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Leaf size and thickness are related to frost damage in ground layer species of Neotropical savannas

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ABSTRACT

Savannas in southeastern Brazil are frequently exposed to frost events, causing the death of leaves and branches in many woody and herbaceous species. Frost events are frequent in these regions, with one relatively stronger than usual event every 5 years. Our experimental site at São Paulo State, Southeastern Brazil, was affected by strong frost events during June–July 2021, when temperatures reached -4 °C, causing aboveground dieback in most ground layer species, although we observed some species were not affected and maintained a fully green canopy. We used this opportunistic frost event to study and report these damages and measured leaf traits that could explain our observations, as well as point directions to ecological understanding of frost on savanna vegetation. We measured morphological leaf traits such as leaf shape (width, length, width to length ratio), leaf area, specific leaf area and leaf thickness, and we also quantified canopy and leaf damage in 17 species (5 non-affected by frost and 12 that were visually affected). We found that species with larger and thicker leaves were more prone to leaf and canopy damage (70–100% of damage) than those with smaller and thinner leaves (0% damage). These results suggest that leaf morphology may provide resistance against frost and could ultimately act as a filter favoring species that can support extreme frost events, if those became more frequent and stronger under future climatic changes.

1. Introduction

Tropical savannas are perhaps the most studied open canopy vegetation in the tropics, given their diversified structure and composition formed by a mosaic of relatively small trees and herbaceous species (mainly grasses) varying in their density and dominance (Furley, 2006; Veldman et al., 2015). Detailed studies addressed to understand how biological and environmental factors drive savanna ecology and function were performed in all three major continents of the southern hemisphere harboring savanna vegetation, especially focused on the effects of herbivory (Staal et al., 2018), seasonality and edaphic aspects (Lehmann et al., 2011) as well as fire (Lehmann et al., 2014). Of all of these biotic and abiotic factors, there is a consensus that fire is considered the most important driver of savanna ecology (Bond et al., 2003), determining species persistence and stable states maintenance, thus affecting vegetation structure and ecosystem functioning (Midgley et al., 2010; February et al., 2019).

While fire is a major research topic on tropical savanna studies (Mistry, 1998; Van Wilgen et al., 2007; Beringer et al., 2015), frost is an important but still an incipient studied topic in the southern hemisphere, and especially, the non-temperate regions deserve much more attention (Brando and Durigan, 2005; Holdo, 2006; Bannister, 2007; De Antonio et al., 2020, 2021; Pilon et al., 2022). The reason for this pattern is probably due to its rarity and less frequent occurrence in relation to fire (Muller et al., 2016; Hoffmann et al., 2019) or even because, at a first glance, low temperatures are not expected to occur in tropical and sub-tropical regions with higher frequency when compared to temperate and polar regions (Kreyling, 2010).

Recent studies show that these events have become frequent in Neotropical savannas, especially at their southeastern limit of distribution in South America (Hoffmann et al., 2019). Frost events are mainly driven by strong polar winds coming from Antarctica from time to time

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(Risbey et al., 2019), causing relatively rapid drops in temperature to below 0 °C for more than 5 days during the night (Hoffmann et al., 2019). Studies about the effects of low temperatures on savannas were performed especially in the Neotropics, showing patterns of leaf and branch mortality in susceptible and non-susceptible tree species (Hoffmann et al., 2019; De Antonio et al., 2020, 2021), which ultimately affects community composition in woody and ground layer (Hoffmann et al., 2019; Pilon et al., 2022). However, the use of species traits to understand such responses to frost is largely underexplored (Franco and Álvaréz-Yépiz, 2021), especially in ground layer species which are the primary and dominant component of savanna vegetation and that are drastically affected by frosts (Pilon et al., 2022).

Frost events on southern savannas are classified as radiation frost, a condition in where there is surface cooling due to extreme heat loss to the atmosphere, with the cooling being most extreme just above the soil surface, but decreasing with increasing vegetation height and cover (Inouye, 2000; Pilon et al., 2022). Thus, such events have a bigger impact on plants growing near the ground (forbs, grasses, subshrubs and even shrubs) in comparison to trees, which display their leaves and canopy distant from the soil surface. Several leaf traits are related to resistance or susceptibility to frost: studies published on temperate and polar ecosystems showed that smaller, thinner and acicular leaves showed higher tolerance to low temperatures compared to species with larger, thicker and ovate leaves (Campitelli et al., 2013; Lusk et al., 2018). Thus, traits such as leaf thickness, specific leaf area and leaf shape may be good proxies to understand frost resistance on plant species (Hekneby et al., 2006; Pescador et al., 2016).

Since savannas of Southeastern Brazil are prone to frequent frost, we took the opportunity to record and quantify leaf and canopy damage in 17 ground layer species from Cerrado during a strong radiation frost event that occurred in July of 2021. We also measured morphological leaf traits (leaf width and length, leaf area, damaged area, thickness and specific leaf area) in affected (varying for some degree to total leaf damage) and unaffected (no sign of leaf damage) species. Our aim was to

Fig. 1. Frost effect on Cerrado ground layer plants. A – General view of the grassland with white and black arrows pointing to the unaffected *Allagoptera campestris* palms and *Baccharis linearifolia*, respectively; B – *Pradosia brevipes* individual showing frost damage with partial damaged leaves; C – *Eugenia anomala* individual with no signs of damage by frost; D – *Anacardium humile* individual with leaves top part killed; E – dead fruits and dead leaves of *Jacaranda decurrens*; F – the dead canopy of *Psidium grandifolium*; G – damaged and non-damaged leaves of *Eugenia bimarginata*, note the arrows indicating previously lost biomass; H – dead leaves of *Moquiniastrum barrosae*.

understand how leaf morphological traits are related to damage resistance caused by low temperatures. As found in temperate and polar systems, we expected to find that species that did not suffer any damage were those with smaller and thinner leaves, showing a more acicular shape than those with thicker and round leaves.

2. Material and methods

2.1. Study site

This study was conducted at Águas de Santa Bárbara Ecological Station (EESB), with delimitation at 22° 46′ through 22° 41′ S and 49° 16′ through 49° 10′ W (Fig. S1), located in São Paulo State, Southeastern Brazil, in elevations between 600 and 680 m a.s.l. (Melo and Durigan, 2011). Average annual precipitation is around 1300 mm in the area, with the dry season occurring from June through September, with mean temperature ranging from 18 to 22 °C (Melo and Durigan, 2011). Soils are sandy and well drained Oxisols, with lower water retention during the dry season (Melo and Durigan, 2011). Vegetation at EESB is classified as a typical savanna physiognomy (regionally named cerrado *sensu stricto*), having 10 to 40% tree cover.

2.2. Frost events and species selection

During our monthly observation routine to evaluate vegetative phenology and measure leaf gas exchange (part of PhD thesis of A.C. Antonio) in July 24–26/2021, we were surprised to find out that the majority of our herbaceous and shrubby plants were severely damaged by recurrent frost events (Fig. 1 and Fig. S1). Almost all ground layer plants exhibited clear signs of damage, such as dead branches, brownish leaves, and dead flowers and fruits still attached to the plant body (Fig. 1B-H). Different from fire events, which consume aerial biomass and increase ash deposition at the soil surface as well as increase the bare soil cover (Coutinho, 1990; Pilon et al., 2021), these frost events did not consume biomass, nor deposited ash but in fact, it increased the amount of dead standing aboveground biomass (Pilon et al., 2022). After data retrieving at the nearest meteorological station, we were able to determine that our study site suffered four strong frost events previously to our visit: the first on June 30th, where the lowest temperature was −5.1 °C, and three subsequent frost events during 19–21th of July, reaching −4.3, −4.2 and −1.9 °C respectively (see Table 1). Additional three severe frost events further occurred in the region after we left the field, during 28–30th of July, with temperatures reaching −2.4, −3.9 and −3.6 °C (Table 1), totaling 7 frost events in 30 days.

In the studied area, six 20 $m \times 50$ m plots of savanna and grassland vegetation were established and monitored to study effects of fire in the vegetation (see Abreu et al., 2017). We observed damages in species we were collecting phenological data, then we decided to use it to measure leaf traits. Specifically, we sampled species in the 3 plots used as control (i.e., without fire for more than 20 years), since in these plots we observed that the landscape attained a brownish tone (Fig. 1), while some species stood up still showing a green canopy without signs of injuries (Fig. 1A–C). We detected five species without any signs of damage: the palms *Allagoptera campestris* (Fig. 1A) and *Syagrus flexuosa*, the Asteraceae *Baccharis linearifolia* (Fig. 1A), the grass *Axonopus pressus* and the Myrtaceae *Eugenia anomala* (Fig. 1C); and twelve species showing different degrees of damage: *Anacardium humile* (Anacardiaceae), *Duguetia furfuracea* (Annonaceae), *Moquiniastrum pulchrum* and *M. barrosae* (Asteraceae), *Jacaranda decurrens* (Bignoniaceae), *Tontelea micrantha* (Celastraceae), *Licania humilis* (Chrysobalanaceae), *Byrsonima subterranea* (Malpighiaceae), *Eugenia bimarginata* and *Psidium grandifolium* (Myrtaceae), *Pradosia brevipes* (Sapotaceae) and *Smilax fluminensis* (Smilacaceae).

Table 1
Minimum and maximum temperatures, and daily total rainfall records at the study site during frost events between June and July 2021. Records from a nearby meteorological station (Manduri – SP, 30 km apart from the ecological station) were obtained from http://www.ciiagro.org.br/ema/index.php?id=41.

Month	Day	Min (°C)	Max (°C)	Rainfall (mm)
June	30	−5.1	21.0	0
July	19	−4.3	23.7	0.5
July	20	−4.0	19.2	0
July	21	−1.9	20.8	0
July	28	−2.4	16.2	1.5
July	29	−3.9	14.7	0
July	30	−3.6	17.0	0

2.3. Sampling and leaf traits

All 12 affected and the 5 unaffected species were selected. We haphazardly sampled 3 leaves from 5 to 9 individuals of each species, distancing at least 10 m from each other in the 3 control plots. These leaves were scanned and leaf length (cm), leaf width (cm) and leaf area (cm^2) were measured using ImageJ software (Abrámoff et al., 2004). We calculated the ratio between leaf width and length (W/L) to understand if the leaf had a more acicular (W/L values near 0) or oval shape (W/L values near 1). Leaf thickness was measured with a digital caliper (mm), leaves were oven dried for 48 h at 70 °C, weighed and specific leaf area was calculated as the ratio between leaf area and leaf dry mass. Damaged area (%) was calculated using the scanned images, since for the affected species, it was possible to visualize the part of the leaf that died (brown color) and the part that was still functioning (green color) (see Fig. 1B–D for example). For canopy damage estimation, we adapted the Fournier index (Fournier, 1974) to quantify the percentage of canopy with damaged leaves, with 0 – no damage; 1 – 1–25% damage; 2 – 26–50% damage; 3 – 51–75% damage and 4 – 76–100% damage.

2.4. Statistical analysis

To understand how the studied traits were related to the frost-driven leaf damage, we performed a principal component analysis (PCA) to verify if species differed in their syndrome of leaf traits (5 studied functional traits). All data were standardized (z-transformation) before applying the analysis. For the PCA, we used the Euclidian distance with the correlation matrix method (Gotelli and Ellison, 2016). We also used pairwise comparison between affected and unaffected species, using t-tests after checking for data normality and variance homeoscedasticity. R v. 4.0.2 (RCore Team, 2019) was used for all analyses.

3. Results

Unaffected species showed no leaf or canopy damage, and affected species showed high leaf damage, ranging between 75 and 100% affected leaf area and between 90 and 100% of canopy area (Table 2). The first and second axes of the PCA explained, respectively, 49.8% and 25.4% of the variation (Fig. 2, Table S1). The first axis was mostly driven by leaf thickness and width, being possible to define two clear groups: one on the left comprised by the unaffected plants showing more acicular thinner leaves (lower W/L and lower leaf thickness; Figs. 2 and 3), and a group on the right comprised by the affected plants, showing thicker damaged leaves, with an oval shape (higher W/L and leaf thickness; Figs. 2 and 3).

These results were corroborated by the pairwise comparisons (Fig. 3). No differences between affected and unaffected species were found for leaf length and specific leaf area (Fig. 3); however, we found significant differences between affected and unaffected species for leaf width, leaf length, Width/Length ratio, leaf thickness and leaf area (Fig. 3). All these traits were higher in affected than unaffected plants (t-tests, $P < 0.05$).

Table 2

Species studied in this work, as well as their families, growth forms (GF) and the mean values (± standard error) of their morphological leaf traits ($n = 5$–9 individuals per species). G – grass; P – palm; S – shrub; SS – subshrub, V – vine. W/D – ratio between width and length of the leaf; LA – leaf area (measured in leaflets of species showing composite leaves*); THI – thickness and SLA – specific leaf area.

Family	Species	GF	Height (m)	% of leaf damage	% canopy damage	W/D	LA (cm^2)	THI (mm)	SLA ($g.cm^{-2}$)
				Affected					
Anacardiaceae	*Anacardium humile*	SS	0.15	80.85±20.2	90	0.21	40.39	0.23	63.32
Annonaceae	*Duguetia furfuracea*	S	1.15	100	100	0.37	27.77	0.27	51.66
Asteraceae	*Moquiniastrum pulchrum*	SS	1.20	100	100	0.32	13.40	0.20	52.32
Asteraceae	*Moquiniastrum barrosae*	S	1.30	100	100	0.68	49.97	0.34	67.55
Bignoniaceae	*Jacaranda decurrens**	SS	0.11	100	100	0.23	10.32	0.18	59.93
Celastraceae	*Tontelea micrantha*	SS	0.07	100	100	0.26	15.41	0.37	35.45
Chrysobalanaceae	*Licania humilis*	SS	0.08	100	100	0.42	16.75	0.23	56.86
Malpighiaceae	*Byrsonima subterranea*	SS	0.09	100	100	0.31	114.16	0.22	85.96
Myrtaceae	*Eugenia bimarginata*	S	1.20	85.81±14.5	90	0.78	21.01	0.34	35.17
Myrtaceae	*Psidium grandifolium*	S	1.10	100	100	0.45	35.11	0.30	44.79
Sapotaceae	*Pradosia brevipes*	SS	0.12	74.01±20.3	100	0.28	44.06	0.25	53.79
Smilacaceae	*Smilax fluminensis*	V	1.20	100	100	0.51	64.33	0.34	62.47
	Mean ± SE		0.64±0.16	94.3 ± 10.2	98.3 ± 3.89	0.42±0.2	37.7 ± 29.2	0.27±0.06	51.57±14.03
				Unaffected					
Arecaceae	*Allagoptera campestris**	P	0.40	0	0	0.08	12.42	0.09	63.72
Arecaceae	*Syagrus flexuosa**	P	2.00	0	0	0.03	35.97	0.21	37.75
Asteraceae	*Baccharis linearifolia*	SS	1.10	0	0	0.12	0.29	0.06	40.43
Myrtaceae	*Eugenia anomala*	SS	0.08	0	0	0.11	4.69	0.06	60.72
Poaceae	*Axonopus pressus*	G	0.10	0	0	0.04	9.70	0.07	134.01
	Mean ± SE		0.73±0.3	0	0	0.07±0.04	12.6 ± 15.8	0.09±0.07	67.32±13.45

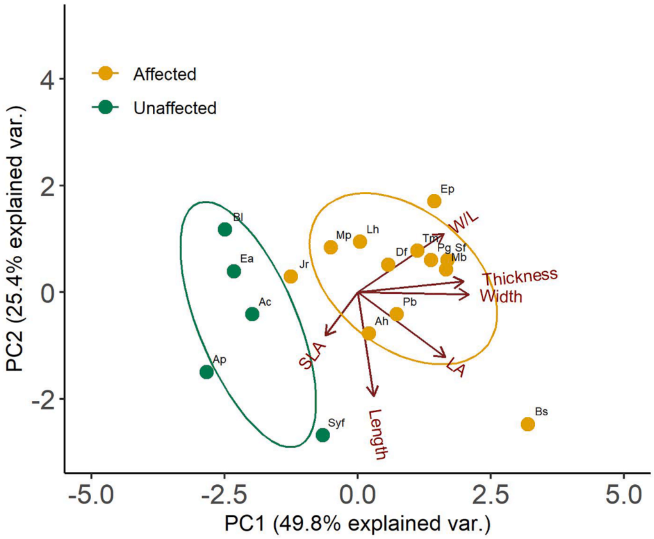

Fig. 2. Principal component analysis of leaf morphological traits from ground layer savanna species grouped into affected (yellow circles at right) and unaffected (green circles at left) by recurrent frost events. Solid lines indicate weighing of vectors representing the five leaf traits considered. SLA: specific leaf area; LA: leaf area; W/L: ratio between leaf width and leaf length; Width: leaf width; Length: leaf length and Thickness: leaf thickness. Loadings are available in Supplementary Material. Unaffected species: Ac: *Allagoptera campestris*; Ap: *Axonopus pressus*; Bl: *Baccharis linearifolia*; Ea: *Eugenia anomala*; Syf: *Syagrus flexuosa*. Affected species: Ah: *Anacardium humile*; Bs: *Byrsonima subterranea*; Df: *Duguetia furfuracea*; Eb: *Eugenia bimarginata*; Jd: *Jacaranda decurrens*; Lh: *Licania humilis*; Mb: *Moquiniastrum barrosae*; Mp: *Moquiniastrum pulchrum*; Pb: *Pradosia brevipes*; Pg: *Psidium grandifolium*; Sf: *Smilax fluminensis*; Tm: *Tontelea micrantha*.

4. Discussion

We corroborated our expectations since leaf morphology from species with no leaf damage differs from affected species. Unaffected species shared morphological traits such as smaller thinner leaves with acicular shape (i.e. higher length than width). Although we were not able to measure biochemical and physiological leaf traits, which should be important to understand the complete scenario concerning plant responses to frost events (Müller et al., 2016; Pommerrenig et al., 2018; de Antonio et al., 2021), we believe that leaf shape and morphology is a good proxy to understand species response to frost.

Leaf shape is an important trait concerning protection to frost, since it can drive the leaf thermal balance, providing higher or lower protection against low or high temperatures (Raschke, 1960; Lusk et al., 2018). This should be especially the case in non-woody ground layer species, which normally are smaller in height and have a higher exposure to low temperatures at the ground surface (Hoffmann et al., 2019), the region more affected by radiation frost events (Inouye, 2000). Such observations of a more acicular leaf shape were already reported for plants under temperate and polar environments (Givnish, 1979); additionally, this response was also confirmed for a series of species in forest environments subjected to frost in sub-tropical regions (Lusk et al., 2018), and first time reported for savanna species here.

We did not find differences for specific leaf area between affected and non-affected species; however, differences in leaf thickness were found, suggesting that leaf dry matter content (LDMC) and leaf density should differ between affected and non-affected species. LDMC is clearly related with freezing-resistant traits (Pescador et al., 2016). Although we did not measure LDMC, given leaf thickness differences and the known relationship with SLA and LDMC (Vile et al., 2005), we can speculate about differences in LDMC between non-affected and affected species, which in turn will provide more physical resistance to freezing of water in the symplast, which is the main cause of cellular damage after frost events (Grossnickle, 1992). We thus suggest that LDMC might be a key trait to be measured when studying frost resistance.

Such studied traits (SLA, LT, leaf shape) are also reported to enhance plant survival and persistence during droughts (Di Francescantonio et al., 2020), and resistance against drought can allow resistance against frost (Hofmann et al., 2013). Although savanna environments possess a clear dry season during the southern winter (Franco, 2002), it is reported in the literature that majority of plants has access to deep ground water layers or even possesses mechanisms do decrease drought effects (Goldstein et al., 2008; Rossatto et al., 2013). Thus, morphophysiological responses to fire and to elevated irradiances should be related to such responses to frost events, instead of responses to drought.

We observed that responses to frost are mainly binary: the ground layer species studied here are highly affected with over 70% leaf area

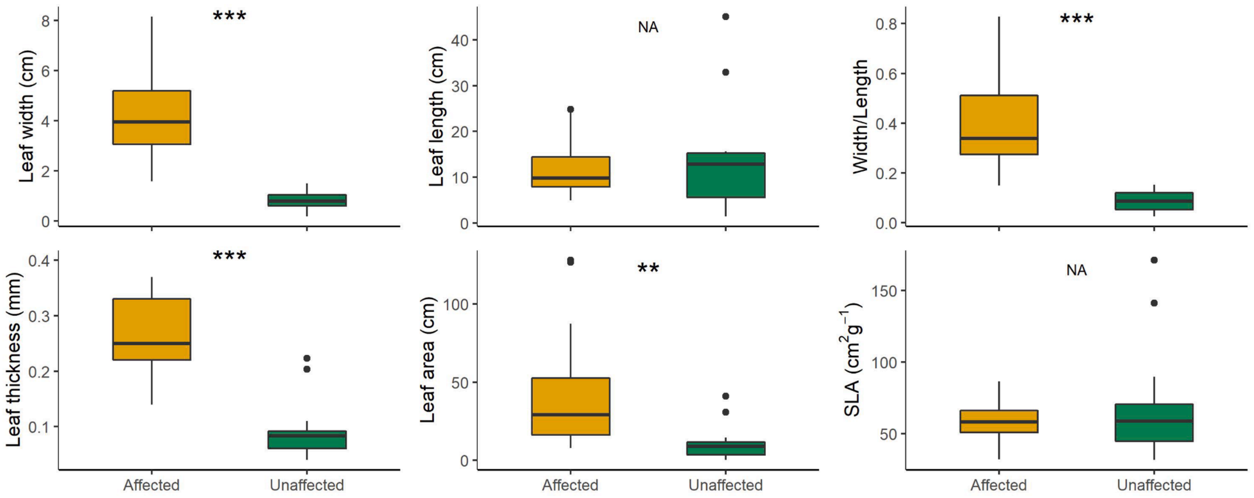

Fig. 3. Pairwise comparison of leaf morphological traits between affected (yellow boxes at left) and unaffected (green boxes at right) ground layer species by recurrent frost events in Cerrado (t-tests). Boxplots showing median and 25% and 75% percentiles, and asterisks show statistical differences, as follow: ***, $P < 0.001$; **, $P < 0.05$; NA when there was no significant difference between affected and unaffected plants.

damaged and over 90% of canopy damaged, or non-affected at all. These results differ drastically from that found for tree species, which are reported to suffer different canopy and leaf damage after frost events, with leaf damage widely varying from 0 to 100% (Brando and Durigan, 2004; Hoffmann et al., 2019). Previous studies reported that the majority of savanna woody species are able to persist after frost events in Southeastern Brazilian savannas (Hoffmann et al., 2019; De Antonio et al., 2021). However, our findings suggest that, compared to woody species, frost may have a stronger effect in the ground layer, where the majority of species, accordingly with our results, are highly sensitive to its effect. In contrast, only few species species could be classified as highly tolerant to frost. We suggest that there is no in-between strategy for the ground layer species: either they resist frost, or their aboveground biomass will be completely lost.

For non-woody species it seems that leaf morphology may explain why some species did not suffer any damage after frost, while others species lost their entire canopy and leaf surface. Ground layer savanna species are known for their high capacity to resprout after fire events given their well-developed underground organs and great amount of bud banks (Ferraro et al., 2021). Considering our results and previous studies focused on woody species (Hoffmann et al., 2019; De Antonio et al., 2021), affected ground layer species may have the strategy to resprout the entire lost biomass after frost events (Pilon et al., 2022), while the unaffected species may persist. This suggests that persistence under recurrent frost events may require the consumption of carbohydrate reserves, a strategy similar to that found under fire events (de Moraes et al., 2016).

Although our study was observational and data were collected opportunistically after a frost event, we believe we provide interesting information on how ground layer species are affected or not, according to their traits, by these events. Previous studies performed in savannas reported only the presence or absence of damage to ground layer species, not relating it to functional traits (Pilon et al., 2022). Responses of ground layer species to frost, however, are well known for alpine and temperate environments (Taschler and Neuner, 2004). In contrast, response of woody species to frost are becoming more frequently in the literature for savanna species (Holdo, 2006; Hoffmann et al., 2019; De Antonio et al., 2021, 2022), and are well explored for temperate trees (Hufkens et al., 2012; D'Andrea et al., 2020).

While controlled experiments with frost events can be performed in situ (Taschler and Neuner, 2004), given elevated diversity and higher structural complexity of savannas it would be somewhat a challenge to perform such controlled experimental studies in the field to a better understanding of how species respond to frost; however, the understanding of tissues / organs survival under low temperatures can be easily done in laboratory controlled conditions, exposing branches and leaves to below zero temperatures (De Antonio et al., 2020, 2021). Another possibility to increase our understanding on the relationship between species traits and frost events would be tracking the meteorological forecast during the dry season (especially during June and July), which may help to prepare for field trips on the exact day to visualize direct effects in the field, or even protect leaves from the lower temperatures to compare its physiological responses with unprotected leaves. After the frost event, it is important to revisit affected plants to visualize whether they will be able to resprout or not (Hoffmann et al., 2019; de Antonio et al., 2021).

To a full understanding of savanna plants response to frost, we suggest initial screenings about resistance, tolerance or sensitivity (*sensu* Agrawal et al., 2004), as well as understanding whether these traits are conserved phylogenetically, which was studied, for example,concerning fire disturbance (Simon and Pennington, 2012 and Giroldo et al., 2017). Additionally, as frost maintains the aboveground biomass standing, studies focusing on space competition, plant resprout, effects on reproduction (especially on fruits and seeds, which can be severely damaged by frost – see supplementary material) and on the amount of litter deposition after frost events should be performed to understand how ecosystem ecology is affected. These approaches will be of utmost importance to understand the role of frost (and maybe its interaction with fire) in determining species persistence and community composition, as well as vegetation physiognomies and ecosystem functioning.

Extreme climatic events are expected to increase in their frequency (IPCC, 2019), including frost events in the southern hemisphere (Rusticucci, 2012; Crimp et al., 2016; Risbey et al., 2019), which should be mainly related to increases in polar air jets reaching tropical regions (Müller et al., 2017). Although a significant amount of information showing the importance of frost events on savanna ecology has been produced in the past 15 years (Gottsberger and Silberbauer-Gottsberger, 2006; Whitecross et al., 2012; Duker et al., 2015; Muller et al., 2016; Hoffmann et al., 2019; de Antonio et al., 2020; Botha et al., 2020, 2021), we must direct efforts on understanding morpho-physiological responses that are behind such observations, aiming to understand how plants respond and are affected by frost events (Franco and Álvaréz-Yépiz, 2021). Our work here suggests that, in terms of the ground layer component, the majority of species within this functional group will be affected.

CRediT authorship contribution statement

Ariadne Cristina de Antonio: Data curation. **Marina Corrêa Scalon:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Davi Rodrigo Rossatto:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2022.152208.

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