



UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOLOGIA
Programa de Pós-Graduação em Ecologia, Conservação e
Biodiversidade



Como a suplementação de nitrogênio e fósforo pode alterar a
fisiologia de plantas nativas do Cerrado?

Denny Fernandes Eduardo

Uberlândia

2025

Denny Fernandes Eduardo

Como a suplementação de nitrogênio e fósforo pode alterar a
fisiologia de plantas nativas do Cerrado?

Tese apresentada à Universidade Federal de Uberlândia,
como parte das exigências para a obtenção de título de
Doutor em Ecologia, Conservação e Biodiversidade.

Orientadora: Profa. Dra. Ana Silvia Franco Pinheiro Moreira

Uberlândia

Novembro de 2025

Dados Internacionais de Catalogação na Publicação (CIP)
Sistema de Bibliotecas da UFU, MG, Brasil.

E24c
2024

Eduardo, Denny Fernandes, 1989-
Como a suplementação de nitrogênio e fósforo podem alterar a
fisiologia de plantas nativas do Cerrado? [recurso eletrônico] / Denny
Fernandes Eduardo. - 2024.

Orientadora: Ana Sílvia Franco Pinheiro Moreira.
Tese (Doutorado) - Universidade Federal de Uberlândia, Programa de
Pós-graduação em Ecologia, Conservação e Biodiversidade.
Modo de acesso: Internet.
Disponível em: <http://doi.org/10.14393/ufu.te.2024.5068>
Inclui bibliografia.
Inclui ilustrações.

1. Ecologia. I. Moreira, Ana Sílvia Franco Pinheiro, 1980-, (Orient.).
II. Universidade Federal de Uberlândia. Programa de Pós-graduação em
Ecologia, Conservação e Biodiversidade. III. Título.

CDU: 574

André Carlos Francisco
Bibliotecário Documentalista - CRB-6/3408



ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Ecologia, Conservação e Biodiversidade				
Defesa de:	Tese, número 100, PPGECEB				
Data:	dezenove de dezembro de dois mil e vinte e quatro	Hora de início:	14:16	Hora de encerramento:	19:40
Matrícula do Discente:	12023ECR003				
Nome do Discente:	Denny Fernandes Eduardo				
Título do Trabalho:	Como a suplementação de nitrogênio e fósforo pode alterar a fisiologia de plantas nativas do Cerrado?				
Área de concentração:	Ecologia				
Linha de pesquisa:	Ecologia de populações				
Projeto de Pesquisa de vinculação:	Ecofisiologia de Plantas do Cerrado				

Reuniu-se por videoconferência a Banca Examinadora designada pelo Colegiado do Programa de Pós-graduação em Ecologia, Conservação e Biodiversidade assim composta pelos doutores: Luzia Valentina Modolo - UFMG; Gustavo Habermann - UNESP; Tiago Vilas Boas da Silva - UFMG; Daniela Boaneres de Souza - UERJ e Ana Silvia Franco Pinheiro Moreira - INBIO/UFU, orientadora do candidato.

Iniciando os trabalhos a presidente da mesa, Dra. Ana Silvia Franco Pinheiro Moreira, apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir a senhora presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores que passaram a arguir o candidato. Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o candidato:

Aprovado.

Esta defesa faz parte dos requisitos necessários à obtenção do título de Doutor.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.



Documento assinado eletronicamente por **Ana Silvia Franco Pinheiro Moreira, Professor(a) do Magistério Superior**, em 19/12/2024, às 19:46, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Luzia Valentina Modolo, Usuário Externo**, em 20/12/2024, às 15:48, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Gustavo Habermann, Usuário Externo**, em 20/12/2024, às 16:23, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Daniela Boaneres de Souza, Usuário Externo**, em 03/01/2025, às 11:37, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



Documento assinado eletronicamente por **Tiago Vilas Boas da Silva, Usuário Externo**, em 03/01/2025, às 14:03, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do [Decreto nº 8.539, de 8 de outubro de 2015](#).



A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **5887951** e o código CRC **1F2D9259**.

Dedicatória

Dedico esse trabalho ao meu avô Sebastião Eduardo, por incentivar minha curiosidade pelos processos da natureza.

Agradecimentos

Primeiramente, quero agradecer à minha orientadora pela confiança e por me incentivar a explorar o fascinante mundo da fisiologia vegetal e nutrição de plantas do Cerrado.

Gostaria também de expressar minha gratidão aos meus colegas do Programa de Pós-Graduação em Ecologia, Conservação e Biodiversidade pela convivência e apoio durante minha jornada do doutorado.

À Camila Bosenbecker, Patrícia e Lucas Issa que me acolheram nos primeiros dias em Uberlândia até eu conseguir moradia.

Aos colegas do LAFIVE, em especial à Amanda e à Jessica, pelos momentos de discussão do projeto e pela ajuda em laboratório e campo.

Agradeço também à minha família, em especial ao meu avô, pelos sábios conselhos “na hora exata”. À minha irmã pelo apoio emocional durante o desenvolvimento da tese. Ao meu pai por me ajudar em campo e me apoiar como pode, e à minha mãe por me ajudar financeiramente quando precisei.

Ao meu amigo Vinícius Dezute, por facilitar a aquisição das mudas de ipês na secretaria Municipal de Meio Ambiente de Catalão.

Gratidão também pela Ana Luísa Nunes, por me ajudar nas aventuras de campo, laboratório e a me organizar em Belo Horizonte.

Agradeço também ao professor Marcel, por me aceitar no laboratório de Fisiologia Vegetal da UFMG durante o período de mobilidade acadêmica, onde realizei o experimento do terceiro capítulo. Também agradeço à Aldinéia, por me apresentar a UFMG e pela assistência nas análises de clorofila. À Ana Paula Faria, agradeço pelo auxílio na implementação de uma parte do meu experimento na UFMG.

À família Mendonça, agradeço pelos momentos de descontração na comunidade rural Caipora. Em especial à Angie Mendonça, pela parceria e por me apresentar e compartilhar o Arboreto “pequeno mundo das plantas”.

Por fim, agradeço ao CNPq, pelo financiamento da bolsa de estudo, e ao PELD-TMSG (CNPq e CAPES) e MTP pelo suporte logístico, pelo recurso financeiro e por disponibilizar o transporte para as campanhas de campo.

Lista de abreviaturas e acrônimos

CP - Clube Caça e Pesca Itororó

GA - RPPN Galheiros

GL - Fazenda Experimental Glória

PE - Parque Estadual da Serra de Caldas Novas

PF - Parque Estadual do Pau-Furado

REP - Reserva Ecológica do Panga

SB - Fazenda Santa Bárbara

SJ - Fazenda São José

Chl *a/b* - Razão entre clorofila *a/b*

Chl *a+b* - Clorofila total *a+b*

Chl/carot – Razão entre clorofila total (*a + b*) e carotenoides do PSII após adaptação à luz

F_v/F_m - Rendimento quântico potencial

MS – Massa seca

MT – Massa fresca

NPQ - *quenching* não fotoquímico durante a adaptação a luz

QY_{max} - Rendimento quântico máximo do PSII

Rfd – Razão do declínio da fluorescência

RWC – Conteúdo relativo de água

SWC – Conteúdo de saturação hídrica

^{14}N - Isótopo estável de nitrogênio mais comum na crosta terrestre

^{15}N - Isótopo estável de nitrogênio que contém em seu núcleo 7 prótons e 8 nêutrons

^{13}C - Isótopo natural e estável de carbono que contém em seu núcleo 6 prótons e 7 nêutrons

Índice

1. Resumo geral	10
2. Introdução geral.....	14
3. Capítulo 1	22
Variação no Enriquecimento de ^{15}N em Fragmentos do Cerrado: Relação entre Efeitos de Borda e a Fisiologia de <i>Bauhinia holophylla</i> (Bongard) Stuedel.....	22
3.1 Introduction	25
3.2 Material and Methods.....	27
3.3 Statistical analysis	31
3.4 Results	32
3.5 Discussion.....	32
3.6 Conclusion.....	36
3.7 References	37
3.8 Figures and table.....	45
4. Capítulo 2	52
How nitrogen and phosphorus supplementation can influence the physiology of <i>Bauhinia holophylla</i> (Bong.)?	53
4.1 Introduction	54
4.2 Materials and methods.....	57
4.3 Statistical analysis	59
4.4 Results	60
4.5 Discussion.....	61
4.6 Conclusion.....	63
4.7 References	64
Tables and figures.....	71
5. Capítulo 3	75
Como <i>Handroanthus impetiginosus</i> (Mart. ex DC.) responde à suplementação por N e P?	75

5.1	Introdução	77
5.2	Material e métodos	78
5.3	Análises dos dados	80
5.4	Resultados.....	80
5.5	Discussão	81
5.6	Conclusão	83
5.7	Referências	85
5.8	Tabelas e figuras	90
5.9	Conclusão Geral	94
	Anexo	96

1. Resumo geral

As atividades humanas têm modificado globalmente o ciclo dos nutrientes essenciais para as plantas, podendo afetar a fisiologia de espécies nativas. Na América do Sul especificamente no Cerrado brasileiro grandes áreas têm-se transformado em áreas agrícolas ao longo do tempo e devido as condições oligotróficas do bioma, é amplamente utilizado a suplementação fertilizantes industriais para fornecer condições ideais o crescimento e desenvolvimento de plantas cultivadas. De uma forma geral, entre os nutrientes essenciais minerais, o nitrogênio é o mais requisitado em quantidade, assim a quantidade desse elemento tem aumentado ao longo dos anos nos ecossistemas naturais em áreas próximas a monoculturas que fazem suplementação de fertilizantes nitrogenados. Em decorrência do escoamento superficial, lixiviação e volatilização parte do nitrogênio pode ter concentrações mais elevadas principalmente nas bordas dos remanescentes de ecossistemas naturais. O que pode influenciar não apenas a dinâmica do nitrogênio no solo e nas plantas, mas também a disponibilidade de carbono. Isótopos estáveis não radioativos de ^{15}N e ^{13}C tem sido usado em trabalhos ecológicos para determinar a origem e o transporte do N e C em ambientes naturais. No primeiro capítulo, foi verificado a presença de ^{15}N no solo no interior e nas bordas de oito fragmentos de Cerrado, juntamente ao ^{15}N nas folhas de *Bauhinia holophylla* durante dois anos. Também foi analisado as relações entre ^{15}N e ^{13}C entre a borda e interior dos fragmentos. Os fragmentos de Cerrado intermediários apresentaram maiores valores de ^{15}N nas folhas das plantas encontradas nas bordas. Em um dos fragmentos que o ^{15}N foi maior na borda, também foi analisada a fisiologia de *B. holophylla*, e foram encontrados valores mais altos de área foliar específica (SLA) e conteúdo relativo de água (CRA), nas bordas. Por outro lado, os resultados de fotossíntese sugerem que no interior do fragmento as plantas tem uma maior capacidade de dissipação de energia luminosa em excesso e mantém um aparato fotossintético mais preservado, o que pode ser interpretado como um indicativo de estresse nas bordas. No segundo capítulo, foi realizada uma suplementação de N, P e N+P em 25 parcelas de 100m² permanentes e foi analisado os efeitos fisiológicos em *B. holophylla*. A espécie mostrou aumento na SLA e CRA no tratamento com suplementação de N+P, enquanto o rendimento quântico potencial (Fv/Fm) foi maior no tratamento com suplementação de P isoladamente. Por outro lado, a suplementação de P apresentou menores capacidades de dissipação de energia luminosa em excesso e menor proteção contra o excesso de luz. O terceiro capítulo, abordou as respostas fisiológicas

em função da suplementação de N, P ou N+P em *Handroanthus impetiginosus* (Mart. ex DC.) cultivado em casa de vegetação. A suplementação de N ou P, promoveu um aumento na área foliar e crescimento, enquanto a combinação de N+P aumentou a eficiência foliar para captação de luz. Contudo, suplementação combinada de alto N+P (2,44 g de N e 2,50 g de P), as plantas não apresentaram alteração no tamanho total e na fluorescência da clorofila, por outro lado, diminuíram a eficiência fotossintética e a condutância estomática, sugerindo toxicidade. Esses resultados indicam que altas disponibilidade de N+P pode comprometer a saúde fisiológica de *H. impetinosus*.

Palavras chave: Alterações antropogênicas, isótopos de nitrogênio, metabolismo fotossintético, savana tropical.

General Summary

Human activities have globally modified the cycle of essential nutrients for plants, potentially affecting the physiology of native species. In South America, specifically in the Brazilian Cerrado, large areas have been transformed into agricultural lands over time. Due to the oligotrophic conditions of the biome, the use of industrial fertilizers is widely adopted to provide ideal conditions for the growth and development of cultivated plants. In general, among the essential mineral nutrients, nitrogen is the most required in quantity by plants. Consequently, the concentration of this element has increased over the years in natural ecosystems near monocultures that use nitrogen fertilizers. Due to surface runoff, leaching, and volatilization, part of the nitrogen may reach higher concentrations, especially at the edges of remaining natural ecosystems. This can influence not only the dynamics of nitrogen in the soil and plants but also the availability of carbon. Non-radioactive stable isotopes of ^{15}N and ^{13}C have been used in ecological studies to determine the origin and transport of N and C in natural environments. In the first chapter, the presence of ^{15}N in the soil and leaves of a Cerrado plant species was examined at the interior and edges of eight Cerrado fragments was verified, along with ^{15}N in the leaves of *Bauhinia holophylla* over two years. The relationships between ^{15}N and ^{13}C the edges and interior of the fragments were also analyzed. Intermediate Cerrado fragments showed higher values of ^{15}N in the leaves at the edges. In one of the fragments where ^{15}N was higher at the edge, the physiology of *B. holophylla* was analyzed, revealing higher values of specific leaf area (SLA) and relative water content (RWC) at the edges. On the other hand, the results of photosynthesis suggest that, inside the fragment, plants have a greater capacity to dissipate excess light energy and maintain a more preserved photosynthetic apparatus, which may indicate stress at the edges. In the second chapter, N, P, and N+P supplementation was applied to 25 permanent plots of 100m² each, and the physiological effects of *B. holophylla* were analyzed. The specie showed an increase in SLA and RWC in the treatment with N+P supplementation, while the potential quantum yield (Fv/Fm) was higher in the treatment with P supplementation alone. On the other hand, P supplementation showed lower capacity to dissipate excess light energy and less protection against excess light. The third chapter addressed the physiological responses to supplementation with N, P, or N+P in *Handroanthus impetiginosus* (Mart. ex DC.) grown in a greenhouse. Supplementation with N or P promoted an increase in leaf area and growth, while the combination of N+P increased leaf efficiency for light capture.

However, in the combined supplementation of high N+P (2.44 g of N and 2.50 g of P), the plants did not show changes in total size and of chlorophyll fluorescence and exhibited decreased photosynthetic efficiency and stomatal conductance. These results indicate that high availability of N+P may compromise the physiological health of *H. impetiginosus*.

Keywords: Anthropogenic changes, nitrogen isotopes, photosynthetic metabolism, tropical savanna.

2. Introdução geral

Pesquisas pioneiras realizadas no século XVI por John Woodward, mostraram que as plantas cresciam de forma diferenciada quando submetidas a diferentes substâncias dissolvidas na água. Woodward previu que, quando fossem descobertas quais substâncias e em quais quantidades são necessárias para o crescimento e desenvolvimento das plantas, o uso do solo para o cultivo se tornaria dispensável (Stanhill, 1986). Posteriormente, no século XIX, Justus Von Liebig começou a especular sobre a existência de elementos químicos essenciais para o desenvolvimento ideal das plantas. Liebig formulou a “Lei do Mínimo”, que afirma que as plantas necessitam de uma quantidade mínima de determinados elementos químicos e que é desnecessária a adição excessiva de nutrientes, porque há uma quantidade máxima que as plantas conseguem absorver e utilizar. Além disso, o aumento excessivo de um nutriente poderia desfavorecer a disponibilidade e uso de outros elementos (van der Ploeg et al., 1999).

Entre os anos de 1770 e 1810, outros pesquisadores descobriram e demonstraram a essencialidade dos elementos nitrogênio (N) fósforo (P), enxofre (S), potássio (K), cálcio (Ca), magnésio (Mg), ferro (Fe), manganês (Mn), cobre (Cu), zinco (Zn), molibdênio (Mo), boro (B) e cloro (Cl) (Faquin, 2005). Já em 1939, Arnold e Stout, propuseram que os elementos minerais essenciais são componentes intrínsecos na estrutura ou metabolismo de uma planta cuja ausência causa anormalidades graves no crescimento, desenvolvimento e reprodução. Uma planta deve ser incapaz de completar seu ciclo de vida na ausência do elemento mineral e a função do elemento não deve ser substituível por outro elemento mineral. Os critérios de essencialidade de nutrientes propostos por Arnold e Stout foram revisados por Epstein (1999), e foram definidos 14 elementos essenciais para as plantas. Atualmente, estes critérios têm sido revistos e existem propostas que incluam outros elementos benéficos para o desenvolvimento vegetal, como por exemplo o alumínio para algumas plantas (Brown et al., 2022).

Os minerais essenciais foram classificados conforme suas concentrações no tecido vegetal como macro e micronutrientes (Epstein, 1999). Posteriormente, uma nova classificação foi proposta de acordo com o papel bioquímico e sua função fisiológica, sendo divididos em grupos tais como o daqueles que constituem substâncias orgânicas, os que contribuem para o armazenamento de energia e integridade estrutural, os elementos que se mantêm em formas iônicas e aqueles que assistem reações redox

(Mengel and Kirkby 2001). Dentro dessas classificações, o N é um macro nutriente mais requisitado pela maioria das plantas (Conant et al., 2013; Galloway et al., 2004). Faz parte do grupo dos compostos de carbono, envolvido na produção de aminoácidos, proteínas, nucleotídeos e parte das bases nitrogenadas (Taiz et al., 2017).

O N apresenta diversos estados de oxidação ao longo de seu ciclo nos ecossistemas. Aproximadamente 78% da atmosfera é composta por nitrogênio molecular (N_2), mas essa forma não é diretamente assimilável pelas plantas. Para que o nitrogênio se torne disponível, a tripla ligação presente na molécula de N_2 precisa ser quebrada, gerando formas mais reativas, como o amônio (NH_4^+) e o nitrato (NO_3^-), que são assimiláveis pelos vegetais e incorporados em seus tecidos (Berdanier and Grace, 2013). Esse processo de conversão pode ocorrer por meio de mecanismos naturais ou industriais (Humphreys et al., 2021; James, 2016). Nos processos naturais, a quebra da tripla ligação do N_2 pode ser realizada por descargas elétricas (raios), por bactérias de vida livre no solo ou por associações simbióticas com raízes de leguminosas (James, 2016). Já no processo antropogênico, a fixação do nitrogênio ocorre por meio do método Haber-Bosch, no qual as moléculas de N_2 são submetidas a altas temperaturas e pressões para a produção de amônia (NH_3) (Humphreys et al., 2021).

Como produtos do processo de Haber-Bosch, são produzidos fertilizantes amplamente utilizados em monoculturas com o intuito de aumentar a produtividade de plantas cultivadas (Boschiero et al., 2020; Galloway et al., 2004). No entanto, nas monoculturas, a aplicação desses fertilizantes muitas vezes excede as necessidades ideais das plantas cultivadas, porque o N pode ser perdido para o ambiente por meio de diferentes vias. Uma das vias de perda de N nos ecossistemas é a volatilização, na qual o N pode ser convertido em NH_3 e liberado para a atmosfera. Outra via de perda é a infiltração no lençol freático, onde o N na forma de NO_3^- pode ser lixiviado para camadas mais profundas do solo. Por fim, o escoamento superficial durante eventos de chuva, o N pode ser carregado pela água da superfície (Good and Beatty, 2011). Podendo alcançar as plantas que se estabeleceram nas bordas das monoculturas (Pocewicz et al., 2007), e consequentemente pode mudar atributos funcionais fisiológicos específicos ao longo do tempo e influenciar na diversidade de espécies locais (Lannes et al., 2020). Uma das formas do nitrogênio que é utilizado em trabalhos de ecologia para rastrear o caminho do elemento nos ecossistemas é o ^{15}N , encontrado em altas concentrações nos fertilizantes industrializados nitrogenados (Hastings et al., 2013; Kusano et al., 2011; Liu et al., 2021)

e também pode ser encontrado altos valores de ^{15}N nas bordas de remanescentes florestais que tiveram um histórico ou atual suplementação de fertilizantes nitrogenados (Pocewicz et al., 2007).

O P também é um macronutriente essencial para os organismos vivos, participando da composição de moléculas fundamentais como DNA, RNA, nucleotídeos, coenzimas e fosfolipídios. Tem um papel central em reações que envolvem ATP, além de sinalizador e ativador de enzimas de fosforilação (Taiz et al., 2017). Naturalmente o fósforo é liberado em pequenas quantidades no solo a partir do intemperismo de rochas fosfatadas, sendo assimilado pelas plantas na forma de íons de fosfato (PO_4^{3-}). A maior parte do PO_4^{3-} está incrustada em rochas, onde forma complexos com óxidos de cálcio, ferro, magnésio ou alumínio. O desgaste natural de rochas solubiliza esse fosfato muito lentamente. Muitas plantas e microrganismos associados exsudam ácidos orgânicos que aceleram esse processo (Epstein and Bloom., 2005). De uma forma geral, o P é um fator limitante de produtividade para os organismos e ecossistemas (Vance et al., 2003). Entretanto, em florestas tropicais as plantas podem se adaptar à baixa disponibilidade de P alterando sua alocação foliar para atender às demandas da fotossíntese (Vance et al., 2003). Por exemplo, espécies típicas de florestas tropicais no leste Asiático demonstraram a capacidade de manter a taxa fotossintética mesmo sob limitações de fósforo (Mo et al., 2019). Por outro lado, a suplementação em altas quantidades pode chegar a níveis tóxicos para algumas espécies (Moeneclaey et al., 2022; Lambers, 2022) e também levar à redução na diversidade de espécies de uma área ao longo do tempo (Zemunik et al., 2015).

Na América do Sul, especialmente na savana Brasileira, a qual chamamos de Cerrado, os ciclos do nitrogênio e fósforo também vêm sofrendo alterações (Filoso et al., 2006; Gomes et al., 2019; Nóbrega et al., 2017), devido à progressiva conversão de áreas naturais em monoculturas de soja, algodão, cana de açúcar, sorgo e milho, entre outras (Silva, 2018). Concomitantemente, para manter a alta produtividade destas cultivares agrícolas, comumente é utilizada a suplementação de altas dosagens de N e P, podendo ser aplicados mais de uma vez durante a mesma colheita (Simão et al., 2020). O excesso destes nutrientes acaba chegando nas espécies nativas próximas a monoculturas por lixiviação, volatilização e chuvas (Pocewicz et al., 2007). Assim, podem ser incorporados nas folhas ao longo do tempo e, influenciando na seleção de atributos funcionais fisiológicos e no desenvolvimento das espécies nativas (Güsewell, 2004).

Com base no exposto, no primeiro capítulo dessa tese buscamos investigar se fragmentos menores de Cerrado apresentam uma quantidade semelhante de ^{15}N no solo e nas folhas (sendo influenciados por inteiro) e se as áreas com maior extensão apresentam maior quantidade de ^{15}N nas bordas, devido a maior distância da aplicação de nitrogênio nas monoculturas em relação ao interior das áreas maiores (sendo mais influenciados nas bordas). Um dos 8 fragmentos utilizados neste estudo foi utilizado como modelo para avaliar se a fisiologia de uma espécie nativa também apresentaria diferenças em atributos funcionais foliares relacionados à produtividade e fotossíntese entre as bordas e o interior do fragmento. No segundo capítulo, procuramos entender em campo o efeito destes nutrientes sobre plantas nativas. Para tal, propusemos um estudo em área experimental de Cerrado suplementado por fertilizantes industriais nitrogenados e fosfatados nas proporções 45–00–00 e 00–44–00, respectivamente da marca *EQCOAT equilibrio fertilizantes* durante a estação chuvosa (janeiro e fevereiro de 2022 e 2023), em uma dosagem que considera o aumento de nitrogênio para previstas por Galloway et al. (2004) e de aumento para P por Crous et al. (2015) para o ano de 2050. Posteriormente, foram avaliados o efeito desta suplementação, isolada e combinada, sobre a fisiologia de *Bauhinia holophylla* (Bong.) Steud. após 15 dias da suplementação. Por fim, em um terceiro capítulo avaliamos o efeito da suplementação por N e P, isolados ou combinados, sobre a fisiologia de *Handroanthus ipetiginosus* (Mart. ex DC.). Desta forma, esperamos nesta tese confirmar se os fertilizantes nitrogenados atingem os fragmentos remanescentes de Cerrado, afetando não somente a borda como também seu interior, e se podem influenciar em atributos funcionais específicos ligados a produtividade de plantas nativas.

Referências

- Arnon DI, Stout PR. 1939. The Essentiality of Certain Elements in Minute Quantity for Plants with Special Reference to Copper. *Plant Physiol.* 1939 Apr;14(2):371-5. <https://doi.org/10.1104/pp.14.2.371>
- Berdanier, A.B., Grace, P.R., 2013. Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture. *Global Biogeochem. Cycles* 27, 558–566. <https://doi.org/10.1002/gbc.20053>
- Boschiero, B.N., Mariano, E., Torres-Dorante, L.O., Sattolo, T.M.S., Otto, R., Garcia, P.L., Dias, C.T.S., Trivelin, P.C.O., 2020. Nitrogen fertilizer effects on sugarcane growth, nutritional status, and productivity in tropical acid soils. *Nutr. Cycl. Agroecosystems* 117, 367–382. <https://doi.org/10.1007/s10705-020-10074-w>
- Brown, P.H., Zhao, F.J., Dobermann, A., 2022. What is a plant nutrient? Changing definitions to advance science and innovation in plant nutrition. *Plant Soil* 476, 11–23. <https://doi.org/10.1007/s11104-021-05171-w>
- Conant, R.T., Berdanier, A.B., Grace, P.R., 2013. Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture. *Global Biogeochem. Cycles* 27, 558–566. <https://doi.org/10.1002/gbc.20053>
- Epstein E. 1999. Silicon. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:641–64 52.
- Epstein, E., and Bloom, A. J. 2005. *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd ed. Sinauer Associates, Sunderland, MA. p-381.
- Epstein, E., 1975 *Nutrição mineral de plantas: princípios e perspectivas*. São Paulo / Rio de Janeiro, Ed. da Universidade de São Paulo e Livros Tec. e Cient. Editora, 1975. 34p.
- Faquin, V., 2005. *Nutrição mineral de plantas*. Editora: UFLA/FAEPE. p-8
- Filoso, S., Martinelli, L.A., Howarth, R.W., Boyer, E.W., Dentener, F., 2006. Human activities changing the nitrogen cycle in Brazil. *Biogeochemistry* 79, 61–89. <https://doi.org/10.1007/s10533-006-9003-0>
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen

cycles: Past, present, and future, *Biogeochemistry*. <https://doi.org/10.1007/s10533-004-0370-0>

- Gomes, L., Simões, S.J.C., Dalla Nora, E.L., de Sousa-Neto, E.R., Forti, M.C., Ometto, J.P.H.B., 2019. Agricultural expansion in the Brazilian Cerrado: Increased soil and nutrient losses and decreased agricultural productivity. *Land* 8, 1–26. <https://doi.org/10.3390/land8010012>
- Good, A.G., Beatty, P.H., 2011. Fertilizing nature: A tragedy of excess in the commons. *PLoS Biol.* 9. <https://doi.org/10.1371/journal.pbio.1001124>
- Güsewell, S., 2004. N:P ratios in terrestrial plants: Variation and functional significance. *New Phytol.* 164, 243–266. <https://doi.org/10.1111/j.1469-8137.2004.01192.x>
- Hastings, M.G., Casciotti, K.L., Elliott, E.M., 2013. Stable isotopes as tracers of anthropogenic nitrogen sources, deposition, and impacts. *Elements* 9, 339–344. <https://doi.org/10.2113/gselements.9.5.339>
- Humphreys, J., Lan, R., Tao, S., 2021. Development and Recent Progress on Ammonia Synthesis Catalysts for Haber–Bosch Process. *Adv. Energy Sustain. Res.* <https://doi.org/10.1002/aesr.202000043>
- James, E.K., 2016. Nitrogen Fixation, in: *Encyclopedia of Applied Plant Sciences*. Elsevier Inc., pp. 271–277. <https://doi.org/10.1016/B978-0-12-394807-6.00124-6>
- Kusano, M., Fukushima, A., Redestig, H., Saito, K., 2011. Metabolomic approaches toward understanding nitrogen metabolism in plants. *J. Exp. Bot.* 62, 1439–1453. <https://doi.org/10.1093/jxb/erq417>
- Lambers, H., 2022. Phosphorus Acquisition and Utilization in Plants. *Annu. Rev. Plant Biol.* 73, 17–42. <https://doi.org/10.1146/annurev-arplant-102720-125738>
- Lannes, L.S., Karrer, S., Teodoro, D.A.A., Bustamante, M.M.C., Edwards, P.J., Olde Venterink, H., 2020. Species richness both impedes and promotes alien plant invasions in the Brazilian Cerrado. *Sci. Rep.* 10, 1–9. <https://doi.org/10.1038/s41598-020-68412-5>
- Liu, M., Han, G., Li, X., 2021. Using stable nitrogen isotope to indicate soil nitrogen dynamics under agricultural soil erosion in the Mun River basin, Northeast Thailand. *Ecol. Indic.* 128. <https://doi.org/10.1016/j.ecolind.2021.107814>

- Mengel, K., and Kirkby, E. A. 2001. Principles of Plant Nutrition, 5th ed, Kluwer Academic Publishers, Dordrecht, Netherlands. <https://doi.org/10.1007/978-94-010-1009-2>
- Mo, Q., Li, Z., Sayer, E.J., Lambers, H., Li, Y., Zou, B., Tang, J., Heskell, M., Ding, Y., Wang, F., 2019. Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. *Funct. Ecol.* 33, 503–513. <https://doi.org/10.1111/1365-2435.13252>
- Moeneclaey, I., Schelfhout, S., Vanhellemont, M., DeCock, E., Van Coillie, F., Verheyen, K., Baeten, L., 2022. Species ecological strategy and soil phosphorus supply interactively affect plant biomass and phosphorus concentration. *Basic Appl. Ecol.* 62, 1–11. <https://doi.org/10.1016/j.baae.2022.03.013>
- Nóbrega, R.L.B., Guzha, A.C., Torres, G.N., Kovacs, K., Lamparter, G., Amorim, R.S.S., Couto, E., Gerold, G., 2017. Effects of conversion of native cerrado vegetation to pasture on soil hydro-physical properties, evapotranspiration and streamflow on the Amazonian agricultural frontier. *PLoS One* 12. <https://doi.org/10.1371/journal.pone.0179414>
- Pocewicz, A., Morgan, P., Kavanagh, K., 2007. The effects of adjacent land use on nitrogen dynamics at forest edges in northern Idaho. *Ecosystems* 10, 226–238. <https://doi.org/10.1007/s10021-007-9015-1>
- Silva, C.M. da, 2018. Between fenix and ceres: The great acceleration and the agricultural frontier in the Brazilian Cerrado. *Varia Hist.* 34, 409–444. <https://doi.org/10.1590/0104-87752018000200006>
- Simão, E.D.P., de Resende, Á.V., Neto, M.M.G., da Silva, A.F., Campos Godinho, V. de P., Galvão, J.C.C., Borghi, E., de Oliveira, A.C., Giehl, J., 2020. Nitrogen Fertilization in Off-Season Corn Crop in Different Brazilian Cerrado Environments. *Pesqui. Agropecu. Bras.* 55, 1–11. <https://doi.org/10.1590/S1678-3921.PAB2020.V55.01551>
- Stanhill, G., 1986. John woodward - a neglected 17th century pioneer of experimental botany. *Isr. J. Bot.* 35, 225–231. <https://doi.org/10.1080/0021213X.1986.10677056>
- Taiz, L., Zeiger, E., Moller, I. M. Murphy, A. 2017. Fundamentos de fisiologia vegetal.

Porto Alegre. Artmed, p-120.

- Van der Ploeg, R.R., Böhm, W., Kirkham, M.B., 1999. On the Origin of the Theory of Mineral Nutrition of Plants and the Law of the Minimum. *Soil Sci. Soc. Am. J.* 63, 1055–1062. <https://doi.org/10.2136/sssaj1999.6351055x>
- Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157, 423–447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>
- Zemunik, G., Turner, B.L., Lambers, H., Laliberté, E., 2015. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nat. Plants* 1, 1–4. <https://doi.org/10.1038/nplants.2015.50>

3. Capítulo 1

Variação no Enriquecimento de ^{15}N em Fragmentos do Cerrado: Relação entre Efeitos de Borda e a Fisiologia de *Bauhinia holophylla* (Bongard) Stuedel.

Resumo

O uso de áreas agrícolas em larga escala alterou os ecossistemas naturais, afetando a disponibilidade de nutrientes necessários para o desenvolvimento de plantas nativas do Cerrado. Dentre os nutrientes minerais essenciais, o nitrogênio é o mais requisitado em quantidade para as plantas e, devido às características oligotróficas do solo do Cerrado, é comum a suplementação de fertilizantes industriais nitrogenados. No entanto, grande parte do nitrogênio é perdida no ambiente por volatilização e lixiviação, podendo atingir áreas adjacentes onde ocorre a suplementação e, conseqüentemente, pode ser incorporada nas folhas de plantas nativas. No entanto, plantas do Cerrado historicamente crescem em condições de baixa disponibilidade de nitrogênio e podem não responder tão rapidamente quanto as espécies invasoras; além disso, alterações na relação C/N resultantes da suplementação, podem desencadear efeitos deletérios nas plantas nativas, e pode desequilibrar a disponibilidade de outros elementos essenciais. Isótopos estáveis e não radioativos tais como ^{15}N e ^{13}C podem ser usados em estudos ecológicos para determinar a origem do nitrogênio e do carbono em ambientes naturais, sendo que altas concentrações de ^{15}N podem ser detectadas em fertilizantes nitrogenados e relacionadas ao ^{13}C , reverberando nas respostas fisiológicas de plantas nativas. Esse estudo verificou a presença de ^{15}N em fragmentos do interior e nas bordas de Cerrado ao longo de dois anos para determinar os efeitos de borda no metabolismo de *Bauhinia holophylla* (Bongard) Stuedel. Em 2022, apenas o menor fragmento, de 16 ha, apresentou valores mais altos de ^{15}N no solo nas bordas; no entanto, em 2023, os fragmentos que apresentaram valores mais altos de ^{15}N nas bordas foram os de 127 ha e 2200 ha. Somente fragmentos de 127 ha e 409 ha apresentaram valores relativamente altos de ^{15}N nas folhas, indicando que o tamanho dos fragmentos não pareceu afetar a disponibilidade de ^{15}N entre os fragmentos do interior e das bordas. As bordas apresentaram uma relação negativa entre o ^{15}N nas folhas e o ^{15}N no solo, o que pode indicar estresse ambiental causado pelo efeito de borda. Além disso, em *B. holophylla*, foram encontrados valores mais altos de ^{15}N nas folhas dos indivíduos na borda, assim como valores mais altos de área foliar específica (SLA) e conteúdo relativo de água (CRA). Também, no interior do fragmento, as plantas apresentaram uma maior diminuição na razão do declínio da fluorescência (Rfd) e maior

quenching não-fotoquímico (NPQ) em comparação com as plantas na borda. Este resultado sugere que as plantas no interior têm uma capacidade maior de dissipar o excesso de energia luminosa e, assim mantêm um aparato fotossintético mais preservado.

Abstract

The use of large-scale agricultural land has altered natural ecosystems, affecting the availability of nutrients for the development of native plants in the Cerrado. Among the essential mineral nutrients, nitrogen is the most necessary for plants, and due to the oligotrophic characteristics of the Cerrado soil, it is common to supplement industrial nitrogen chemical fertilizers. However, much of the nitrogen is lost in the environment by volatilization and leaching, is able to reach adjacent areas where supplementation occurs, and consequently can be incorporated into the leaves of native plants. However, Cerrado plants historically grow under conditions of low nitrogen availability and may not respond as quickly as invasive species, or even, changes in the C/N balance resulting from supplementation can trigger deleterious effects on native plants, unbalancing the amount of other nutrients. Stable isotopes of ^{15}N and ^{13}C can be used in ecological studies to determine the origin of nitrogen and carbon in natural environments, and high concentrations of $\delta^{15}\text{N}$ can be detected in nitrogen fertilizers and can be related to ^{13}C and consequently reverberate in the physiological responses of native plants. This study verified the presence of $\delta^{15}\text{N}$ in the interior and edge of Cerrado fragments over two years to determine the effects of the edge on *Bauhinia holophylla* (Bongard) Steudel metabolism. In 2022, only the smallest fragment, 16 ha, presented higher values of ^{15}N in the soil at the edges; however, in 2023, the fragments that presented higher values of ^{15}N at the edges were those with 127 ha and 2200 ha. Only fragments of 127 ha and 409 ha had relatively high values of ^{15}N in the leaves, so the size of the fragments did not seem to affect the availability of ^{15}N between the interior and edges. The edges show a negative relationship between the ^{15}N in the leaves as a function of the ^{15}N in the soil, which may indicate environmental stress caused by the edge effect. In *B. holophylla*, higher values of ^{15}N were found in the leaves of the individuals at the edge, as were higher values of specific leaf area (SLA) and relative water content (CRA). In addition, in the interior of the Cerrado fragment, the plants presented a greater fluorescence decline ratio in steady-state (Rfd) and greater steady-state quenching non-photochemical (NPQ) for the plants in the interior of fragment. This result suggests that plants in the interior have a greater ability to dissipate excess light energy and, thus, a more preserved photosynthetic apparatus, which can be interpreted as indicative of stress at the edge.

3.1 Introduction

The Brazilian neotropical savanna, known as the Cerrado, is considered the second-largest biome in South America and the most biodiverse globally (Nóbrega et al. 2017). Despite the importance of the Cerrado as a refuge for a wide variety of endemic species (Mendonça 2008), it is one of the most threatened biomes in Brazil due to the expansion of the agricultural frontier, which involves the conversion of native areas into lands for agriculture and livestock (Strassgurg et al. 2017). According to MapBiomas (2024), the Cerrado lost approximately 53% of its native vegetation between 1985 and 2022, leaving only 47% of its original area. This process of devastation is intensified by the fragmentation of native areas into smaller, disconnected portions, potentially leading to environmental homogenization (Gámez-Virués et al. 2015). These intensifications of edge effects and disturbances in ecosystem processes over time cause changes in the input of essential mineral nutrients and can reverberate in the functional leaf traits, composition and dynamics of populations and communities. (Honnay et al. 2002; Harper et al. 2024). These environmental changes can affect the responses of leaf functional traits related to productivity, where responses to environmental conditions imposed at the edges of fragments differ from those in the interior (Dillon et al. 2018). The edge effect alters the microclimate at the edges of forest fragments, subjecting plants to severe biotic and abiotic factors, such as higher incidence of direct light, winds, and greater temperature variation, potentially altering environmental equilibrium and influencing the availability of nutrients such as carbon (Magnago et al. 2015), and nitrogen (Smith et al. 2018). This phenomenon is more intense in small, isolated fragments, once smaller areas have a proportionally greater edge effect, which can even affect them in a generalized manner (Ewers and Didham 2006).

Around the fragments, the implementation and maintenance of monocultures in Cerrado areas usually include the supplementation of chemical fertilizers, mainly nitrogen, phosphorus, and potassium, to meet the essential requirements for the development of agricultural plants (Simão et al. 2020; Ferreira et al. 2022). However, nitrogen fertilizers can leach and reach forest areas (Jordan et al. 1993), potentially concentrating mainly at the edges (Honnay et al. 2002) and gradually decreasing with increasing distance from the edge (Rivera-Cubero et al. 2023). The nitrogen input has also been recorded in areas elevated relative to the application site (Pocewicz et al. 2007).

Nitrogen is structurally essential for plant growth and development because it is a component of DNA, enzymes, and the electron transport chain of photosynthesis (Vega et al. 2019). However, high concentrations can be toxic and reduce photosynthetic rates (Berghetti et al. 2021). Nitrogen can naturally be found in the soil in various chemical forms according to its solubility, mobility, and availability to plants (Bouwman et al. 2009). The isotope ^{15}N is used as a tool to indicate the nitrogen dynamics in terrestrial environments. This isotopic chemical form occurs naturally in the atmosphere, water, and soil, and indicate different nitrogen sources in the environment, such as biological nitrogen fixation or atmospheric deposition (Craine et al. 2015).

On the other hand, higher concentrations can be detected in industrial nitrogen chemical fertilizers (Hastings et al. 2013; Liu et al. 2021). Consequently, physiological processes affecting plant growth and development are altered, favoring the productivity of certain specific groups of native plants as well as invasive ones such as *Melinis minutiflora* P. Beauv., and *Urochloa decumbens* Stapf (Lannes et al. 2020). These changes alter the composition and structure of plant communities (Zemunik et al. 2015), leading to a reduction in diversity over time and an indirect negative effect on the ecosystem (Jacobson et al. 2011).

In addition to ^{15}N , carbon dynamics are addressed in edge effect studies, indicating changes in ecosystem productivity (Tolbert 1997). The carbon isotopes used in ecological studies are ^{13}C and ^{12}C , whose values provide information about metabolic flows in plants, help to understand how carbon is absorbed, and are used in photosynthesis and respiration processes (Wingate et al. 2010). Changes in ^{13}C isotopic composition indicate that plants respond to variations in environmental conditions, such as climate change and anthropogenic impacts that influence the input and balance of C/N (Amundson et al. 2003; Yan et al. 2020). Furthermore, ^{13}C is also related to the availability of ^{15}N and organic matter in soil (Yan et al. 2020). Data on ^{13}C and ^{15}N can provide insights into root nodulation, mycorrhizal associations, and how plants utilize these nutrients (Sprent et al. 1996).

Although there is evidence that edge effects can result in increased nutrient availability for native plants, influencing their physiology and community diversity over time (Kozovits et al. 2007; Jacobson et al. 2011), few studies have addressed this topic. Research on the dynamics and origins of nutrients available to plants as well as their physiological responses to increased nutrients are relevant topics for projecting the future

of biodiversity and the conservation of Cerrado remnants. In this context, considering the agricultural expansion in this biome, the use of nitrogen fertilizers, and the possible changes in plant physiology, we formulated the following hypotheses: 1) Preservation areas adjacent to monocultures exhibit $\delta^{15}\text{N}$ enrichment in the soil at the edges compared to the soil in the interior of the fragments; 2) Larger fragments should show a greater difference between $\delta^{15}\text{N}$ enrichment in the edge soil and the interior soil, indicating that smaller preservation areas are more affected overall; 3) Plants at the edges of the fragments show greater $\delta^{15}\text{N}$ enrichment in their leaf tissues compared to individuals found in the interior of the preservation areas; and 4) Plants growing at the edges of the fragments should exhibit physiological variations in their leaves that can be related to increased foliar nitrogen.

3.2 Material and Methods

3.2.1 Sample areas

Eight preservation areas of varying sizes adjacent to agricultural monocultures were selected (Fig. 1). The climate is classified as tropical (AW) for all fragments according to the Köppen climate classification, with a dry season from May to September (Alvares et al. 2013). The sizes of the fragments and the usual monocultures in each area are described in Table 1, which highlights that soybean monocultures traditionally receive less nitrogen supplementation than other monocultures, as biological nitrogen fixation can provide all the necessary N for the efficient growth and development of soybeans (Hungria et al. 2006). On the other hand, fertilization can depend on factors such as soil fertility. For example, maize monocultures in the Cerrado can require the supplementation of 100 kg N per hectare, 30 kg ha⁻¹ N applied at planting and 70 kg ha⁻¹ N applied as a side-dressing (Coser et al. 2016) and sugarcane can require the application of 50 kg ha⁻¹ to 100 kg ha⁻¹ N (Boschiero et al. 2020).

Fazenda Santa Bárbara (SB): This is a private property with a fragment of Cerrado sensu stricto and Cerradão (approximately 16 ha), located in the municipality of Orizona, Goiás, at coordinates 16°59'00.0"S, 48°16'00.0"W. This area is subjected to a rotational production of maize, soybean, and sorghum during the rainy season, and extensive cattle farming during the dry season. The average annual temperature ranges between 19°C and 25°C, and the average annual precipitation is 1,400 mm, which is concentrated between October and March (INMET 2024).

Fazenda São José (SJ): The preservation area is approximately 20 ha, predominantly covered by semideciduous forest, with its surroundings consisting of eucalyptus cultivation and agro-pastoral activities. Located in the urban area in Uberlândia, Minas Gerais (18°51'35.0"S, 48°13'53.0"W), (Vargas and Araújo 2014).

Fazenda Experimental Glória (GL): This is an area within the urban perimeter that has a Cerrado remnant (30 ha) with a seasonal semideciduous forest (Lopes et al. 2011), located at coordinates 18°58'15.7"S, 48°12'29.6"W. The fragment is surrounded by areas designated for agriculture and the planting of maize and eucalyptus.

Reserva Ecológica do Panga (REP): This is a private natural heritage site (RPPN) of 409 ha, located at coordinates 19°11'40.0"S, 48°19'06.0"W. The site features mesophytic forest, Cerradão, Cerrado *sensu stricto*, Campo Cerrado, Campo Sujo, and Veredas (Gonçalves et al. 2021). To the west of the reserve, there is an extensive sugarcane plantation.

Clube Caça e Pesca Itorotó (CP): This area has a fragment (127 ha) with Cerrado phytogeographies, ranging from Cerrado *sensu stricto* to gallery forest (Silva et al. 2022), located at coordinates 18°59'00.0"S, 48°18'00.0"W, adjacent to soybean monoculture.

Reserva Particular do Patrimônio Natural Unidade de Conservação de Galheiros (GA): The vegetation cover is dominated by Cerrado *sensu stricto* formation, along with areas of Campo Limpo, Campo Cerrado, Cerradão, seasonal semideciduous forest, and evergreen gallery forest (Landa et al. 2010). Located at coordinates 19°13'21.14"S, 47°08'58.03"W. The RPPN have 2,694 ha area, surrounded by soybean monoculture

Parque Estadual do Pau-Furado (PF): This is a state conservation unit for full protection. The park contains a 2,200ha fragment of the Cerrado biome, located at coordinates 18°46'00.0"S, 48°10'19.4"W, with some occurrences of Atlantic Forest, featuring seasonal deciduous forest, seasonal semideciduous forest, riparian forest, gallery forests, Cerrado *sensu stricto*, and Cerradão (IEF 2011). The surroundings of the park are predominantly soybean monocultures.

Parque Estadual da Serra de Caldas Novas (PE): This area spans 12,500 ha and contains a vegetation mosaic that varies along a topographic gradient, located at coordinates 17°44'40.8"S, 48°39'00.0"W. It features forest formations such as Cerradão and gallery forest, as well as grassland formations such as rupestrian Cerrado and the typical Cerrado found on the plateau of the mountain range (Lima et al. 2010). In the

areas surrounding the park, the rotational planting of soybeans, sorghum, and maize is the most common.

3.2.2 Isotopic Signature of Nitrogen and Carbon

Soil samples to determine the presence of ^{15}N were collected during the rainy season (January and February), when most monocultures had already received agricultural inputs. The analyses were carried out for two consecutive years (2022 and 2023). To determine the possible presence of industrial nitrogen in the soil, samples were taken at a depth of 10 cm from both sites of the fragments and dried at 45°C until a constant weight was reached. The incorporation of ^{15}N by the plants was assessed in two leaves from each individual collected from both sites of each of the eight Cerrado fragments. Approximately 50 µg of each sample was weighed in tin capsules and taken to a mass spectrometer for element discrimination. The isotopic ratio was calculated using the formula $\delta^{15}\text{N} = [(\text{R}_{\text{sample}} - \text{R}_{\text{standard}}) / (\text{R}_{\text{standard}})] \times 1000$, where R represents the ratio between ^{15}N and ^{14}N , and the standard refers to the nitrogen isotope values in the atmosphere. The unit used was parts per thousand (‰) (Craine et al. 2015). The carbon isotopic ratio R ($^{13}\text{C}/^{12}\text{C}$) in the soil was determined following the methodology of Ehleringer and Osmond (1989). Two soil samples were collected from both the interior and the edge of all forest fragments and dried at 50°C for three days. The samples were analyzed using a continuous flow isotope ratio mass spectrometer (CF-IRMS) at the Stable Isotopes Center, UNESP (Botucatu, Brazil). The CF-IRMS equipment comprises an elemental analyzer-EA (Flash 2000 – Thermo Scientific, Germany) coupled to an isotope ratio mass spectrometer (IRMS) (Delta V Advantage – Thermo Scientific Germany) through a gas interface (ConFlo IV – Thermo Scientific, Germany). Samples weighing between 0.10 and 0.12 mg were placed in tin capsules and inserted into the EA for conversion into CO_2 by combustion. The R ($^{13}\text{C}/^{12}\text{C}$) ratio of the CO_2 was determined via IRMS, and the results were converted to $\delta^{13}\text{C}$ (‰), representing the relative differences in isotopic ratios, based on the international VPDB standard.

3.2.3 Species and Site as a Model for Leaf Physiology Study

Bauhinia holophylla (Bongard) Steudel (Fabaceae) was used as a model for the study in the Reserva Ecológica do Panga (REP). This species has a wide geographical distribution, including in Brazil, Bolivia, and Paraguay. Minas Gerais is well represented and can be found both at the edges and in the interior of the REP, allowing comparisons

between these two sites. Additionally, physiological analyses of the interior and edge of the REP revealed differences in ^{15}N enrichment during the year of sampling. Ten individuals were marked at both sites (edge and in the interior of the fragment). To date, there have been no reports of this species exhibiting root nodules.

3.2.4 Specific Leaf Area and Relative Water Content

In both the interior and the edge of the fragment, a fully expanded leaf of *B. holophylla* was collected and leaves from the third node that did not show visible injuries were selected (n=10 for each location). The total leaf area was measured using the software ImageJ®, and the leaves were weighed (fresh weight) using an analytical balance (Shimadzu, AUY, Japan). The material was dried in an oven (Novatecnica, NT513, Brazil) at 60°C for three days and weighed again (dry weight). The specific leaf area was calculated using the ratio between the total area and the dry weight of each leaf (Cornelissen et al. 2003). The relative water content (RWC) was determined in the morning using two 0.4 cm² samples of leaves collected from ten *B. holophylla* individuals in the interior and edge of the Cerrado fragment. The RWC was calculated using the following equation: $\text{RWC} = [(\text{FW}-\text{DW})/(\text{TW}-\text{DW})] \times 100$, where FW is the fresh weight, TW is the turgid weight after 24 hours of distilled water immersion, and DW is the dry weight after the samples were dried at 60°C until it reached a constant weight (Bars and Watherly, 1962).

3.2.5 Photosynthetic Parameters

The chloroplast pigment contents were quantified using two leaf fragments of 0.4 cm² of ten individuals of the interior and the edges in the fragment. The samples were immersed in 80% (v/v) acetone for 48 hours, macerated, and centrifuged at 1,000 rpm for 5 min. The absorbance of the supernatant was measured at 470, 646, and 663 nm in a spectrophotometer (Bioespectro, SP-2020). The contents of chlorophyll a and b and carotenoids were determined according to Lichtenthaler and Wellburn (1983).

Six individuals from the edge and interior of the fragment were selected to evaluate the efficiency of the photochemical phase of photosynthesis between 8:00 and 10:00 am. One leaf from each individual was dark-adapted for 30 min and analyzed using a Handy FluorCam imaging fluorometer (PSI, version 2.0 Italy). The Kautsky effect was evaluated from the images obtained following the quenching protocol (Fluorcam7® software). Twenty fields of view of 50 mm were considered, extracting data from the maximum quantum yield of PSII after light adaptation (F_v/F_m), the ratio of fluorescence decline

(Rfd), and the nonphotochemical quenching during light adaptation (NPQ) (Genty et al. 1989).

3.3 Statistical analysis

To evaluate the effect of monocultures on the availability of $\delta^{15}\text{N}$ in the soil and leaves of the interior and edge of the Cerrado fragments, considering their sizes, a two-way analysis of covariance (ANCOVA) was performed, assuming the assumptions of normality, they were transformed to a logarithmic scale. The analyses were conducted using R software version 4.3.1 (2022), and graphs were generated using GraphPad Prism 8.0.2 software.

To evaluate the effect of ^{15}N in the soil on ^{15}N in the leaves, simple linear regressions were performed considering the response variable ($\delta^{15}\text{N}$ in the leaves) as a function of the predictor variable ($\delta^{15}\text{N}$ in the soil). The effect of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the soil was evaluated using simple regressions, assuming the normality of the residual distribution and homogeneity of variances. The data were analyzed, and graphs were generated using the R version 4.3.1 (2022).

For the analysis and comparison of physiological parameters, generalized linear models (GLM) were generated, to test the overdispersion of the data. The Poisson distribution was assumed due to the nonnormality of the data. The predictors considered were the edge and interior of the Cerrado fragment of the REP, and the response variables were chloroplast pigment content, relative water content, specific leaf area, FvFm, QYmax, Rfd, and NPQ. To perform the test, the dispersion variation for each analysis was checked, and graphs (violin plots) showing the data distribution through a density curve were generated using the software GraphPad Prism 8.0.2.

To determine the existence of clustering patterns in the physiological responses observed between the edge and interior of the Panga Ecological Station, MG, Brazil, a multivariate analysis was conducted using principal component analysis (PCA). The PCA function from the FactoMineR package was applied (Le et al. 2008). The data were standardized beforehand using a correlation matrix to highlight the relationships between variables (Gotelli and Ellison 2004). For visualization of the results, graphs were generated using the ggplot2 (Wickham 2016) and FactorExtra packages with the fviz_pca_biplot function (Kassambra and Mundt 2020). The analyses and plots were generated using R version 4.3.1 (2022).

3.4 Results

3.4.1 Isotopic signature analysis of nitrogen

In the first sampling year (2022), the $\delta^{15}\text{N}$ fraction in the soil showed an interaction between the size of the fragments and the position where the samples were collected (interiors and edges) ($p=0.01$, $F=6.14$, $df=4.55$). Only the preservation area of Fazenda Santa Bárbara (the smallest area) had higher $\delta^{15}\text{N}$ values at the edge (average of 5.1‰ at the edge and 2.1‰ in the interior) (Fig. 2 A). In 2023, the fragments that showed significant differences were Caça e Pesca and Parque Pau-Furado, with higher $\delta^{15}\text{N}$ values at the edges (Fig. 2B). In 2023, when the concentrations of ^{15}N in *B. holophylla* leaves were compared with the fragment size of individuals found at the edges of Clube Caça e Pesca Itororó and Panga Ecological Station greater $\delta^{15}\text{N}$ values ($p<0.001$) were detected (Fig. 2C). The $\delta^{15}\text{N}$ values in the leaves had a positive relationship with the $\delta^{15}\text{N}$ values in the soil when collected from the interior of the fragment, and a negative relationship when collected from the edges of the fragments (Fig. 3A and B, respectively). When the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the soil were compared, no relationships were found in the interiors of the fragments. However, a strong relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was observed at the edges, considering all the forest fragments studied (Fig. 3C and D).

3.4.2 Aspects of leaf physiology of *Bauhinia holophylla*

The leaves of *B. holophylla* at the edge of the fragments showed greater specific leaf area ($p<0.001$, $T=-4.824$) and greater relative water content ($p<0.001$, $T=-3.338$) than those from the interior (Fig. 4 A and B). On the other hand, the chlorophyll a to b ratios and the total chlorophyll and carotenoid contents did not significantly differ ($p=0.282$, $T=-1.108$; $p=0.98$, $T=-0.021$; $p=0.664$, $T=0.441$, respectively) (Fig. 4c-f). The leaves of individuals in the interior of the fragments had higher values for the Rfd and NPQ ($F=11.08$, $p<0.01$ and $F=5.126$, $p=0.047$, respectively) (Fig. 5). The QYmax and the Fv/Fm did not significantly differ ($F=1.255$, $p=0.206$ and $F=1.19$, $p=0.206$) between individuals from the interior and the edge.

When all the physiological parameters of the leaves of *B. holophylla* were used in a PCA, we observed a separation of 26.9% along axis 1 (Fig.6). The parameters that most influenced individuals at the edge were Fv/Fm, QYmax, $\delta^{15}\text{N}$ in the leaves, and CRA, while individuals from the interior were strongly influenced by Rfd.

3.5 Discussion

3.5.1 Variation in the ^{15}N composition of soil and leaves of *Bauhinia* sp. grown in the interior and at the edge of Cerrado fragments

In general, although an enrichment of $\delta^{15}\text{N}$ at the edges of some fragments compared to the interior soil has been observed, the size of the studied fragments does not seem to influence this relationship. This is evidenced by the fact that both smaller and larger fragments may show variable differences in soil $\delta^{15}\text{N}$ values. However, some studies have shown that agricultural nitrogen inputs are responsible for the enrichment of soil $\delta^{15}\text{N}$ and the high concentration of nitrogen at the edges of forests adjacent to agricultural areas (Bateman and Kelly 2007; Hastings et al. 2013; Pocewicz et al. 2007; Liu et al. 2021). This enrichment may result from recent supplementation or reflect historical fertilization of the area (Pocewicz et al. 2007). On the other hand, other studies have shown that the variation in the ^{15}N in different ecosystems can also be due to factors such as the presence of nitrogen-fixing legumes, associations with mycorrhizal fungi, variability in the ^{15}N in soil organic matter with depth, fire events, seasonality of nitrogen immobilization and mineralization processes (Bustamante et al. 2004), climate, topography (Amundson et al. 2003), and even precipitation (Goulden et al. 2019). Additionally, the leaching and absorption of nitrogen compounds contribute to the detection of ^{15}N in the soil, so all these factors together explain the observed variation between the soil at the edges and the interior of the Cerrado fragments over the two years of the experiment. Moreover, the different amounts and frequencies of nitrogen fertilization, associated with other chemical elements, depend on the type of monoculture under cultivation, which can also affect the input of ^{15}N into the soil and, consequently, its absorption and concentration in plant leaves (Sainju et al. 2020; Liu et al. 2024).

In the study areas with forest fragments smaller than 100 ha and the largest tree fragments, the plants collected did not show differences in ^{15}N values in the leaves between the edges and interior. These results suggest that factors other than the size of the Cerrado fragments must influence the isotopic signature of ^{15}N in the leaves of these locations. On the other hand, the fragments with 127 and 409 ha demonstrated enrichment of ^{15}N in the leaves at the edge due to the application of industrial fertilizers on the ^{15}N values in the leaves. As shown by Pocewicz (2007), plants in forest areas adjacent to monocultures can have leaves with approximately 57% more enriched $\delta^{15}\text{N}$ at the edges than in their interior. Additionally, a historical analysis of the fragments should be performed. Some of the fragments, such as PE, GA, PF, and CP, had agricultural areas

predominantly cultivated soybeans, a monoculture in which nitrogen supplementation is usually not used or is used at low concentrations (Zuffo et al. 2022).

From a general perspective of $\delta^{15}\text{N}$ composition, the studied areas showed $\delta^{15}\text{N}$ variation in leaves between -0.99‰ and +3.2‰. This range is in line with what is expected for Cerrado areas, as the biome exhibits wide variation in leaf $\delta^{15}\text{N}$, with values ranging from -5‰ to +7.9‰ (Bustamante et al. 2004). From a continental perspective, the concentration of ^{15}N in soil and leaves found in this study shows a variation similar to the patterns found in ecosystems in South America and high-altitude locations of the Northern Hemisphere (Sena-Souza et al. 2020).

In the interior of fragments, a positive relationship was observed between $\delta^{15}\text{N}$ in leaves and $\delta^{15}\text{N}$ in the soil, indicating the direct uptake of $\delta^{15}\text{N}$ by plants from the soil (Nel et al. 2018; Goulden et al. 2019). Additionally, the interior of the fragments generally shows more consistent soil conditions, such as higher fertility, stable organic matter content, and well-structured soil, which favor nutrient cycling and plant growth (Craine et al. 2015). These conditions contrast with the edges of fragments, where soil degradation and nutrient leaching are more common due to external disturbances (Pereira et al. 2021). On the other hand, the edges showed a negative relationship between the values of $\delta^{15}\text{N}$ in the leaves and $\delta^{15}\text{N}$ in the soil, suggesting the absorption of $\delta^{15}\text{N}$ by leaves that are present in the soil at some time, and a possible stress related to soil alteration. For example, the decrease in nitrogen and carbon available at the edge could be due to greater utilization and incorporation by perennial grasslands (Aguilar et al. 2023).

A strong correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was observed at the edges of the fragments. Variations in the C/N ratio resulting from the edge effect (lower C/N at the edges of a fragment) have already been observed in previous studies, regardless of land use around the fragment or even in pastures that have never been fertilized (Devlaeminck et al. 2005; Pociwicz et al. 2007), with this relationship being more evident in agricultural areas (Pociwicz et al. 2007). There is evidence that the carbon stock at the edges of forest fragments may be lower than that at more preserved locations (Li et al. 2009; Aynekulu et al. 2021), and it is expected that sites with lower carbon concentrations will have higher ^{15}N values (Mendonça et al. 2010). The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ may be correlated due to metabolic processes and nutrient cycling by plants (Yan et al. 2020). However, we did not find studies showing this relationship in tropical savannas.

3.5.2 Foliar metabolism of *B. holophylla* found in a Cerrado fragment where the edges are enriched with $\delta^{15}\text{N}$

Bauhinia holophylla showed higher $\delta^{15}\text{N}$ values in the foliar tissues of individuals growing at the edge of the fragment, as observed for other plant species (Pocewicz et al. 2007). In addition to $\delta^{15}\text{N}$ enrichment in the leaves, higher values of specific leaf area (SLA) and relative water content (RWC) were also observed in edge individuals. Generally, higher SLA values indicate a greater ability of the plant to capture light per unit of leaf area, resulting in faster growth and greater productivity (Cornelissen et al. 2003; Riva et al. 2016). In turn, RWC is an indicator of the plant's water status and can reflect a greater ability of the plant to retain water in its leaves, which is advantageous for the plant under water stress conditions (Bars and Watherly, 1962).

There were no significant differences in the chloroplast pigment content between the leaves of *B. holophylla* collected from the interior and the edge of the fragment or in the ratios of chlorophyll *a* to *b*, total chlorophyll content, or carotenoids. Similarly, no changes were detected in the maximum quantum yield (QY_{max}) or potential quantum yield (Fv/Fm) of the plants from the interior and edge of the Cerrado fragment. Indeed, a relationship between chlorophyll production and photosynthetic performance is expected (Tatarko and Knops 2018). These results suggest that despite the edge effect and ^{15}N enrichment, the plants found at the edges and in the interior of the fragment do not show differences in photosynthetic efficiency (Genty et al. 1989). On the other hand, when comparing the ratio of fluorescence decline (Rfd) and nonphotochemical quenching (NPQ) during light adaptation, the plants from the interior of the Cerrado fragment showed higher values. This result suggests that the plants in the interior have a greater capacity to dissipate excess light energy (higher NPQ values) and, therefore, have a more preserved photosynthetic apparatus (higher Rfd values), as a decrease in the Rfd ratio can be interpreted as an indicator of stress (Lichtenthaler et al. 2005). Considering that the data were collected during the rainy season in the Brazilian savannas these results may confer an advantage to the plants in the interior of the fragment, potentially placing the edge plants in more vulnerable conditions during dry periods. From a general perspective of the results, the PCA showed clusters that separated the individuals from the interior and the edge of the Cerrado fragment based on physiological functional leaf traits and photochemical responses, even though not all evaluated parameters are differentiated separately.

3.6 Conclusion

This study demonstrates that the edge effect in Cerrado *sensu stricto* fragments significantly influences the nitrogen dynamics and physiological responses of *Bauhinia holophylla*. The observed enrichment of $\delta^{15}\text{N}$ in leaves at the edges, compared to the interior, suggests that agricultural inputs and edge-related disturbances play a critical role in shaping nitrogen availability and assimilation. However, the size of the fragments did not consistently influence $\delta^{15}\text{N}$ patterns, indicating that other factors, such as historical land use, soil conditions, and local environmental variability, are equally important in determining nitrogen dynamics. The positive correlation between $\delta^{15}\text{N}$ in leaves and soil in the interior highlights the stability of nutrient cycling in these areas, while the negative correlation at the edges reflects the impact of external disturbances, such as nutrient leaching and soil degradation.

Physiologically, *B. holophylla* exhibited distinct responses to edge and interior conditions. Higher specific leaf area (SLA) and relative water content (RWC) at the edges suggest adaptations for light capture and water retention, which may confer advantages in more open and stressful environments. However, the lack of significant differences in chlorophyll content and quantum yield (Fv/Fm) between edge and interior plants indicates that photosynthetic efficiency is maintained despite edge effects. The higher non-photochemical quenching (NPQ) and fluorescence decline ratio (Rfd) in the interior suggest that plants in these areas are better to dissipate excess light energy, potentially providing a physiological advantage during periods of environmental stress. These findings underscore the complexity of edge effects in Cerrado fragments and their implications for plant physiology and ecosystem functioning. The results highlight the need for conservation strategies that consider the interplay between fragment size, historical land use, and local environmental conditions to mitigate the impacts of edge effects on nutrient cycling and plant adaptation.

Author contributions: Research work was designed by Ana Silvia FP Moreira (ASFPM), and Denny Fernandes Eduardo (DFE) and fieldwork was performed by DFE, Amanda de Paula Colzani (APC), and Jéssica Ferreira de Lima (JFL). The experimental work and data interpretation were performed by ASFPM, and DFE. DFE has written the manuscript and it has been edited by ASFPM, APC, and JFL.

3.7 References

- Aguiar M, Conway AJ, Bell JK, Stewart KJ (2023). Agroecosystem edge effects on vegetation, soil properties, and the soil microbial community in the Canadian prairie. PLoS One 18: 1–21. <https://doi.org/10.1371/journal.pone.0283832>
- Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2013). Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22: 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT (2003). Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochem Cycles 17(1): 1031. <https://doi.org/10.1029/2002GB001903>
- Aynekulu E, Sileshi GW, Rosenstock TS, Noordwijk M, Tsegaye D, Koala J, Sawadogo L, Milne E, Leeuw J, Shepherd K (2021). No changes in soil organic carbon and nitrogen following long-term prescribed burning and livestock exclusion in the Sudan-savanna woodlands of Burkina Faso. Basic Appl Ecol 56: 165–175. <https://doi.org/10.1016/j.baae.2021.07.007>
- Barrs HD, Weatherley PE (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 15: 413–428. <https://doi.org/10.1071/BI9620413>
- Bateman AS, Kelly SD (2007). Fertilizer nitrogen isotope signatures. Isotopes Environ Health Stud 43: 237–247. <https://doi.org/10.1080/10256010701550732>
- Berghetti ALP, Araujo MM, Tabaldi LA, Turchetto F, Tassinari A, Bernardy D, Griebeler AM, Barbosa FM, Aimi SC, Brunetto G (2021). Effects of nitrogen fertilization on the growth and on photochemical efficiency in plants of *Handroanthus heptaphyllus*. J Plant Nutr 44: 2464–2475. <https://doi.org/10.1080/01904167.2021.1899216>
- Boschiero BN, Mariano E, Torres-Dorante LO, Sattolo TMS, Otto R, Garcia PL, Dias CTS, Trivelin PCO., (2020). Nitrogen fertilizer effects on sugarcane growth, nutritional status, and productivity in tropical acid soils. Nutr Cycl Agroecosyst 117: 367–382. <https://doi.org/10.1007/s10705-020-10074-w>
- Bouwman AF, Beusen AHW, Billen G., (2009). Human alteration of the global nitrogen and phosphorus soil balances for the period 1970–2050. Global Biogeochem Cycles 23: GBOA04. <https://doi.org/10.1029/2009GB003576>
- Bustamante MMC, Martinelli LA, Silva DA, Camargo PB, Klink CA, Domingues TF,

- Santos RV (2004). ^{15}N natural abundance in woody plants and soils of Central Brazilian savannas (cerrado). *Ecol Appl* 14: 200–213. <https://doi.org/10.1890/01-6013>
- Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege HT, Morgan HD, Heijden MGA, Pausas JG, Poorter H (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust J Bot* 51: 335–380. <https://doi.org/10.1071/BT02124>
- Coser TR, Ramos MLG, Figueiredo C, Urquiaga S, Carvalho AM, Barros FV, Mendonça MT (2016). Nitrogen uptake efficiency of maize in monoculture and intercropped with *Brachiaria humidicola* and *Panicum maximum* in a dystrophic red-yellow latosol of the Brazilian Cerrado. *Crop Pasture Sci* 67: 47–54. <https://doi.org/10.1071/CP15077>
- Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E, Wang L (2015). Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* 396: 1–26. <https://doi.org/10.1007/s11104-015-2542-1>
- Devlaeminck R, Schrijver A, Hermy M (2005). Variation in throughfall deposition across a deciduous beech (*Fagus sylvatica* L.) forest edge in Flanders. *Sci Total Environ* 337: 241–252. <https://doi.org/10.1016/j.scitotenv.2004.07.005>
- Dillon WW, Lieurance D, Hiatt DT, Clay K, Flory SL (2018). Native and invasive woody species differentially respond to forest edges and forest successional age. *Forests* 9: 381. <https://doi.org/10.3390/f9070381>
- Ehleringer JR, Osmond CB (1989). Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW, Eds., *Plant Physiological Ecology: Field Methods and Instrumentation*, Kluwer, London, 281. https://doi.org/10.1007/978-94-009-2221-1_13
- Ewers RM, Didham RK (2006). Confounding factors in the detection of species responses to habitat fragmentation. *Biol Rev Camb Philos Soc* 81:117–142. <https://doi.org/10.1017/S1464793105006949>
- Ferreira ACB, Borin ALDC, Lamas FM, Ferreira GB, Resende ÁV (2022). Exchangeable potassium reserve in a Brazilian savanna oxisol after nine years under different cotton production systems. *Sci Agric* 79: e20200339. <https://doi.org/10.1590/1678-992x-2020-0339>

- Gámez-Virués S, Perović DJ, Gossner MM, Börschig C, Blüthgen N, Jong H, Simons NK, Klein AM, Krauss J, Maier G, Scherber C, Steckel J, Rothenwöhrer C, Steffan-Dewenter I, Weiner CN, Weisser W, Werner M, Tschardt T, Westphal C (2015). Landscape simplification filters species traits and drives biotic homogenization. *Nat Commun* 6: 8568. <https://doi.org/10.1038/ncomms9568>
- Genty B, Briantais JM, Baker NR (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta Gen Subj* 990: 87–92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9)
- Gonçalves RVS, Cardoso JCF, Oliveira PEAM, Oliveira DC (2021). Changes in the Cerrado vegetation structure: insights from more than three decades of ecological succession. *Web Ecol* 21: 55-64. <https://doi.org/10.5194/WE-21-55-2021>
- Gotelli NJ, Ellison AM (2011). *Princípios da Estatística em Ecooga*. Artmed, Porto Alegre.
- Goulden SKE, Ohkouchi N, Freeman KH, Chikaraishi Y, Ogawa NO, Suga H, Chadwick O, Houlton BZ (2019). Strong correspondence between nitrogen isotope composition of foliage and chlorin across a rainfall gradient: implications for paleo-reconstruction of the nitrogen cycle. *Biogeosciences* 16: 3869–3882. <https://doi.org/10.5194/bg-16-3869-2019>
- Harper KA, Yang JR, Dazé Querry N, Dyer J, Alves RSC, Ribeiro MC (2024). Limited influence from edges and topography on vegetation structure and diversity in Atlantic Forest. *Plant Ecol* 225: 361–371 (2024). <https://doi.org/10.1007/s11258-023-01353-x>
- Hastings MG, Casciotti KL, Elliott EM (2013). Stable isotopes as tracers of anthropogenic nitrogen sources, deposition, and impacts. *Elements* 9: 339–344. <https://doi.org/10.2113/gselements.9.5.339>
- Honnay O, Verheyen K, Hermy M (2002). Permeability of ancient forest edges for weedy plant species invasion. *For Ecol Manage* 161: 109–122. [https://doi.org/10.1016/S0378-1127\(01\)00490-X](https://doi.org/10.1016/S0378-1127(01)00490-X)
- Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, Sibaldelli RNR, Mendes IC, Arihara J (2006). Nitrogen nutrition of soybean in Brazil: contributions of biological N₂ fixation and N fertilizer to grain yield. *Canad J Plant Sci* 86. <https://doi.org/10.4141/P05-098>

- IEF (2011) <http://www.ief.mg.gov.br/unidades-de-conservacao/205?task=view>
- INMET (2024). Estação meteorológica de Ipameri-GO.
<https://clima.inmet.gov.br/GraficosClimatologicos/DF/83377>
- Jacobson TKB, Bustamante MMDC, Kozovits AR (2011). Diversity of shrub tree layer, leaf litter decomposition and N release in a Brazilian Cerrado under N, P and N plus P additions. *Environ Pollut* 159: 2236–2242.
<https://doi.org/10.1016/j.envpol.2010.10.019>
- Jordan TE, Correll DL, Weller DE (1993). Nutrient interception by a riparian forest receiving inputs from adjacent cropland. *J Environ Qual* 22: 467–473.
<https://doi.org/10.2134/jeq1993.00472425002200030010x>
- Kassambara A, Mundt F (2020). factoextra: extract and visualize the results of multivariate data analyses. Rpackage version 1.0.7 URL <https://cran.r-project.org/package=factoextra>.
- Kozovits AR, Bustamante MMC, Garofalo CR, Bucci S, Franco AC, Goldstein G, Meinzer FC (2007). Nutrient resorption and patterns of litter production and decomposition in a Neotropical Savanna. *Funct Ecol* 21: 1034–1043.
<https://doi.org/10.1111/j.1365-2435.2007.01325.x>
- Landa, G.G., Rezende, J.L.P., Lima, F. d. S., Werneck, M. de S., 2010. Fitossociologia de uma área de Cerrado na Estação de Pesquisa e Desenvolvimento Ambiental Galheiro, Perdizes (MG): uma comparação entre métodos. *Rev. Sinapse Ambient.* 7, 71–85.
- Lannes LS, Karrer S, Teodoro DAA, Bustamante MMC, Edwards PJ, Venterink HO (2020). Species richness both impedes and promotes alien plant invasions in the Brazilian Cerrado. *Sci Rep* 10: 1–9. <https://doi.org/10.1038/s41598-020-68412-5>
- Le J, Josse J, Husson F (2008). FactoMinerR: An R package for multivariate analysis. *Journal of Statistical Software* 25: 1–18. <https://doi.org/10.18637/jss.v025.i01>
- Li FR, Zhao WZ, Liu JL, Huang ZG (2009). Degraded vegetation and wind erosion influence soil carbon, nitrogen and phosphorus accumulation in sandy grasslands. *Plant Soil* 317: 79–92. <https://doi.org/10.1007/s11104-008-9789-8>
- Lichtenthaler HK, Buschmann C, Knapp M (2005). How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. *Photosynthetica* 43: 379–393. <https://doi.org/10.1007/s11099-005-0062-6>

- Lichtenthaler HK, Wellburn AR (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans* 11: 591-592. <https://doi.org/10.1042/bst0110591>
- Lima TA, Pinto RRJ, Lenza E, Pinto AS (2010). Floristic and structure of woody vegetation of a “cerrado rupestre” area in serra de caldas novas state park, Goiás. *Biota Neotrop* 10: 159–166. <https://doi.org/10.1590/S1676-06032010000200020>
- Liu G, Wang Q, Chen J, Yan G, Wang H, Xing Y (2024). Natural $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ abundance of plants and soils under long-term N addition in a temperate secondary forest. *J Soil Sci Plant Nutr*. <https://doi.org/10.1007/s42729-024-01770-5>
- Liu M, Han G, Li X (2021). Using stable nitrogen isotope to indicate soil nitrogen dynamics under agricultural soil erosion in the Mun River basin, Northeast Thailand. *Ecol Indic* 128: 107814. <https://doi.org/10.1016/j.ecolind.2021.107814>
- Lopes SF, Schiavini I, Prado-Júnior JA, Gusson AE, Souza-Neto AE, Vale VS, Dias-Neto OC., 2011. Caracterização ecológica e distribuição diamétrica da vegetação arbórea em um remanescente de floresta estacional semidecidual, na fazenda experimental do Glória, Uberlândia, MG. *Biosci J* 27: 322-335.
- Machado DN, Novais RF, Silva IR, Loureiro ME, Milagres JJ, Soares EMB (2011). Enriquecimento e alocação de ^{13}C em plantas de eucalipto. *Rev Bras Cienc Solo* 35: 857–866. <https://doi.org/10.1590/S0100-06832011000300020>
- Magnago LFS, Rocha MF, Meyer L, Martins SV, Meira-Neto JAA (2015). Microclimatic conditions at forest edges have significant impacts on vegetation structure in large Atlantic Forest fragments. *Biodivers Conserv* 24: 2305–2318. <https://doi.org/10.1007/s10531-015-0961-1>
- Mendonça LAR, Frischkorn H, Santiago MF, Camargo PB, Lima JOG, Mendes-Filho J (2010). Identificação de mudanças florestais por ^{13}C e dos solos da Chapada do Araripe, Ceará. *Rev Bras Eng Agric Ambient* 14: 314–319. <https://doi.org/10.1590/S1415-43662010000300012>
- Mendonça RC, Felfili JM, Walter BMT, Silva Júnior MC, Rezende AV, Filgueiras TS, et al. (2008). Flora vascular do cerrado. In: Sano SM, Almeida SP, Ribeiro JF. (eds). *Cerrado, ecologia e flora*. EMBRAPA-CPAC, Brasília, pp 29–47.
- Nel JA, Craine JM, Cramer MD (2018). Correspondence between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in soils suggests coordinated fractionation processes for soil C and N. *Plant Soil* 423: 257–271. <https://doi.org/10.1007/s11104-017-3500-x>

- Nóbrega RLB, Guzha AC, Torres GN, Kovacs K, Lamparter G, Amorim RSS, Couto E, Gerold G (2017). Effects of conversion of native cerrado vegetation to pasture on soil hydro-physical properties, evapotranspiration and streamflow on the Amazonian agricultural frontier. *PLoS One* 15(7): e0236236 <https://doi.org/10.1371/journal.pone.0179414>
- Pereira, F.C., Guilherme, F.A.G., Marimon, B.S., (2021). Edge Effects on Successional Dynamics of Forest Fragments in the Brazilian Cerrado. *Floresta e Ambient.* 28, 1–8. <https://doi.org/10.1590/2179-8087-floram-2020-0063>
- Pocewicz A, Morgan P, Kavanagh K (2007). The effects of adjacent land use on nitrogen dynamics at forest edges in northern Idaho. *Ecosystems* 10: 226–238. <https://doi.org/10.1007/s10021-007-9015-1>
- R Core Team (2022). R: Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL <https://www.r-project.org/>.
- Riva EG, Olmo M, Poorter H, Ubera JL, Villar R (2016). Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 mediterranean woody species along a water availability gradient. *PLoS One* 11: 1–18. <https://doi.org/10.1371/journal.pone.0148788>
- Rivera-Cubero LD, Downton AL, Rothstein DE (2023). Nitrate and ammonium deposition in the midwestern fragmented forest. *Forests* 14 (3): 512. <https://doi.org/10.3390/f14030512>
- Sainju UM, Ghimire R, Pradhan GP (2020). Nitrogen fertilization I: impact on crop, soil, and environment. In: Rigobelo EC, Serra A, Surguchov A (eds) *Nitrogen Fixation*. IntechOpen, pp 1–24. <https://doi.org/10.5772/intechopen.86028>
- Sena-Souza JP, Houlton BZ, Martinelli LA, Nardoto GB (2020). Reconstructing continental-scale variation in soil $\delta^{15}\text{N}$: a machine learning approach in South America. *Ecosphere* 11: e03223. <https://doi.org/10.1002/ecs2.3223>
- Silva LMS, Dias AG, Nakajima JN (2022). As árvores do cerrado no Clube Caça e Pesca Itororó, Uberlândia, Estado de Minas Gerais, Brasil. *Hoehnea* 49: e72-2021. <https://doi.org/10.1590/2236-8906-72-2021>
- Simão EDP, Resende ÁV, Gontijo-Neto MM, Silva AF, Godinho VPC, Galvão JCC, Borghi E, Oliveira AC, Giehl J (2020). Nitrogen fertilization in off-season corn crop in different Brazilian Cerrado environments. *Pesqui Agropecu Bras* 55: e01551.

<https://doi.org/10.1590/s1678-3921.pab2020.v55.01551>

- Smith IA, Hutrya LR, Reinmann AB, Marrs JK, Thompson JR (2018). Piecing together the fragments: elucidating edge effects on forest carbon dynamics. *Front Ecol Environ* 16(4): 213-221. <https://doi.org/10.1002/fee.1793>
- Sprent JI, Geoghegan IE, Whitty PW, James EK (1996). Natural abundance of ^{15}N and ^{13}C in nodulated legumes and other plants in the cerrado and neighboring regions of Brazil. *Oecologia* 105(4): 440–446. <https://doi.org/10.1007/BF00330006>
- Strassburg BBN, Brooks T, Feltran-Barbieri R, Iribarrem A, Crouzeilles R, Loyola R, Latawiec AE, Oliveira Filho FJB, Scaramuzza CAM, Scarano FR, Soares-Filho B, Balmford A (2017). Moment of truth for the Cerrado hotspot. *Nat Ecol Evol* 1(4): <https://doi.org/10.1038/s41559-017-0099>
- Tatarko AR, Knops JMH (2018) Nitrogen addition and ecosystem functioning: both species abundances and traits alter community structure and function. *Ecosphere* 9(1): e02087. <https://doi.org/10.1002/ecs2.2087>
- Tolbert NE (1997) The C_2 oxidative photosynthetic carbon cycle. *Annu Rev Plant Biol* 48: 1–25. <https://doi.org/10.1146/annurev.arplant.48.1.1>
- Turner NC (1981) Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* 58(1): 339-366. <https://doi.org/10.1007/BF02180062>
- Vargas BC, Araújo GM (2014) Florística de trepadeiras em fragmentos de florestas semidecíduais em Uberlândia, Minas Gerais, Brasil. *Rodriguésia* 65(1): 49–59. <https://doi.org/10.1590/S2175-78602014000100004>
- Vega A, O'Brien JA, Gutiérrez RA (2019) Nitrate and hormonal signaling crosstalk for plant growth and development. *Curr Opin Plant Biol* 52: 155-163. <https://doi.org/10.1016/j.pbi.2019.10.001>
- Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer-Verlag New York.
- Wingate L, Ogée J, Burlett R, Bosc A, Devaux M, Grace J, Loustau D, Gessler A (2010) Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration. *New Phytol* 188(2): 576-589. <https://doi.org/10.1111/j.1469-8137.2010.03384.x>
- Yan G, Han S, Zhou M, Sun W, Huang B, Wang H, Xing Y, Wang Q (2020) Variations in the natural ^{13}C and ^{15}N abundance of plants and soils under long-term N addition and precipitation reduction: interpretation of C and N dynamics. *For Ecosyst* 7: 49. <https://doi.org/10.1186/s40663-020-00257-w>

- Zemunik G, Turner BL, Lambers H, Laliberté E (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nat Plants* 1(5): 1–4. <https://doi.org/10.1038/nplants.2015.50>
- Zuffo AM, Ratke RF, Steiner F, Aguilera JG (2022) Agronomic characteristics of soybean cultivars with late-season nitrogen application in supplementation to the inoculation of *Bradyrhizobium* spp. *Cienc Agrotecnologia* 46: e022521. <https://doi.org/10.1590/1413-7054202246022521>

3.8 Figures and table

3.8.1 Table 1: Cerrado fragments, size, and type of culture of the adjacent areas to the Cerrado fragments used as sampling areas.

Cerrado fragments	Area (ha)	Type of culture	Bibliographic reference
		Agricultural	
Fazenda Santa Bárbara (FSB)	16	turnover (corn, soybean, sorghum) and extensive livestock	Google earth and personal observation
Fazenda São José (FSJ)	20	Planting of Eucalyptus and livestock	Vargas-Araujo (2014) Personal observation
Fazenda Experimental Gloria (FEG)	30	Planting of corn, Eucalyptus and livestock	Lopes et al. (2011) Google earth and personal observation
Clube Caça e Pesca Itororó (CCP)	127	Soybean monoculture	Silva et al. (2022) Google earth and personal observation
Estação Ecológica do Panga (PANGA)	409	Sugarcane monoculture	Cardoso et al. (2010)
Parque Estadual do Pau Furado, MG	2200	Soybean monoculture	Google earth and personal observation IEF (2011) Personal observation
RPPN Galheiros	2694	Soybean monoculture	Landa et al. (2010) Google earth and

Parque Estadual da Serra de Caldas Novas (PESCaN), GO	12500	Rotary planting of soybeans, sorghum and corn	personal observation Lima et al. (2010) Google earth and personal observation
---	-------	---	---

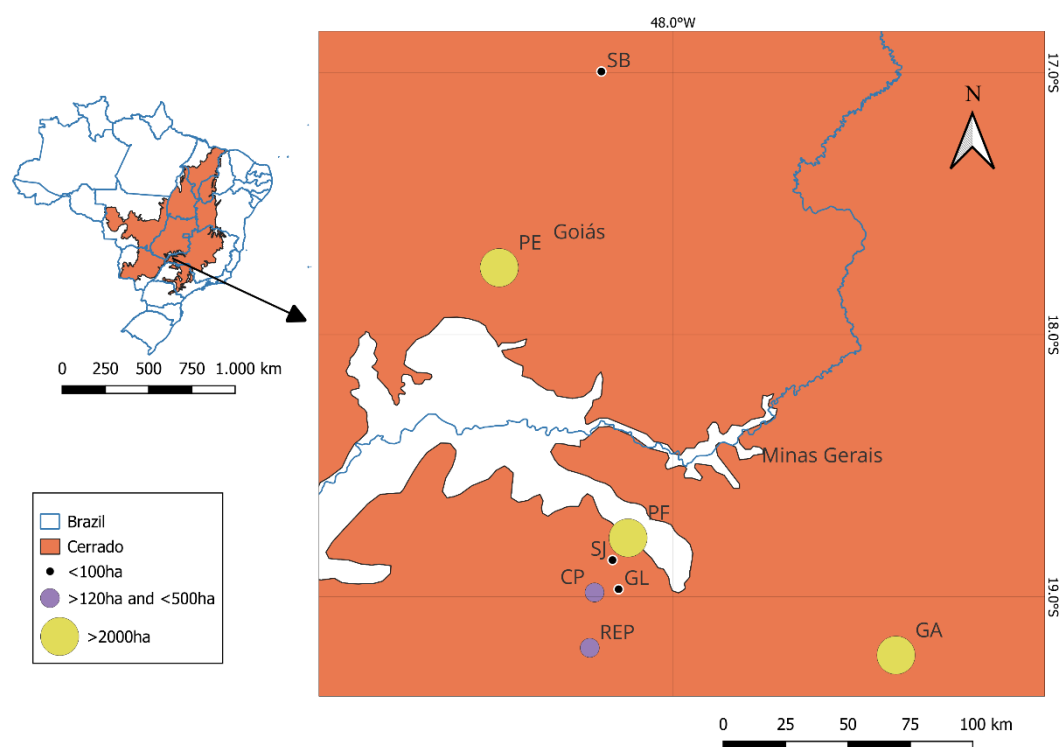


Fig. 1. Location and size of eight Cerrado remnants used for analysis of the isotopic signature of ^{15}N soil and leaves of *B. holophylla*. The maps were generated using QGIS (v. 3.30.1, DATUM SIGAS 2000). Legends: (SB) Fazenda Santa Bárbara, (SJ) Fazenda São José, (GL) Fazenda Experimental do Gloria, (CP) Clube Caça e Pesca Itororó, (REP) Estação Ecológica do Panga, (PF) Parque Estadual dos Pau Furado, (GA) RPPN Galheiros, (PE) Parque Estadual da Serra de Caldas Novas.

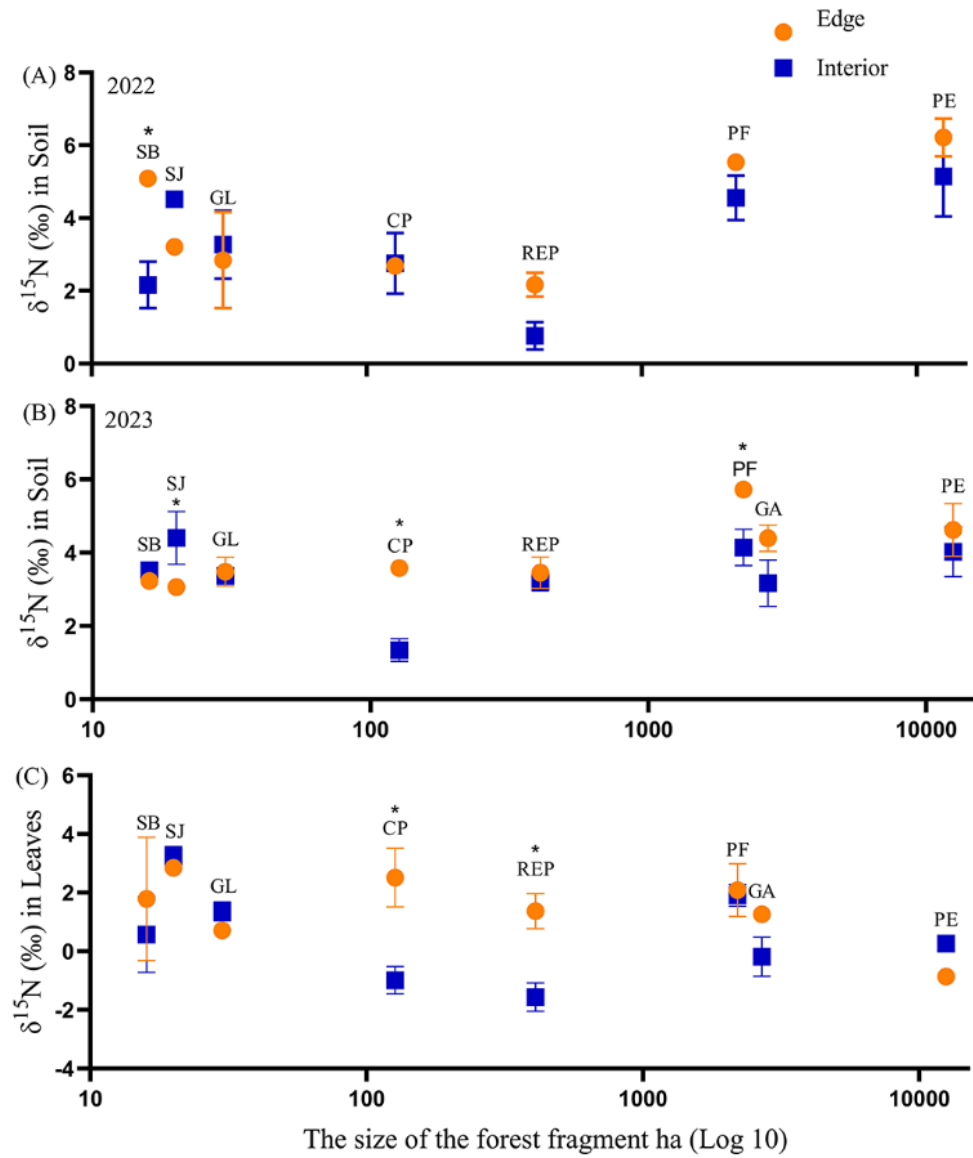


Fig. 2. Relationship between the presence of ^{15}N (‰) in the soil and leaves of *B. holophylla* considering the size of Cerrado fragments. Soil samples were collected in January 2022 (A) and 2023 (B), while leaf samples (C) were collected in 2023 during the rainy season. Legends: in blue (inside the fragments), in orange (the edge of the fragments). (SB) Fazenda Santa Bárbara, (SJ) Fazenda São José, (GL) Fazenda Experimental do Gloria, (CP) Clube Caça e Pesca Itororó, (REP) Estação Ecológica do Panga, (PF) Parque Estadual dos Pau Furado, (GA) RPPN Galheiros, (PE) Parque Estadual da Serra de Caldas Novas * Means significant difference $p < 0.05$.

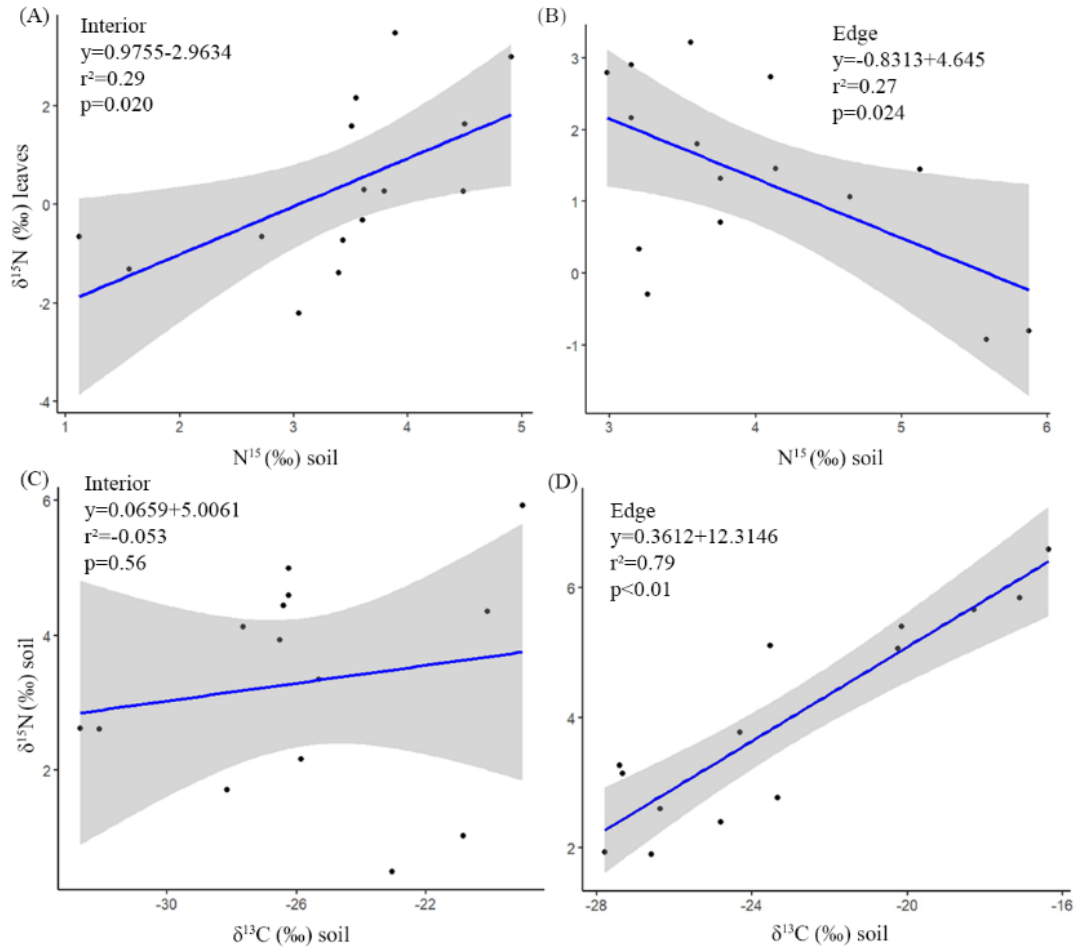


Fig. 3. Relation between the values of $\delta^{15}\text{N}$ in the leaves of *B. holophylla* and the values of $\delta^{15}\text{N}$ in the soil of the edge (A) and the interior (B) of eight Cerrado fragments (see Fig. 1). Relation between the values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the soils of the interior (C) and of the edges (D) in the fragments of Cerrado.

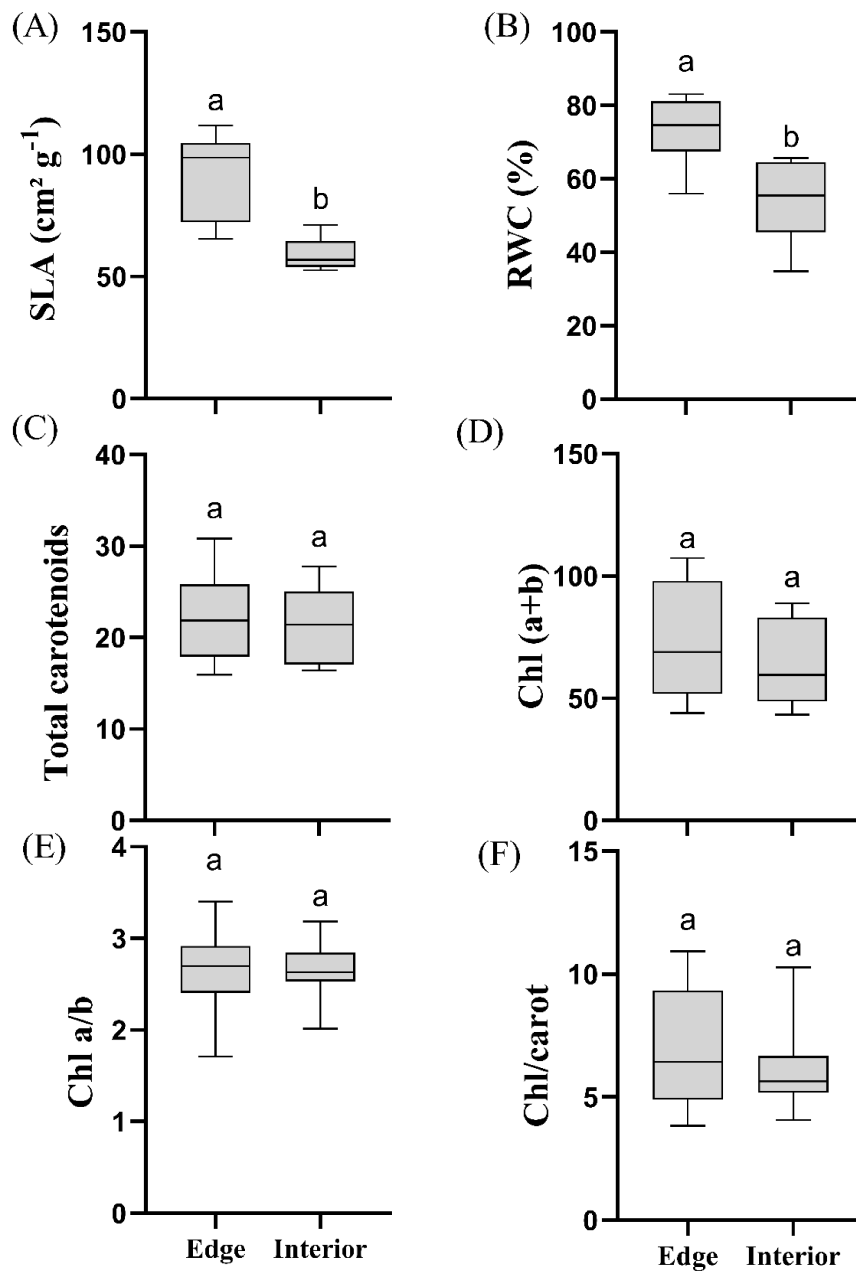


Fig. 4. Comparison of physiological parameters among individuals of *B. holophylla* found at the edge and inside the Panga Ecological Station, MG, Brazil. (A) Specific leaf area (SLA); (B) Relative water content (RWC); (C) Total carotenoids; (D) total chlorophyll ($a+b$), (E); chlorophyll a and b ratio (a/b); (F) total chlorophyll and carotenoids ratio (Chl/carot). Different letters indicate significant differences considering $p < 0.05$.

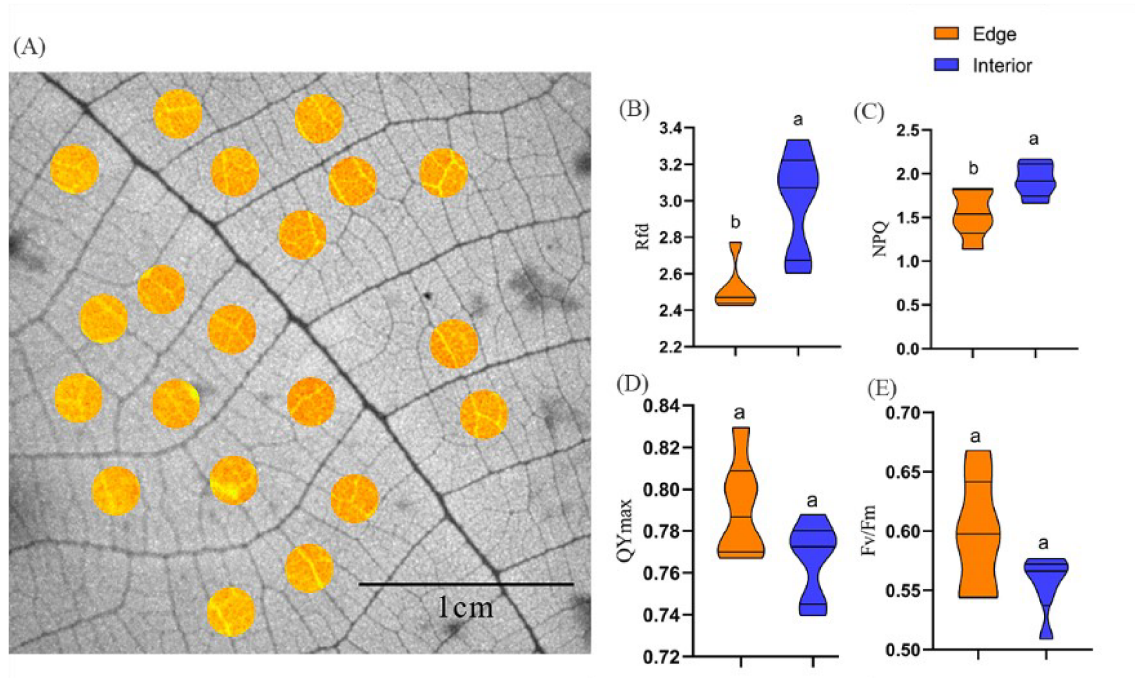


Fig. 5. Fluorescence of chlorophyll a in leaves of *B. holophylla*. (A) Model of the 20 visualization fields used to measure the fluorescence parameters of chlorophyll. For the analysis, the measurements were obtained in leaves of *B. holophylla* a fragment of Cerrado in the Panga Ecological Station, MG, Brazil. Legend: in blue (inside the fragments), and in orange (the edge of the fragments). (B) (Rfd) Reason of fluorescence decline in PSII; (C) (NPQ) non-photochemical quenching during light adaptation; (D) (QYmax) maximum quantum yield of PSII; and (E) (Fv/Fm) potential quantum yield of PSII after dark adaptation.

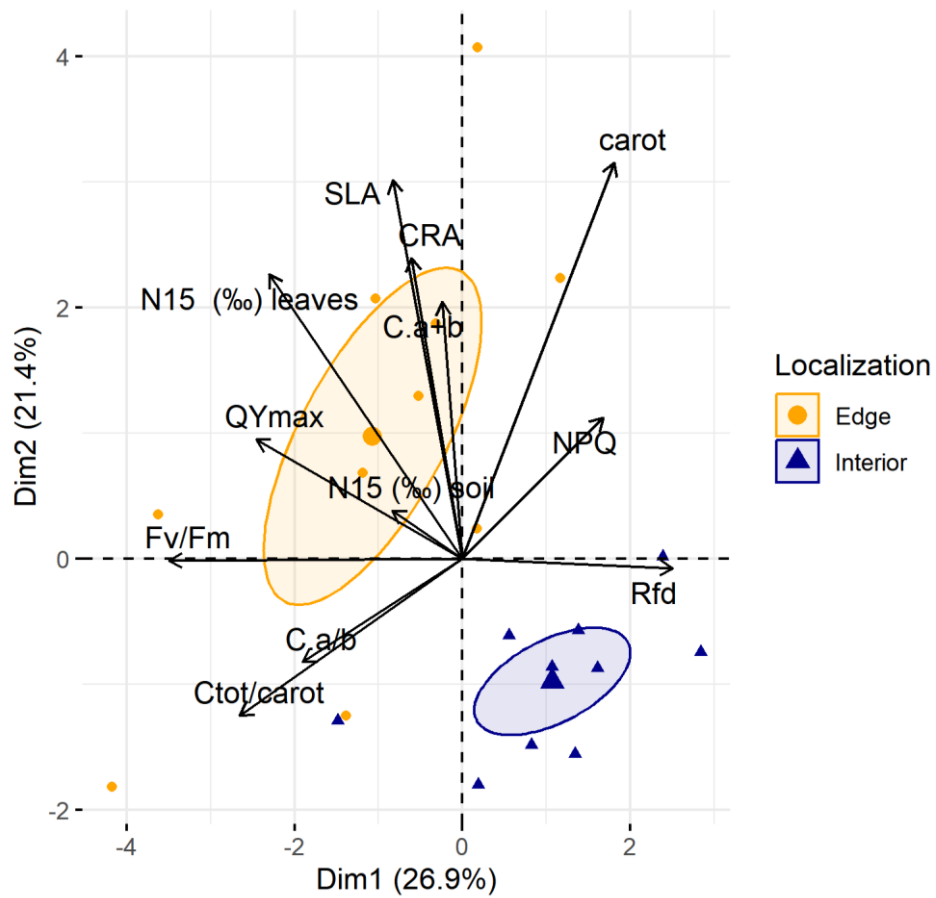


Fig. 6. Principal component analysis (PCA- Bitplot) of physiological parameters and values of $\delta^{15}\text{N}$ in the leaves and soil of the interior and edge of the Cerrado fragment of the Panga Ecological Station, MG, Brazil. Legend: in blue (inside the fragments), and in orange (the edge of the fragments). (Rfd) Reason of fluorescence decline in PSII; (NPQ) non-photochemical quenching during light adaptation; (QYmax) maximum quantum yield of PSII; (Fv/Fm) potential quantum yield of PSII after light adaptation.

4. Capítulo 2

Como a suplementação de nitrogênio e fósforo pode influenciar na fisiologia de *Bauhinia holophylla* (Bong.)?

Resumo

A entrada dos nutrientes minerais essenciais nitrogênio (N) e fósforo (P) no ambiente tem sido alterada principalmente por ações antrópicas relacionadas à conversão de vastas áreas do Cerrado em monoculturas. O N e P industrializados são aplicados em grandes quantidades para garantir a alta produtividade das culturas agrícolas. Consequentemente, esses nutrientes podem ser lixiviados ou volatilizados, alcançando remanescentes do Cerrado e influenciando as respostas fisiológicas de plantas nativas. Pesquisas preveem que as entradas de N e P têm aumentado ao longo do tempo nos ecossistemas e, com base nesses estudos, foi suplementado em uma área experimental de Cerrado fertilizantes nitrogenados e fosfatados. O objetivo desta pesquisa foi entender como o aumento desses nutrientes influencia respostas fisiológicas relacionadas ao rendimento foliar de *Bauhinia holophylla* (Bongard) Steaudel em campo. Foram avaliadas cinco parcelas de (100m²) para cada tratamento, considerando a suplementação de N, P e N+P combinados e parcelas controle. A espécie apresentou valores relativamente altos de área foliar específica e conteúdo relativo de água no tratamento com N+P, mas não diferiu significativamente entre os tratamentos em termos de área foliar, clorofila total (a+b), clorofila (a/b) e clorofila total e carotenoides. A rendimento quântico potencial do PSII (F_v/F_m) foi maior no tratamento com fósforo em comparação com o grupo controle, sugerindo maior eficiência fotossintética com a adição de fósforo. Por outro lado, o *quenching* não fotoquímico durante a adaptação a luz (NPQ) e a razão do declínio da fluorescência (R_{fd}) foram menores no tratamento com fósforo do que no grupo controle, indicando que, nessas condições, *B. holophylla* apresenta menor capacidade de dissipação de energia não utilizada na fotossíntese, menor proteção contra excesso de luz e menor vitalidade. No entanto, o rendimento quântico máximo do PSII (QY_{max}) não diferiu entre os tratamentos, indicando que a espécie não alterou o potencial máximo de captura de energia do PSII. Além disso, a análise do índice de distância de plasticidade relativa (RDPI) mostrou que *B. holophylla* possui alta capacidade de ajuste fenotípico em resposta as variações na disponibilidade de N e P em campo.

Palavras chave: fisiologia foliar, fotossíntese, Cerrado, plasticidade fisiológica.

How nitrogen and phosphorus supplementation can influence the physiology of *Bauhinia holophylla* (Bong.)?

Abstract

The entry of the essential mineral nutrients nitrogen (N) and phosphorus (P) into the environment has been altered mainly by anthropic actions related to the conversion of vast areas of the Cerrado into monocultures. Industrialized N and P are applied in large amounts to ensure the high productivity of agricultural cultivars. Consequently, these nutrients can be leached or volatilized, arriving in Cerrado remnants and influencing the physiology of native plants. Research has predicted that N and P have increased over time, and on the basis of these studies, we supplemented an experimental area of Cerrado with industrial nitrogen and phosphate fertilizers. We sought to understand how the increase in these nutrients influences physiological responses related to the leaf yield of *Bauhinia holophylla* (Bongard) Steudel in the field. Five plots (100 m²) of each treatment were evaluated, considering N and P supplementation and N+P combined, in addition to the control plots. The species presented relatively high values of specific leaf area and relative water content in the N+P treatment but did not significantly differ between the treatments in terms of leaf area, total chlorophyll (*a+b*), chlorophyll *a* and *b*, or total chlorophyll and carotenoids. The maximum quantum yield of PSII (F_v/F_m) was greater in the P supplementation treatment group than in the control group, suggesting greater photosynthetic efficiency with phosphorus addition. On the other hand, the nonphotochemical *quenching* during light adaptation (NPQ) and the fluorescence decline ratio (R_{fd}) were lower in the treatment with phosphorus than in the control, suggesting that under these conditions, *B. holophylla* has a lower energy dissipation capacity. that is not used in photosynthesis, lower protection against excess light and lower vitality. However, the quantum yield of PSII (QY_{max}) did not differ among the treatments, indicating that the species did not affect the maximum potential of energy capture by PSII. In addition, analysis of relative distances plasticity index (RDPI) revealed that *B. holophylla* has a high capacity for phenotypic adjustment in response to variations in the availability of N and P in field.

Keywords: leaf physiology, photosynthesis, Cerrado, physiological plasticity.

4.1 Introduction

Nitrogen (N) and phosphorus (P) are essential mineral nutrients for plant growth and are considered to limit plant productivity when their concentrations in soil availability are less than necessary (Elser et al., 2007; Vitousek et al., 2010). The N is involved in the synthesis of amino acids, proteins, chlorophylls, nucleic acids, lipids and other metabolites (Kusano et al., 2011). In turn, P plays crucial roles in the production of ATP, in the electron transport chain, and in several other physiological functions (Carstensen et al., 2018). Despite the importance of these nutrients for the efficient development of plants, excessive concentrations of N and P can cause toxicity and decrease their photosynthetic capacity (Kayoumu et al., 2023). Therefore, the availability of N and P and the stoichiometric relationship between these elements in an environment can alter the selection and filtering of leaf functional attributes linked to productivity, such as the specific leaf area, concentration of chloroplast pigments and other photosynthetic parameters (Bubier et al., 2011).

For plants to use N, it is necessary to fix it in ecosystems in a process that can occur through abiotic, biological or anthropogenic mechanisms. Naturally, approximately 3 to 4% of the of atmospheric is performed by electrical discharges (lightning). However, biological nitrogen fixation (whether through free-living fixatives or symbiotic relationships) plays an essential role in this process. This fixation is catalyzed by the enzyme nitrogenase, which reduces atmospheric nitrogen (N_2) into ammonia (NH_3). In the presence of water, ammonia is rapidly converted into ammonium ions (NH_4^+) (Crawford et al., 1998; Thamdrup, 2012). It is important to note that nitrate (NO_3^-) is not a direct product of nitrogenase activity. Instead, nitrate is formed through the oxidation of ammonium (NH_4^+) in the soil. Plants can absorb both ammonium (NH_4^+) and nitrate (NO_3^-), but only after these forms are available in the soil. Once absorbed, nitrate is assimilated into organic compounds within the plant (Crawford et al., 1998).

One form of anthropogenic nitrogen fixation is the Haber–Bosch process, which consists of the synthesis of NH_3 from hydrogen (H_2) and N_2 , increasing the number of molecules to high temperatures and pressures (Humphreys et al., 2021). The NH_3 produced in the Haber–Bosch process is present in nitrogen fertilizers widely used in monocultures to ensure the high productivity of agricultural cultivars (James, 2016). On the other hand, NH_3 is a gas and it can easily disperse in air. The volatility of ammonia allows it to move quickly into the atmosphere, where it can be deposited on soil and water

surfaces in natural ecosystems (Behera et al., 2013; Lan et al., 2024), including Cerrado remnants (see chapter 1).

The intensification of monocultures to meet the growing global food protein and caloric demand, especially in response to population growth, has contributed to the increase in soil nutrient levels, especially nitrogen, over time (Tilman et al., 2011). The intensive use of nitrogen fertilizers can result in excessive increases in nutrients, causing acidity and soil saturation, or can overload nutrients in rivers, lakes and oceans, creating “dead zones” (Good and Beatty, 2011; Lai et al., 2018; Sutton et al., 2014). In addition to anthropogenic supplementation, large-scale cultivation of legumes with the ability to fix atmospheric nitrogen may increase soil nitrogen availability. However, N from biological fixation in legume monocultures, combined with the application of nitrogen fertilizers, may contribute to excessive nitrogen accumulation (Schmidt et al., 2021). At the end of the 20th century, human nitrogen fixation exceeded natural nitrogen fixation, and in 2004, global N deposition varied between 1 and 10 kg N ha⁻¹ per year. It is estimated that this fixation may increase the amount of N deposited in natural areas fivefold by 2050, reaching 50 kg N ha⁻¹ per year (Galloway et al., 2004).

The P is also an essential macronutrient for plants and is available in natural environments, mainly through the weathering of phosphate rocks, which release phosphate particles and ions into the soil solution. This element has lower mobility in the soil than does N, and when phosphate fertilizers are supplemented in natural environments, they tend to increase the concentration of P at the soil surface but may also facilitate its loss through the surface runoff process (Messiga et al., 2013). In the atmosphere, phosphorus has a natural origin, such as mineral dust, aerosol particles, and ash from volcanoes and forest fires. On the other hand, anthropogenic sources can be released from the burning of fossil fuels and agricultural activities, including phosphorus supplementation and deforestation. This P can be transported over large distances in dust particles (Diao et al., 2023), as shown by the flow between Africa and the Amazon, which consequently can increase the productivity of native plants (Yu et al., 2015). Similarly, a relatively high concentration of this element is expected in Cerrado fragments close to monocultures that supplement this nutrient.

Plant physiology and, consequently, productivity are altered in plants subjected to different concentrations of nutrients. In the case of P, changes in the production of photosynthesis-related enzymes and pigments are expected, as observed with its

application in monocultures of *Zea mays* (Verlinden et al., 2022). Excess P can have several negative effects on plant physiology, such as decreased resistance to pathogens (Campos-Soriano et al., 2020), increased biomass with dilution of P in plant tissues, the accumulation of inorganic P in vacuoles altering nutrient allocation patterns and an increase in the proportion of metabolically inactive tissues, which may impact the efficiency of the use of other nutrients and plant health (Moenclaey et al., 2022). On the other hand, insufficient P content can lead to reduced photosynthetic rates, carbon fixation and plant growth. This effect is attributed to the deficiency of P in the electron transport chain and the reduction in ATP synthase activity in the chloroplast (Khan et al., 2023). However, each species may respond differently in terms of photosynthesis and leaf functional attributes to different levels of P availability and N+P combinations (Mo et al., 2019).

Despite the evidence of changes in functional leaf traits, the most studies involving P supplementation in the natural environment focus on changing the diversity and abundance of species in response to the limitation of this element (Zemunik et al., 2015). However, few studies have investigated how the physiology of plants native to the Cerrado is affected by P supplementation and its combination with N, being important to emphasize that the type of soil can filter functional characteristics that are linked to the acquisition of nutrients. Most aspects of the geochemical cycle and its implications for biodiversity are discussed (Kozovits et al., 2007; Jacobson et al. 2011; Bustamante et al. 2012). Considering the high species diversity of the Cerrado and that different species may respond differently to increases in N and P, studies on this topic are still scarce, and there is no robustness in how these nutrients influence the leaf physiology of shrub species in the Cerrado, including changes in productivity and photosynthetic processes. As a study model, we used *Bauhinia holophylla* (Bongard) Steudel (Fabaceae), a species with a wide distribution in the sample area. We sought to understand how *B. holophylla* responds to N, P and N+P combined supplementation, evaluating mainly leaf functional attributes linked to photosynthesis.

Few studies have investigated how the physiology of plants native to the Cerrado is affected by phosphorus (P) supplementation and its interaction with nitrogen (N). This gap is particularly relevant given that the type of soil can act as a filter for functional traits related to nutrient acquisition, such as root morphology, phosphatase activity, and mycorrhizal associations (Abrahão et al., 2019). Understanding these interactions is

crucial for predicting how Cerrado species respond to nutrient availability in different soil contexts, especially in light of increasing anthropogenic nutrient inputs. Furthermore, most aspects of the geochemical cycle and its implications for biodiversity, including nutrient dynamics and their effects on plant communities, have been extensively discussed in the literature (Kozovits et al., 2007; Jacobson et al., 2011; Bustamante et al., 2012).

The high species diversity of the Cerrado and the potential for varied responses to increases in nitrogen (N) and phosphorus (P) highlight the need for more comprehensive studies on this topic. Currently, research on how these nutrients influence the leaf physiology of shrub species, including changes in productivity and photosynthetic processes, remains scarce and lacks robustness. This knowledge gap limits our understanding of how nutrient enrichment affects Cerrado ecosystems, particularly in the context of increasing anthropogenic inputs. To address this, we used *Bauhinia holophylla* (Bongard) Steudel (Fabaceae), a widely distributed species in the study area, as a model. Our aim was to evaluate how *B. holophylla* responds to N, P, and combined N+P supplementation, with a focus on leaf functional attributes linked to photosynthesis. We hypothesized that the supplementation of P and N should increase leaf functional attributes such as specific leaf area, relative water content and also influence photosynthetic parameters.

4.2 Materials and methods

4.2.1 Study area and sample design

The study was conducted in the Panga Ecological Reserve, MG, Brazil (Fig. 1). The reserve has typical Cerrado phytophysiognomies, ranging from sparse cerrado, typical cerrado, dense cerrado, dense cerrado woodland, evergreen forest, gallery forest and *vereda* palm swamp (Gonçalves et al., 2021). The present study was conducted in an area of dense cerrado woodland. The local climate is tropical (Aw) according to the Köppen classification with two well-defined seasons: a rainy season in the summer, which occurs from November to April, and a dry winter season, which occurs from May to October. (Alvares et al., 2013). Twenty fixed plots of 100 m² (10 × 10 m) were established completely randomized for application by free throw of fertilizers containing N and P, at proportions of 45–00–00 and 00–44–00, respectively, of the brand *EQCOAT equilibria fertilizers* during the rainy season (January and February 2022 and 2023).

Supplementation in the experimental area has occurred annually since 2007, and the N dosages were defined according to the predictions proposed by Galloway et al. (2004) for the year 2050. The P plots were implemented in 2017, and the P dosages were proportional to those proposed by Crous et al. (2015). After 15 days of supplementation, individuals randomly distributed into the five plots of each treatment, avoiding the edge of each plot (up to 1 m from the edge), were sampled: the control group (no nutrient application), supplementation with 1.111 kg of N per plot, supplementation with 1.136 kg of P per plot, and N + P combined supplementation of 1.111 kg of N and 1.136 kg of P per plot.

4.2.2 Specific leaf area and relative water content

A mature leaf of ten individuals from each treatment (control, N, P and N+P) was collected, cooled and taken to the laboratory. The total leaf area was measured via ImageJ® software. The leaves were weighed using a precision scale (Shimadzu, AUY, Japan) to obtain the dry weight, placed in an oven (Novatecnica, NT513, Brazil) at 60°C for three days and weighed again. The specific leaf area (SLA) was calculated as the difference between the total area and the dry weight of each leaf (Witkowski and Lamont, 1991). To evaluate the water status of the leaves, the relative water content (RWC) was determined in the morning from two fragments per 0.4 cm² area of leaf (n = 10 individuals per treatment). The RWC was calculated via the following equation: $RWC = [(FM - DM) / (TM - DM)] * 100$, where FM is the fresh mass, TM is the turgid mass after hydration for 24 hours, and DM corresponds to the dry mass after drying the material in an oven at 60°C until it reaches a constant weight (Bars and Watherly, 1962).

4.2.3 Evaluation of photosynthetic performance

The investment in photosynthetic pigments was evaluated in ten leaves of ten individuals in two leaf fragments of approximately 0.4 cm² in area. The samples were immersed in 10 ml of dimethyl sulfoxide (PA) for 72 h, and the extracts were taken at wavelengths of 480, 649 and 665 nm in a spectrophotometer (Biospec, Brazil). The

contents of chlorophyll *a* and *b* and carotenoids were calculated according to the formulas proposed by Wellburn (1994).

To evaluate the photochemical efficiency, one leaf from every six individuals in each treatment (control, N, P and N+P) was dark-adapted for 30 min. Chlorophyll *a* fluorescence was assessed between 8:00 am and 10:00 am using a Handy FluorCam imaging fluorometer (version 2.0, Photon Systems Instruments/PSI, Czech Republic). The Kautsky effect was determined from the images obtained according to the *quenching* protocol (Fluorcam7® software). Twenty 50-mm fields of view were considered, extracting data from the maximum quantum yield of PSII after light adaptation (QY_{max}) (Oxborough, 2004), potential quantum yield of PSII (F_v/F_m) (Genty et al., 1989), nonphotochemical *quenching* during light adaptation (NPQ) (Horton & Ruban, 1992), and the fluorescence decay ratio (R_{fd}) as an empirical parameter to assess plant vitality (Lichtenthaler and Miehe, 1997).

4.2.4 Relative distances plasticity index (RDPI)

The RDPI was used to quantify the variation in physiological parameters between treatments (Valladares et al., 2006) of six individuals of *B. holophylla* under four different nutrient treatments (control, N, P and N+P). The RDPI is a measure that varies between 0 (no variation) and 1 (maximum variation) and was calculated on the basis of a matrix X_{ij} , where *i* (rows) are the individuals analyzed, and *j* (columns) refers to the treatments for each parameter. The IDR values were calculated considering $IDR = \sum (d_{ij} \rightarrow i'j') / (x_{ij} + x_{j'i'}) / n$, where $j \rightarrow j'$ represents the number of treatments and $i \rightarrow i'$ represents the number of treatments. individuals, with the total number of distances being the *x* characteristic to be analyzed. The parameters used to obtain the RPI were QY_{max} , F_v/F_m , NPQ, R_{fd} , SLA, RWC, total chlorophyll (*a+b*), chlorophyll *a/b*, and total chlorophyll/carotenoids.

4.3 Statistical analysis

Generalized linear models (GLMs) were used considering the treatments (control, N, P and N+P) as predictive variables and the following parameters as response variables: QY_{max} , F_v/F_m , NPQ, R_{fd} , SLA, CRA, total chlorophyll (*a+b*), the ratio between chlorophyll *a* and *b* (*a/b*) and the ratio between total chlorophyll and carotenoids

(*chl/carot*) and multiple comparisons of IDR. The overdispersion of the data was subsequently checked, and when necessary, the quasi-Poisson family of distributions was used. The data and graphs were analyzed and generated via GrapPrism 8.0.2 software (country).

4.4 Results

4.4.1 Physiological parameters associated with productivity

The leaf area of *B. holophylla* was similar among the treatments N, P and N+P supplementation (Fig. 2). On the other hand, the specific leaf area in the N+P treatment was greater than that in the control ($p<0.01$) (Fig. 2B). In terms of the relative water content, the N+P treatment resulted in greater values than did the control ($p<0.01$) and the P supplementation treatment ($p=0.04$) (Fig. 2C). On the other hand, the parameters that compare the investment in chloroplast pigments, such as total chlorophyll ($a + b$), the ratio between chlorophyll *a* and *b* (a/b), and the ratio between total chlorophyll and carotenoids (*chl/carot*), were not different between the treatments.

The analysis of the parameters linked to chlorophyll *a* fluorescence in PSII revealed that the PSII maximum quantum yield values after light adaptation (QY_{max}) were greater in the treatment with P supplementation ($p<0.05$) (Fig. 3A). Nonphotochemical quenching during light adaptation (NPQ) was lower in the P treatment than in the control and supplementation with N only ($p = 0.03$) (Fig. 3B). The potential quantum yield of PSII (F_v/F_m) did not differ among the treatments (Fig. 3C), whereas the fluorescence decay ratio (R_{fd}) was lower in the treatment with P supplementation than in the control ($p<0.01$).

4.4.2 Relative distances plasticity index (RDPI)

The RDPI results are presented in Table 1. A comparison of the chlorophyll *a* fluorescence data revealed that the greatest distances were observed between the control and P supplementation groups, and for N and P (for F_v/F_m , NPQ and R_{fd}) and between P and N+P supplementation groups (QY_{max} and F_v/F_m). With respect to the parameters that compare leaf structure, the highest RDPI values for SLA were between the control and N+P supplementation groups, whereas for RWC, the greatest distances were observed between P and N+P supplementation groups. Finally, considering the content of chloroplast pigments, the highest RDPI values for *Chl a/b* were between the control and

the different supplementations (control and N; control and P; control and N+P) compared to the distances obtained for P and N+P. In contrast, the greatest relative distance for the *Chl/carot* ratio was found when the distance between W and N+P was compared with the distances between the control and the different supplementations (control and N; control and P; control and N+P) and between N supplementation and P.

4.5 Discussion

The results obtained for *B. holophylla* show that this species responds differently from a physiological point of view to N, P and N+P supplementation. These results show possible changes over time in response to predictions that indicate an increase in these nutrients in the environment due to anthropic actions (Bobbink et al., 2010; Dentener et al., 2006; Galloway et al., 2004; Güsewell, 2004; Zemunik et al., 2015). In general, the results of the present study indicate that P or N+P supplementation have the greatest effects on the functional attributes of *B. holophylla*. These results corroborate studies that show the importance of an adequate N/P balance for the regulation of plant physiological processes. The availability of P influences the ability of a plant to use N; promote growth and leaf development; regulate stomatal opening and closing; act in the formation of chloroplasts; increase the production of carbohydrates; and act in the regulation of several enzymes (Khan et al. al., 2023; Lambers, 2022; Malhotra et al., 2018). However, high P concentrations can be toxic to native plants (Lambers, 2022). In addition, considering the changes in ecosystems, the addition of combined fertilizers has a relatively small impact on natural environments. When nutrients are supplied separately, there is a greater loss of N with the addition of N and greater loss of P with the addition of P than with the combined N+P supplementation. This fact indicates that if N supplementation is performed separately, there may be N losses through leaching and gas emissions, causing other environmental impacts (Jacobson et al., 2011).

The results of the chlorophyll *a* fluorescence parameter, indicate that P supplementation has a positive effect on the photochemical efficiency of plants, particularly on the increase in the quantum yield after light adaptation (QY_{max}), the reduction in energy dissipation in the form of heat (nonphotochemical quenching - NPQ) and the rate of decrease in fluorescence (R_{fd}). The improvement in PSII efficiency indicates a greater photosynthetic capacity of these plants (Terada et al., 2020); therefore, the results suggest that P supplementation improves PSII efficiency after light adaptation,

which reverberates to a better capacity. Naturally, higher photochemical efficiency may lead to a reduction in NPQ, since this parameter indicates a lower dissipation of excess light energy by plants (Jin et al., 2023; Lichtenthaler et al., 2005). NPQ helps in the regulation of photosynthesis and protection against damage caused by excessive light, contributing to photosynthetic efficiency (Zhang et al., 2021). In contrast to what was expected, the values of the fluorescence decay ratio (R_{fd}) were lower in the treatment with P supplementation. The R_{fd} is an empirical parameter used to evaluate plant vitality and indicates the efficiency of the use of available light energy (Lichtenthaler & Miehé 1997; Lichtenthaler et al., 2005). High values of R_{fd} indicate high photosynthetic efficiency and plant health, whereas low values may suggest limitations in the ability to use light energy or greater energy dissipation in nonphotochemical forms. Stressed plants exhibit a slower reduction in plastoquinones (F_p), which is reflected in the reduction in the R_{fd} values (Lichtenthaler & Miehé 1997). For example, plants that are subjected to stress, such as drought, may have low values of this parameter (Jin et al., 2023). Therefore, the results suggest that despite the improvement in PSII efficiency shown by the QY_{max} values in the treatment with P, *B. holophylla* has an indication of stress. In general, NPQ and R_{fd} were the photosynthetic parameters that presented the greatest variation (higher values of RDPI), indicating the sensitivity of the photochemical parameters to variations in P supplementation.

In more infertile soils, greater species diversity and functional diversity are expected to survive under these conditions (Lambers et al., 2011; Zemunik et al., 2015). The Cerrado soil is considered dystrophic (Klink and Machado, 2005) with low natural N (Coser et al., 2016) and P availability. Deficiencies in the availability of organic P can be greater than 60% under natural conditions, and industrial P_i supplementation only increases the levels of inorganic P (Neufeldt et al., 2000). This factor is important for cultivated plants because of the need for faster P uptake for rapid growth (George et al., 2018). On the other hand, the conversion of the Cerrado into monocultures further decreases the chemical fertility of the soil, requiring the application of greater amounts of industrial fertilizers to maintain monocultures over time (Dias et al., 2019).

In experiments in the Cerrado, the addition of separate or combined N and P fertilizers showed that the physiological response of herbs and shrubs varies among species. For example, the addition of N may favored the production of the native grass *Echinoleana inflexa*, whereas the exotic grass *Melinis minutiflora* was favored by the

addition of P, either alone or in combination with N. These results suggest that the increase in nutrients may affect competitiveness between native and exotic species, resulting in negative impacts on the diversity of native species over time (Bustamante et al., 2012). In this context, as the studied species is a legume and with the dosages provided, it does not respond to an increase in photosynthetic pigments; it may be that other species of herbaceous and shrub plants respond faster to the increase in nitrogen and phosphorus predicted in the future and consequently affect the abundance of species in the study area. In addition, the study area is undergoing a process of forestry densification, which may modify the structure of the community, reducing the number of Cerrado areas (Goncalves et al., 2021).

4.6 Conclusion

This study demonstrates that *Bauhinia holophylla* exhibits distinct physiological responses to nitrogen (N), phosphorus (P), and their combined supplementation (N+P), reflecting the species' plasticity in environments with varying nutrient availability. The combined N+P supplementation promoted higher specific leaf area (SLA) and relative water content (RWC), indicating an adaptive capacity for rapid growth and water retention under conditions of increased nutrient availability. However, the absence of differences in the maximum quantum yield of PSII (QY_{max}) among the treatments suggests that the maximum potential for energy capture by PSII is not affected by N and P supplementation at the provided concentrations. The analysis of the Relative Distance Plasticity Index (RDPI) revealed that *B. holophylla* has a high capacity for phenotypic adjustment in response to variations in N and P availability. This plasticity may be crucial for the species' survival in a scenario of environmental changes, such as increased nutrient availability due to anthropogenic activities. However, the reduction in the fluorescence decline ratio (R_{fd}) and non-photochemical quenching (NPQ) under P supplementation suggests possible signs of stress, despite the improvement in photochemical efficiency. These results highlight the importance of considering the effects of nutrient supplementation on the physiology of native Cerrado plants, especially in a context of increased nutrient availability in the environment due to intensive agriculture. The ability of *B. holophylla* to adjust phenotypically may confer competitive advantages in altered environments, but it also underscores the need for future studies to understand how these

responses affect the dynamics of plant communities and the long-term biodiversity of the Cerrado.

4.7 References

- Abrahão, A., Costa, P.B., Lambers, H., Andrade, S.A.L., Sawaya, A.C.H.F., Ryan, M.H., Oliveira, R.S., 2019. Soil types select for plants with matching nutrient-acquisition and -use traits in hyperdiverse and severely nutrient-impoverished campos rupestres and cerrado in Central Brazil. *J Ecol.* 107(3), 1302-1316. <https://doi.org/10.1111/1365-2745.13111>
- Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. *Meteorol. Zeitschrift* 22, 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>
- Barrs HD, Weatherley PE., (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences* 15: 413–428. <https://doi.org/10.1071/BI9620413>
- Behera, S.N., Sharma, M., Aneja, V.P., Balasubramanian, R., 2013. Ammonia in the atmosphere: A review on emission sources, atmospheric chemistry and deposition on terrestrial bodies. *Environ. Sci. Pollut. Res.* 20, 8092–8131. <https://doi.org/10.1007/s11356-013-2051-9>
- Bubier, J.L., Smith, R., Juutinen, S., Moore, T.R., Minocha, R., Long, S., Minocha, S., 2011. Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs. *Oecol.* 167, 355–368. <https://doi.org/10.1007/s00442-011-1998-9>
- Bustamante, M.M.C., Brito, D.Q., Kozovits, A.R., Luedemann, G., Mello, T.R.B., Pinto, A.S., Munhoz, C.B.R., Takahashi, F.S.C., 2012. Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). *Plant Ecol.* 213, 795–808. <https://doi.org/10.1007/s11258-012-0042-4>
- Campos-Soriano, L., Bundó, M., Bach-Pages, M., Chiang, S.F., Chiou, T.J., San Segundo, B., 2020. Phosphate excess increases susceptibility to pathogen infection in rice. *Mol. Plant Pathol.* 21, 555–570. <https://doi.org/10.1111/mpp.12916>
- Carstensen, A., Herdean, A., Schmidt, S.B., Sharma, A., Spetea, C., Pribil, M., Husted, S., 2018. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant Physiol.* 177, 271–284. <https://doi.org/10.1104/pp.17.01624>
- Carvalho, A.P.F., Bustamante, M.M.C., Kozovits, A.R., Asner, G.P., 2007. Variações

- sazonais nas concentrações de pigmentos e nutrientes em folhas de espécies de cerrado com diferentes estratégias fenológicas. *Rev. Bras. Bot.* 30, 19–27. <https://doi.org/10.1590/S0100-84042007000100003>
- Coser, T.R., Ramos, M.L.G., Figueiredo, C.C., Urquiaga, S., Carvalho, A.M., Barros, F.V., Mendonça, M.T., 2016. Nitrogen uptake efficiency of maize in monoculture and intercropped with *Brachiaria humidicola* and *Panicum maximum* in a dystrophic red–yellow latosol of the Brazilian Cerrado. *Crop Pasture Sci.* 67, 47–54. <https://doi.org/10.1071/CP15077>
- Crawford, M.N., Anthony, D.M.G., Blackburn, T.H., 1998. Transport Mechanisms, In: Crawford N.M. & Glass, A.D.M. *Molecular and Physiological Aspects of Nitrate Uptake in Plants*. Elsevier Science, pp. 389–395. <https://doi.org/10.1016/B978-0-12-415836-8.00002-5>
- Crous, K.Y., Ósváldsson, A., Ellsworth, D.S., 2015. Is phosphorus limiting in a mature Eucalyptus woodland? Phosphorus fertilization stimulates stem growth. *Plant Soil* 391, 293–305. <https://doi.org/10.1007/s11104-015-2426-4>
- Diao, X., Widory, D., Ram, K., Tripathi, L., Bikkina, S., Kawamura, K., Gao, Shaopeng., Wan, X., Wu, G., Pei, Q., Wang, X., Cong, Z., 2023. Atmospheric phosphorus and its geochemical cycling: fundamentals, progress, and perspectives. *Earth-Science Reviews* 243, 104492. <https://doi.org/10.1016/j.earscirev.2023.104492>
- Dias, F.P.M., Hübner, R., Nunes, F.J., Leandro, W.M., Xavier, F.A.S., 2019. Effects of land-use change on chemical attributes of a Ferralsol in Brazilian Cerrado. *Catena* 177, 180–188. <https://doi.org/10.1016/j.catena.2019.02.016>
- Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen cycles: Past, present, and future, *Biogeochemistry*. <https://doi.org/10.1007/s10533-004-0370-0>

- Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* 990, 87-92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9)
- George TS, Giles CD, Menezes-Blackburn D, Condon LM, Gama-Rodrigues AC, Jaisi D, Lang F, Neal AL, Stutter MI, Almeida DS DS, Bol R Cabugao KG, Celi L., Cotner JB, Feng G., Goll DS, Hallama M., Krueger J., Plassard C., Rosling A., Darch T. Fraser T. Giesler R. Richardson AE Tamburini F Shand CA Lumsdon DG Zhang H Blackwell MSA Wearing C Mezeli MM Almås R Audette Y., Bertrand, I., Beyhaut, E., Boitt, G., Bradshaw, N., Brearley, CA, Bruulsema, TW, Ciais, P., Cozzolino, V., Duran, PC, Mora, ML. de Menezes, AB, Dodd, RJ, Dunfield, K., Engl, C., Frazão, JJ, Garland, G., González Jiménez, JL, Graca, J., Granger, SJ, Harrison, AF, Heuck, C. Hou EQ, Johnes PJ, Kaiser K, Kjær HA, Klumpp E, Lamb AL, Macintosh KA, Mackay EB, McGrath J, McIntyre C, McLaren T. Mészáros, E., Missong, A., Mooshammer, M., Negrón, CP, Nelson, LA, Pfahler, V., Poblete-Grant, P., Randall, M., Seguil, A., Seth, K., Smith, AC, Smits, MM, Sobarzo, JA, Spohn M. Tawaraya K., Tibbett M. Voroney P., Wallander H. Wang L. Wasaki J. Haygarth PM 2018. Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. *Plant Soil* 427, 191–208. <https://doi.org/10.1007/s11104-017-3391-x>
- Goncalves, R.V.S., Cardoso, J.C.F., Eugênio Oliveira, P., Coelho Oliveira, D., 2021. Changes in the Cerrado vegetation structure: Insights from more than three decades of ecological succession. *Web Ecol.* 21, 55–64. <https://doi.org/10.5194/we-21-55-2021>
- Good, A.G., Beatty, P.H., 2011. Fertilizing nature: A tragedy of excess in the commons. *PLoS Biol.* 9. <https://doi.org/10.1371/journal.pbio.1001124>
- Horton, P., Ruban, A.V., 1992. Regulation of photosystem-II. *Photos Res.* 34, 375-385. <https://doi.org/10.1007/BF00029812>
- Humphreys, J., Lan, R., Tao, S., 2021. Development and Recent Progress on Ammonia Synthesis Catalysts for Haber–Bosch Process. *Adv. Energy Sustain. Res.* <https://doi.org/10.1002/aesr.202000043>
- Jacobson, T.K.B., Bustamante, M.M.D.C., Kozovits, A.R., 2011. Diversity of shrub tree layer, leaf litter decomposition and N release in a Brazilian Cerrado under N, P and

- N plus P additions. *Environ. Pollut.* 159, 2236–2242. <https://doi.org/10.1016/j.envpol.2010.10.019>
- James, E.K., 2016. Nitrogen Fixation, in: *Encyclopedia of Applied Plant Sciences*. Elsevier Inc., pp. 271–277. <https://doi.org/10.1016/B978-0-12-394807-6.00124-6>
- Jin, E.J., Yoon, J.H., Lee, H., Bae, E.J., Yong, S.H., Choi, M.S., 2023. Evaluation of drought stress level in Sargent’s cherry (*Prunus sargentii* Rehder) using photosynthesis and chlorophyll fluorescence parameters and proline content analysis. *PeerJ* 11. <https://doi.org/10.7717/peerj.15954>
- Kayoumu, M., Iqbal, A., Muhammad, N., Li, X., Li, L., Wang, X., Gui, H., Qi, Q., Ruan, S., Guo, R., Zhang, X., Song, M., Dong, Q., 2023. Phosphorus Availability Affects the Photosynthesis and Antioxidant System of Contrasting Low-P-Tolerant Cotton Genotypes. *Antioxidants* 12. <https://doi.org/10.3390/antiox12020466>
- Khan, F., Siddique, A.B., Shabala, S., Zhou, M., Zhao, C., 2023. Phosphorus Plays Key Roles in Regulating Plants’ Physiological Responses to Abiotic Stresses. *Plants* 12. <https://doi.org/10.3390/plants12152861>
- Kusano, M., Fukushima, A., Redestig, H., Saito, K., 2011. Metabolomic approaches toward understanding nitrogen metabolism in plants. *J. Exp. Bot.* 62, 1439–1453. <https://doi.org/10.1093/jxb/erq417>
- Lai, M., He, S., Yu, S., Jin, G., 2018. Effects of experimental N addition on plant diversity in an old-growth temperate forest. *Ecol. Evol.* 8, 5900–5911. <https://doi.org/10.1002/ece3.4127>
- Lambers, H., 2022. Phosphorus Acquisition and Utilization in Plants. *Annu. Rev. Plant Biol.* 73, 17–42. <https://doi.org/10.1146/annurev-arplant-102720-125738>
- Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D., 2011. Plant mineral nutrition in ancient landscapes: High plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant Soil.* <https://doi.org/10.1007/s11104-011-0977-6>
- Lan, Z., Lin, W., Zhao, G., 2024. Sources, Variations, and Effects on Air Quality of Atmospheric Ammonia. *Curr. Pollut. Reports.* <https://doi.org/10.1007/s40726-023-00291-6>
- Lichtenthaler, H.K., Miehe, J.A., 1997. Fluorescence imaging as a diagnostic tool for plant stress. *Trend Plant Sci.* 2, 316–320. [https://doi.org/10.1016/S1360-1385\(97\)89954-2](https://doi.org/10.1016/S1360-1385(97)89954-2)

- Lichtenthaler, H.K., Buschmann, C., Knapp, M., 2005. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer. *Photosynth.* 43, 379–393. <https://doi.org/10.1007/s11099-005-0062-6>
- Malhotra, H., Vandana, Sharma, S., Pandey, R., 2018. Phosphorus nutrition: Plant growth in response to deficiency and excess, In: Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (eds) *Plant Nutrients and Abiotic Stress Tolerance*. Springer, Singapore. https://doi.org/10.1007/978-981-10-9044-8_7
- Messiga, A.J., Ziadi, N., Bélanger, G., Morel, C., 2013. Soil Nutrients and Other Major Properties in Grassland Fertilized with Nitrogen and Phosphorus. *Soil Sci. Soc. Am. J.* 77, 643–652. <https://doi.org/10.2136/sssaj2012.0178>
- Mo, Q., Li, Z., Sayer, E.J., Lambers, H., Li, Y., Zou, B., Tang, J., Heskell, M., Ding, Y., Wang, F., 2019. Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. *Funct. Ecol.* 33, 503–513. <https://doi.org/10.1111/1365-2435.13252>
- Moeneclaey, I., Schelfhout, S., Vanhellemont, M., DeCock, E., Van Coillie, F., Verheyen, K., Baeten, L., 2022. Species ecological strategy and soil phosphorus supply interactively affect plant biomass and phosphorus concentration. *Basic Appl. Ecol.* 62, 1–11. <https://doi.org/10.1016/j.baae.2022.03.013>
- Neufeldt, H., Da Silva, J.E., Ayarza, M.A., Zech, W., 2000. Land-use effects on phosphorus fractions in Cerrado oxisols. *Biol. Fertil. Soils* 31, 30–37. <https://doi.org/10.1007/s003740050620>
- Oxborough, K., 2004. Imaging of chlorophyll *a* fluorescence: theoretical and practical aspects of an emerging technique for the monitoring of photosynthetic performance. *J Exp Bot.* 55, 1195–1205. <https://doi.org/10.1093/jxb/erh145>
- Schmidt, M., Corre, M.D., Kim, B., Morley, J., Göbel, L., Sharma, A.S.I., Setriuc, S., Veldkamp, E., 2021. Nutrient saturation of crop monocultures and agroforestry indicated by nutrient response efficiency. *Nutr. Cycl. Agroecosystems* 119, 69–82. <https://doi.org/10.1007/s10705-020-10113-6>
- Sutton, M.A., Mason, K.E., Sheppard, L.J., Sverdrup, H., Haeuber, R., Hicks, W.K., 2014. Nitrogen deposition, critical loads and biodiversity: Proceedings of the international nitrogen initiative workshop, linking experts of the convention on

- long-range transboundary air pollution and the convention on biological diversity. Nitrogen Depos. Crit. Loads Biodivers. Proc. Int. Nitrogen Initiat. Work. Link. Expert. Conv. Long-Range Transbound. Air Pollut. Conv. Biol. Divers. 1–535. <https://doi.org/10.1007/978-94-007-7939-6>
- Terada, R., Yuge, T., Watanabe, Y., Mine, T., Morikawa, T., Nishihara, G.N., 2020. Chronic effects of three different stressors, irradiance, temperature, and desiccation, on the PSII photochemical efficiency in the heteromorphic life-history stages of cultivated *Pyropia yezoensis* f. *narawaensis* (Bangiales) from Japan. J. Appl. Phycol. 32, 3273–3284. <https://doi.org/10.1007/s10811-020-02152-x>
- Thamdrup, B., 2012. New pathways and processes in the global nitrogen cycle. Annu. Rev. Ecol. Evol. Syst. 43, 407–428. <https://doi.org/10.1146/annurev-ecolsys-102710-145048>
- Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. U. S. A. 108, 20260–20264. <https://doi.org/10.1073/pnas.1116437108>
- Turner, N.C., 1981. Techniques and experimental approaches for the measurement of plant water status. <https://doi.org/10.1007/BF02180062>
- Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol 94:1103–1116. <https://doi.org/10.1111/j.1365-2745.2006.01176.x>
- Verlinden, M.S., Abdelgawad, H., Ven, A., Verryckt, L.T., Wieneke, S., 2022. Phosphorus stress strongly reduced plant physiological activity, but only temporarily, in a mesocosm experiment with *Zea mays* colonized by arbuscular mycorrhizal fungi 2353–2364. <https://doi.org/10.5194/bg-19-2353-2022>
- Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. Ecol. Appl. 20, 5–15. <https://doi.org/10.1890/08-0127.1>
- Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313. [https://doi.org/10.1016/S0176-1617\(11\)81192-2](https://doi.org/10.1016/S0176-1617(11)81192-2)
- Witkowski, E.T.F., Lamont, B.B., 1991. Leaf specific mass confounds leaf density and thickness. Oecol. 88, 486–493. <https://doi.org/10.1007/BF00317710>

- Yu, H., Chin, M., Yuan, T., Bian, H., Remer, L.A., Prospero, J.M., Omar, A., Winker, D., Yang, Y., Zhang, Y., Zhang, Z., Zhao, C., 2015. The fertilizing role of African dust in the Amazon rainforest: A first multiyear assessment based on data from Cloud-Aerosol Lidar and Infrared Pathfinder Satellite Observations. *Geophys. Res. Lett.* 42, 1984–1991. <https://doi.org/10.1002/2015GL063040>
- Zemunik, G., Turner, B.L., Lambers, H., Laliberté, E., 2015. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nat. Plants* 1, 1–4. <https://doi.org/10.1038/nplants.2015.50>
- Zhang, J.Y., Zhang, Q.H., Shuang, S.P., Cun, Z., Wu, H.M., Chen, J.W., 2021. The responses of light reaction of photosynthesis to dynamic sunflecks in a typically shade-tolerant species *Panax notoginseng*. *Front. Plant Sci.* 12, 1–16. <https://doi.org/10.3389/fpls.2021.718981>

Tables and figures

Table 1. Results of the relative distances plasticity index (RDPI) comparing various physiological parameters of *Bauhinia holophylla* leaves found in 20 plots of 100 m² established in a Cerrado area (dense cerrado woodland), with 5 plots assigned to each of the control treatments, N supplementation, P supplementation and N+P combined supplementation.

	Controle e N	Controle e P	Controle e N+P	N e P	N e N+P	P e N+P
SLA	0.106 ± 0.08 b	0.109 ± 0.08 ab	0.142 ± 0.08 a	0.053 ± 0.04 c	0.082 ± 0.04 bc	0.084 ± 0.05 bc
RWC	0.157 ± 0.12 b	0.186 ± 0.14 b	0.255 ± 0.14 ab	0.173 ± 0.15 b	0.192 ± 0.11 b	0.347 ± 0.28 a
Chl <i>a</i> + <i>b</i>	0.101 ± 0.08 abc	0.054 ± 0.03 bc	0.078 ± 0.05 abc	0.091 ± 0.08 abc	0.11 ± 0.10 ab	0.07 ± 0.05 bc
Chl <i>a</i> / <i>b</i>	0.11 ± 0.07 a	0.09 ± 0.06 a	0.098 ± 0.06 a	0.073 ± 0.05 ab	0.063 ± 0.04 ab	0.042 ± 0.03 b
Chl/carot	0.048 ± 0.03 b	0.063 ± 0.05 b	0.078 ± 0.014 b	0.05 ± 0.03 b	0.091 ± 0.05 ab	0.13 ± 0.09 a
F _v /F _m	0.028 ± 0.01 b	0.051 ± 0.03 a	0.029 ± 0.02 b	0.044 ± 0.03 ab	0.021 ± 0.013 bc	0.049 ± 0.03 a
QY _{max}	0.063 ± 0.43 bc	0.125 ± 0.07 a	0.095 ± 0.07 ab	0.112 ± 0.07 a	0.084 ± 0.07 ab	0.127 ± 0.11 a
NPQ	0.169 ± 0.11 b	0.330 ± 0.264 a	0.203 ± 0.14 b	0.295 ± 0.24 a	0.164 ± 0.11 b	0.187 ± 0.16 b
R _{fd}	0.18 ± 0.12 b	0.379 ± 0.257 a	0.237 ± 0.17 ab	0.335 ± 0.24 a	0.231 ± 0.14 ab	0.211 ± 0.01 ab

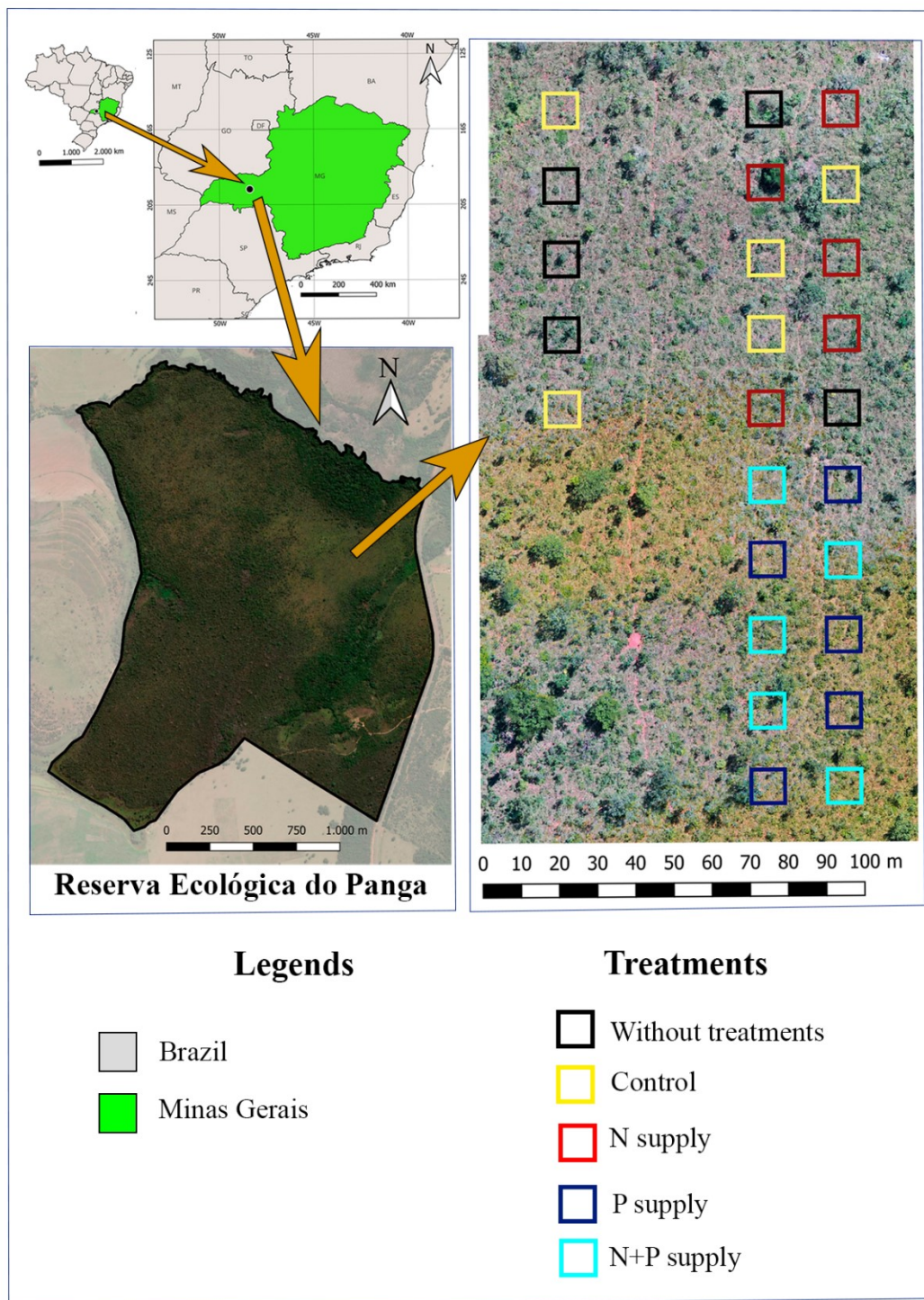


Figure 1: Location of the experimental area (Estação Ecológica do Panga, MG, Brazil) and experimental design. Twenty plots of 100 m² were established in a Cerrado area (dense cerrado woodland), with 5 plots assigned to each treatment (control, N, P and N+P combined supplementations). Source: Qqgis, DATUM SIGAS 2000 and photo by the Mavic 2 Pro drone.

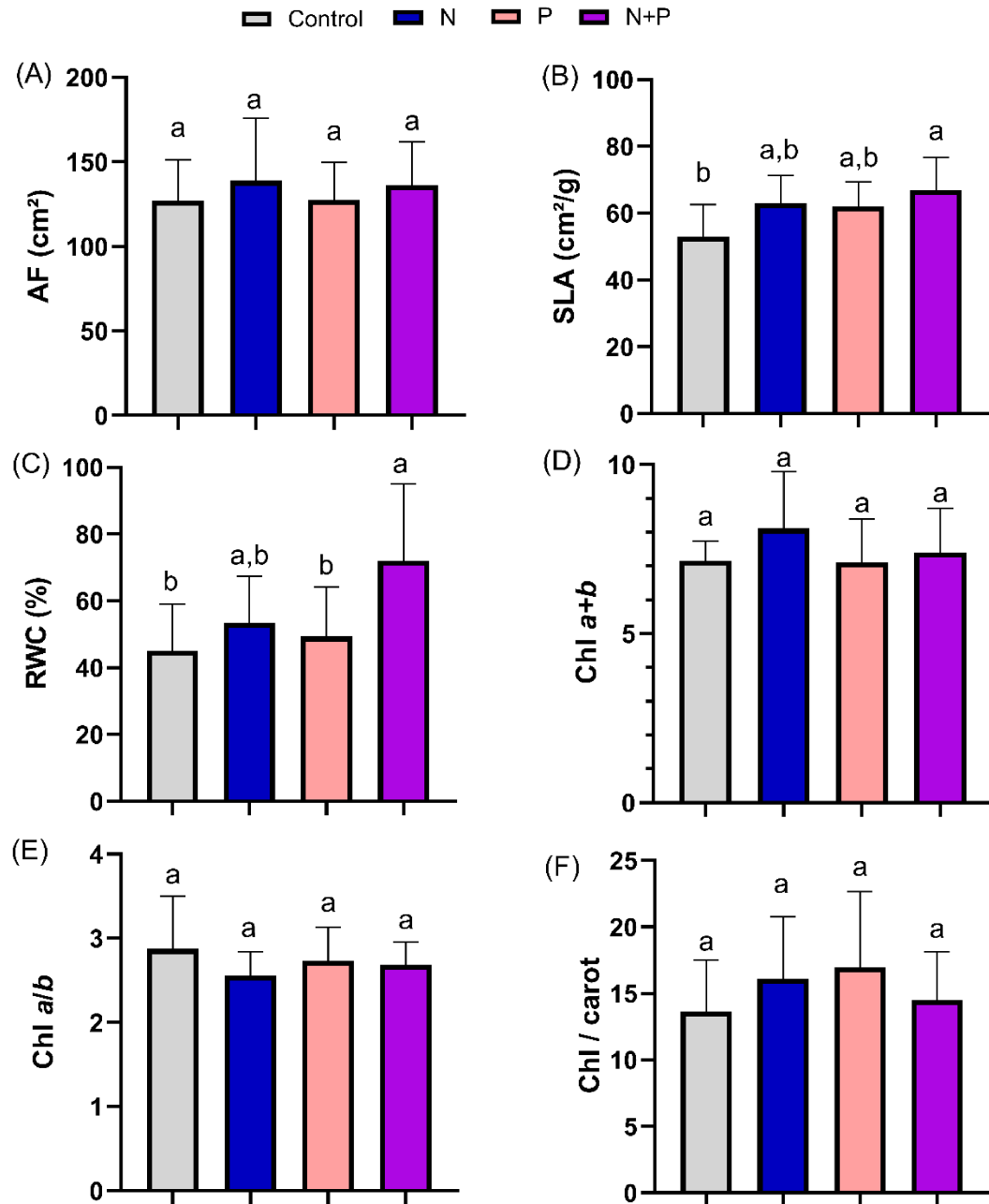


Figure 2: Leaf area, specific leaf area, and relative content of water and chloroplast pigments in the leaves of *Bauhinia holophylla* found in 20 plots of 100 m² established in a Cerrado area (dense cerrado woodland), with 5 plots assigned to each of the treatment groups: the control, N supplementation, P supplementation and N+P combined supplementation groups. Legends: (A) AF - total leaf area, (B) SLA - specific leaf area, (C) RWC - relative water content, (D) Chl a+b - total chlorophyll (a+b), (E) Chl a/b - chlorophyll a and b ratio (a/b), (F) Chl / carot - total chlorophyll and carotenoids ratio (Chl/carot).

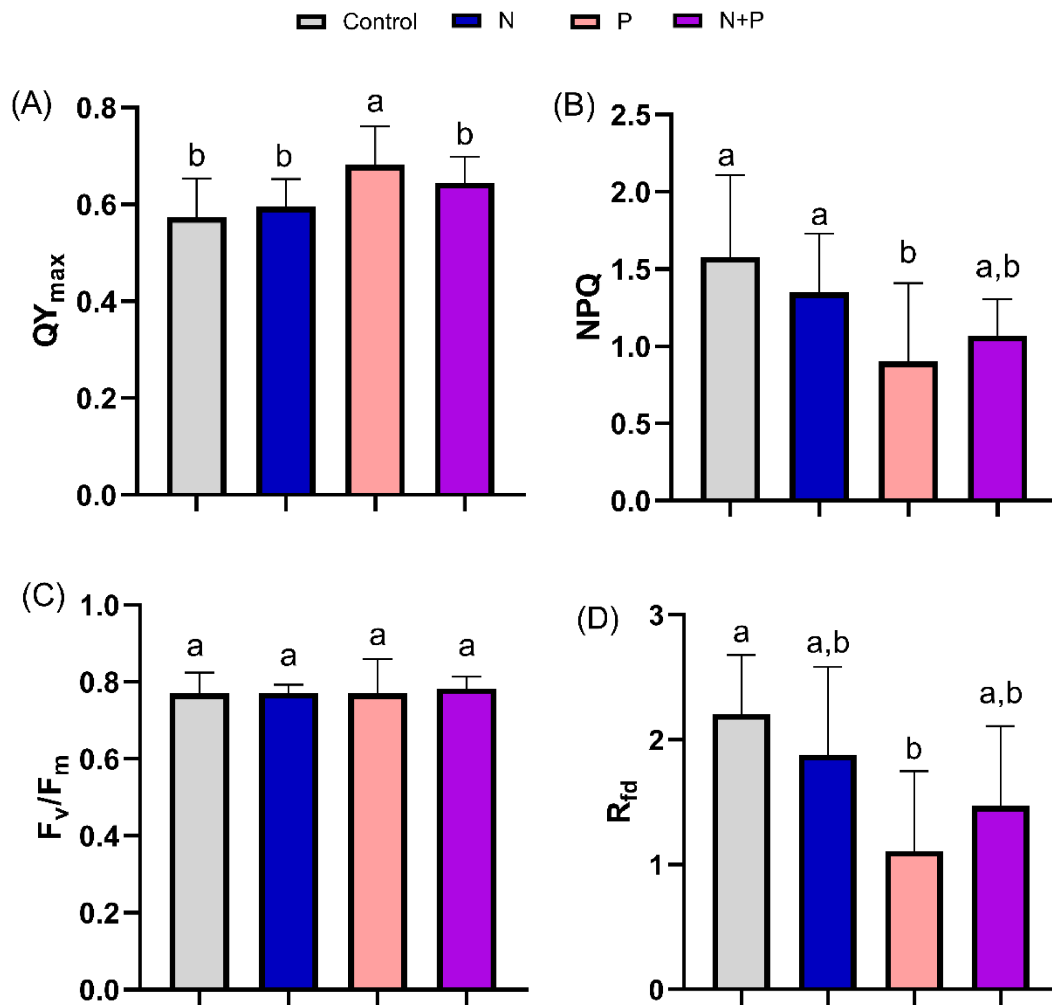


Figure 3: Parameters derived from the chlorophyll *a* fluorescence analysis of *Bauhinia holophylla* leaves found in 20 plots of 100 m² established in a Cerrado area (dense cerrado woodland), with 5 plots assigned to each of the control, N supplementation, P supplementation and combined N+P supplementation. Legends: gray (control), blue (N supplementation), pink (P supplementation) and purple (N+P combined supplementation). (A) Quantum yield of PSII after light adaptation (QY_{max}), (B) nonphotochemical quenching (NPQ), (C) maximum quantum yield of PSII (F_v/F_m) and (D) ratio of decreased fluorescence (R_{fd}).

5. Capítulo 3

Como *Handroanthus impetiginosus* (Mart. ex DC.) responde à suplementação por N e P?

Resumo

Compreender como espécies de plantas nativas respondem a variações na disponibilidade de nutrientes minerais essenciais é contribui para um melhor manejo e conservação de ecossistemas como o Cerrado. Esse bioma tem sido intensamente transformado em áreas agrícolas que utilizam elevadas quantidades de nitrogênio (N) e fósforo (P) em busca de uma alta produtividade, o que afeta diretamente nas plantas nativas em áreas adjacentes às monoculturas. No caso de *Handroanthus impetiginosus*, a resposta fisiológica à suplementação de N e P ainda é pouco explorada, principalmente em relação a suplementação combinada desses nutrientes. Neste estudo, foram avaliados os efeitos de diferentes concentrações de N e P, isoladamente e em combinação, sobre os parâmetros fisiológicos de *H. ipetiginosus*, como área foliar específica (SLA), pigmentos fotossintéticos, fluorescência da clorofila, assimilação de CO₂ e condutância estomática. Os resultados mostram que a suplementação isolada de N ou P promoveu maior crescimento e um aumento na área foliar, enquanto a combinação de N+P levou ao aumento da SLA, sugerindo uma maior eficiência no uso da área foliar para captação de luz. No entanto, essa suplementação combinada em alto teor não resultou diferenças no tamanho das plantas e na fluorescência da clorofila e acarretou em uma diminuição na eficiência fotossintética e na condutância estomática, sugerindo um efeito de toxicidade na quantidade dos nutrientes fornecidos. Esses resultados mostram que, embora a suplementação de N e P seja benéfica em baixo teor, doses elevadas poderiam comprometer a saúde fisiológica de *H. impetinosus*.

Palavras-chave: Cerrado, fisiologia de plantas nativas, nutrição de plantas.

Abstract

Understanding how native plant species respond to variations in the availability of essential mineral nutrients contributes to better management and conservation of ecosystems such as the Cerrado. This biome has been intensely transformed into agricultural areas that use high amounts of nitrogen (N) and phosphorus (P) in pursuit of high productivity, which directly affects native plants in areas adjacent to monocultures. In the case of *Handroanthus impetiginosus*, the physiological response to N and P supplementation is still poorly explored, especially regarding the combined supplementation of these nutrients. In this study, the effects of different concentrations of N and P, both individually and in combination, were evaluated on the physiological parameters of *H. impetiginosus*, such as specific leaf area (SLA), photosynthetic pigments, chlorophyll fluorescence, CO₂ assimilation, and stomatal conductance. The results show that isolated supplementation of N or P promoted greater growth and an increase in leaf area, while the combination of N+P led to an increase in SLA, suggesting greater efficiency in the use of leaf area for light capture. However, this combined supplementation at high levels did not result in differences in plant size and chlorophyll fluorescence and led to a decrease in photosynthetic efficiency and stomatal conductance, suggesting a toxicity effect due to the amount of nutrients supplied. These results indicate that, although N and P supplementation is beneficial at low levels, high doses could compromise the physiological health of *H. impetiginosus*.

Keywords: Cerrado, native plant physiology, plant nutrition.

5.1 Introdução

Tentativas de compreender como as espécies de plantas nativas respondem fisiologicamente às variações na disponibilidade de nutrientes têm sido o foco de diversos estudos (Cordeiro et al., 2024; Faria et al., 2015; Mota et al., 2023). Essas alterações influenciam características morfológicas, anatômicas e metabólicas de plantas impactando diretamente ecossistemas naturais, uma vez que selecionam espécies ao longo do tempo e modificam a diversidade de locais específicos (Hetherington and Woodward, 2003; Tatarko and Knops, 2018; Zemunik et al., 2015). Na principal savana neotropical da América do Sul, a que chamamos de Cerrado, tem sido observado um rápido processo de conversão em monoculturas que requerem suplementação com fertilizantes industriais, especialmente de nitrogênio (N) e fósforo (P), para manter a alta produtividade de plantas cultivadas (Boschiero et al., 2020; Conant et al., 2012; Messiga et al., 2013). Esse processo expõe principalmente plantas nativas nos fragmentos remanescentes adjacentes às áreas de monoculturas a diferentes entradas de N (ver capítulo 1) (Pocewicz et al., 2007).

Plantas nativas que recebem diferentes aportes de nutrientes minerais, especialmente de N e P, podem responder de forma variável alterando seus parâmetros fisiológicos. Muitos destes parâmetros são frequentemente utilizados como indicadores de crescimento e saúde vegetal, como a área foliar específica e parâmetros relacionados à fluorescência da clorofila *a* (Diaz et al., 1998; Jin et al., 2023; Somavilla et al., 2014) (ver capítulo 2). Apesar das plantas em geral necessitarem de uma alta demanda de N e P, algumas espécies são mais sensíveis, portanto, menos tolerantes a variações desses nutrientes (Kayoumu et al., 2023). Entre as espécies consideradas chave para conservação do Cerrado, o gênero *Handroantus* apresenta espécies que são de interesse comercial, com finalidades farmacológicas, para a produção de madeira (Macêdo et al., 2022), paisagismo e arborização urbana (Martins et al., 2018). Além disso, as espécies de ipês têm a capacidade de se desenvolver em diversas condições nutricionais, o que as torna adequadas para projetos de recuperação ambiental (Scarpa et al., 2022).

Handroantus impetiginosus (ipê-roxo) é uma árvore que pode chegar de oito a 30 metros de altura com 60 a 100 cm de diâmetro do caule. A espécie pode ser encontrada na Índia, Tailândia, Austrália e é amplamente distribuída no México, América Central, Guiana Francesa, Bolívia, Chile, Argentina, Paraguai. No Brasil é bem representativa no Norte, Nordeste, Centro-Oeste e Sudeste (iNaturalist, 2024). Pode ser utilizada em

projetos paisagísticos, urbano e rural (Camillo and Salomão, 2018) e no uso medicinal (Ryan et al., 2021). Portanto, pesquisas relacionadas ao melhor desenvolvimento dessas espécies tornam-se relevantes. Embora estudos mostrem alterações no tamanho das plantas e nos processos fotossintéticos com adição de N, até o momento não foram encontradas pesquisas que explorem de forma abrangente a suplementação isolada com diferentes concentrações de N e P, e com a combinação desses elementos para *H. impetiginosus*. Neste contexto, o objetivo deste trabalho foi analisar quais parâmetros fisiológicos de *H. ipetiginosus* ligados à produtividade são influenciados pela maior disponibilidade de N, P e pela combinação destes dois elementos. Neste contexto, espera-se que a adição de N, P e principalmente destes dois elementos em conjunto aumente o tamanho das plantas, área foliar específica, conteúdo relativo de água, o teor de clorofilas, a eficiência fotossintética (fotoquímica e assimilação de CO₂) e a condutância estomática.

5.2 Material e métodos

Foram utilizadas 48 plântulas de *Handroanthus impetiginosus* (Mart. ex DC.) fornecidas pela Secretaria Municipal de Catalão-GO. O experimento foi realizado em uma casa de vegetação, onde as plantas foram transplantadas para potes com tamanho com capacidade de 1,7 L. As plantas foram aclimatadas durante 60 dias e regadas de dois em dois dias de forma uniforme em cada pote. Posteriormente, utilizando o nitrogênio em forma de ureia granulada protegida 46-00-00 (Agroadubo, Brasil) e fósforo encapsulado 00-44-00 (EQCOAT equilíbrio fertilizantes, Brasil). As plantas foram submetidas a suplementação de acordo com o indicado com a quantidade fornecida pelo fabricante para produção de cultivares, e foram adaptadas de uma forma proporcional para os potes. Os tratamentos foram divididos em (n=7 plantas para cada tratamento): (tratamento 1) suplementação baixa de nitrogênio (cada pote recebeu 1,22 g de N); (tratamento 2) suplementação com alta concentração de nitrogênio (cada pote recebeu 2,44 g de N); (tratamento 3) suplementação com baixo fósforo (cada pote recebeu 1,25 g de P); (tratamento 4) suplementação de alto fósforo (cada pote recebeu 2,50 g de P); (tratamento 5) suplementação combinada de N+P com as dosagens baixas (1,22 g de N e 1,25 g de P) (tratamento 6) suplementação combinada de alto N+P (2,44 g de N e 2,50 g de P), e controle, onde não foi realizada nenhum tipo de suplementação.

5.2.1 Tamanho das plantas, área foliar e área foliar específica

O tamanho das plantas foi tomado antes da suplementação pelos nutrientes e após 30 e 60 dias. No final do experimento foi mensurada a área total foliar, e a área foliar específica. A área foliar total foi obtida utilizando-se o programa ImageJ (<http://rsb.info.nih.gov/ij>). A área foliar específica foi obtida considerando-se a razão entre a área de um disco de 0,4 cm² (A) sua massa seca (MS) ($AFE = A/MS$). A MS foi obtida colocando-se os fragmentos em estufa até atingirem peso constante.

5.2.2 Conteúdo dos pigmentos cloroplastídicos

Dois fragmentos de 0,4 cm² de cada indivíduo foram submersos em 10 ml de dimetilsulfóxido PA por 72h. Os extratos foram realocados em microplacas de ELISA e lidos em um leitor de placas utilizando o Software SKanlttm (versão 6.1.2020, Thermo Scientific). Foram realizadas as leituras nos comprimentos de onda de 480, 649 e 665 nm. O conteúdo de clorofilas *a*, *b* e carotenoides foram calculados de acordo com as fórmulas propostas por Wellburn (1994).

5.2.3 Fluorescência da clorofila *a*

A taxa de transporte de elétrons (ETR) foi mensurada em uma folha de seis indivíduos em cada tratamento entre 8:00 e 18:00, de 2 em duas horas, utilizando-se um fluorômetro modulado (MINI-PAM, Walz). As curvas de luz foram obtidas após adaptação ao escuro por 30 minutos. O valor do exponencial platô foi utilizado para calcular os seguintes pontos cardinais: taxa máxima de transporte de elétrons (ETR_{max}), a metade da taxa máxima de transporte de elétrons ($\frac{1}{2} ETR_{max}$), a radiação fotossinteticamente ativa na taxa máxima de transporte de elétrons ($PAR_{ETR_{max}}$), a radiação fotossinteticamente ativa na metade da taxa máxima de transporte de elétrons ($PAR_{\frac{1}{2} ETR_{max}}$) e a radiação fotossinteticamente ativa de saturação (PAR_{sat}) (Rascher et al., 2000).

5.2.4 Assimilação de CO₂ e condutância estomática

Em cada indivíduo em cada tratamento foram avaliadas as trocas gasosas, considerando-se a condutância estomática, a taxa de transpiração e a assimilação de CO₂. As medidas foram tomadas em intervalos de duas horas entre 8:00 e 18:00, utilizando-se o analisador de gases por infravermelho (Infrared Gas Analyzer) (IRGA, LI6400XT LI-

COR, EUA) (Capelin et al., 2017). As leituras foram tomadas após estabilização do equipamento considerando-se como referência o ar coletado por uma antena móvel posicionada em área aberta (concentração de CO₂ de aproximadamente ~400 µmol mol⁻¹). Foi utilizada uma fonte de luz do LI-COR anexado à câmara de medição, com os valores de densidade de fluxo de fótons fotossintéticos (PPFD) de 1.200, 2.000 e 1.200 µmol m⁻² s⁻¹, a câmara foliar foi operada com umidade fixa, temperatura de 26°C, pressão de vapor de 1,11 kPa e fluxo interno de ar de 300 µmol s⁻¹.

5.3 Análises dos dados

Inicialmente, foi verificado se os dados apresentam superdispersão e independências dos erros, utilizando a transformação quasi-poisson quando necessário. Para avaliar o efeito da suplementação de N, P e N+P sobre os parâmetros fisiológicos obtidos foi gerado um Modelo Linear Generalizado (GLM) através do pacote emmeans no software R.studio 4.3.1 (Lenth 2024).

5.4 Resultados

5.4.1 Tamanho das plantas, área foliar e área foliar específica

Após 60 dias de suplementação, as plantas nos tratamentos alto N e baixo P foram maiores em comparação com o tamanho inicial ($T=4.418$, $p<0.01$ e $T=-3.059$, $p=0.01$, respectivamente) (Fig. 1). Os indivíduos submetidos às suplementações com baixo e alto N apresentaram maior área foliar em relação ao grupo controle ($T=3.183$, $p=0.02$ e $t=6.437$, $p<0.01$, respectivamente) (Fig. 2), sendo os maiores valores encontrados para o alto N. As plantas do tratamento N+P em alta suplementação apresentaram maiores valores de SLA do que o grupo controle ($T=-3.25$, $p=0.04$) (Fig. 3A).

5.4.2 Pigmentos fotossintéticos

O conteúdo de clorofilas totais ($a+b$) apresentou maiores valores nos tratamentos com alta suplementação de N (77.5 ± 13.06 µg cm⁻²) e alta suplementação de P ($70,5 \pm 6,23$ µg cm⁻²) em relação ao grupo controle (40.6 ± 6.69 µg cm⁻²) ($p<0.01$ e $T=2.74$, $p<0.01$ $T=2.94$, respectivamente) (Fig. 3B). A razão entre o conteúdo de clorofilas a e b (a/b) apresentou maiores valores de baixa suplementação por N e baixo N+P em relação ao grupo controle ($T=3.132$, $p<0.01$ e $T=2.195$, $p<0.01$, respectivamente) (Fig. 3C). A

razão entre clorofilas totais e os carotenoides não apresentou diferenças significativas entre os tratamentos (Fig. 3D).

5.4.3 Fluorescência da clorofila a

Avaliando-se os dados obtidos a partir da curva de luz para o ETR, os valores de ETR_{max} , e $\frac{1}{2} ETR_{max}$ foram maiores nos tratamentos com alta suplementação de P e baixo N com relação ao tratamento com baixa suplementação de P ($p < 0.01$ e $T = 4.124$, $p < 0.01$ e $T = 3.98$, respectivamente). Os maiores valores para o PAR ($PAR_{ETR_{max}}$, $PAR_{\frac{1}{2} ETR_{max}}$ e PAR_{sat}) foram encontrados para os tratamentos com alta suplementação de P ($p < 0.01$ e $T = 4.336$), baixa suplementação de N ($p < 0.01$ e $T = 4.419$) e baixa suplementação de N+P ($p < 0.01$ e $T = 3.255$) com relação à baixa suplementação de fósforo $PAR_{ETR_{max}}$, ao $PAR_{\frac{1}{2} ETR_{max}}$ e ao PAR_{sat} , respectivamente (Tabela 1).

5.4.4 Assimilação de CO₂ e condutância estomática

A assimilação de CO₂ foi maior nas plantas submetidas ao tratamento com baixa suplementação de N+P combinados ($7.66 \pm 0.521 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) e com baixa suplementação por P ($7.12 \pm 0.475 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) com relação ao tratamento com alta suplementação de N+P combinados ($4.03 \pm 0.521 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) ($p < 0.01$ e $T = -4.937$; $p < 0.01$ e $T = -4.394$ e $p = 0.01$ e $T = 3.687$) (Fig. 4A). A condutância estomática apresentou maiores valores na suplementação de baixo N ($0.93 \pm 0.01 \text{ mol}^{-2} \text{ s}^{-1}$), baixo P ($0.094 \pm 0.009 \text{ mol}^{-2} \text{ s}^{-1}$) e baixo N+P ($0.971 \pm 0.01 \text{ mol}^{-2} \text{ s}^{-1}$) em relação a suplementação de alto N+P ($p > 0.01$ e $T = -4.043$, $p < 0.01$ e $T = -4.241$, $p < 0.01$ e $T = -4.025$, respectivamente) (Fig. 4 B).

5.5 Discussão

Os resultados desse estudo indicam que a suplementação de N e P, tanto dos nutrientes isolados quanto combinados, gera melhorias em atributos fisiológicos de uma forma distinta em *H. impetiginosus*, influenciando no crescimento da planta, na eficiência fotoquímica e na assimilação do carbono via fotossíntese. Após 60 dias da suplementação, as plantas submetidas aos tratamentos com alto N apresentaram maior tamanho e maior quantidade de clorofila total. No tratamento baixo N apresentaram maior área foliar total e maiores valores da relação entre clorofila a/b. Estes resultados corroboram com estudos que mostram uma correlação positiva entre o aumento da disponibilidade do N com a

síntese de pigmentos fotossintéticos que maximizam a captação de luz e promovem o aumento das taxas fotossintéticas e consequentemente de biomassa (Berghetti et al., 2021; Bubier et al., 2011; Santos et al., 2020).

As plantas submetidas ao tratamento com suplementação de altas concentrações de P apresentaram maiores valores de clorofila total ($a+b$) e o tratamento com baixo fósforo também apresentou maior tamanho em relação ao tempo inicial. Tais resultados também corroboram com pesquisas que mostraram a importância do P para o crescimento, pois este elemento está relacionado com a formação de nucleotídeos e ácidos nucleicos (fundamentais para divisão celular), (Moeneclaey et al., 2022) e a produção de proteínas e fosfolipídios (Raghothama and Karthikeyan, 2005), além de ser crucial para a maioria das enzimas e no transporte de energia, na produção de carboidratos, respiração e na fotossíntese (Jin et al., 2015). Porém, a resposta da biomassa ao aumento do P pode variar entre as diferentes espécies de plantas, dependendo de suas características de crescimento e estratégias de uso de nutrientes. Espécies de crescimento rápido (aquisitivas) tendem a responder mais positivamente ao aumento de P, enquanto espécies mais conservativas (Edwards et al., 2014; Wigley et al., 2016) podem não responder tão rapidamente na biomassa ao aumento deste nutriente (Moeneclaey et al., 2022). Além disso, a assimilação de CO₂ pode ser mantida em solos com baixa disponibilidade de P em algumas espécies (Mo et al., 2019) e também a adição de P pode ser prejudicial para plantas adaptadas à baixa disponibilidade de P (Lambers et al., 2008). Em nosso estudo foi observado uma maior eficiência fotoquímica para as altas concentrações de N e P isolados. A combinação sobre baixas concentrações aumentou a inclinação da reta, de forma que estas plantas alcançam o ETR_{max} primeiro, mas não alterou a eficiência fotossintética. O aumento da eficiência fotoquímica nos tratamentos com altas concentrações de N e P isoladamente coincide com o maior conteúdo de clorofilas a e b (principalmente clorofila a , como pode ser observado pela relação clorofila a/b). O tratamento baixo N+P também apresentou maior diferença entre clorofila total (a/b).

De uma forma geral, altas concentração de N e P são associadas a uma redução de massa foliar específica, atributo funcional inversamente proporcional à SLA (Hulshof et al 2013; Westoby and Wright, 2006). Em um estudo que adicionaram soluções de Hoagland em rejeitos de mineradora contendo N e P, mostraram que a alta disponibilidade de N e P elevou a massa seca e assimilação de CO₂ taxa de fotossíntese em *H. impetiginosus* (Scarpa et al., 2022). Em outras espécies do mesmo gênero, mostraram que

a suplementação com N aumenta o tamanho de *H. serratifolius* (Vahl) S. Grose (Goulart et al., 2017), o diâmetro do caule, a concentração de nitrogênio nas folhas e a eficiência fotossintética em *Handroanthus heptaphyllus* (Vell.) Mattos (Berghetti et al., 2021). Em nosso estudo, as plantas com suplementação por altas concentrações de N+P apresentaram maior área foliar específica, o que indicaria maior investimento em área por massa seca (Rossatto and Kolb, 2013). Entretanto, não houve alteração na área foliar total, indicando que as alterações nos valores de SLA são decorrentes da baixa assimilação de CO₂ e condutância estomática.

Em espécies cultivadas, por exemplo o milho, a combinação de N+P favorece a eficiência fotossintética (Tofa et al., 2022), o que pode aumentar seu potencial competitivo. Em áreas de Cerrado, tudo indica que o efeito da adição de N+P pode alterar atributos funcionais fisiológicos das plantas nativas, uma vez já mostrou alterar a densidade, dominância, riqueza e padrões de diversidade das espécies em comparação com a adição isolada de N ou P (Jacobson et al., 2011). Esta alteração na comunidade local pode levar à perda de diversidade no Cerrado (Bustamante et al., 2012; Kozovits et al., 2007). Nessa pesquisa, os resultados evidenciam que, apesar do N e P isolados mostrarem relações positivas nos atributos funcionais, crescimento e fotossíntese, a suplementação por altas concentrações de N e P combinados é prejudicial para *H. impetiginosus* por influenciar negativamente sua condutância estomática e taxa fotossintética. Em outro contexto, apesar de uma suplementação adequada poder otimizar o crescimento de mudas de *H. impetiginosus* (Cunha et al., 2022), estudos recentes mostram que esta espécie também cresce de forma eficiente com a suplementação de biofertilizantes em conjunto com NPK industrial, demonstrando que a aplicação dos fertilizantes industriais em alta quantidade é desnecessário (Oliveira et al., 2023).

5.6 Conclusão

Os resultados evidenciam que *H. ipetiginosus* responde positivamente a suplementação isolada de nitrogênio e fósforo, promovendo aumento da área foliar e eficiência na captação de luz, especialmente quando o nitrogênio é fornecido em quantidades mais baixas. No entanto, a suplementação combinada de N e P, em concentrações elevadas, não resultou em maior crescimento das plantas, com uma redução na taxa fotossintética e condutância estomática. Esses resultados sugerem que, apesar dos nutrientes serem essenciais para o crescimento e desenvolvimento das plantas,

o excesso de N e P suplementados simultaneamente, pode ter efeitos negativos, em alguns atributos funcionais foliares. Portanto no caso de suplementação com o intuito de aumentar a produtividade de *H. impetiginosus* para utilização de urbanização paisagística ou em recuperação ambiental é necessária uma dosagem adequada destes nutrientes para a espécie. Por outro lado, quando altas quantidades de N e P, são escoados nas margens de monoculturas e acabam mudando o aporte desses nutrientes, *H. impetiginosus*, é uma das espécies que pode ter efeitos negativos na fotossíntese e pode acabar sendo suprimida por espécies que tem a capacidade adaptativa de utilizar esses nutrientes em altas quantidades, inclusive espécies invasoras, podendo diminuir a diversidade de espécies ao longo do tempo.

5.7 Referências

- Berghetti, Á.L.P., Araujo, M.M., Tabaldi, L.A., Turchetto, F., Tassinari, A., Bernardy, D., Griebeler, A.M., Barbosa, F.M., Aimi, S.C., Brunetto, G., 2021. Effects of nitrogen fertilization on the growth and on photochemical efficiency in plants of *Handroanthus heptaphyllus*. J. Plant Nutr. 44, 2464–2475. <https://doi.org/10.1080/01904167.2021.1899216>
- Boschiero, B.N., Mariano, E., Torres-Dorante, L.O., Sattolo, T.M.S., Otto, R., Garcia, P.L., Dias, C.T.S., Trivelin, P.C.O., 2020. Nitrogen fertilizer effects on sugarcane growth, nutritional status, and productivity in tropical acid soils. Nutr. Cycl. Agroecosystems 117, 367–382. <https://doi.org/10.1007/s10705-020-10074-w>
- Bubier, J.L., Smith, R., Juutinen, S., Moore, T.R., Minocha, R., Long, S., Minocha, S., 2011. Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs. Oecologia 167, 355–368. <https://doi.org/10.1007/s00442-011-1998-9>
- Bustamante, M.M.C., de Brito, D.Q., Kozovits, A.R., Luedemann, G., de Mello, T.R.B., de Siqueira Pinto, A., Munhoz, C.B.R., Takahashi, F.S.C., 2012. Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). Plant Ecol. 213, 795–808. <https://doi.org/10.1007/s11258-012-0042-4>
- Camillo, J., Salomão, A.N., 2018. *Handroanthus impetiginosus*. Ipê-roxo. Plantas para o futuro. Embrapa Recursos Genéticos e Biotecnologia. 984-991.
- Capelin, D., Daneluzzi, G.S., de Oliveira, R.F., Bressan, D.F., Corrêa, C.V., Joca, T.A.C., Alves, M. da S., Broetto, F., 2017. Utilização do IRGA - Analisador de gases por infravermelho para avaliação de trocas gasosas em plantas: check list de preparação. O Estresse das Plantas -Teoria Prática, 187–194.
- Conant, R.T., Berdanier, A.B., Grace, P.R., 2013. Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture. Global Biogeochem. Cycles 27, 558–566. <https://doi.org/10.1002/gbc.20053>
- Cordeiro, Á.R., Duarte, A.A., Della-Torre, F., França, F.A.N., França, M.G.C., 2024. Do invasive and native grasses in Brazilian Savanna differ in their photosynthetic performance under natural light radiation? Flora Morphol. Distrib. Funct. Ecol. Plants 315. <https://doi.org/10.1016/j.flora.2024.152523>
- Cunha, Fernanda, L., Arcanjo dos Santos, J., Carvalho Diniz, P., Amaral de Melo, L.,

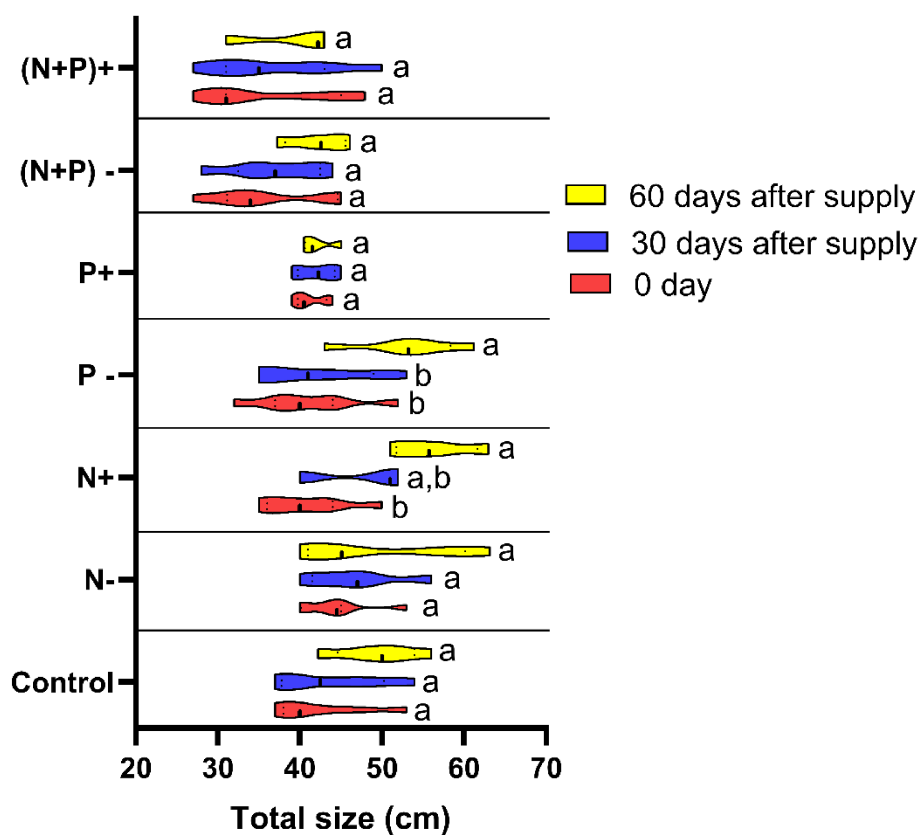
- Venturin, N., 2022. Uso de adubos de liberação lenta na produção de mudas de *Handroanthus impetiginosus*. Adv. For. Sci. 9, 1885–1891. <https://doi.org/10.34062/afs.v9i4.13072>
- De Oliveira, A.P., De Oliveira, E.F., Oliveira, A.J. de P., Lemos, J.L.F.G., Camargos, C.E.M., Lopes, M.C., 2023. Utilização De Biofertilizante No Desenvolvimento Da *Handroanthus Impetiginosus*. Rev. Foco 16, e1428. <https://doi.org/10.54751/revistafoco.v16n3-124>
- Diaz, S., Cabido, M., Casanoves, F., 1998. Plant functional traits and environmental filters at a regional scale. J. Veg. Sci. 9, 113–122. <https://doi.org/10.2307/3237229>
- Edwards, E.J., Chatelet, D.S., Sack, L., Donoghue, M.J., 2014. Leaf life span and the leaf economic spectrum in the context of whole plant architecture. J. Ecol. 102, 328–336. <https://doi.org/10.1111/1365-2745.12209>
- Faria, A.P., Fernandes, G.W., França, M.G.C., 2015. Physiological approaches to determine the impact of climate changes on invasive African grasses in the savanna ecoregion of Brazil. Environ. Earth Sci. 74, 3077–3088. <https://doi.org/10.1007/s12665-015-4343-5>
- Goulart, L.M.L., de Paiva, H.N., Leite, H.G., Xavier, A., Duarte, M.L., 2017. Produção de Mudas de Ipê-amarelo *Tabebuia serratifolia* em resposta a fertilização nitrogenada. Floresta e Ambient. 24. <https://doi.org/10.1590/2179-8087.137315>
- Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901–908. <https://doi.org/10.1038/nature01843>
- Hulshof, C.M., Violle, C., Spasojevic, M.J., McGill, B., Damschen, E., Harrison, S., Enquist, B.J., 2013. Intra-specific and inter-specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. J. Veg. Sci. 24, 921–931. <https://doi.org/10.1111/jvs.12041>
- iNaturalist., 2024. Acesso em 19 de novembro de 2024. Disponível em: https://www.inaturalist.org/observations?subview=map&taxon_id=281274
- Jacobson, T.K.B., Bustamante, M.M.D.C., Kozovits, A.R., 2011. Diversity of shrub tree layer, leaf litter decomposition and N release in a Brazilian Cerrado under N, P and N plus P additions. Environ. Pollut. 159, 2236–2242. <https://doi.org/10.1016/j.envpol.2010.10.019>
- Jin, E.J., Yoon, J.H., Lee, H., Bae, E.J., Yong, S.H., Choi, M.S., 2023. Evaluation of drought stress level in Sargent's cherry *Prunus sargentii* Rehder using

- photosynthesis and chlorophyll fluorescence parameters and proline content analysis. PeerJ 11. <https://doi.org/10.7717/peerj.15954>
- Jin, J., Lauricella, D., Armstrong, R., Sale, P., Tang, C., 2015. Phosphorus application and elevated CO₂ enhance drought tolerance in field pea grown in a phosphorus-deficient vertisol. Ann. Bot. 116, 975–985. <https://doi.org/10.1093/aob/mcu209>
- Kayoumu, M., Iqbal, A., Muhammad, N., Li, X., Li, L., Wang, X., Gui, H., Qi, Q., Ruan, S., Guo, R., Zhang, X., Song, M., Dong, Q., 2023. Phosphorus Availability Affects the Photosynthesis and Antioxidant System of Contrasting Low-P-Tolerant Cotton Genotypes. Antioxidants 12. <https://doi.org/10.3390/antiox12020466>
- Kozovits, A.R., Bustamante, M.M.C., Garofalo, C.R., Bucci, S., Franco, A.C., Goldstein, G., Meinzer, F.C., 2007. Nutrient resorption and patterns of litter production and decomposition in a Neotropical Savanna. Funct. Ecol. 21, 1034–1043. <https://doi.org/10.1111/j.1365-2435.2007.01325.x>
- Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E., 2008. Plant nutrient-acquisition strategies change with soil age. Trends Ecol. Evol. 23, 95–103. <https://doi.org/10.1016/j.tree.2007.10.008>
- Leth, R., 2024. Estimated Marginal Means _emmeans, aka Least-Squares Means_. R package version 1.10.2, <<https://CRAN.R-project.org/package=emmeans>>.
- Macêdo, F.A. de A., Coêlho, M.L., Ribeiro, M.C., Barros, E.D.S., Gomes, J.P. da S., Batista, C.L., Lima, L.K.F., Sousa, R.P. de, 2022. Análise das atividades farmacológicas da *Handroanthus serratifolius* (Vahl) S.Grose. Res. Soc. Dev. 11, e5611222891. <https://doi.org/10.33448/rsd-v11i2.22891>
- Martins, W.B., De Oliveira, V.P., Roque Lima, M.D., 2018. Use of morphometry in the arborization of Paragominas city, Pará, Brazil, with *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae). Rev. agro@mbiente on-line 12, 213. <https://doi.org/10.18227/1982-8470ragro.v12i3.4975>
- Messiga, A.J., Ziadi, N., Bélanger, G., Morel, C., 2013. Soil Nutrients and Other Major Properties in Grassland Fertilized with Nitrogen and Phosphorus. Soil Sci. Soc. Am. J. 77, 643–652. <https://doi.org/10.2136/sssaj2012.0178>
- Mo, Q., Li, Z., Sayer, E.J., Lambers, H., Li, Y., Zou, B., Tang, J., Heskell, M., Ding, Y., Wang, F., 2019. Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. Funct. Ecol. 33, 503–513. <https://doi.org/10.1111/1365-2435.13252>

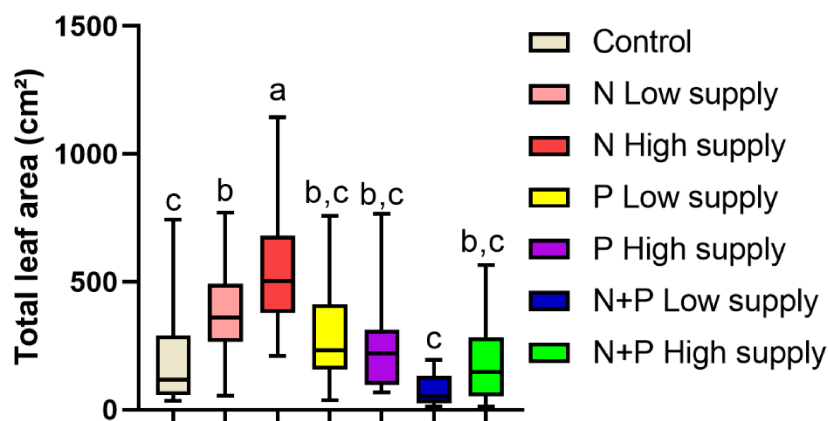
- Moeneclaey, I., Schelfhout, S., Vanhellemont, M., DeCock, E., Van Coillie, F., Verheyen, K., Baeten, L., 2022. Species ecological strategy and soil phosphorus supply interactively affect plant biomass and phosphorus concentration. *Basic Appl. Ecol.* 62, 1–11. <https://doi.org/10.1016/j.baee.2022.03.013>
- Mota, N.M., Meira-neto, J.A.A., Brito, C., 2023. Spatial and temporal variation in nutrient availability under ambient and rainfall reduction conditions in the Brazilian Savanna. *Res. Sq.* 2, 1–21. <https://doi.org/10.21203/rs.3.rs-3238052/v1>
- Pocewicz, A., Morgan, P., Kavanagh, K., 2007. The effects of adjacent land use on nitrogen dynamics at forest edges in northern Idaho. *Ecosystems* 10, 226–238. <https://doi.org/10.1007/s10021-007-9015-1>
- Raghothama, K.G., Karthikeyan, A.S., 2005. Phosphate acquisition. *Plant Soil* 274, 37–49. <https://doi.org/10.1007/s11104-004-2005-6>
- Rascher, U., M., L., U., L., 2000. *Plant Cell Environment* - 2008 - Rascher - Evaluation of instant light-response curves of chlorophyll fluorescence.
- Rossatto, D.R., Kolb, R.M., 2013. Leaf anatomical traits are correlated with tree dominance in a neotropical deciduous forest. *New Zeal. J. Bot.* 51, 242–251. <https://doi.org/10.1080/0028825X.2013.795904>
- Ryan, R.Y.M., Fernandez, A., Wong, Y., Miles, J.J., Cock, I.E., 2021. The medicinal plant *Tabebuia impetiginosa* potently reduces pro-inflammatory cytokine responses in primary human lymphocytes. *Sci. Rep.* 11, 1–12. <https://doi.org/10.1038/s41598-021-85211-8>
- Santos, C.C., Da Cruz Ortega, R.C., Silvério, J.M., Poyer, H.C., De Paula Quintão Scalon, S., Do Carmo Vieira, M., 2020. Nitrogen in the initial growth and photosynthesis photochemical in *Alibertia sessilis* (vell.) K. Schum. *Floresta* 50, 1379–1388. <https://doi.org/10.5380/rf.v50i2.64044>
- Scarpa, A.L.M., da Cunha Cruz, Y., Duarte, V.P., de Castro, E.M., Pasqual, M., de Oliveira, J.P.V., Pereira, F.J., 2022. Growth Response, Gas Exchange, and Leaf Anatomy of *Handroanthus* spp. Seedlings in Mine Tailings Enriched with Nutrient Solution. *J. Soil Sci. Plant Nutr.* 22, 3774–3787. <https://doi.org/10.1007/s42729-022-00926-5>
- Somavilla, N.S., Kolb, R.M., Rossatto, D.R., 2014. Leaf anatomical traits corroborate the leaf economic spectrum: a case study with deciduous forest tree species. *Rev. Bras. Bot.* 37, 69–82. <https://doi.org/10.1007/s40415-013-0038-x>

- Tatarko, A.R., Knops, J.M.H., 2018. Nitrogen addition and ecosystem functioning: Both species abundances and traits alter community structure and function. *Ecosphere* 9. <https://doi.org/10.1002/ecs2.2087>
- Tofa, A.I., Kamara, A.Y., Babaji, B.A., Aliyu, K.T., Ademulegun, T.D., Bebeley, J.F., 2022. Maize yield as affected by the interaction of fertilizer nitrogen and phosphorus in the Guinea savanna of Nigeria. *Heliyon* 8. <https://doi.org/10.1016/j.heliyon.2022.e11587>
- Wellburn, A.R., 1994. The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Physiol.* 144, 307–313. [https://doi.org/10.1016/S0176-1617\(11\)81192-2](https://doi.org/10.1016/S0176-1617(11)81192-2)
- Westoby, M., Wright, I.J., 2006. Land-plant ecology on the basis of functional traits. *Trends Ecol. Evol.* 21, 261–268. <https://doi.org/10.1016/j.tree.2006.02.004>
- Wigley, B.J., Slingsby, J.A., Díaz, S., Bond, W.J., Fritz, H., Coetsee, C., 2016. Leaf traits of African woody savanna species across climate and soil fertility gradients: evidence for conservative versus acquisitive resource-use strategies. *J. Ecol.* 104, 1357–1369. <https://doi.org/10.1111/1365-2745.12598>
- Zemunik, G., Turner, B.L., Lambers, H., Laliberté, E., 2015. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nat. Plants* 1, 1–4. <https://doi.org/10.1038/nplants.2015.50>

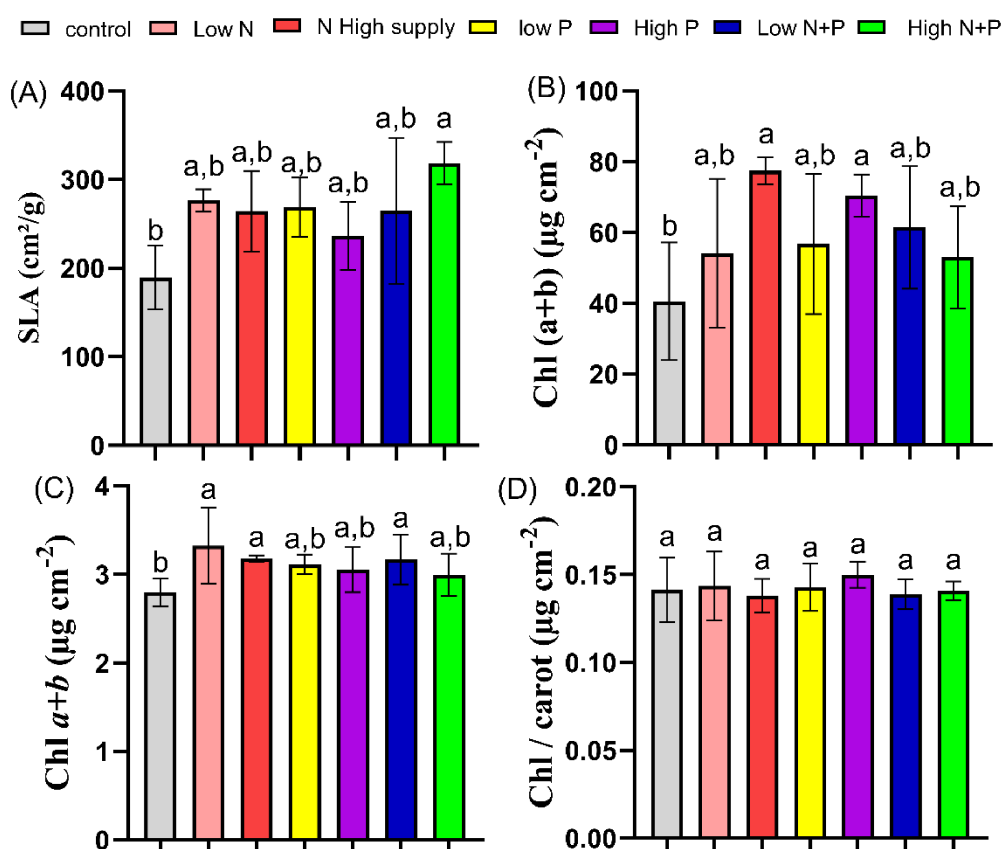
5.8 Tabelas e figuras



5.8.1 Figura 1: Tamanho das plantas *Handroanthus impetiginosus* submetidos à suplementação de fertilizantes industriais nitrogenados e fosfatados, no momento da suplementação e após 30 e 60 dias. As plantas foram submetidas aos tratamentos com baixas concentrações de N (N-), altas concentrações de N (N+), baixas concentrações de P (P-), altas concentrações de P (P+), suplementação sinérgica de N+P com as dosagens baixas (N+P) e altas (N+P). As letras comparam os diferentes tempos em cada tratamento. Letras diferentes indicam diferenças significativas $p < 0.05$ entre o tamanho de *H. impetiginosus* em cada tratamento separadamente.



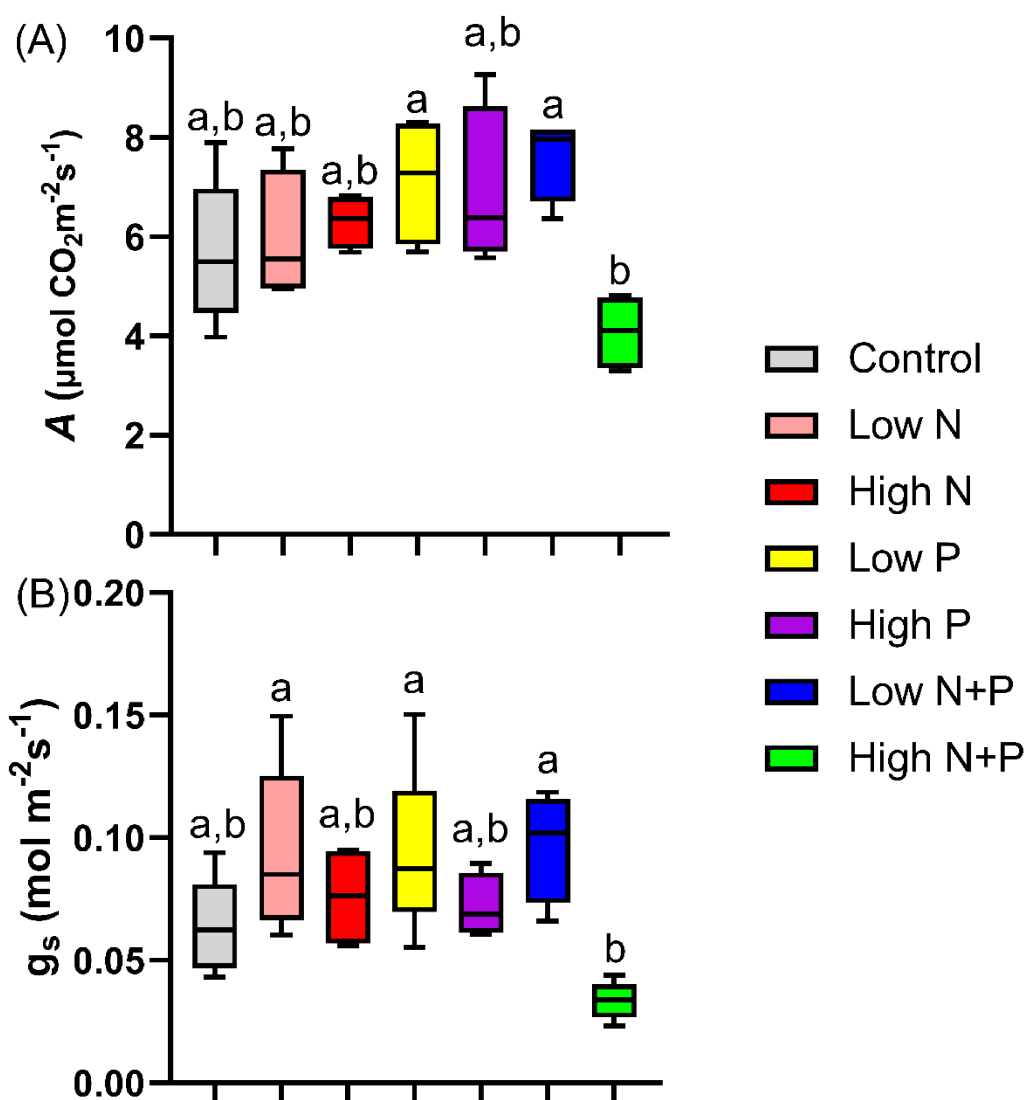
5.8.2 Figura 2: Area foliar de *Handroanthus impetiginosus* submetidos à suplementação de fertilizantes industriais nitrogenados e fosfatados. As plantas foram submetidas aos tratamentos com baixas concentrações de N (rosa), altas concentrações de N (vermelho), baixas concentrações de P (amarelo), altas concentrações de P (roxo), suplementação sinérgica de N+P com as dosagens baixas (azul) e altas (verde). Letras diferentes indicam diferenças significativas entre os tratamentos.



5.8.3 Figura 3: Área foliar específica (SLA) e dados relativos ao conteúdo de pigmentos cloroplastídicos em folhas de *Handroanthus impetiginosus* Clorofila total $a+b$ (Chl $a+b$), razão entre clorofila total a/b (Chl a/b) e razão entre clorofila total e carotenoides (Chl/carot) submetidos à suplementação de fertilizantes industriais nitrogenados e fosfatados. As plantas foram submetidas aos tratamentos com baixas concentrações de N (rosa), altas concentrações de N (vermelho), baixas concentrações de P (amarelo), altas concentrações de P (roxo), suplementação sinérgica de N+P com as dosagens baixas (azul) e altas (verde). Letras diferentes indicam diferenças significativas $p<0,05$ entre os tratamentos.

5.8.4 Tabela 1: Valores médios e erro padrão dos pontos cardinais calculados a partir da fluorescência da clorofila a . Os dados foram obtidos pela curva de luz para a taxa transportadora de elétrons ($ETR \mu\text{mol m}^{-2} \text{s}^{-1}$), sendo eles: taxa máxima de transporte de elétrons (ETR_{max}); metade da taxa máxima de transporte de elétrons ($\frac{1}{2} ETR_{\text{max}}$); radiação fotossinteticamente ativa na taxa máxima de transporte de elétrons ($PAR_{ETR_{\text{max}}}$); radiação fotossinteticamente ativa na metade da taxa máxima de transporte de elétrons ($PAR_{\frac{1}{2} ETR_{\text{max}}}$) e radiação fotossinteticamente ativa de saturação ($PAR_{\text{sat.}}$). Os dados foram comparados por GLM, e as médias de cada tratamento foram contrastadas pelo teste de Tukey ($p<0.05$). Médias seguidas pelas mesmas letras não diferem significativamente.

Treatment	ETR_{max}	$\frac{1}{2} ETR_{\text{max}}$	$PAR_{ETR_{\text{max}}}$	$PAR_{\frac{1}{2} ETR_{\text{max}}}$	$PAR_{\text{sat.}}$
Control	$89 \pm 6.44^{\text{ab}}$	$44.5 \pm 3.22^{\text{ab}}$	$543 \pm 38.8^{\text{ab}}$	$164 \pm 11.7^{\text{ab}}$	$488 \pm 34.9^{\text{ab}}$
Low N	$115.3 \pm 7.89^{\text{a}}$	$57.7 \pm 3.95^{\text{a}}$	$716 \pm 47.5^{\text{a}}$	$216 \pm 14.3^{\text{a}}$	$644 \pm 42.7^{\text{a}}$
High N	$101.8 \pm 7.89^{\text{ab}}$	$50.9 \pm 3.95^{\text{ab}}$	$626 \pm 47.5^{\text{ab}}$	$189 \pm 14.3^{\text{ab}}$	$564 \pm 42.7^{\text{ab}}$
Low P	$74.8 \pm 6.44^{\text{b}}$	$37.4 \pm 3.22^{\text{b}}$	$445 \pm 38.8^{\text{b}}$	$134 \pm 11.7^{\text{b}}$	$400 \pm 34.9^{\text{b}}$
High P	$120.8 \pm 9.11^{\text{a}}$	$60.4 \pm 4.56^{\text{a}}$	$736 \pm 54.8^{\text{a}}$	$222 \pm 16.5^{\text{a}}$	$662 \pm 49.3^{\text{a}}$
Low N+P	$99.5 \pm 7.89^{\text{ab}}$	$49.8 \pm 3.95^{\text{ab}}$	$644 \pm 47.5^{\text{a}}$	$194 \pm 14.3^{\text{a}}$	$580 \pm 42.7^{\text{a}}$
High N+P	$96.2 \pm 7.89^{\text{ab}}$	$47.1 \pm 3.95^{\text{ab}}$	$624 \pm 47.5^{\text{ab}}$	$188 \pm 14.3^{\text{ab}}$	$562 \pm 42.7^{\text{ab}}$



5.8.5 Figura 5: Taxa fotossintética (A) e condutância estomática (B) em folhas de *Handroanthus impetiginosus* submetidos à suplementação de fertilizantes industriais nitrogenados e fosfatados. As plantas foram submetidas aos tratamentos com baixas concentrações de N (rosa), altas concentrações de N (vermelho), baixas concentrações de P (amarelo), altas concentrações de P (roxo), suplementação sinérgica de N+P com as dosagens baixas (azul) e altas (verde). Letras diferentes indicam diferenças significativas $p < 0,05$ entre os tratamentos.

5.9 Conclusão Geral

Os ciclos do nitrogênio (N) e do fósforo (P) têm sido modificados, com aumento destes nutrientes no ambiente. No Brasil central, atividades humanas associadas à transformação de grandes áreas de Cerrado em monoculturas têm fragmentado as áreas nativas. Neste estudo, mostramos que estas áreas recebem resíduos de fertilizantes das monoculturas adjacentes. Valores de $\delta^{15}\text{N}$ encontrados em análises no solo das bordas e interior mostraram que o tamanho das reservas não impede estes nutrientes alcançarem todo o fragmento, sugerindo que as plantas em ambas as localidades estão sujeitas ao aumento destes nutrientes. Tal resultado pode ser explicado pela dependência do histórico de aplicação de fertilizantes das monoculturas adjacentes a cada fragmento estudado, além dos fatores bióticos e abióticos. Esses fatores também podem estar relacionados com a interpretação da concentração de ^{15}N no solo e nas folhas e sua relação com ^{13}C . Considerando os $\delta^{15}\text{N}$ nas folhas, os fragmentos menores não apresentaram diferenças entre borda e interior, enquanto os fragmentos de tamanho intermediários apresentaram maiores valores nas bordas. Porém os três fragmentos maiores não apresentaram diferenças de ^{15}N nas folhas entre a borda e interior. Esses resultados dos maiores fragmentos devem ser pelo fato de que essas reservas são circundadas por monoculturas de soja, e nesse tipo de monocultura a suplementação de fertilizantes nitrogenados de menor ou isenta.

O efeito deste maior incremento de N na borda sobre espécies nativas foi avaliado em um fragmento utilizado como modelo de estudo, a Reserva Ecológica do Panga. As folhas de *Bauhinia holophylla* apresentaram maiores valores de ^{15}N nos indivíduos crescidos nas bordas quando comparadas com os indivíduos do centro do fragmento. As plantas da borda também apresentaram maiores valores de SLA e CRA, resultado similar aos obtidos no capítulo 2 para esses parâmetros em uma área experimental sujeita à suplementação por N+P. Os resultados dos dois primeiros capítulos também não apresentaram diferenças para o conteúdo de pigmentos cloroplastídicos entre a borda e interior ou quando as plantas foram submetidas à suplementação por estes nutrientes.

As análises fisiológicas em *B. holophylla* mostraram que aspectos do seu metabolismo fotossintético podem ser afetados disponibilidade de N e P. No capítulo 1 as plantas no interior do fragmento de Cerrado mostraram maiores Rfd e NPQ do que as plantas na borda. Este resultado sugere que as plantas no interior têm uma maior capacidade de dissipar o excesso de energia luminosa e, assim, um aparato fotossintético

mais preservado. Esses resultados indicam que, apesar do efeito de borda no Cerrado sensu stricto não apresentar as mesmas características de uma área de floresta densa, as condições ambientais específicas no interior e na borda dos fragmentos modulam os valores de $\delta^{15}\text{N}$ no solo e nas folhas e influencia na fisiologia de *B. holophylla*. Além disso, no segundo capítulo o tratamento com fósforo apresentou maior QY_{max} e menor NPQ e R_{fd} , sugerindo que a adição de fósforo na quantidade fornecida influencia negativamente na capacidade de dissipação de energia luminosa e prejudica o aparato fotossintético. Tais resultados mostram possíveis efeitos negativos na fisiologia de *B. holophylla* próximos a locais que recebem suplementação de fertilizantes fosfatados e nitrogenados.

Por fim, cultivamos uma espécie nativa sobre diferentes concentrações de P e N, isolados e combinados. Não encontramos mudas de *B. holophylla* e, por isso, utilizamos *Handroanthus impetiginosus* como modelo de estudo. As plantas responderam positivamente à suplementação isolada de N e P, promovendo aumento da área foliar e eficiência na captação de luz, especialmente quando o nitrogênio é fornecido em quantidades mais baixas. No entanto, a suplementação de baixo P apresentou menor taxa de transporte de elétrons, e a suplementação combinada de N e P, resultou em uma redução na taxa fotossintética e condutância estomática. Esses resultados sugerem que, apesar dos nutrientes serem essenciais para o crescimento e desenvolvimento das plantas, o excesso de N e P suplementados simultaneamente, pode ter efeitos negativos, possivelmente tóxicos, na fisiologia da espécie.

Apesar de as espécies estudadas terem histórias de vida diferente, em ambas apresentaram respostas negativas na fotossíntese na suplementação de P, sendo na capacidade de dissipação de excesso de energia para *B. holophylla* e na taxa de transporte de elétrons para *H. impetiginosus*. De uma forma geral, os resultados mostram que são necessárias práticas sustentáveis do uso de fertilizantes nitrogenados e fosfatados como forma de mitigar os efeitos negativos em espécies nativas do Cerrado.

Anexo



Figura 6. *Handroanthus impetinosus* em casa de vegetação.