UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS

BRUNO PAGANELI

ESTRATÉGIAS NUTRICIONAIS EM ESPÉCIES CONGENÉRICAS DE CERRADO E FLORESTA ESTACIONAL SEMIDECÍDUA

SÃO CARLOS - SP

[2019]

BRUNO PAGANELI

ESTRATÉGIAS NUTRICIONAIS EM ESPÉCIES CONGENÉRICAS DE CERRADO E FLORESTA ESTACIONAL SEMIDECÍDUA

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Ambientais da Universidade Federal de São Carlos, como parte dos requisitos para obtenção do título de Mestre em Ciências Ambientais.

Orientador: Dr. Marco A.P.L. Batalha

Linha de pesquisa: Sistemas Ecológicos

São Carlos - SP

[2019]

Paganeli, Bruno

ESTRATÉGIAS NUTRICIONAIS EM ESPÉCIES CONGENÉRICAS DE CERRADO E FLORESTA ESTACIONAL SEMIDECÍDUA / Bruno Paganeli. -- 2019.
78 f. : 30 cm.

Dissertação (mestrado)-Universidade Federal de São Carlos, campus São Carlos, São Carlos
Orientador: Marco Antonio Portugal Luttembarck Batalha
Banca examinadora: Andréa Lúcia Teixeira de Souza, Carmen Sílvia Fernandes Boaro
Bibliografia

1. Ecofisiologia vegetal. 2. Nutrição mineral. 3. Ecologia vegetal. I. Orientador. II. Universidade Federal de São Carlos. III. Título.

Ficha catalográfica elaborada pelo Programa de Geração Automática da Secretaria Geral de Informática (SIn).
DADOS FORNECIDOS PELO(A) AUTOR(A)
Bibliotecário(a) Responsável: Ronildo Santos Prado – CRB/8 7325

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ciências Ambientais

Folha de Aprovação

Assinaturas dos membros da comissão examinadora que avaliou e aprovou a Defesa de Dissertação de Mestrado do candidato Bruno Paganeli, realizada em 05/09/2019:

Prof. Dr. Marco Antonio Portugal Luttembarck Batalha
UFSCar

Profa. Dra. Andrea Lucia Teixeira de Souza
UFSCar

Profa. Dra. Carmen Sílvia Fernandes Boaro
UNESP

Agradecimentos:

Agradeço pelo pleno apoio financeiro referente aos processos n.os 2017/04937-8 e 2018/11557-0, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), que tornou possível a execução deste projeto. Sendo importante ressaltar que as opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade dos autores e não necessariamente refletem a visão da FAPESP. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Aos meus pais, cujos ensinamentos são imprescindíveis para meu constante desenvolvimento como ser social.

Ao apoio de toda minha família, que acredita e incentiva a permanência em meu plano de vida, resultando na minha dedicação em direção aos meus objetivos.

A todos os professores e professoras que contribuíram para o meu processo de aprendizagem. Ao Professor Caique e ao técnico Casali, que me auxiliaram na implementação do experimento em casa de vegetação. Em especial, gostaria de agradecer ao meu orientador Marco, que contribuiu imensamente e de maneira multidimensional para o capital cultural adquirido nestes anos. Ao Professor Kyle, que me acolheu muito bem em seu grupo de pesquisa na Universidade de Edimburgo, criando condições para meu desenvolvimento como pesquisador.

Ao Programa de Pós-Graduação em Ciências Ambientais e ao Departamento de Botânica da UFSCar São Carlos, pela oportunidade de realizar o estudo.

Aos meus amigos e amigas que foram excepcionais comigo durante todo o processo de formação.

Resumo

O cerrado, apesar de ser alvo prioritário de conservação, ainda está sob pressões. Uma delas se relaciona às práticas de restauração. Este hotspot apresenta grande diversidade vegetacional, porém, com predominância de fisionomias savânicas. Estas podem ser definidas como biomas tropicais e subtropicais em que os componentes herbáceo-subarbustivos são quase contínuos, com arbustos e árvores em densidades variáveis e em que os padrões de crescimento e reprodução estão relacionados com a estacionalidade climática e a ocorrência de queimadas. Uma vez que, sob o clima em que ela se encontra, há também florestas, apenas o clima não é suficiente para explicar sua ocorrência. Um dos fatores postulados para explicar a ocorrência de savanas em áreas cujo clima comporta floresta é a fertilidade do solo, especialmente em relação ao nitrogênio e o fósforo. Posto isso, analisamos as estratégias apresentadas por espécies congenéricas, uma típica do cerrado, e outra, de floresta estacional, cultivadas em variações de disponibilidade nutricional, notadamente nitrogênio e fósforo, em sistema hidropônico com quatro tratamentos: (1) solução de Hoagland completa, (2) solução de Hoagland sem nitrogênio, (3) solução de Hoagland sem fósforo e (4) solução de Hoagland sem nitrogênio e fósforo. Acompanhamos os indivíduos medindo o tempo de duração dos cotilédones, o tempo para o surgimento do primeiro par de folhas, a altura, a área foliar, a área foliar específica, a relação raiz:parte aérea e a biomassa total. Todas nossas comparações foram feitas usando análises de variâncias. Plântulas congenéricas savânicas e florestais são limitadas por nitrogênio, mas, as alterações em seus traços devido a oferta de fósforo, evidenciaram suas estratégias nutricionais distintas. Enquanto as florestais mostraram incremento nos traços funcionais em presença de fósforo — evidenciando a co-limitação e maior demanda nutricional —, algumas espécies do cerrado expressaram sinais de toxicidade ao fósforo.

Palavras chave: cerrado; savanas; floresta; germinação; hidroponia; nutrição mineral; traços funcionais.

Abstract

The Brazilian cerrado, although is one of the hotspots for the biology conservation, is still under pressure. One of these is related to restoration practices. This hotspot presents a huge vegetative diversity, however with predominance of savannas physiognomies. These can be defined as tropical and subtropical biomes, where the herbaceous layer is almost continuous, interrupted only by shrubs and trees at varying densities, with growth and reproductive patterns linked to climatic seasonality and to the occurrence of fires. Since savannas appear in climates that also harbour forests, only the climate is not enough to explain the occurrence of the former. One of the factors postulated to explain the occurrence of savannas in areas whose climate allows forests is nutrient-poor soils, especially regarding nitrogen and phosphorus. We analyzed the strategies presented by congeneric species, one typical of the cerrado, and the other, seasonal forest through four treatments in hydroponic system: (1) complete Hoagland solution, (2) Hoagland solution but nitrogen, (3) Hoagland solution but phosphorus, and (4) Hoagland solution but nitrogen and phosphorus. We followed the individuals measuring how long cotyledons persist, the time for the appearance of the first pair of leaves, height, leaf area, specific leaf area, root:shoot ratio, aboveground biomass and total biomass. All tests were done using variance analyses. Our results suggest that cerrado and forest species are functionally distinct, already in the early stages of their development. Both, savanna and forest congeneric seedlings are limited by nitrogen. However, changes in functional traits due to phosphorus supply evidenced their distinct nutritional strategies. While the forest species improved their functional traits — evidencing the co-limitation and higher nutritional demand —, savannas species displayed signs of toxicity, expressed by the decrease of some attributes.

Keywords: cerrado; forest; functional traits; germination; hydroponics; mineral nutrition; savanna.

Lista de tabelas

Lista de figuras

Sumário

Estrutura da dissertação

A redação da dissertação foi elaborada na forma de publicações científicas, conforme previsto no regimento do programa de Pós-graduação em Ciências Ambientais da Universidade Federal de São Carlos. Dessa maneira, o trabalho apresenta uma introdução geral, três capítulos e uma conclusão. A introdução geral contextualiza a temática central da dissertação, apresentando as justificativas e objetivos do estudo.

O primeiro capítulo é representado por uma nota científica, cujo título é "*Expanded clay: efficient substrate to Solanum lycocarpum germination*", oriunda de um aperfeiçoamento no método para germinação da fruta-de-lobo, uma espécie típica e importante do cerrado.

O segundo capítulo é composto por um artigo intitulado como *"Nutritional strategies in two congeneric pairs of savanna and forest species"* em que testamos se dois pares de espécies características de formações savânicas e florestais apresentam alterações distintas em seus traços funcionais em condições nutricionais diferenciadas. Em cultura hidropônica, realizamos quatro tratamentos com presença e ausência de nitrogênio e fósforo ou de ambos. Após 45 dias, medimos alguns traços funcionais para visualizarmos a consequência da oferta dos macronutrientes para o desenvolvimento das plântulas. Para aumentar o poder estatistico, usamos dois gêneros distintos, *Solanum* e *Enterolobium*, e para cada um deles duas espécies, uma tipica de fisionomia savânica e outra da florestal.

O terceiro trabalho é outro artigo intitulado *"Early growth in a congeneric pair of savanna and seasonal forest trees under different nitrogen and phosphorus availability"*, que buscou entender as estratégias nutricionais de plantas características de formações savânicas e florestais em ofertas diferenciais de nitrogênio e fósforo. Para isso, usamos espécies arbóreas congenéricas do gênero *Handroanthus*, família Bignoniaceae: *Handroanthus aureus*, típica de formações savânicas, e *H. impetiginosus*, característica de floresta estacional semidecídua, nas quais medimos alguns traços funcionais que serviram como base para nossas interpretações à luz do conhecimento científico.

Por fim, apresentamos uma conclusão geral do trabalho, que mostrou uma alternativa para a germinação de sementes de *Solanum lycocarpum* e resumiu as respostas de espécies congenéricas características de formações savânicas e florestais frente às ofertas diferenciadas de nitrogênio e fósforo.

Introdução Geral

O coeficiente latitudinal de diversidade prevê que regiões tropicais comportam os ambientes mais biodiversos e complexos do planeta (Willig 2003, Kinlock 2017), provendo vários serviços ambientais (Brandon 2014). As maiores médias anuais de temperatura e pluviosidade dos trópicos propicia um ambiente pouco restritivo a muitos organismos fotossintetizantes e, consequentemente, aos outros níveis tróficos (Gurevitch et al. 2009). Na América do Sul, África, Austrália e Ásia — áreas com latitudes similares —, ocorre grande variação fisionômica, onde formações savânicas e florestais frequentemente coexistem em mosaico (Cuni-Sanchez 2016, Lehmann 2011, Miatto et al. 2016). Considerando apenas ambientes terrestres, estes biomas cobrem cerca de 20% das paisagens mundiais (Murphy & Bowman 2012), sendo, além do maior ecótono tropical, o mais produtivo ambiente do globo (Oliveras & Malhi 2016), responsável por mais de 60% dos processos de sequestro e fixação de CO_2 (Beer et al. 2010).

No Brasil, o cerrado tem sido colocado como um dos ambientes prioritários para conservação (Myers et al. 2000). Entretanto, ainda permanece esquecido e sob pressões (Marris 2005, Strassburg et al. 2017). Um dos entraves para seu restabelecimento está na restauração, já que a produção de mudas nativas ainda é baixa (Cava et al. 2016). Seu gradiente fisionômico apresenta predominância de formações savânicas, como o campo sujo, o campo cerrado e cerrado sensu stricto (Coutinho 1990) — as mais ricas e diversas do mundo (Silva & Bates 2002) — além do cerradão, uma fisionomia florestal. Além disto, ocorrem outros tipos vegetacionais como florestas estacionais decíduas, semidecíduas e ripícolas em áreas de mesmo clima (Coutinho 1990). Diversos fatores atuam de maneira conjunta e criam condições para a predominância de formações savânicas ou florestais (Lehmann 2011). Dentre eles, os mais relevantes são a frequência e intensidade de chuvas, incêndios e herbivoria, além do estado de trofia dos solos (Viani et al. 2011). Acredita-se que a gama fisionômica existente no cerrado possa ser explicada pelas diferenças nas características do solo (Goodland & Pollard 1973, Silva et al. 2015), principalmente no que tange à disponibilidade nutricional (Pellegrini 2016).

No cerrado, os solos são predominantemente arenosos, mais ácidos e com maiores concentrações de alumínio. Já os solos florestais são menos ácidos e férteis, além de terem menor disponibilidade de alumínio (Ruggiero et al. 2002). Tais características edáficas podem mudar intensamente em pequenas distâncias (Souza & Martins 2004), permitindo o mosaico vegetacional frequentemente existente no cerrado. Entretanto, os nutrientes geralmente limitantes em ambientes terrestres — nitrogênio e fósforo — possuem ciclos biogeoquímicos distintos (Vitousek & Howarth 1991) e vêm sendo alterados de maneira intensa (Bouwman et al. 2011). Previsões a respeito da deposição antrópica de nitrogênio sugerem que esse elemento tenha sua concentração duplicada ainda neste século (Wooliver et al. 2016, Stevens 2018). O fósforo, por exibir um ciclo mais lento, pode exaurir já nas próximas décadas (Cordell et al. 2009), gerando um desbalanço nutricional nos solos de maneira global (Peng et al. 2019).

Além das savanas e florestas apresentarem notáveis diferenças fisionômicas, são funcionalmente distintas (Hoffmann & Franco 2003, Rossatto et al. 2009). Sendo assim, podemos inferir que as espécies típicas dessas formações possam ter passado por pressões ambientais distintas ao longo do processo evolutivo. De fato, espécies características desses tipos vegetacionais são limitadas por diferentes fatores (Hoffmann e Franco 2008). Em ambientes florestais, geralmente o recurso limitante é a incidência luminosa (Gignoux et al. 2016), já em ambientes savânicos, as maiores restrições se encontram no solo (Dantas et al. 2015). Isto pode ter criado diferentes comprometimentos entre os biomas, onde mecanismos evolutivos poderiam ter culminado na diferenciação de nicho ou especiação, diminuindo a competição e favorecendo sua coexistência (Rueffler et al. 2006).

A fim de analisar o desempenho de espécies frente a diferentes padrões nutricionais, a análise de traços funcionais têm sido um caminho muito utilizado em estudos ecológicos que procuram entender a dinâmica entre fisionomias savânicas e florestais (Brouillette et al. 2014, Hoffmann et al. 2012). Nesse sentido, o estudo de espécies congenéricas é especialmente desejável para entender essas relações porque propicia maior independência filogenética e aumenta o poder estatístico (Hoffmann 2008).

Sendo assim, objetivando identificar as estratégias nutricionais de espécies de cerrado e floresta, analisamos o desenvolvimento inicial das seguintes espécies: *Solanum lycocarpum* St. Hil., *Enterolobium gummiferum* (Mart.) J. F. Macbr. e *Handroanthus aureus* Mattos do cerrado e, *S. grandiflorum* Ruiz & Pav., *E. contortisiliquum* (Vell.) Morong e *H. impetiginosus* (Mart. Ex DC.) Mattos de floresta. Os pares de espécies pertencem respectivamente às familias Solanaceae, Fabaceae e Bignoniaceae. Medimos alguns traços funcionais nas plântulas que cresceram em soluções que compunham um modelo fatorial completo com os macronutrientes nitrogênio e fósforo. Apresentamos também uma técnica que, apesar de não usar hormônios, proporcionou boas taxas de germinação para *S. lycocarpum*, espécie de grande relevância ecológica no cerrado.

Referências

Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carval-hais N, Rödenbeck C, Arain MA, Baldocchi D, Bonan GB, Bondeau A, Cescatti A, Lasslop G, Lindroth A, Lomas M, Luyssaert S, Margolis H, Oleson, KW, Roupsard O,Veenendaal E, Viovy N, Williams C, Woodward FI, Papale D (2010) Terrestrial gross carbon dioxide uptake: Global dis-tribution and covariation with climate. Science. 329: 834-838.

Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E (2011) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900- 2050 period. Proceedings of the National Academy of Sciences. USA.

Brandon K (2014) Ecosystem services from tropical forests: review of current science. CGD Working Paper #380. Center for Global Development. Washington, DC.

Brouillette LC, Mason CM, Shirk RY, Donovan LA (2014) Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient. New Phytologist. 201: 1316-1327.

Cava, M.G.B.; Isernhagen, I.; Mendonça, A.H.; Durigan, G. 2016. Comparison of techniques for woody vegetation restoration of Cerrado in abandoned pastures Hoehnea 43: 301-315.

Cordell D, Drangert JO, White, S (2009) The story of phosphorus: global food security and food for thought. Global Environmental Change. 19: 292-305.

Coutinho LM (1990) Fire in the ecology of the Brazilian cerrado. In: Goldammer, J. G. Fire in the tropical biota. Springer. Berlin. pp. 82-105.

Cuni-Sanchez A, White LJT, Calders K, Jeffery KJ, Abernethy K, Burt A, Disney M, Gilpin M, Gomez-Dans JL, Lewis SL (2016) African savanna-forest boundary dynamics: A 20-year study. PLoS One. 11.

Dantas VL, Batalha MA, França H, Pausas JG (2015) Resource availability shapes fire-filtered savannas. Journal of vegetation Science 26: 395-403.

Gignoux J, Konaté S, Lahoreau G, Roux X, Simioni G (2016) Allocation strategies of savanna and forest tree seedlings in response to fire and shading: Outcomes of a field experiment. Scientific Reports. 6: 1-15.

Goodland R and Pollard R (1973) The Brazilian cerrado vegetation: a fertility gradient. Journal of Ecology 61: 219-224.

Gurevitch J, Scheiner SM, Fox GA (2009) Ecologia vegetal. Porto Alegre: Artemed. 574p.

Haridasan M (2008) Nutritional adaptations of native plants of the cerrado biome in acid soils. Brazilian Journal of Plant Physiology. 20: 183-195.

Hoffmann WA and Franco AC (2008) The importance of evolutionary history in studies of plant physiological ecology: Examples from cerrados and forest of central Brazil. Brazil Journal of Plant Physiology. 20: 247-256.

Hoffmann WA, Geiger EL, Gotsch SG, Rossatto DR, Silva LC, Lau OL, Haridasan M, Franco AC (2012) Ecological thresholds at the savanna-forest boundary: How plants traits, resources and fire govern the distribution of tropical biomes. Ecology Letters. 15: 759-768.

Hoffmann, WA and Franco AC (2003) Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts. Journal of Ecology. 91: 475-484.

Kinlock NLL, Prowant EM, Herstoff CM, Foley M, AkinFajiye N, Bender M, Umarani HY, Ryu B, Sxen J, Gurevitch J, Pither J (2017) Explaining global variation in the latitudinal diversity gradient: Meta-analysis confirms known patterns and uncovers new ones. Global Ecology and Biogeography. 27: 125-141.

Lehmann CER, Archibald SA, Hoffmann WA, Bond WJ (2011) Deciphering the distribution of the savanna biome. New Phytologist. 191: 197-209.

Miatto RC, Wright IJ, Batalha, MA (2016) Relationships between soil nutrient status and nutrient-related leaf traits in Brazilian cerrado and seasonal forest communities. Plant and Soil. 404: 12-33.

Murphy BP and Bowman DMJS (2012) What controls the distribution of tropical forest and savanna? Ecology Letters. 15: 748-758.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature, 403: 853-858.

Oliveras I and Malhi Y (2016) Many shades of green: The dynamic tropical forest–savannah transition zones. Philosophical Transactions of the Royal Society of London. Series B, Biological Science. 371: 20150308.

Pellegrini AFA (2016) Nutrient limitation in tropical savannas across multiple scales and mechanisms. Ecology 97: 313-324.

Peng Y, Peng Z, Zeng X, Houx HJ (2019) Effects of nitrogen-phosphorus imbalance on plant biomass production: a global perspective. Plant and Soil. 436: 245-252.

Rossatto DR, Hoffmann WA, Franco AC (2009) Differences in growth patterns between co-occuring forest and savanna trees affect the forest savanna boundary. Functional Ecology. 23: 689-698.

Rueffler C, Van Dooren, TJM, Leimar O, Abrams PA (2006) Disruptive selection and then what? Trends in Ecology & Evolution. 21: 238-245.

Ruggiero PGC, Batalha MA, Pivelo VR, Meireles ST (2002) Soil-vegetation relationships in cerrado (Brazilian savanna) and semideciduous forest, Southeastern Brazil. Plant Ecology. 160: 1-16.

Silva JM and Bates JM (2002) Biogeographic patterns and conservation in the South American Cerrado: A tropical savanna hotspot. Bioscience. 52: 225-233.

Silva RBM, Francelino MR, Moura PA, Moura TA, Pereira MG, Oliveira CP (2015) Relação solo/vegetação em ambiente de cerrado sobre influência do grupo Urucuia. Ciência. Florestal. 25: 363-373.

Souza AF and Martins FR (2004) Microsite specialization and spatial distribution of Geonoma brevispata, a clonal palm in south-eastern Brazil. Ecology Research. 19: 521-532.

Stevens BM, Propster J, Wilson GWT, Abraham A, Ridenour C, Doughty C, Johnson NC (2018) Mycorrhizal symbioses influence the trophic structure of the Serengeti. Journal of Ecology. 106: 536-546.

Strassburg, B.B.N.; Brooks, T.; Feltran-barbieri, R.; Iribarrem, A.; Crouzeilles, R.; Loyola, R.; Latawiec, A.E.; Oliveira filho, F.J.B.; Scaramuzza, C.A.M.; Scarano, F.R.; Soares-filho, B.; Balmford, A. 2017. Moment of truth for the cerrado hotspot. Nature Ecology & Evolution 1: 1-3.

Viani RAG, Rodrigues RR, Dawson TE, Oliveira RS (2011) Savanna soil fertility limits growth but not survival of tropical forest tree seedlings. Plant and Soil. 349: 341-353.

Vitousek PM and Howarth RW (1991) Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry. 13: 87-115.

Willig MR, Kaufman DM, Stevens RD (2003) Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annual Review of Ecology, Evolution, and Systematics. 34: 273-309.

Wooliver R, Pfennigwerth AA, Bailey JK, Schweitzer JA (2016) Plant functional constraints guide macroevolutionary trade-offs in competitive and conservative growth responses to nitrogen. Functional Ecology. 30: 1099-1108.

1. Expanded clay: efficient substrate for *Solanum lycocarpum* germination

ABSTRACT: One of the main issues in restoring cerrado areas is achieving high germination rates for species typical to this vegetation type, such as the wolf-apple, *Solanum lycocarpum*. To optimize the germination rate of this species, we tested two substrates, paper filter and expanded clay, using scarified seeds grown on Petri dishes. Whereas the paper filter did not promote germination, the expanded clay led to a germination rate of 64%. Thus, expanded clay seems to be an efficient substrate to induce the germination of *Solanum lycocarpum*.

Keywords: cerrado, dormancy, restoration, savanna, seed.

1.1 Introduction

Although the cerrado is one of the hotspots for the conservation of biological diversity (Myers et al. 2000), it remains "forgotten" (Marris 2005) and threatened (Strassburg et al. 2017). Attempts to restore the cerrado vegetation clashes with the little knowledge about seed dormancy breaking and the difficulty in seedling production (Zaidan & Carreira 2008; Ferreira et al. 2015; Cava et al. 2016).

The wolf-apple, *Solanum lycocarpum* A. St.-Hil., is one of the typical cerrado species for which information about seed dormancy breaking is scarce. *S. lycocarpum* is a shrub, 1-3 m tall, that occurs especially in open cerrado physiognomies (Cuevas-Reyes et al. 2013, Tavares et al. 2016). This species facilitates the establishment of other species, mainly in disturbed environments (Passos et al. 2014), and, thus, is important for the restoration of cerrado areas. Its large, fleshy fruits serve as food for several animals, including the threatened maned-wolf, *Chrysocyon brachyurus* (Pinto et al. 2007).

The seeds of the wolf-apple are dispersed after passing through the digestive tract of the animals that consume the fruits (Motta-junior 1993). However, only that is not enough for the seed to germinate occur (Taiz & Zieger 2006). Frequently, the seed presents adaptive mechanisms of dormancy, which requires certain environmental conditions or even hormonal changes to be overcome to reactivate the seed metabolism (Taiz & Zieger 2006). In vitro, the use of hormones to enhance the germination rate is common, but this process increases the price (Shi et al. 2017).

As a consequence, few individuals are able to overcome the seed stage, the most critical phase in plant development (Kitajima 2000). Among those that succeed, few establish themselves and replace the senescent individuals in a given plant community (Mesa-Sierra 2017). Studies on seed germination of native cerrado species, are still neglected (Lima et al. 2014), since many studies have focused on vegetative reproduction (Zaidan & Carreira, 2008). Thus, to contribute to the filling of this gap, we tested two substrates in the *S. lycocarpum* germination.

1.2 Materials and Methods

We collected freshly fallen fruits from individuals present in the cerrado area located in São Carlos (21°59'01"S, 47°52'50"W; southeastern Brazil), in February and March 2019. We depulped them and selected 200 seeds using two criteria: absence of injuries and similar size. We gently scarified the seeds with roughening sandpaper 120 and immersed them in distilled water for 10 h, favoring adequate imbibition and consequent metabolic reactivation (Bahry et al., 2017.). We placed the seeds in 10 Petri dishes, 15 cm in diameter, with 20 seeds each. We separated the 10 Petri dishes randomly in two treatments.

In the five dishes of the first treatment, we used paper filter as a substrate. On each dish, we placed two sheets of paper, moistened with 6 mL of distilled water. We replaced these sheets every five days. We sterilized them in an ultraviolet chamber for 15 minutes, covered the dishes with plastic film, and took them to a germination chamber.

In the five dishes of the second treatment, we used expanded clay as a substrate. We sieved the clay with a 2 mm soil sieve. Then we sieved the content again with a 1 mm sieve. Thus, we standardized the grain size between 1 and 2 mm. We washed the substrate repeatedly and abundantly with running water in the first times and with distilled water in the last. On each dish, we put 55 g of clay, the 20 seeds on top of this layer, and finally another layer of 55 g of clay. After sterilized them in an ultraviolet chamber for 15 minutes, we moistened each dish with 45 mL of distilled water. We covered the dishes with plastic film, and took them to the germination chamber. The photoperiod of both treatments was 12h, modifying light/dark with high/low temperatures, respectively 30 and 20 °C (Pinto et al. 2007).

We followed the dishes for 60 days. During this period, we counted the number of germinated seeds per dish, using the botanical germination criterion, that is, the emission of the radicle (Castellani et al. 2009), reaching at least 5 mm (Figure 1). At the end of the experiment, we obtained the percentage of germination per dish. Since in one of the treatments there was no germination, it was not necessary to apply any statistical test.

1.3 Results and Discussion

When we used paper filter as a substrate, there was no germination. When we used expanded clay, the germination rate per dish was 64% ± 18% (mean ± standard deviation). In this second treatment, the first germination event occurred on the seventh day and the last on the sixteenth day.

S. lycocarpum seeds display endogenous and exogenous dormancy (Pinto et al. 2007). Abscisic acid is a plant hormone with several functions, among them the inhibition of the germination process (Taiz & Zieger 2006). By using fresh fruits, recently fallen from the mother plant, we ensured a low concentration of abscisic acid in seeds and, consequently, a possible increase in the germination rate (Pinto et al. 2007). By scarifying them, we also guaranteed the overcoming of the integumentary dormancy, essential for the occurrence of the germinative process in this species (Zaidan & Carreira 2008).

However, using filter paper as substrate, even carrying out such procedures, no seed emitted radicle. In about two-thirds of the seeds, a white structure appeared, which is confused often with the radicle (Castellani et al. 2009). By using as germination criterion the emission of radicle with at least 5 mm long, we avoided the mistake of considering the seeds with such structure as germinated (Castellani et al. 2009).

The expanded clay on its turn gave appropriate conditions for seed germination, since 64% of germination can be considered a good rate (Sampaio et al. 2015). Expanded clay may have created a microenvironment similar to that found naturally in the soils. Expanded clay has a high retention of moisture and good oxygenation among its grains, creating appropriate conditions for embryo imbibition and respiration, which culminate in the metabolic reactivation and growth of the embryonic axis (Kerbauy 2008).

Although paper filter is widely used as a substrate in germination tests on native cerrado species (Lima et al. 2014), its use did not give good results. Moreover, it is also more expensive (Baskin & Baskin 2001). Not only did the use of expanded clay as substrate ensure a good germination rate, but it also avoided the use of hormones. Additionally, it is a simple technique and the substrate can be reused, decreasing both the cost and the environmental impact. These improvements may reduce some of the gaps in the challenging and indispensable process of restoration of the cerrado vegetation (Cava et al. 2016).

Acknowledgments

We are grateful for the financial support provided by the São Paulo Research Foundation (FAPESP), grant 2017/04937-8 and the National Council for Scientific and Technological Development, process 305912 / 2013-5.

References

Bahry CA, Perboni AT, Nardino M, Zimmer PD (2017) Physiological quality and imbibitions of soybean seeds with contrasting coats. Revista Ciência Agronômica. 48: 125-133.

Baskin CC and Baskin JM (2001) Seeds. Ecology, biogeography, and evolution of dormancy and germination. Academic Press. San Diego, CA, USA. 2.ed. p. 1600.

Castellani ED, Aguiar IB, Paula RC (2009) Basis for standardization of the germination test in three *Solanum l.* species. Revista Brasileira de sementes 31: 77-85.

Cava MGB, Isernhagen I, Mendonça AH, Durigan G (2016) Comparison of techniques for woody vegetation restoration of Cerrado in abandoned pastures Hoehnea 43: 301-315.

Cuevas-Reyes, P.; Gilberti, L.; González-rodríguez, A.; Fernandes, G.W. 2013. Patterns of herbivory and fluctuating asymmetry in *Solanum lycocarpum* St. Hill (Solanaceae) along an urban gradient in Brazil. Ecological Indicators 24: 557-561.

Ferreira MC, Walter BMT, Vieira DLM (2015) Topsoil translocation for Brazilian savanna restoration: propagation of herbs, shrubs, and trees. Restoration Ecology 23: 723-728.

Kerbauy GB (2008) Fisiologia vegetal. Guanabara Koogan. Rio de Janeiro: 2.ed. p. 452.

Kitajima K, Fenner M (2000) Ecology of seedling regeneration. In Fenner, M. (Ed.). Seeds: The ecology of regeneration in plant communities. CAB Publishing. 331-359.

Lima VBC, Durigan G, Souza FM (2014) Germinação de 15 espécies vegetais do cerrado sob diferentes condições de luz. Bioscience Journal 30: 864-1872.

Marris E (2005) The forgotten ecosystem. Nature 437: 944-945.

Mesa-sierra N, Laborde J (2017) Insights for the conservation of native tree species gleaned from the advance regeneration community in a seasonally dry tropical landscape. Tropical Conservation Science 10: 1-15.

Motta-junior JC, Lombardi JA (1993) Seed dispersal of *Solanum lycocarpum* St.Hil. (Solanaceae) by the Maned Wolf, *Chrysocyon Brachyurus* (Mammalia: Canidae). Ciência e Cultura 45: 126-127.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

Passos FB, Lopes CM, Aquino FG, Ribeiro JF (2014) Nurse plant effect of *Solanum lycocarpum* A. St.-Hil. in area of Brazilian Savanna undergoing a process of restoration. Brazilian Journal of Botany 37: 251-259.

Pinto LVA, Silva EAA, Davide AC, Jesus VAM, Toorop PE, Henk WM, Hilhorst HWM (2007) Mechanism and control of *Solanum lycocarpum* seed germination. Annals of Botany 100: 1175-1187.

Sampaio AB, Vieira DLM, Cordeiro AOO, Aquino FG, Sousa AP, Albuquerque LB, Schmidt IB, Ribeiro JF, Pellizaro KF, Sousa FS, Moreira AG, Santos ABP, Rezende GM, Silva RRP, Alves M, Motta CP, Oliveira MC, Cortes CA, Ogata R (2015) Guide to cerrado restoration. Direct Seeding. Guia de restauração do cerrado. Semeadura Direta. Rede de sementes do cerrado, Brasília, DF, Brazil. p.40.

Shi TQ, Peng H, Zeng SY, Ji RY, Shi K, Huang H, Ji XJ (2017) Microbial production of plant hormones: opportunities and challenges. Bioengineered 8: 124-128.

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. Nature Ecology & Evolution 1: 1-3.

Taiz L and Zeiger E (2006) Plant physiology. Sinauer Associates. Inc. Publishers. Massachusetts, USA. 4 ed. p.764.

Tavares PRA, Alves-junior VV, Morais GA (2016) Does Atta laevigata (Smith, 1858) act as *Solanum lycocarpum* seed dispersers? Sociobiology 63: 682-687.

Zaidan LBP and Carreira RC (2008) Seed germination in cerrado species. Brazilian Journal of Plant Physiology 20: 167-181.

2. Nutritional strategies in two congeneric pairs of savanna and forest species

Abstract

In the tropical region, savannas and seasonal forests, both highly diverse biomes, occur side by side, under the same climate. If so, that mosaic cannot be explained solely by climatic variables, but also by fire, water availability and soil status. Nutrient availability in the soil, especially nitrogen and phosphorus, has been postulated to explain the abrupt transitions between savannas and seasonal forests in tropical regions. Plants from these two biomes may present different nutritional strategies to cope with nitrogen and phosphorus limitation. We used two congeneric pairs — each pair with a species from the savanna and another from the neighbouring seasonal forest — to test whether savanna and forest species presented different nutritional strategies during their early development. We cultivated 14 individuals from each of these species in a hydroponics system with four treatments: (1) complete Hoagland solution, (2) Hoagland solution without nitrogen, (3) Hoagland solution without phosphorus, and (4) Hoagland solution without nitrogen and phosphorus. After 45 days, we harvested the plants and measured total biomass, root to shoot ratio, height, leaf area, and specific leaf area. Overall, savanna species were lighter, shorter, with smaller leaves, higher specific leaf areas, and higher root to shoot ratios when compared to the forest species. Nitrogen increased the performance of species from both biomes. Phosphorus improved the performance of the forest species and caused toxicity symptoms in the savanna species. Hence, savanna and forest species presented different nutritional strategies and were functionally distinct already as seedlings.

Keywords: savanna, forest, nitrogen, phosphorus; functional diversity.

2.1 Introduction

Not only do tropical regions harbour a high biodiversity, but also some of the most threatened biomes (Kinlock 2017), including the savanna (Beerling & Osborne 2006). Savannas are characterised by the presence of grasses, sub-shrubs, shrubs, and tress, in varying proportions, and temporal patterns related to seasonality and fire (Maurin et al. 2014). The occurrence of savannas and their physiognomic gradient are related not only to climate and fire, but also to herbivory, water availability, carbon dioxide, and soil nutrients (Beerling & Osborne 2006).

Border dynamics between savannas and forests, despite being intensively studied, remains poorly understood (Murphy & Bowman 2012). However, savanna and forest species belong to different functional groups (Silva et al. 2013): whereas the former present a conservative nutritional strategy, the latter present an acquisitive one (Maracahipes et al 2018). Thus, they respond differently to environmental changes (Barbosa et al. 2014). For instance, whereas savanna species tend to be water- and nutrient-limited, forest species tend to be limited by light (Dantas et al. 2015, Stark et al. 2015). Thus, climate change, soil eutrophication, soil impoverishment, and fire regime may affect that dynamics and favor one biome over the other (Hill & Southworth 2016).

Soils from these two adjoining biomes are very different (Ruggiero et al. 2002). Savanna soils are usually older and sandy, whereas forest soils are usually younger and clayey (Ruggiero et al. 2002). As a consequence, they present different leaching patterns and nutrient availability, especially concerning nitrogen and phosphorus (Tahir & Marschner 2017). In savannas, the more intense leaching, the strength of ion adsorption, the frequent fires that volatizes some nutrients, and the older age make the soil more acid, richer in Al^{3+}, and poorer in nitrogen and phosphorus than in forests (Pinheiro & Monteiro 2010, Vitousek et al. 2010). Since these features can intensely change over short distances, it allows a mosaic in the spatial distribution of these two biomes

(Souza & Martins 2004).

Nitrogen and phosphorus are strong environmental filters and key factors for the establishment and prevalence of either savannas or forests (Almeida et al. 2018, Pellegrini 2016). As long as their cycles are being intensively modified (Bouwman et al. 2011), it may pose new challenges to plants (Sardans & Peñuelas 2012). Their availabilities can also change functional attributes and trade-offs in biomass allocation (Poorter et al. 2012, Zhang et al. 2018). Since savanna and forest species evolved under different environmental scenarios, nutritional conditions may impose distinct pressures for the species of these two biomes in their early development (Hoffmann and Franco 2008, Poorter et al. 2012).

Studies that aim to understand the effects of different nutritional conditions on species strategies may be useful to predict the dynamics of savanna-forest borders in future scenarios of nutrients inputs (Peng et al. 2019, Viani et al. 2011). In this sense, we tested whether two congeneric pair of savanna and forest species had contrasting trade-offs under different nitrogen and phosphorus conditions. Thus, we tried to answer the following questions: (1) Are forest species heavier than savanna species?; (2) Do savanna species allocate more photoassimilated to the root system than forest species?; (3) Are forest species taller than savanna species?; and (4) Do forest species present larger leaves, with higher specific areas, than savanna species?

2.2 Materials and methods

To account for phylogenetic relatedness (Hoffmann & Franco 2008), we used two congeneric pairs of species, each pair with one species from the savanna and another from the neighbouring seasonal forest. The first pair belonged to the genus *Solanum* L. (Solanaceae), with *S. lycocarpum* St. Hil. from the savanna and *S. grandiflorum* Ruiz & Pav. from the forest; the second pair belonged to the genus *Enterolobium* Mart. (Fabaceae), with *E. gummiferum* (Mart.) J. F. Macbr. from the savanna and *E. contortisiliquum* (Vell.) Morong from the forest. All species are common in central Brazil, in their respective vegetation types.

We purchased the seeds from nurseries and germinated them in the laboratory. We moistened germitest paper sheets with 10 ml of distilled water (Lessa et al. 2015). We scarified the *Enterolobium* seeds and placed 20 of them on Petri dishes with two germitest paper sheets above the seeds and two below. We wrapped the dishes with plastic film and took them to an incubator, at 25°C and 12-hour photoperiod (Malavasi & Malavasi 2004). For *S. grandiflorum*, we used the same procedure, but, for, *S. lycocarpum*, we were able to germinate the seeds only using expanded clay as substrate (see Chapter 1). We followed the seeds until they present radicles with 1 cm long. After that, we randomly picked 56 seedlings of each species and placed each of them in a 180-cm^3 plastic tube, filled with expanded clay and exposed the seedlings to experiment for 45 days.

During the summers of 2018 and 2019, we took the plastic tubes to a greenhouse located in São Carlos (21°59'01"S, 47°52'50"W; southeastern Brazil), where cooling, humidification, ventilation, and exhaustion were automatically controlled, so that the temperature was kept between 20°C and 28°C, the air relative humidity between 60% and 80% under natural light regime. To test the effect of nitrogen and phosphorous depletion on plant growth, we cultivated the seedlings in closed hydroponic systems, in which the nutrient solutions were recycled (Prado & Casali 2006, Jensen 2007). We used four treatments: (1) complete Hoagland solution, (2) Hoagland solution without nitrogen, (3) Hoagland solution without phosphorus, and (4) Hoagland solution without nitrogen and phosphorus. Their pH and electric conductivity were respectively, 5.9 and 824 µs/cm, 5.7 and 845 µs/cm, 6.8 and 864 µs/cm, and in the treatment with nitrogen and phosphorus absence were 6.5 and 855 µs/cm. All treatments had their ionic strength reduced by 50% (Table 1). These initial values were weekly measurements. We added distilled water or solution as necessary whenever the initial values changed. We did a complete exchange of all solutions after 23 days.

For each congeneric pair, we placed eight boxes on aluminium stands. For each treatment, we used two boxes, one for the savanna species and the other for the forest species. To prevent algae proliferation, we covered the boxes with aluminised thermal blankets to avoid light in the solutions. We filled each box with 40 l of its respective solution. Then, we placed a support for 14 plastic tubes. The two boxes of each treatment were connected to a 100 L reservoir by a silicone hose, at one end with a T-connector and at the other end with a submersible motor pump that remained inside the reservoir. The solution was propelled by the motor pump, going through the hose, to the T-connector, and to the two boxes. We set a timer to propel the solution in five cycles daily: at 06:30, 10:30, 12:00, 14:30, and 17:30. The timer remained on for 3 min, the time necessary to move the solutions, homogenize them, and moisten the clay. At the end of each cycle, the solution returned to the reservoir by gravity.

After 45 days, we harvested 14 individuals of each species and each treatment, ovendried them at 80°C for 72 hours, and measured total dry biomass. Total dry biomass is an important functional trait, since it is considered the best predictor of community functioning (Grime 1998). We also separated the below from the aboveground portion and weighed their dry masses. Dividing the below by the aboveground portion, we obtained the root to shoot ratio, which is appropriate to assess biomass allocation (Poorter et al. 2012). Plants adapted in oligotrophic conditions are expected to present higher biomass allocation to roots, so that the ratio is a good indicative of resource limitation (Poorter et al. 2012). We measured height by taking the distance from the hypocotyl to the apical bud. We scanned the leaves and used ImageJ (Rueden et al. 2017) to measure their areas. We divided leaf area by leaf dry biomass to calculate the specific leaf area. Height and leaf traits play an important role in plant growth by improving the light interception, serving as a proxy of performance (Rodriguez et al. 2016).

To answer the four questions posed, we used covariance analyses (Gotelli & Ellison 2011). In a given model, the response variable was “total dry biomass”, "root to shoot ratio", "height", “leaf area” or “specific leaf area”; the explanatory variables were "biome" (savanna or forest),“nitrogen” (presence or absence), and "phosphorus" (presence or absence). We also included four interaction terms as explanatory variables: (1) between "nitrogen" and "phosphorus", to assess whether the effect of one nutrient on the growth of seedlings depended on the other, (2) “nitrogen” and “biome”, (3) “phosphorus” and “biome”, and (4) “nitrogen”, “phosphorus”, and “biome”, to test whether one nutrient was more relevant than other and whether its effect depended on the biome. In the case of specific leaf area, we also tested whether it was related to height, root to shoot ratio, and biome. We carried out all analyses in R (R Core Team 2018).

2.3 Results

Total biomass was significantly related to the explanatory variables considered together (R^2 = 0.40; $P < 0.001$), particularly to nitrogen ($P < 0.001$), phosphorus ($P = 0.005$), biome ($P < 0.001$), the interaction term between nitrogen and biome ($P < 0.001$), and the interaction term between nitrogen and phosphorus ($P = 0.003$). Individuals of forest species (1.73 g ± 1.59, mean ± sd) were heavier than individuals of savanna species (0.66 g ± 0.77). Savanna species were heavier in the complete solution (1.27 ± 0.80), then without phosphorus (0.67 ± 0.95), without nitrogen and phosphorus (0.42 ± 0.40), and without nitrogen (0.28 ± 0.34). Forest species were heavier in the complete solution (3.05 ± 1.91), then without phosphorus (1.99 g ± 1.36), without nitrogen (0.98 ± 1.02), and without both (0.90 g ± 0.84).

Root to shoot ratio was related to the explanatory variables considered together (R^2 = 0.47; $P <$ 0.001), higher in savanna (0.68 ± 0.30) than in forest species (0.38 ± 0.12). Both nitrogen ($P <$

0.001) and phosphorus ($P < 0.001$) affected this trait significantly. Savanna species presented higher ratio in the solution without both nutrients (0.90 ± 0.16), then in the complete solution (0.48 ± 0.17), without nitrogen only (0.40 ± 0.73), and without phosphorus only (0.25 ± 0.59). Forest species presented higher ratio in the solution without phosphorus only (0.42 ± 0.09), then without both nutrients (0.39 ± 0.12), without nitrogen only (0.38 ± 0.13), and in the complete solution (0.30 ± 0.09). The interaction term between nitrogen and biome was also significant ($P < 0.001$).

Individuals of the forest (23.14 cm ± 18.6) were taller ($R^2 = 0.28$; $P < 0.001$) than those of the savanna (11.25 cm ± 6.4). Height was related to nitrogen ($P < 0.001$), but not to phosphorus ($P = 0.154$). The effect of nitrogen on height depended on the biome ($P = 0.001$). Individuals of the savanna species grew taller in the complete solution (14.48 ± 6.15), then without phosphorus only (11.12 ± 6.04), without nitrogen only (9.92 ± 6.68), and without both nutrients (9.5 ± 5.81). Individuals of the forest species grew taller in the complete solution (33.53 ± 21.71), then without phosphorus only (27.05 ± 20.16), without both nutrients (16.15 ± 12.49), and without nitrogen only (15.82 ± 12.98).

Leaves were larger ($R^2 = 0.75$; $P < 0.001$) in forest (201.40 cm^2 ± 157.16) than in savanna species (72.90 cm^2 ± 58.23). All explanatory variables were significant ($P < 0.01$ in all cases). Savanna species presented larger leaves in the complete solution (142.34 ± 29.15), then without phosphorus only (67.09 ± 54.75), without nitrogen only (43.95 ± 42.35), and without both nutrients (37.74 ± 32.25). Forest species presented larger leaves in the complete solution (399.91 ± 95.99), then without phosphorus only (225.30 ± 107.14), without nitrogen only (90.92 ± 91.09), and without both nutrients (89.45 ± 73.75).

Specific leaf area was not related to the explanatory variables taken all together ($R^2 = 0.05$; $P = 0.113$), but it was marginally different between the biomes ($P = 0.055$), higher in savanna (552.06 $\text{cm}^2\ \text{g}^{-1}$ ± 1050.62) than in forest species (360.32 $\text{cm}^2\ \text{g}^{-1}$ ± 198.16). The interaction terms between nitrogen and phosphorus (*P* = 0.069) and among nitrogen, phosphorus, and biome (*P* = 0.046) were, respectively, marginally significant and significant. Savanna species presented higher specific leaf areas in the solution without nitrogen (791.12 ± 1560.89), then in the solution without phosphorus (695.36 ± 1285.85), in the complete solution (416.03 ± 505.46), in the solution without both nutrients (300.60 ± 179.51). Forest species presented higher specific leaf areas in the solution without nitrogen (393.6 ± 245.36), then in the complete solution (372.50 ± 202.14), in the solution without both nutrients (358.88 ± 217.64), and in the solution without phosphorus (302.30 ± 108.29). Specific leaf area was related to height, root to shoot ratio, and biome ($R^2 = 0.35$; $P < 0.001$).

2.4 Discussion

Concerning their growth under different nitrogen and phosphorus supply, congeneric savanna and forest species displayed distinct strategies from the very early stages of their life cycles, as predicted, for example by Poorter (2012) and Zhang et al. (2018). Although species from these biomes live under different scenarios of nutrient availability (Ruggiero et al. 2002), both congeneric pairs we analysed were limited by nitrogen. Overall, when they were supplied with that element, they grew faster and higher. Phosphorus, on its turn, increased the performance, but generally speaking, the improvement was less pronounced than those provided by nitrogen.

Such pattern was evident, for instance, in total biomass, with species from the two biomes showing different responses to nutrient addition. Biomass increment provided by nitrogen was enhanced by phosphorus in species from both biomes. However, in the savanna species, when there was no nitrogen, the addition of phosphorus decreased growth rate (Figure 2) – perhaps an indication of phosphorus toxicity (Silber et al. 2002, Hasmah et al. 2015). As phosphorus availability in savannas tend to be low (Matzek & Vitousek 2009), species from this biome may present strategies to cope with such hindrance. Forest species, on the other hand, displayed high nutritional demand, shown by intense biomass reduction when growing without nitrogen or phosphorus.

These patterns could be fit into divergent plant nutritional strategies, the conservative and the acquisitive ones, which allow savanna and forest species to live in contrasting habitats in neighbouring sites (Maracahipes et al 2018). The conservative strategy is usually adopted by the less demanding savanna species, with lower growth rates and nutrient retranslocation from senescent to younger organs, appropriate to survive on oligotrophic soils (Massi 2015, Chacón-Madrigal et al. 2018, Maracahipes et al. 2018). The acquisitive strategy is often adopted by the more demanding forest species, with higher absorption and growth rates (Caplan et al. 2017). Both strategies are consistent with our results and the evolutionary history of savanna and forest species. It makes possible the prevalence of one vegetation type over the other concerning competition under different nutrient supply, changing not just species composition, but also functional diversity of a given community (Grime 1998, Hill & Southworth 2016).

Since biomass comes from the photosynthetic process, the amount of carbon dioxide sequestrated by these biomes was very different. There is a gradient in carbon stock according to physionogmic differences from savannas to forests (Abreu et al. 2017). Environmental quality and community functioning are complex and cannot be defined solely by total biomass (Norberg 2004). Regarding plant biomass, the root to shoot ratio can be interpreted as where – below or aboveground – the incorporated organic matter was preferentially allocated, allowing species to be more adapted to forage with the roots or the aerial organs (Tuller et al. 2018). It also implies differences in vegetation structure (Potter & Klooster 1997) and, consequently, provides information about ecological interaction below or aboveground (Schuldt et al. 2017).

Since savanna soils are usually poorer, we expected a higher root to shoot ratio in savanna species. As a matter of fact, we found higher ratios in savanna species when compared to forest ones. The different patterns of biomass allocation in forest species can be indirectly visualised by their bigger leaves and tallest individuals in comparison with congeneric savannas species. It also can be interpreted as an evolutionary response, since natural selection on forest species may have acted in favour of another resource, light (Dantas et al. 2015, Stark et al. 2015), and, thus, in aerial growth (Gignoux et al. 2016, Rodriguez et al. 2016). Plant height, though, depended on nutrient availability, what may be related to leaf area, since the leaves are the photosynthetically active organ. If there are more nutrients available, plants can produce more energy molecules and proteins, enhancing its metabolism (Theobald et al. 1998, Niinemets 2010).

An important allometric relationship used to predict light interception and its efficiency is the specific leaf area, which is usually higher in forest species than in the savannas (Hoffmann & Franco 2003, Hoffmann et al. 2005, Hoffmann and Franco 2008). However, our results were different, since the savannas species presented higher specific leaf areas. This difference may have come from our method, since the greatest physiological problem for plants with large leaf areas is high water loss due to transpiration (Taiz et al. 2015), what did not happen in our experiment, because we used the hydroponics technique. Whereas savanna species displayed the smallest specific leaf area in the treatment with shorter individuals, they also had higher biomass allocation to roots, showing that they were able to keep their morphological characteristics that provide better competition capacity to aboveground resources, even in a life phase in which light incidence is scarce (Hoffmann and Franco 2008).

The forest species, on their turn, were totally different, since in the same treatments that they presented the shorter individuals, they also presented the highest specific leaf areas. It can mean that if there are not enough nutrients to support the growth in height, they invest more in specific leaf area and enhance their ability to compete for light. When specific leaf area is higher, water loss by transpiration increases (Liu et al. 2017), but it is not a problem in forest environments, where water availability is also higher (Ellison et al. 2017). Thus, when the forest species present less efficient functional attributes in light capture, they still try to display another mechanism that optimises not only plant growth but also efficiency in light capture (Prado et al. 2015, Liu et al. 2017), both good strategies to survive in closed environments.

Taking all functional traits together, savanna and forest species presented contrasting strategies concerning nutritional conditions, highlighting the different pressures experienced by these different functional groups throughout their evolutionary history (Hoffmann and Franco 2008, Silva et al. 2013). Our results pointed out that nitrogen was the most important nutrient, shaping the functional traits in seedlings from both savanna and forest species. As nutrient availability — especially nitrogen — changes, one biome may be favoured over the other, altering the structure and functioning of the savanna and forest mosaic (Bouwman et al. 2011, Hill & Southworth 2016, Touboul et al. 2018). In fact, forest encroachment in savannas landscapes is a global phenomenon already described by changes in fire regime (Staver et al. 2011, Stevens et al. 2017). It may also be related to changes in nitrogen and phosphorus availability.

References

Almeida HA, Bahia TO, Gélvez-Zúñiga I, Fernandes GW (2018) Together yet separate: variation in soil chemistry determines differences in the arboreal-shrub structure of two contiguous rupestrian environments. Acta Botanica Brasilica. 32: 578-587.

Abreu RCR, Hoffmann WA, Vasconcelos HL, Pilon NA, Rossatto DR, Durigan G (2017) The biodiversity cost of carbon sequestration in tropical savanna. Science Advances. 3(8): e1701284.

Barbosa ERM, Langevelde F, Tomlinson KW, Carvalheiro LG, Kirkman K, Bie S, Prins HHT (2014) Tree species from different functional groups respond differently to environmental changes during establishment. Oecologia. 174: 1345-1357.

Beerling DJ, Osborne CP (2006) The origin of the savanna biome. Global Change Biology. 12: 2023-2031.

Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E (2011) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900-2050 period. Proceedings of the National Academy of Sciences. 110: 20882-20887.

Caplan JS, Stone BWG, Faillace CA, Lafond JJ, Baumgarten JM, Mozdzer TJ, Dighton J, Meiners SJ, Grabosky JC, Ehrenfeld JG (2017) Nutrient foraging strategies are associated with productivity and population growth in forest shrubs. Annals of Botany. 119(6): 977-988.

Chacón-Madrigala E, Wanekc W, Hietzd P, Dullinger S (2018) Traits indicating a conservative resource strategy are weakly related to narrow range size in a group of neotropical trees. Perspectives in Plant Ecology, Evolution and Systematics. 32: 30-37.

Dantas VL, Batalha MA, França H, Pausas JG (2015) Resource availability shapes fire-filtered savannas. Journal of Vegetation Science. 26: 395-403.

Ellison D, Morris CE, Locatelli B, Sheil D, Cohen J, Murdiyarso, Gutierrez V, Noordwijk M, Creed IF, Pokorny J, Gaveau D, Spracklen DV, Tobella AB, Ilstedt U, Teuling AJ, Gebrehiwot SG, Sands DC, Muys B, Verbist B, Springgay E, Sugandi Y, Sullivan CA (2017) Trees, forests and water: cool insights for a hot world. Global Environmental Change. 43: 51-61.

Gottsberger G and Silberbauer-Gottsberger I (2006) Life in the Cerrado - a South American tropical seasonal ecosystem. v.1: origin, structure, dynamic and plant use. Reta Verlag, Ulm.

Gotelli NJ, Ellison AM (2011) Princípios de ecologia em estatística. Artmed, Porto Alegre. p. 528.

Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology. 86: 902–910.

Gignoux J, Konaté S, Lahoreau G, Roux X, Simioni G (2016) Allocation strategies of savanna and forest tree seedlings in response to fire and shading: Outcomes of a field experiment. Scientific Reports. 6: 1-15.

Hasmah M, Hanafi MM, Rafii MY, Abdullah SNA, Idris AS, Man S, Idris J, Sahebi M (2015) Determination of optimum levels of nitrogen, phosphorus and potassium of oil palm seedlings in solution culture. Bragantia. 74: 247-254.

Hill MJ, Southworth J (2016) Anthropogenic change in savannas and associated forest biomes. Journal of Land Use Science. 11: 1-6.

Hoffmann WA, Franco AC (2003) Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts. Journal of Ecology. 91: 475-484.

Hoffmann WA, Franco AC (2008) The importance of evolutionary history in studies of plant physiological ecology: Examples from cerrados and forest of central Brazil. Brazilian Journal of Plant Physiology. 20: 247-256.

Hoffmann WA, Franco AC, Moreira MZ, Haridasan M (2005) Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees. Functional Ecology. 19: 932- 934.

Jensen MH (1997) Hydroponics. Hortscience. 32: 1018-1021.

Kinlock NLL, Prowant EM, Herstoff, CM, Foley M, AkinFajiye N, Bender M, Umarani HY, Ryu Sxen B, Gurevitch J, Pither J (2017) Explaining global variation in the latitudinal diversity gradient: meta-analysis confirms known patterns and uncovers new ones. Global Ecology and Biogeography. 27: 125-141.

Lessa BFT, Almeida JPN, Pinheiro CL, Gomes FM, Medeiros FS (2015) Germinación y crecimiento de plántulas de *Enterolobium contortisiliquum* en función del peso de lasemilla y las condiciones de temperatura y luz. Agrociência. 49: 315-327.

Liu M, Wang Z, Li S, Lü X, Wang X, Han X (2017) Changes in specific leaf area of dominant plants in temperate grasslands along a 2500-km transect in northern China Scientific Reports. 7: Article number 10780.

Malavasi UC, Malavasi MM (2004) Dormancy breaking and germination of *Enterolobium contortisiliquum* (Vell.) morong seed. Brazilian Archives of Biology and Technology. 47(6): 851-854.

Maracahipes L, Carlucci MB, Lenza E et al. (2018) How to live in contrasting habitats? Acquisitive and conservative strategies emerge at inter- and intraspecific levels in savanna and forest woody plants. Perspectives in Plant Ecology, Evolution and Systematics. 34: 17–25.

Massi KG (2015) Effects on mineral fertilization on seed production, germination and seedling biomass of *Dalbergia miscolobium* Benth. Revista Árvore, Viçosa-MG 39: 1039–1046.

Matzek V, Vitousek PM (2009) N:P stoichiometry and protein: RNA ratios in vascular plants: an evaluation of the growth-rate hypothesis. Ecology Letters. 12: 765-771.

Maurin O, Davies TJ, Burrows JE, Daru BH, Yessoufou K, Muasya AM, Bank M, Bond W (2014) Savanna fire and the origins of the “underground forest” of Africa. New Phytologist. 204: 201-214.

Murphy BP, Bowman DMJS (2012) What controls the distribution of tropical forest and savanna? Ecology Letters. 15: 748-758.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature. 403: 853-858.

Norberg J (2004) Biodiversity and ecosystem functioning: a complex adaptive systems approach.

American Society of Limnology and Oceanography. 49: 1269-1277.

Niinemets Ü (2010) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. Ecological Research. 25: 693–714.

Pellegrini AFA (2016) Nutrient limitation in tropical savannas across multiple scales and mechanisms. Ecology. 97: 313-324.

Peng Y, Peng Z, Zeng X, Houx HJ (2019) Effects of nitrogen-phosphorus imbalance on plant biomass production: a global perspective. Plant and Soil. 436: 245–252.

Pinheiro M, Monteiro R (2010) Contribution to the discussions on the origin of the cerrado biome: Brazilian savanna. Brazilian Journal of Biology. 70: 95-102.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analysis of interspecific variation and environmental control. New Phytologist 193: 30-50.

Potter CS & Klooster SA (1997) Global model estimates of carbon and nitrogen storage in litter and soil pools: response to changes in vegetation quality and biomass allocation, Tellus B: Chemical and Physical Meteorology. 49(1): 1-17.

Prado CHBA, Casali C (2006) Fisiologia Vegetal: práticas em relações hídricas, fotossíntese e nutrição mineral. Manole, São Carlos.

Prado JJ, Schiavini I, Vale V, Lopes S, Arantes C, Oliveira AP (2015) Functional leaf traits of understory species: strategies to different disturbance severities. Brazilian Journal of Biology. 75: 339-346.

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rodriguez HG, Maiti R, Kumari CA (2016) Biodiversity of leaf traits in woody plant species in northeastern Mexico: a synthesis. Forest Research. 5: 169.

Rueden CT, Schindelin, J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) "ImageJ2: ImageJ for the next generation of scientific image data", BMC Bioinformatics. 18:529, PMID 29187165.

Ruggiero PGC, Batalha MA, Pivelo VR, Meireles ST (2002) Soil-vegetation relationships in cerrado (Brazilian savanna) and semideciduous forest, southeastern Brazil. Plant Ecology 160: 1-16.

Sardans J, Penuuelas J (2012) The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant–soil system. Plant Physiology. 160: 1741-1761.

Schuldt A, Bruelheide H, Buscot F, Assmann T, Erfmeier A, Klein A, Ma K, Scholten T, Staab M, Wirth C, Zhang J, Wubet T (2017) Belowground top-down and aboveground bottom-up effects structure multitrophic community relationships in a biodiverse forest. Scientific Reports. 7: 4222.

Silva LCR, Hoffmann WA, Rossatto DR, Haridasan M, Franco AC, Horwath WR (2013) Can savannas become forests? A coupled analysis of nutrient stocks and fire thresholds in central Brazil. Plant and Soil. 373: 829-842.

Souza AF, Martins FR (2004) Microsite specialization and spatial distribution of Geonoma brevispata, a clonal palm in south-eastern Brazil. Ecological Research. 19: 521-532.

Stark SC, Enquist BJ, Saleska SR, Leitold V, Schietti J, Longo M, Alves LF, Camargo PB, Oliveira RC (2015) Linking canopy leaf area and light environments with tree size distributions to explain Amazon forest demography. Ecology Letters. 18: 636-645.

Staver AC, Archibald S, Levin SA (2011) The global extent and determinants of savanna and forest as alternative biome states. Science. 334: 230-232.

Stevens N, Lehmann CE, Murphy BP, Durigan G (2017) Savanna woody encroachment is widespread across three continents. Global Change Biology. 23: 235-244.

Silber A, Ben-Jaacov J, Ackerman A, Bar-Tal A, Levkovitch I, Matsevitz-Yosef T, Swartzberg D, Riov J, Granot D (2002) Interrelationship between phosphorus toxicity and sugar metabolism in *Verticordia plumosa* L. Plant Soil. 245: 249–260.

Taiz L, Zeiger E, Moller IM, Murphy A (2015) Plant Physiology and Development. 6th Edition, Sinauer Associates, Sunderland, CT.

Tahir S & Marschner P (2017) Clay addition to sandy soil reduces nutrient leaching — Effect of clay concentration and ped size. Communications in Soil Science and Plant Analysis. 48: 1813–1821.

Theobald JC, Mitchell RA, Parry MA, Lawlor DW (1998) Estimating the excess investment in ribulose-1,5-bisphosphate Carboxylase/ Oxygenase in leaves of spring wheat grown under elevated CO2. Plant Physiology. 118: 945–955.

Tuller J, Marquis RJ, Andrade SMM, Monteiro AB, Faria LDB (2018) Trade-offs between growth, reproduction and defense in response to resource availability manipulations. Plos One. 13: 1-12.

Touboul JD, Staver AC, Levin SA (2018) On the complex dynamics of savanna landscapes. Proceedings of the National Academy of Sciences. 115: 1336-1345.

Viani RAG, Rodrigues RR, Dawson TE, Oliveira RS (2011) Savanna soil fertility limits growth but not survival of tropical forest tree seedlings. Plant and Soil. 349: 341-353.

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation:

mechanisms, implications, and nitrogen–phosphorus interactions. Ecological Applications. 20: 5-15.

Zhang H, Li W, Adams HD, Wang A, Wu J, Jin C, Guan D, Yuan F (2018) Responses of woody plant functional traits to nitrogen addition: A meta-analysis of leaf economics, gas exchange, and hydraulic traits. Frontiers in Plant Science. 9: 683.

3. Early growth in a congeneric pair of savanna and seasonal forest trees under different nitrogen and phosphorus availability

Abstract

Aims Most of the physiognomies of the Brazilian cerrado fall within the definition of tropical savanna. Interspersed with the cerrado, the seasonal forest appears. The occurrence of savannas in areas whose climate allows forests may be related to the nutrient-poor soil, especially nitrogen and phosphorus. We analysed the initial development of a congeneric pair, *Handroanthus aureus*, a savanna species, and *H. impetiginosus*, a seasonal forest species, to test whether they present different nutritional strategies.

Methods We used a hydroponic system to provide to the plants: (1) complete Hoagland solution, (2) Hoagland solution but nitrogen, (3) Hoagland solution but phosphorus, and (4) Hoagland solution but nitrogen and phosphorus. We followed the plants for three months and measured: total biomass, aboveground biomass, root to shoot ratio, height, cotyledon persistence, appearance of the first pair of leaves, and leaf area.

Results Except for root to shoot ratio and height which were higher in the forest species, the values for the other traits were consistently higher in the savanna species.

Conclusions The savanna species was more efficient overcoming nutritional deficiencies. The two species were already ecologically distinct at early stages of development, which may be related to different nutritional strategies in savanna and forest woody species in general.

Keywords functional traits, hydroponics, mineral nutrition, savanna, tropical forest.

3.1 Introduction

The Brazilian cerrado presents a wide range of physiognomies (Coutinho 1990), going from grassland to forest, but with most of its physiognomies within the range of tropical savanna (Bourlière & Hadley 1983). Interspersed with the cerrado, other vegetation types appear, such as riparian forest, palm forest, deciduous seasonal forest, semi-deciduous seasonal forest, rocky grassland, and floodplain grassland (Coutinho 1990). Since these vegetation types occur as a mosaic, under the same climatic zone, the climate is not sufficient to explain the variety of physiognomies (Bueno et al. 2018). Other factors, such as fire frequency, water availability, and soil features, have been postulated to explain it (Viani et al. 2011). The occurrence of savanna or seasonal forest in a given area may be related to a "fertility gradient" — the former on poorer soils and the latter on richer ones (Goodland & Pollard 1973; Bond 2010).

Nitrogen is an essential resource for plants and is usually limiting in terrestrial communities (Templer et al. 2012), since it constitutes electron donating molecules and nucleic acids and acts on respiratory and photosynthetic processes (Epstein & Bloom 2005). Nitrogen is the mineral element in the highest demand for plants, and its deficiency can be harmful for their development, survival, and defense (Epstein & Bloom 2005). However, phosphorus may be equally important and can also be a limiting factor (Carstensen 2018). In communities whose soils have been widely leached, phosphorus tends to become the most limiting resource (Matzek & Vitousek 2009). Species adapted to infertile soils tend to present strategies to reduce their nutritional losses (Vergutz et al. 2012) and may respond negatively to the addition of nitrogen and phosphorus, since fertilisation with one or both nutrients can lead to loss of diversity due to acidification and other toxic effects (Flynn & O'Connor 2005; Lambers et al. 2008).

Since nutrient availability is one of the main soil attributes related to the presence and predominance of either savanna or forest (Sarmiento & Monasterio 1975; Pellegrini 2016), species occurring in either vegetation type may present different adaptive mechanisms to deal with dystrophic environments (Sarmiento 1984). Although much has been discussed about savanna soil deficiencies in the cerrado, especially in relation to nutrient availability, little is known about the strategies of savanna species to overcome this problem (Haridasan 2008). Considering that savanna and seasonal forest species may present different nutritional strategies (Sarmiento 1984, Miatto et al. 2016), they may respond differently to the depletion of nitrogen, phosphorus, or both. Since savanna species tend to be adapted to poorer soils, we expected them to be less affected by nutrient depletion when compared to seasonal forest species (Bustamante et al. 2012).

Functional trait variation can be interpreted as a reflection of the different adaptive strategies of plants under distinct resource availability (Brouillette et al. 2014; Lambers et al. 2010; Pérez-Harguindeguy et al. 2013), being an important tool for the understanding of the dynamics of savanna-forest borders (Hoffmann et al. 2012). Although the limiting factors for native species is a complex issue (Haridasan 2008), the savannas environmental presents limitation in the soil resources (Dantas et al. 2015), and the forests are usually limited by light (Gignoux et al. 2016). Thus, obtaining resources in the savanna versus the seasonal forest could result in conflicting strategies of biomass allocation for different plant organs depending on which resource is most limiting (Poorter et al. 2012; Tuller et al. 2018), leading to trade-offs in biomass allocation.

Although total biomass may be an excellent predictor of community functioning (Grime 1998), the root to shoot ratio has also been frequently used to indicate biomass allocation strategies and, consequently, adaptation for foraging above or belowground. Root to shoot ratios are often higher under conditions of nutrient limitation (Poorter et al. 2012) and higher in savanna than in forest tree species (Hoffmann et al. 2004; Hoffmann and Franco 2003; Loiola et al. 2015; Miatto et al.

2016). Similarly, previous works with congeneric savanna and forest species found taller individuals with larger leaves in the forest than in the savanna (Hoffmann & Franco 2003; Hoffmann & Franco 2008; Hoffmann et al. 2005), probably related to improved light interception (Onoda et al. 2014). Seedlings can obtain both nutrients and photoassimilates via cotyledons; however, specialised organs for these functions, such as roots and true leaves, are more efficient (Zhang et al. 2008 a, b). Since forest species grow faster than savannas ones (Gignoux et al. 2016; Viani 2011), it is expected the exhaustion and loses of the cotyledon and the appearance of the first pair of leaves to occur first in the former.

We studied the relationships between mineral nutrition and initial development in a congeneric pair of species occurring in the Cerrado Domain, one from the savanna and the other from the semi-deciduous seasonal forest. Based on previous work, we postulated that the savanna species would grow slower, investing more in belowground biomass. Thus, we tried to answer the following questions: (1) do cotyledons last longer in the savanna species than in the forest one?; (2) does the appearance of the first pair of leaves take longer in the savanna species?; (3) does the cerrado species grow slower than the forest one?; (4) are the leaves in the forest species larger when compared to the savanna species?; and (5) is the root to shoot ratio higher in the savanna species?

3.2 Material and methods

To compare nutritional strategies in woody species of two adjoining vegetation types, the savanna and the semi-deciduous seasonal forest, we used as a model a congeneric pair, *Handroanthus aureus* Mattos and *Handroanthus impetiginosus* (Mart. Ex DC.) Mattos, belonging to the Bignoniaceae family. *H. aureus*, popularly known as “yellow ipê”, occurs in savanna, reaching up to 15 m in height, whereas *H. impetiginosus*, popularly known as "purple ipê", occurs in the neighbouring semi-deciduous seasonal forest, where it can reach 30 m (Lorenzi 1992). Both species are common in their respective vegetation types, have ornamental, medicinal, and construction applications, and are used in the restoration of degraded areas (Cabral et al. 2004; Oliveira et al. 2005).

We purchased hermetically sealed seeds from a nursery. In the 2018 summer, we germinated the seeds in an incubator, set for a 12-hour photoperiod, and at 30°C. After approximately 10 days, when the radicles were 1 cm long, we randomly picked 72 seedlings of each species and placed each seedling in a 180-cm^3 plastic tube, filled with expanded clay that had been previously washed and sieved in two stages: initially with a 2-mm sieve and, then, the sifted material with a 1-mm sieve, standardising grain size between 1 mm and 2 mm.

We took the plastic tubes to a greenhouse, where cooling, humidification, ventilation, and exhaustion were automatically controlled, so that the temperature was kept between 20°C and 28°C and the air relative humidity between 60% and 80% under natural light. To test the effect of nutrient depletion on plant growth, we cultivated the seedlings in closed hydroponic systems, in which the nutrient solutions were recycled (Jensen 2007; Prado & Casali 2006). We had four treatments: (1) complete Hoagland solution, (2) Hoagland solution without nitrogen, (3) Hoagland solution without phosphorus, and (4) Hoagland solution without nitrogen and phosphorus. And their pH and electric conductivity were respectively: 5.9 and 824 µs/cm, 5.7 and 845 µs/cm, 6.8 and 864 µs/cm, and 6.5 and 855 µs/cm. weekly. We measured these values weekly and added distilled water or solution whenever the initial values changed. All treatments had their ionic strength reduced by 50% (Table 1). We carried out a complete exchange of all solutions monthly.

We placed eight boxes on aluminium stands. For each combination of nutrients, we used two boxes, one for the savanna species and the other for the forest species. To avoid algae proliferation, we covered the boxes with aluminised thermal blankets to prevent the entrance of light. Each box had a volume of 40 L, was filled with its respective solution, and had a support for 18 plastic tubes.

The two boxes of each treatment were connected to a 100 L reservoir by a silicone hose, at one end with a T-connector and at the other end with a submersible motor pump that remained inside the reservoir. The solution was propelled by the motor pump, going through the hose, to the T-connector, and to the two boxes. We set a timer to propel the solution in five cycles daily: at 06:30, 10:30, 12:00, 14:30, and 17:30. The timer remained on for 3 min, the time necessary to move the solutions, homogenise the volumes of the reservoirs, and moisten the clay. At the end of each cycle, the solution returned to the reservoir by gravity.

We observed the growth and development of the plants monthly, for three months. For each species, each treatment, and each month, we harvested and measured six individuals. We separated the below- from the aboveground portion, oven dried them at 80°C for 72 hours, and weighed their dry mass. We measured the total biomass and calculated the root to shoot ratio, dividing the below- by the aboveground portion, which is appropriate to assess biomass allocation (Poorter et al. 2012) and is used as a proxy for plant vigour (Ros et al. 2003). We recorded the duration of the cotyledons and the time for the appearance of the first pair of leaves for the plants that were sampled at the end of the experiment. At the end of each month, we also measured plant height and leaf area.

To achieve normality of the residuals and minimise heteroscedasticity, we log-transformed "total biomass", "aboveground biomass", and "height". Were squared "root to shoot ratio", "time for the first pair of leaves", and "leaf area". To assess how the growth patterns of the two species varied over time and across nutrient treatment, we used general linear models. In a given model, the response variable was "total biomass", "aboveground biomass", "root to shoot ratio", "plant height", "duration of cotyledons", "time for the first pair of leaves", or "leaf area". The explanatory variables were "species", "month" as a quantitative variable, "nitrogen" (presence/absence), and "phosphorus" (presence/absence). We included an interaction term between "nitrogen" and "phosphorus" to assess whether the effect of one nutrient on the growth of seedlings depended on the presence of the other. As our main goal was to assess species differences, we also included interaction terms for "species" with "month" and "treatment" variables (nitrogen, phosphorous and their interaction). We carried out all analyses in R (R Core Team 2018).

3.3 Results

Concerning total biomass, individuals of *H. aureus* (0.69 g ± 0.39, mean ± sd) were heavier than those of *H. impetiginosus* (0.37 g ± 0.25) ($P < 0.001$). On average, individuals responded positively to nitrogen addition ($P = 0.037$), but there was a significant interaction between species and N ($P = 0.015$), with the forest species responding more positively to nitrogen addition (Figure 3). There was a strong interaction between species and phosphorus ($P = 0.001$), because the savanna species responded negatively to phosphorus addition whereas the forest species responded positively (Figure 3). This opposite pattern resulted in a non-significant effect of phosphorus addition on total biomass ($P = 0.786$).

Individuals of *H. aureus* (0.37 g ± 0.19) had larger aboveground biomass ($P < 0.001$) than those of *H. impetiginosus* (0.18 g ± 0.09). Aboveground growth over time was higher in *H. aureus* than in *H. impetiginosus* ($P = 0.093$). On average, individuals responded positively to nitrogen addition ($P < 0.001$). Although phosphorus was not significantly influential on aboveground biomass ($P = 0.336$), there was a significant interaction between species and phosphorus ($P = 0.005$), because the savanna species responded negatively and the forest species, positively (Figure 4). The two species grew more in the presence of nitrogen, but, when phosphorus was added, the growth rate of *H. aureus* decreased, whereas the growth rate of *H. impetiginosus* increased (Figure 4).

Root to shoot ratio was higher (*P* = 0.004) in *H. impetiginosus* (1.0 ± 0.6) than in *H. aureus* (0.8 ± 0.6). Both species showed significant increases in root to shoot ratio over the course of the experiment, with the preferential allocation to the root system most evident in the last month (*P* < 0.001). The increase in root to shoot ratio was higher in the forest species (*P* < 0.001; Figure 5) Higher ratios were observed in both nitrogen (*P* < 0.001) and phosphorus (*P* = 0.002) depleted solutions. Although both species presented similar strategies in the presence of either nitrogen (*P* = 0.061) or phosphorus (*P* = 0.214), there was a significant interaction between species and the presence of both nutrients (*P* = 0.002; Figure 5).

Individuals of *H. impetiginosus* (78.7 mm ± 25.4) grew taller (*P* < 0.001) than those of *H. aureus* (19.1 mm ± 3.6), with the time being significant for height increase (*P* = 0.011). Plant height was not affected by either nitrogen (*P* = 0.425) or phosphorus (*P* = 0.451). However, there was a significant interaction between species and phosphorus (*P* = 0.004), since phosphorus addition decreased the growth of *H. aureus*, but increased that of *H. impetiginosus* (Figure 6). *H. aureus* grew taller in the presence of nitrogen, but once again, when phosphorus was available, it had a negative effect on height (Figure 6). When we added both nutrients, *H. impetiginosus* showed a large growth in height, suggesting nitrogen and phosphorus co-limitation.

Cotyledons lasted longer in *H. aureus,* whose individuals kept theirs until the last day of the experiment, than in *H. impetiginosus*, whose individuals lost their cotyledons during the third month. There was no significant difference concerning the nutrients or the interaction terms. The first pair of leaves appeared later (*P* < 0.001) in *H. aureus* (19.5 days ± 2.6) than in *H. impetiginosus* (15.6 days ± 4.2; Figure 7). Neither nitrogen (*P* = 0.839) nor phosphorus (*P* = 0.974) had an effect on the appearance of the first leaves. Leaves of *H. aureus* (44.3 cm^2 ± 27.3) were larger (*P* < 0.001) than those of *H. impetiginosus* (25.8 cm^2 ± 11.5). Leaf area increase over time was higher in the former than in the latter (*P* = 0.007). Overall, individuals responded positively to nitrogen addition (*P* < 0.001) and did not respond to phosphorus (P = 0.069). Both species responded the same way to the nitrogen addition (*P* = 0.746), but differently to phosphorus (*P* < 0.001), with *H. aureus* responding negatively and *H. impetiginosus* positively (Figure 8).

3.4 Discussion

Even though they share an evolutionary history and occur in neighbouring vegetation types, the savanna (*H. aureus*) and the forest (*H. impetiginosus*) tree species had markedly different nutritional strategies. Although nitrogen was the limiting element for almost all traits in both species, the nutrient availability altered the values of these traits distinctly, since *H. aureus* required less phosphorus than *H. impetiginosus*, indicating that, in the early growth, these species have different nutritional demands. Not only did the savanna species present higher total and aboveground biomass, but higher rates of biomass accumulation over time. Since total biomass may be considered the best predictor of community functioning (Grime 1998), *H. aureus* and *H. impetiginosus* seem to be ecologically distinct, corroborating the idea that savanna and forest woody species belong to distinct functional groups (Rossatto et al. 2009; Silva et al. 2013). Nevertheless, both species accumulated more biomass in the presence of nitrogen — irrespective of the presence of phosphorus — indicating that even species from different vegetation types may be limited by the same element (Templer et al. 2012).

Root to shoot ratio is related to nutrient foraging ability and biomass allocation (Poorter et al. 2012). This ratio increased over time, an indication that the need for belowground resources increased more, relative to resources acquired by aerial organs (Mašková & Herben 2018). This strategy was similar in both species, which presented a preferential allocation of biomass to the root system in the third month. However, contrary to previous studies, that ratio here was higher in the forest species (Hoffmann & Franco 2003; Hoffmann et al. 2004; Loiola et al. 2015), which is particularly surprising given that it grew taller. Although the preferential allocation biomass to roots obviously brings an improvement in the nutrient intake of both species, these previous studies used environment conditions in which water was also scarce. In these studies, the higher root to shoot ratio in savanna species may be related to water foraging. In fact, savanna tree species have a deep root system that, even in the dry season, enables them to access underground water (Gottsberger & Silberbauer-Gottsberger 2006). Since, in our case, water was a non-limiting factor, the trade-off concerning resource uptake between the two vegetation types was changed (Fan et al. 2017).

Neverthless, *H. impetiginosus* showed lower root to shoot ratios when growing without both nutrients, indicating low foraging ability under harsh conditions. *H. aureus* in turn presented a higher ratio when growing without both nutrients and invested more in root growth in extreme nutritional conditions. Both strategies are consistent with the edaphic characteristics of the two vegetation types, which during the evolutionary process may have acted as an ecological filter in species attributes (Pellegrini 2016). Although the forest species invested less in aboveground biomass, it grew taller. Investment in height has been proposed as the best trait to overcome limitation for light in closed environments (Gignoux et al. 2016; Moles et al. 2009). Even though *H. impetiginosus* grew more in the presence of both nutrients, phosphorus was more relevant to plant height, which may be related to different functions and consequently distinct nutritional demands in the plant's organs (Yang 2014). Shorter but heavier individuals in the savanna species may be a consequence of investment in bark thickness (Dantas et al. 2015). In savanna, fire is a recurring ecological factor and, thus, individuals with thick barks are able to protect better their meristems and present a competitive advantage (Hoffmann et al. 2012; Maurin et al. 2014).

Although the biomass of *H. aureus* and *H. impetiginosus* seemed to be mainly affected by nitrogen, demand for phosphorus was different, since the savanna species did not show a decrease in biomass when in a solution without phosphorus. Cotyledon reserves may provide an adequate supply of this element and, consequently, ensure metabolic efficiency. Apparently, in the initial phase of their lives, individuals of *H. impetiginosus* assimilated a greater amount of organic matter in the presence of both nutrients, whereas *H. aureus* did so only in the presence of nitrogen. As previously observed, forest species have greater nutritional demand and perform better in environments with higher nutrient availability (Bond 2010; Goodland & Pollard 1973; Pellegrini 2016; Silva et al. 2013). The savanna species, on the contrary, was less sensitive to nutrient depletion, which could improve its chances of overcoming nutritional limitations imposed by an oligotrophic environment (Bustamante et al. 2012).

Given that, in all treatments, the cotyledons remained connected to the plant in the savanna species through the whole experiment, whereas they were dropped by the third month in the forest species, we may postulate that either the reserves were larger or the consumption was slower in the savanna species. In both cases, the need for external sources of nutrients — especially phosphorus in our study — would be reduced, which seems to be an advantageous strategy for the recruited individuals. Although this potential strategy does not alter the total amount of phosphorus in the soil, nutrients present in absorbable forms would be available to already established individuals, decreasing competition also at the community level in an environment that, due to its high leaching rate, has a phosphorus shortage (Matzek & Vitousek 2009). In fact, the *H. aureus* seeds are heavier than those of *H. impetiginosus* (Felix et al. 2018), and because all individuals of *H. aureus* retained their cotyledons supports the idea of less external nutritional dependence in the savanna than in the forest species, and the great relevance of these structures in early growth (Ferreira et al. 2017).

Since cotyledons lasted less time and leaves appeared earlier in *H. impetiginosus*, this species showed faster development than the savanna species. If so, this species had an early increase not only in photosynthetic rate, but also in nutrient demand (Gignoux et al. 2016; Viani et al. 2011).

Similarly to traits previously analysed, fertilisation with one or both nutrients led to an increase in leaf area for *H. impetiginosus*, whereas, for *H. aureus*, this only took place in the presence of nitrogen. These results are indicative not only of different nutritional requirements between the two species, but also a probable phosphorus toxicity in the initial development of *H. aureus*, since the fertilisation with phosphorus decreased its leaf area. Relating leaf physiology of the savanna species with biomass acquisition, we may postulate that the leaf area was the main characteristic that provided the highest acquisition of organic matter displayed in the previous traits. According to Aerns (1958), the morpho and physiological characteristics of savanna tree leaves are mainly a consequence of oligotrophic soils, including macrophily (Aerns 1958; Coutinho 2002). However, in comparative studies with congeneric forest and savanna species, leaf area was found to be higher in forest species (Hoffmann et al. 2005; Hoffmann & Franco 2003; Hoffmann & Franco 2008), even in studies that used the same species than us (Capuzzo et al. 2012). Since water loss through transpiration is the main physiological limitation in plants with large leaf areas (Taiz & Zeiger 2006), leaf attributes may be altered by water availability (Wright et al. 2001).

Since savannas and forests appear in a mosaic are more sensitive to environmental drivers such as nutrient availability (Oliveras & Malhi 2016). Thus, the intense and unbalanced inputs of nitrogen and phosphours in terrestrial communities (Peng et al. 2019) may influence the dynamics of the boundaries between these formations. If other typical species from savannas and forests present patterns similar to those found here — that is, different demands and strategies to cope with the addition and depletion of one or both nutrients — in a scenario of global change, this can act as an environmental filter by altering the composition, structure, and functioning of these already threatened biodiversity hotspots (Myers et al. 2000).

Acknowledgments

We are grateful for the financial support provided by the São Paulo Research Foundation (FAPESP), grants 2017/04937-8 and 2018/11557-0. The third author is grateful to the National Council for Scientific and Technological Development (CNPq) for the productivity grant (305912/2013-5). We are grateful to the School of GeoSciences at the University of Edinburgh for hosting the first author during a visit to the second author's research group.

References

Arens K. (1958) O cerrado como vegetação oligotrófica. Boletim da Faculdade de Filosofia, Ciências e Letras da USP. Botânica, 224 (15): 57-78.

Bond WJ (2010) Do nutrient-poor soils inhibit development of forests? A nutrient stock analysis. Plant and Soil 334:47-60.

Bourlière F and Hadley M (1983) Present-day savannas: an overview. In Goodall DW. Ecosystems of the world – tropical savannas. Elsevier 13: 1-17.

Brouillette LC, Mason CM, Shirk RY, Donovan LA (2014) Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient. New Phytologist 201(4):1316-1327.

Bueno ML, Dexter KG, Pennington RT, Pontara V, Neves DM, Ratter JA, Oliveira-Filho AT (2018) The environmental triangle of the Cerrado Domain: Ecological factors driving shifts in tree species composition between forest and savannas. Journal of Ecology 106 (5): 2109-2120.

Bustamante MMC, Brito DQ, Kozovitz AR, Luedmann G, Mello TRB, Pinto AS, Munhoz CBR, Takahashi FSC (2012) Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). Plant Ecology 213: 795-808.

Cabral EL, Barbosa DCA, Simabukuru EA (2004) Crescimento de plantas jovens de Tabebuia aurea (Manso) Benth. and Hook. F. Ex. S. Moore submetidas a estresse hídrico. Acta Botânica Brasílica 18(2): 241-251.

Capuzzo JP, Rossatto DR, Franco AC (2002) Differences in morphological and physiological leaf characteristics between Tabebuia aurea and T. impetiginosa is related to their typical habitats of occurrence. Acta Botanica Brasilica 26(3): 519-526.

Carstensen A, Herdean A, Schmidt SB, Sharma A, Spetea C, Pribil M, Husted S (2018) The impacts of phosphorus deficiency on the photosynthetic electron transport chain. Plant Physiology 177: 271–284.

Coutinho LM (1990) Fire in the ecology of the Brazilian cerrado. In: Goldammer, J. G. Fire in the tropical biota. Springer, Berlin. 84: 82-105.

Coutinho LM (2002) O bioma cerrado. In: Klein AL (Ed), Eugen Warming e o cerrado brasileiro: um século depois. Editora UNESP, Imprensa Oficial do Estado. São Paulo. p. 77-91.

Dantas VL, Batalha MA, França H, Pausas JG (2015) Resource availability shapes fire-filtered savannas. Journal of vegetation Science 26(2): 395-403.

Epstein E and Bloom AJ (2005) Mineral nutrition of plants: principles and perspectives. Sinauer, Sunderland. 2 ed. p. 400.

Fan Y, Miguez-Macho G, Jobbágy EG, Jackson RB, Otero-Casal C. (2017) Hydrologic regulation of plant rooting depth. Proceedings of the National Academy of Sciences of the United States of America. 114(40): 10572–10577.

Felix FC, Medeiros JAD, Pacheco MV (2018) Morfologia de sementes e plântulas de *Handroanthus impetiginosus* (Mart. ex DC.) Mattos. Revista de Ciências Agrárias [online]. 41(4): 161-170.

Ferreira JVA, Meiado, MV, Siqueira-Filho JA (2017) Efeito da predação de sementes por microlepidópteros na germinação e no desenvolvimento inicial de Handroanthus spongiosus (Rizzini) S. Grose (Bignoniaceae), uma espécie arbórea endêmica da Caatinga e ameaçada de extinção. Gaia Scientia 11: 79-87.

Flynn RWS and O'Connor TG (2005) Determinants of community organization of a South African mesic grassland. Journal of Vegetation Science 16: 93-102.

Gignoux J, Konaté S, Lahoreau G, Roux X, Simioni G (2016) Allocation strategies of savanna and forest tree seedlings in response to fire and shading: Outcomes of a field experiment. Scientific Reports 6: 1-15.

Goodland R and Pollard R (1973) The Brazilian cerrado vegetation: a fertility gradient. Journal of Ecology 61: 219-224.

Gottsberger G and Silberbauer-Gottsberger I (2006) Life in the Cerrado - a South American tropical seasonal ecosystem. v.1: origin, structure, dynamic and plant use. Reta Verlag, Ulm.

Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86:902–910.

Haridasan M (2008) Nutritional adaptations of native plants of the cerrado biome in acid soils. Brazilian Journal of Plant Physiology 20:183-195.

Hoffmann WA, Orthen B, Franco AC (2004) Constraints to seedling success of savanna and forest trees across the savanna-forest boundary. Oecologia 140(2): 252-260.

Hoffmann WA, Franco AC, Moreira MZ, Haridasan M (2005) Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees. Functional Ecology 19(6): 932-940.

Hoffmann, WA, Geiger EL, Gotsch SG, Rossatto DR, Silva LC, Lau OL, Haridasan M, Franco AC (2012) Ecological thresholds at the savanna-forest boundary: How plants traits, resources and fire govern the distribution of tropical biomes. Ecology Letters 15(7): 759-768.

Hoffmann, WA and Franco AC (2003) Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts. Journal of Ecology 91(3): 475-484.

Hoffmann WA and Franco AC (2008) The importance of evolutionary history in studies of plant physiological ecology: Examples from cerrados and forest of central Brazil. Brazil Journal of Plant Physiology 20(3): 247-256.

Jensen MH (1997) Hydroponics. Hortscience 32: 1018-1021.

Lambers H, Raven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23: 95-103.

Lambers H, Brundrett MC, Raven JA, Hopper SD (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant and Soil 334: 11-31.

Loiola PP, Scherer-Lorenzen M, Batalha, MA (2015) The role of environmental filters and functional traits in predicting the root biomass and productivity in savannas and tropical seasonal forest. Forest Ecology and Management. 342: 49-55.

Lorenzi H (1992) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Plantarum, Nova Odessa.

Matzek V and Vitousek, PM (2009) N:P stoichiometry and protein: RNA ratios in vascular plants: an evaluation of the growth-rate hypothesis. Ecology Letters 12: 765-771.

Maurin O, Davies TJ, Burrows JE, Daru BH, Yessoufou K, Muasya AM, Bank M, Bond W (2014) Savanna fire and the origins of the “underground forest” of Africa. New Phytologist 204(1) :201-214.

Mašková T and Herben T (2018) Root:shoot ratio in developing seedlings: how seedlings change their allocation in response to seed mass and ambient nutrient supply. Ecology and Evolution 8: 7143–7150.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. (2000) Biodiversity hotspots for conservation priorities. Nature, 403: 853-858.

Miatto RC, Wright IJ, Batalha, MA (2016) Relationships between soil nutrient status and nutrient-related leaf traits in Brazilian cerrado and seasonal forest communities. Plant and Soil 404(1-2): 12-33.

Moles AT, Warton DI, Warman L, Swenson GN, Laffan SW, Zanne AE, Pitman A, Hemmings FA, Leishman MR (2009) Global patterns in plant height. Journal of Ecology 97: 923–932.

Oliveira LM, Carvalho MLM, Silva TTA, Borges DI (2005) Temperatura e regime de luz na germinação de *Tabebuia impetiginosa* (Martius ex A. P. de Candolle) Standley e *T. serratifolia* Vahl Nich. Bignoniaceae. Ciência e Agrotecnologia 29(3): 642-648.

Oliveras I and Malhi Y (2016) Many shades of green: The dynamic tropical forest–savannah transition zones. Philosophical Transactions of the Royal Society of London. Series B, Biological Science. 371(1703): 20150308.

Onoda Y, Saluñga JB, Akutsu K, Aiba S, Yahara T, Anten NPRA (2014) Trade-off between light interception efficiency and light use efficiency: implications for species coexistence in one-sided light competition. Journal of Ecology 102(1): 167-175.

Pellegrini AFA (2016) Nutrient limitation in tropical savannas across multiple scales and mechanisms. Ecology 97(2): 313-324.

Peng Y, Peng Z, Zeng X, Houx HJ (2019) Effects of nitrogen-phosphorus imbalance on plant biomass production: a global perspective. Plant and Soil. 436: 245–252.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico LJG, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier FJG, Hodgson JG, Thompson K, Morgan HD, Steege H, Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany 61: 167–234.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analysis of interspecific variation and environsmental control. New Phytologist 193(1): 30-50.

Prado CHBA and Casali C (2006) Fisiologia Vegetal: práticas em relações hídricas, fotossíntese e nutrição mineral. Manole, São Carlos.

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ros C, Bell RW, White PF (2003) Seedling vigour and the early growth of transplanted rice. Plant and Soil 252(2): 325-337.

Rossatto DR, Hoffmann WA, Franco AC (2009) Differences in growth patterns between co-occuring forest and savanna trees affect the forest savanna boundary. Functional Ecology 23: 689-698.

Sarmiento G (1984) The ecology of neotropical Savannas. Harvard University, Cambridge.

Sarmiento G and Monasterio M (1975) A critical consideration of the environmental conditions associated with the occurrence of savanna ecosystems in tropical America. In Golley, F. B. Medina, E. Tropical Ecological Systems, Springer, New York.

Silva LCR, Hoffmann WA, Rossatto DR, Haridasan M, Franco AC, Horwath WR (2013) Can savannas become forests? A coupled analysis of nutrient stocks and fire thresholds in central Brazil. Plant and Soil 373: 829–842.

Taiz L and Zeiger E (2006) Plant physiology. Sinauer Associates. Inc. Publishers. Massachusetts, USA. 4 ed. p.764.

Templer PH, Mack MC, Chapin FS, Christenson LM, Compton JE, Crook HD, Currie WS, Curtis CJ, Dail DB, D'Antonio CM, Emmett BA, Epstein HE, Goodale CL, Gundersen P, Hobbie SE, Holland K, Hooper DU, Hungate BA, Lamontagne S, Nadelhoffer KJ, Osenberg CW, Perakis SS, Schleppi P, Schimel J, Schmidt IK, Sommerkorn M, Spoelstra J, Tietema A, Wessel WW, Zak DR (2012) Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of 15N tracer field studies. Ecology 93: 1816-1829.

Tuller J, Marquis RJ, Andrade SMM, Monteiro AB, Faria LDB (2018) Trade-offs between growth, reproduction and defense in response to resource availability manipulations. Plos One 13(8): 1-12. https://doi.org/10.1371/journal.pone.0201873

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecological Monographs 82: 205-220.

Viani RAG, Rodrigues RR, Dawson TE, Oliveira RS (2011) Savanna soil fertility limits growth but not survival of tropical forest tree seedlings. Plant and Soil 349 (1-2): 341-353.

Wright IJ, Reich PB, Westoby M (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. Functional Ecology 15: 423–434.

Yang X, Tang Z, Ji C, Liu H, Ma W, Mohhamot A, Shi Z, Sun W, Wang T, Wang X, Wu X, Yu S, Yue M, Zheng C (2014) Scaling of nitrogen and phosphorus across plant organs in shrubland biomes across Northern China. Scientific Reports 4: 5448.

Zhang H, Wu Y, Matthew C, Zhou D, Wang P (2008) Contribution of cotyledons to seedling dry weight and development in *Medicago falcata* L. New Zealand Journal of Agricultural Research 51: 107-114.

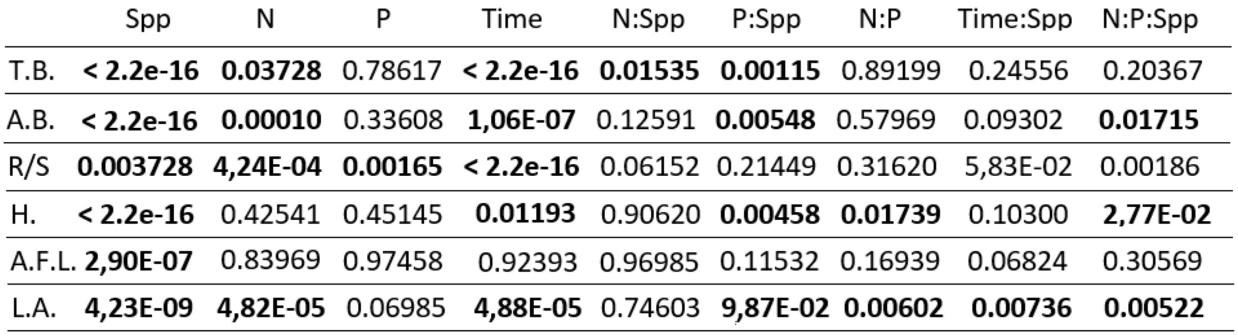
Zhang H, Zhou D, Matthew C, Wang P, Zheng W (2008) Photosythetic contribution of cotyledons to early seedling development in *Cynoglossum divaricatum* and *Amaranthus retroflexus*. New Zealand Journal of Botany 46: 39-48.

Tabelas

Table 1. Treatments (Com = complete Hoagland solution, -N = Hoagland solution without nitrogen, -P = Hoagland solution without phosphorus, -NP = Hoagland solution without nitrogen and phosphorus) used for plant growth, with their respective concentrations. In the cells, there are the volumes (ml) of different 1M stock solutions previously prepared and added to 90 L of distilled water.

	Nutrients							
Treatments	KH_2PO_4	KNO_3	$Ca(NO_3)_2$	$MgSO_4$	KCl	$CaCl_2$	Micronutrients - Fe	Ferric and monosodium EDTA
Com	90	90	135	90	0	0	45	45
-N	90	0	0	90	90	135	45	45
-P	0	90	135	90	90	0	45	45
-NP	0	0	0	90	180	135	45	45

Table 2. In the cells there are the p values referring to chapter 3 manuscript. The statistically significant are in bold. T.B.: Total biomass; A.B.: Aboveground biomass; R/S: Root to shoot ratio; H.: Height; A.F.L.: Appearance of the first pair of leaves; L.A.: Leaves area; Spp: Species; N: Nitrogen; P: Phosphorus; Tim: Time; N:Spp: Interaction between nitrogen and species; P:Spp: Interaction between phosphorus and species; N:P: Interaction between nitrogen and phosphorus; Tim:Spp: Interaction between time and species; N:P:Spp: Interaction between nitrogen, phosphorus and species.



	Spp	N	P	Time	N:Spp	P:Spp	N:P	Time:Spp	N:P:Spp
T.B.	**< 2.2e-16**	**0.03728**	0.78617	**< 2.2e-16**	**0.01535**	**0.00115**	0.89199	0.24556	0.20367
A.B.	**< 2.2e-16**	**0.00010**	0.33608	**1,06E-07**	0.12591	**0.00548**	0.57969	0.09302	**0.01715**
R/S	**0.003728**	**4,24E-04**	**0.00165**	**< 2.2e-16**	0.06152	0.21449	0.31620	5,83E-02	0.00186
H.	**< 2.2e-16**	0.42541	0.45145	**0.01193**	0.90620	**0.00458**	**0.01739**	0.10300	**2,77E-02**
A.F.L.	**2,90E-07**	0.83969	0.97458	0.92393	0.96985	0.11532	0.16939	0.06824	0.30569
L.A.	**4,23E-09**	**4,82E-05**	0.06985	**4,88E-05**	0.74603	**9,87E-02**	**0.00602**	**0.00736**	**0.00522**

Figures

Figure 1. The figure shows the germination process in *Solanum lycocarpum*. In our work, just seeds with radicle at least 5 mm long were considered germinated, that is, the seeds represented by numbers 5, 6, 7, 8, 9, 10, 11, 12 and 13.

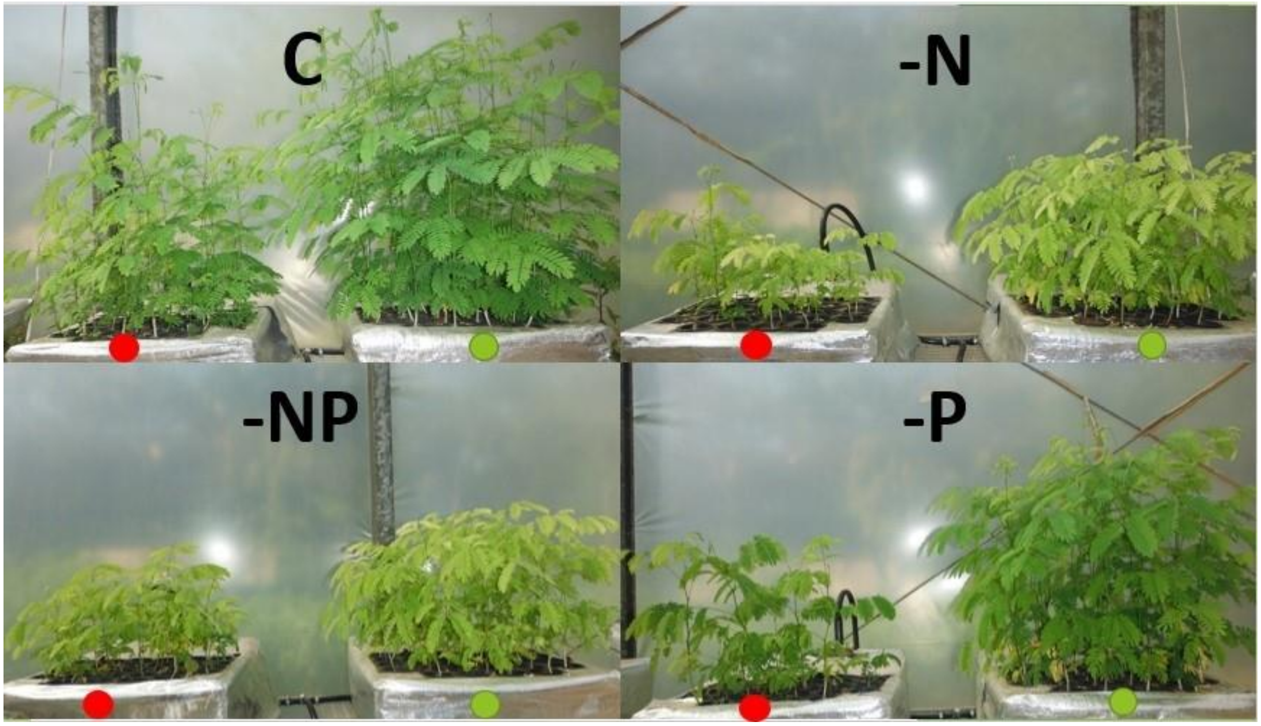


Figure 2. The figure shows the development of a congeneric pair of species after 45 days. The red circles represent *Enterolobium gummiferum,* a savanna species. The green ones represent *E. contortisiliquum,* from forest. The four treatments are represented by: **C** is the complete Hoagland solution; **-N** is the Hoagland solution but nitrogen; **-NP** is Hoagland solution but nitrogen and phosphorus; **- P** is the Hoagland solution but phosphorus.

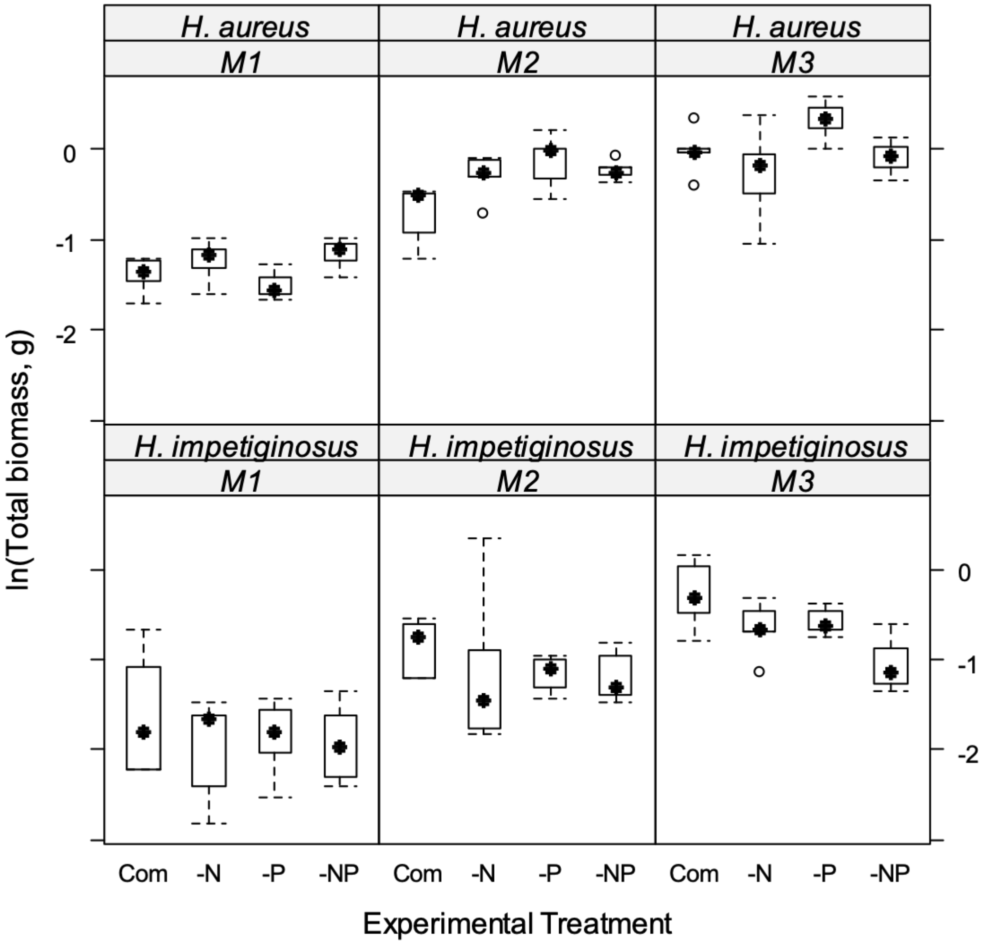


Fig. 3: The figure represents the evolution of total biomass logarithm (grams) over time in *H. aureus*, typical savanna species and *H. impetiginosus*, typical from seasonal forest. The x-axis represents the treatments. The complete solution is represented by **Com**; The solution without nitrogen by **-N**; The solution without phosphorus by **-P**; The solution without both nutrients by **-NP**. **M1**, **M2** and **M3** respectively mean: the first, second and third months from the beginning of the experiment.

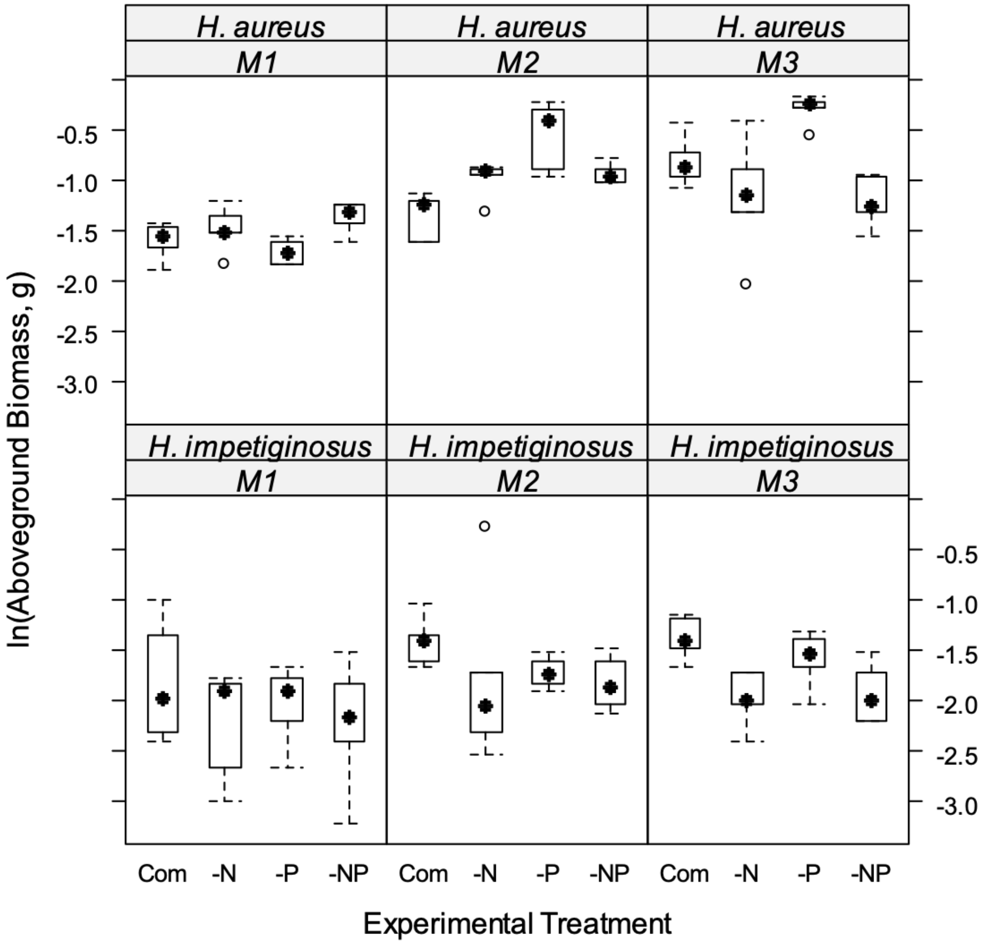


Fig. 4: The figure represents the evolution of aboveground biomass logarithm (grams) over time in *H. aureus*, typical savanna species and *H. impetiginosus*, typical from seasonal forest. The x-axis represents the treatments. The complete solution is represented by **Com**; The solution without nitrogen by **-N**; The solution without phosphorus by **-P**; The solution without both nutrients by **-NP**. **M1**, **M2** and **M3** respectively mean: the first, second and third months from the beginning of the experiment.

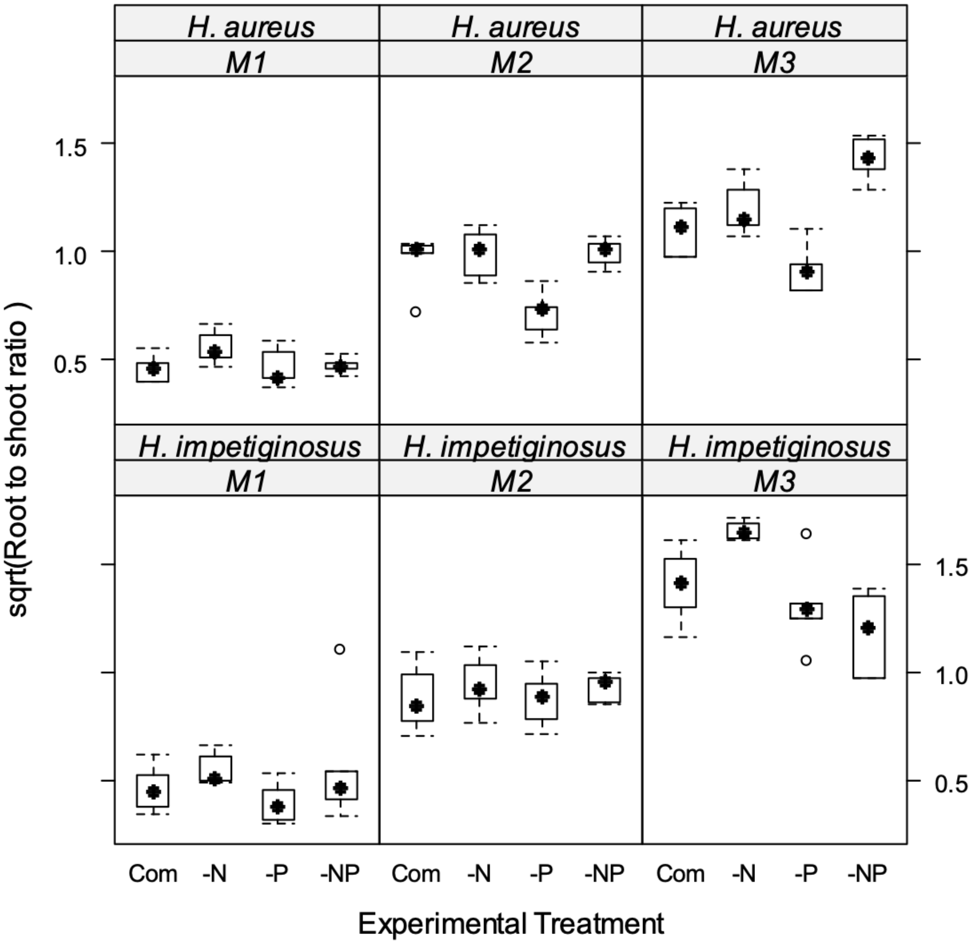


Fig. 5: The figure represents the evolution of root/shoot ratio square root (sqrt) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the treatments. The complete solution is represented by **Com**; The solution without nitrogen by **-N**; The solution without phosphorus by **-P**; The solution without both nutrients by **-NP**. **M1**, **M2** and **M3** respectively mean: the first, second and third months from the beginning of the experiment.

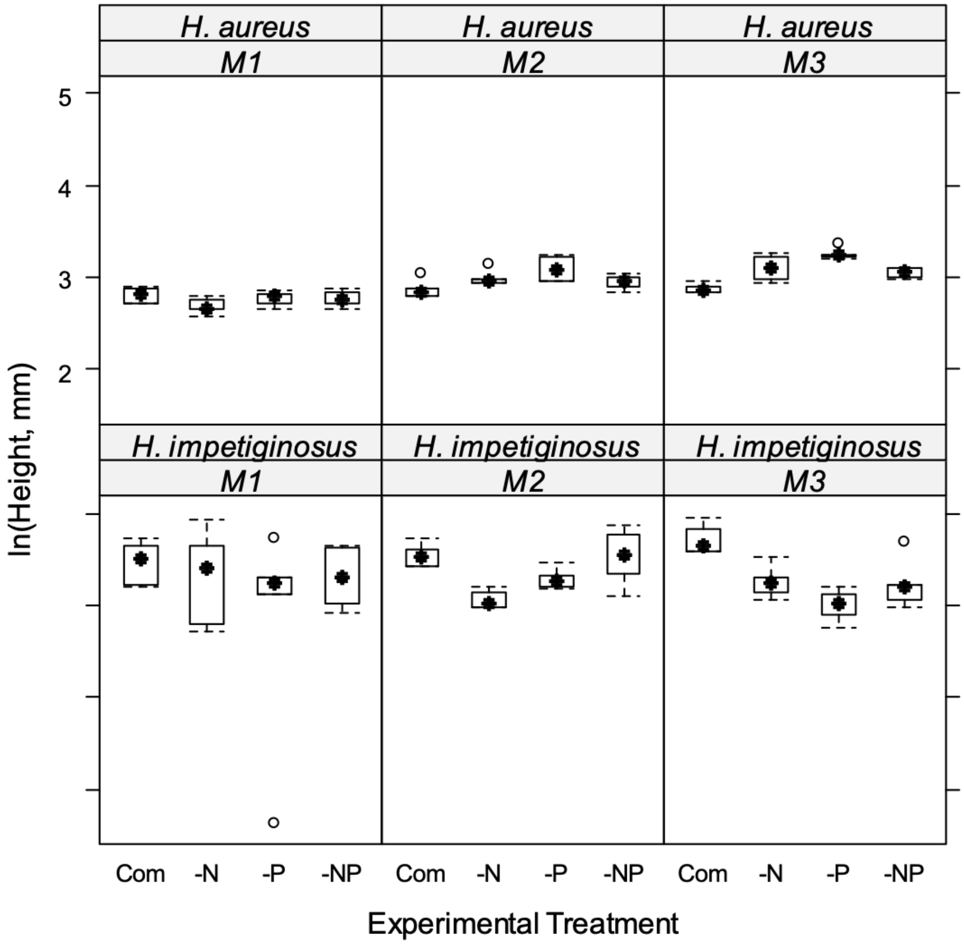


Fig. 6: The figure represents the evolution of heights ln (mm) over time in *H. aureus*, typical savanna species and *H. impetiginosus*, typical from seasonal forest. The x-axis represents the treatments. The complete solution is represented by **Com**; The solution without nitrogen by **-N**; The solution without phosphorus by **-P**; The solution without both nutrients by **-NP**. **M1**, **M2** and **M3** respectively mean: the first, second and third months from the beginning of the experiment.

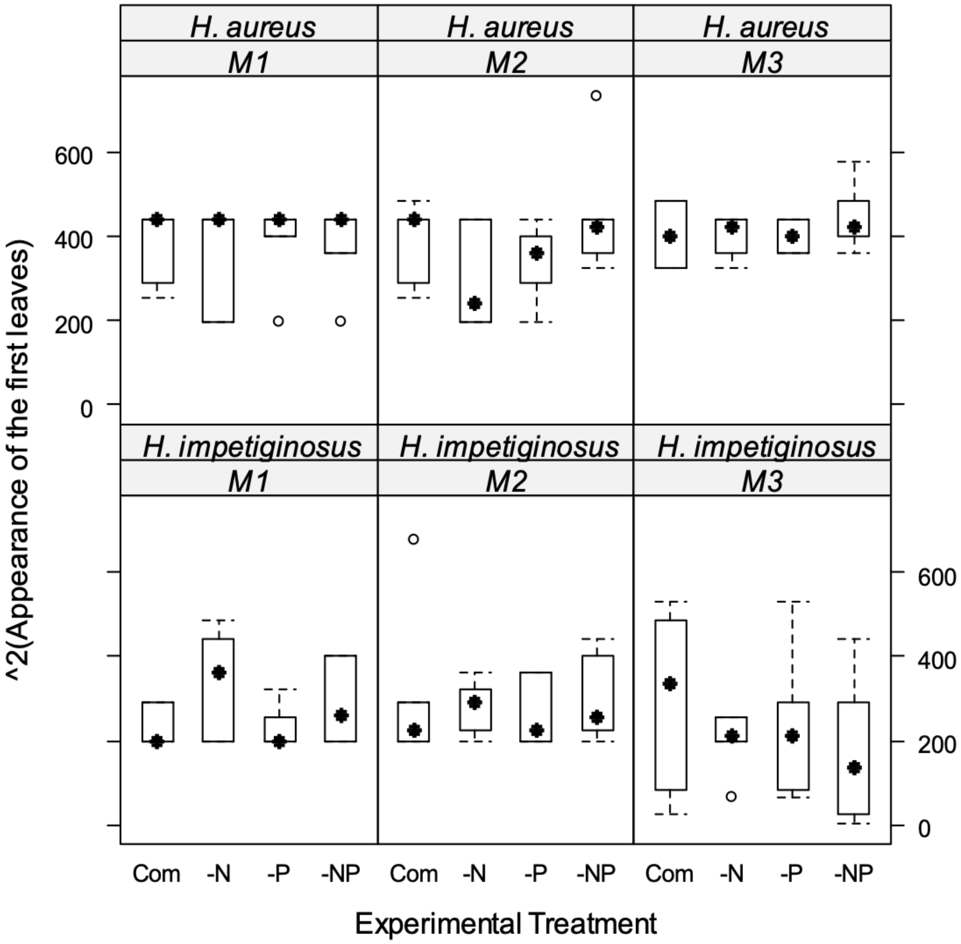


Fig. 7: The figure represents the evolution of appearance of the first leaves squared (^{2}) (days) over time in *H. aureus*, typical savanna species and *H. impetiginosus*, typical from seasonal forest. The x-axis represents the treatments. The complete solution is represented by **Com**; The solution without nitrogen by **-N**; The solution without phosphorus by **-P**; The solution without both nutrients by **-NP**. **M1**, **M2** and **M3** respectively mean: the first, second and third months from the beginning of the experiment.

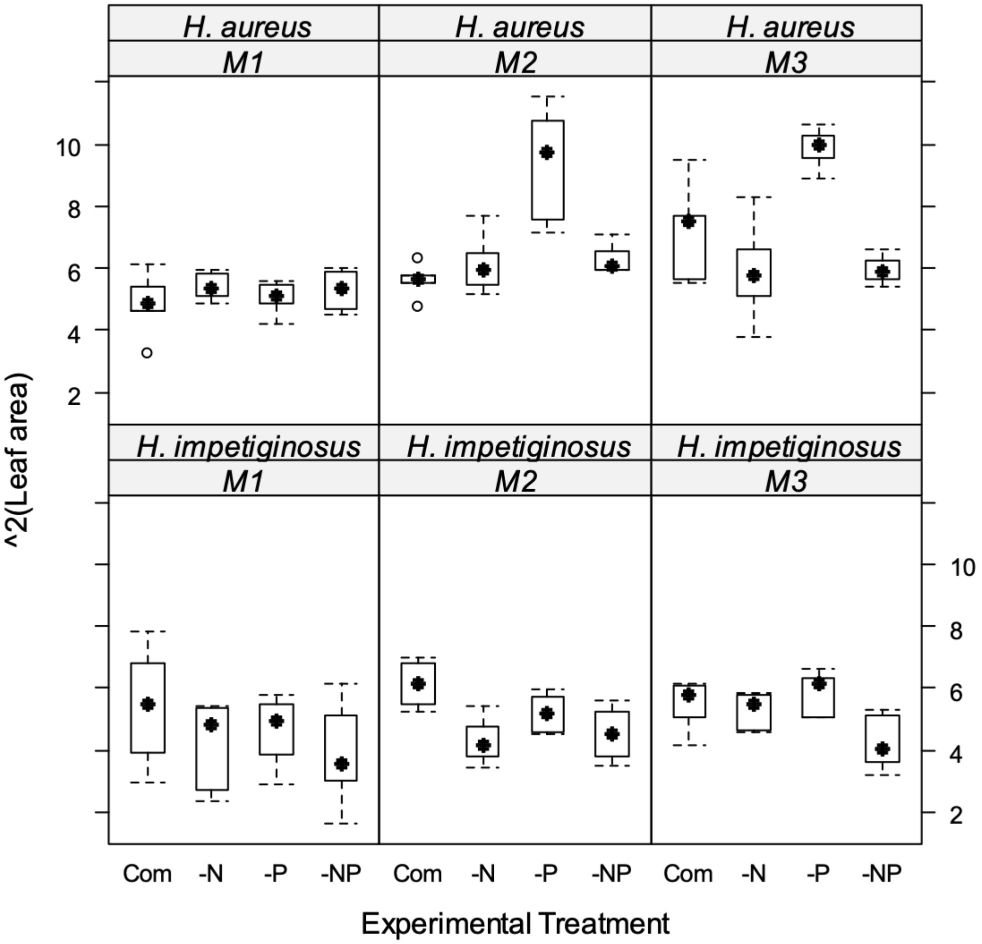


Fig. 8: The figure represents the evolution of leaf areas squared (^{2}) (cm^2) over time in *H. aureus*, typical savanna species and *H. impetiginosus*, typical from seasonal forest. The x-axis represents the treatments. The complete solution is represented by **Com**; The solution without nitrogen by **-N**; The solution without phosphorus by **-P**; The solution without both nutrients by **-NP**. **M1**, **M2** and **M3** respectively mean: the first, second and third months from the beginning of the experiment.

Conclusão geral

Apresentamos um aprimoramento da técnica de germinação de sementes na espécie *S. lycocarpum*. Ao testarmos o uso de argila expandida como substrato germinativo, conseguimos boas taxas de germinação. Uma vez que este substrato é barato, pode ser reutilizável e ainda é eficiente, isto pode contribuir para a prática de restauro no cerrado. Mostramos também que espécies típicas de floresta estacional semidecídua e aquelas características de fisionomias savânicas do cerrado, podem ser funcionalmente distintas já nas fases iniciais de desenvolvimento. Mesmo usando espécies congenéricas — que compartilham boa parte de suas histórias evolutivas —, elas apresentaram diferentes estratégias nutricionais para lidar com a depleção de nitrogênio, fósforo ou ambos macronutrientes. Conforme previamente postulado, a depleção nutricional teve efeitos mais nocivos às espécies florestais, adaptadas a ambientes com maiores disponibilidades nutricionais. Já as espécies do cerrado, mostraram-se adaptadas a ambientes com depleção nutricional, ambas estratégias coerentes com seus ambientes. Em relação ao nitrogênio, ele parece ser o nutriente limitante para espécies de ambos biomas. Quanto ao fósforo, sua oferta trouxe incrementos em traços funcionais de plântulas florestais, mas, pode acarretar em sintomas de toxicidade para plântulas de espécies savânicas. Como tais espécies são típicas de tais fisionomias, acreditamos que suas estratégias nutricionais possam ser extrapoladas para outras espécies que, durante o processo evolutivo, vivenciaram pressões evolutivas semelhantes.