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

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Cover Letter

Dr. Gustavo Habermann
Editor-in-Chief
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Dear Dr. Gustavo Habermann

We are submitting the manuscript entitled “Early growth in a congeneric pair of savanna and seasonal forest trees under different nitrogen and phosphorus availability”, by Bruno Paganeli, Kyle Graham Dexter, and Marco Antonio Batalha, to be considered for publication in Theoretical and Experimental Plant Physiology.

In this paper, we report the initial development of two congeneric species, one typical to savanna physiognomies of the Brazilian cerrado and the other to the neighbouring seasonal forest. Our work used an innovative methodology in plant cultivation. We also obtained results regarding phosphorus toxicity on savanna seedlings. The species displayed significant differences in all analyzed traits, with the savanna species performing better under nitrogen and phosphorus depletion and the forest one presenting higher nutrient demand.

We believe that this manuscript is appropriate for publication in Theoretical and Experimental Plant Physiology, because it includes aspects related to plant mineral nutrition, ecology and instrumentation in plant physiology, all included in the scope of the journal.

There are no conflicts of interest to disclose and the paper has never been published. We appreciate your kindness and are looking forward to hearing from you soon.

Sincerely,

Bruno Paganeli

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

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Running title Nutritional conditions and savanna and seasonal forest seedlings development

2 tables; 6 figures; 26 pages; 7413 words.

The dynamics between savanna and forest borders is not completely understood.
Typical species from these biomes are ecologically different even as seedlings.
The savanna species grew slower and had lower N and P demands than the forest one.

Abstract

Most of the physiognomies of the Brazilian cerrado fall within the definition of tropical savanna. However, patches of seasonal forest are interspersed within the cerrado. The occurrence of savannas in areas whose climate allows forests may be related to the nutrient-poor soil, especially nitrogen and phosphorus. We analysed the initial development of a congeneric pair, *Handroanthus aureus*, a savanna species, and *H. impetiginosus*, a seasonal forest species, to test whether these nutrients shape their functional traits differently. We used a hydroponic system with four treatments: (1) Complete Hoagland solution cointaining N, P, K, Ca, Mg, S, O, H, Cl, B, Mn, Zn, Cu, Mo and Fe (2) Hoagland solution without phosphorus, (3) Hoagland solution without nitrogen, and (4) Hoagland solution without both nutrients. We followed the plants for three months and measured total biomass, aboveground biomass, root to shoot ratio, height, cotyledon persistence, appearance of the first pair of leaves, and leaf area. Growth of both species was sensitive to nitrogen availability. Supplemental phosphorus increased values of all traits in forest species but decreased some trait values in the savanna species. Except for root to shoot ratio and height which were higher in *H. impetiginosus*, the values for the other traits were consistently higher in *H. aureus*. The savanna species was more efficient overcoming N and P deficiencies. The two species were already ecologically distinct at early stages of development, which may be related to their evolutionary history regarding nutrients availability.

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

Introduction

The Brazilian cerrado presents a wide range of physiognomies (Coutinho 1990), going from grassland to forest, but with most of its physiognomies within the range of tropical savanna (Bourlière and Hadley 1983). Patches of other vegetation types are interspersed within the cerrado, such as riparian forest, palm forest, deciduous seasonal forest, semi-deciduous seasonal forest, rocky grassland, and floodplain grassland (Coutinho 1990). Since these vegetation types occur as a mosaic, under the same climatic zone, climate is not sufficient to explain the variety of physiognomies (Bueno et al. 2018). Other factors, such as fire frequency, water availability, and soil features, have been postulated to explain it (Viani et al. 2011, Dantas et al. 2013, Oliveras & Malhi 2016). The occurrence of savanna or seasonal forest in a given area may be related to a "fertility gradient" – the former on poorer soils and the latter on richer ones (Goodland and Pollard 1973; Bond 2010).

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

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

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

Although total biomass may be an excellent predictor of community functioning (Grime 1998), the root to shoot ratio has also been frequently used to indicate biomass allocation strategies and, consequently, adaptation for foraging above or belowground. Root to shoot ratios are often higher under conditions of nutrient limitation (Poorter et al. 2012) and higher in savanna than in forest tree species (Hoffmann and Franco 2003; Hoffmann et al. 2004; Loiola et al. 2015; Miatto et al. 2016). Similarly, previous works with congeneric savanna and forest species found taller trees with larger leaves in the forest than in the savanna (Hoffmann and Franco 2003; Hoffmann et al. 2005; Hoffmann and Franco 2008), probably related to improved light interception (Onada et al. 2014). Admittedly, the nutritional reserves stored in seeds and consequently on the cotyledons can meet all demands for the early seedlings development, since it may also have a photosynthetic role (Gogosz and Boeger 2019; Green and Juniper 2004). However, specialised organs for these functions, such as roots and true leaves, are more efficient (Zhang et al. 2008 a, b). Since forest species grow faster than savannas ones (Viani 2011; Gignoux et al. 2016), it is expected the exhaustion and lose of the cotyledon and the appearance of the first pair of leaves to occur first in the forest species.

We studied the relationships between mineral nutrition and initial development in a congeneric pair of species occurring in the Brazilian Cerrado Domain, one from the savanna and the other from the semi-deciduous seasonal forest. Based on previous work, we postulated that the savanna species would grow slower, investing more in belowground biomass being shorter, and presenting smaller leaves. Thus, we tried to answer the following questions: (1) do cotyledons last longer in the savanna species than in the forest one?; (2) does the appearance of the first pair of leaves take longer in the savanna species?; (3) does the cerrado species grow slower than the forest one?; (4) is the root to shoot ratio higher in the savanna species?; and (5) are the leaves and heights in the forest species larger when compared to the savanna species? (6) Do these characteristics differ distinctly in both species according to nitrogen and phosphorus supply?

Materials and methods

To compare the early development in woody species of two adjoining vegetation types, the savanna and the semi-deciduous seasonal forest, we used as a model a congeneric pair, *Handroanthus aureus* Mattos and *Handroanthus impetiginosus* (Mart. Ex DC.) Mattos, belonging to the Bignoniaceae family. *Handroanthus aureus*, popularly known as “yellow ipê”, occurs in savanna, reaching up to 15 m in height, whereas *H. impetiginosus*, popularly known as “purple ipê”, occurs in the neighbouring semi-deciduous seasonal forest, where it can reach 30 m (Sano et al. 2008). Both species are common in their respective vegetation types, have ornamental, medicinal, and construction applications, and are used in the restoration of degraded areas (Cabral et al. 2004; Oliveira et al. 2005).

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

We took the plastic tubes to a greenhouse located in São Carlos (21°59'01"S, 47°52'50"W; southeastern Brazil), where cooling, humidification, ventilation, and exhaustion were automatically controlled, so that the temperature was kept between 20°C and 28°C, the air relative humidity between 60% and 80% under natural light regime. To test the effect of nutrient depletion on plant growth, we cultivated the seedlings in closed hydroponic systems, in which the nutrient solutions were recycled (Prado and Casali 2006; Jensen 2007). We had four treatments: (1) complete Hoagland solution, (2) Hoagland solution without nitrogen, (3) Hoagland solution without phosphorus, and (4) Hoagland solution without nitrogen and phosphorus. All treatments had their ionic strength reduced by 50% (Table 1). We measured the initial values and carried out weekly measurements of pH and electrical conductivity. We added distilled water or solution whenever the initial values changed. We carried out a complete exchange of all solutions monthly.

We placed eight boxes on aluminium stands. For each combination of nutrients, we used two boxes, one for the savanna species and the other for the forest species. To avoid algae proliferation, we covered the boxes with aluminised thermal blankets to prevent the entrance of light. Each box had a volume of 40 L, was filled with its respective solution, and had a support for 18 plastic tubes. The two boxes of each treatment were connected to a 100 L reservoir by a silicone hose, at one end with a T-connector and at the other end with a submersible motor pump, SB 1000c model, that remained inside the reservoir. The solution was propelled by the motor pump, going through the hose, to the T-connector, and to the two boxes. We set a timer to propel the solution in five cycles daily: at 06:30am, 10:30am, 12:30pm, 2:30pm, and 5:30pm. The timer remained on for 3 min, the time necessary to move the solutions, homogenise the volumes of the reservoirs, and moisten the clay. At the end of each cycle, the solution returned to the reservoir by gravity.

We observed the growth and development of the 144 individuals monthly, for three months. For each species, each treatment, and each month, we harvested and measured six individuals. We separated the below- from the aboveground portion, oven dried them at 80°C for 72 hours and weighed their dry mass. We measured the total biomass and calculated the root to shoot ratio, dividing the below by the aboveground portion, which is appropriate to assess biomass allocation (Poorter et al. 2012) and is used as a proxy for plant vigour (Ros et al. 2003). Although harvest took place over three months, the emergence was accounted as soon as it occurred. Similarly, we recorded the duration of the cotyledons. At the end of each month, we measured height by taking the distance from the hypocotyl base to the apical bud. We scanned the leaves and used ImageJ (Rueden et al. 2017) to measure their areas.

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

Results

Except for root to shoot ratio and height which were higher in forest species, values for other functional traits were consistently higher savanna species. In the presence of nitrogen all functional traits increased, in both species. The phosphorus on its turn, brought increment in values of all functional traits regarding forest species but decrease some in the savanna.

Concerning total biomass, the savanna individuals (0.69 g ± 0.39, mean ± sd) were heavier than those from forest (0.37 g ± 0.25) ($P < 0.001$). On average, individuals responded positively to nitrogen addition ($P = 0.037$), but there was a significant interaction between species and nitrogen ($P = 0.015$), with the forest species responding more positively to nitrogen addition (Figure 1). There was a strong interaction between species and phosphorus ($P = 0.001$), because the savanna species responded negatively to phosphorus addition whereas the forest species responded positively (Figure 1). This opposite pattern resulted in a non-significant effect of phosphorus addition on total biomass ($P = 0.786$).

Savanna individuals (0.37 g ± 0.19) had larger aboveground biomass ($P < 0.001$) than those forest ones (0.18 g ± 0.09). Aboveground growth over time was higher in savanna species than in forest one ($P = 0.093$). On average, individuals responded positively to nitrogen addition ($P < 0.001$). Although phosphorus was not significantly influential on aboveground biomass ($P = 0.336$), there was a significant interaction between species and phosphorus ($P = 0.005$), because the savanna species responded negatively and the forest species, positively (Figure 2).

Root to shoot ratio was higher ($P = 0.004$) in the forest (1.0 ± 0.6) than in savanna species (0.8 ± 0.6). Both species showed significant increases in root to shoot ratio over the course of the experiment, with the preferential allocation to the root system most evident in the last month ($P < 0.001$). The increase in root to shoot ratio was higher in the forest species ($P < 0.001$; Appendix 1). Higher ratios were observed in both nitrogen ($P < 0.001$) and phosphorus ($P = 0.002$) depleted solutions. Although both species presented similar strategies in the presence of either nitrogen ($P = 0.061$) or phosphorus ($P = 0.214$), there was a significant interaction between species and the presence of both nutrients ($P = 0.002$; Figure 3).

Individuals of the forest species (78.7 mm ± 25.4) grew taller ($P < 0.001$) than those of the savanna (19.1 mm ± 3.6), with the time being significant for height increase ($P = 0.011$). Plant height was not affected by either nitrogen ($P = 0.425$) or phosphorus ($P = 0.451$). However, there was a significant interaction between species and phosphorus ($P = 0.004$), since phosphorus addition decreased the growth of savanna species, but increased that of forest one (Figure 4). The savanna species grew taller in the presence of nitrogen, but once again, when phosphorus was available, it had a negative effect on height (Figure 4). When we added both nutrients, the forest species showed a large growth in height, suggesting nitrogen and phosphorus co-limitation.

Cotyledons lasted longer in the savanna species, whose individuals kept theirs until the last day of the experiment, than in the forest one, whose individuals lost their cotyledons during the third month. There was no significant difference concerning the nutrients or the interaction terms. The first pair of leaves appeared later ($P < 0.001$) in the savanna species (19.5 days ± 2.6) than in the forest one (15.6 days ± 4.2; Figure 5). Neither nitrogen ($P = 0.839$) nor phosphorus ($P = 0.974$) had an effect on the appearance of the first leaves. Leaves of the savanna species (44.3 cm2 ± 27.3) were larger ($P < 0.001$) than those from forest (25.8 cm2 ± 11.5). Leaf area increase over time was higher in the former than in the latter ($P = 0.007$). Overall, individuals responded positively to nitrogen addition ($P < 0.001$) and did not respond to phosphorus ($P = 0.069$). Both species responded the same way to the nitrogen addition ($P = 0.746$), but differently to phosphorus ($P < 0.001$), with savanna species responding negatively and the forest one positively (Figure 6).

Discussion

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

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

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

Although the biomass of the two species seemed to be mainly affected by nitrogen, demand for phosphorus was different, since the savanna species did not show a decrease in biomass when in a solution without phosphorus. Cotyledon reserves may provide an adequate supply of this essential element and, consequently, ensure metabolic efficiency. Apparently, in the initial phase of their lives, individuals from the forest assimilated a greater amount of organic matter in the presence of both nutrients, whereas those from savanna did so only in the presence of nitrogen. As previously observed, forest species have greater nutritional demand and perform better in environments with higher nutrient availability (Bond 2010; Goodland and Pollard 1973; Pellegrini 2016; Silva et al. 2013). The savanna species, on the contrary, was less sensitive to nutrient depletion, which could improve its chances of overcoming nutritional limitations imposed by an oligotrophic environment (Bustamante et al. 2012). These patterns could be fit into divergent plant nutritional strategies which allow savanna and forest species to live in contrasting edaphic characteristics in neighbouring sites (Maracahipes et al 2018).

Given that, in all treatments, the cotyledons remained connected to the plant in the savanna species through the whole experiment, whereas they were dropped during the third month in the forest species, we may postulate that either the reserves were larger or the consumption was slower in the savanna species. In both cases, the need for external sources of nutrients — especially phosphorus in our study — would be reduced, which seems to be an advantageous strategy for the recruited individuals. Although this potential strategy does not alter the total amount of phosphorus in the soil, nutrients present in absorbable forms would be available to already established individuals. Since the seedlings have a high mortality rate (Collet & Moguedec 2007) and this resource consume make it momentarily unavailable, this performances decreasing competition also at the community level, a good strategy in an environment that, due to its high leaching rate, has a phosphorus shortage (Matzek and Vitousek 2009).

Admittedly, *H. aureus* — savanna species — seeds are heavier than those of *H. impetiginosus* — forest species (Felix et al. 2018), and because all individuals of savanna retained their cotyledons, it supports the idea of less external nutritional dependence in the savanna than in the forest species, and the great relevance of these structures in early growth (Ferreira et al. 2017). Since cotyledons lasted less time and leaves appeared earlier in forest species, it showed faster development and early increase in nutrient demand than the savanna species (Gignoux et al. 2016; Viani et al. 2011).

Similarly to traits previously analysed, fertilisation with one or both nutrients led to an increase in leaf area for forest the species, whereas, for the savanna one, this only took place in the presence of nitrogen. These results are indicative not only of different nutritional requirements between the two species, but also a probable phosphorus toxicity (Silber et al. 2002, Hasmah et al. 2015) in the initial development of the savanna species*,* since the fertilisation with phosphorus decreased its leaf area. Relating leaf physiology of the savanna species with biomass acquisition, we may postulate that leaf area was the main characteristic that provided the highest acquisition of organic matter displayed in the previous traits. Morphological and physiological characteristics of savanna tree leaves are mainly a consequence of oligotrophic soils, including macrophily (Aerns 1958; Coutinho 2002). However, in comparative studies with congeneric forest and savanna species, leaf area was found to be higher in forest species (Hoffmann et al. 2005; Hoffmann and Franco 2003; Hoffmann and Franco 2008), even in studies that used the same species than us (Capuzzo et al. 2012). Since water loss through transpiration is the main physiological limitation in plants with large leaf areas (Taiz and Zeiger 2002), leaf attributes may be altered by water availability (Wright et al. 2001).

According to our results, the demand and acquisition of nitrogen and phosphorus in a typical congeneric pair of savanna and forest species are different already in their early stages. Nitrogen-free solutions led to a reduction in growth in both species. However, the extra cotyledonary phosphorus improved the growth in the forest species but decrease the values of some functional traits in savanna seedlings. Thus, if other typical species from these biomes present patterns similar to those found here, the intense and unbalanced inputs of nitrogen and phosphorus in terrestrial communities (Peng et al. 2019) may became an environmental driver on those susceptible mosaics (Oliveras and Malhi 2016).

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

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

Table 2. In the cells there are the p values. The statistically significant are in bold. **T.B.**: Total Biomass; **A.B.**: Aboveground Biomass; **R/S**: Root to Shoot ratio; **HEI**; Height; **A.F.L.**: Appearance of the First pair of Leaves; **L.A.**: Leaves area; **Spp**: Species; **N**: Nitrogen; **P**: Phosphorus; **Tim**: Time; **N:Spp**: Interaction between Nitrogen and Species; **P:Spp**: Interaction between Phosphorus and Species; **N:P**: Interaction between Nitrogen and Phosphorus; **Tim:Spp**: Interaction between Time and Species; **N:P:Spp**: Interaction between Nitrogen, Phosphorus and Species.

	Spp	N	P	Time	N:Spp	P:Spp	N:P	Time:Spp	N:P:Spp
T.B.	**<0.00001**	**0.03728**	0.78617	**<0.00001**	**0.01535**	**0.00115**	0.89199	0.24556	0.20367
A.B.	**<0.00001**	**0.00010**	0.33608	**<0.00001**	0.12591	**0.00548**	0.57969	0.09302	**0.01715**
R/S	**0.00372**	**<0.00001**	**0.00165**	**<0.00001**	0.06152	0.21449	0.31620	**<0.00001**	**0.00186**
HEI	**<0.00001**	0.42541	0.45145	**0.011937**	0.90620	**0.00458**	**0.01739**	0.10300	**<0.00001**
A.F.L.	**<0.00001**	0.83969	0.97458	0.92393	0.96985	0.11532	0.16939	0.06824	0.30569
L.A.	**<0.00001**	**<0.00001**	0.06985	**<0.00001**	0.74603	**<0.00001**	**0.00602**	**0.00736**	**0.00522**

Fig1 The figure represents the evolution of total biomass logarithm (grams) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the time. T1, T2 and T3 respectively mean: the first, second and third months from the beginning of the experiment. The complete solution is represented by the black solid line; The solution without nitrogen by the dark grey dashed line; The solution without phosphorus by the medium grey dotted line; The solution without both nutrients by light grey dotdash line. The biggest points are the treatment mean and the smallest ones, the individuals' values.

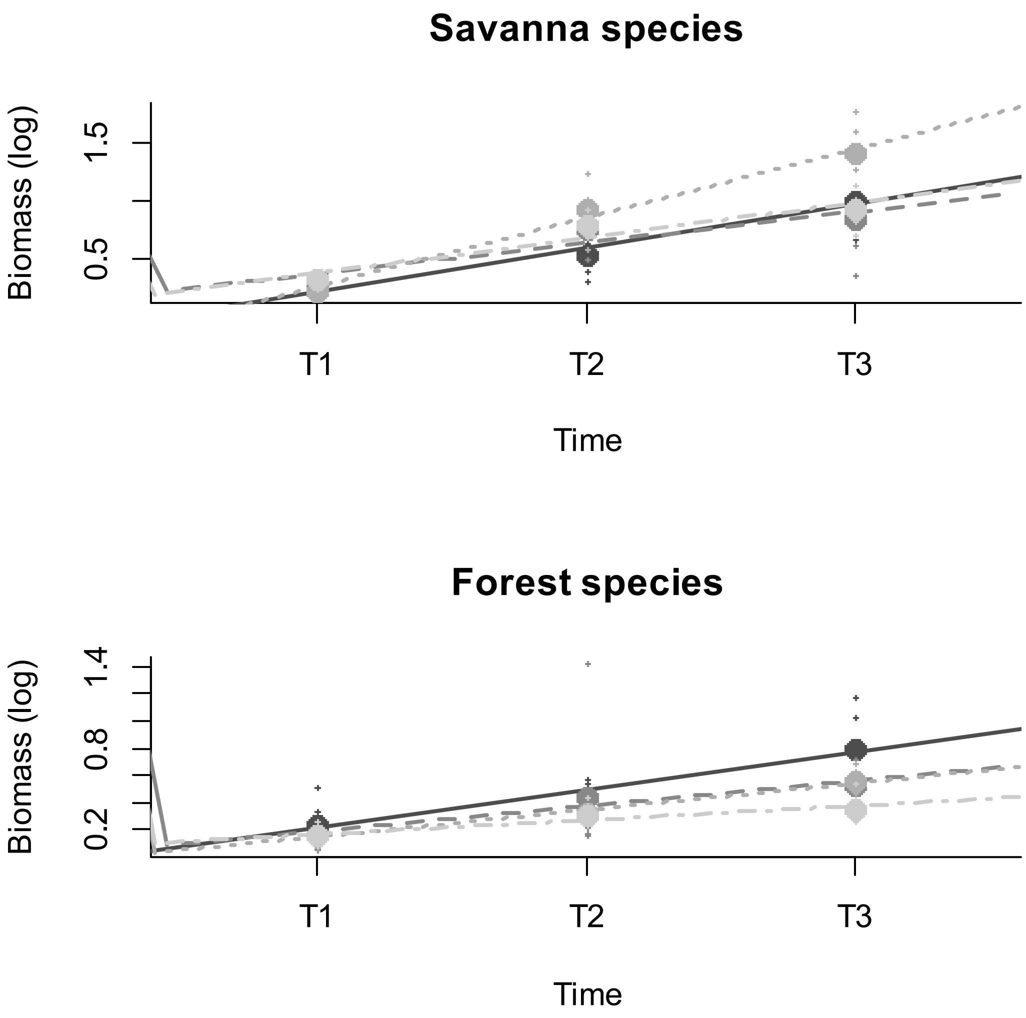

Fig2 The figure represents the evolution of aboveground biomass logarithm (grams) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the time. T1, T2 and T3 respectively mean: the first, second and third months from the beginning of the experiment. The complete solution is represented by the black solid line; The solution without nitrogen by the dark grey dashed line; The solution without phosphorus by the medium grey dotted line; The solution without both nutrients by light grey dotdash line. The biggest points are the treatment mean and the smallest ones, the individuals' values.

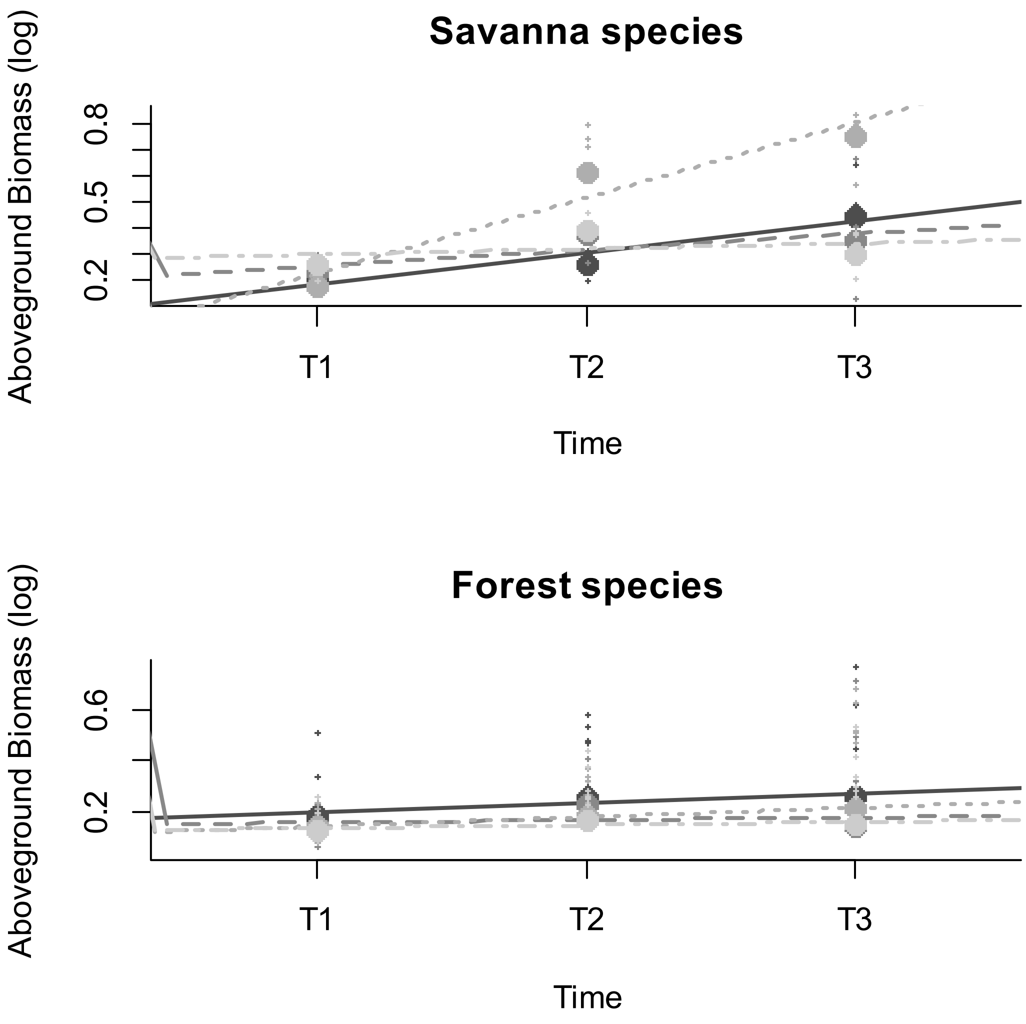

Fig3 The figure represents the evolution of root/shoot ratio square root (sqrt) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the time. T1, T2 and T3 respectively mean: the first, second and third months from the beginning of the experiment. The complete solution is represented by the black solid line; The solution without nitrogen by the dark grey dashed line; The solution without phosphorus by the medium grey dotted line; The solution without both nutrients by light grey dotdash line. The biggest points are the treatment mean and the smallest ones, the individuals' values.

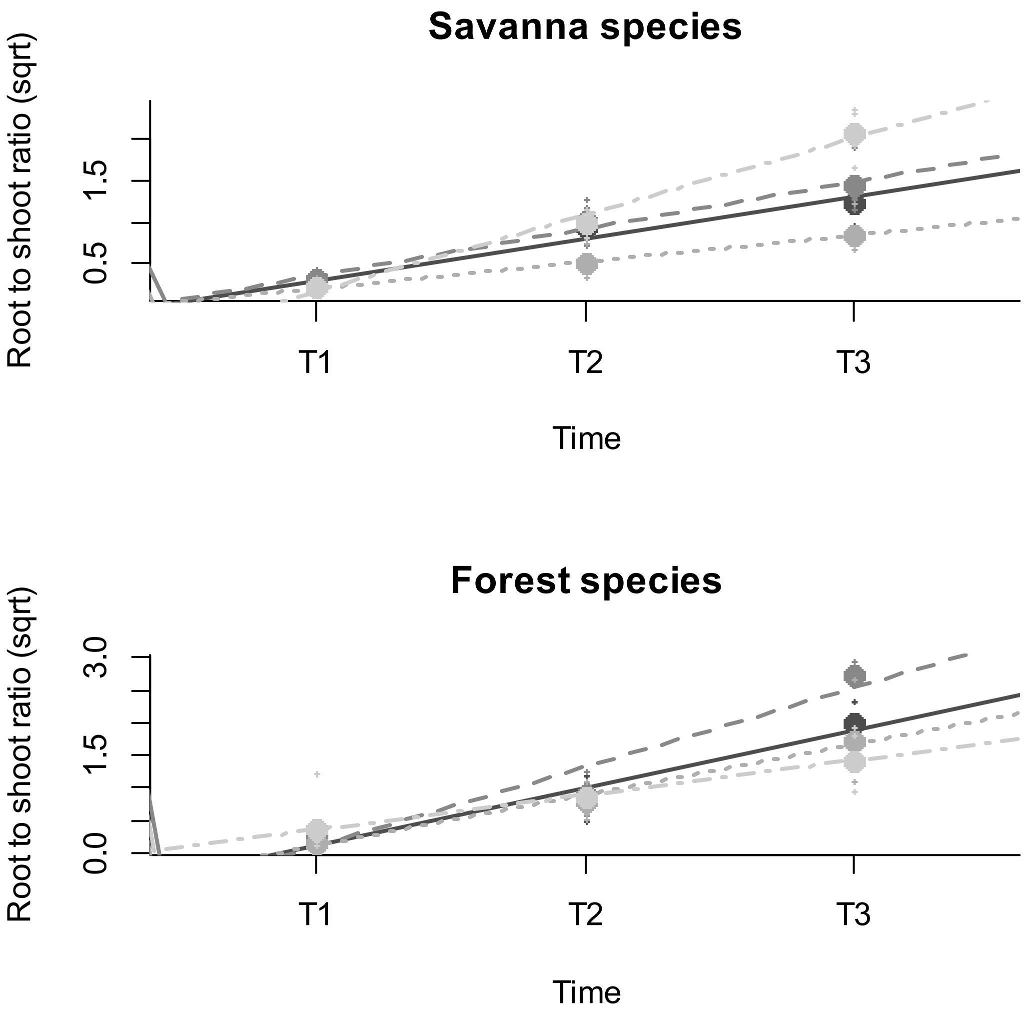

Fig4 The figure represents the evolution of heights ln (mm) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the time. T1, T2 and T3 respectively mean: the first, second and third months from the beginning of the experiment. The complete solution is represented by the black solid line; The solution without nitrogen by the dark grey dashed line; The solution without phosphorus by the medium grey dotted line; The solution without both nutrients by light grey dotdash line. The biggest points are the treatment mean and the smallest ones, the individuals' values.

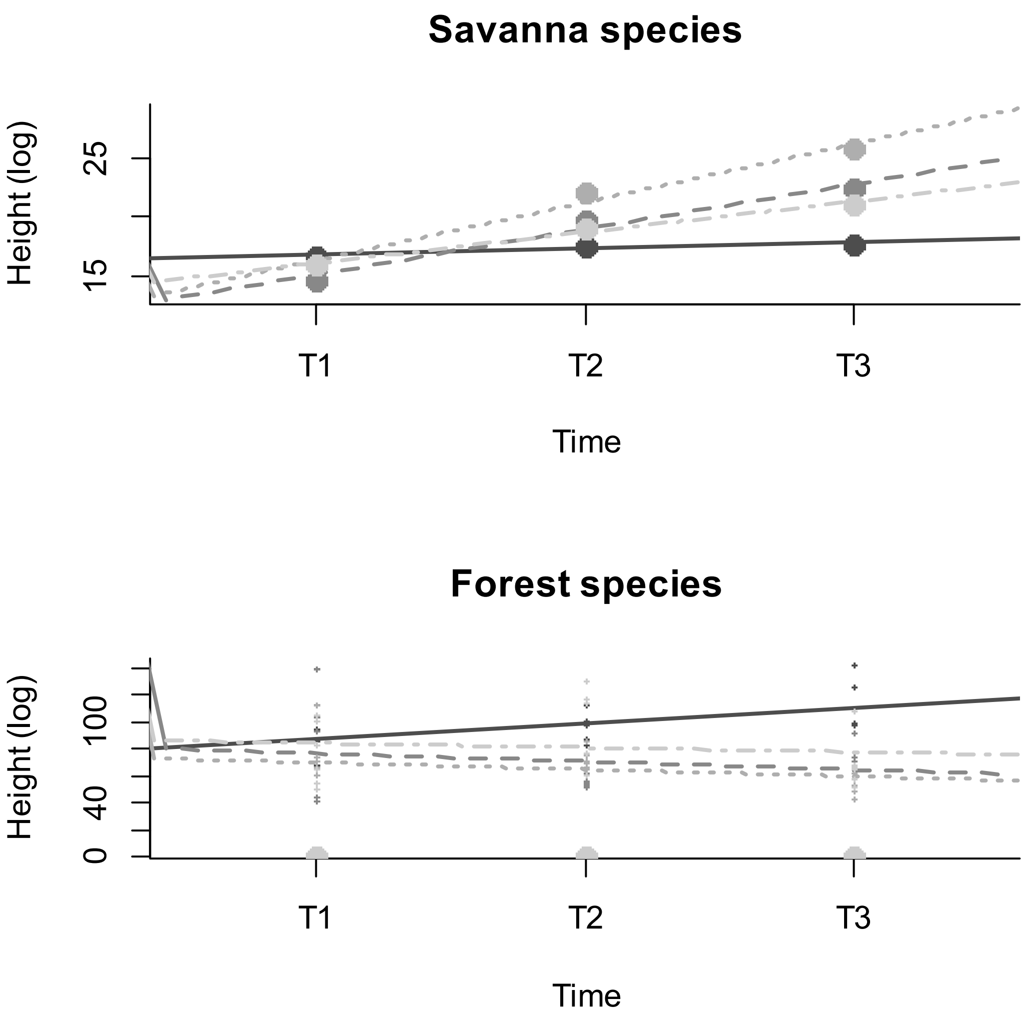

Fig5 The figure represents the evolution of appearance of the first leaves squared (^{2}) (days) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the time. T1, T2 and T3 respectively mean: the first, second and third months from the beginning of the experiment. The complete solution is represented by the black solid line; The solution without nitrogen by the dark grey dashed line; The solution without phosphorus by the medium grey dotted line; The solution without both nutrients by light grey dotdash line. The biggest points are the treatment mean and the smallest ones, the individuals' values.

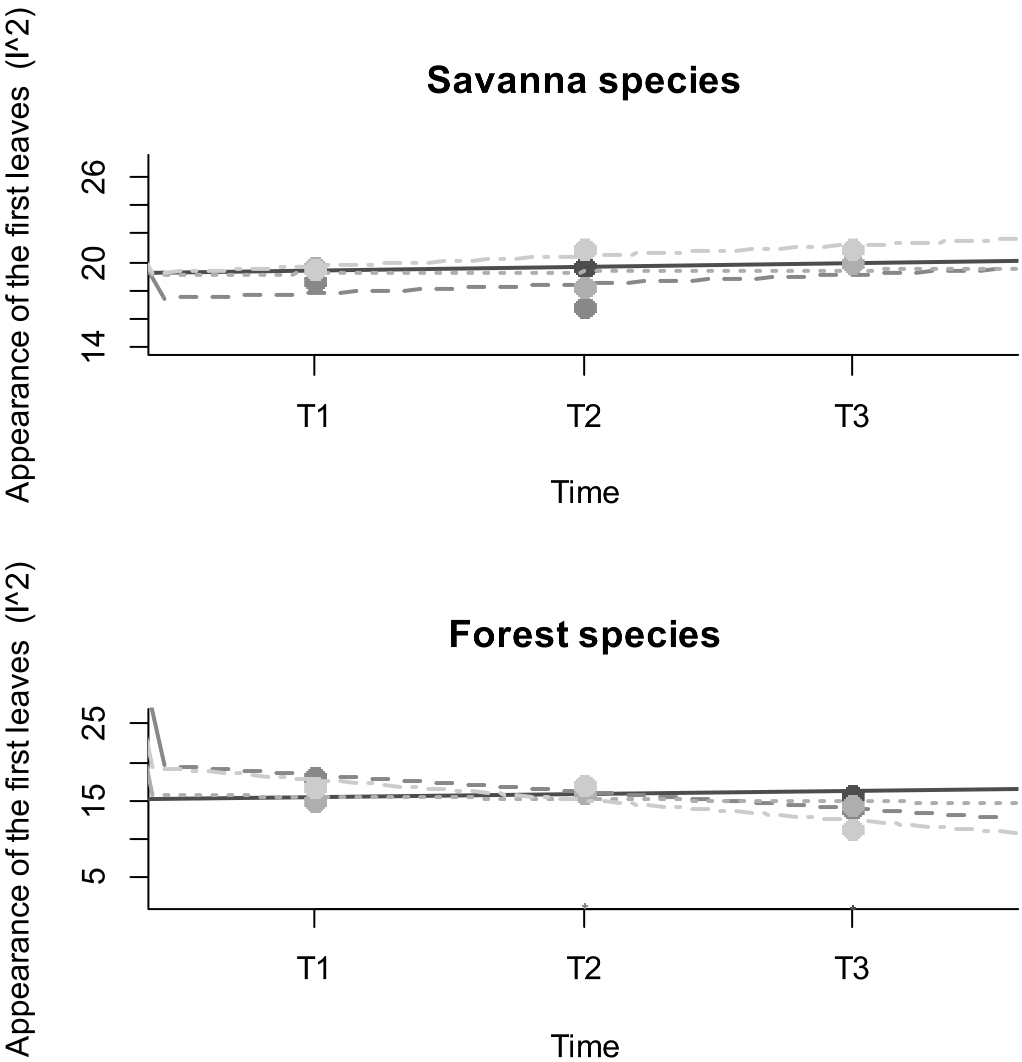

Fig6 The figure represents the evolution of leaf areas squared (^{2}) (cm^2) over time in *H. aureus*, typical savanna species and *H. impetiginosus,* typical from seasonal forest. The x-axis represents the time. T1, T2 and T3 respectively mean: the first, second and third months from the beginning of the experiment. The complete solution is represented by the black solid line; The solution without nitrogen by the dark grey dashed line; The solution without phosphorus by the medium grey dotted line; The solution without both nutrients by light grey dotdash line. The biggest points are the treatment mean and the smallest ones, the individuals' values.