# Pequenas plantas, grandes estratégias: adaptações e sobrevivência no Cerrado

Aelton Biasi Giroldo

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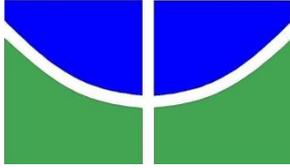
Universidade de Brasília
Instituto de Ciências Biológicas
Departamento de Ecologia
Programa de Pós-Graduação em Ecologia

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Aelton Biasi Giroldo

Orientador Dr. Aldicir Scariot

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Aelton Biasi Giroldo

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Banca Examinadora:

_____

Dr. Aldicir O. Scariot

Presidente / Orientador

Embrapa – Cenargen

_____   _____

Dr. Marcelo F. Simon   Dra. Alessandra T. Fidelis

Membro Titular   Membro Titular

Embrapa - Cenargen   UNESP – Rio Claro

_____   _____

Dr. Fabian Borghetti   Dra. Heloísa S. Miranda

Membro Titular   Membro Titular

UnB   UnB

_____

Dr. Eduardo R. M. Barbosa

Membro Suplente

UnB

Brasília, DF

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Apresentação

A abundância e a diversidade do estrato herbáceo em formações savânicas chamam a atenção de qualquer observador mais atento. Estas formas de vida chegam a representar mais de 60% da diversidade do bioma Cerrado. A questão levantada neste trabalho é o porquê desta dominância. Estudos realizados com pares congenéricos verificaram as características ecofisiológicas que favoreceriam as espécies de savana em detrimento às espécies de floresta em ambientes com fogo, entretanto no que se refere à comparação entre formas de vida no ambiente savânico, pouco foi investigado. Utilizando a abordagem de pares congenéricos, no capítulo 1 deste trabalho, foram selecionados pares de subarbustos e árvores, que ocorrem nas formações savânicas do bioma Cerrado. Foram examinadas as diferenças na biomassa de sementes, germinação, sobrevivência, capacidade de rebrota, investimento em biomassa e na capacidade fotossintética entre os pares congenéricos, e se essas características eram filogeneticamente conservadas.

No capítulo 2, por meio de uma extensa revisão da literatura e checagem de dados de todos os herbários disponíveis no speciesLink, juntamente com os dados do herbário do IBGE, foi criada uma lista de todas as espécies capazes de se reproduzir após a passagem do fogo, tanto em formações savânicas quanto florestais. O interessante desta lista é que ela começa como um teste no speciesLink, e acaba por se tornar um grande trabalho. Utilizando a ajuda de especialistas, juntamente com o esforço de parceiros, todas as espécies foram catalogadas e seus nomes foram checados no site Flora do Brasil. Além disso, as espécies foram classificadas quanto ao habitat - de formações florestais ou savânicas, família botânica, síndromes de polinização, dispersão e forma de vida. Para testar as hipóteses de que as subarbustivas possuem maior capacidade reprodutiva após a passagem do fogo, se comparadas às árvores, foi necessário utilizar dados relativos à todas as espécies do bioma.

No capítulo 3, foi testado o efeito competitivo de gramíneas sobre pares congenéricos de subarbustos e árvores, e dos pares congenéricos entre si. Os resultados deste capítulo trazem à tona a importância da biodiversidade em atenuar as relações competitivas e ao mesmo tempo indica a importância do "root-gap" para o estabelecimento de árvores no bioma Cerrado.

Por fim, os principais resultados dos três capítulos foram consolidados em um resultado geral, no qual tentou-se explicar a dominância dos subarbustos nas savanas; a relação fogo-biodiversidade-evolução, e a importância das políticas de manejo de fogo para a conservação da biodiversidade do bioma Cerrado.

O autor

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Sumário

1 **Pequenas plantas, grandes estratégias: adaptações e sobrevivência no**

2 **Cerrado**

Resumo Geral

4 A diversificação da flora da savana brasileira ocorreu há 10 Ma, e envolveu a seleção de

5 características como casca espessa, desenvolvimento de órgãos subterrâneos, além de

6 adaptações ecofisiológicas (ex. acúmulo de reservas, capacidade de rebrota) e redução das

7 formas de vida. As formas de vida menores, como os subarbustos, representariam o extremo de

8 adaptação a ambientes pirofíticos. Embora a biomassa aérea das formas de vida menores seja

9 mais consumida durante a passagem de fogo do que das formas de vida maiores, como as

10 árvores, elas hipoteticamente possuiriam mais reservas, maior capacidade fotossintética, além

11 de se reproduzirem mais cedo e manterem a capacidade de rebrota por toda a vida. Embora

12 esses argumentos tenham sido levantados como centrais para o sucesso das espécies

13 subarbustivas em relação às arbóreas, nenhum estudo verificou a presença destas diferenças,

14 com exceção das relações de fotossíntese. Em experimentos em casa de vegetação com pares

15 congenéricos de subarbustos e árvores da savana brasileira, foram investigadas as diferenças

16 no peso de sementes, germinação, sobrevivência, alocação de carbono estrutural e de reservas,

17 capacidade de rebrota, capacidade competitiva e capacidade fotossintética. Por meio de revisão

18 de literatura e checagem de dados em herbários foram verificadas as espécies capazes de se

19 reproduzir após a passagem de fogo, verificando se alguma forma de vida é reprodutivamente

20 favorecida após a passagem de fogo, além de verificar se alguma síndrome de dispersão e

21 polinização se destaca em espécies que se reproduzem após o fogo.

22 Contrariando a hipótese levantada de que subarbustos representam o extremo evolutivo de

23 adaptação ao bioma savânico, muitas das características verificadas se mostraram semelhantes

24 entre árvores e subarbustos. Entretanto, as pequenas diferenças observadas poderiam se

25 acumular ao longo da vida, culminando em histórias de vida bastante distintas quando no estágio

26 adulto. Os subarbustos, de fato, investem menos em desenvolvimento aéreo e mais em

27 armazenamento de energia na forma de reservas, além de possuírem um peso de sementes

28 menor que as espécies arbóreas. Um total de 2058 espécies são capazes de se reproduzir após

29 a passagem de fogo e, refutando a hipótese de supremacia reprodutiva dos subarbustos, foi

30 encontrada que a razão de chance de uma árvore florescer após a passagem do fogo é maior

31 que a de um subarbusto. Isso não significa que após o fogo um observador verá mais árvores

32 florescendo, o que só aconteceria se a abundância das formas de vida fosse semelhante. O fogo

33 não favorece nenhuma síndrome de polinização específica, mas favorece a dispersão zoocória

34 em detrimento da autocórica. Quanto à capacidade competitiva, tanto subarbustos quanto

35 árvores são prejudicados pela presença de gramíneas. Entretanto, a presença de árvores atenua

36 as relações competitivas das gramíneas para os subarbustos, favorecendo-os. O oposto,

37 entretanto, não é verdadeiro, e as árvores são, de forma sinérgica, negativamente afetadas pela

38 presença de subarbustos e gramíneas. Em relação à competição par-a-par, as árvores exercem

39 efeito negativo no crescimento e sobrevivências das plantas subarbustivas, mas o contrário não
40 é verdadeiro, e subarbustos não afetam o crescimento e sobrevivências das árvores.

41 A longa história evolutiva das plantas do Cerrado na presença do fogo selecionou diferentes
42 formas de vida, igualmente aptas a sobreviverem e reproduzirem neste ambiente. O grande
43 número de espécies capazes de se reproduzir após o fogo, distribuídas em diferentes famílias,
44 corrobora a ideia de que a aquisição de caracteres adaptativos para o fogo envolve simples
45 rearranjo de material genético e raramente mutações, o que norteia parte da hipótese de que o
46 fogo pode moldar as características funcionais de um ecossistema. A dominância dos
47 subarbustos (abundância e diversidade) pode ser explicada principalmente pela grande
48 quantidade de energia armazenada, o que favoreceria a sua sobrevivência em períodos
49 desfavoráveis e em locais em que o fogo é um evento frequente. Além disso, como possuem
50 estatura menor, atingiriam a fase adulta mais rapidamente (*Mimosa foliolosa* floriu 1 ano e meio
51 após o plantio em casa de vegetação) e produziriam sementes menores, que são facilmente
52 enterradas, reduzindo a predação e exposição à altas temperaturas causadas pelo fogo, o que
53 asseguraria o sucesso no ambiente savânico. As árvores, por outro lado, possuem como maior
54 limitante ao recrutamento para o estágio adulto, a necessidade de escapar do *fire-trap*, evitando
55 a mortalidade da parte aérea e alcançando o estágio reprodutivo. Desta forma, a redução da
56 frequência de fogo nas savanas aumenta o recrutamento de árvores para o estágio adulto,
57 favorecendo a formação de fisionomias florestais. As formações florestais reduzem a camada de
58 gramíneas, e juntamente com ela a de subarbustos, que são competidores inferiores se
59 comparadas às formas arbóreas. Portanto, para assegurar a manutenção da biodiversidade nos
60 ecossistemas savânicos é necessário que políticas de manejo controlado de fogo sejam
61 empregadas em todo o bioma, principalmente em unidades de conservação.

62

Introdução Geral

A savana tropical brasileira, Cerrado, é caracterizada por um estrato rasteiro dominante, com presença de gramíneas, subarbustos e ervas, e um estrato arbóreo descontínuo, relativamente baixo (Gottsberger & Silberbauer-Gottsberger 2006; Ratnam *et al.* 2011). O bioma Cerrado é a savana mais biodiversa do mundo, com mais de 12.000 espécies de angiospermas, 4.252 delas endêmicas (BFG 2015), e, diferentemente das outras savanas, não possui uma família dominante, sendo que poucas espécies (38 espécies) estão distribuídas em toda a extensão do bioma (Ratter *et al.* 2003). O bioma possui síndromes globais de regime de fogo (piromas), caracterizadas por eventos frequentes (1 – 4 anos de intervalo de retorno de fogo), com o fogo podendo ter intensidade e altura de chama variável (ver Archibald *et al.* 2013). O regime de fogo pode ser definido como as características de intensidade[1], frequência[2], severidade[3], sazonalidade, tamanho e extensão do fogo (Bond & Keeley 2005), e englobar os processos mais antigos como as mudanças no clima e integrar também as influências humanas (Whitlock *et al.* 2010).

Os eventos de fogo provavelmente se tornaram mais frequentes no final do Mioceno, coincidindo com a expansão global das gramíneas C_4 (Pennington *et al.* 2006) e com a diversificação da flora do Cerrado (Simon *et al.* 2009). Desta forma, a flora do bioma evoluiu em um ambiente muito inflamável, levando à seleção de caracteres adaptativos ao regime de fogo. A maioria das espécies selecionadas a este ambiente possui casca espessa, órgãos de reserva e capacidade de rebrota (Hoffmann 2002; Gottsberger & Silberbauer-Gottsberger 2006; Miranda *et al.* 2009). De forma geral, a dinâmica da vegetação do Cerrado é governada pelo regime de fogo, e não pela mortalidade (Hoffmann *et al.* 2009; Hoffmann *et al.* 2012a), sendo influenciada pelo clima regional e pelas características alométricas específicas das espécies lenhosas, de forma que a recuperação após a passagem de fogo depende das características de crescimento das mesmas (Lehmann *et al.* 2014).

Intervalos curtos entre queimadas (alta frequência) e alta intensidade de fogo geram vegetações mais abertas, com mais herbáceas, e um ambiente mais inflamável; e, inversamente, ambientes com baixa frequência de fogo permitem que os indivíduos da comunidade adquiram tamanho suficiente para resistir às chamas, de forma que o ambiente se torna mais fechado e com menos biomassa combustível (Hoffmann *et al.* 2012a; Just *et al.* 2015). A menor quantidade de biomassa combustível, referente à baixa abundância de herbáceas, juntamente com a alta umidade e menor incidência de ventos garantem que as fisionomias florestais sejam menos susceptíveis às queimadas (Hoffmann *et al.* 2012b).

Nas matas de galeria, por exemplo, a maioria das espécies não teve uma história evolutiva influenciada pelo regime de fogo e, portanto, não desenvolveram caracteres de

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[1] A intensidade de fogo se refere a energia liberada pelo fogo na sua passagem, e na prática, pode ser definida como a altura das chamas e a taxa de propagação. [2] A frequência de fogo é a ocorrência de fogo em um determinado período de tempo em uma área. [3] A severidade do fogo seria uma medida de impacto no ecossistema, como por exemplo a mortalidade de árvores em florestas ou o consumo de biomassa (Bond & Keeley 2005; Keeley 2009).

98 resistência e tolerância ao mesmo. Desta forma, é bem elucidado na literatura que,
99 comparativamente às espécies de cerrado, as espécies de mata possuem uma menor razão
100 raiz:parte aérea (82% menor), maior investimento em copa e menor investimento em casca e em
101 reservas de raízes, o que se reverte em uma menor capacidade de rebrota e sobrevivência a
102 eventos de fogo (Hoffmann 2000; Hoffmann *et al.* 2003; Hoffmann & Solbrig 2003; Hoffmann *et*
103 *al.* 2004; Hoffmann *et al.* 2005).

104   Dentro do próprio cerrado, as espécies podem responder de forma distinta ao regime de
105 fogo. A literatura sugere que as formas de vida menores (subarbustos e ervas) seriam
106 favorecidas em detrimento das formas de vida maiores (árvores) em áreas com alta frequência
107 e intensidade de fogo (Gottsberger & Silberbauer-Gottsberger 2006; Simon & Pennington 2012).
108 Isso ocorreria em decorrência de sincronismo de floração em relação ao fogo e uma melhor
109 exploração dos nutrientes deixados pelo consumo da biomassa nas camadas superficiais do solo
110 (cinzas), gerando transferência indireta de energia das espécies arbóreas e arbustivas para os
111 subarbustos e gramíneas. Ademais, as formas de vida menores teriam maior capacidade de
112 rebrota e menor perda de produtividade (flor e frutos) (Coutinho 1982; Gottsberger & Silberbauer-
113 Gottsberger 2006; Miranda *et al.* 2009). Além disso, para atingirem o estágio reprodutivo, as
114 espécies arbóreas precisam de maiores intervalos sem fogo do que as subarbustivas e
115 herbáceas (Hoffmann & Solbrig 2003) e analogamente, as formas menores precisam de menos
116 nutrientes para alcançarem o estágio adulto (Bond 2010).

117   Embora o debate sobre favorecimento de uma forma de vida em detrimento doutra em
118 eventos de fogo exista, trabalhos com enfoque nas diferenças de caracteres adaptativos ao fogo
119 são escasssos. Entender quais características da história de vida de uma espécie garantem
120 sucesso em um ambiente é importante para inferir como os ecossistemas naturais serão afetados
121 frente às perturbações antrópicas e mudanças climáticas. Desta forma, este trabalho tem como
122 objetivo principal verificar as diferenças e semelhanças de caracteres adaptativos ao fogo em
123 espécies arbóreas e subarbustivas do Cerrado. Para tanto, o trabalho será organizado em três
124 capítulos: O primeiro com enfoque nas características fotossintéticas, alocação e uso de
125 reservas, sobrevivência e capacidade de rebrota; o segundo com foco na capacidade reprodutiva
126 entre as formas de vida, seleção de síndromes de polinização e dispersão; e o terceiro nas
127 relações de competição interespecífica entre árvores e subarbustos e destes com a gramínea
128 nativa do Cerrado *Paspalum atratum*.

129

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194 **Small plants, great strategies: adaptations and survival of trees and subshrubs in**
195 **a fire-prone ecosystem**
196

197 Giroldo, A.B.[1,2], Scariot, A.[2], Hoffmann, W. A.[3]

198 1. Departamento de Ecologia, Universidade de Brasília, Caixa Postal 04457, 70919-970 Brasília, DF,
199 Brazil; Corresponding author: aeltonbg@gmail.com; 2. Embrapa Recursos Genéticos e Biotecnologia, Laboratório
200 de Ecologia e Conservação, Caixa Postal 02372, 70770-900 Brasília, DF, Brazil; 3. Department of Plant and
201 Microbial Biology, North Carolina State University, 2115 Gardner Hall, Box 7612, Raleigh, NC 27695, USA

202

203 **Abstract:**

204 Over the past 10 million years, tropical savanna environments have selected for small growth forms within
205 woody plant lineages. The result has been the evolution of subshrubs (geoxyles) within many lineages of
206 predominantly tree species, presumably as an adaptation to frequent fire. The main objective of this study
207 was to evaluate the traits that favor subshrubs over trees in fire-prone ecosystems. We compared seed
208 biomass, germination, survival, resprout capacity, biomass allocation, and photosynthesis between
209 congeneric trees and subshrubs, and quantified the strength of phylogenetic conservatism. Despite the
210 large differences in adult morphology between trees and subshrub species, as seedlings the differences
211 are modest, and most of the variation in seedling traits was explained by phylogeny. Regardless, seedlings
212 of tree species invested more heavily in aboveground growth, compared to subshrubs, which is consistent
213 with the adult strategy of trees which depends on a large resistant-fire stem. Subshrub seedlings also invest
214 in greater non-structural carbohydrate reserves, likely as an adaptation to the high fire frequencies typical
215 of Neotropical savannas. These modest differences as seedlings suggest that selective pressures during
216 early development may not have contributed substantially to the evolution of the subshrub growth form.
217 Instead the distinct allocation and life history appear to arise later, and as adults, subshrubs differ from
218 trees by reaching maturity at a small stem size, allowing them to reproduce despite repeated fire-induced
219 topkill, but they produce smaller seeds than trees. The convergent evolution of subshrubs within multiple
220 tree lineages reaffirms the importance of fire in the origin and diversification of the flora of mesic savannas.
221 Furthermore, this assembled guild of subshrubs retains a strong phylogenetic signal of these diverse
222 lineages, resulting in a Cerrado subshrub flora that is functionally quite diverse.

223 *Key words:* subshrub vs tree; Brazilian Savanna; savanna evolution; fire adaptations; traits phylogenetically
224 conserved

225

Introduction:

Evidence suggests that diversification of the tropical savanna woody flora began in the late Miocene, following the expansion of C4 grasses and accompanying an increase in fire frequency (Simon et al. 2009, Maurin et al. 2014). This flora was assembled from multiple lineages, many of which originated as trees from evergreen or deciduous forests. Many of these lineages retained a tree growth form, but underwent natural selection for multiple traits, including thick bark, increased investment in belowground biomass and reserves, thick leaves, and reduced adult height. A more extreme example of this reduction in adult height is observed in subshrubs (geoxyles), which have diminutive aerial stems and large investment in belowground organs (see Gottsberger and Silberbauer-Gottsberger 2006, Simon and Pennington 2012). As adults, the belowground allocation of subshrubs is often so great these subshrubs are called underground trees (Warming 1908, White 1976, Simon and Pennington 2012, Bond 2016).

Trees and subshrubs represent starkly different outcomes of natural selection within the savanna environment. Although morphological differences between these growth forms are substantial as adults, it is not clear how these differences extend to physiological traits and seedling ecology, and consequently we lack a complete picture of the shift in life-history strategies that has accompanied the evolution of the subshrub habit. One possibility is that subshrubs represent an extreme endpoint in a continuum of woody plant adaptation to the savanna environment, with savanna trees being intermediate between forest trees and savanna subshrubs. Under this hypothesis, savanna subshrubs should exhibit more extreme values of a suite of traits shown to be typical of savanna trees, with seedlings exhibiting, for example, substantially lower values of specific leaf area (SLA), higher root-shoot ratio, and higher allocation to carbohydrate reserves, compared to trees of the same environment.

An alternative scenario is that savanna trees and subshrubs represent distinct life-history strategies that differ primarily during later development, as distinct strategies for ensuring reproduction under frequent burning. That is, adult savanna trees are able to maintain reproductive size in spite of fire because their large, fire-resistant stems are largely immune to fire (Hoffmann et al. 2009, Dantas and Pausas 2013). In contrast, the aerial biomass of subshrubs is totally consumed by fire, but they can resprout vigorously and reach reproductive size quickly after fire (Zizka et al. 2014). These differing strategies could be manifested largely as differences in size at maturity and investment in aboveground biomass of established plants, and might not involve differences in seedling traits or in leaf physiology. In fact, seedlings of trees and subshrubs are exposed to similar stresses and disturbances, and their stems should be equally vulnerable to fire, imposing similar needs for resprout capacity.

To better understand the suite of plant adaptations associated with the subshrub growth form, we compared congeneric trees and subshrubs of the Brazilian Cerrado to test for convergence in seedling and leaf traits in subshrubs across multiple lineages. Furthermore, we examined the strength of phylogenetic conservatism across lineages because of its potential to shape the functional diversity of subshrub communities. That is, if phylogeny has a strong influence on species traits, as is commonly documented

7

262 (Verdú and Pausas 2007, Pausas and Verdú 2008, Souza-Neto et al. 2016), then the functional diversity
263 of subshrubs should closely mirror the underlying diversity present in their ancestral tree species.
264 Considering that subshrubs arose independently from multiple and diverse lineages of tropical trees, trait
265 conservatism should ensure high functional diversity across subshrubs species. Furthermore, this large
266 diversity may obscure differences between trees and subshrubs, making it important to account for
267 phylogenetic effects (see Hoffmann and Franco 2008).

268

Materials and Methods:

270   -   *Species selection and growth form classification*

271 We selected 16 pairs of species, each containing one subshrub and one tree species from the same genus
272 and present in Brazilian Savanna. The use of these congeneric pairs allows us to confirm that similarities
273 found within the growth forms can be interpreted as convergent evolution, which is strong evidence of
274 natural selection, and not because of shared ancestral traits (Hoffmann and Franco 2008, de Bello et al.
275 2015). These genera represent 12 different families, which are well distributed across the angiosperm
276 phylogeny (Figure 1). We classified species as trees if they commonly possess a single, well-developed
277 and persistent stem of over 2 m tall. Subshrubs had an herbaceous-like morphology, with poorly developed
278 stems, commonly without apical dominance and usually smaller than 1 m.

279

280   -   *Seed collection and sowing*

281 We were able to collect seeds of 11 of the species pairs. Most were collected in the vicinity of Brasília, DF,
282 Brazil, but seeds of *Copaifera oblongifolia* were collected in Chapada Gaúcha, MG, *Anacardium humile*
283 was collected in Grão Mogol, MG and *Mimosa foliolosa* was collected in Serra da Canastra, São Roque de
284 Minas, MG. We collected seeds between November, 2013 and July, 2014. We weighed 50 fresh seeds of
285 each species using a precision scale (0.0001 g) to obtain mean seed mass. We sowed the species in
286 polyethylene sacks containing approximately 12.5 l of soil (20 cm diameter x 40 cm deep), with three seeds
287 per sack, and 60 sacks per species. Most were sown in February 2014, but *Mimosa heringeri* and *M.*
288 *speciosissima* were sown in April, 2014 and the *Stryphnodendron* spp. were sown in July, 2014. *Mimosa*
289 and *Stryphnodendron* seeds were immersed in sulfuric acid for 5 minutes to break physical dormancy. We
290 used a substrate 70% oxisol subsoil (40 cm deep or more) mixed with 30% of washed sand, without added
291 nutrients. The experiment was conducted in a greenhouse, with an automatic irrigation system supplying ~
292 7 mm/day.
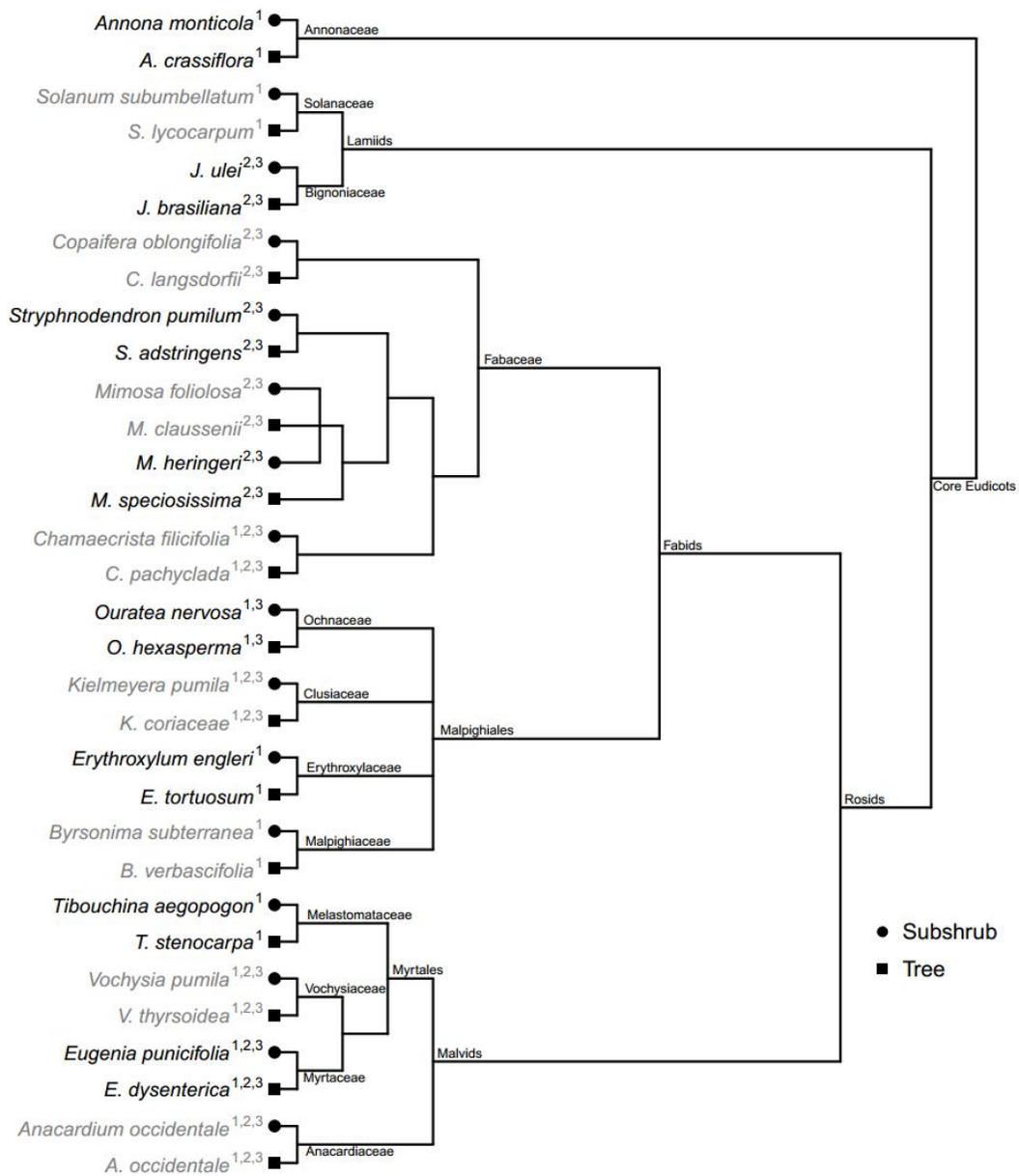
Figure 1. Phylogenetic tree for congeneric pairs of subshrubs and trees that occur in Brazilian Savanna. Phylogenetic relation was based on the current Phylomatic tree (tree R20120829 – Stevens (2001 onwards)). We improved the *Mimosa* clade using a more recent phylogeny (Simon et al. 2011). Alternating colors indicate congeneric pairs. Superscripts indicate data the data that were collected: [1] photosynthesis, respiration, and SLA of adult plants were measured; [2] germination and seedling traits were measured; [3] seed mass was measured.

299   -   *Germination, survival, resprouting, biomass and reserves analysis*

300 We monitored germination and survival at monthly intervals. At 10 months after sowing, we randomly placed
301 the plants into three groups. One group was kept as control, the second group was clipped at soil level to
302 assess resprout capacity, and the third group was harvested to quantify biomass and reserves. For clipped
303 plants, the number of individuals that resprouted in each sack was assessed monthly and resprout height
304 was measured after six months. Harvested plants were washed in a sieve (2 mm) to remove all soil and
305 were then divided into shoot and roots. Roots were submerged in liquid nitrogen to stop metabolic activity
306 and were then lyophilized and stored with silica gel until analyzed for root carbohydrate reserves. Shoots
307 were dried in a forced air chamber at 70°C for 72 h, separated into leaves and stems (including petiole for
308 species with compound leaves), and weighed on a precision scale (0.0001 g).

309   To analyze the root reserves we randomly selected six of the harvested individuals of each species,
310 except *Kielmeyera pumilum*, for which only five individuals were selected. When there was more than one
311 surviving individual per sack, we analyzed the largest. Samples were ground, and soluble sugar and starch
312 were extracted and measured using the protocol of Amaral et al. (2007).

313

314   -   *Photosynthesis, respiration and specific leaf area*

315 We located six adult individuals of each species in natural areas of Brasília, and using a portable
316 photosynthesis system LCpro-SD (BioScientific Ltd.) we measured maximum photosynthesis rate and
317 respiration on a leaf area basis. Measurements were performed on a fully expanded, healthy leaf, which
318 was exposed to direct sunlight during part of the day. We performed measurements on one leaf per
319 individual and recorded five measurements of photosynthesis per leaf at 1 min intervals after the exchange
320 rate stabilized. Measurements were taken between 8:30 am and 12:30 pm with a photosynthetic photon
321 flux density (PPFD) of 1,600 µmol $m^{-2}s^{-1}$, chamber temperature at 30°C and open-flow mode (CO_2
322 concentration mean ± sd was 373 ± 5 µmol mol^{-1}). We then measured the dark respiration of the same leaf
323 by turning off the LED (PPFD = 0) and covering the leaf chamber with aluminium foil. Specific leaf area
324 (SLA) was measured in six individuals per species.

325

326   -   *Statistical analysis*

327 We used mixed-effect models with interactions to verify differences between growth forms on
328 ecophysiological traits: seed biomass, plant germination, survival, root reserves, photosynthesis rate,
329 respiration rate, SLA, and resprout capacity. The models were built using growth form as a fixed factor, and
330 genus as a random factor. The Gaussian distribution was used in most of the variables, exception was the
331 resprout capacity that we used a Binomial distribution. When necessary, the response variable was log
332 transformed to ensure the normality of residuals. To verify if traits were phylogenetically conserved we

333 calculated the fraction of the total variance that was explained by growth form (fixed effect) and by genus
334 (random effect) using the conditional R^2_{GLMM}, which describes the variance explained by fixed and random
335 factors as a proportion of the sum of all the variance components (see Nakagawa and Schielzeth 2013,
336 Johnson 2014).

337   While the previous analysis provides a test of phylogenetic signal across genera, we also tested
338 for trait conservatism over the entire phylogeny using the Blomberg's *K* and Pagel's *λ* (Pagel 1999,
339 Freckleton et al. 2002, Blomberg et al. 2003). Blomberg's *K* is defined as the ratio between mean squared
340 error (MSE) of the tip data divided by the MSE of data calculated using the variance-covariance matrix
341 derived from the phylogenetic tree (see Blomberg et al. 2003 for details) and quantifies the degree of
342 variation in a trait that can be explained by the phylogeny. If Blomberg's *K* < 1, this indicates overdispersion,
343 and traits have less phylogenetic signal than expected from Brownian motion (BM) model. If *K* > 1, there is
344 more phylogenetic signal than expected from BM model (Crisp and Cook 2012). The Pagel's *λ* compares
345 the distribution of a trait to that expected by BM. Low *λ* values indicates little phylogenetic signal in a trait
346 given, and high *λ* values indicates a strong phylogenetic signal (Münkemüller et al. 2012, Swenson 2014).

347   To realize the Blomberg's K and Pagel's λ tests, we first constructed a phylogenetic tree with all 32
348 species. The current Phylomatic tree (R20120829) was used to estimate phylogenetic distances among
349 taxa. The tree resolution was improved using data from Hedges and Kumar (2009). We dated the nodes
350 using the "branch length adjustment" algorithm in Phylocom (Webb et al. 2008), and we obtained the age
351 for major nodes in the tree from Hedges and Kumar (2009).

352   To verify the relationship between seed biomass and plant reserves or plant biomass in different
353 growth forms we used Analysis of Covariance (ANCOVA), in this case the seed biomass was used as
354 covariate, growth form as variable independent and plant reserves or plant biomass as dependent variable.
355 The same approach was used to verify the relationship between SLA and photosynthesis or respiration
356 rate, and in this case, SLA was included as a covariate. All analyses were conducted in *R program* (R
357 Development Core Team 2015), with the packages *lme4* (Bates et al. 2015), *car* (Fox and Weisberg 2011),
358 brranching (Chamberlain 2016), phytools (Revell 2012), rncl (Michonneau et al. 2015), and ape (Paradis et
359 al. 2004).

360

361 **Results:**

362   - *Seed and Plant Biomass*

363 On average, seeds of trees were 36% heavier than seeds of subshrubs ($F_{1,10}$ = 6.015, p = 0.034, Figure
364 2A). At an age of 10 months, total seedling biomass was 72% higher in trees than in subshrubs ($F_{1,9}$ =
365 5.242, p = 0.048, Figure 2D). Similarly, trees had greater biomass of stems ($F_{1,9}$ = 8.968, p = 0.015),
366 leaves ($F_{1,9}$ = 8.921, p = 0.038), and shoots ($F_{1,9}$ = 7.031, p = 0.026), but not of roots ($F_{1,9}$ = 4.071, p =

367   0.074). When compared in context of biomass allocation, the only significant difference between tree and

368   subshrub was stem mass ratio ($F_{1,9}$ = 32.788, $P < 0.001$, Figure 2G), which was 31% higher in trees than

369   in subshrubs. Root mass ratio ($F_{1,9}$ = 0.426, p = 0.531), leaf mass ratio ($F_{1,9}$ = 0.093, p = 0.767) and

370   root:shoot ratio ($F_{1,9}$ = 0.476, p = 0.508) were not significantly different between growth forms. Seed

371   biomass did not affect total plant biomass ($F_{1,16}$ = 2.001, p = 0.176), and marginally affected the root

372   biomass ($F_{1,16}$ = 4.040, p = 0.062).

373

374   - *Germination and Survival*

375   Germination success did not differ significantly between subshrubs and trees ($F_{1,9}$ = 3.171, p = 0.109); with

376   means of 73 ± 17 % (mean ± sd) and 62 ± 27%, respectively (Figure 2B). Survival after 10 months was

377   similar between forms ($F_{1,9}$ = 0.656, p = 0.439), with survival of 81 ± 18% for trees and 76 ± 22% for

378   subshrubs (Figure 2C). Resprout capacity did not consistently differ between life forms (X^2 = 0.023, p =

379   0.878, Figure 2J), with mean resprouting of 57.5% for subshrubs and 53.0% for trees.

380

381   - *Carbohydrate reserves*

382   Root carbohydrate mass did not differ between the growth forms ($F_{1,9}$ = 1.944, p = 0.197, Figure 2E), but

383   nonstructural carbohydrate:structural biomass ratio of roots was 37% higher in subshrubs than trees ($F_{1,9}$

384   = 5.830, p = 0.039, Figure 2I). Root carbohydrate mass:total plant mass ratio was marginally significantly

385   ($F_{1,9}$ = 3.479, p = 0.095, Figure 2H). There was a relationship between root carbohydrate mass and seed

386   biomass ($F_{1,16}$ = 13.457, p = 0.002), and root carbohydrate concentration was marginally correlated with

387   seed biomass ($F_{1,16}$ = 3.209, p = 0.092).

388

389   - *Photosynthesis, Respiration and Specific Leaf Area (SLA)*

390   There was no difference between growth forms in light-saturated photosynthesis ($F_{1,10}$ = 0.053, p = 0.823)

391   dark respiration rate ($F_{1,10}$ = 0.001, p = 0.968) or SLA ($F_{1,10}$ = 0.077, p = 0.787), and no correlation was

392   detected between rates of photosynthesis ($F_{1,18}$ = 0.519, p = 0.481) or respiration ($F_{1,18}$ = 0.040, p = 0.843)

393   with SLA.

394

395   - *Phylogenetic conservatism*

396   Overall, phylogeny explained much more of trait variation than did growth form. That is, genus explained

397   more than 67% of the total variance in most traits. In contrast, the maximum variance explained by growth

398   form was 6% for the traits total biomass and germination rate (Table 1). Even so, not all traits had a

399 significant phylogenetic signal when the full phylogeny was considered. While seed mass, stem mass ratio,

400 root:shoot ratio and root carbohydrate biomass were phylogenetically conserved, the others 12 analyzed

401 traits were not (Table 1).

402 Table 1. Analyses of phylogenetic conservationism for ecophysiological traits in two differents growth forms

403 of Brazilian Savanna. The R^2_{GLMM} was calculated using the Johnson (2014) and Nakagawa and Schielzeth

404 (2013) approach and represent total variance explained by genus and growth form in linear mixed models.

405 Variables with asterisks were log transformed. The K represent the values in Blomberg's K, λ the value of

406 Pagel's λ test, P_1 and P_2 the p value in Blomberg's K test and Pagel's λ, respectively.

Trait	$R^2_{GLMM\,Genus}$	$R^2_{GLMM\,Growth\,form}$	K	p_1	λ	p_2
Seed mass*	0.94	0.02	0.97	0.004	1.01	<0.001
Germination rate	0.76	0.06	0.25	0.338	<0.01	>0.999
Survival rate	0.76	0.01	0.51	0.047	<0.01	>0.999
Total plant mass*	0.64	0.06	0.59	0.036	0.91	0.089
Root mass ratio	0.69	<0.01	0.64	0.012	0.84	0.101
Stem mass ratio	0.68	0.05	0.79	0.003	0.97	0.012
Leaf mass ratio	0.78	<0.01	0.80	0.005	0.85	0.070
Root : Shoot ratio*	0.70	<0.01	0.74	0.010	0.96	0.020
Resprout rate*	0.57	<0.01	0.61	0.009	0.820	0.167
Photosynthesis rate	0.42	0.02	0.16	0.543	<0.01	>0.999
Dark respiration rate	0.42	<0.01	0.34	0.263	0.37	0.370
Specific leaf area	0.68	<0.01	0.12	0.856	<0.01	>0.999
Nonstructural carbohydrate : structural biomass*	0.68	0.04	0.68	0.080	0.72	0.202
Root carbohydrate mass*	0.66	0.03	0.67	0.136	0.83	0.022
Root carbohydrate concentration*	0.68	0.04	0.42	0.146	0.71	0.269
Root carbohydrate mass : Total plant mass*	0.75	0.02	0.34	0.246	0.79	0.251

407

Discussion:

409 Although the differences between trees and subshrubs are large and evident as adults (Poorter et al. 2012,

410 Díaz et al. 2016), we found surprisingly few consistent differences in seedling characteristics (Figure 2).

411 Instead, interspecific variation in seedling traits was consistently found to be most strongly determined by

412 genus (Table 1) than by growth form. Similar patterns were observed for leaf traits of adult plants.

413 Of all the seedling traits studied, investment in above-ground biomass and carbohydrate reserves

414 were the only ones to hint at the remarkable divergence between trees and subshrubs that develops later

415 in life. As seedlings, trees consistently invested a modestly larger fraction of their biomass in stems (13%

416 versus 9%, Figure 2G) while subshrubs have greater investment in carbohydrate reserves (39% versus

417 29% of root structural mass, Figure 2I). These differences, particularly in stem biomass, obviously must
418 accrue further as plants approach maturity, when subshrubs and trees exhibit starkly different strategies.
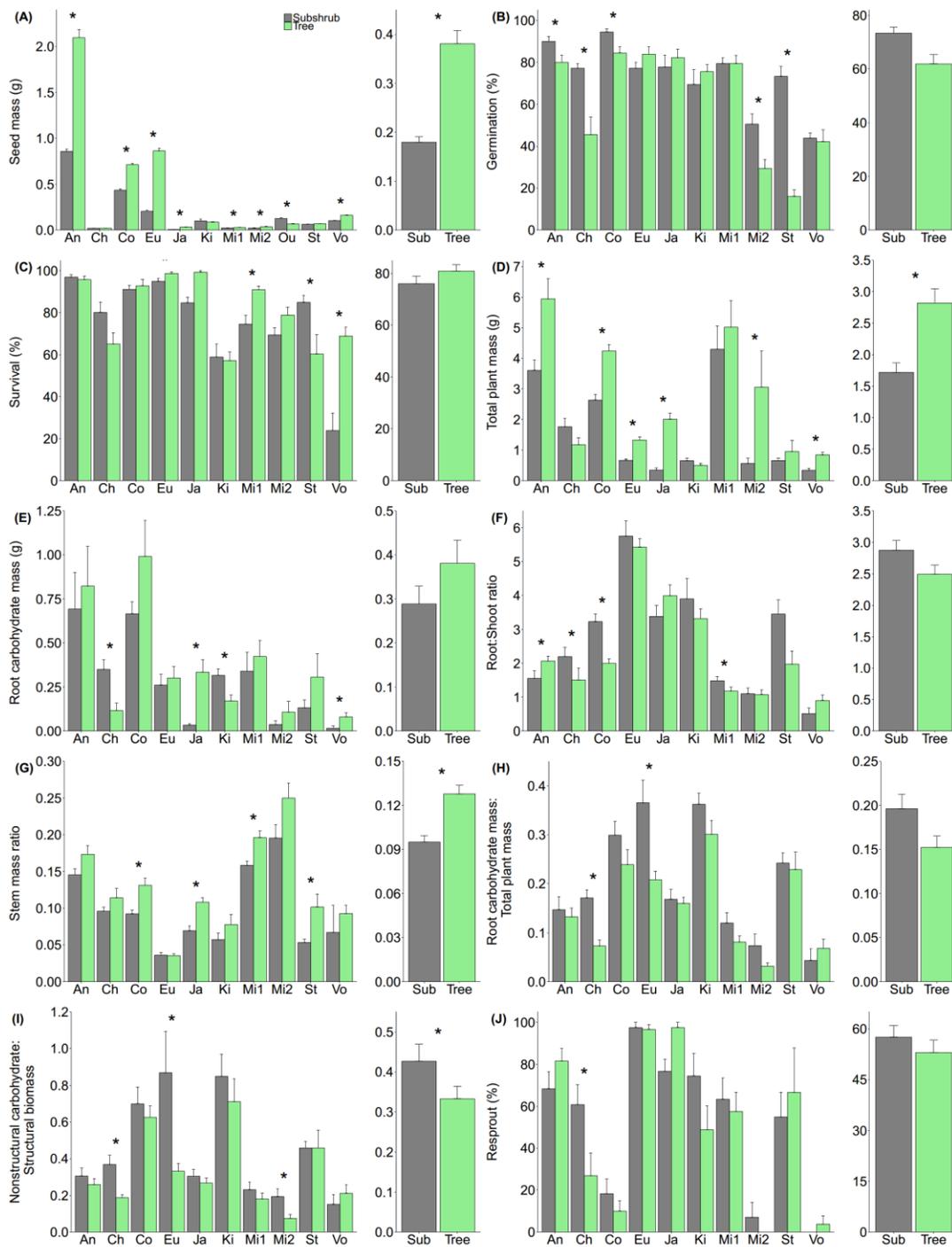
419

420 Figure 2. Seed and seedling traits (mean±SE) of congeneric pairs of subshrubs and trees in Brazilian Savanna. An
421 asterisk indicates a significant difference (p < 0.05). The differences between congeneric pairs was calculated using t
422 test. An = *Anacardium*, Ch = *Chamaecrista*, Co = *Copaifera*, Eu = *Eugenia*, Ja = *Jacaranda*, Ki = *Kielmeyera*, Ou =
423 *Ouratea*, Mi1 and Mi2 = *Mimosa*, St = *Stryphnodendron*, Vo = *Vochysia*, Sub = Subshrub.

14

424   Savanna trees exhibit an escape strategy (Zizka et al. 2014) that consist of early investment in height
425   growth or early bark growth (Dantas and Pausas 2013), allowing them to become fire resistant. If stem
426   growth is not sufficiently rapid, juvenile trees can be maintained indefinitely in a suppressed juvenile state
427   due to repeated topkill by fire, thereby precluding sexual reproduction. Subshrubs, in contrast, are able to
428   reproduce sexually despite repeated loss of aboveground biomass.

429   The modest differences in seedling allocation between savanna trees and subshrubs suggests that
430   natural selection during establishment and early development has not been the primary factor driving the
431   divergence of these growth forms. At this stage, seedlings of both growth forms are subject to an
432   environment characterized by long dry seasons, low nutrient availability, and high risk of fire. In particular,
433   fire is a particularly important factor in savannas, and may occur about once every 2-5 years (Gottsberger
434   and Silberbauer-Gottsberger 2006, Archibald et al. 2013). The herbaceous aboveground layer is reduced
435   by more than 90% after fire (Miranda et al. 2002), along with seedlings of trees and subshrubs, and must
436   regrow from soil level. We found seedlings of both subshrubs and trees to recover similarly well following
437   biomass loss, and exhibit similar functional traits. As plants develop, however, eventually a stem size is
438   reached at which subshrubs are reproductively mature, while trees are neither mature nor large enough to
439   resist fire (Figure 3). Is not clear whether both growth forms would maintain similar growth rates until this
440   time, nor if they would exhibit similar allocation patterns. More importantly, however, is that this point marks
441   the divergence between growth forms in their demographic responses to frequent fire. At this point, trees
442   are susceptible to being maintained in a fire-trap of repeated topkill and resprouting, and may be maintained
443   indefinitely in this suppressed state without an opportunity to reach reproductive maturity. Meanwhile
444   subshrubs are generally able to recover reproductive size quickly between fires, thereby being able to
445   produce seeds in the intervals between fire (Figure 3). So although allocation patterns may have diverged
446   substantially prior to reaching this size, perhaps the most essential difference between these growth forms
447   is the stem size at sexual maturity. However, this may have indirect effects on evolution of maximum size
448   because evidence suggests a tradeoff between maximum size and ability to reproduce at small size
449   (Aarssen 2015). Thus, the ability to reproduce while suppressed by frequent fires may preclude the ability
450   to become a large, fire-resistant tree.

451   The ability to reproduce sexually at a small stem size allows successful reproduction despite
452   frequent topkill, but imposes a cost upon the potential reproductive output. An adult tree may produce a
453   vast number of seeds, while an adult subshrub has a much smaller amount of resources to invest in
454   reproduction. Available resources can be invested in many small offspring or few large offspring (Henery
455   and Westoby 2001, Muller-Landau et al. 2008, Díaz et al. 2016), and small growth forms tend to smaller
456   seeds (Díaz et al. 2016). Selective pressure towards smaller offspring was detected as significantly smaller
457   seeds and seedlings in subshrubs than in trees (Figure 2A). Yet, the number of offspring produced per unit
458   plant size, per unit time describe the evolutionary fitness of a plant, and the most parsimonious way for a

459 plant to make more seeds is to make smaller ones (Aarssen et al. 2006), and in ecosystems with limited
460 time availability for plant growth, small reproductive plant size is favored (Aarssen 2015).

461 Other factors likely contributed to evolution of seed size in the Cerrado, but it is not clear whether
462 they would consistently contribute to smaller seeds of subshrubs. Their smaller reserves results in smaller
463 seedlings (Westoby et al. 2002) that would be more vulnerable to fire and drought, but such habitats with
464 low seedling survival may favor production of many small seeds with high dispersal to enhance arrival at
465 suitable sites (Smith and Fretwell 1974, Leishman et al. 2000, Moles and Westoby 2004). Furthermore,
466 small seeds have been associated with lower capacity to withstand heat shock, such as that produced by
467 fire (Ribeiro et al. 2015). However, tree recruitment in the Brazilian Savanna has been shown to be seed
468 limited (Salazar et al. 2012) so predation in this environment has a substantial effect on seedling abundance
469 by reducing the seed supply (Campbell and Clarke 2006, Salazar et al. 2012). Small seeds are easily
470 buried, reducing predation and exposure to high temperatures in fire events, which would favor small seeds.

471 One remarkable feature of subshrub evolution is the fact that they arose independently within a
472 large number of tropical tree lineages. These multiple origins suggest that the conquest of fire-prone
473 ecosystems may require simple, easily acquired, genetic changes, perhaps involving gene regulation and
474 rather than structural mutation (Simon and Pennington 2012). This could involve, for example,
475 downregulation of gibberellin pathways, which is known to induce both dwarfism and flowering at smaller
476 stem sizes (Davies 2010, Gupta and Chakrabarty 2013), two characteristics of subshrubs (Figure 3).

477 Regardless of the genetic mechanism, the evolutionary shift from tree to subshrub has relatively
478 little impact on many species traits. In fact, for most traits, genus explained >65% of the total interspecific
479 variation across the study species, revealing strong phylogenetic conservatism of these traits. Each of these
480 independent origins occurred within a lineage that possesses a unique suite of plant functional traits. This,
481 combined with substantial conservatism of these traits at the genus level, has given rise to a functionally
482 diverse community of subshrubs in the Cerrado, which largely mirrors the diversity of lineages from which
483 the subshrubs evolved.

484

485 **Conclusions:**

486 These findings have multiple implications for the ecology and evolution of the subshrub (geoxyle) growth
487 form, which is a diverse component of mesic savannas of South America and Africa. As seedlings,
488 functional traits among our study species were more strongly determined by phylogeny than by growth
489 form, but presumably this pattern is inverted later in development as plants differentiate into the highly
490 distinctive tree and subshrub growth forms. Even so, leaf traits of adult plants continued to show little
491 difference between growth forms, emphasizing the importance of the allocation and reproductive traits that
492 characterize these growth forms.

493
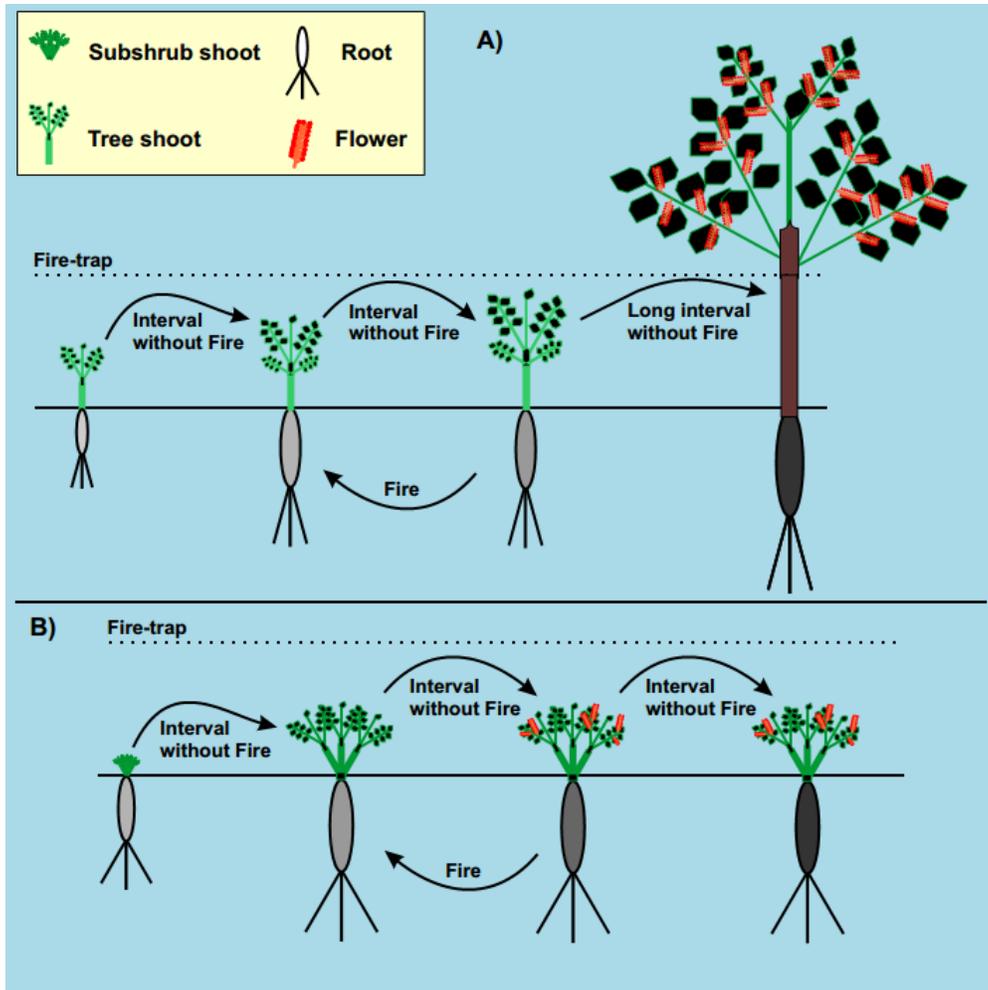
Figure 3. Schematic of the life histories of A) a tree and B) a subshrub, showing the differences in development and reproduction and response to the frequent fire.

Nevertheless, there were several significant differences in several traits between subshrubs and trees across multiple independent lineages, revealing an influence of natural selection on these traits. Overall subshrubs had significantly smaller seeds, greater investment in carbohydrate reserves and less investment in aboveground growth during early development, all of which are consistent with a life history strategy involving adults that invest little in permanent aboveground structures and reproduce quickly after fire. On the other hand, trees are comparatively less adapted to habitats with high fire frequency. Although, they can remain "*gullivers*", this life-history strategy depends on longer free-fire intervals or alternatively many years of accumulating reserves for fueling sustained rapid growth to become fire resistant. These two different life-history strategies reaffirm the importance of fire events in the origin of savanna biodiversity during the recent evolution and diversification of Brazilian savanna flora. Subshrubs comprise an important component of this flora, which by means of extreme reduction in growth form have converged on an effective strategy for the savanna environment.

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Plant reproduction in a fire-prone ecosystem: traits selected

628 Giroldo, A.B.[1,2]; Scariot, A.[2]; Massi, K.G.[1]; Walter, B.M.T.[2]; Hoffmann, W.A.[3]

629 1. Departamento de Ecologia, Universidade de Brasília, Caixa Postal 04457, 70919-970 Brasília, DF, Brazil. Corresponding author:
630 aeltonbg@gmail.com; 2. Embrapa Recursos Genéticos e Biotecnologia, Laboratório de Ecologia e Conservação, Caixa Postal
631 02372, 70770-900 Brasília, DF, Brazil; 3. Department of Plant and Microbial Biology, North Carolina State University, 2115 Gardner
632 Hall, Box 7612, Raleigh, NC 27695, USA

633

634 **Abstract:**

635 How many species are able to reproduce after a fire event? Are some reproductive traits selected in fire-
636 prone ecosystems? These questions have intrigued researchers for a long time. We knew that savanna
637 diversification occurred in the last 10 Mya and involved development of underground organs, thick terminal
638 branches and bark, and resprout capacity. Small life forms represent, theoretically, the extreme of
639 adaptation in fire-prone ecosystems, and most of them are able to reproduce after fire events. Using
640 herbarium and literature data, we found that 2,058 species (16.6%) in *Cerrado* biome are able to reproduce
641 until one year after fire. The species represented 61% of the *Cerrado* families, and subshrubs represent
642 60% of the reproductive species after a fire, and trees 12%. Although subshrubs are the dominant
643 reproductive growth form in the *Cerrado* biome, surprisingly, the trees have higher chances of reproduction
644 after a fire event. "The biased point of view" could explain why the naturalist thought, that the subshrubs
645 reproduction, despite trees, are positively influenced by fire. We explained why trees reproduction are not
646 negatively influenced by fire, using "fire as a pruning", and all traits correlated with tree survival in fire-prone
647 ecosystems. We explored the importance of pollination and dispersion syndromes in plants that reproduce
648 after fire. Reproduction of anemophilous species is not favored after fire events, however the number of
649 pollinators is not negatively affected by fire, so there is not any reason to natural selection favor the wind-
650 pollinated species after fire. Yet, we found an odds ratio in favor of zoochory species, and against autochory.
651 The wind-dispersed species is not significantly favored in species able to reproduce after a fire, probably
652 because there is a fast recovery of burnt areas, not favoring the long dispersion by wind.

653 **Keywords:** post-fire reproduction, pyrophytic species, Savanna evolution, Brazilian Savanna, *Cerrado*,
654 pollination and dispersion syndromes.

655

656 **Introduction:**

657 "In October 8, 1864, a Cerrado grassland was burned… in October 25 the Cerrado's soil was covered with
658 countless buds, and fresh and new flowers, I have never seen more beautiful grassland" (Warming 1908 -
659 page 90). The event related by Warming (1908) is a life history strategy of species in fire-prone ecosystems
660 (Figure 1). Savanna diversification occurred in the Miocene, together with the spread of C_4 grasses and

661　enhancement of fire frequency (Simon *et al.* 2009). During the savanna diversification, species with thick
662　bark, thick terminal branches, underground organs and resprout capacity were selected (Gottsberger &
663　Silberbauer-Gottsberger 2006a; Simon & Pennington 2012; Maurin *et al.* 2014), and some of them seem
664　to depend on fire for reproduction (Lamont & Downes 2011; Rissi 2016).

665　Post-fire reproduction events are most reported for small growth forms, because, theoretically, herbs and
666　subshrubs are more adapted to fire-prone ecosystems than large growth forms (Simon & Pennington 2012;
667　Maurin *et al.* 2014). The small growth forms invest proportionally large amount of energy in belowground
668　reserves (xylopodia) (Gottsberger & Silberbauer-Gottsberger 2006a), have a lower investment in
669　aboveground growth and have an early investment in reproduction (Zizka *et al.* 2014). Apparently, the
670　induction of reproduction is not the result of thermal action or fertilization by ashes or gases emanating from
671　combustion, but from the effect of plant pruning at soil level (although, efficiently different, see Fidelis &
672　Blanco (2014)) (Coutinho 1990). While herbaceous forms increase flower production after fire (Munhoz &
673　Felfili 2005), woody forms usually decrease sexual reproduction (Coutinho 1982; Hoffmann 1998; García-
674　Nunez *et al.* 2001).

675　A high percentage of woody species are zoochorous, when compared with herbaceous species that are
676　mostly anemochorous and autochorous (Batalha & Martins 2004; Ishara & Maimoni-Rodella 2011).
677　Anemochorous species could be favored after a fire event because the biomass reduction in the ground-
678　layer allow seeds to disperse longer distances than when fire does not occur (Coutinho 1982, 1990).
679　Besides that, independently of the dispersal syndrome, the reduction of seed predators after fires can have
680　a positive effect on species fitness (Salazar *et al.* 2012) that are able to reproduce after fire. Understanding
681　species traits and growth forms that have high fitness in fire-prone ecosystems can highlight evidences of
682　the role of fire as an evolutionary force shaping plant adaptations (Bond & Scott 2010; Keeley *et al.* 2011;
683　Pausas & Schwilk 2012), and contribute to improve management in fire-prone ecosystems. In this context,
684　we quantified and identified all species able to reproduce in the Brazilian Savanna biome after fire events.
685　We asked: a) Which are the most able species to reproduce after the fire: savanna or forest species? b)
686　Are the small or large growth forms the most reproductive after a fire event? c) Is there any type of
687　pollination syndrome selected in species able to reproduce after a fire event? d) and dispersion syndrome?
688　We hypothesized that: a) most species that are able to reproduce after a fire event would be savanna
689　species, b) small growth forms, c) wind pollinated species and d) wind dispersed species. Yet, we tested if
690　there are some families with significant number of species reproducing after fire. The results are discussed
691　regarding savanna evolution and conservation.

Figure 1. (a) A natural grassland in the Brazilian Savanna a few months after a fire event in Distrito Federal, Brazil; (b) *Vellozia* sp. (Velloziaceae), (c) *Habranthus irwinianus* (Amaryllidaceae), (d) *Eriosema prorepens* (Fabaceae), (e) *Lessingianthus* sp. (Asteraceae), (f) *Stryphnodendron pumilum* (Fabaceae), (g) *Lippia pumila* (Verbenaceae), (h) *Bulbostylis paradoxa* (Cyperaceae), (i) *Monnina* sp. (Polygalaceae), (j) *Vochysia pumila* (Vochysiaceae), (k) *Palicourea rigida* (Rubiaceae), (l) *Kielmeyera pumila* (Clusiaceae), (m) *Mimosa speciosissima* (Fabaceae), (n) *Anacardium humile* (Anacardiaceae) with fruits, (o) *Hippeastrum goianum* (Amaryllidaceae), (p) *Mimosa radula* (Fabaceae). Photographs: A.B. Giroldo (a,j-l,p), M.F. Simon (b-i,o), H. Moreira (m) and I.L.P. Lima (n).

Able to reproduce after fire, stimulated by fire or fire-dependent flowering species:

700

701 To define if a species is fire stimulated it is necessary to conduct experiments with and without fire. These

702 type of studies were conducted in the Brazilian Savanna (see Araújo *et al.* 2013; Fidelis & Blanco 2014),

703 and produced valuable information about the effect of fire in flower production of some species. Using this

704 approach it is possible to affirm if the species produce more flowers in areas with fire than without fire (fire-

705 stimulated flowering species), if it does not produce flowers (fire-inhibited flowering species) or even if the

706 production of flowers occurs just in areas with fire (a fire-dependent flowering species) (see Lamont &

707 Downes 2011). Although with our data was not possible to verify if a species is stimulated or dependent of

708 fire to reproduce, we can affirm that the species is not inhibited and is able to reproduce after a fire event.

709 We therefore considered here only species able to reproduce after fire.

Data acquisition and classification:

710

711 To create a list of species able to reproduce after a fire event (SAR) we checked two sources. We

712 accessed herbariums data using the speciesLink network (speciesLink 2015), and searched the English

713 words "fired", "fire", "burn" and "burned", the Portuguese words "queimada", "fogo", "incêndio", "queima",

714 "queimou", "queimado". We also searched for these words in the dataset from the large fire project

715 developed in the IBGE reserve, in Brasilia, Brazil (Miranda *et al.* 2011) for IBGE herbarium data is not

716 included at speciesLink network. We also verified if the species occurred in the Brazilian Savanna biome

717 (*Cerrado*) checking each name in the Brazilian Flora Website (List of Species of the Brazilian Flora 2015)

718 and replaced synonyms by accepted names. We considered as species able to reproduce after fire,

719 species that were found with reproductive structure (fruits or flowers) until one year after the fire event.

720 The second source was the literature; we did an exhaustive search checking all available papers,

721 seminars, short communications, and annals of events with data of species that reproduce after a fire

722 event. We stopped searching when the effort to find plants was exacerbated compared with the results.

723 As we collected the data from labels of herbarium specimens, we could not control the identification

724 errors, common in herbariums, however the voucher number was kept in the list, and the identification

725 can be checked in herbarium.

726 We verified the growth form of each species using the Brazil Flora website. As a species can be included

727 in more than one growth form type (Warming 1908), we conducted analyses using growth forms as

728 dummies, and classified them as subshrubs (herbs and subshrubs), shrubs, trees and vines. With

729 information from labels of herbarium specimens and specialists knowledge (in special the specialist B.M.T.

730 Walter) we classified species to either savanna/grassland or forest habitat. Savanna species were those

731 with predominant occurrence in fire-prone formations of Brazilian Savanna biome, including savannic and

732 grassland physiognomies. The forest species were those occurring mainly in forest physiognomies of the

733 Brazilian Savanna biome (see Ribeiro & Walter 2008). We classified the pollination and dispersal

734 syndromes of each genus with information from scientific literature. We considered genus as an appropriate

735 proxy of the species when data was not available for a given species. Plant pollination syndromes were

736   simplified into anemophily, entomophily and zoophily, and the dispersal syndromes into autochory,
737   anemochory and zoochory.

738 **Data Analysis:**

739   We used basic statistics to describe the SAR patterns. To test if the families with higher number of species
740   also had more species reproducing after a fire event we used a zero-inflated regression model with negative
741   binomial distribution. Ignoring zero-inflation may generate an estimated parameter and standard error
742   biased, and the excessive number of zeros can cause overdispersion (Zuur *et al.* 2009). Subsequently, we
743   tested if the percentage of SAR by family was correlated with the number of species within the family using
744   a generalized linear model (GLM), with binomial distribution (link logit), and calculated the D^2 that is the
745   equivalent of R^2 (coefficient of determination) for generalized linear models (GLMs) (Guisan & Zimmermann
746   2000). To test the hypotheses: a) that plants in savanna are more likely to reproduce after fire than forest
747   species; b) that the small growth forms are most able to reproduce after fire than large forms; c) that the
748   wind pollination and d) wind dispersion syndromes are selected in species able to reproduce after a fire,
749   we built a generalized linear model (GLM), using the binomial distribution with link logit. The independent
750   variable in the model was transformed in dummies. This analysis allow us to include in the model species
751   occurring in more than one habitat, growth form, pollination and dispersal syndrome, and test the main
752   characteristics of species that reproduce after a fire in only one model. All epiphytic species were removed
753   from this model. The analyses were done with R program (R Development Core Team 2015), using the
754   packages pscl (Jackman 2015), car (Fox & Weisberg 2011) and modEva (Barbosa *et al.* 2016).

755 **Brazilian Savanna biome or Savanna physiognomies:**

756   The expression savanna is used to refer to physiognomies with trees and shrubs sparsed in a continuous
757   grassy layer, without closed canopy (Ribeiro & Walter 2008), and fire-prone. Yet, we included in this concept
758   grassland formations (Ribeiro & Walter 2008), so the expression savanna refers to fire-prone ecosystems
759   (Figure 2). This expression differs from the expression Brazilian Savanna biome (named "*Cerrado*"), that
760   includes forested physiognomies, like *Cerradão*, gallery forest, deciduous and semi-deciduous forests
761   (Eiten 1972; Eiten 1978; Ribeiro & Walter 2008). The forest physiognomies (hereafter *Forest*) have closer
762   canopy if compared to savanna physiognomies, that suppresses the development of a grassy cover, mainly
763   C_4 grasses, the major determinant of fire spread/suppress along the savanna-forest gradient (Hoffmann *et*
764   *al.* 2012b; Just *et al.* 2015). Fire in Central Brazil forests is a casual event, and fire adaptations are poorly
765   developed in species there (Hoffmann *et al.* 2003), culminating in an environment tree/shrub species
766   dominated (Figure 2). Savanna species have an odds to reproduce after a fire 1400% higher than forest
767   species (Figure 3A), corroborating in part our first hypotheses, because forest species were not adversely
768   affected by fire, and did not reduce the reproductive chances ($z = 0.097$, $p = 0.922$) as we expected.
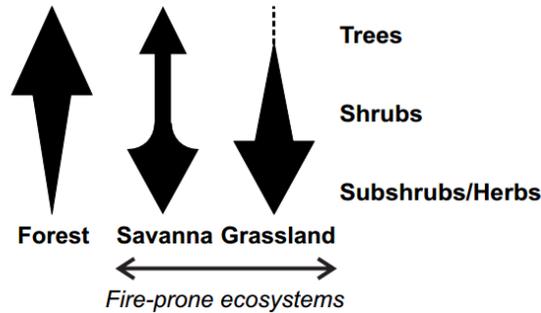
769

770 Figure 2. Differences in species number and abundance of growth forms in different vegetation formations within the
771 Brazilian savanna biome (*Cerrado*). The fire-prone ecosystems represent the savanna, where fire is one of the main
772 factors determining species abundance and richness.

773 **How many species can reproduce after a fire event?**

774 We found that 2,058 species are able to reproduce until one year after a fire event in *Cerrado* biome
775 (Appendix 1), 1,942 species present in savanna, 455 in the *Forest*, and 342 common to both *Forest* and
776 savana. The number of reproductive species after a fire is 5 times higher than the number of species
777 reported in South Africa and Australia Savannas together (see Lamont & Downes 2011), and represent
778 more than 16% of the *Cerrado* Biome species. There are 181 families of Angiosperms in the *Cerrado* biome,
779 and at least 61% of them are able to reproduce after a fire event. The families Fabaceae, Asteraceae,
780 Poaceae and Orchidaceae had more than 100 species flowering after fire, and the ten families with the
781 highest number of SAR comprise 56.6% of total reproductive species (Table 1). The total number of species
782 reproducing after fire within a family is correlated with species richness within the family ($z = -2.321$, $p = $
783 0.020) (Figure 4A), and although the results for the percentage of SAR by family was significant ($X^2 = $
784 22.997, $p < 0.001$), the model had a poor adjust ($D^2 = 0.034$), and do not represent correctly our data (Figure
785 4B). Yet, when considering the relative number of reproductive species and families with more than 10
786 species, it is possible to observe the families that are very well adapted to fire-prone ecosystems.
787 Polygalaceae is an example (Table 1), with 46% of the species flowering after fire, and some of them just
788 flowering after fire events (see Pastore & Cavalcanti 2008); the diversification of the family happened near
789 the tips and rather it is related to the roots of the tree (Forest *et al.* 2007), which could means recent
790 diversification. Other prominent family is Vochysiaceae, with species very well distributed into *Cerrado*
791 biome (see Ratter *et al.* 2003), and 34% of them can reproduce after fire; this family is originated from
792 South American and did not diversify until ca. 60 m.yr. (Sytsma et al. 2004, Forest and Chase 2009).
793 Oxalidaceae and Malpighiaceae had about 32% of SARs. The high number of families able to reproduce
794 after fire could push up the evolution process in favor of these families, once the sexual reproduction is
795 linked directly with the process of adaptation and fitness enhancement (Barton & Charlesworth 1998; Otto
796 2009), mainly in ecosystems like the Brazilian Savanna where the fire normally occurs once every five years
797 (Gottsberger & Silberbauer-Gottsberger 2006a).

Is reproduction between growth forms differentially influenced by fire?

798

799   The small-sized growth forms are dominant in the group of species able to reproduce after fire, and almost
800   60% of the SARs could be classified as subshrubs, and only 12% as trees in *Cerrado* biome (Figure 5A).
801   However, the dominance of small growth forms is similar to that showed in the *Cerrado* biome (Figure 5B),
802   and when we investigated savanna physiognomies, surprisingly, we found that 1/3 of trees were able to
803   reproduce after a fire event, while just 1/4 of subshrubs had reproduced. In *Forest*, the differences were
804   higher, but not distinct, and less than 1/6 of trees and only 1/16 of subshrubs were able to reproduce until
805   one year after fire. The results of logistic regression with dummies point out same trend (Table 2), showing
806   that trees had an odd ratio higher than subshrubs, although both growth forms had high chances of
807   reproducing after a fire event (Figure 3B). These results mean that if an area has species equally distributed
808   in all life forms, the chances to find a tree reproducing after a fire is higher than to find a subshrub. This
809   statement did not corroborate the second hypothesis (b), that small life forms are most able to reproduce
810   than large growth forms after a fire event. We postulated some explanations about this result.

811   The first explanation could be called "the biased point of view", and could happen because when a naturalist
812   walks in a recently burnt field, the subshrubs are the most abundant growth form; they usually dominate
813   almost the whole landscape, covering all soil surface with vegetative parts and colorful flowers (Figure 1).
814   This pronounced cover could bias the naturalist view, who believes that subshrubs reproduction is positively
815   influenced by fire event, while the trees, that are sparse elements in the landscape, could be seen as
816   negatively influenced. The second explanation shows why trees reproduction was influenced by fire. If we
817   consider fire as an herbivorous (see Bond & Keeley 2005), analogously we could consider it as a pruning
818   agent, despite the fact that fire could kill more plants than pruning (see Kelly et al. 1997). Pruning plants is
819   a common practice in agriculture and gardening, which enhances the productivity, by the production of new
820   leaves with high photosynthesis capacity, reduction of dead parts, and production of new branches. Yet,
821   although fire can destroy the buds and promote top-kill, the savanna trees which had escaped from the fire-
822   trap had thick terminal branches (Simon & Pennington 2012) and thick bark that protect the buds and
823   cambium, allowing a vigorous epicornic resprout after the fire event (Hoffmann et al. 2009; Lawes et al.
824   2011; Pausas 2015); these characteristic could allow trees to recovery their vegetative part and reproduce
825   after a fire event. However, this does not mean that trees are stimulated to reproduce after a fire (as we
826   have mentioned in the second section); the fire regime intensification reduces the relative importance of
827   sexual reproduction, and increases the importance of suckers in the vegetation and the most woody
828   individuals are kept in a non-reproductive state (the "gullivers") (Hoffmann 1998; Bond & Midgley 2001).

Families	# Species	Relative to Family	Relative to SAR
Fabaceae	245	0.198	0.119
Asteraceae	223	0.180	0.108
Poaceae	153	0.206	0.074
Orchidaceae	129	0.177	0.063
Euphorbiaceae	87	0.224	0.042
Malpighiaceae	74	0.318	0.036
Myrtaceae	68	0.260	0.033
Lamiaceae	63	0.211	0.031
Melastomataceae	62	0.127	0.030
Cyperaceae	61	0.184	0.030
Rubiaceae	57	0.137	0.028
Apocynaceae	56	0.189	0.027
Polygalaceae	52	0.473	0.025
Eriocaulaceae	40	0.087	0.019
Malvaceae	37	0.110	0.018
Convolvulaceae	35	0.136	0.017
Bignoniaceae	31	0.183	0.015
Lythraceae	28	0.170	0.014
Bromeliaceae	26	0.098	0.013
Amaranthaceae	25	0.219	0.012
Acanthaceae	24	0.159	0.012
Turneraceae	23	0.247	0.011
Velloziaceae	23	0.122	0.011
Verbenaceae	23	0.154	0.011
Solanaceae	22	0.204	0.011
Arecaceae	16	0.170	0.008
Vochysiaceae	16	0.340	0.008
Iridaceae	15	0.221	0.007
Oxalidaceae	15	0.326	0.007
Ochnaceae	13	0.176	0.006

829

830 Figure 3. Odds ratio of species that reproduce after
831 fire in *Forest* and savanna (Savanna/Grassland)
832 (A), growth forms (B), pollination (C), and dispersal
833 syndromes (D) in the *Cerrado* biome. The dots
834 represent the mean and the arrows the confidence
835 interval (95%).

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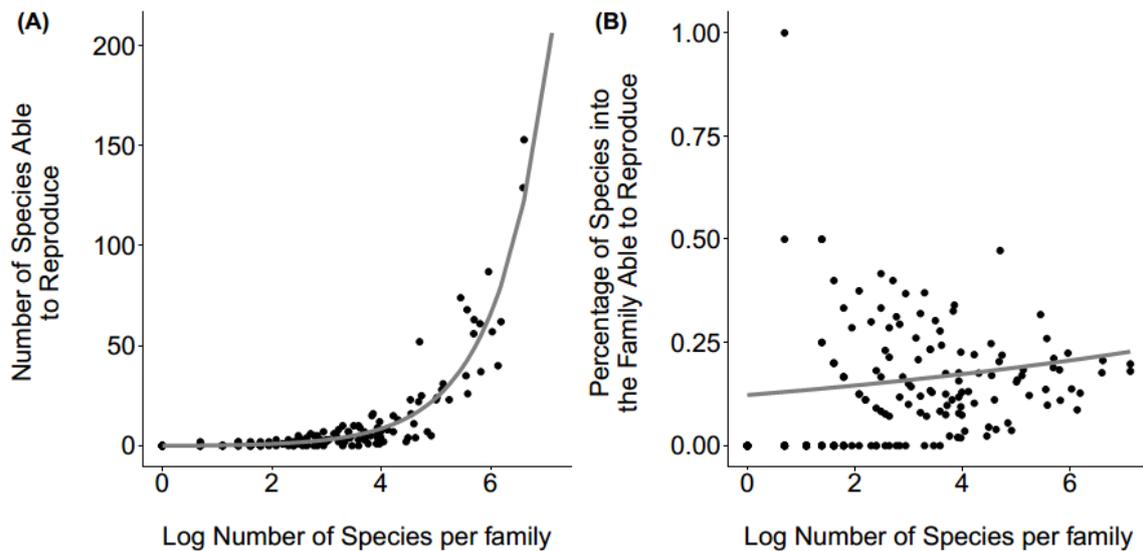
847

848 Figure 4. Number of species able to reproduce after fire in *Cerrado* biome (A), and percentage of species

849 within the family able to reproduce per number of species per family (B). The dots represent observed data

850 and the lines the predict zero-inflated model (A), and logistic model (B).

851 Table 2. Result from the logistic regression model, with species able to reproduce after fire as a dependent

852 variable, and independent variable the physiognomies in *Cerrado* biome, *Forest* and savanna/ grassland

853 (savanna); growth forms, subshrub, shrub, tree and vine; pollination syndromes, anemophily, entomophily

854 and zoophily; and dispersal syndromes, anemochory, autochory and zoochory of plants in *Cerrado* biome.

Independent Variable	Estimate ± SE	z value	p
Forest	0.008 ± 0.078	0.097	0.922
Savanna/grassland	2.719 ± 0.127	21.366	< 0.001
Subshrub	0.844 ± 0.097	8.693	< 0.001
Shrub	0.142 ± 0.073	1.942	0.052
Tree	1.158 ± 0.112	10.377	< 0.001
Vine	0.262 ± 0.129	2.038	0.041
Anemophily	0.183 ± 0.116	1.579	0.114
Entomophily	0.110 ± 0.114	0.965	0.334
Zoophily	-0.148 ± 0.927	-1.602	0.109
Anemochory	-0.089 ± 0.068	-1.312	0.189
Autochory	-0.184 ± 0.064	-2.891	0.004
Zoochory	0.169 ± 0.070	2.401	0.016
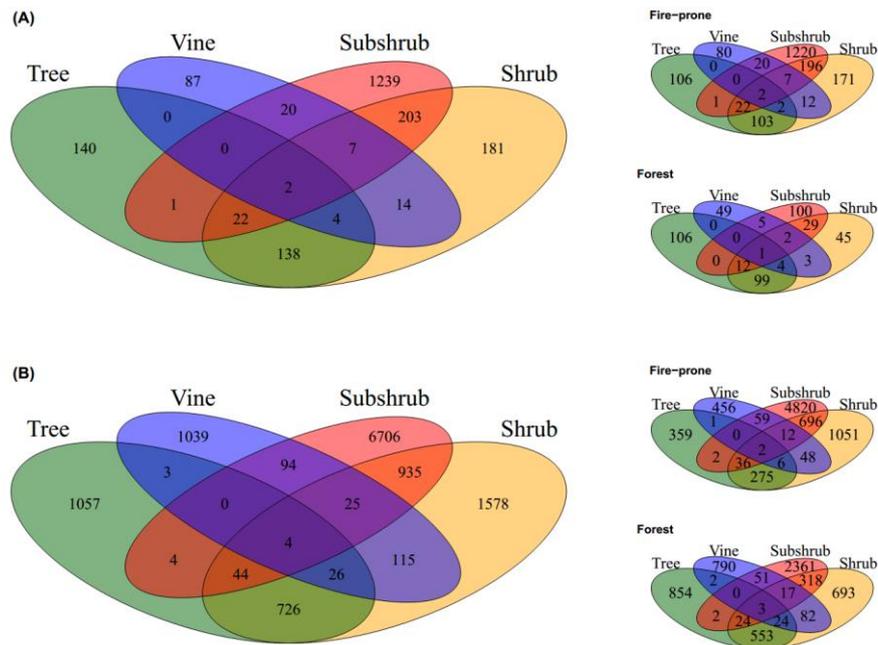
Figure 5. Number of species able to reproduce after a fire in each growth form (A); and in *Cerrado* biome (B), divided by fire-prone habitats (savanna) and *Forest*.

Pollination syndromes related with fire

The dominance of animal pollinated species (zoophily and entomophily species) and the reduction of the anemophily syndrome found here (Figure 6A) may reflect the rainforest origin of the Brazilian Savanna (Oliveira & Gibbs 2002). Although some families have different pollination syndromes in the *Forest* and savanna physiognomies, generally the pollination traits are conservative (see Gottsberger 1986) (Figure 6A); and many plants in the "*Cerrado*" have polyphilic flowers, which are pollinated by taxa of totally unrelated groups (egg. by different bees and flies or beetles), so the pollinators may be substituted depending upon the fauna composition at a particular place (Gottsberger & Silberbauer-Gottsberger 2006b). Yet, there were not differences in the odds ratio of pollination syndrome in species able to reproduce after a fire (Table 2 – Figure 3C and 6B), meaning that no syndrome is benefited in the availability of plants flowering after fire events. Even when we built the model using just savanna (Savanna/Grassland) species no syndrome appears with high or low odds ratio (Figure 3C), although the anemophily syndrome was marginally significant ($z = 1.706$, $p = 0.088$). Moreover, using just *Forest* species, the zoophily syndrome had an odds 37% smaller to reproduce than by chance ($z = -2.099$, $p = 0.036$). These results did not corroborate the hypothesis (c), that the wind-pollinated species were selected in species able to reproduce after fire. However, investigating the data by growth forms we found that anemophilous species had an odd ratio significantly higher than the chance in vines ($z = 2.246$, $p = 0.025$) and shrubs ($z = 3.811$, $p < 0.001$), and the odd ratio of entomophilous ($z = -0.079$, $p = 0.038$) and zoophilous trees ($z = -2.422$, $p = 0.015$) was smaller than by chance (Figure 7A). The evolution process could really favor the wind-pollinated plants if the fire negatively affected the pollination guilds. However, the main effect of fire in pollination is the enhancement

880   of it, because usually the number of plants that produce new flowers, mainly in herbaceous
881   stratum, increases (see Campbell *et al.* 2007; Van Nuland *et al.* 2013), and the rewards for
882   pollinators (pollen and nectar) are smaller in mature areas than in freshly burnt areas (Potts *et al.*
883   2003). However, probably this pattern is kept mainly because *Cerrado* species have polyphilic
884   flowers, and can be pollinated by multiple pollinators. Species that depends on solitary bees could
885   be negatively affected, since these type of pollinator is more specialist, and usually decrease in
886   abundance in recently burnt areas (see Ne'eman *et al.* 2000). The effects on species dependence
887   of specialist pollinators need to be investigated, as well as the effect of fire in all guilds of pollinator,
888   mainly in neotropical savanna, where fire frequency and species diversity are high.

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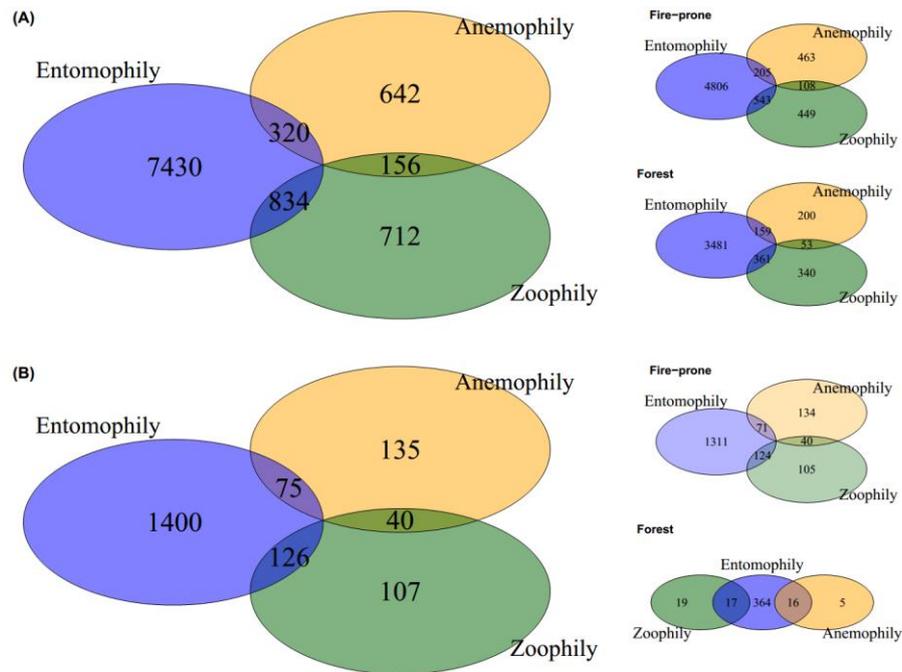
890

891   Figure 6. Number of species in each pollination syndrome in *Cerrado* biome (A); able to reproduce into
892   pollination syndromes (B), all of them separated by savanna (fire-prone) and *Forest* physiognomies.
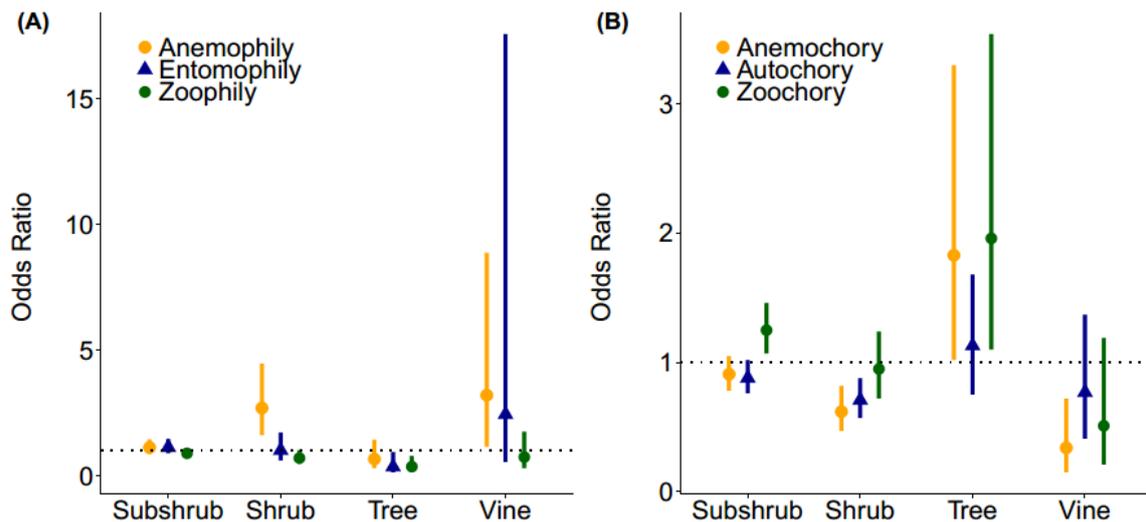
Figure 7. Odds ratio of species able to reproduce after fire in pollination syndromes (A) and dispersal syndromes (B) of different growth forms in *Cerrado* biome.

Dispersal syndromes related with fire:

The environmental gradient affect the dispersal syndromes. At landscape level the wind dispersion is related with nutrient and light availability, wherein the increased nutrient results in the reduction of species wind dispersed, and increased light increases anemochorous species. The zoochorous species have complex relations; while mammals dispersed species increase with light availability, bird dispersed species decrease with light availability (Ozinga *et al.* 2004). As a result of these relationships, and given the vegetation structure differences between *Forest* and Brazilian savana physiognomies (see Ribeiro & Walter 2008), the dispersal syndromes are not conserved between them. Instead, *Forest* physiognomies are dominated by zoochorous species, while savanna physiognomies are dominated by anemochorous species (Figure 8A) (further examples Gottsberger & Silberbauer-Gottsberger 2006b; Jacobi & Carmo 2011). However, when referring to species able to reproduce after fire in *Cerrado* biome, zoochorous species had an odds ratio higher than by chance, and autochorous species had a reduction in the odds ratio (Table 2 – Figure 3D and 8B). These results are kept even in savanna (Autochory: z = -2.712, p = 0.007; Zoochory: z = 2.257, p = 0.024), but not in *Forest*, where the anemochorous species had an odd ratio higher than by chance (z = 2.262, p = 0.024), autochorous lower than by chance (z = -4.820, p < 0.001) and zoochorous were not significantly affected (z = -0.628, p = 0.530). We did not find any pattern of dispersal syndromes with growth form; each growth form had a predominant type of dispersion in SARs. Subshrubs showed high odds chance for zoochorous species (z = 2.865, p = 0.004); shrubs had low odds ratio for anemochorous (z = - 3.396, p = 0.001) and autochorous (z = -3.108, p = 0.002); trees had high odds ratio in anemochorous (z = 2.030, p = 0.042) and zoochorous species (z = 2.252, p = 0.024); and vine had lower odds ratio than by chance for anemochorous species (z = -2.745, p = 0.006 – Figure 7B).
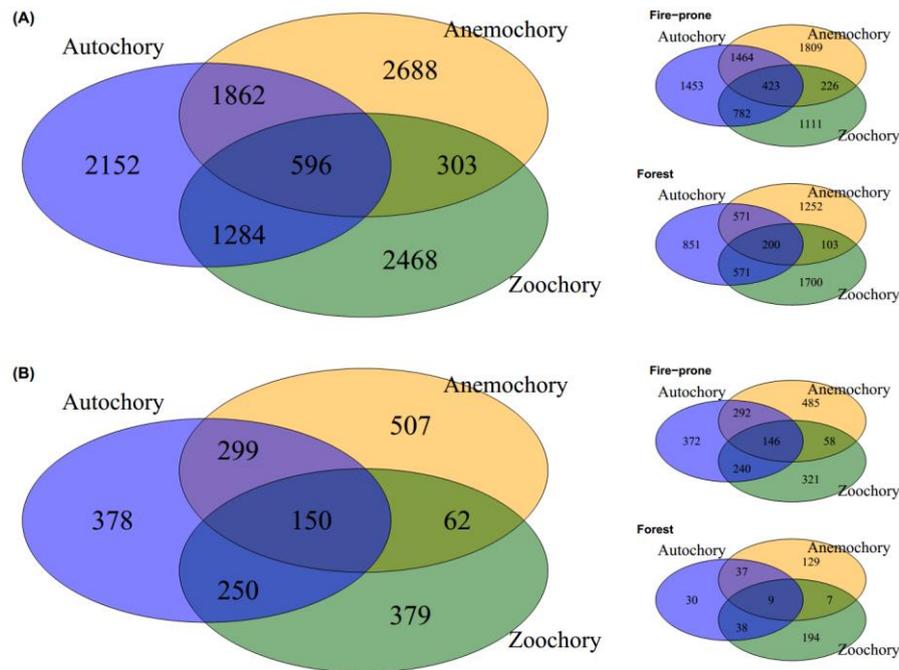
Figure 8. Number of species in each dispersal syndrome in *Cerrado* biome (A); able to reproduce after fire into dispersal syndromes (B), all of them separated by savanna (fire-prone) and *Forest* physiognomies.

The dispersal strategy has a direct impact on species abundance, distribution and evolution. When the quality of the environment varies in space and time, like in savannas, there is a selective pressure for increased dispersion (Levin *et al.* 2003). The dispersion distance is widely different between the dispersal modes; autochory dispersion do not favor long dispersion distance, while the dispersion distance in zoochory is greatest, and wind dispersal syndrome is intermediate (Thomson *et al.* 2011). Yet, the off-spring dispersal is also influenced by the community structure (density), and it is more obvious in wind dispersed seeds, wherein if there is little physical obstruction imposed by community architecture the seeds could be carried away from the parental plant. In the *Cerrado* biome, usually after a fire event, there is a simplification of the landscape structure; however the recovery of the structure is farther fast in savanna physiognomies than in *Forest*, due to the adaptive differences between the physiognomies (see Hoffmann *et al.* 2003). As the time between plant bloom and plant seed ripening is long for some species (for instance, more than 9 months in *Stryphnodendron pumilum*, *Andira vermifuga* and *Mimosa foliolosa*, A. B. Giroldo personal observation), usually the structure of the community in savanna have been almost recovered (see Rissi 2016), so could not have selective pressure to favor anemochorous species in this physiognomy. Instead of wind-dispersion syndrome, the zoochorous syndrome, that ensure high dispersal distance, was favored there. On the other hand, the forest habitat is not resilient to fire effects (see Hoffmann *et al.* 2012a; Pellegrini *et al.* 2015), requiring more time for recovery the community structure, allowing long dispersal distance in species wind-dispersed, that is favored in this physiognomy (Figure 6D).

Conservation efforts in fire-prone ecosystems and general conclusions:

The density of trees in the *Cerrado* biome varies greatly between dense and open physiognomies, and fire, together with moisture and soil fertility is the major driver of vegetation type in savannas (Haridasan 2008; Rossatto *et al.* 2012; Archibald *et al.* 2013; Lehmann *et al.* 2014). Many species are dependent on fire to reproduce (Rissi 2016), or, at least, are not inhibited by it (Figure 5A). On the other hand, fire suppression promotes savanna encroachment, with a reduction in biodiversity, mainly in the herbaceous stratum, that is the most species rich (Figure 5B) (BFG 2015) and usually photophobic; changes the structure, with the arrival of fire-sensitive species; and modifies the functioning of the *Cerrado* ecosystem (Durigan & Ratter 2016). Although, there is not a single recommendation for fire management, the *Cerrado* biome, as well as others neotropical savannas, needs to implement a fire management policy (Durigan & Ratter 2016). This ecosystems have a history with fire, which promoted the diversification of many *Cerrado* lineages (Simon *et al.* 2009) selecting many traits (Pausas & Schwilk 2012). Although we were expecting a supremacy of subshrubs against trees in species that were able to reproduce after a fire, we surprisingly discovered that trees are equally qualified and selected to reproduce after a fire event. Trees, like subshrubs, are shaped and selected in fire-prone ecosystems. The persistence of this growth form is ensured by many different traits that could assure their reproduction. Our hypotheses that anemophily and anemochorous species were the syndromes selected in species able to reproduce after a fire was not corroborated. Pollinators enhancement after fire, and the fast recovery of the community structure could be the main causes of the absence of selection pressure in favor of the wind pollination and dispersion syndrome. Many plant families were able to reproduce after a fire, and some of them are good candidates to better study the relations between fire-traits selection and evolution.

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1130   Appendix 1. Species able to reproduce after a fire in Brazilian *Cerrado* with growth form,

1131   physiognomies, pollination and dispersion syndromes, type of fruit and references where the

1132   dispersions syndromes where found.

1133

1134   As data is too large, we attached it in a server. To access it contact the main author.

1135

1136

Small plants, great strategies: a complex history about congeneric pairs and grass competition in a Neotropical savanna

Giroldo, A.B.[1,2,3]; Scariot, A.[3]; Ferreira, J.B.[1,3]; Moser, P.[1,3], Hoffmann, W.A.[4]

1. Departamento de Ecologia, Universidade de Brasília, Caixa Postal 04457, 70919-970 Brasília, DF, Brazil;
2. Corresponding author: aeltonbg@gmail.com; 3. Embrapa Recursos Genéticos e Biotecnologia, Laboratório de Ecologia e Conservação, Caixa Postal 02372, 70770-900 Brasília, DF, Brazil; 4. Department of Plant and Microbial Biology, North Carolina State University, 2115 Gardner Hall, Box 7612, Raleigh, NC 27695, USA

Abstract:

Competition, facilitation and niche partition drive ecosystem biodiversity and allow the coexistence of multiple growth forms. The models that investigated this coexistence in Neotropical savanna focus primarily in tree-grass coexistence, and although subshrubs and dicot herbs represent more than 50% of all species, they normally are ignored in coexistence models or incorporated as part of grass stratum. We investigated the effect of grasses in tree and subshrub survival and growth, including the interaction of congeneric pairs of trees and subshrubs, in an experiment split-plot designed. Congeneric pairs are phylogenetic correlated, usually sharing traits that enhance the competitive forces. We found that congeneric pairs competition and grass competition changed growth and survival of the species. Although the effect of grass and congeneric pair competitive forces had been synergetic for tree survival, they were attenuated for trees and subshrubs growth when they were together and no effect was detected. We suggest that trees and subshrubs could establish in savanna by the presence of root gaps. Once the trees are established in these gaps and out of the fire-trap they change the conditions under canopy and facilitate the establishment of new trees. The subshrubs could have an advantage against trees because they can reproduce earlier, and they are more fertile, with more chances to arise in patches with root gap. Yet, the fire frequency plays an important effect in biodiversity, particularly subshrubs, because it avoids savanna encroachment.

Keywords: *grass-subshrub-tree, Brazilian Savanna, coexistence, niche partition.*

Introduction:

Competition both intraspecific and interspecific is one of the major forces determining the abundance and distribution of plant species and the plant communities biodiversity (Tilman 2009). The competitive exclusion principle says that even small advantages may result in species exclusion when two species occupy the same niche (Gauze 1934; Hardin 1960). Savannas, that cover 20% of the world land, are formed by different coexistent growth forms (see Gottsberger & Silberbauer-Gottsberger 2006; Maurin *et al.* 2014), some of them are closely related species, usually sharing morphological and physiological traits because of phylogenetic relatedness (Blomberg et al. 2003), which enhances the resource competition.

Scientific literature has widely demonstrated the process that could reduce the competition in savannas and that mediate tree-grass coexistence (see Scholes & Archer 1997; Rodríguez-Iturbe et al. 1999; Higgins et al. 2000; Accatino et al. 2010; Dantas et al. 2013; Accatino et al. 2016). In summary, these models highlight that tree-grass coexistence is mediated by fire regime and could be influenced by climate, soil and grazing. Usually, the reduction of fire frequency promotes the savanna encroachment, and the established trees could change the microclimate (Rossatto & Rigobelo 2016), contributing to avoid fire spread. On the other hand, increased fire frequency could promote grass spread, reducing tree cover in savanna ecosystems (Hoffmann *et al.* 2012).

Although, these models explain very well the tree-grass coexistence, savannas cannot be summarized only by trees and grasses, instead the herbaceous strata (hereafter subshrubs) is the most important element in open habitats, covering the entire ground, with a high richness and density of species (Filgueiras 2002). Current data for the *Cerrado* indicates that at least 7,654 species are subshrubs and dicots herbs (51%), excluding 3,380 shrubs, 1,276 vines and 742 grasses species, and trees represent just 1,790 of all species (BFG 2015). Therefore, tree-grass models have incorporated just 19% of the total biodiversity in savannas, although some models consider subshrubs as part of the grass strata. The incorporation of subshrubs in the same category of grasses could make sense, since 97% of the herbaceous strata (subshrubs + grasses) is consumed in fire events (Miranda et al. 2002). However, grass, subshrubs and trees differ in some leaf anatomical traits (Rossatto *et al.* 2015; Díaz *et al.* 2016) and in some ecophysiological traits related to water access (Nippert & Knapp 2007; Rossatto et al. 2013), biomass allocation and seed biomass (Díaz et al. 2016).These differences indicate that subshrubs and grasses have different life-history strategies and therefore should be considered as separate units.

Trees and subshrubs when adults could mediate their establishment by facilitation or difficult it by competition. Usually, both growth forms can modify the microclimate and nutritional conditions under canopy (Scholes & Archer 1997; Rossatto & Rigobelo 2016), and it has been reported that adult trees can facilitate the establishment of subshrubs (Barnes & Archer 1999). However once established, they would compete with each other, and when the adult tree dies a new one could not be established under the canopy of subshrubs (Barnes & Archer 1999). This inhibition of establishment happens because most of the trees are shade intolerant, differently from subshrubs that can tolerate some levels of shade. Although, the literature describes at least the effects of adults in seedling establishment, we do not know the effect of seedling competition of both growth forms. We expected that the growth forms compete with each other because they share common traits since they are phylogenetically correlated, but we do not know who the superior competitor is.

We conducted an experiment to test the effect of grass competition on tree and subshrub establishment and growth. Furthermore, we tested the effect of tree and subshrubs competition, and the combination of their competition with grass competition in a split-plot design experiment. We know that seedling recruitment is a critical life-history stage, mainly for plants with long life

1213   (Scholes & Archer 1997; Sankaran et al. 2004), and we had different assumptions for the effect
1214   of grass in trees and subshrubs. First, we hypothesize that the trees and subshrubs seedlings will
1215   not differ against grass competition because the growth forms are very similar in this ontogenetic
1216   stage, and both will be negatively affected. The negative effect would happen because grasses
1217   can reduce the available water, nutrient and photosynthetic active radiation (PAR), as it has been
1218   reported in other studies (Jurena & Archer 2003; Wardle & Peltzer 2003; Riginos 2009). The
1219   second hypotheses is that trees are able to grow and develop a deep root system (Kambatuku et
1220   al. 2013), even in the beginning of development (Silveira et al. 2013), so they will be less affected
1221   by grass competition than subshrubs, that compete with grass in the upper soil layers.
1222

1223   **Methods:**

1224   -   *Species selection and experimental design*

1225   We selected three congeneric pairs that represent subshrub and tree growth forms in the *Cerrado*
1226   biome (Table 1). We used the native grass *Paspalum atratum* in the experiment, which can be
1227   found in almost all *Cerrado* biome (Maciel *et al.* 2009). Grass seeds were obtained from the
1228   Brazilian Agricultural Research Corporation (Embrapa), and seeds of most congeneric pairs were
1229   collected in Brasília, Distrito Federal, Brazil. Exceptions were the seeds of *C. oblongifolia*
1230   collected in Chapada Gaúcha, Minas Gerais and *A. humile* collected in Grão Mogol, Minas Gerais.
1231   We collected seeds between November 2013 and July 2014. We sown them in polyethylene bags
1232   with 20 cm diameter and 40 cm deep. The grass was sown five months before the other species,
1233   on February 2014. Separation between plots with and without grass ensured that shadow effect,
1234   promoted by grass, occurred just in plots with grass (Figure 1). *Stryphnodendron* spp. seeds were
1235   immersed in sulfuric acid for 5 minutes to break the physical dormancy. We used as substrate
1236   70% oxisol subsoil (40 cm deep or more) mixed with 30% of washed sand, without any other
1237   nutrient. The experiment was conducted in a green house, with automatic irrigation system (~ 7
1238   mm/day).

1239 Table 1. Species sown in a competition experiment between grass vs subshrub vs tree in Cerrado biome.

Family	Species	Growth Form	Habitat
Anacardiaceae	*Anacardium humile*	Subshrub	savanna
Anacardiaceae	*A. occidentale*	Tree	forest/savanna
Fabaceae	*Stryphnodendron pumilum*	Subshrub	savanna
Fabaceae	*S. adstringens*	Tree	forest/savanna
Fabaceae	*Copaifera oblongifolia*	Subshrub	forest/savanna
Fabaceae	*C. langsdorffii*	Tree	forest/savanna
Poaceae	*Paspalum atratum*	Grass	savanna

1240

1241 The experiment design was split-plot, with and without grass competition (G+; G-); with and
1242 without congeneric pair competitor (C+; C-) (Figure 1); and with two growth forms, tree and
1243 subshrubs (T; S). Each species was sown 16 times alone in each plot and together with its
1244 congeneric pair 16 times, in both cases with and without grass.
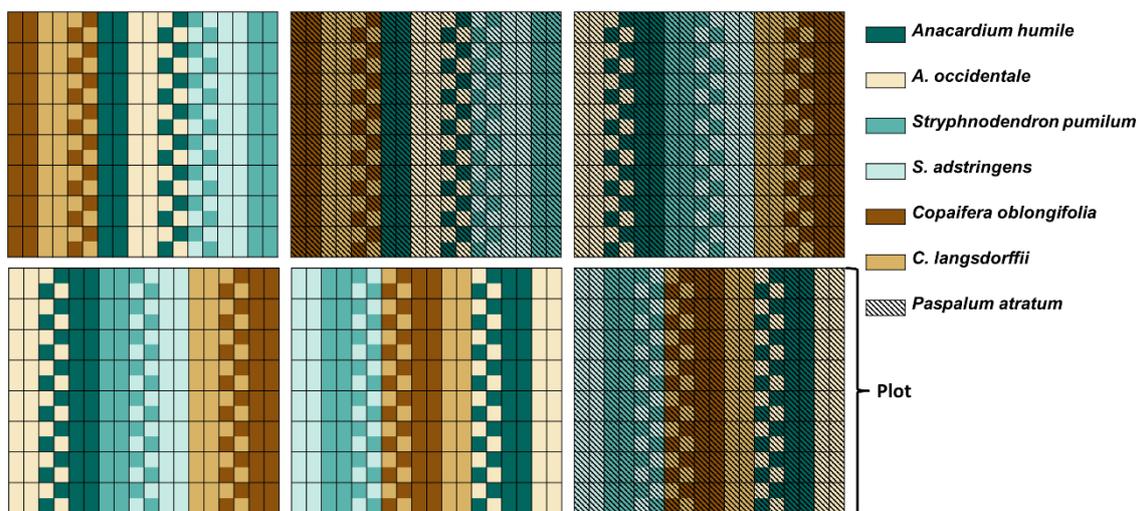
1246 Figure 1. Scheme of competition experiment including six species of trees and subshrubs. Each rectangle
1247 represents a bag, colors represent species, and rectangles with mixed colors represent a combination of
1248 congeneric pairs. The grass (*Paspalum atratum*) was sown in three plots.

1249

1250   -   *Data collection and statistical analysis:*

1251 After one year, we collected the biomass in two bags per plot. The subshrub and tree seedlings
1252 were divided in shoot and root part, dried in a dry chamber at 70ºC per 72 h (Pérez-Harguindeguy
1253 et al. 2013), and weighed in a precision scale (0.0001 g). After a year and a half, we counted all
1254 plants alive in each bag to verify if competition had affected trees and subshrubs survival. We
1255 used a linear mixed model to verify if the root:shoot ratio, shoot and root and total plant biomass
1256 were different between growth forms (S; T), and were affected by grass (G+; G-) or by congeneric
1257 pair (C+; C-) competition. The genus was used as a random factor in the models. To verify if the
1258 survival rate was affected by grass and congeneric pair competition, and the difference between

1259   the growth forms we used a generalized linear mixed model, with a binomial distribution. The

1260   genus was used as a random factor. All analyses were done in R program (R Development Core

1261   Team 2015) using the packages lme4 (Bates et al. 2015) and car (Fox & Weisberg 2011).

1262

1263 **Results:**

1264   - *Survival rate:*

1265   We found that the survival rate was affected by congeneric pair competition (χ^2 = 11.416, p <

1266   0.001), but was not affected by grass (χ^2 = 0.224, p = 0.621), or between growth forms (χ^2 = 434,

1267   p = 0.510). There was interaction between some factors (Table 1), and after exploring them we

1268   found that grass competition affected trees survival (χ^2 = 4.375, p = 0.036), but not subshrubs (χ^2

1269   = 1.886, p = 0.170). Yet, the effect of grass just exist together with congeneric pair competition,

1270   and only for tree growth form (χ^2 = 19.700, p < 0.001 – Figure 2A).

1271

1272   Table 2. Effect of congeneric competition (CC), grass competition (GC), growth form (GF) on the survival

1273   rate and biomass of three congeneric pairs of species from the Brazilian Savanna. Statistic χ^2 is from

1274   generalized linear model (GLM) with binomial distribution, and F is from ANOVA.

Factors	Survival rate	Plant Total Biomass	Root Biomass	Shoot Biomass	Shoot:Root Ratio
GC	χ^2 = 0.224	$F_{1,118}$ = 21.557***	$F_{1,119}$ = 20.232***	$F_{1,116}$ = 13.755***	$F_{1,116}$ = 4.223*
CC	χ^2 = 11.416***	$F_{1,118}$ = 4.323*	$F_{1,119}$ = 5.725*	$F_{1,116}$ = 1.036	$F_{1,116}$ = 1.351
GF	χ^2 = 0.434	$F_{1,2}$ = 0.489	$F_{1,2}$ = 2.362	$F_{1,2}$ = 0.535	$F_{1,2}$ = 0.891
GC:CC	χ^2 = 4.033*	$F_{1,118}$ = 9.748**	$F_{1,119}$ = 8.162**	$F_{1,116}$ = 8.423**	$F_{1,116}$ = 0.836
GC:GF	χ^2 = 6.214*	$F_{1,120}$ = 0.136	$F_{1,120}$ = 0.001	$F_{1,116}$ = 0.770	$F_{1,116}$ = 3.711
CC:GF	χ^2 = 0.578	$F_{1,120}$ = 6.551*	$F_{1,120}$ = 4.921*	$F_{1,116}$ = 2.960	$F_{1,116}$ = 1.857
GC:CC:GF	χ^2 = 6.406*	$F_{1,116}$ = 0.031	$F_{1,116}$ = 0.017	$F_{1,117}$ = 0.358	$F_{1,117}$ = 0.745

1275   Significance statistical values: * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

1276   - *Plant Biomass*:

1277   Grass and congeneric pairs competition affected negatively the plant biomass in almost all the

1278   factors analyzed (Table 2 – Figure 2B-E), and there were interactions between the congeneric

1279   pairs and grass competition, and between congeneric pairs competition and growth forms.

1280   Congeneric pair competition affected subshrubs plant total biomass ($F_{1,60}$ = 10.455, p = 0.002)

1281   and root biomass ($F_{1,60}$ = 10.996, p = 0.001), but not the tree plant biomass ($F_{1,60}$ = 0.135, p =

1282   0.714) or tree root biomass ($F_{1,60}$ = 0.001, p = 0.999). Moreover, the negative effect of grass and

1283   congeneric pair competition just occurred when applied alone. When grass competition was

1284   analised as a treatment the congeneric pair competition did not affect plant total biomass ($F_{1,52}$ =

1285   0.120, p = 0.731), root biomass ($F_{1,53}$ = 0.399, p = 0.530) or shoot biomass ($F_{1,52}$ = 0.115, p =

1286   0.735). The inverse was equally true, when the congeneric pair competition was present the grass

1287   did not exert any effect on plant total biomass ($F_{1,54}$ = 0.266, p = 0.608), root plant biomass ($F_{1,47}$

1288   = 0.015, p = 0.903) or shoot biomass ($F_{1,52}$ = 0.036, p = 0.850) (Figure 2B-D).

1289
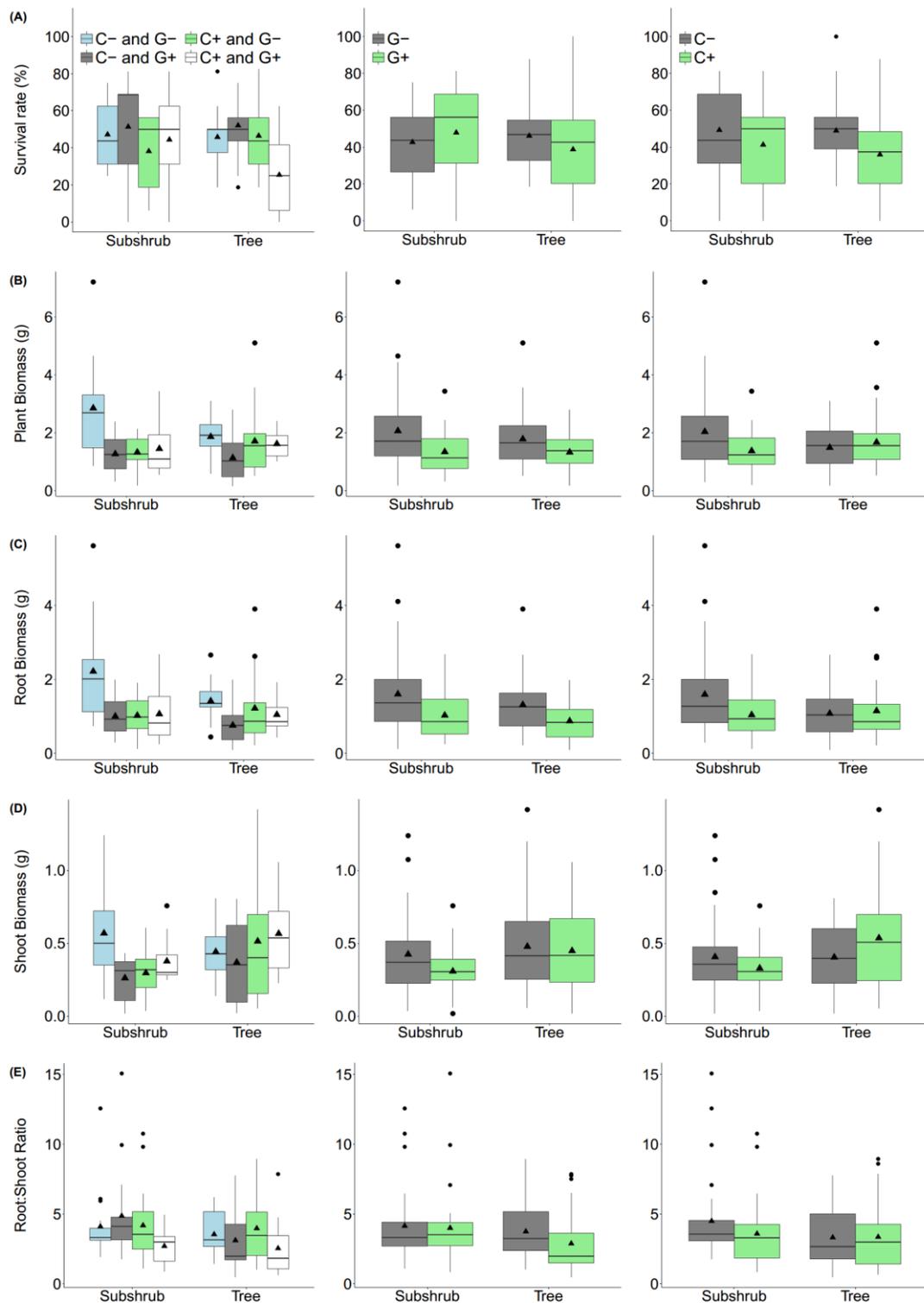
Figure 2. A) Survival rate, B) Plant Biomass, C) Root Biomass, D) Shoot Biomass and E) Shoot:Root ratio of subshrubs and trees separated by grass competition (presence - G+, absent - G-), and congeneric pair competition (presence - C+, absent - C-). The box plots indicate median, quartiles, and data range. Dots denote outliers and triangles means.

Discussion:

Coexistence of multiple growth forms in savannas is possible by a combination of niche segregation, intra and inter growth forms competition, asymmetry of competition and frequent disturbance, particularly fire (Scholes & Archer 1997; Rossatto et al. 2015; Accatino et al. 2016). Yet, the multiple competitive forces carried out by a mix of species can attenuate the intensity that exist in pairwise competition, and ensure the coexistence (Aschehoug & Callaway 2015). The attenuated effect seems to happen in our study when the competitive negative effect of grass inhibits the effect of congeneric pair competition, and vice-versa, in plant biomass (Figure 2B-D). Oppositely, the effect of both competitive factors seems to be synergetic when related with tree survival (Figure 2A).

The grass competition reduced trees and subshrubs growth (Figure 2), probably because grass cover can reduce light, water and nutrient resources availability (Scholes & Archer 1997). The reduction in biomass allocation pattern can result in a lower resources exploration by both growth forms (Wardle & Peltzer 2003), and consequently reduce root reserves content, that is necessary to survive after perturbation, and root depth, mainly in trees, that is a requirement to extract water in depth soil during the dry season. Although grass competition can decrease the chance of establishment and growth of both growth forms, this effect is probably not strong enough to generate total competitive exclusion (Scholes & Archer 1997). We believe that the coexistence of multiple growth forms in savannas could be ensured by environmental heterogeneity and ecophysiological differences that exist between growth forms.

As tree survival is negatively affected by a synergic effect of congeneric pairs and grass competition, and growth better in the presence of both together, but not in the presence of one or another, the tree life-history is complex and the tree establishment is dependent on root gaps at least in the beginning of development. The root gap hypothesis postulates that the absence of grass roots in patches within the landscape is ensured by environmental heterogeneity. In these patches trees could be able to establishment and grow normally (Cramer et al. 2012; Wakeling et al. 2015). The formation of root gaps is related with a reduction in fire frequency and intensity, that is related with rainfall, grazing and animal trampling (Van Langevelde et al. 2003; Bond & Keeley 2005; Accatino et al. 2010; Koerner & Collins 2014). Once the tree is established and can escape from the fire-trap (see Wakeling et al. 2011; Dantas & Pausas 2013), it can alter the conditions under canopy, allowing the establishment of more trees and the savanna encroachment (Scholes & Archer 1997; Accatino et al. 2016; Rossatto & Rigobelo 2016).

Subshrub survival and growth are mitigated when both grass and tree competition are together (Figure 2), however when alone with the tree congeneric pair the subshrubs lose the competition and grow less. The negatively competitive effect of trees against subshrubs could explain why the subshrubs are less abundant in forest than in savanna ecosystems (BFG 2015), since in forests the grasses are not present, and against trees they are inferior competitors. Yet, the root gap hypotheses could explain the establishment of subshrubs in savannas, with the advantage that this growth form has high fertility (Westoby et al. 2002), since their seeds are smaller (Díaz et al. 2016), that allow them to reach more patches than trees. Subshrubs are also able to

1335   reproduce early (Zizka et al. 2014), and can colonize patches were trees and grasses were

1336   present together, enhancing their fitness in neotropical savanna.

1337   The Neotropical savanna burn, on average, once every five years (Gottsberger & Silberbauer-

1338   Gottsberger 2006), and the main effect of this fire frequency is the absence of savanna

1339   encroachment, with an abundant grass layer, and scattered trees in the landscape (Bond &

1340   Keeley 2005; Bond 2008). This type of landscape is able to keep biodiversity, indicating how fire

1341   regime is important to maintain the biodiversity, mainly subshrubs, in savannas. If fire frequency

1342   is reduced, probably the trees abundance will increase, and consequently, as trees are able to

1343   suppress the grass development, the subshrubs will be reduced, as they are inferior competitors

1344   in a pairwise relation with trees. In conclusion, the mitigated effect of multiple competitive forces,

1345   together with fire regime are able to keep biodiversity in Neotropical savanna, and if natural areas

1346   are not correctly fire managed, the biodiversity could be reduced.

1347

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Conclusão Geral

O principal motivo da realização deste estudo foi verificar os motivos das pequenas plantas, ervas e subarbustos serem tão abundantes no ambiente savânico. No primeiro capítulo foram investigadas as diferenças nas características de peso de semente, taxa de germinação, taxa de sobrevivência, taxa de fotossíntese e respiração, área foliar especifica, capacidade de rebrota e alocação de biomassa. Essas características foram investigadas com o intuído de se testar a hipótese de que as espécies de subarbusto representariam o ponto final da história evolutiva no bioma Cerrado, possuindo menor área foliar específica, maior investimento em raízes e reservas, e maior capacidade de rebrota. Embora árvores e subarbustos sejam muito diferentes quando adultos, surpreendentemente a maioria destas características são semelhantes entre plântulas de subarbustos e árvores. Entretanto acredita-se que as pequenas diferenças encontradas na alocação de biomassa e peso de sementes possam resultar, com o tempo, em grandes diferenças nas histórias de vida de subarbustos e árvores.

No segundo capítulo, foram investigadas as plantas capazes de se reproduzir após o fogo, incluindo as diferenças entre as formas de vida e as síndromes de polinização ou dispersão. Os resultados não corroboraram as hipóteses levantadas de que subarbustos seriam as espécies mais favorecidas em reprodução após o fogo, ou mesmo que as síndromes de polinização e dispersão por vento ocorreriam mais frequentemente após o fogo. Foram encontradas 2.058 espécies, distribuídas em mais de 111 famílias, capazes de se reproduzir após o fogo, sendo 60% delas subarbustos. As árvores tiveram uma razão de chance de se reproduzir maior do que o esperado ao acaso, razão essa superior à dos subarbustos, embora não significativamente diferente. No que se refere à polinização, novamente os resultados não corroboraram as hipóteses estabelecidas, e nenhuma síndrome foi favorecida em plantas que se reproduzem após o fogo. Provavelmente, o não favorecimento de nenhuma síndrome de polinização ocorre devido a um aumento dos dispersores, o que não gera pressão de seleção a favor de uma ou outra síndrome. A rápida recuperação da estrutura das formações savânicas após a passagem de fogo indica que há baixa pressão de seleção em favor da dispersão anemocórica.

No último capítulo foram testadas hipóteses nas quais subarbustos e árvores diferiam quanto a capacidade competitiva contra o estrato graminoso. Além disso, uma hipótese alternativa era de que as duas formas de vida eram igualmente afetadas pela grama. Também se testou o efeito da competição de subarbustos contra gramas, e a interação gramas e presença de outra forma de vida. Os resultados indicam que não há simetria entre a competição exercida entre subarbustos e árvores. Árvores exercem efeito negativo em subarbustos, mas o inverso não ocorre. Gramíneas influenciam negativamente as duas formas de vida, mas a competição exercida sobre os subarbustos é atenuada pela presença concomitante de gramíneas e árvores. Em árvores, o efeito competitivo não é atenuado, ao contrário, subarbustos e gramíneas atuam de forma sinérgica e reduzem o estabelecimento das árvores. Desta forma, o estabelecimento de árvores é dependente de "*root gaps*" e o de subarbustos poderia ocorrer tanto nos "*root gaps*" quanto na presença de árvores com gramíneas.

1499 A síntese destes três capítulos fomenta a hipótese de que a diferença encontrada na abundância
1500 de subarbustos em relação às árvores poderia se dar pelas diferenças nas características de
1501 alocação de reservas, o que poderia conferir uma maior chance de sobrevivência em eventos
1502 sucessivos de fogo, ou na escassez de nutrientes. Além disso, o próprio peso das sementes
1503 poderia resultar em aumento de "*fitness*" por parte das plantas menores, uma vez que uma
1504 semente menor seria facilmente enterrada, e, portanto, menos predada e susceptível ao fogo.
1505 Embora possa parecer que existem diferenças nas chances reprodutivas de subarbustos e
1506 árvores, ambas as formas de vida são igualmente capazes de se reproduzir após o fogo.
1507 Acredita-se que a aquisição precoce da capacidade reprodutiva de espécies subarbustivas possa
1508 garantir um maior "*fitness*" em ambientes pirofíticos, e a demora em atingir o tamanho reprodutivo
1509 por parte das árvores acaba por ser um limitante à proliferação sexuada dessa forma de vida.
1510 Além disso, as formas arbóreas teriam maior chance de se estabelecer em locais onde há a
1511 presença de "*root gaps*", reduzindo assim as oportunidade de estabelecimento das mesmas,
1512 enquanto as formas subarbustivas se estabeleceriam na presença de gramíneas e árvores.

1513 As adaptações apresentadas pelas diferentes formas de vida ao ambiente pirofítico demonstram
1514 a importância do fogo como modelador das comunidades. A grande quantidade de espécies
1515 capazes de se reproduzir, distribuídas em diferentes famílias e formas de vida, mostra que além
1516 das características que favorecem a sobrevivência, as características reprodutivas poderiam ser
1517 vistas como uma características que favorece a persistência em ambientes pirofíticos.