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## No evidence of positive feedback between litter deposition and seedling growth rate in Neotropical savannas

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**Litter addition in the soil have negative or none effect in the growth rates of forest seedlings growing in savanna dystrophic soil.**

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**Key-Words:** Litter decomposition, forest-savanna transition, soil respiration, Cerrado, microorganisms activity.

## **Abstract**

### *Aims*

Plant-soil feedbacks are important drivers of ecosystem dynamics, and have been hypothesized to affect woody encroachment in savannas. Woody encroachment is expected to increase savanna soil fertility through increased deposition of dead organic matter which, once decomposed, release immobilized nutrients, favoring further establishment and growth of woody individuals. In this context, we tested if litter deposition promotes forest seedling growth in dystrophic savanna soils, and if this was parallel by an increase in microbial activity.

### *Methods*

In a greenhouse, we planted woody seedlings of three forest species in savanna soils either mixed or not (control) with litter from closely related savanna or forest species (10 species in total). We evaluated woody seedlings growth as well as the response of the soil microbiota, in terms of activity and biomass, to litter addition.

### *Results*

We found that litter addition had either none or negative effects on seedling growth rates, and that different seedling species responded differently to litter addition. However, we did found microbial activity to increase in response to litter addition , especially if phosphorus (P) and carbon (C) are also mixed in the soil.

### *Conclusions*

Litter addition stimulated soil microbial activity. However, seedling response was not consistent with an effect associated with increased soil mineralization. Instead, plant responses to seedling addition was species-specific, depending both on litter and on

seedling species, and their interactions, but was never positive. Moreover, while the effects of litter input in seedlings growth was potentially mediated by soil microorganism activity, the specific effect was more consistent with a negative or neutral response. Thus, our results did not support the hypothesis that litter deposition trigger positive feedbacks with woody encroachment.

## **Introduction**

In the Brazilian Cerrado, savannas and forests dynamically coexist under similar climatic and edaphic conditions (Hirota et al. 2011; Staver et al. 2011; Bueno et al. 2018). The encroachment of open ecosystems by forests (and vice versa) in savanna-forest mosaics is regulated by fire, resource availability and their feedbacks (Hoffmann et al. 2012; Dantas et al. 2016; Bueno et al. 2018), and dependent on forest species colonization and growth rates, and how it promotes grass exclusion and fire inhibition (i.e. the fire suppression threshold; Hoffmann et al. 2012). The time required to achieve this threshold is dependent on tree community productivity, which is influenced by soil nutrient availability (Hoffmann et al. 2012; Murphy and Bowman 2012). Therefore, by controlling the rate at which forest tree grow and inhibit fire, soil fertility is a key factor influencing the dynamics of forest encroachment in open ecosystems.

It was recently suggested that woody encroachment could be triggered or hindered by feedback mechanisms related to increased litter deposition with woody encroachment and increased soil nutrient mineralization. This would positively affect forest tree growth, accelerating the replacement of savanna by forest (Hoffmann et al. 2012; Dantas et al. 2016; Bueno et al. 2018.. It is well-known that the vegetation influences soil fertility through litter input (Schimel and Bennett 2004; Freschet et al. 2013; Hobbie 2015). Yet, in many cases, the exact mechanism is unclear. Litter input affects soil abiotic conditions

influencing both soil physical structure or chemical composition (Facelli and Pickett 1991; Veen et al. 2019). Litter chemical composition can both positively or negatively affect nutrient availability to plants, depending on the availability of both nutrients (which increases nutrient availability for plant uptake), and secondary metabolites (which is related to the immobilization of nutrients and inhibition of root growth; Meier and Bowman 2008; Lopez-Iglesias et al. 2014; Veen et al. 2019). Moreover, litter physical structure affects seed germination, since it filters the amount of radiation that reaches the soil and increases soil humidity and temperature; it may also act as a mechanical barrier for seedlings emergence (Facelli and Pickett 1991; Hovstad and Ohlson 2008; Veen et al. 2019). Therefore, depending on litter traits and how they influence soil physical structure and chemical composition litter accumulation can have both positive, negative or even neutral effects.

Litter accumulation have also biological effects in the soil, affecting decomposer community composition, activity and biomass (Veen et al. 2019). Litter is the main substrate used by saprophytic microorganism growth whose activity is directly related to litter chemical composition (Zechmeister-Boltenstern et al. 2015; Bani et al. 2018; Veen et al. 2019). Litter addition stimulates soil microbiota growth and activity through the addition of limiting resources (Zechmeister-Boltenstern et al. 2015). Overall, soil microbiota is nutrient-limited, mainly by nitrogen (N) and phosphorus (P), and its activity is usually positively related to litter nutrients concentration (Zechmeister-Boltenstern et al. 2015). However, in specific areas, such as in tropical regions, soil microbiota has been reported to be energy limited, whose activity is positively related to the litter concentration of easily-degradable carbon (C) compounds (Hättenschwiler and Jørgensen 2010; Whitaker et al. 2014; Fanin et al. 2014). On the other hand, the concentration of recalcitrant molecules in litter, such as lignin, is negatively related to soil microbial activity, since recalcitrant molecules are energetically costly to degrade and demands specific enzymes that are

produced by a limited range of microorganism species (Bani et al. 2018). Therefore, whether litter addition has a positive, neutral or negative effect in plant growth will depend both: 1) on the total amount of nutrients available in the litter and that can be readily decomposed; and 2) on the balance between these nutrients and the fraction that is retained in the microbial biomass and is also not available for plants.

The effects of litter addition in seedlings and soil microbiota growth are related to litter decomposition rates (Freschet et al. 2013). Litter decay rates are determined by decay agent activity, which is responsible for the physical degradation of litter and the release of molecules and elements immobilized in dead tissues (Cotrufo et al. 2013; Bani et al. 2018). Since the activity of saprophytic soil microbiota is influenced by litter traits, litter decay rates are best predicted by litter chemical composition (Zhang et al. 2008; Cornwell et al. 2008; Makkonen et al. 2012). Overall, litter with higher concentration of limiting elements to soil microbial activity and lower concentration of recalcitrant compounds – i.e. high-quality litter – decomposes faster than litter with lower concentration of limiting elements and higher concentration of recalcitrant compounds – i.e. low-quality litter (Zhang et al. 2008; Cornwell et al. 2008; Hättenschwiler and Jørgensen 2010). On the other hand, it is expected that litter decay rates influence the response of plants to litter addition, since the physical and chemical effects of litter on plant growth and development are dependent on the maintenance of its physical structure or on the degradation of molecules and release of elements immobilized in dead plant tissue (Freschet et al. 2013).

The amount and the characteristics of litter produced by an ecosystem are closely related to plant species' resource-use strategy (Freschet et al. 2013). In the Cerrados, resource-acquisitive woody species predominantly occurs in seasonally dry forests and gallery forests (hereafter forest species; Dantas et al. 2013; Viani et al. 2014; Miatto and Batalha 2016; Maracahipes et al. 2018). Resource-acquisitive species produce litter of high nutrient concentration and low carbon and phytotoxic compounds concentration

(Freschet et al. 2013). On the other hand, resource-conservative woody species predominantly occurs in savannas (hereafter savanna species; Dantas et al. 2013; Viani et al. 2014; Miatto and Batalha 2016; Maracahipes et al. 2018) and produces litter of low nutrient concentration and high carbon and phytotoxic concentration (Freschet et al. 2013). The differences in litter production and characteristics of forests and savannas woody species leads to differences in patterns of nutrient cycling between these ecosystems. For instance, there is a higher input of nutrients and organic matter through litter in forests than in savannas, which is expected to have consequences in the dynamics and distribution of the dominant vegetation type (Silva et al. 2008, 2013; Paiva et al. 2015). It has been hypothesized that the high input of nutrients and organic matter through litter by woody forest species increases soil fertility and favors the further development and expansion of forests into savanna areas (Silva et al. 2008, 2013; Paiva et al. 2015).

Therefore, in this study, we evaluated how litter addition is related to the activity and biomass of savanna soil microbiota, and how litter addition influences the response of forest seedlings growing in savanna soils. Specifically, we tested if: (1) litter addition increases soil microbiota activity and biomass through the addition of limiting resources, either energy or nutrients; (2) litter addition affects seedlings growth either positively, through the release of nutrients in soil, or negatively, through the release of secondary metabolites; (3) savanna and forest species litter differ in its effects in seedlings growth rates and soil microbiota activity and biomass, due to its differences in resource-use strategies.

## **Material and Methods**

### *Study area*

Litter collection was carried out at two Cerrado reserves located in the State of São Paulo, Southeastern Brazil: one at the Municipality of São Carlos (21° 58' S and 47° 52'

W) and the other at a reserve comprising the Municipalities of Itirapina and Brotas (Experimental and Ecological Station of Itirapina at 22° 00' S and 47° 45' W). The decomposition experiment was set in the later reserve. The Cerrado is an ecoregion of the tropical savanna biome consisting of savanna-dominated landscapes scattered with grasslands, closed-canopy woodlands and strips of riparian forests along river courses. The first reserve has approximately 125 ha and the second has approximately 2,300 ha, and are formed by gallery forest, typical savanna (locally called '*cerrado sensu stricto*'), wooded grassland (known as '*campos sujos*') and short closed-canopy woodland (dense cerrados or '*cerrado denso*', consisting of short statured trees and lacking a typical continuous grass cover). The regional climate of both areas is Cwa, according to the Köppen classification system, with mean annual precipitation of 1450 mm, with well-defined wet (October to March) and dry (April to September) seasons, and mean annual temperature of 20.8 °C.

Litter was collected in dense cerrado sites, a transitional vegetation of forest and savanna, which harbors a mixed woody flora containing typical shade-intolerant savanna, shade-tolerant forest, as well as more generalist shade-tolerant plant species (Bueno et al. 2018) all growing in the same environment (e.g., soil, microclimate). We collected litter from 10 woody species: five shade-intolerant savanna species and five shade-tolerant species (three forest and two generalist species, hereafter called "forest species" for simplicity), classified according to occurrence records in Sano et al. (2008; see Table 1). We chose pairs of shade tolerant and intolerant species from the same genus or family to minimize phylogenetic influences. This approach allowed the inclusion of leaves spanning a large range of light niches, and was directed at maximizing differences in litter traits.

We measured litter traits from freshly-senesced leaves (litter) collected from at least 20 individuals of each species. Individuals were selected systematically along a trail as the first visualized individuals, but respecting a distance of at least 10 m between individuals of



the same species. The litter collection was performed at the end of the dry season (August, September and October, 2014), after the placement of collection bags in the branches of the selected plants. We also used litter fallen from the plant (when we could unambiguously assign the litter to the selected plant) to complete the sample. We sampled the litter at the end of the dry season in order to collect freshly-senesced leaves and avoid the sampling of litter in late stages of decomposition. The collected material was dried at 25°C for 72 hours, and stored in sealed paper bags until use.

### *Litter effects on seedling growth*

In order to evaluate the effects of litter accumulation on seedling growth rates, we performed a greenhouse experiment at the Biology Institute of the State University of Campinas (UNICAMP), Brazil. We used 15 L pots of 45 cm height, filled with soil collected in dense cerrado area at the Experimental and Ecological Station of Itirapina (see Appendix Table S1 for soil features). For this, we prepared a plot of 9 m<sup>2</sup> of which we discarded the top layer of soil (0 – 20 cm) to avoid the collection of the organic layer. Then, we collected the soil from deep layer (20 – 50 cm) and used it for pot preparation.

We prepared pots for 10 treatments (with litter of each species) and a control (without the addition of litter). For each treatment, we prepared 5 pots in which we mixed the top 10 cm soil with 100 g of litter, whose litter specie varied according to the treatment. Since our aim was to investigate whether woody encroachment can specifically influence seedling growth by affecting soil fertility, the litter material was manually fragmented prior to mixing in order to simulate a more advanced stage of physical fragmentation. At the center of each pot we planted a seedling of one of three fast growing forest species also found in dense cerrado: *Croton floribundus* Spreng. (Euphorbiaceae), *Tapirira guianensis* Aubl. (Anacardiaceae) and *Inga vera* Willd. (Fabaceae). We obtained the plants from a seedling nursery, and all the plants had a similar time since germination. We did not use

savanna species because we were particularly interested in the consequences of litter accumulation in the encroachment of savanna by forest trees and because savanna species are less responsive to differences in soil nutrient availability (Viani et al. 2011). The experiment consisted of 10 treatments (10 litter species) and one control (no litter added), for each of the three seedling species. We used five replicates per group (combinations of litter and seedling species, including control), resulting in 165 pots, set in a complete randomized and balanced design. Plants were grown for approximately six months and were watered daily, simulating an entire growth season. During the experiment, temperature and relative air humidity were monitored using a HOBO® U23 temperature and humidity data logger, protected inside a solar radiation shield. The mean greenhouse air temperature was  $23.7^{\circ}\text{C} \pm 5.5$ , and the mean relative air humidity was  $68\% \pm 19.4$ . Every two weeks we measured seedling stem height (from the surface to the apical meristem) and diameter at soil height (DSH) on each individual. We subsequently used this information to estimate seedling biomass and seedling growth rate during the study period based on DSH, height and dry total biomass from the seedlings harvested at the end of the experiment. We calculated biomass by building allometric equations and selecting the best fitted model for each species using Akaike's Information Criterion (AIC; Fig. S1 and Table S2).

### *Mechanisms of seedling responses*

To study potential mechanisms explaining seedling responses to the litter addition, we measured litter traits related to species nutrient acquisition strategy and degradability (litter phenol, lignin, carbon and nutrient concentrations). For this, we ground 200 g of dried litter collected from five individuals of each plant species using a ball mill (Geno/Grinder 2010 SPEX SamplePrep). The samples were sieved through 5 mm meshes, and stored. Phenol concentration was determined using the Folin-Ciocalteu

extraction method (Graça et al. 2007). Lignin concentration was determined using Acid-Detergent method, obtaining the Klason lignin values following Graça et al. (2007). Carbon concentration was determined colorimetrically after oxidation with potassium dichromate ( $K_2Cr_2O_7$ ; Nelson and Sommers 1996). Nutrient analyses were based on subsamples of 10 g. Nitrogen concentration was determined colorimetrically after Kjeldahl digestion (Bremner and Mulvaney 1982). Phosphorus concentration was determined by spectrophotometry after nitric perchloric acid digestion (Motomizu and Oshima 1987). Manganese, K and Ca concentrations were determined by atomic absorption spectrophotometry (Malavolta et al. 1997). The means of the five samples per species analyzed were determined and are shown in Table S3.

We also measured the decomposition rates of litter of each species, since decomposition rates are related to the release of nutrients immobilized in dead tissue. To determine the litter decomposition rates of each species, we placed litterbags in dense cerrado sites at the Experimental and Ecological Station of Itirapina in December 2014, following Graça et al. (2007). For each species, we added approximately 5 g of senesced leaves from different individuals into 10 x 10 cm litterbags of 5 mm mesh. This mesh size allowed the access of litter by microorganisms, as well as meso and macro fauna (Makkonen et al. 2012). To maximize environmental heterogeneity, the bags were laid out in a randomized block design, setting five blocks with 50 litterbags (five replicates per species), in a total of 250 litterbags. We collected a bag in each block from each species after approximately 3, 4, 6, 8 and 10 months from the beginning of the decomposition experiment. After the collection of the litterbags, their contents were washed and dried at 70 °C for 48 h to constant mass, and the dry mass recorded. We determined the decomposition rates for each species by applying the Olson (1963) model:  $X_t = X_0 * e^{(-k * t)}$ ; to the data, in which  $X_t$  is the predicted mass at time  $t$ ;  $X_0$  is the initial mass converted to

equivalent dried mass at 70°C; and  $k$  is the Olson decomposition rate constant, a dimensionless parameter (Fig. S2).

We also measured the effects of litter addition in the soil microbiota activity and biomass. We used soil respiration rates as a proxy of soil microbiota activity, which is also related to the rate of C mineralization (Singh and Gupta 1977). For this, we prepared pots adopting the same experimental design as described in text (see *Litter effects on seedling growth*), but without planting the seedlings, to estimate soil respiration as a function of the addition of litter for each of the 10 litter species. Soil respiration was estimated based on CO<sub>2</sub> evolution (Singh and Gupta 1977) by inserting PVC tubes at the center of each pot to a depth of 5 cm and measuring CO<sub>2</sub> efflux every three weeks using an infra-red gas analyzer EGM-4 (PP Systems®). Soil temperature in each pot was measured using the temperature probe of EGM-4 (PP Sytems®). Since soil moisture was kept high under constant and controlled greenhouse conditions, and was similar among pots, we did not monitor this variable. All measurements were performed between mid-day and 3:00 pm. We adjusted a Non-Linear Mixed Effect Model (NLME) on soil respiration rates against time for each litter species using a negative exponential model ( $R_t = R_i * \exp(-R * T)$ ). In this model,  $R_i$  was the initial soil respiration rate,  $R_t$  was the soil respiration rate in day  $t$ ,  $R$  was the soil respiration rate through time, and  $T$  was the incubation time. We then calculated the total CO<sub>2</sub> - referred now on as total C respired - that evolved from soil during the experiment, by plotting the soil respiration rate by time and calculating the area under the adjusted NLME model curve (Meier and Bowman 2008b; Fig. S3).

To determine the effects of the litter deposition on soil microbial biomass, we collected, at the end of the experiment, the top 10 cm soil and sieved it, separating the soil from the remaining litter. Sieved soil was used to quantify C and N in the microbial biomass by the fumigation-extraction method (Vance et al. 1987). The microbial C

biomass was determined by potassium sulfate extraction (Vance et al. 1987), and microbial N biomass by the ninhydrin method (Joergensen and Brookes 1990).

### *Statistical analyses*

To evaluate the effects of litter addition in seedlings biomass and whether these effects were dependent on litter and seedling species identity, we estimated the biomass relative growth rate of each individual. Since relative growth rate was non-linear and did not reach a plateau, we fitted the exponential model:  $X_t = X_0 * e^{(t*r)}$  (Paine et al. 2012) to the data, in which  $X_t$  is the estimated biomass of the seedlings at time  $t$ ,  $X_0$  is the initial seedling estimated biomass,  $t$  is time in days and  $r$  is the relative growth rate. Then, we performed an Analysis of Variance (ANOVA), considering the seedling biomass relative growth rate as response variable, and litter identity and seedling specie as predictive variables. For the ANOVAs in which we have found significant influence of the predictive variables over the response variables, we performed a *post-hoc* Tukey's honestly significant test (Tukey HSD) in order to identify which litter significantly affected seedlings growth rate in relation to control seedlings. To evaluate the effects of litter addition on soil microbial activity, we also performed an ANOVA, considering the total C respired as response variable and litter identity as predictive variable. Then we performed a Tukey HSD to identify which litter significantly affected the microbial biomass in relation to the control soil. Similar analyses were performed in order to evaluate the effects of litter addition in soil microbial C and N biomass. We also performed a principal component analysis (PCA) to evaluate differences in litter trait among litter species and if litter from forest and savanna were significantly different.

To identify potential mechanisms of litter addition on plants growth and soil microbiota, we evaluated the relations between seedlings growth rate, soil microbiota activity – i.e. soil respiration and litter decay rates – with litter traits. For this, we initially

performed a hierarchical partitioning analysis (Chevan and Sutherland 1991), to quantify the average independent contribution of each litter trait in either plant growth or soil microbiota activity. Then, we performed linear regressions between the litter traits with the highest contribution with plant growth rate, the total C respired from soil and litter decay rates. We did not consider the control seedlings in these analyses.

The analyses were performed in R v.4.0.0 (R Core Team 2020), using the packages 'nlme' (Pinheiro et al. 2020), 'hier.part' (Nally and Walsh 2004), 'emmeans' (Lenth 2020) and 'ggplot2' (Wickham 2016).

## Results

The addition of litter to the soil either had no effects in seedlings growth or affected it negatively in comparison with the control seedlings (i.e. no litter addition; Table 2, Fig. 1), depending on seedling species. *I. vera* seedlings were negatively affected by the addition of litter from six of eight litter species, while the seedlings of *C. floribundus* and *T. guianensis* were not affected by litter addition, that is, no seedling species were positively affected by litter addition. Despite of these effects in seedlings growth rate, litter addition also affected soil microbial activity (but not soil microbial biomass; Table 3, Fig. 2). The addition of litter increased the total C respired from soil for all litter species and the strongest effect was observed after the addition of *T. formosa* litter (Table 3, Fig. 2). The effects of litter addition on seedlings growth and soil microbial activity were not related to litter species habitat (i.e. forest vs. savanna species; Fig. 1-3).

Seedlings response to litter addition were mainly related to litter initial N concentration (Fig. 4a), but this relation was dependent on the seedling species considered (Table 4, Fig. 5). We observed that the growth of *I. vera* seedlings was positively related to litter initial N concentration (Table 4; Fig. 5b). However, the growth of control plants was still higher than the growth of plants grown with N-rich litter (i.e. litter

with high initial concentration of N had less intense negative effect on *I. vera* seedlings growth). On the other hand, the growth of *C. floribundus* and *T. guianensis* seedlings was not related to litter initial concentration of N (Fig. 5a, c).

The total C respired from soil was positively related to both litter initial P and C concentration (Table 4, Fig. 6a, b), showing a distinct pattern from the observed for seedlings response. A similar pattern was observed for litter decay rate, which was mainly related to the litter initial C concentration (but not P; Fig. 4c). Overall, litter decay rate was positively related to litter initial C concentration (Table 4, Fig. 6c).

## **Discussion**

### *The effects of litter addition in soil microbial activity and biomass*

Litter addition significantly increased soil respiration in relation to pots in which litter was not added – control pots (Fig. 2). During the experiment, the respiration rates of the control pots was close to zero, consistent with resource limitation of the microbial activity (Fig. 2, Fig S3). The same was not observed when litter was added, consistent with previous studies showing that litter is an important source of resources to soil microbiota, stimulating microbial activity and soil respiration (Wang et al. 2013; Fang et al. 2015; Peng et al. 2020). Therefore, the negative and neutral effect of litter addition in seedling growth was not mediated by a potential inhibition of microbial activity.

The stimulation of soil respiration by litter addition was correlated to litter chemical composition (Fig. 4b, Fig. 6a, b). Overall, soil respiration was positively related to litter initial C and P concentration (Fig. 6a, b). Soil microbial activity is stimulated by the addition of limiting resources by litter (Zechmeister-Boltenstern et al. 2015). The correlation between soil respiration and litter C and P concentration might be related to two main mechanisms: (1) soil microbial community is energetically limited and activity is stimulated by the input of C-rich organic matter. The stimulated soil microbial activity increases the

turnover of organic matter, increasing the C mineralization and soil respiration (Whitaker et al. 2014; Zechmeister-Boltenstern et al. 2015); (2) soil microbial community is P-limited, and the addition of P-rich litter increases the turnover of organic matter, also increasing the C mineralization and soil respiration (Manzoni et al. 2010; Zechmeister-Boltenstern et al. 2015). Both energetic and P limitation of Cerrado soil microbiota is consistent with previous evidence. The energetic limitation of soil microbiota is consistent with findings from other tropical forest? regions, in which the addition of litter with high concentration of labile C increased soil heterotroph respiration and litter decay rates (Hättenschwiler and Jørgensen 2010; Makkonen et al. 2012; Whitaker et al. 2014). Also, soil microbiota P-limitation was expected, since communities occurring in old and highly weathered soils are expected to be P limited (Reich and Oleksyn 2004), which is the case of several Cerrado sites (Kozovits et al. 2007; Jacobson et al. 2011; Bustamante et al. 2012; Dionizio et al. 2018; Abrahão et al. 2019). Therefore, our results indicate a key role of C and P on soil microbiota activity in Cerrado soils.

Litter chemical traits were also related to litter decomposition rates (Fig. 4c, Fig. 6c). Litter chemical composition plays a key role in litter decay dynamics, since litter rich in limiting resources stimulates soil microbiota activity and the litter degradation process (Zhang et al. 2008; Cornwell et al. 2008; Makkonen et al. 2012; Bani et al. 2018). We found that the relation between litter decay rates and litter chemical traits had a similar pattern to that of the relationship between soil respiration and litter chemical traits (positively related to C and P and negatively to Phenol concentration; Fig. 4b, c). This similarity suggests that the observed increase in soil microbiota activity was directly associated to the decomposition process. Combined, these results suggest that litter addition to pots in our experiment did promote litter degradation during the studied period. Moreover, these results highlight the key role of litter traits in regulating carbon cycling in



savanna ecosystems, especially under the low fire conditions found once a savanna crosses the fire suppression threshold (i.e. the conditions simulated by our experiment).

Despite stimulating soil microbial activity, we did not observe a higher microbial C and N biomass in soil in which litter was added in relation to control pots (Fig. 2a, b). The similarity in soil microbial biomass observed among treatments and control could be related to the remineralization, which is the mineralization of elements in microbial biomass (Bengtson and Bengtsson 2005). The addition of substrate stimulates microbial activity and growth, increasing the immobilization of elements in microbial biomass. However, the depletion of substrate due its consumption could lead to microbial death by starvation and the remineralization of its biomass (Bengtson and Bengtsson 2005). In our experiment, the addition of litter at the beginning of the experiment stimulated microbial activity (Fig. S3) and, potentially, stimulated its growth. However, since no substrate was added further, the resources could have been depleted by the end of the experiment, reducing microbial activity (Fig. S3) and microbial biomass, resulting in the observed pattern.

#### *The effects of litter addition in growth rates of woody seedlings*

Litter addition had either no or negative effect in seedlings growth rates (Table 2, Fig. 1), which is in accordance with previous short-term studies (Xiong and Nilsson 1999; Meier and Bowman 2008; Lopez-Iglesias et al. 2014; Gavinet et al. 2018). The neutral effects of litter addition on seedlings growth can be related to the small liberation of nutrients and secondary metabolites during decomposition caused by a short-term decomposition of litter, which is insufficient to cause significative modifications in soil properties. Alternatively, it could be related to a neutralizing balance between the litter negative and positive effects in plants' growth, caused by the release of secondary

metabolites and the liberations of nutrients, respectively (Lopez-Iglesias et al. 2014; Gavinet et al. 2018).

In addition, the effects of litter addition in seedlings growth is dependent on the seedling sensitivity to variations in soil characteristics, which is related to seedling characteristics (Bonanomi et al. 2017; Gavinet et al. 2018). For instance, experimental evidence suggest that slow growing savanna species respond less to variation in nutrient availability compared to fast growing forest species (Viani et al. 2011). For this reason, we avoided including savanna species as seedling and because we wanted to simulate the conditions experienced by a forest species that colonize a savanna under fire suppression. Yet, even forest species can differ in resource strategy, and evidence suggest that *I. Vera* is a faster growing species compared to *T. guianensis* and *C. floribundus* (references). We observed that slow-growing seedlings of *T. guianensis* and *C. floribundus* were not affected by litter addition, while fast-growing seedlings of *I. vera* were negatively affected by the addition of litter from six of litter species in relation to control seedlings (Fig. 1). Indeed, fast-growing plants are more responsive to variations in resource availability than slow-growing plants (Aerts and Chapin 1999; Viani et al. 2011). Additionally, positive effects of litter addition in seedlings growth are usually related to litter physical structure (Hovstad and Ohlson 2008; Gavinet et al. 2018), which was not evaluated in our experiment. Therefore, short-term litter chemical composition has overall no effect or negative effect in seedlings growth, and the negative effects seems to be related to seedlings characteristics.

The growth rate of *I. vera* seedlings were positively related to litter initial N concentration, however the growth rate of control seedlings was still higher than the seedlings in which litter was added (Fig. 4). This result indicates that higher litter N only alleviates the negative effects of litter in *I. vera* seedlings growth rate.

The negative effects of litter input in plant growth is usually related to the litter concentration of secondary metabolites, which inhibits root growth and immobilizes nutrient in soil (Meier and Bowman 2008; Bonanomi et al. 2011; Lopez-Iglesias et al. 2014). However, while we found evidence that Phenolic compounds limited decomposition, a low leaf Phenolics was not associated with faster seedling growth rate, even in the most fast-growing most sensitive species, suggesting that something else was responsible for the lack of a positive effect after litter addition.

The negative effects of litter input in plant growth is related to the stimulus of litter addition on microbial activity and growth, which have been reported to increase the immobilization of limiting nutrients in microbial biomass, which are able to outcompete the plants in nutrients uptake, at least in the short term (Čapek et al. 2018). On the other hand, litter nutrient concentration is usually positively related to plant growth (Veen et al. 2019) and no effect was observed in relation of litter of different quality. Even-though *I. vera* seedlings growth rate was positively related to litter N concentration, the effects of litter addition was not positive in relation to control seedlings. In this case, we hypothesize that the negative effects of litter input in *I. vera* seedlings growth rate were related to the immobilization of nutrients in microbial biomass. The immobilization of nutrients in microbial biomass is more intense when the concentration of these nutrients in the substrate is low, while when the concentration of limiting nutrients in substrate is high, the resource requirements by decomposers is rapidly achieved and the release of nutrients in soil solution occurs (Manzoni et al. 2010; Zechmeister-Boltenstern et al. 2015). This mechanism can be related to the observed positive relation between litter initial N concentration with seedlings growth rates. Further investigation should be conducted to evaluate this hypothesis.

*Differences in litter traits and effects between forest and savanna species*

Despite the structural and environmental differences between savanna and forests, which select for plants with contrasting resource-use strategies (Dantas et al. 2013; Maracahipes et al. 2018; Abrahão et al. 2019), we did not find significant differences between the litter chemical traits of

#### *Implications for forest encroachment in dystrophic savannas*

Our results showed that, while decomposition is clearly stimulated by the addition of litter to the soil, this did not result in faster forest seedling growth. This should not be observed if seedling growth was controlled by a positive feedback mechanism relating litter deposition, decomposition and increased nutrient release. Thus, other process must be operating (candidate hypotheses were already addressed in previous paragraphs). Importantly, while some of these effects were affected by litter traits, they did not depend on litter species habitat preferences and their related adaptations at the leaf level (no litter trait differences could be detected). Thus, whether the canopy is dominated by forest or savanna species do not seem to influence how the litter is decomposed by microbes (in the absence of fire) and forest seedling success. While previous studies have suggested greater forest seedling success under a forest canopy in the Cerrado region (Hoffmann et al. 2004), many alternative mechanisms could underlie these previous results. A forest canopy buffers microclimatic conditions against climatic fluctuations outside the forest. Moreover, humus accumulation is a potential alternative fertilizing mechanism which depends on the amounts of undecomposable litter mass. Further studies should address these alternative mechanisms in order to provide a more comprehensive understanding of the role of vegetation-soil feedbacks in regulating biome dynamics in the tropics. In addition, considering that these systems are dynamic and regulated by tipping points, longer term studies should address if the patterns reported here persist through longer time periods until the transition to the sapling stage or are temporary.

## *Conclusions*

In regions in which forest and savanna coexist, forming mosaics, forests have been shown to produce higher amounts of litter than savannas and with higher concentration of nutrients (Silva et al. 2008, 2013; Paiva et al. 2015). This high input of nutrients and organic matter through litter by forests has been suggested to increase soil fertility, which could favor woody encroachment in savannas areas (Silva et al. 2008, 2013; Paiva et al. 2015). Our study is the first to test this mechanism experimentally and contributes to a better understanding of the effects of litter accumulation in the dynamics of nutrients.

Overall, our results show a key role of C and P in nutrient cycling dynamics in the Brazilian Cerrado. The positive relation between litter C and P with soil microbial activity, either measured as soil respiration or litter decay rates, indicates a potential energetic or P-limitation of soil microbial community. Furthermore, our results show that litter addition alone does not represent a direct input of nutrients for plant uptake, and that litter addition does not benefit woody seedlings growth, at least in the short term. Instead litter addition has neutral or hinders the growth of seedlings. In this case, woody seedlings remain vulnerable or could even become more vulnerable to top-kill by fire (Hoffmann et al. 2012). In this scenario, our study indicates that litter accumulation leads to a complex set of interaction involving soil microbial community, which influences in the mineralization rate of litter and also resource competition, and also the characteristics of woody individuals.

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