

RESEARCH PAPER

The role of bud protection and bark density in frost resistance of savanna trees

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ABSTRACT

- Frost events occur with a significant frequency in savannas of the Southern Hemisphere, especially in the Cerrados of Brazil. One of the main strategies to deal with such events is to invest in thick and dense bark, which can insulate internal branch tissues and protect buds, essential to ensure resprouting if frost damage causes plant canopy die-back. Such strategies may be fundamental to determine the persistence of savanna species in regions where low temperatures and frost events are recurrent.
- Here we describe bud protection and bark strategies of 53 woody species growing in typical savanna vegetation of central Brazil. In addition, we used an experimental approach exposing branches to 0 °C to measure temperature variation in internal branch tissue and test its relationship to bud protection and bark properties.
- We found that the majority of species (69%) showed medium to high bud protection against extreme temperatures; however, the degree of bud protection was not clearly related to bark properties, such as bark thickness and density. Bark density is a fundamental trait in determining protection against low temperatures (0 °C), since species with low bark density showed lower temperature variation in their internal branch tissues, independently of the bud protection degree.
- Bark properties and bud protection are two different (albeit related) strategies for the protection and persistence of savanna trees under extreme environmental temperatures and can explain ecological observations related to savanna tree responses after frost events.

INTRODUCTION

Chilling and frost are relatively frequent disturbances that damage and kill aboveground plant tissues and organs, such as leaves, branches and stems, in various temperate and polar ecosystems, thus affecting plant functioning and vegetation structure in these regions (Fernández-Palacios & Nicolás 1995; Inouye 2000). The persistence of plant species under such conditions depends on a series of functional strategies at the organ or individual level: plants subjected to low temperatures can enter dormancy to minimise damage, or display certain morphological and physiological traits that can confer protection, avoidance or tolerance to this factor (Sakai & Larcher 1987).

Morphological traits to deal with frost occurrence include the insulation of the vital internal tissues of the trunk and branches, such as parenchyma and vascular tissues, through production of thicker and softer bark (Van Mantgem & Schwartz 2003; Bannister 2007), which confers a high degree of insulation against external temperature variations (Van Mantgem & Schwartz 2003; Clarke *et al.* 2013). The bark insulation capacity is derived from its morphological structure, based on the relatively low ratio of tissue mass:volume, and chemical aspects, such as the presence of certain compounds that can together decrease the thermal diffusivity of its composition

(Van Mantgem & Schwartz 2003; Bauer *et al.* 2010). Additionally, the protection of buds in branches is essential because resprout capacity may be fundamental to recover the lost plant biomass when temperatures decrease significantly and branch mortality occurs (McLeester *et al.* 1969; Tolvanen 1997).

Plant responses to frost, despite being well studied in polar and temperate areas (Hänninen 1991), are particularly little studied in tropical biomes, which may also be exposed to frost when plants occur at high elevations or high latitudes, especially in the Southern Hemisphere (Silberbauer-Gottsberger *et al.* 1977; Vuono *et al.* 1982; Childe & Walker 1987; Filgueiras & Pereira 1987; Bannister 2007). Tropical plants are normally reported as having high tolerance to elevated temperatures (Sastry & Barua 2017), but their capacity to tolerate lower temperatures is not well understood. In fact, for tropical plants, frost damage may prevail because their tissues and organs are easily injured by chilling (Lyons 1973; Burke *et al.* 1976), causing plant death when the temperature is near or below 0 °C (Sakai & Larcher 1987).

Although not expected, many reports of frost damage in tropical plants can be easily found in the literature (Silberbauer-Gottsberger *et al.*, 1977; Childe & Walker 1987; Filgueiras & Pereira 1987; Brando & Durigan 2005; Hoffmann *et al.* 2019). Most of these reports usually refer to frost in

savanna regions, especially the Brazilian savanna (Hoffmann *et al.* 2019). In its wide distribution within the Brazilian territory, the savannas of Brazil (Cerrado) can be exposed to frost and low temperatures (0–10 °C) at high altitudes (Filgueiras & Pereira 1987) and at the southern limit of their distribution (Silberbauer-Gottsberger *et al.* 1977; Hoffmann *et al.* 2019). Frost can be considered a recurrent event in southern Brazilian savannas, occurring almost annually, but with a very low intensity. In turn, severe frost events are less frequent, recurring every 25 years on average (Brando & Durigan 2005; Hoffmann *et al.* 2019), but affecting a much larger area, and potentially reaching even the savannas in the central region of Brazil (Filgueiras & Pereira 1987). Such events have been shown to reinforce the dynamics imposed by fire, since frost injuries share important features with fire and herbivore damage (Hoffmann *et al.* 2019), especially concerning the death of internal stem, branch and leaf tissues (Brando & Durigan 2005), which affect plant resprouting, growth and persistence (Hoffmann *et al.* 2019).

Evaluating plant traits related to species persistence is fundamental to understand how tropical savanna species will respond under lower temperatures, which would also have consequences for species occurrence and vegetation changes at a geographic scale under a scenario of climate change (Hoffmann *et al.* 2019). In fact, some scenarios report an increase in severity of frost events in the Southern Hemisphere (Crimp *et al.* 2016), which may drastically affect the functioning and structure of tropical ecosystems. The presence of thick bark and protected buds in the majority of savanna tree species (Burrows *et al.* 2010; Charles-Dominique *et al.* 2015) might suggest that such species could have their branch tissues protected and insulated against extreme temperatures, as an adaptation to fire that might become useful to deal with low temperatures.

In this study, we aimed to evaluate the role of bud position and bark properties in 53 woody savanna species experiencing low temperatures. First, we analysed the frequency of the degree of bud protection (high, medium or low) in savanna tree species and used the degree of bud protection to test for differences in bark thickness and density. Second, we experimentally evaluated the role of bark thickness and density in insulating internal branch tissues at low temperatures (0 °C). We expected to find a positive relationship between bark thickness and the degree of bud protection, so that species that invest in building thicker bark will also have a higher degree of bud protection. We also expected to find a positive relationship between bark density and internal tissue temperature variation, meaning that species with softer bark will show lower temperature variation in internal branch tissues.

MATERIAL AND METHODS

Study site

This study was conducted at the IBGE Ecological Reserve, located 33 km south of Brasília in central Brazil (15°56' S, 47°53' W). The reserve is at an average altitude of 1100 m. The predominant soils at the study site are deep well drained Oxisols, and the average annual precipitation is approximately 1500 mm, with a pronounced dry season from May to

September. Mean monthly temperatures range from 19 to 23 °C throughout the year. Lower temperatures appear during the dry season, especially during May, June and July (Fig. 1), where air temperature can reach as low as 2–9 °C (IBGE meteorological station, data from 1980 to 2014 obtained from <https://recor.org.br/pesquisas-cientificas/estrutura-para-pe-squisa/estacao-meteorologica/>). The last severe frost event in the region was reported by Filgueiras & Pereira (1987), where temperatures in that year dropped below 0 °C. In the past 25 years, 21 events of low (2–8 °C) temperatures were recorded in the area (Fig. 1).

Species selection

In November 2017, during the wet season, we randomly sampled 53 tree species from different families (Table S1) occurring in a typical savanna vegetation (Cerrado *sensu stricto*), in a radius of 100 m around the coordinate point 15°57'27.77" S and 47°52'51.69" W. Our selection included species with a variety of strategies regarding bud protection and bark thickness.

Describing bud protection and branch bark properties

To describe the degree of bud protection on epicormic buds from branches of the studied species, we collected one branch (approximately 40 ± 2.3 cm in length and 1.8 ± 0.33 cm in diameter, ranging from 0.57 to 2.00 cm) from three individuals of each species. The sampled branches were completely healthy, without any signs of fire damage or herbivory, and were located at the canopy periphery. Based on our previous experience with studies dealing with branch growth (Rossatto & Franco 2008; Rossatto *et al.* 2009), we tried to sample branches of similar age, taking as reference the leaf cohort position: we discarded the most recent cohort, collecting branch parts appearing between the new and the oldest cohort. Branches collected in the field were stored in black bags and brought to the

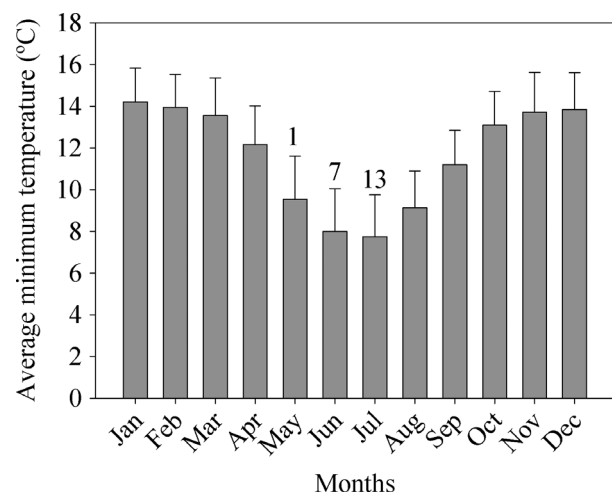


Fig. 1. Distribution of average minimum temperatures during the year for a period of 25 years (1980–2014) in the IBGE ecological reserve. Numbers above bars indicate the number of days in which the temperature dropped below 5 °C, 1980–2014.

laboratory, where we used pruning shears to cut branch pieces of 5 cm in length. To determine the degree of bud protection we used the semi-quantitative categories proposed by Charles-Dominique *et al.* (2015) and Burrows *et al.* (2010): 0 for completely exposed buds; 1 for emerging buds with protected meristem; 2 for buds within bark depressions; and 3 for buds completely protected by bark. Bud position was checked in transverse sections and at radial longitudinal orientation of branches using a stereomicroscope at a 0.7× magnification (Model SZ-B; Bel Photonics, Monza, Italy), where we identified the epicormic buds by small irregularities on the bark surface, according to Burrows *et al.* (2010).

To determine branch bark properties, we cut pieces of branches of similar size and diameter (approximately 5.00 cm in length and 1.80 ± 0.33 cm in diameter, ranging from 0.57 to 2.06 cm), controlling for similar age of branches among species, and measured inner and outer bark thickness using a digital caliper (0.001 mm resolution), following definitions adopted by Hoffmann *et al.* (2009). After bark thickness determination, a piece of bark was stripped from the branch and its fresh volume determined in a graduated beaker using the water displacement method (Smith 1954). The sample was oven-dried and weighed on an analytical scale to calculate bark density (*i.e.* the ratio of fresh bark volume and bark dry weight, $\text{g}\cdot\text{cm}^{-3}$). For bark density analysis, we use only the outer bark.

Exposing branches to low temperatures

We used a drilling machine (1-mm drill bit) to drill a hole in the central part of the branches. In this hole, thermocouples (copper-constantan, T-type thermocouples; Omega, Norwalk, CT, USA) were attached and isolated from the exterior to monitor the internal branch temperature. The branches were exposed to ambient temperature (25 °C) for 3 h prior to the experiment start. The initial temperature (T_i) was measured (mean \pm SD: 24.6 ± 0.50 °C) before the branches were exposed to 0 ± 0.5 °C in a BOD (Biochemical Oxygen Demand) chamber (Model EL202/4LED; Eletrolab, São Paulo, Brazil). After 1 h in the chamber under low temperature, the final temperature (T_f) reached was recorded (T_f). Temperature (T_i and T_f) was measured using a data logger (Omega Model HH506RA; Omega) and T_f and T_i were used to quantify the modular value of temperature change ($|\Delta T| = |T_f - T_i|$). The 1-h period was chosen based on the time experienced by typical savanna vegetation (basal area $> 10\text{-m}^2\cdot\text{ha}^{-1}$) under 0 °C during a frost event (Hoffmann *et al.* 2019).

Statistical analysis

To test for differences in bark thickness and density between the distinct bud protection categories we used analysis of covariance (ANCOVA), with the stem diameter as a covariate, followed by Fisher LSD test. To test the relationship between the degree of bud protection and bark properties (inner and outer bark thickness and bark density), we performed a Spearman rank order classification test. To determine the effect of inner and outer bark thickness and bark density in the temperature variation, we used non-linear regression. For all analyses, differences were considered significant when $P \leq 0.05$. Data were checked for normality and homogeneity

of variances using Kolmogorov–Smirnov test, and \log_{10} transformation was used when necessary (outer bark thickness). All analyses were performed using R software version 3.4.4 (R Core Team 2018).

RESULTS

Bud protection

Of the 53 woody species analysed, the majority (69%) showed medium to high bud protection (categories 2 and 3; Fig. 2, Table S1). Twelve species showed buds emerging from the stem surface, *i.e.* low bud protection (category 1), while only four out of the 53 species showed buds at the bark surface, with completely exposed buds.

Bark properties

Despite inner bark thickness having a positive relationship with branch diameter ($F_{1,48} = 9.179$, $P = 0.004$), we did not find differences in inner bark thickness ($F_{3,48} = 2.479$, $P = 0.072$) between species with distinct bud protection degrees (Fig. 3A). For outer bark, branch diameter did not have a significant effect ($F_{1,48} = 0.32$, $P = 0.810$), and outer bark did not differ ($F_{3,48} = 0.22$, $P = 0.8881$) among species with distinct bud protection degrees (Fig. 3B). However, species with unprotected buds showed higher bark density ($F_{3,48} = 3.49$, $P = 0.0023$; Fig. 3C), as branch diameter did not have a significant effect ($F_{1,48} = 1.175$, $P = 0.284$). We did not find any relationship between bud protection and inner ($r = 0.061$, $P > 0.05$) or outer ($r = 0.021$, $P > 0.05$; Fig. 4) bark thickness. Bark density was also not related to bud protection category ($r = -0.081$, $P > 0.05$; Fig. 4).

Bark properties and temperature variation

Branches that acclimated to the ambient temperature had initial temperatures around 25 °C, however, after the 1-h period exposed to 0 °C, the modular values of temperature variation ($|\Delta T|$) inside the branches ranged between 21 to 25 °C (Fig. 5), with no significant differences between the groups with different bud protection strategies ($F_{3,49} = 1.64$, $P = 0.1922$). While we did not find any significant relationship between temperature variation and inner or outer bark thickness ($r^2 < 0.02$, $P > 0.30$ for all comparisons), temperature variation was positively related to bark density ($r^2 = 0.497$, $P < 0.001$), which means that a higher temperature variation corresponds to a lower bark insulation capacity. Nevertheless, plants with different strategies of bud protection showed diverse responses regarding branch temperature variation ($r = -0.085$, $P > 0.05$); the four species having unprotected buds had high bark density and consequently higher temperature variation (Fig. 5).

DISCUSSION

The majority of Neotropical savanna woody species studied here show a medium to high degree of bud protection. However, contrary to our expectations, bark properties were not related to bud position. We found a clear and positive relationship between bark density and

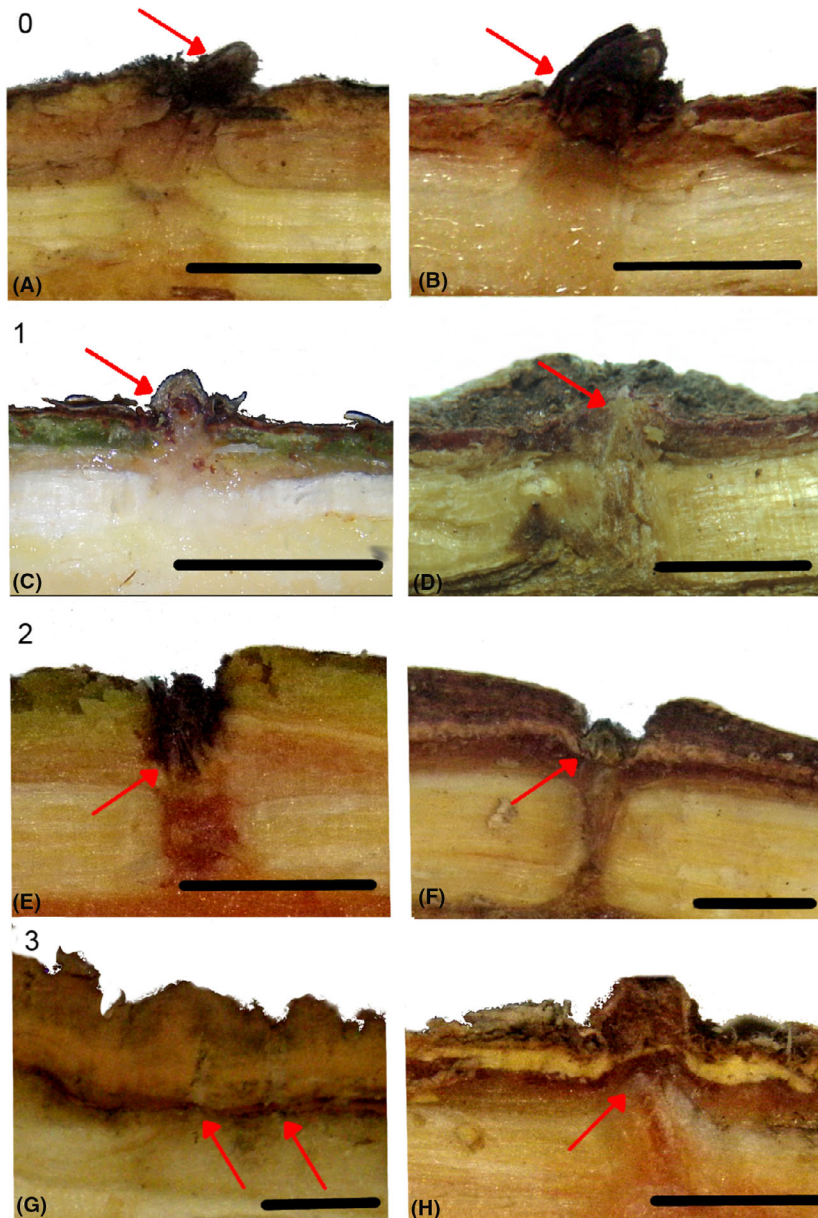


Fig. 2. Epicormic buds from twigs of six out of the 53 Cerrado species used in this study (Table S1) divided into bud protection categories: 0 for completely exposed buds; 1 for emerging buds but protected meristem; 2 for buds allocated inside depressions in the bark; and 3 for buds completely protected under the bark. Left to right, depicted species are 0 – *Annona coriacea* (a), *Caryocar brasiliense* (b); 1 – *Hymenaea stignocarpa* (c), *Qualea grandiflora* (d); 2 – *Byrsonima coccolobifolia* (e), *Qualea multiflora* (f); 3 – *Aspidosperma tomentosum* (g), *Erythroxylum suberosum* (h). Scales 5 mm. Arrows point to the position of the buds.

the temperature variation of internal branch tissue, which was again not related to bud position. Our results suggest that irrespective of bud protection strategy, branch bark density may be the main factor determining the degree of insulation of internal branch tissues against low temperatures, and that bark density and bud protection are complementary strategies that can help plants to resist and persist under disturbances such as frost events.

Degree of bud protection and bark properties

The proportion of species with medium to high bud protection found here (69%) was higher in comparison with a study performed in a savanna community sampled in Africa (42%) (Charles-Dominique *et al.* 2015), which analysed buds from

trees, but also included shrub species. Species with unprotected buds were more commonly found in Africa (20%) than in Brazil (7%). The higher proportion of species showing medium to high bud protection should be related to the fire frequency history of Neotropical savannas, which are reported to be more frequent than in African savannas (Lehmann *et al.* 2014). Additionally, an important aspect driving such differences in bud protection in the studied plant communities could be species composition. Around 50% of the species sampled in Charles-Dominique *et al.* (2015) were from plants in four botanical families: *Fabaceae* (especially plants from *Acacia* genus), *Ebenaceae*, *Rubiaceae* and *Euphorbiaceae*, while in our study the families *Fabaceae*, *Vochysiaceae*, *Apocynaceae*, *Malpighiaceae* and *Melastomataceae* represent 50% of the species sampled.

The degree of bud protection at branch level seems not to be related to branch bark properties for the studied savanna

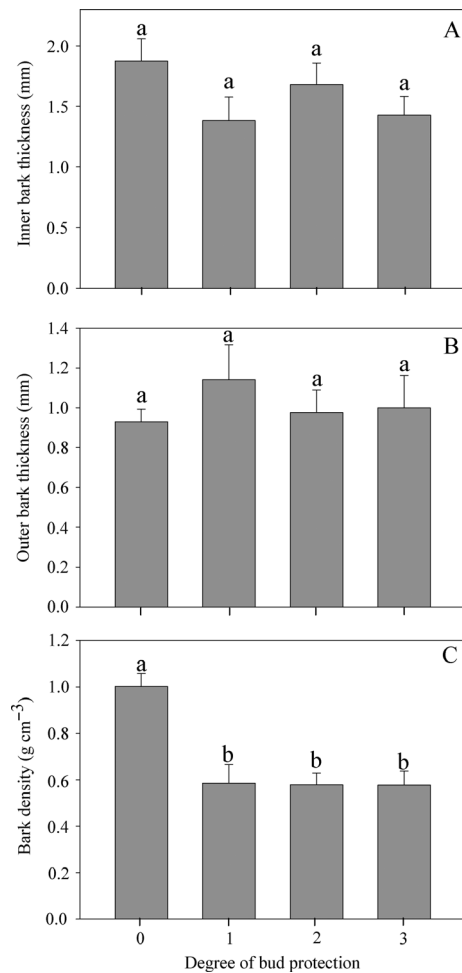


Fig. 3. Comparison between species with different bud protection strategies for inner (A) and outer (B) bark thickness and for bark density (C). Bark protection increases from 0 to 3. Vertical bars indicate SE (number of species: 0 = 3; 1 = 10; 2 = 21 and 3 = 19). Means denoted by a different letter are significantly different according to Fisher LSD test ($P < 0.05$).

plants, as we did not find any significant difference, nor a relationship, between inner and outer bark thickness across the different bud protection strategies. Although bud protection is defined as the location of buds in relation to the bark layer (Burrows *et al.*, 2010; Burrows & Chisnall, 2016), bud protection is not only conferred by bark, but also by bud scales (Clarke *et al.* 2013). However, some studies have reported the importance of bark thickness in protecting buds, allowing plants an increased resprout capacity (Burrows & Chisnall 2016). Resprouting will only occur if branch tissues are not damaged (Palacio *et al.* 2015), especially the vascular tissues that are responsible for translocating carbohydrates stored in subterranean systems, and which provide the energy necessary to vigorously resprout (Malanson & Trabaud 1987; de Moraes *et al.* 2016).

Bark properties and low temperatures

Contrary to our expectations, species with more protected buds did not always have thicker and less dense bark (Figs

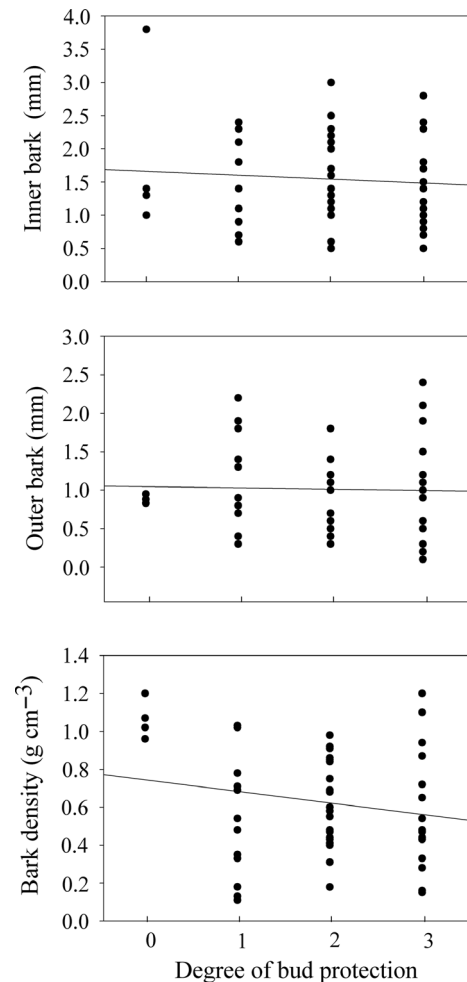


Fig. 4. The lack of relationship between the degree of bud protection and measured bark properties (outer/inner bark thickness and bark density) in branches of woody species from the Brazilian Cerrado in Brasília – DF.

3 and 4). Bark thickness and density in branches should be important to protect parenchyma and conductive tissues, critical to water and carbohydrate translocation, which supply the buds and allow them to resprout and develop (Wenger 1953; Del Tredici 2001). The size of a tree or branch is important in determining protection against frost (Whitecross *et al.* 2012), given size should strongly affect the thermal mass, bark thickness (Rosell 2016) and changes in internal tissue temperature (Hengst & Dawson 1994). However, our analysis of co-variance showed a lack of effect from branch diameter, which supports the primary effect of bark density in protection of internal branch tissues. Thus, for a similar branch size among species, we found that bark density should be the most important driver of branch protection under low temperatures in the Cerrado. The strong and positive relationship found between bark density and temperature variation showed that the higher the bark density the lower the thermal insulation capacity.

Brando & Durigan (2005) found that the Cerrado species *Stryphnodendron* sp. and *Dimorphandra mollis* were the species most affected by frost, with high branch mortality.

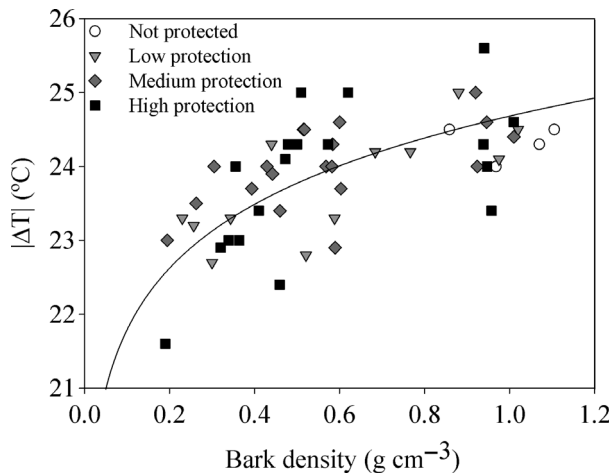


Fig. 5. Influence of bark density on thermal insulation of branches (exponential adjust, equation $y = 24.69x^{0.054}$). Each point represents species mean ($n = 3$ for each species). Different symbols indicate the different degree of bud protection. Δ temperature = $|t_{\text{final}} - t_{\text{initial}}|$.

Accordingly, in our study, these were the two species with the highest internal branch temperature variation and with denser bark (Table S1). In contrast, species that did not show branch mortality in the study of Brando & Durigan (2005) (e.g. *Styrax* sp. and *Erythroxylum* sp.) were considered the most tolerant species, and our data for bark density confirms this ecological observation, because these species have bark with lower density and lower temperature variation. Differences in bark density could also be related to bark nutritional composition. For instance, Jönsson *et al.* (2001) report that phosphorus and magnesium bark concentrations affected resistance to frost. They found that plants with higher bark concentrations of phosphorus and magnesium were less sensitive to frost; indicating that softer barks had a higher nutrient content. However, very little information is available on nutritional aspects of bark in Neotropical savanna species and more detailed studies should be conducted to assess the relationship between extreme temperature protection and bark nutrient concentration.

Under frost events, adult savanna trees are more exposed to lower temperatures than adult established forest trees,

especially because the former are short and most of their branches and leaves are 1–2 m above ground level (Hoffmann *et al.* 2019). If there is no strategy to isolate internal branch tissues, the frost may affect tree survival and may create a ‘frost trap’, similarly to the well-studied ‘fire trap’ (Hoffmann *et al.* 2012). The ‘frost trap’ might maintain plants in a suppressed state through repeated cycles of stem loss and resprouting (Hoffmann *et al.* 2019). Individual traits, such as low bark density, could confer protection against these low temperatures, insulating the branch tissues against damage. Detailed studies, similar to ours, should consider the use of branches of different diameters. This approach would provide a better understanding of how bark and bud traits vary across branch ontogeny and how these traits could have been selected in the plant community to protect savanna plants from frost, a recurrent event in most of the subtropical savannas.

Here, for the first time, we provide important and new information about the role of bark properties, especially bark density, in Brazilian savanna species, showing their function in protecting inner tissues against lower temperatures, a recurrent event in Neotropical savannas at their southern limit of distribution. Our results indicate that bark properties and bud protection may be two different (albeit related) strategies for the protection and persistence of savanna trees under extreme environmental temperatures and can explain ecological observations related to savanna tree responses to frost events.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Bud protection classification (protection increases from 0 to 3), branch diameter (cm), bark density ($\text{g}\cdot\text{cm}^{-3}$) and inner and outer bark thickness (mm) for the studied species in a cerrado area of Brasília, DF, Brazil.

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