{2+} (cmol$_c$ dm^{-3})	0.13	1.07
Fe (mg dm^{-3})	19.9	33.1
Mn (mg dm^{-3})	6.7	26
Zn (mg dm^{-3})	0.45	0.64
Cu (mg dm^{-3})	0.17	0.45
Cr (mg dm^{-3})	0	0
Ni (mg dm^{-3})	0.92	0
Cd (mg dm^{-3})	0.44	0.23
Pb (mg dm^{-3})	0.77	1.13
Al^{3+} (cmol$_c$ dm^{-3})	1.07	0
H + Al (cmol$_c$ dm^{-3})	10.3	0.6
SB (cmol$_c$ dm^{-3})	0.53	0.32
ECEC$_t$ (cmol$_c$ dm^{-3})	1.6	0.32
CEC$_T$ (cmol$_c$ dm^{-3})	10.83	0.92
V (%)	4.9	34.8
m (%)	66.9	0
res-P (mg L^{-1})	4.7	17.2
pH H_2O	4.39	5.48
Coarse sand (kg kg^{-1})	0.145	0.085
Fine sand (kg kg^{-1})	0.066	0.347
Silt (kg kg^{-1})	0.06	0.493
Clay (kg kg^{-1})	0.729	0.075
Textural classification	Very clay	Loam

Legend: H + Al. potential acidity; SB sum of exchangeable bases; ECEC$_t$ effective cation exchange capacity; CEC$_T$ cation exchange capacity at pH 7; V base saturation index; m aluminum saturation index; res-P residual phosphorus.

2.6. Characterization of growth functional attributes

Destructive morphological evaluations were performed at time zero, when the seedlings were transferred to plastic pots containing the reference soil and waste, and at the end of the experiment after 326, 469 and 301 days for *J. caroba*, *M. splendens* and *P. mediterranea*, respectively. Five replicates were used for morphological analyses. Leaf area was obtained using a portable scanner and the Image J Area Measurement Software. Curved leaves were cut into smaller pieces and positioned so that the surface was completely scanned. The dry biomass of roots, stems and leaves was determined with an analytical balance (accurate to 0.01 mg) after 96 hours of drying in an oven at 60º C. With these results, the leaf mass per area (LMA) was calculated as LMA = W_L/A_F; it was also calculated the accumulation of total dry matter (W_T) = $W_R+W_S+W_L$; the relative growth rate (R) $=(1/W_T)*(dW_T/dT)$; the net assimilation rate (*NAR*) = $(1/\ LA)*(dW_T/dT)$ and the leaf area ratio (F_A) = LA / W_L according to Hunt (1979). In which: LA = leaf area; W_L = leaf dry mass; W_R = dry root mass; W_S = stem dry mass; W_T = total dry mass; and $dW_T = W_{T2}-W_{T1}$ and $dT = T_2-T_1$. At the end of the experiment, the number of nodules present in the roots of *P. mediterranea* was quantified.

The number of leaves, stem length (Daughtry *et al.*) and diameter (Daughtry *et al.*) were measured weekly throughout the experimental period. A total of 5 individuals for each species were sampled under control substrate and iron ore tailing.

2.7. Characterization of the functional attributes of leaf phenology

In order to evaluate the leaf development of *J. caroba*, *M. splendens* and *P. mediterranea*, two buds from each replicate were marked before leaf emergence to determine the leaf expansion interval (LEI, days to full expansion), leaf expansion rate (LER, leaf area divided by LEI, mm^2 $days^{-1}$) and leaf life span (LLS, days). The length and width of each leaf were recorded weekly until growth stopped, at which time the LER was calculated. The LLS was recorded weekly, and the leaves were considered finished when ~90% of the total leaf area was yellowish or when the leaf underwent natural plant abscission. Leaf phenology analyses were performed with 10 replications.

2.8. Characterization of photosynthetic attributes

At the end of the experiment, after 326, 469 and 301 days for *J. caroba*, *M. splendens* and *P. mediterranea*, respectively, total chlorophyll, chlorophyll *a* and chlorophyll *b* indices were evaluated with the ClorofiLOG portable meter (CFL1030, Falker, Brazil). Three measurements were taken on the middle portion of the third fully expanded leaf, and the average of the three measurements was calculated as a replicate. Chlorophyll *a* fluorescence was also evaluated in the same leaves used for chlorophyll measurements, using a Mini-PAM fluorometer (Heinz Walz, Effeltrich, Germany). Initial fluorescence (F_0) and maximum quantum efficiency of photosystem II (PSII) (F_v/F_m) were determined in dark-adapted leaves for at least 30 minutes (Genty et al. 1989). Posteriorly, the leaves were exposed to the light conditions where plants grown for at least 10 min and the rapid light response curves were obtained by using the Mini-PAM fluorometer to determine the instantaneous capacity of photosynthetic response to excessive solar radiation. The leaves were exposed to increasing actinic light intensities (0–2000 µmol m^{-2} s^{-1}) during nine 10^{-s} intervals, and the steady-state fluorescence (F_s) and maximum light-adapted fluorescence (F_m') were measured. At each light intensity, we measured the effective quantum yield of PSII (ϕ_{PSII}); apparent electron transport rates through PSII (Manzoni *et al.*) and non-photochemical quenching (NPQ).

2.9. Nitrogen concentration in plant tissues

At the end of the experiment, after 326, 469 and 301 days for *J. caroba, M. splendens* and *P. mediterranea*, respectively, the seedlings were separated into root and shoot, washed and dried in an oven at 75 °C for 72 hours. Nitrogen (N) concentrations were determined using the Kjeldahl method (Tedesco *et al.*, 1995; de Oliveira, 1997).

2.10. Statistical analysis

The experiment followed a randomized block design with factorial arrangement (3×2) consisting of two substrates (control and iron ore tailing) and three species (J. *caroba*, *M. splendens* e *P. mediterranea*). We calculated the mean and standard error for all physiological, morphological, and morphometrical variables. An analysis of variance was used to test differences between substrates treatments and also its interaction with species. A posteriori Tukey’s test was applied a 5% significance level. Correlation analysis was performed using the corrplot, Hmisc and Performance Analytics statistical packages. Correlations with "x" are non-significant interactions with p>0.005. The color intensity and the size of the circles represent the correlation coefficients, with blue being positive and red being negative.The program R was utilized to perform all statistical analyses (R Core Team, 2014).

3. RESULTS

3.1. The seed germination percentage was not affected by the iron ore tailing substrate

Seeds of the three species showed a germination percentage above 73%, and there was no difference between the substrates. From the 4th day after sowing (DAS) all the seeds had already started to germinate, however, until the 10th day the germination percentage of *J. caroba* seeds in the control was higher, reaching an equal percentage at the end of the evaluated period (Fig. S1). Only *J. caroba* seeds showed differences between substrates for the germination speed index, with higher speed in the control substrate (Table 3).

Table 3. Germination speed index and germination percentage germination of *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) in control and iron ore tailings treatments

	Germination speed index		**Germination percentage**	
Species	**Control**	**Iron ore tailing**	**Control**	**Iron ore tailing**
J. caroba	43.84±1.30*	36.66±1.58	95.00±17.11	97.5±17.85
M. splendens	41.82±1.21	37.82±1.67	90.00±5.54	96.67±6.28
P. mediterranea	19.72±2.01	25.61±1.37	73.33±6.13	86.67±4.75

* It means statistical difference between substrates according to Tukey's test at 5% probability ± standard error.

3.2. Species in tailings substrate showed decreases in functional growth attributes

When growing in the control treatment, all species showed a greater gain in dry matter, leaf area and relative growth rate (Fig. 1). The net assimilation rate (*NAR*) was higher in plants from the control substrate only for *M. splendens* and *P. mediterranea*. Regarding the leaf area ratio and leaf mass per area, there was no difference between treatments (Fig. 1). The morphological variables showed a greater number of leaves, plant height and stem diameter for plants grown in the control treatment over time. Prolonged exposure to tailings caused a drop in these variables mainly at the end of the experiment (Fig. S2).

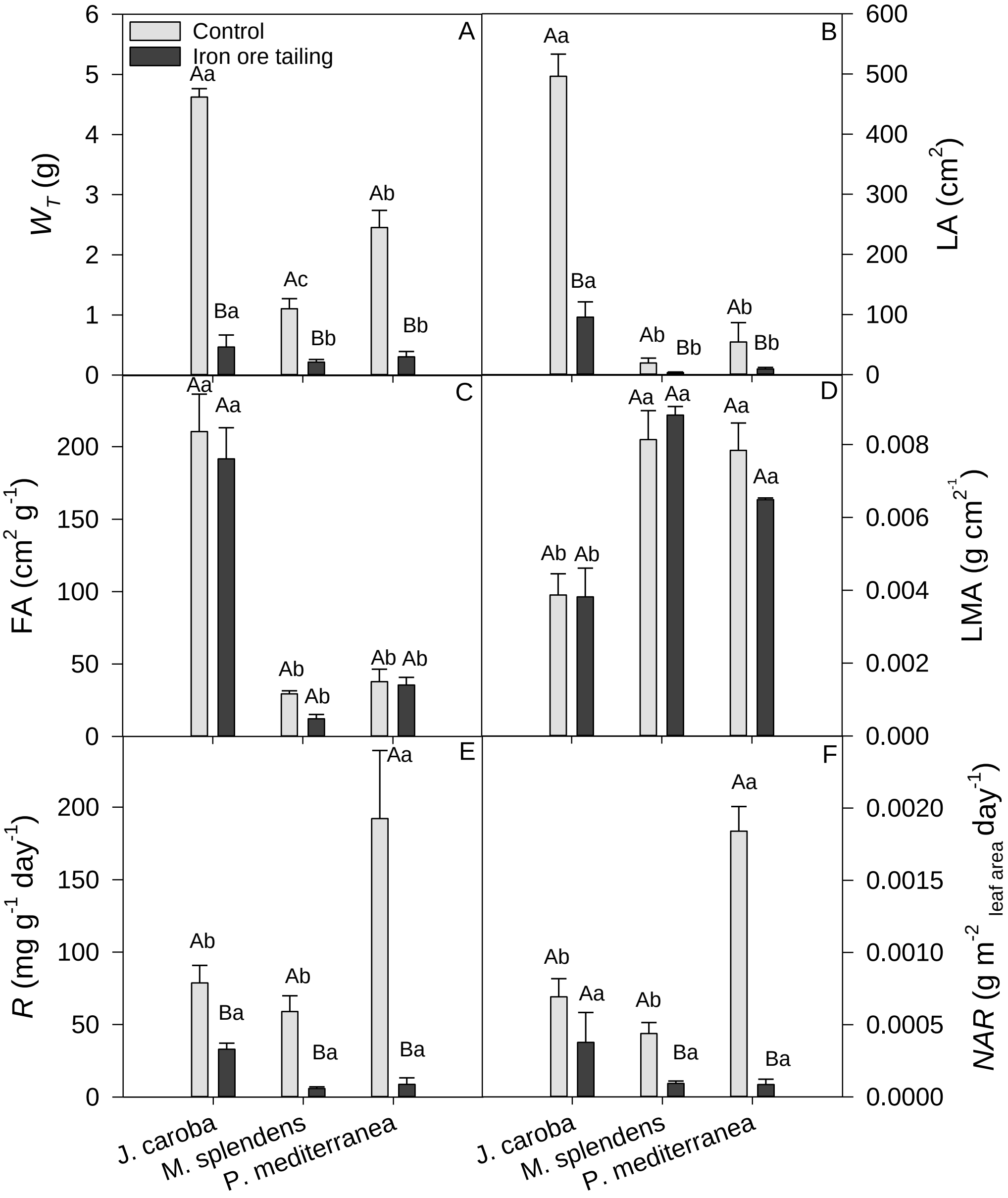

Figure 1. Functional attributes of growth of *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) in iron ore tailing and control treatments: total dry matter accumulation (W_T; A), leaf area (LA; B), leaf area ratio (F_A; C), leaf mass per leaf area (LMA; D), relative growth rate (*R*; E) and net assimilation rate (*NAR*; F). Capital letters means statistical difference between substrates for each species and lowercase letters mean statistical difference between species for each substrate, according to Tukey's test at 5% probability.

3.3 Differences in functional attributes of leaf phenology were observed between species

When grown in the control treatment, *J. caroba* showed the highest rate of leaf expansion (LER), followed by the species *P. mediterranea* and *M. splendens*, respectively. When in tailings, the three species did not differ (Fig. 2A). *J. caroba* and *P. mediterranea* had higher LER in the control soil treatment, *M. splendens* had no difference between substrates. The substrates in which all evaluated species were exposed did not changed the leaf life span (LLS). However, there was a significant difference in LLS between the species, being greater for *M. splendens*, followed by *P. mediterranea* and *J. caroba*, respectively (Fig. 2B). The leaf expansion interval (LEI) was on average 26 days, not differing between the species and treatments analyzed, except for *J. caroba* in tailings, in which the lowest values were observed when compared to the other species and when compared to the results of the species in the control (Fig. 2C).

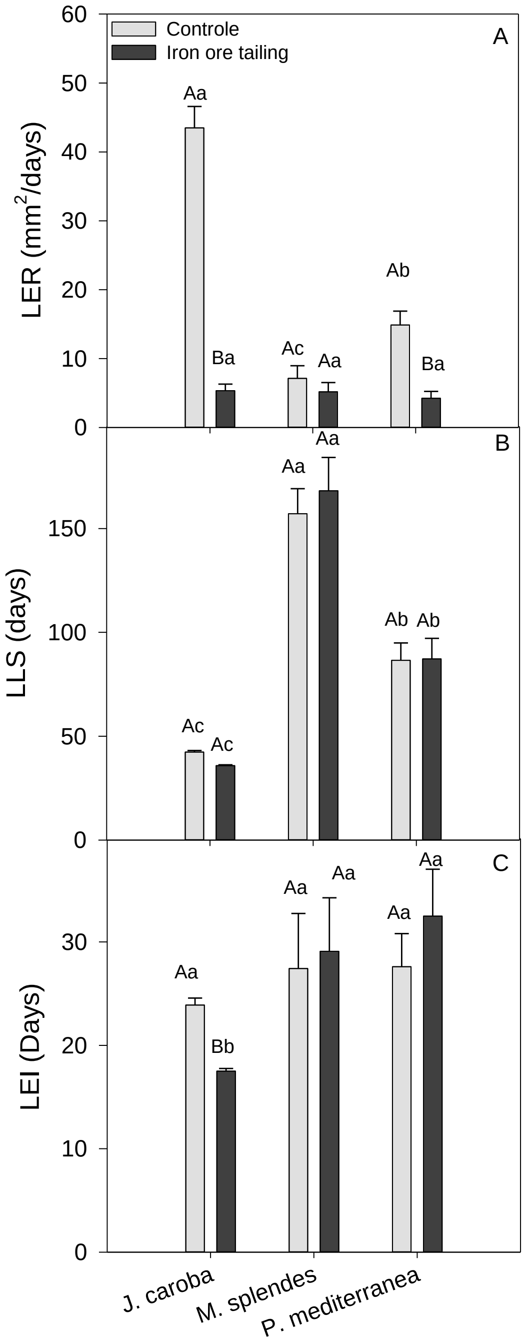

Figure 2. Functional attributes of leaf phenology of *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) in iron ore tailing and control treatments: leaf expansion rate (LER; A), leaf lifetime (LLS; B) and leaf expansion interval (LEI; C). Capital letters mean

statistical difference between substrates for each species and lowercase letters mean statistical difference between species for each substrate, according to Tukey's test at 5% probability.

3.4. The iron ore tailings caused a decrease in photochemical responses of the analyzed functional groups

A reduction in the total chlorophyll content of *M. splendens* and *P. mediterranea* species was observed when in the tailings. When comparing the species, the highest values of total chlorophyll were observed for *M. splendens* and *P. mediterranea* grown in the control substrate. The opposite was observed when the species were grown in the tailings, that is, the greatest increments of total chlorophyll were observed for *J. caroba* (Fig. 3A). The iron ore tailings caused a reduction in initial fluorescence only for *J. caroba* (Fig. 3B). However, there were no consequences on the maximum quantum efficiency of the PSII, since the F_v/F_m values remained constant in both substrates, with the exception of *M. splendens* when grown in iron ore tailings (Fig. 3C). However, *J. caroba* showed higher effective quantum yield of PSII when exposed to tailings and consequently reduced energy dissipation in a regulated manner. On the other hand, *M. splendens* despite reducing ϕ_{PSII} when subjected to tailings, still showed an efficient strategy, which consists of greater energy dissipation in a regulated manner (Figs. 3D and E). Finally, for *P. mediterranea* there is no difference between the substrates for any energy partitioning parameter (F_v/F_m, F_0, ϕ_{PSII}, ϕ_{NPQ} and ϕ_{NO}). The quantum yield of non-photochemical energy dissipation in an unregulated manner was equal between substrates for all species (Fig. 3F).

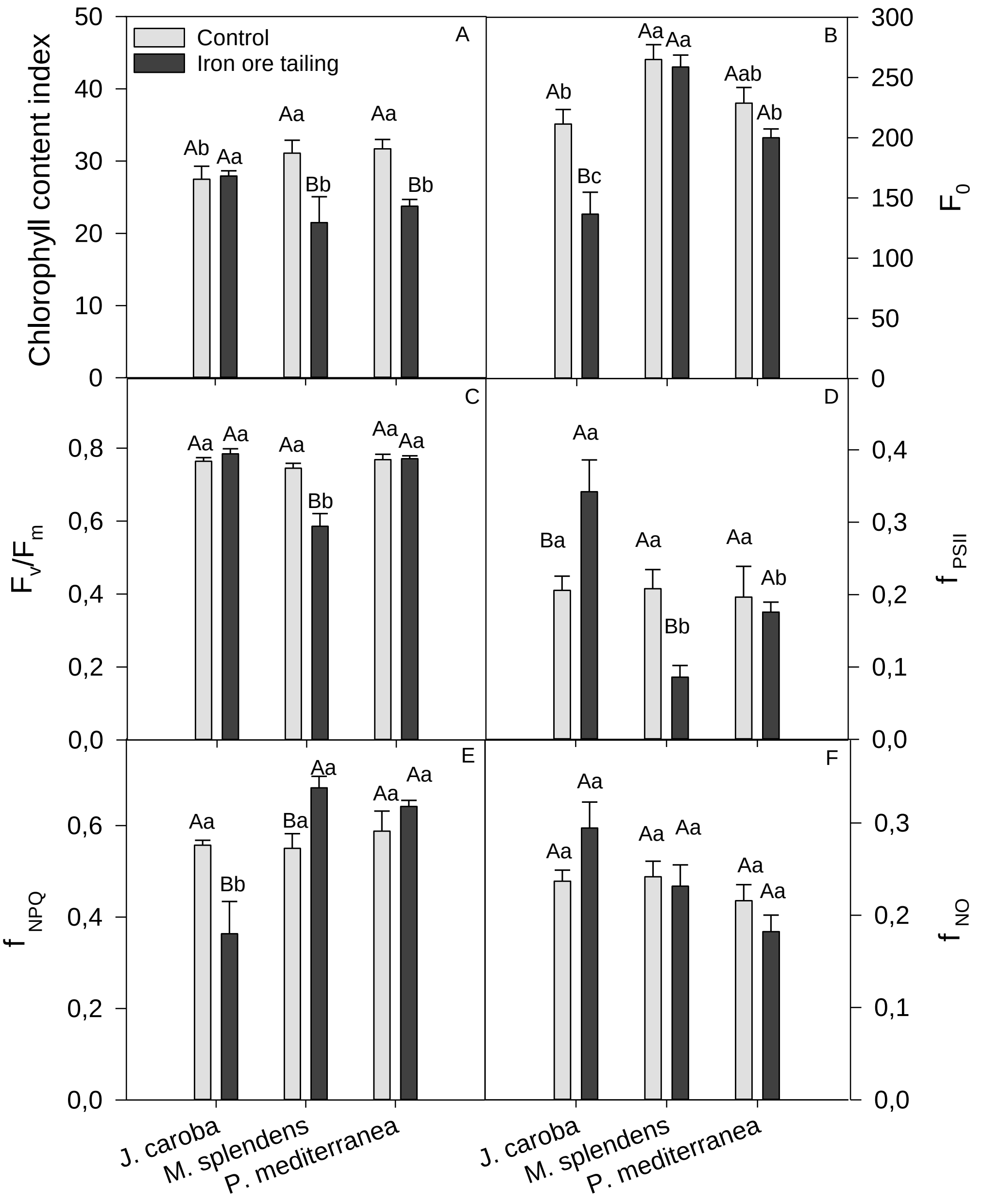

Figure 3. Analysis of chlorophyll content index (A), initial fluorescence (F_0; B), maximum quantum efficiency of the PSII (F_v/F_m; C), effective quantum yield of the PSII (ϕ_{PSII}; D), quantum yield of regulated form non-photochemical energy dissipation of PSII (ϕ_{NPQ}; E) and quantum yield of non-photochemical energy dissipation in an unregulated form of PSII (ϕ_{NO}; F), performed at the end of the experiment *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) in iron ore tailings and control treatments. Capital letters mean statistical difference between substrates for each species and lowercase letters mean statistical difference between species for each substrate, according to Tukey's test at 5% probability.

3.5. Functional groups present different photoprotection capacity when exposed to progressive increase in light intensity

By increasing the light intensity, a reduction in the effective quantum yield of PSII was observed for all species in both substrates. *J. caroba* showed differences between treatments only when exposed to high light intensities (above 730 µmol photons m^{-2} s^{-1}), with the highest values of ϕ_{PSII} observed in the tailings plants. On the other hand, *M. splendens* showed differences between treatments during exposition to all irradiances, with higher ϕ_{PSII} observed in plants from the control treatment (Figs. 4A - C). There was a significant difference between treatments for non-photochemical quenching (NPQ) only for the species *P. mediterranea*, with greater values for plants in the tailing substrate along all points of the curve (Figs. 4D - F). When in the tailing, *J. caroba* showed increases in the electron transport rate (Manzoni *et al.*) in all irradiances. *M. splendens* in the control substrate showed higher ETR values. For *P. mediterranea*, no difference was observed between the substrates for the ETR values (Fig. 4G - I).

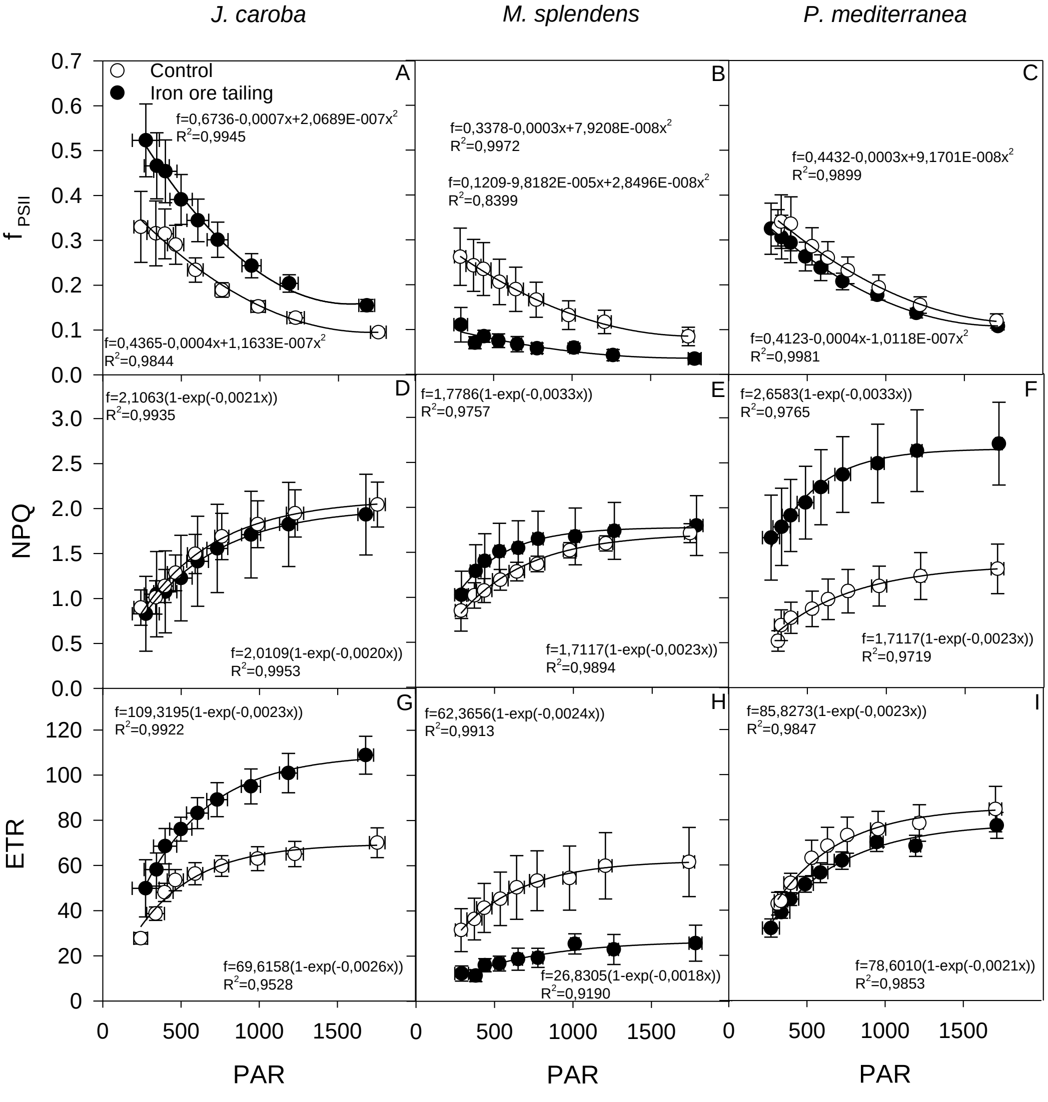

Figure 4. Light curve analysis performed at the end of the experiment with *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) in iron ore tailing and control

tailings treatments.

3.6. Exposure to tailings caused a reduction in N uptake for some species

As for N concentration in shoots, was higher for *J. caroba* in the tailings and for *M. splendens* when in control substrate (Fig. 5A). In the roots, the N concentration was higher for *P. mediterranea*, mainly for the control substrate (Fig. 5B). For the N-fixing species, *P. mediterranea*, the formation of nodules was observed in the roots, with an average of 1.8 and 6 nodules per roots in the iron ore tailing and control treatments, respectively. Despite the smaller number of nodules present in the roots from plants of the tailing treatment, these were larger, unlike the control treatment, where small and numerous nodules were observed.

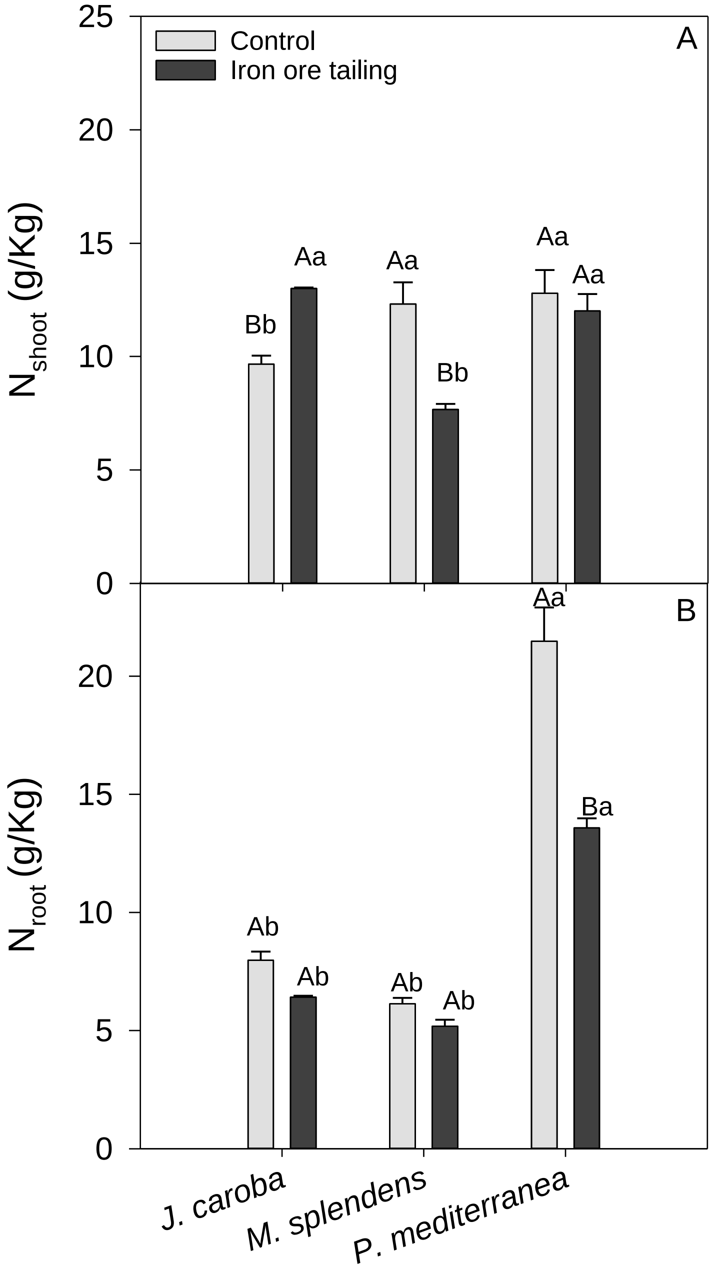

Figure 5. Nitrogen concentration in the shoot (A) and root (B) of *J. caroba* (deciduous shrub), *M. splendens* (widespread semideciduous) and *P. mediterranea* (nitrogen-fixing) in iron ore tailing and control treatments. Capital letters mean statistical difference between substrates for each species and lowercase letters mean statistical difference between species for each substrate, according to Tukey's test at 5% probability.

3.7. Correlation between morphofunctional and photochemical attributes of plants in control substrate and iron ore tailing

The plants from the tailings treatment showed a greater correlation between the functional and photochemical attributes. In the iron ore tailings, a positive correlation was observed between the following attributes: LA, *FA* and *NAR* with ϕ_{PSII} and ETR_{max}; W_T with

ETR_{max} and N_{shoot} and negative correlation between LMA with ϕ_{PSII}; LLS with F_v/F_m, ϕ_{PSII}, ETR_{max} and N_{shoot}. In the control treatment, there was a positive correlation between LA and *FA* with the ratio of chlorophyll *a/b*; *NAR* with N_{root}. Negative correlation was observed between W_T and LER with chlorophyll *b* indices (Fig. 6).

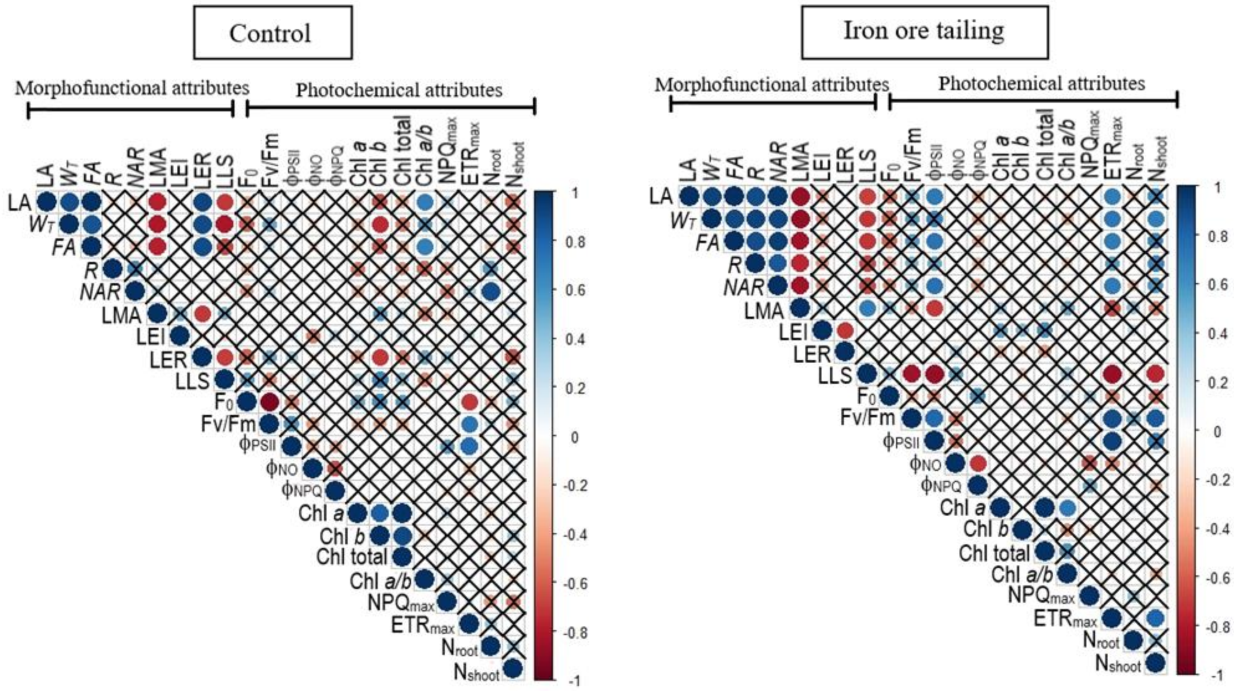

Figure 6. Correlation matrix between morphofunctional attributes (LA = leaf area; W_T = total dry matter accumulation; *FA* = leaf area ratio; *R* = relative growth rate; *NAR* = net assimilation rate; LMA = leaf mass per leaf area; LEI = leaf expansion interval; LER = leaf expansion rate; LLS = leaf lifetime) and photochemical attributes (F_0 = initial fluorescence; F_v/F_m = maximum quantum efficiency of the PSII; ϕ_{PSII} = effective quantum yield of PSII; ϕ_{NO} = quantum yield of unregulated dissipation of non-photochemical energy from PSII; ϕ_{NPQ} = quantum yield of regulated dissipation of non-photochemical energy from PSII; Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*; Chl total = total chlorophyll; Chl *a/b* = ratio between chlorophyll a/b; NPQ_{max} = maximum non-photochemical extinction; ETR_{max} = apparent rate of maximum electron transport; including also N_{root} = nitrogen concentration in the root; N_{shoot} = nitrogen concentration in the shoot). Blue circles represent positive correlations and red circles negative correlations, while the greater size of the circle and the greater intensity of the color represent stronger correlations. X represents non-significant interactions (>5% probability).

4. DISCUSSION

The analyzed species from different functional groups showed satisfactory germination and distinct responses during development when cultivated in mining substrate. The difference in functional traits between species was directly related to the functional group to which it belongs. However, regardless of the functional group, there was a decrease in the values of the variables of biomass, growth rate and leaf area. All individuals in the iron ore tailings substrate had slower growth, thus, they could not reach the same level of biomass accumulation, height, leaf area, number of leaves and stem diameter, when compared to plants from the control treatment. The semideciduous widespread species, *M. splendens*, with longer LLS was not affected regarding the functional attributes of leaf phenology during its development in the tailings. However, a reduction in photosynthetic attributes was observed, but with greater dissipation of non-photochemical energy in a regulated manner, contributing to the photoprotection mechanism. The endemic deciduous species, *J. caroba*, presenting morphofunctional attributes that included leaves with large LA and lower LMA, shorter LLS and faster LEI, showed unchanged net assimilation rate even when in tailings. It is also related to the satisfactory photosynthetic attributes of this species, with high values of total chlorophyll, and effective and maximum quantum efficiency of PSII, even when exposed to iron mining tailings. Finally, the symbiosis with nitrogen-fixing bacteria allowed the leguminous species *P. mediterranea* to present a higher concentration of nitrogen in plant tissues. However, for the plants in the tailings substrate, despite the reduction in the LER and *NAR*, the photosynthetic attributes were not altered, except for the chlorophyll content and increased non-photochemical dissipation of excess light.

The nutritional conditions imposed by the iron mining tailings substrate, such as acidic pH, presence of metals, including a high concentration of Fe and low nutritional contents, limited plant growth (Oliveira *et al.*, 2015; Silveira *et al.*, 2016). The negative effects of the tailings on the analyzed species were observed, such as lower biomass production, lower plant height, lower leaf area, lower relative growth rate, lower net assimilation rate and lower chlorophyll content indicating a probable nutritional deficit, phytotoxic effects and possible water stress present in the tailings. In a study developed by Cruz *et al.* (2022), when adequate levels of the main nutrients for plant growth were guaranteed, fertilization did not reverse the negative effect of the tailings. Despite the limiting nutritional conditions of the substrate, the concentration of N present in the plant tissues of *P. mediterranea* was higher than the other species, even when in tailings. The association of symbiosis with N-fixing bacteria probably provided the highest concentration of N. The same is observed in studies using inoculum as an ecological alternative to inorganic additives directly in the area affected by iron ore tailings (Santos *et al.*, 2022). For the other species, decreases in N concentration in plant tissues when grown in tailings were observed only for *M. splendens*, showing that the nutritional conditions of the tailings did not compromise the absorption of this essential nutrient.

In addition to the soil attributes, the functional characteristics of the seeds are also very important during the process of ecological restoration of degraded areas, since germination will be the initial process and its success guarantees satisfactory results in the future. Seed size is directly related to its germination (Leishman *et al.*, 2000), as well as environmental factors such as temperature, humidity, light, soil characteristics and dispersion time (Khurana & Singh, 2001). The studied species have small seeds, being the winged seeds of *J. caroba* were the smallest and lightest, which favors their anemochoric dispersion (Dácio *et al.*, 2021). Because they are small seeds, they have little mass and reserve, however they germinated well and relatively quickly. The establishment of seedlings may be more compromised, but for germination, once dormancy is overcome, only water, light and adequate temperature are needed (Garcia *et al.*, 2020). The small size of the seeds corroborates what was expected, since disturbed habitats tend to have species with small seeds that need light to germinate (Leishman *et al.*, 2000; Milberg *et al.*, 2000; Fenner *et al.*, 2005). Garcia *et al.* (2020) also verified this predominance in species from *campo rupestre*. Such characteristics bring advantages to the restoration processes of mined areas as it facilitates the collection, handling and sowing.

In addition to easy applicability, the species selected for restoration processes must be resistant and able to survive these degraded environments (Figueiredo *et al.*, 2018). However, it is initially necessary that the selected species can be able to germinate under these conditions since seed development is influenced by both the genotype and the environment (Bewley *et al.*, 2013). The studied species, even being native to the *canga* environment, showed great germination capacity and seed viability. In severe habitats with nutritional deficit, such as *campos rupestres*, the presence of species with empty, dormant and unviable seeds is common. Seed germination and dormancy are complex physiological processes that are strongly affected by environmental factors and regulated by several endogenous hormones (Garcia *et al.*, 2020). However, herbaceous species of *campo rupestre* present a great diversity of germination strategies, with some groups of species capable of producing non-dormant seeds with rapid germination (Le Stradic *et al.*, 2015). The germination success of the studied species is of particular importance, since, in the context of recovery of degraded areas, germination is a key component, which provides subsequent development and a future establishment of the species.

Despite the success of seed germination in the tailings, the functional growth attributes throughout the development of the species were affected. The larger area and smaller leaf thickness of the endemic deciduous species, *J. caroba*, is relevant in the light interception process and is related to energy absorption and plant water balance (Wright *et al.*, 2017). On the other hand, *M. splendens* and *P. mediterranea* invested in smaller and thicker leaves, a functional characteristic common to species from other nutrient-poor habitats (Poorter & Remkes, 1990). Even with functional differences, when cultivated in tailings the responses were similar. The smaller height and leaf area associated with the reduced number of leaves resulted in a lower net assimilation rate of the mining tailings species, which is positively correlated with the effective quantum yield of PSII and electron transport. However, this reduction in photosynthetic efficiency of plants in tailings was observed only for *M. splendens*. Different from the morphofunctional characteristics, the physiological responses related to photosynthetic adjustments differed among the species cultivated in the tailings. Emphasizing the importance of this responses as a tool in the process of selection of species to be used in initiatives for the recovery of degraded areas (Rios *et al.*, 2022).

Deciduous plant species, such as *J. caroba*, are characterized by the complete loss of leaves throughout their development, so most of the time they do not invest in energetically expensive leaves (Castro-Díez *et al.*, 2000; Poorter *et al.*, 2009; Sancho-Knapik *et al.*, 2021). These species have predominantly lower LMA content and shorter leaf life. On the other hand, semideciduous species, such as *M. splendens*, invest in thicker leaves with higher LMA and LLS (Wright *et al.*, 2005; Kikuzawa *et al.*, 2013; de la Riva *et al.*, 2016). However, the functional groups showed different responses when in mining tailings. There were decreases in LER only for the species *J. caroba* and *P. mediterranea* and in LEI for *J. caroba*. These species have higher growth rates (R) and biomass (W_T). The semideciduous, arboreal and slower growing species, *M. splendens*, did not have the leaf phenology parameters affected by the tailings. The physical characteristics of the mine tailings are limiting the growth of plants, the low concentration of clay, as well as the high concentration of silt and fine sand providing the tailings substrate with greater drainage and consequently less water retention (Da Silva *et al.*, 2014; de Matos *et al.*, 2020). As well as the nutritional characteristics of the tailings, such as high concentrations of iron oxide, silica and aluminum as well as the presence of some heavy metals such as cadmium, chromium, arsenic and mercury, even when in very small concentrations (Zheng *et al.*, 2016; Geng *et al.*, 2020).

Although the three studied species belong to different functional groups, growth data and morphofunctional variables were similarly affected when in tailings. However, *J. caroba, M. splendens* and *P. mediterranea* presented different physiological strategies when they were developed in tailings substrate, in this way the physiological adjustments were important to differentiate the representative responses of each functional group. Although species have distinct physiological characteristics, all strategies are beneficial and guarantee their adaptive success. The ability to safely dissipate excess energy through protective regulatory mechanisms or by photochemistry was demonstrated by NPQ values statistically equal between substrates for *J. caroba* and *M. splendens* or increased for *P. mediterranea* after development in tailings.

The lower concentration of N present in the leaves of *M. splendens* when in tailings resulted in lower levels of chlorophyll. As nitrogen is a structural element of chlorophyll and protein molecules, its lower absorption affects the formation of chloroplasts and the accumulation of chlorophylls (Daughtry *et al.*, 2000). The reduced chlorophyll index after development in tailings for the species *M. splendens* is related to the reduction in the values of F_v/F_m, ϕ_{PSII} and ETR, indicating a loss in the energy of transfer of the antenna pigments to the reaction center and greater susceptibility to photoinhibition with reduced effectiveness of PSII in primary photochemical reactions. These are ultimately related to lower relative growth rate, lower net assimilation rate and longer leaf life span, functional attributes characteristic of slow-growing semideciduous tree species (Souza *et al.*, 2009). However, the absence of difference in the values of ϕ_{NO} as well as the increase of ϕ_{NPQ} when growing in tailings indicate the capacity for maintenance and resilience, common characteristics that allow the *M. splendens* to occupy extensive geographic areas.

Deciduous species are characterized by the formation of plagiotropic branches and larger leaf area when compared to semideciduous species. This distribution of branches prevents self-shading and allows the irradiance to be evenly distributed throughout the plant (Kikuzawa *et al.*, 1996; Kikuzawa, 2003) and the maximum net photosynthesis to be equivalent between the basal and distal leaves (Souza *et al.*, 2009). They are also species with rapid leaf expansion and shorter leaf life span (Wright *et al.*, 2005; Kikuzawa *et al.*, 2013). Such functional characteristics describe the deciduous endemic species *J. caroba*, even when in tailings, the greater leaf area associated with the lower LLS are related to higher chlorophyll contents and higher yield and maximum quantum efficiency of PSII. As the increases in the values of ETR and ϕ_{PSII} for *J. caroba* in the tailings indicate the ability of the species to assimilate N when exposed to gradual increases in irradiance, they reinforce the ability of the species to maintain its photosynthetic performance even when exposed to area soils degraded by mining.

P. mediterranea, as an herbaceous legume species (Funch & Barroso, 1999; Viana & Lombardi, 2007), presents greater initial growth capacity, as observed by the functional attributes, relative growth rate, net assimilation rate, height, number of leaves and stem diameter. The symbiosis with nitrogen-fixing species promoted a greater accumulation of this nutrient in plant tissues, demonstrating that the main source of N is represented by biological nitrogen fixation via *P. mediterranea* (Nogueira *et al.*, 2019). However, the absence of difference in the concentration of N in the shoot as well as the reduction of levels in the roots when exposed to mining tailings resulted in lower chlorophyll contents (Daughtry *et al.*, 2000). However, the other photosynthetic characteristics were not affected.

The use of resistant native species suitable for water and nutrient management, imposed by climatic seasonality and soil conditions, respectively, constitutes the most economical and efficient way to recover degraded environments by iron mining. However, the choice of species that present satisfactory development and adaptive resistance strategies requires knowledge about their germination, development and physiology responses under tailings conditions. Despite belonging to different functional groups, when in tailings, the different functional characteristics cancel each other out, in this way the physiological responses better explained the adaptive strategies developed by the species. The correlation between some morphofunctional and photochemical attributes, especially for plants grown in tailings, allows the use of photochemical variables in species selection projects, monitoring, and development of management strategies, among others. However, this correlation may change according to the species and functional group studied.

5. CONCLUSION

The substrate from iron ore mining tailings areas did not affect the germinability of *J. caroba*, *M. splendens* and *P. mediterranea*. However, the chemical and physical characteristics of the tailings limited the growth of the species.

The widespread *M. splendens*, was the species with development most affected by the conditions imposed by the iron ore mining tailings. The symbiosis with N-fixing species favored the accumulation of this nutrient by the species *P. mediterranea,* which provided satisfactory growth and maintenance of photosynthetic attributes even when in iron ore tailing. And *J. caroba* even being an endemic species, had its growth in the tailings favored by its adaptive photochemical characteristics.

The physiological analyses showed different adaptive strategies in the use of light energy for the species when exposed to the stress conditions imposed by the tailings. The endemic species, *J. caroba* in the tailings increased the effective quantum yield of PSII. The widespread semideciduous species, *M. splendens*, increased energy dissipation in a regulated manner. And *P. mediterranea*, a species associated with N-fixers, showed no difference between the substrates.

The correlation between the morphofunctional and photochemical variables in the tailings plants allows us, in species selection processes to be used in projects for the recovery of degraded areas, to suggest the use of methods in plant ecophysiology capable of evaluating physiological responses, mainly photochemical, because they are able to better explain the adaptive strategies developed by the species, guaranteeing the success of revegetation.

6. DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

7. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. All authors approved the manuscript.

8. DECLARATION OF FUNDING

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SUPPLEMENTARY MATERIAL

Functional Plant Biology

Photochemical rather than morphofunctional attributes are decisive in the responses of native plant species from ferruginous outcrops when grown in iron mining substrates

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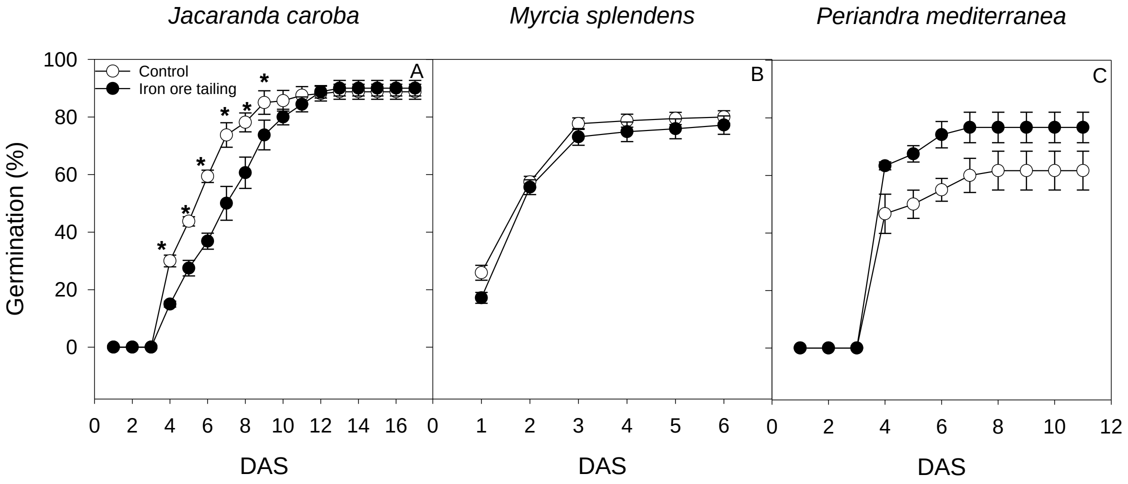

Figure S1. Percentage of germination performed during the experiment with *J. caroba* (deciduous shrub), *M. splendens* (widespread evergreen) and *P. mediterranea* (nitrogen-fixing) in control and iron ore tailings treatments. * means that there was statistical interaction between substrates and DAT, according to Tukey's test at 5% probability.

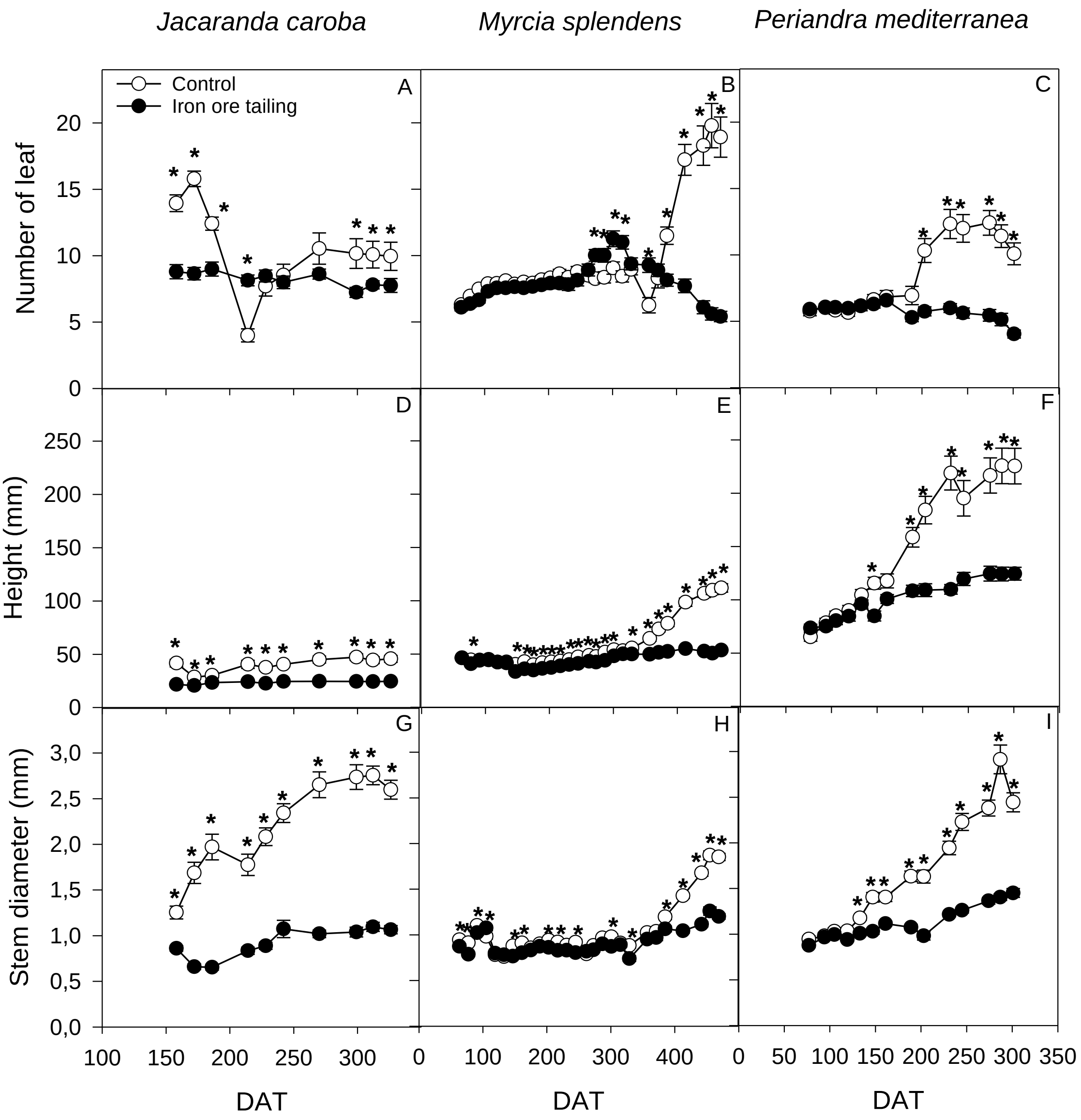

Figure S2. Non-destructive morphological assessments. Leaf number, plant height and stem diameter performed throughout the experiment with *J. caroba* (deciduous shrub), *M. splendens* (widespread evergreen) and *P. mediterranea* (nitrogen-fixing) in control and iron ore tailings treatments. * means that there was statistical interaction between substrates and days after treatment (DAT), according o Tukey's test at 5% probability.

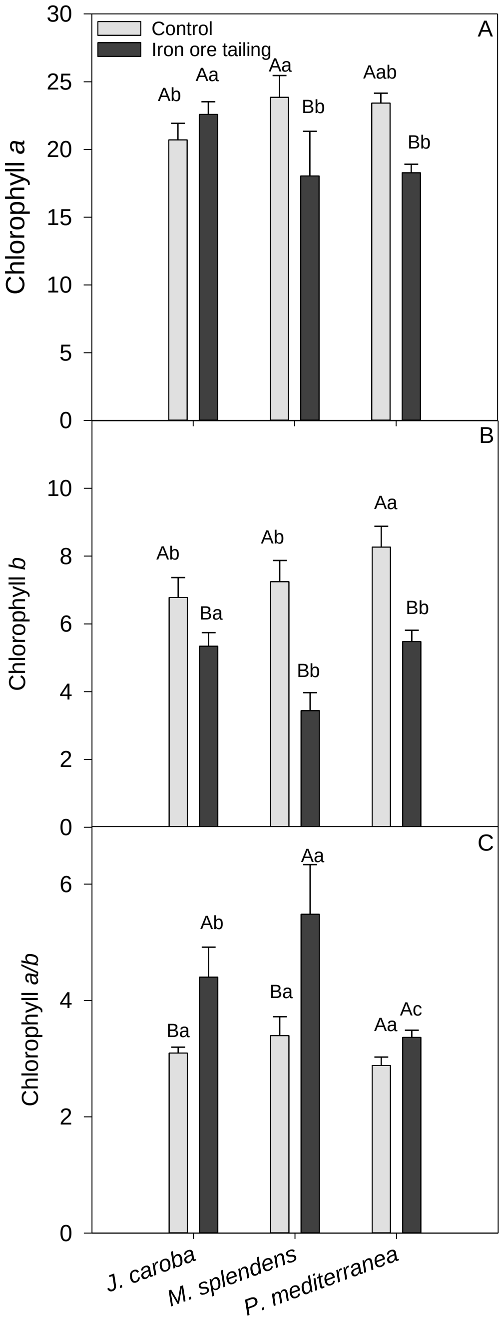

Figure S3. Chlorophyll *a*, *b* and *a/b* indices performed at the end of the experiment with *J. caroba* (deciduous shrub), *M. splendens* (widespread evergreen) and *P. mediterranea* (nitrogen-fixing) in control and iron ore tailings treatments. Capital letters mean statistical difference between substrates for each species and lowercase letters mean statistical difference between species for each substrate, according to Tukey's test at 5% probability.

CONSIDERAÇÕES FINAIS

Nesta tese, demonstramos que as condições abióticas, tais como escassez de recursos edáficos e ambientes sazonalmente secos, atuam como filtros ambientais selecionando características morfofuncionais e fotoquímicas, que promovem maior dissipação do excesso de energia e evitam danos oxidativos. Mudanças na utilização da energia luminosa apontam para diferentes estratégias adaptativas das espécies vegetais da canga e permitem o estabelecimento sobre condições abióticas severas. Os parâmetros, F_v/F_m, índices de clorofila, ϕ_{PSII}, ϕ_{NPQ} e acúmulo de amido são eficazes na seleção de espécies a serem utilizadas na recuperação de áreas degradadas. Uma vez que:

- As espécies do campo rupestre ferruginoso mostraram capacidade de responder a múltiplus estressores, através da manutenção ou incremento de parâmetros chave como F_v/F_m, índices de clorofila, ϕ_{PSII} e ϕ_{NPQ}.
- O acúmulo de amido na parte aérea foi uma das respostas utilizadas pelas espécies estudadas durante o déficit hídrico, o que contribuiu para os aumentos nos valores de LMA e LSI
- Durante período de menor precipitação as espécies demonstraram estratégias de competição e tolerância ao estresse.

Mudanças nos traços funcionais e mecanicistas das espécies, ao longo da sazonalidade climática, indicaram que as características particulares do solo de cada microambiente são determinantes na resiliência das espécies e estas apresentam diferentes níveis de correlação com o ambiente. Uma vez selecionadas em campo, espécies de diferentes grupos funcionais apresentaram respostas diversas quando em rejeito de mineração de ferro. A correlação entre as variáveis morfofuncionais e fotoquímicas nas plantas de rejeitos nos permite, sugerir o uso de métodos em ecofisiologia vegetal para explicar as estratégias adaptativas desenvolvidas pelas espécies, garantindo o sucesso da revegetação.

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