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MATEUS CARDOSO SILVA

USANDO ATRIBUTOS FUNCIONAIS DE PLANTAS PARA AVALIAR OS RESULTADOS DA RESTAURAÇÃO EM UM CAMPO TROPICAL

USING PLANT TRAITS TO ASSESS RESTORATION OUTCOMES IN A TROPICAL GRASSLAND

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Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Mestre em Ecologia

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Orientador: Rafael Silva Oliveira

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Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

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“*That’s all Folks*”

RESUMO

1. O Cerrado é um *hotspot* de biodiversidade global. Restaurar esse bioma é fundamental para conservar a natureza e o bem-estar das pessoas. Várias espécies de plantas do Cerrado podem ser restabelecidas por métodos de restauração, no entanto não temos uma avaliação dos aspectos funcionais de ecossistemas restaurados. Nosso objetivo foi preencher essa lacuna de conhecimento, comparando as condições do solo e os atributos das plantas entre uma área restaurada de 3 anos de idade com uma área antiga no Cerrado.
2. Realizamos o estudo em (i) um experimento de restauração de campo via semeadura direta e (ii) em na vegetação antiga mais próxima no Brasil Central (14°05’ S, 47°38’ O). Em cada local de estudo, medimos as condições do solo (fertilidade, acidez, umidade e textura) e atributos relacionados ao uso de água em plantas (potencial hídrico mínimo, Ψ_{min}; condutância mínima, g_{min}; e ponto de perda de turgor, π_{tlp}) e produtividade (altura da planta e massa foliar por área, LMA) entre as espécies graminóides dominantes, incluindo espécies nativas e exóticas (*Urochloa decumbens*).
3. A área restaurada teve uma concentração menor de alumínio, solos mais secos e níveis mais baixos de fósforo e nitrogênio. Os atributos relacionados à tolerância à seca (g_{min} e π_{tlp}) foram significativamente menos variáveis na comunidade restaurada em comparação com a de referência. O campo restaurado foi dominado por espécies com atributos associadas à produtividade (baixo LMA) e sensibilidade à seca (alto π_{tlp}) (e.g., *U. decumbens*) em contraste com o campo antigo.
4. A umidade do solo menor na área restaurada em comparação com a referência pode ser explicada pela textura do solo, enquanto os legados do solo da invasão e do manejo da terra prévios podem ter conduzido às diferenças na fertilidade entre as áreas. A recuperação da diversidade e composição funcional pode depender do reestabelecimento de um conjunto diversificado de espécies em proporções semelhantes às dos locais de referência. Além disso, a re-invasão de *U. decumbens* no Cerrado restaurado pode ser favorecida pelo rápido uso de recursos dessa gramínea invasora.
5. No presente estudo, descobrimos que a diversidade funcional das plantas após a restauração ainda é muito diferentes da comunidade de referência a curto prazo. Estudos futuros podem abordar a eficácia de restaurar os níveis de alumínio e uma estrutura de abundância desigual no direcionamento do ecossistemas restaurados a um estado funcionalmente diverso e resistente à invasão no bioma Cerrado.

Palavras-chave: restauração por semeadura direta, atributos funcionais de plantas, invasão biológica, variáveis edáficas, ecofisiologia de gramíneas, dinâmica do fogo, tolerância à seca, produtividade da campos

ABSTRACT

1. The Brazilian *Cerrado* is a global biodiversity hotspot. Restoring this tropical grassy biome is critical to conserving nature and human well-being. Several *Cerrado* plant species can be re-established by restoration methods yet we lack an assessment of the functional outcomes of restored ecosystems. Here, we aimed to fill this knowledge gap by comparing the soil and traits of native and invasive plants between a 3-years-old restored site and an old-growth site in the *Cerrado*.
2. We carry out this study in a (i) direct-seeding grassland restoration experiment and (ii) the nearest well-conserved grassland in Central Brazil (14°05' S, 47°38' W). In each study site, we measured soil conditions (fertility, acidity, moisture, and texture) and traits related to plant water-use (leaf minimum water potential, Ψ_{min}; minimum conductance, g_{min}; and turgor-loss point, π_{tlp}) and productivity (plant height and leaf mass per area, LMA) of the dominant graminoid native and exotic species (*Urochloa decumbens*).
3. The restored area had a lower concentration of aluminum, drier soils, and lower phosphorus and nitrogen levels. Traits related to drought tolerance (g_{min} and π_{tlp}) were significantly less variable in the restored community compared to the old-growth one. The restored grassland was dominated by species with traits associated with higher productivity (low LMA) and higher drought-sensitivity (high π_{tlp}) (e.g., *U. decumbens*) in contrast to the old-growth counterpart.
4. Lower soil moisture in the restored area compared to the old-growth area can be explained by soil texture, while soil legacies from prior invasion and land management might have determined the differences in fertility between the sites. Recovering functional diversity and composition may rely on re-assembling a diverse set of species in mimicking the dominance-rarity structure of an old-growth *Cerrado* grassland. Moreover, re-invasion of the African grass *U. decumbens* in the restored site might be favored by the fast resource-use of this invasive species.
5. Here we found that plant functional diversity following restoration is still very different from the reference community in the short-term. Future studies could address the effectiveness of restoring soil properties and an uneven abundance structure in steering restored ecosystems to a functionally-diverse and invasion-resistant state in the *Cerrado* biome.

Key-words: direct-seeding restoration, plant functional trait, old-growth savannah, biological invasion, edaphic variables, grass ecophysiology, fire dynamics, drought tolerance, grassland productivity

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INTRODUCTION

The Brazilian *Cerrado* is the most biodiverse savanna worldwide and probably also the most threatened (Silva and Bates, 2006). *Cerrado* harbor more than 12 thousand plant species, of which one-third are endemic, but has less than 20 % of its original cover currently conserved (INPE, 2015; Zappi *et al.*, 2015). Furthermore, the three greatest South American watersheds lie within the *Cerrado* territory highlighting the role of this biome in water resources. Land-use changes threaten the *Cerrado* iconic biodiversity and the water safety of more than 25 million people that live within this region (Spera *et al.*, 2016; Strassburg *et al.*, 2017). One of the most pervasive land-use changes in the native vegetation is the creation of pastures and crops. Pastures cannot spontaneously recover to the pre-perturbed *Cerrado* state of diversity, structure, and function even 25 years after abandonment (Cava *et al.*, 2018, 2020). Also, pastures have lower water infiltration rates than old-growth *Cerrado*, increasing the probability of groundwater depletion and stream flooding (Hunke *et al.*, 2015; Nóbrega *et al.*, 2017; Falcão *et al.*, 2020). Therefore, active ecological restoration is needed to recover the *Cerrado* biodiversity and ecosystem services after land degradation (Buisson *et al.*, 2020).

Direct-seeding restoration (i.e., reintroducing plants from seeds) has proved to be a promising method to recover *Cerrado* biodiversity on a large-scale (Sampaio *et al.*, 2019). Up to 75 native species can be re-established by direct-seeding (Pellizzaro *et al.*, 2017). Additionally, direct-seeding is more cost-effective than traditional methods (e.g., passive restoration and seedling planting) (Raupp *et al.*, 2020) and provides income to the local population by the seed collection, engaging them in the conservation practice (Schmidt *et al.*, 2019). About 2 thousand hectares of *Cerrado* are expected to be restored in private properties by the next decades (Soares-Filho *et al.*, 2014). Then, improving the direct-seeding method and restoration practices is important to achieve Brazil’s conservation goals. A key step is to evaluate the outcomes of *Cerrado* restoration efforts in terms of ecosystem functioning. The soil features and plant functional traits (i.e., organism characteristics that shape their fitness or ecosystem processes) can provide key insights into ecosystem functioning (Carlucci *et al.*, 2020). While old-growth well-conserved ecosystems in the vicinity of restored areas can be used as a reference to assess restoration functional achievements (Buisson *et al.*, 2020).

It is well known that soil conditions (e.g., nutrient availability and physical structure) affect the trajectory of the restored vegetation (Maron and Jefferies, 2001; Antonsen and Olsson, 2005; Williams, Jackson and Smith, 2007; Valliere *et al.*, 2019). For instance, the soils within the *Cerrado* biome are among the oldest soils worldwide and are commonly infertile, acid, and aluminum-rich (Silveira *et al.*, 2016; Morellato and Silveira, 2018). These harsh soil conditions shaped the evolution of the *Cerrado* flora into a slow-growing, conservative, and stress-tolerant strategy (Oliveira *et al.*, 2015). However, degraded lands usually possess neutral and fertile soils due to past intensive soil management including fertilization, liming, and mowing (Hunke *et al.*, 2015). The increase of soil pH and nutrient availability can threaten *Cerrado* biodiversity and restoration efforts as it favors competitive invasive species rather than stress-tolerant native species (Buisson *et al.*, 2020; Silveira *et al.*, 2020). In Central Brazil, direct-seeding restoration projects are frequently preceded by sequential soil mowing and prescribed fires to de-compact the soil and control biological invasions (Sampaio *et al.*, 2019). Yet, we still do not know how much soils of restored areas resemble the soils of reference areas or whether soil conditions shape the structure and function of restored *Cerrado* areas.

Plant functional traits can help us to uncover the outcomes of restoration efforts in three complementary ways (Buisson *et al.*, 2020; Carlucci *et al.*, 2020). First, the range of trait values within a restored ecosystem can increase its resilience (i.e., resistance and recovery after disturbances [sensu Mitchell et al. 2016]) (Hobbs, Higgs and Harris, 2009; Montoya, Rogers and Memmott, 2012; Timpane-Padgham, Beechie and Klinger, 2017). Communities containing species with complementary trait values are more likely to have at least one species capable to endure or recover from historical or novel disturbances (i.e., insurance effects) (Sakschewski *et al.*, 2016; Anderegg *et al.*, 2018). Second, the average trait value of a restored ecosystem (i.e., community-weighted mean [CWM]) can also affect resilience as well as the ecosystem functions provisioning (Pakeman, Eastwood and Scobie, 2011; Conti and Díaz, 2013; Finegan *et al.*, 2015; Prado-Junior *et al.*, 2016). For instance, vegetation dominated by drought-resistant species is expected to recover faster from droughts than vegetation dominated by drought-sensitive species, even though both vegetations have similar trait variability and insurance effects. Third, unveiling the invasive species traits could explain biotic homogenization and undesirable regime shifts following restoration (Suding, Gross and Houseman, 2004; Funk *et al.*, 2008). Biological invasion is one of the main challenges to *Cerrado* restoration as invaders usually recolonize restored areas and replace native species (Coutinho *et al.*, 2019; Sampaio *et al.*, 2019). Traits conferring fast-growth and competitiveness, to detriment of stress-tolerance, are expected to underlie invasion success (van Kleunen, Dawson and Maurel, 2015). Then, by knowing the traits of key invasive species, future restoration actions may focus on management that increases native species competitiveness over invasive ones.

As *Cerrado* plays a key role in the South American water and carbon cycle, traits related to water-use and productivity could be used to evaluate *Cerrado* restored areas. For instance, the leaf minimum water potential (Ψ_{min}, water potential in the driest period of the year) provides an integrative measure of leaf water status (Bhaskar and Ackerly, 2006). The ability to maintain a high Ψ_{min} (close to zero) may suggest the capacity to avoid dehydration while the opposite denotes dehydration endurance. Also, the ability to keep a low leaf minimum conductance (g_{min}, residual water loss through cuticle or leaky stomata) is another indicator of desiccation avoidance strategy (Duursma *et al.*, 2019). Despite g_{min} being far lower than stomatal conductance, it can lead to leaf dehydration and drought-induced death. On the other hand, species that resist long periods of water deficit may invest in a lower leaf turgor-loss point (π_{tlp}, water potentials in which the leaf loses turgor) (Bartlett, Scoffoni and Sack, 2012). π_{tlp} affects stomatal conductance, photosynthesis, and cell division, impacting long-term leaf productivity (Bartlett *et al.*, 2016; Zhu *et al.*, 2018). An instantaneous proxy of vegetation aboveground productivity can be plant height, which for herbaceous plants is tightly correlated to aboveground biomass (Axmanová *et al.*, 2012). Meanwhile, leaf mass per area (LMA) is suggested as a good proxy of leaf-basis productivity potential (Wright *et al.*, 2004; Onoda *et al.*, 2017).

Here we compared the soil and plant traits between an old-growth and 3-years-old restored grassland in the core of the *Cerrado* biome in Brazil. We focused on (i) graminoid life-form which dominates over *Cerrado* grassland and savannas yet remains largely neglected in the scientific literature; and (ii) traits related to water-use (Ψ_{min}, g_{min}, and π_{tlp}) and productivity (plant height and LMA) due to the role of *Cerrado* on water resources and carbon budget. We addressed the following questions and hypotheses.

1. How does soil conditions vary between restored and old-growth grasslands? We expected soils of the restored grassland to be more fertile and acidic compared to the old-growth counterpart due to previous land-use changes (e.g., fertilization and liming) (Hunke *et al.*, 2015). Yet, we expect similar soil texture and moisture between the study sites due to the spatial proximity of them.
2. Is plant functional diversity recovered by restoration? We expected lower trait variance and distinct CWMs in the restored community compared to the old-growth reference. Species selection in restoration projects usually does not account for functional traits and sometimes uses species in densities different from those found in reference vegetation which can affect the CWMs (Carlucci *et al.*, 2020).
3. Do the invasive species' functional traits differ from the natives? We expected the African grass *Urochloa decumbens* to have traits related to high productivity at the cost of low drought resistance in contrast to native species. Acquisitive resource-use is a major feature of invasive species (van Kleunen, Dawson and Maurel, 2015) and may underpin invasibility in *Cerrado* lands.

MATERIAL AND METHODS

Study site

We carried out this study in a dry grassland at the *Chapada dos Veadeiros* National Park (*Goiás* state, 14°05’ S, 47°38’ W, 1.225 m.a.s.l.) (SI Tab. 1). *Chapada dos Veadeiros* is within one of the most species-rich regions of *Cerrado* (Amaral *et al.*, 2017) and protects about 240,000 ha comprising several vegetation types (e.g., *campo limpo* grasslands, *Cerrado sensu stricto* typical savannas, *campos rupestres* rock outcrops, *matas de galeria* riparian forests). The climate is tropical wet savannah (Aw Köppen classification) with the rain season from October to April (96 % of annual rainfall) and the dry season from May to September. The mean annual cumulative rainfall is 1,324 mm and the temperature is 24 ºC. The predominant soil class is latosol and cambisol.

Within the National Park, we selected two study sites (Fig 1). (i) Restored area: a 0.33 ha restoration experiment implemented in 2016 in abandoned pastures dominated by the African grass *Urochloa decumbens* (Stapf) R.D.Webster (Fig. 1a). Before seed sowing, the area was managed by three prescribed fires followed by soil mowing to reduce the re-establishment of invasive grasses and decompress the soil. Mechanized direct-seeding was used to sow the seeds and the seed mixture had a high proportion of herbaceous species relative to shrubs and trees (Sampaio *et al.*, 2019). (ii) Old-growth area: an undisturbed dry grassland without any invasive species and no previous human use (Fig. 1b) (old-growth concept sensu Veldman et al. 2015). The old-growth area is located 1.14 km next to the restored area in the lower part of the plateau (the difference in elevation between the sites is 18 m). The old-growth site corresponds to the best-conserved region as near as possible to the restored site. Both areas are within the same plateau, located in a similar topographical position (hill slope; the height above the nearest drainage [HAND] is 18 m for the old-growth site and 15 m for the restored site), and facing the same aspect (north).

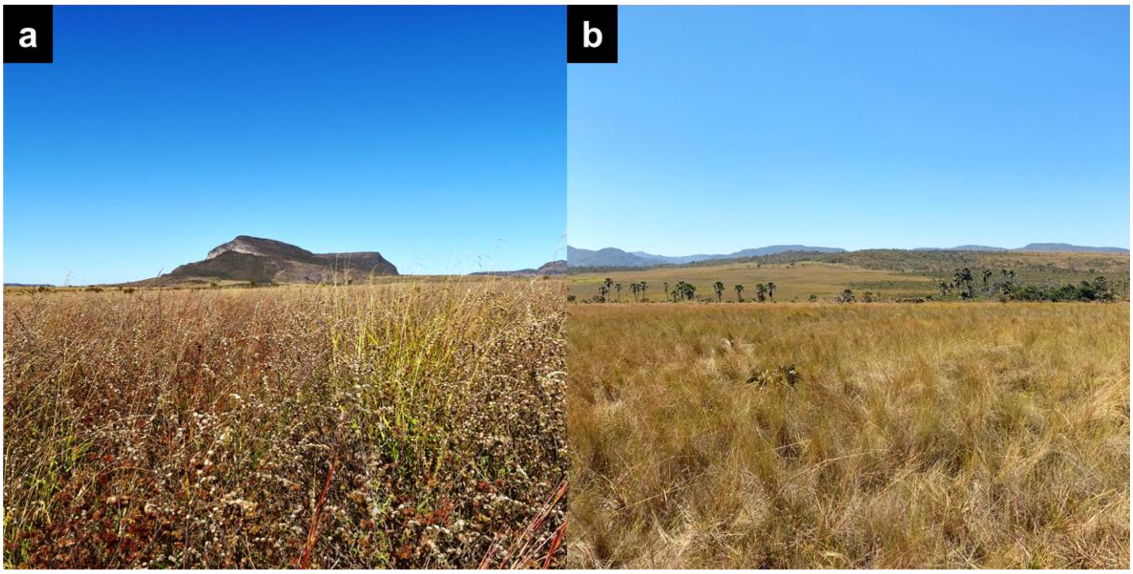

Figure 1. Picture of the (a) restored (b) old-growth study site at the *Chapada dos Veadeiros* National Park, Central Brazil.

Species composition and abundance

We installed five linear plots composed of two perpendicular 20 m lines (cross shape) in each study site 2019. We used the point-intercept method to survey the vegetation which consists of setting a line, putting a pin (or a stick) each 10 cm along the line, and recording each species that touches the pin (see Muñoz, Cassia; Araújo 2011) This methodology provides a tri-dimensional representation of the vegetation composition and structure closely related to the vegetation biomass (Jonasson, 1988). This floristic inventory is part of long-term monitoring of the restored area plant diversity and structure. The relative abundance of the species *i* was measured by the following equation:

$$Relative\ abundance_i = \left(\frac{Abundance_i}{\sum_{i=1}^{n} Abundance_i}\right)$$

Where "abundance" is the number of records of the species *i* and "n" the total number of species. All species were identified at the finest taxonomic resolution as possible with the assistance of experts. In all further analysis, we only kept graminoid species here defined as herbaceous plants belonging to the Poaceae, Cyperaceae, Juncaceae, and Xyridaceae families.

Plant functional traits

In addition to the floristic inventory, we installed five circular plots (20 m of radius) per study site distributed in a 160 m transect and spaced 40 m from each other in 2019 to survey plant functional traits (SI Tab. 1). The trait assessment was made in a subset of graminoid species (Poaceae and Cyperaceae families) from the regional pool of species. We used the floristic inventories to select both dominant and rare species (See SI Tab. 1 for the species list) representing 74.5 and 82.1 % of the total abundance of the old-growth and restored site, respectively. Within the circular plot, we selected tall individuals and preferentially in the mature flowering stage to avoid ontogenetic variation. Unfortunately, it was not possible to measure all traits in the same individual as some traits require the removal of the entire individual off the field or there were not sufficient green fully-expanded healthy leaves for all measurements to be taken simultaneously. The circular plots were placed in the vicinity of the floristic inventory plots respecting a minimum distance of 80 m. This spatial mismatch was required to avoid jeopardizing the restoration monitoring program by trampling or plant removal inside the floristic inventory plots. Within the circular plots, we measured 5 functional traits related to productivity and water relations. Below we described the methodology used to measure the studied traits.

(i) Plant height (“height”, cm): We selected one individual per species per plot, prioritizing healthy and flowering plants. We stretched the tussock to identify the longest organ (usually the inflorescence) and then measured the distance between the ground and the top of this longest organ (in cm). Measurements were made at the peak of the 2019 dry season (July to August).

(ii) Leaf minimum water potential (“Ψ_{min}”, MPa): In the field, we selected a healthy and preferentially flowering individual per plot for each species. We measured leaf water potential in the driest period of the day (midday from 11 a.m. to 1 p.m.) with a Scholander pressure chamber (Model 1000, PMS Instrument Company, USA) in two green full-expanded healthy leaves per individual following the Boyer (2011) protocol. Measurements were made in a sunny week at the peak of the 2019 dry season (July to August).

(iii) Leaf mass per area (“LMA”, g m^{-2}): We followed Pérez-Harguindeguy et al. (2013) protocol to measure LMA. We collected an entire individual per plot of each species by the morning (7:30 to 9 a.m.). We store the individual in black bags to end up photosynthesis, put water on the bag to avoid dehydration, and transported the individuals to the lab. In the lab, we collected five green full-expanded healthy (i.e., without signals of pathogens or herbivory) leaves per individual. We rehydrated the leaves in water-filled plastic bags overnight in a thermal box. After hydration, we scanned the turgid leaves using a desk scanner. We then identified each leaf and stored them in paper bags. We dried the paper bags containing the leaves for at least 72 h under 60 ºC. We measured the dry mass using a precision balance (High Precision Digital Milligram Scale, Smart Weigh, 0.001 g precision). We measured leaf area using the ImageJ software. We measured the LMA dividing the leaf dry mass (in g) per leaf area (in m^2). Measurements were made at the peak of the 2020 rainy season (January to February).

(iv) Leaf turgor loss point ("π_{tlp}", MPa): π_{tlp} was estimated by the pressure-volume (P-V) curve following Sack and Pasquet-Kok (2011) protocol and spreadsheet. Two to three green fully-expanded healthy leaves were taken from the same individual used for LMA analyses. We left the leaves to rehydrated overnight in water-filled plastic bags in a thermal box to make sure to start the P-V curve at the higher water potential. We only removed the leaves out of the bag when we were ready to start the P-V measurements. The curve consisted of several cycles of weighing and measuring the leaf water potential (Ψ_L, MPa). We used a portable balance (High Precision Digital Milligram Scale, Smart Weigh, 0.001 g precision) and a Scholander pressure chamber (Model 1000, PMS Instrument Company, USA) to measure leaf weight and Ψ_L, respectively. At least for the four first cycles, we did not take any time interval as the Ψ_L jumped from less than -0.5 to -1 MPa in the first minutes for the majority of species, thus sequential P-V cycles were needed to capture the initial shape of the P-V curve. After the 5th cycle, we took 10 to 40 min breaks between each cycle according to the variation of Ψ_L over time. We ended the cycles when there was no variation in Ψ_L or extremely low Ψ_L (e.g., less than -5 MPa, an extreme π_{tlp} value according to Bartlett, Scoffoni, and Sack 2012). After ending up P-V cycles, we identified the leaves, put them in paper bags, dry them up by at least 72 h in 60 ºC, and measured the dry weight using the precision balance.

(v) Leaf minimum conductance ("g_{min}", mmol m^{-2} s^{-1}): g_{min} was measured by the weight loss of detached leaves following the Sack and Scoffoni (2011) protocol. The method consists of monitoring leaf water loss (inferred by weight loss) and air vapor pressure deficit (VPD) over time. Previous studies showed that the leaf weight decreases exponentially over time until the stomata closure when the rate of weight loss becomes linear (Duursma *et al.*, 2019). The linear part of the leaf weight loss curve over time can be used to estimate g_{min}. In the lab, we took from two to three green full-expanded healthy leaves of the same individual used in LMA and π_{tlp} measurements. We left the leaves to rehydrated in water-filled plastic bags in a thermal box to avoid starting the measurements after stomata closure. The g_{min} measurement consisted of several cycles of weighting the same leaf and recording time and air moisture and temperature of each measurement. We used a portable precision balance (High Precision Digital Milligram Scale, Smart Weigh, 0.001 g precision) to measure the leaf weight and the cuvette of a porometer system to measure the air moisture and temperature (SC-1 Leaf Porometer, METER Group, Inc. USA). We only removed the leaf from the bag when we were ready to start the procedure. We first dried the leaf surface with paper and measured the first four cycles sequentially (leaf weighting and time and air moisture and temperature recording) and avoiding a long interval between the cycles (i.e., less than 10 minutes). We did at least 7 cycles. The VPD was estimated by the Arden Buck equation. We used Sack and Scoffoni (2011) spreadsheet to estimate g_{min} in which the input was the leaf weight, time, and microclimatic variables. We checked if the weight loss curve reached a linear trend and we selected the last three intervals for g_{min} estimation.

Edaphic variables

We installed five 1.4 m depth holes in the center of each circular plot described before. We used a soil auger to collect soil samples at the following depths: 0–5, 5–10, 10–15, 15–20, 20–40, 60–80, 80–100, 100–120, and 120–140 cm below the soil surface. The sampling was made during the morning (8 to 12 a.m.) at the field and the sampled soil was stored in hermetically closed plastic bags. On the night of the same day that we collected the soil, we opened the soil bags in the lab and measured the fresh weight (FW) of a subsample using a portable precision balance (High Precision Digital Milligram Scale, Smart Weigh, 0.001 g precision). Then, we dried the soil subsamples until the weight of the subsample stabilizes indicating evaporation of the labile water in the subsample. We then weighted the dry soil subsamples (DW) and calculated the soil gravimetric relative water content (soil moisture, %) by the following formula.

$$Soil\ moisture\ (\%) = \left(\frac{FW - DW}{FW}\right) \times 100$$

To investigate soil chemistry, we created a mixed sample of the topsoil by combining about 50 g of the depths 0–5, 5–10, 10–15, and 15–20 cm. The available phosphorus (mg dm^{-3}), total nitrogen (g kg^{-1}), soil organic matter (g kg^{-1}), pH, and aluminum content (cmolc dm^{-3}) of those mixed samples were analyzed at the soils lab of the *Escola Superior de Agricultura Luiz de Queiroz* (ESALQ) linked to the São Paulo University in Brazil. The pH was determined in H_2O and, along with available phosphorus, was measured using the Mehlich method (Silva *et al.*, 1998). The aluminum and organic matter was determined by titrimetry and extracted in KCl and dichromate solution, respectively (Silva *et al.*, 1998; Camargo *et al.*, 2009). Nitrogen was extracted by the Kjeldahl method and determined by electric conductivity (Silva, 2009).

We also analyzed the content of sand and clay in each depth of each sampling plot in the same laboratory.

Data analysis

We ran t-tests and fitted generalized additive models (GAM) to test whether edaphic variables differ between the restored and old-growth area (hypothesis 1). In all analyses, we fitted the model and checked the homoscedasticity and normality assumptions visually. Here and henceforward, we ran Student’s t-test for normal-distributed and variance homogeneous data, Welch’s t-test for normal-distributed and variance heterogeneous data, and Wilcoxon’s t-test (Mann-Whitney) for non-normal-distributed data regardless of its homoscedasticity. We performed a Student’s t-test on phosphorus and nitrogen and a Welch’s t-test on organic matter, pH, and aluminum always contrasting the restored *versus* old-growth topsoil (mixed sample from the first 20 cm depth). We fitted GAMs to investigate the non-linear pattern of soil moisture and clay-to-sand ratio along the soil depth profile. Soil moisture and clay-to-sand ratio were considered response variables, soil depth the predictor variable, and the sample at each depth at each sampling plot the sampling unit. We fitted a GAM without site (restored *versus* old-growth area) as a predictor and another GAM including the site to test whether the study sites had distinct relationships. We then compared the models using the Akaike information criteria (AIC). Meanwhile, to test the effect of the clay-to-sand ratio on soil moisture, we fitted a linear mixed-effect model allowing a random slope and intercept for each sampling plot (the plot was the random effect).

We ran a variance homogeneity test to compare the trait variance between the restored and old-growth vegetation (hypothesis 2). First, we performed a Levene’s homoscedasticity test on Ψ_{min}, g_{min}, π_{tlp}, plant height, and LMA contrasting the restored and old-growth area in which the sampling unit was the individual. Second, we computed the percentage of trait variance explained by the species, study site, or their interaction to explore the drivers of trait variance. For this, we ran an ANOVA on the shared species between the study sites in which the trait values were the response variables and site the predictor variable. We then extracted the sum of squares of each predictor (species identity, study site, or the species-site interaction) and the residuals (unknown source of variation) and divided them by the total sum of squares multiplied by 100. Thereby, we end with the percentage of variance explained by the categories cited above in each trait.

We ran t-tests to compare the community-weighted means (CWM) of the restored *vs*. old-growth vegetation (hypothesis 2). We averaged all traits at the species level and created a matrix where species were in the columns, floristic inventory plots in the rows, and the relative abundance of each species in each plot in the cell values. The trait value of each species was multiplied by its relative abundance and then summed to get only one CWM per sampling plot. We performed Student's t-tests on the CWM g_{min}, π_{tlp}, and plant height and a Welch's t-test on Ψ_{min} and LMA always comparing restored *versus* old-growth areas. As CWMs are sensitive to species dominance we conducted two additional analyses. Firstly, we computed for each study site how exclusive species (i.e., species that occurs only in one site), shared species (i.e., species that occurs in both sites), and unstudied species account for total ground cover. Secondly, we also displayed the relative abundance of the shared species when occurring in the restored or old-growth area.

Finally, we ran t-tests to compare the functional traits of the invasive *vs*. native species (hypothesis 3). As before, each trait was analyzed separately but here the contrast was the species origin (exotic *versus* native), and only the restored site was included as there were no invasive species in the old-growth site. We performed a Wilcoxon's t-test on Ψ_{min} and plant height and a Welch t-test on g_{min}, π_{tlp}, and LMA. We used the *R* environment to perform all the analysis (R Core Team, 2017). The package *FD* was used to calculate the community-weighted means (Laliberté and Shipley, 2011). The package *mgcv* was used to fit the generalized additive models (Wood, 2003). The package *lme4* was used to fit the linear mixed-effect model (Bates *et al.*, 2015).

RESULTS

Edaphic conditions

The restored site had, on average, 2-fold less available phosphorus (P), total nitrogen (N), and soil organic matter (SOM) than the old-growth site in the topsoil (i.e., first 20 cm) at the peak of the dry season (Fig. 2 a) (SI Tab. 3) (P *p*<0.001; N *p*=0.002; SOM *p*=0.02). There was no significant difference between the pH of the old-growth and restored site (Fig. 2d). Aluminum, by its turn, was 1.4-fold lower in the restored site compared to the old-growth one (*p*=0.01) (Fig. 2e).

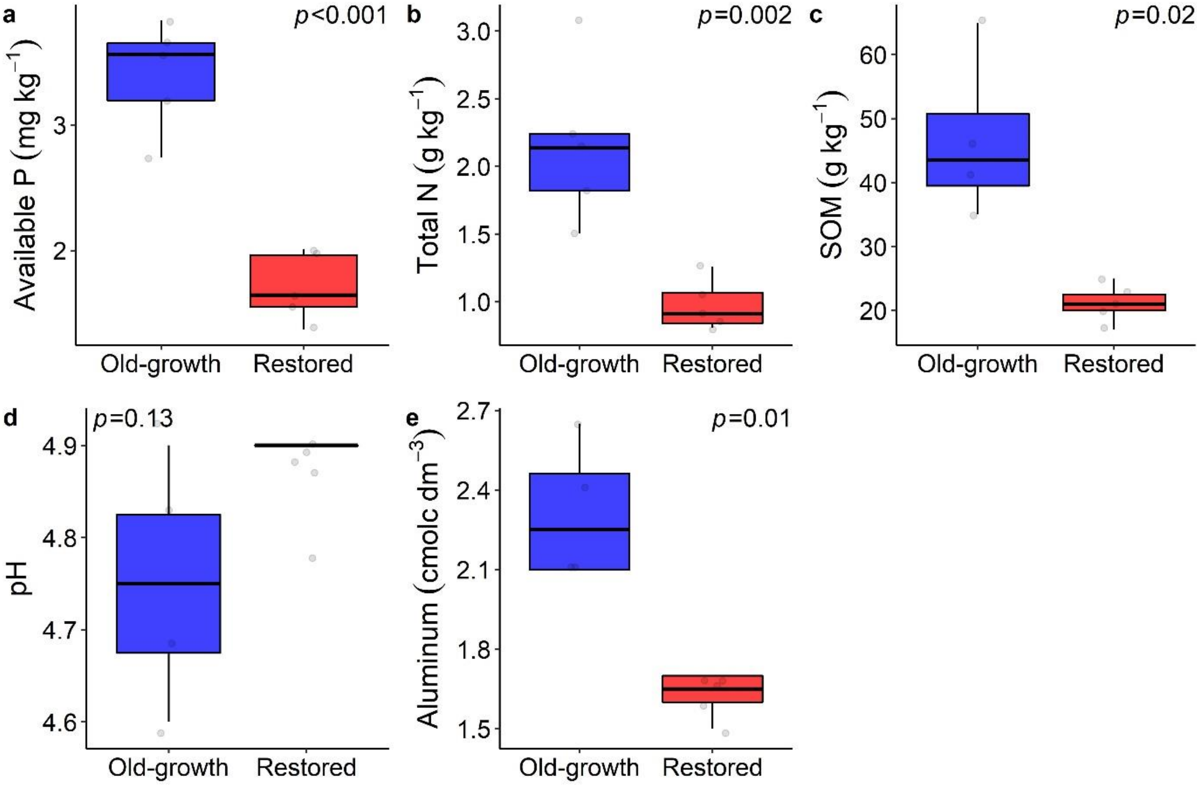

Figure 2. Soil fertility and acidity between an old-growth and restored *Cerrado* grassland. Concentrations of (a) available phosphorus, (b) total nitrogen, (c) soil organic matter, (d) pH in water, and finally (e) aluminum of the topsoil of each study site. Panels display *p*-values according to t-tests and observations represent sampling plots within each site.

Both soil moisture and clay fraction increased along with soil depth at the peak of the dry season (Fig. 3a and b). The relative water content of old-growth soils ranged from 1.1 to 22 % while restored soils ranged from 0.7 to10 % from 2.5 to 130 cm belowground. Overall, soils in the old-growth site were wetter and clayed (mean moisture of 10.8 % and clay-to-sand ratio of 0.88) than restored soils (mean moisture of 6.2 % and clay-to-sand ratio of 0.3). The difference between restored and old-growth soil was significant as the generalized additive model fitted to allow a smooth curve for each site (AIC=321.9, R^2=0.9) outperformed the model fitted to allow a single curve for both sites (AIC=423.8, R^2=0.6). Clay-to-sand ratio affected positively the soil moisture in both sites but the fitted line was sloped in the restored site ($\beta_{restored}$=16.47, p=0.007) compared to the old-growth one ($\beta_{old\text{-}growth}$=11.6, p<0.001) (Fig. 3c).

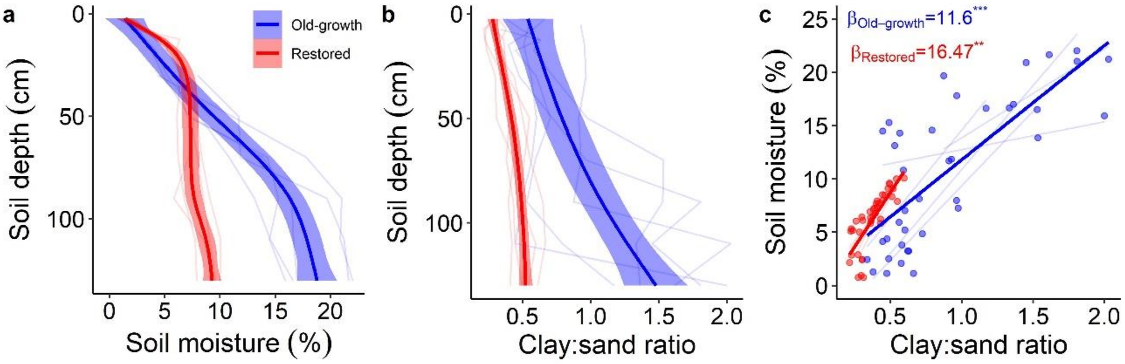

Figure 3. Soil moisture and texture across depth profile in an old-growth and restored *Cerrado* grassland. (a) Moisture is represented by the gravimetric relative water content from the topsoil to 1.3 m depth layers. (b) Texture is represented by the ratio between clay and sand (sandier soils at ratios <1 and clayed soils at ratios >1) in the same vertical profile. (c) Relationship between soil clay:sand ratio and moisture; thicker lines represent the fitted line of each study site (old-growth in blue and restored in red) along with the mixed-effect model slope (β) in the top left corner. Each transparent line in (a), (b), and (c) connect samples of the same sampling plot. **p<0.01, ***p<0.001.

Plant functional traits

The restored community had a similar variability of Ψ_{min}, plant height, and LMA than the old-growth community (Levene's test of homoscedasticity Ψ_{min} p=0.34, height p=0.3, and LMA p=0.06) (Fig. 4a, d, and e) (SI Tab. 4). This pattern did not hold to g_{min} and π_{tlp} as both traits were less variable at the restored community compared to the old-growth reference (Fig.

4b and c). The g_{min} ranged from 7.5 to 30.7 mmol m^{-2} s^{-1} at restored community and from 1.6 to 81.2 mmol m^{-2} s^{-1} in the old-growth one ($p<0.001$); while π_{tlp} ranged from -2.1 to -1.2 MPa in the restored community and -3.8 to -1.4 MPa in the old-growth one ($p=0.002$).

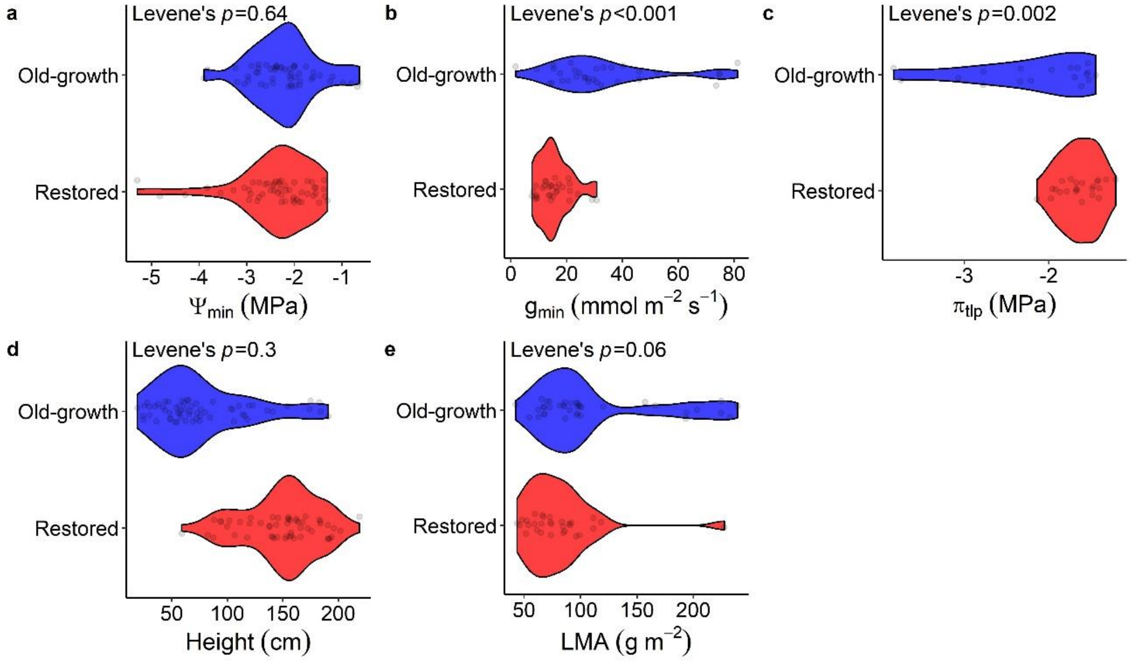

Figure 4. Diversity of trait values of an old-growth and restored *Cerrado* grassland. Violin plots showing the variability of (a) leaf minimum water potential (Ψ_{min}), leaf minimum conductance (g_{min}), (c) leaf turgor loss point (π_{tlp}), (d) plant height, and (e) leaf mass per area (LMA) of each study site. Panels display the *p*-value of Levene's homoscedasticity test; $p<0.05$ means heterogeneous variances while $p>0.05$ homogeneous variances between the sites. Observations represent the trait value of a given individual.

When the variance of the traits was partitioned (Fig. 5), species identity explained from 15.9 to 61.3 % of traits variability, study site from 0.01 to 20.5 %, the interaction between species and site from 2.4 to 12.3 %, and lastly, unknown sources (residuals) accounted from 12.7 to 66.4 % of trait variance.

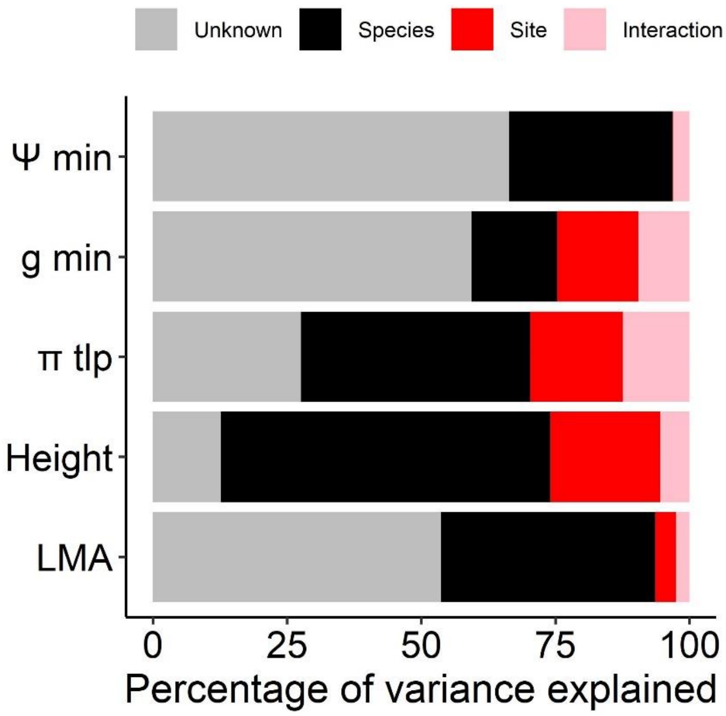

Figure 5. Source of variation of the plant traits among species shared by the old-growth and restored sites. Variance partitioning between the predictor variables extracted from ANOVA tables. “Unknown” (in grey) refers to the model residuals, “species” (in black) to the species which the individual belongs, “site” (in red) to the area where the individual has grown (old-growth or restored), and “interaction” (in light pink) to the interaction between “species” and “site”.

The community-weighted means (CWM) of all traits differed significantly between the restored and old-growth sites (Fig. 6) (SI Tab 5). CWM Ψ_{min} and g_{min} were 0.8- and 2-fold lower in the restored community compared to the old-growth one ($p=0.003$ and <0.001, respectively) (Fig. 6a and b). However, the CWM π_{tlp} was ~2-fold higher in the restored community compared to the old-growth one ($p<0.001$) (Fig. 6c). The restored site had a 1.6-fold higher and 2-fold lower plant height and LMA than the old-growth site, respectively ($p<0.001$ for both) (Fig. 6d and e).

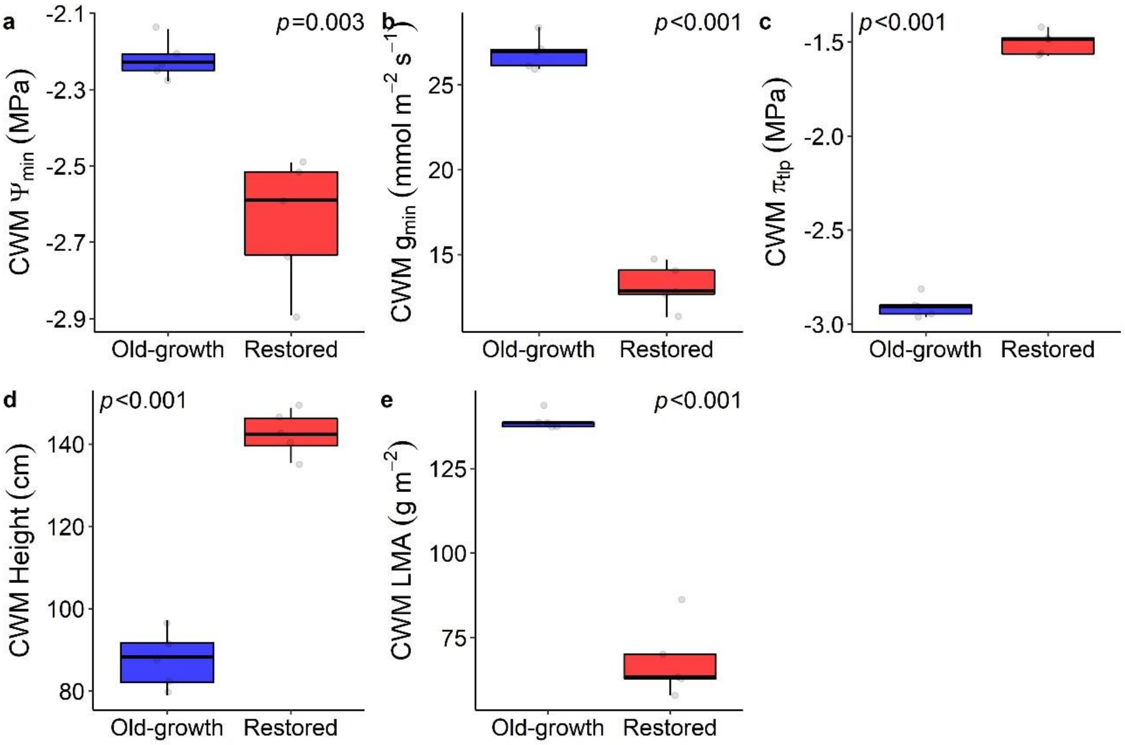

Figure 6. Abundance-weighted traits between an old-growth and restored *Cerrado* grassland. Community-weighted means (CWM) of (a) leaf minimum water potential (Ψ_{min}), leaf minimum conductance (g_{min}), (c) leaf turgor loss point (π_{tlp}), (d) plant height, and (e) leaf mass per area (LMA) of each study site. Panels display the *p*-value according to t-tests. Observations represent the sampling plots within each study site.

Relative abundance, one of the main drivers of CWM, was distributed differently among species within each site. Studied species which include shared species and exclusive species represented 74.5 and 82.21 % of old-growth and restored site plant records (Fig. 7a). Shared species accounted for 65.5 % of the total abundance at the old-growth site and 18.7 % at the restored site, whereas exclusive species accounted for 0.09 % of the total abundance at the old-growth site and 0.63 % at the restored site (Fig. 7a). Looking just at shared species, the most abundant species at the old-growth area, *S. tenerum*, had a relative abundance of 55.8 % at the old-growth site and just 1.3 % at the restored site (Fig. 7b).

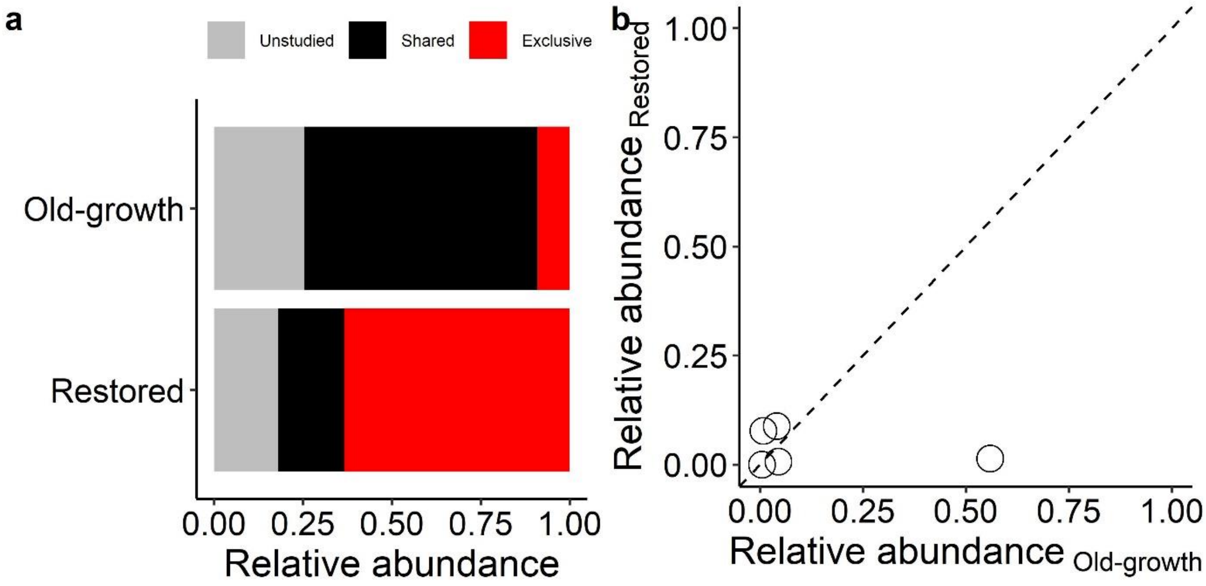

Figure 7. Studied species abundance in an old-growth and restored *Cerrado* grassland. (a) The relative abundance of not-studied species (unstudied, in grey), species occurring both old-growth and restored site (shared, in black), and finally the species restricted to one of these sites (exclusive, in red). (b) The relative abundance of the same species in each study site; species near to the 1:1 line (dashed) had a similar abundance in either site whereas species in the bottom right region were common at the old-growth site yet uncommon at the restored site.

Invasive species traits

The African grass *Urochloa decumbens* had trait values distinct from the remaining grasses in the restored area (Fig. 8). The π_{tlp} of *U. decumbens* (π_{tlp}=-1.3 MPa) was, on average, higher than any of the studied species in the restored site (π_{tlp} range=-2 to -1.4 MPa) (*p*=0.02) (Fig. 8c). Same as LMA, which achieved the lowest values in *U. decumbens* (mean LMA=47.4 g m^{-2}) compared to the remaining species of the restored site (LMA range=55.5 to 169.2 g m^{-2}) (*p*<0.001) (Fig. 8e). The g_{min} of *U. decumbens* was one of the lowest among restored site grasses but did not differ significantly (*p*=0.07) (Fig. 8b). Ψ_{min} and plant height, on the other hand, were statistically similar between the invasive species *U. decumbens* and the native species in the restored site (Ψ_{min} *p*=0.34 and height *p*=0.25) (Fig. 8a and d).

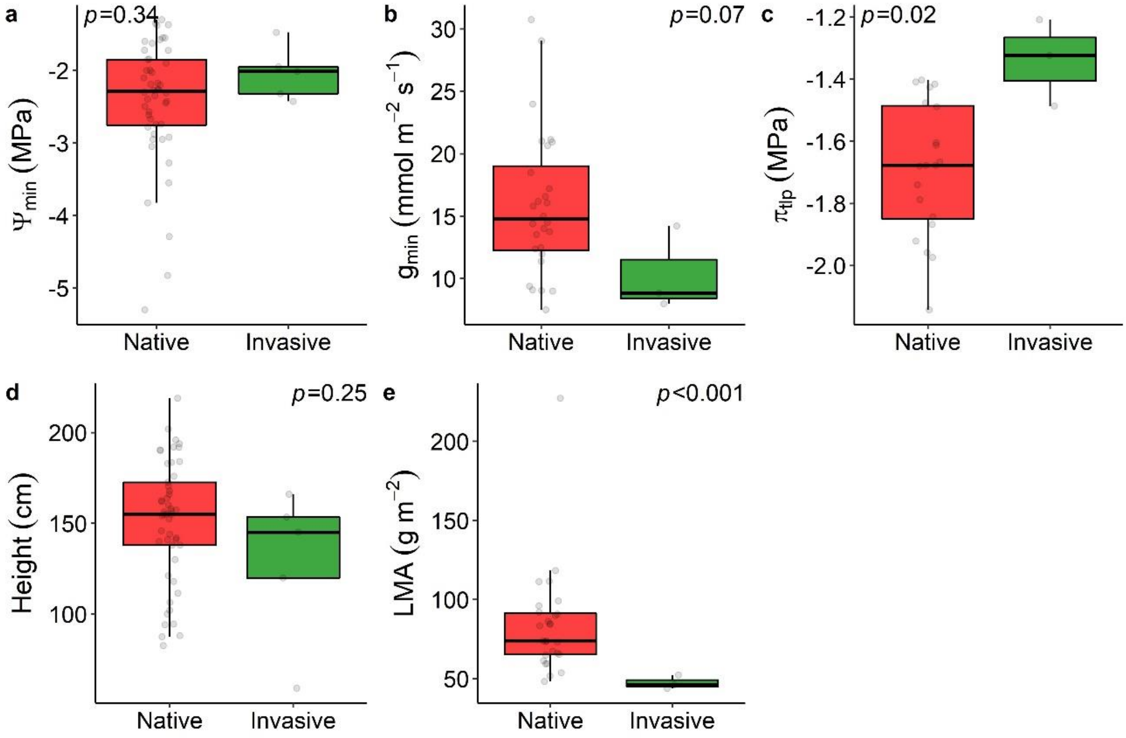

Figure 8. Traits of invasive versus native species in the restored site. (a) Leaf minimum water potential (Ψ_{min}), leaf minimum conductance (g_{min}), (c) leaf turgor loss point (π_{tlp}), (d) plant height, and (e) leaf mass per area (LMA) between native grasses and the African grass *U. decumbens*. Panels display *p*-values according to t-tests and observations represent the trait value of a given individual.

DISCUSSION

Here we unveiled the functional outcomes of direct-seeding restoration in the world's most biodiverse savanna, the Brazilian *Cerrado*. The soil of the old-growth area was wetter and nutrient- and aluminum-richer than the soil of the restored area. Also, the variance of two key traits related to drought coping (g_{min} and π_{tlp}) was limited in the restored community compared to the old-growth counterpart. Although the main cause of trait variation remained unknown, species identity explained a significant part of trait variance between the two areas. Regarding community-weighted trait means, abundant species following restoration had traits related to drought-sensitivity and productivity (higher π_{tlp} and lower LMA) in contrast to old-growth's abundant species. In fact, the old-growth community commonest species became rare in the restored community. The *U. decumbens* presented the highest π_{tlp} and lowest LMA among grasses co-occurring in the restored area pointing for functional differences between exotic and native species. Bellow, we further discuss these results and provide insights into how restoration practitioners can improve *Cerrado* biodiversity recovery in the light of our findings.

Soil fertility and moisture following restoration

Three non-exclusive causes can underlie the lower phosphorus and nitrogen concentrations in restored rather than old-growth areas. Firstly, invasive species legacies on soil can lead to a fast nutrient cycling (i.e., pulses of nutrients rather than immobilization in the soil) even after restoration efforts (Schrama and Bardgett, 2016; Hess, Mesléard and Buisson, 2019; Zenni *et al.*, 2020). Secondly, pre-sowing prescribed fires can reduce nutrient pools by nutrient volatilization (Pivello and Coutinho, 1992; Kauffman, Cummings and Ward, 1994). Although soil and fire management proved to control re-invasion by African grasses in the short-term (Sampaio *et al.*, 2019), the sequential fires within one year undertook at the studied restored site may affect nutrient stocks by releasing nutrients, especially nitrogen, into the atmosphere (Pivello *et al.*, 2010; Bustamante *et al.*, 2012; Oliveras *et al.*, 2013). Lastly, the fast-growing restored plant assemblage may quickly absorb soil nutrients and stock them in the living biomass. Vegetation following restoration was composed of tall plants with acquisitive leaves (low LMA) which can increase the nutrient storage in the aboveground compared to the belowground compartment.

Soil aluminum concentration was higher in old-growth site soils compared to the restored ones in line with our expectations. Past soil liming can explain lower aluminum concentrations following land-use changes found here and elsewhere (Villela and Haridasan, 1994; Castro and Crusciol, 2013; Li *et al.*, 2019). *Cerrado* plant lineages evolved under high aluminum concentrations and possess strategies to tolerate aluminum toxicity and store it in their living tissues (Haridasan, 1982, 2008; Mendonça *et al.*, 2020). African lineages, on the other hand, did not evolve under the same aluminum stress and the low concentration of this element could facilitate African grasses invasions (Zenni *et al.*, 2020). However, African grasses have been invading even conserved Al-rich ecosystems over the *Cerrado* biome (Pivello, Shida and Meirelles, 1999). Thus, although the Al enrichment has the potential to decrease restoration invasibility, additional management actions will probably still be needed to control biological invasions in *Cerrado* restored areas.

The relative proportion of clay to sand affected positively soil moisture, evidencing that soil texture could underlie the differences in water availability between the restored and old-growth areas. Clay soils are known to be capable to hold water more efficiently than sandier soils (An *et al.*, 2018; Lehmann *et al.*, 2018). Moreover, soils in the old-growth area had higher levels of organic matter compared to the restored area which may also increase water holding capacity in addition to the soil clay fraction. Soil texture within the *Cerrado* biome can vary in few meters (Abreu *et al.*, 2012) and is expected to be more influenced by origin rock and topography than historical land-use. Although the study sites are located in the same plateau, we acknowledge that the studied restored and old-growth grassland might have distinct soil textures in the first place. These fine-scale differences in soil texture highlight how challenging it is to select a reference site to contrast with restored areas in highly heterogeneous biomes such as *Cerrado* (White and Walker, 1997; Isabel B. Schmidt *et al.*, 2019).

Recovery of plant functional diversity

Our expectation of lower diversity of trait values held for hydraulic traits (g_{min} and π_{tlp}) whereas leaf water status (Ψ_{min}) and productivity (plant height and LMA) remained as variable in restored communities as in old-growth ones. Similar Ψ_{min} even under distinct soil water availabilities suggest that the native grasses might adjust their water-use to keep leaf water status within an “optimal” range (i.e., isohydry). *Cerrado* trees are known for their isohydric behavior (Bucci *et al.*, 2005, 2008) but Cerrado grasses are expected to be strongly anysohydric based on seasonal variations of the leaf water potential (Rossatto, da Silveira Lobo Sternberg and Franco, 2013). It is possible that grasses vary their leaf water potential along the dry and wet season but kept it fixed within each season, which conciliates our findings and previous evidence. Furthermore, both small *versus* tall grasses and leaf-acquisitive (low LMA) *versus* -conservative (high LMA) grasses coexisted in both sites highlighting the ability of direct-seeding to re-assemble both fast- and slow-growing species (Pellizzaro *et al.*, 2017; Coutinho *et al.*, 2019). However, key traits related to drought resistance such as g_{min} and π_{tlp} remained less variable in the restored community compared to the diverse range of values founded in the old-growth community. As the intensity and frequency of drought events are expected to increase in *Cerrado* in the following decades (see http://pclima.inpe.br/analise/), the lack of diversity in hydraulic traits can jeopardize the resilience of restored vegetation to extreme climatic events (Craine *et al.*, 2013; Timpane-Padgham, Beechie and Klinger, 2017; Sankaran, 2019; Passaretti, Pilon and Durigan, 2020). Overall, traits were more variable between different species than between the studies sites (i.e., restored *versus* old-growth area). This tight relationship between functional traits and species identity means that the failure in restoring g_{min} and π_{tlp} extreme values may lay on missing species. Those may include non-Poaceae graminoids such as Cyperaceae species that was at the end of old-growth's trait spectrum (e.g., *R. consanguinea* with g_{min} of 52.8 mmol m^{-2} s^{-1} and π_{tlp} of -2.1 MPa), possesses unique nutrient acquisition strategies (Oliveira *et al.*, 2015), and represent a key component of the *Cerrado* biodiversity (Silveira *et al.*, 2016).

When the traits are weighted by species abundance, restored vegetation was marked by the dominance of drought-sensitive plants yet productive in contrast to the old-growth vegetation. Even though dehydration avoidance (high Ψ_{min}) and resistance (low Ψ_{min}) can coexist in both sites, grasses dried more in the restored rather than old-growth area as inferred by CWM Ψ_{min}. This difference is probably due to the distinct trait values of the dominant species in each site. Whereas *S. tenerum* with Ψ_{min} of -2.2 MPa dominated in the old-growth vegetation (relative abundance of 55.8%), *A. riparia* with Ψ_{min} of -4 MPa was one of the most abundant species in the restored vegetation (relative abundance of 20.9 %). Besides the water status, grasses lost the leaf turgor faster (high CWM π_{tlp}) in the restored area than in the old-growth one. Based on these, we infer that restored vegetation can experience a striking decrease in productivity throughout prolonged water deficits (Maréchaux *et al.*, 2018). Two trade-offs can simultaneously underlie lower π_{tlp} in the restored vegetation compared to the old-growth one. First, the studied grasses might be allocating resources to either increase leaf productivity (low LMA) or leaf desiccation resistance (high π_{tlp}). Second, studied grasses might be allocating resources to either dehydration avoidance (low g_{min}) or desiccation resistance (high π_{tlp}). The LMA-π_{tlp} relationship has been reported previously (Villagra *et al.*, 2013; Zhu *et al.*, 2018) and also among the investigated species (SI Fig. 1a) whereas the g_{min}-π_{tlp} relationship has not been reported yet (SI Fig. 1b).

Regarding the traits related to productivity, the restored community favored species with a fast resource-use. Specifically, higher CWM plant height and lower CWM LMA may reflect, respectively, a rapid accumulation of aboveground biomass and a higher potential to assimilate carbon (Zuo *et al.*, 2016). Since restored and old-growth vegetation held similar plant height and LMA variance but still opposite CWMs, the major underlying cause of the CWM variation is the differences in species relative abundance. *Cerrado* plant assemblages usually have few dominant and several rare species (Silva, Cianciaruso, and Batalha 2010; Oliveira and Batalha 2005), an abundance structure shared by numerous species-rich communities worldwide and across taxa (McGill *et al.*, 2007; Locey and Lennon, 2016). Just five species that can be found in both restored and old-growth sites accounted for up to 60% of the old-growth plant records and the same set of species represented less than 20% in the restored area. This was mainly due to the drastic decrease in the abundance of *S. tenerum* which represented more than 50 % of the old-growth vegetation and just 1 % of the restored vegetation. Therefore, to recover functional diversity and the associated ecosystem services, *Cerrado* restoration must restore the abundance structure characteristic of the reference sites (see Pilon, Buisson, and Durigan 2018). This can be achieved by increasing the proportion of seeds of some species, managing the soil (lowering the pH and/or increasing Al concentration) to increase the establishment of the target species, and mixing whole-plant transplants of old-growth dominant species with direct-seeding.

The functioning of an invasive grass

The African grass *U. decumbens* had a remarkable higher π_{tlp} and lower LMA compared to the co-occurring native species in the restored community. This result suggests investment in productive and drought-sensitive leaves in the invasive species compared to the natives, consistent with the idea of fast-growing traits underlying invasion success (Funk and Vitousek, 2007; van Kleunen, Dawson and Maurel, 2015; Catford *et al.*, 2019). However, *U. decumbens* was not one of the tallest species possibly because this species is known for allocating a significant amount of resources to belowground (Galdos *et al.*, 2020). Furthermore, the fact that *U. decumbens* lose cell turgor easily while their leaves dehydrate explains a large amount of dry foliage common of vegetation dominated by this invasive grass. As in Neotropical lineages, African grasses evolved tightly with fire recurrence, and invasion by African grasses is recognized by creating new feedback with fire which favors the invasion maintenance (Rossi *et al.*, 2014; Gorgone-Barbosa *et al.*, 2015). Our findings on the higher photosynthetic potential (low LMA) and desiccation sensitivity (high π_{tlp}) in *U. decumbens* may provide a physiological basis for the fire-invasion feedback. The early *U. decumbens* dry off can provide a great amount of high-flammable fuel (low-density leaves due to low LMA) which can feed late dry season intense fires (Rossi *et al.*, 2014; Damasceno and Fidelis, 2020). In the early wet season with the aboveground biomass burned, this invasive species may find a window of opportunity to grow fast and over compete the natives. *U. decumbens* is known to be able to favor a bacteria-dominated soil with a fast nutrient cycling, especially nitrogen (Galdos *et al.*, 2020; Zenni *et al.*, 2020). This microbial composition allows pulses of nitrogen in the first rainfalls after droughts which can also increase the ecosystem invasibility (Schrama and Bardgett, 2016). Therefore, the combination of dehydration-sensitive leaves, late dry season fires, rapid aboveground recovery by productive leaves, and soil nutrient pulses may explain *U. decumbens* success in invading restored and conserved *Cerrado* sites.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Our results suggest that the functional properties of soil and plant community of a restored grassland differ significantly from an old-growth grassland in the *Cerrado*. Restoration favored acquisitive resource-use species which can lead to a fast carbon and nutrient cycling but also a higher drought vulnerability. Soils, on the other hand, had lower phosphorus, nitrogen, organic matter, aluminum, and water availability in the restored area compared to the old-growth contrast. This points to a mismatch between the fast-growing plant community and the dry and impoverished soil following restoration in the studied sites. Alternatively, the higher Al concentration in native soil may be the key factor selecting for a high abundance of stress-tolerant slow-growing species in the old-growth community. Furthermore, the lack of hydraulic diversity in the restored vegetation can limit the long-term success of restoration in the face of extreme climatic events. Therefore, even though the studied restoration re-assembled native-dominated grasslands, these plant assemblages possessed a fast and risky functioning that can affect the recovery of ecosystem function in the *Cerrado* biome. The success of *Cerrado* restoration may lay on steering the soil and plant community to the slow end of resources-use and -cycling.

Future studies can step further on facilitating restoration success by addressing the following outstanding topics. First, it is urgent to develop techniques to restore the soil state typical of an old-growth *Cerrado*. Acidifying the soil and enriching it with aluminum could favor slow-growing native species in the competition with fast-growing invaders. Also, carbon amendments could help to reverse legacy effects, stabilize organic matter and clay particles, and ultimately, increase soil water holding capacity (Hueso-González, Muñoz-Rojas and Martínez-Murillo, 2018; Fehmi, Rasmussen and Gallery, 2020; Zhang, Bhowmik and Suseela, 2020). Second, the restoration seed mix needs to be tailored to re-assemble functional-rich and abundance-structured plant communities. Including a range of herbaceous lineages such as Cyperaceae, Xyridaceae, and Eriocaulaceae in the restoration species list may boost functional diversity. On top of that, as reference communities are not even, seed mix and pre-sowing management can be tailored to increase the dominance of target species to mimic an old-growth *Cerrado* structure and function.

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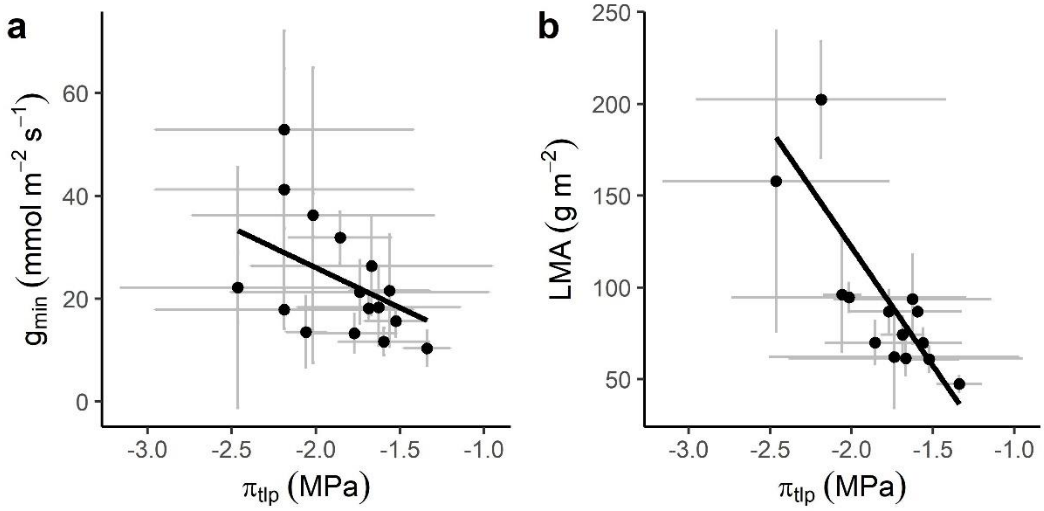
SUPPLEMENTARY INFORMATION

SI Figure 1. Trait correlations among the studied species. (a) The relationship between g_{min} and π_{tlp} (Kendall's τ=-0.41, p=0.02). (b) The relationship between LMA and π_{tlp} (Kendall's τ=0.69, p<0.001). Observations correspond to the trait average per species and the bars the mean ± standard deviation.



SI Table 1. Geographical coordinates and altitude (m.a.s.l.) of the sampling plots. “Functional trait survey” represents the circular plots where trait data were collected and “Floristic inventory” the linear plots where species composition and abundance were recorded.

Study site	**Plot ID**	**Latitude**	**Longitude**	**Altitude (m)**
Functional trait survey				
Old-growth	NA01	14°4'43.41"S	47°38'3.21"W	1,227
Old-growth	NA02	14°4'42.39"S	47°38'4.04"W	1,225
Old-growth	NA03	14°4'41.20"S	47°38'4.64"W	1,224
Old-growth	NA04	14°4'40.07"S	47°38'5.26"W	1,224
Old-growth	NA05	14°4'38.60"S	47°38'5.75"W	1,223
Restored	RE01	14°5'21.33"S	47°38'17.94"W	1,246
Restored	RE02	14°5'20.27"S	47°38'17.15"W	1,247
Restored	RE03	14°5'19.92"S	47°38'15.64"W	1,245
Restored	RE04	14°5'19.60"S	47°38'14.64"W	1,245
Restored	RE05	14°5'18.76"S	47°38'14.09"W	1,245
Floristic inventory				
Old-growth	PLNAT 1	14°4'42.00"S	47°38'2.00"W	1,223
Old-growth	PLNAT 2	14° 4'35.22"S	47°38'3.48"W	1,218
Old-growth	PLNAT 3	14° 4'35.70"S	47°38'8.40"W	1,220
Old-growth	PLNAT 4	14° 4'29.82"S	47°38'12.96"W	1,213
Old-growth	PLNAT 5	14° 4'25.20"S	47°38'12.48"W	1,209
Restored	PLREST 1	14°5'20.97"S	47°38'11.76"W	1,240
Restored	PLREST 2	14°5'22.94"S	47°38'15.78"W	1,243
Restored	PLREST 3	14°5'24.07"S	47°38'12.81"W	1,240
Restored	PLREST 4	14°5'27.69"S	47°38'14.35"W	1,239
Restored	PLREST 5	14°5'33.37"S	47°38'18.30"W	1,241

SI Table 2. Taxonomic and ecological information of the studied species. "Alternative name" displays the different names given to the same species. "Occurrence" classifies species as exclusive of the old-growth area ("Old-growth"), exclusive of the restored area ("Restored"), or present in both areas ("Shared"). "Other" means not-studied species.

Family	**Species**	**Origin**	**Endemism**	**Occurrence**	**Relative cover**	
					Old-growth	Restored
Poaceae	*Trichanthecium cyanescens* (Nees ex Trin.) Zuloaga & Morrone	Native	Non-endemic	Old-growth	0.007	0
Poaceae	*Axonopus siccus* (Nees) Kuhlm.	Native	Non-endemic	Shared	0.004	0
Poaceae	*Echinolaena inflexa* (Poir.) Chase	Native	Non-endemic	Shared	0.04	0.007
Poaceae	*Schizachyrium tenerum* Nees	Native	Non-endemic	Shared	0.55	0.01
Poaceae	*Axonopus chrysoblepharis* (Lag.) Chase	Native	Non-endemic	Shared	0.007	0.07
Cyperaceae	*Rhynchospora confuse* F.Ballard	Native	Endemic	Old-growth	0.002	0
Cyperaceae	*Rhynchospora barbata* (Vahl) Kunth	Native	Non-endemic	Old-growth	0.02	0
Cyperaceae	*Rhynchospora consanguinea* (Kunth) Boeckeler	Native	Non-endemic	Old-growth	0.02	0
Poaceae	*Panicum campestre* Nees ex Trin.	Native	Endemic	Restored	0	0.003
Poaceae	*Aristida setifolia* Kunth	Native	Non-endemic	Restored	0	0.0004
Poaceae	*Andropogon virgatus* Desv.	Native	Non-endemic	Restored	0	0
Poaceae	*Aristida riparia* Trin.	Native	Non-endemic	Restored	0	0.2
Poaceae	*Schizachyrium sanguineum* (Retz.) Alston	Native	Non-endemic	Restored	0	0.13
Poaceae	*Paspalum hyalinum* Nees ex Trin.	Native	Non-endemic	Old-growth	0.02	0
Poaceae	*Loudetiopsis chrysothrix* (Nees) Conert	Native	Non-endemic	Shared	0.03	0.08
Poaceae	*Urochloa decumbens* (Stapf) R.D.Webster	Exotic		Restored	0	0.28
	Other				0.25	0.17

SI Table 3. Mean, standard deviation (SD), and range (min–max) of the edaphic variables between the old-growth and restored area.

Variable	**Unit**	**Old-growth**		**Restored**	
		Mean±SD	Range	Mean±SD	Range
Total nitrogen	g kg^{-1}	2.15±0.59	1.5–3.08	0.97±0.18	0.8–1.26
pH_{H2O}		4.75±0.12	4.6–4.9	4.88±0.04	4.8–4.9
Organic matter	g kg^{-1}	46.75±12.9	35–65	21.1±2.96	17–25
Available phosphorus	mg kg^{-1}	3.39±0.43	2.73–3.83	1.7±0.27	1.36–2
Aluminium	cmolc dm^{-3}	2.31±0.26	2.1–2.65	1.63±0.08	1.5–1.7

SI Table 4. Mean, standard deviation (SD), and range (min-max) of the plant functional traits between the old-growth and restored area.

Trait	**Unit**	**Old-growth**		**Restored**	
		Mean±SD	Range	Mean±SD	Range
Plant height	cm	77.88±43.89	19–191	148.83±34.68	59–219
Ψ_{min}	MPa	-2.2±0.69	-3.9–-0.63	-2.39±0.82	-5.3–-1.3
g_{min}	mmol m^{-2} s^{-1}	32.7±19.91	1.61–81.24	15.36±5.79	7.5–30.72
LMA	g m^{-2}	111.07±56.14	42.41–239.1	80.34±34.27	43.85–227.29
π_{tlp}	MPa	-2.18±0.76	-3.83–-1.44	-1.64±0.23	-2.14–-1.2

SI Table 5. Average of the community-weighted means (CWM) between the old-growth and restored area.

Trait	**Unit**	**Old-growth**	**Restored**
CWM Ψ_{min}	MPa	-2.22	-2.64
CWM g_{min}	mmol m^{-2} s^{-1}	26.9	13.1
CWM π_{tlp}	MPa	-2.91	-1.50
CWM Plant height	cm	87.6	142
CWM LMA	g m^{-2}	139	68

ANEXO 1 – DECLARAÇÃO DE BIOÉTICA E BIOSSEGURANÇA

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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Dissertação de Mestrado, intitulada “***"USING PLANT TRAITS TO ASSESS RESTORATION OUTCOMES IN A TROPICAL GRASSLAND" (USANDO ATRIBUTOS FUNCIONAIS DE PLANTAS PARA AVALIAR OS RESULTADOS DA RESTAURAÇÃO EM UM CAMPO TROPICAL)***”, desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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Data: 20 de abril de 2021

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