



Effects of a megafire on the arbuscular mycorrhizal fungal community and parameters in the Brazilian Cerrado ecosystem

Jadson B. Moura^{1,2}, Rodrigo F. Souza^{1,3}, Wagner G. Vieira-Júnior^{1,4}, Leidiane S. Lucas^{5,6}, Jose M. Santos^{1,2}, Sandro Dutra e Silva^{2,3} and César Marín⁷

¹ Evangelical College of Goianésia, Sedmo. Soil Res. Group, Ecol. & Dynamics of Organic Matter, Av. 2020, Covoá, Goianésia, GO, Brazil. ² Evangelical Univ. of Goiás, Graduate Stud. in Soc., Technol. & Environ. Sci., Av. Universitária, s/n - Cidade Universitária, Anápolis, GO, Brazil. ³ State Univ. of Goiás, Graduate Stud. in Nat. Resour. of the Cerrado, Câmpus Unidade de Senador Canedo, Senador Canedo, GO, Brazil. ⁴ Paulista State Univ. "Júlio de Mesquita Filho", Fac. of Agr. & Vet. Sci., Graduate Stud. in Agr. Microbiol., Rua Quirino de Andrade 215, Centro, São Paulo, SP, Brazil. ⁵ Inst. Federal Goiano, Graduate Stud. in Agr. Sci./Agron., Campus Rio Verde, Rio Verde, Goiás, Brazil. ⁶ Univ. of Brasília, Graduate Stud. in Agron., Campus Universitário Darcy Ribeiro, Brasília, DF, Brazil. ⁷ Universidad Santo Tomás, Centro de Investigación e Innovación para el Cambio Climático (CiiCC), Av. Ejército Libertador 146, Santiago, Chile.

Abstract

Aim of study: To evaluate the effects of a mega-fire on the arbuscular mycorrhizal fungi (AMF) community and parameters in soils under Cerrado vegetation.

Study area: Chapada dos Veadeiros National Park, Goiás, Brazil. This site suffered the biggest fire in its history on October 10, 2017, with an affected area of 66,000 ha.

Materials and methods: We analyzed AMF spore density, roots' mycorrhizal colonization rate, easily extractable glomalin (EEG), as well as the AMF genera present. These parameters were evaluated in burned and unburned areas of five common phytophysiognomies of the region.

Main results: Fire presence immediately affected the mycorrhizal community parameters in Cerrado soils, which tended to increase afterwards. The presence of AMF genera did not differ between burned and unburned areas, with *Acaulospora*, *Claroideglomus*, *Diversispora*, *Glomus*, *Funneliformis*, *Sclerocystis*, and *Gigaspora* being present. The recovery of AMF community conditions in the Cerrado after fire events could also be observed in the mycorrhizal parameters evaluated, as the values of spore density, roots' mycorrhizal colonization rate, and EEG were similar in the burned and unburned areas.

Research highlights: AMF diversity, and especially their community parameters, show great recovery after fire events, since they are crucial in processes like nutrient cycling and soil aggregation.

Additional key words: glomalin; conflagration; arbuscular mycorrhizal fungal diversity; mycorrhizal parameters; spore density.

Abbreviations used: AMF (arbuscular mycorrhizal fungi); EEG (easily extractable glomalin); PVLG (pure polyvinyl lactoglycerol); RDA (redundancy analysis).

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Correspondence should be addressed to César Marín: cesar.marin@postgrado.uach.cl

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Introduction

The Cerrado is the second largest Brazilian biome, extending over an area of 2,045,064 km² and covering eight

States in Central Brazil in addition to the Federal District (Hunke *et al.*, 2015). This biome is crossed by three of the largest hydrographic basins in South America (Amazonia, São Francisco, and Paraná), with constant and high

rainfall levels, and very high levels of animal and plant endemism and biodiversity (Klink *et al.*, 2020), only behind the Amazon in the country. The Cerrado is currently considered as a transitional biome and the last agricultural frontier in the Americas (Hunke *et al.*, 2015; de Moura & Cabral, 2019), with direct geographical contact with other important South American biomes such as the Amazon, Caatinga, Atlantic Forest, Pantanal, and Chacos Bolivianos (de Moura & Cabral, 2019).

Besides strong anthropogenic effects, the environmental history of the Cerrado is determined by a set of natural elements related to its geology and ecology, and their effects on the formation and constitution of the Neotropical savannas that compose this biome (Ratter *et al.*, 2006). Among these elements, natural wildfires stand out as a main factor configuring the Cerrado landscape (de Moura & Cabral, 2019). Nonetheless, intentional fire is the main tool used to expand new agricultural areas in the Cerrado, and as a result, big and irreversible damages have already occurred to this biome, as the fragmentation of previously integrated phytophysiognomies, native biodiversity loss, soil nutrient loss, soil degradation and compaction, soil erosion, pollution of water sources, changes in the natural fire regime, and imbalances in the carbon cycle, all of which contribute to regional climate change (Klink *et al.*, 2020).

The biggest fire event in the history of the Cerrado, suspected as arson, occurred in the Chapada dos Veadeiros National Park in October 10, 2017, destroying about 66,000 ha (de Santana Leite *et al.*, 2017). The Chapada dos Veadeiros National Park was created in 1961 and comprises an area of 240,614 ha, and in 2001 was declared a Natural World Heritage Site by UNESCO (de Santana Leite *et al.*, 2017). The Cerrado ecosystem has several phytophysiognomies, including Cerradão (dense forest formation with several tree species), Cerrado *sensu stricto* (low and twisted trees, shrubs, subshrubs, and herbs), Veredas (hydromorphic soils surrounded by cleaned fields, represented by rows or groups of *Mauritia flexuosa*), Campo Limpo (herbaceous vegetation with few shrubs and no trees), and Campo Sujo (herbaceous-shrub vegetation with shrubs and subshrubs spaced among themselves) (Walter, 2006). Factors as the expansion of the agricultural frontier (especially soybean crops) in the region adjacent to the Chapada dos Veadeiros National Park, and the presence of exotic grasses introduced in the Cerrado since the XVIII century, favor over-combustion, altering the natural fire regime (Simon *et al.*, 2009; Simon & Pennington, 2012), which has harmful consequences to this biome. These intentional fires (in most cases illegally), lead to new areas destined for agricultural production. The harmful consequences of these fires are more accentuated during the dry season (from April to September), where low humidity amplifies the intensity and severity of these events (Simon & Pennington, 2012).

Soil microorganisms are essential for the conservation of biodiversity and for the recovery of ecosystem services, after extreme events as wildfires (Mataix-Solera *et al.*, 2009). Arbuscular mycorrhizal fungi (AMF), are generalists and obligate plant symbionts (Davison *et al.*, 2015), and are extremely sensitive to environmental changes and as such, they are excellent bioindicators of environmental quality and recovery (de Souza *et al.*, 2016). Several studies have shown the resilience of the AMF community and mainly its community parameters after fire events (Martínez *et al.*, 2005, 2018; de Oliveira *et al.*, 2006; Paulino *et al.*, 2009; Rivas *et al.*, 2016). For AMF it is a common response to decrease in biodiversity but sustain or even increase their aggregate parameters such as roots' mycorrhizal colonization, glomalin production, soil aggregation, among others, immediately after disturbances (Paulino *et al.*, 2009; Rivas *et al.*, 2016). For example, some studies have shown a very interesting pattern: a decrease in AMF species richness (as determined by morphological analyses) but no effect on AMF spore density post-fire (Longo *et al.*, 2014).

According to studies already carried out which show the crucial role of AMF in the recovery of the Cerrado ecosystem after disturbance (De Souza *et al.*, 2016), we expected that in this ecosystem the action of fire would promote an increase of roots colonization by AMF, which would cause a population increase of some species, and in return a decrease in biodiversity -not immediately but after a while. Given their environmental similarity, we expect similar results for all phytophysiognomies. This study aimed to evaluate the effects of a megafire on the AMF community in Cerrado soils, by measuring parameters such as spore density, easily extractable glomalin (EEG), and the rate of successful root colonization, comparing burned and unburned areas in five commonly found phytophysiognomies in the region.

Material and methods

Study sites and sampling scheme

The study was carried out in the Chapada dos Veadeiros National Park, Goiás, Brazil (Fig. 1). Five phytophysiognomies were defined according to Walter's characterization (2006): Cerradão, Cerrado *sensu stricto*, Campo Limpo, Campo Sujo, and Veredas. Ten sample plots (20 m × 20 m; 7 in and 3 outside the Park) were selected, and for each phytophysiognomy, one burned and one unburned plot (Fig. 1). Sampling was carried out in four periods: first, on November 10, 2017, right after the containment of the flames; second, on March 11, 2018; the third sampling on June 23, 2018; and the fourth and final sampling was carried out on November 2, 2018. For each plot and sampling period, and after removing organic debris (litter,

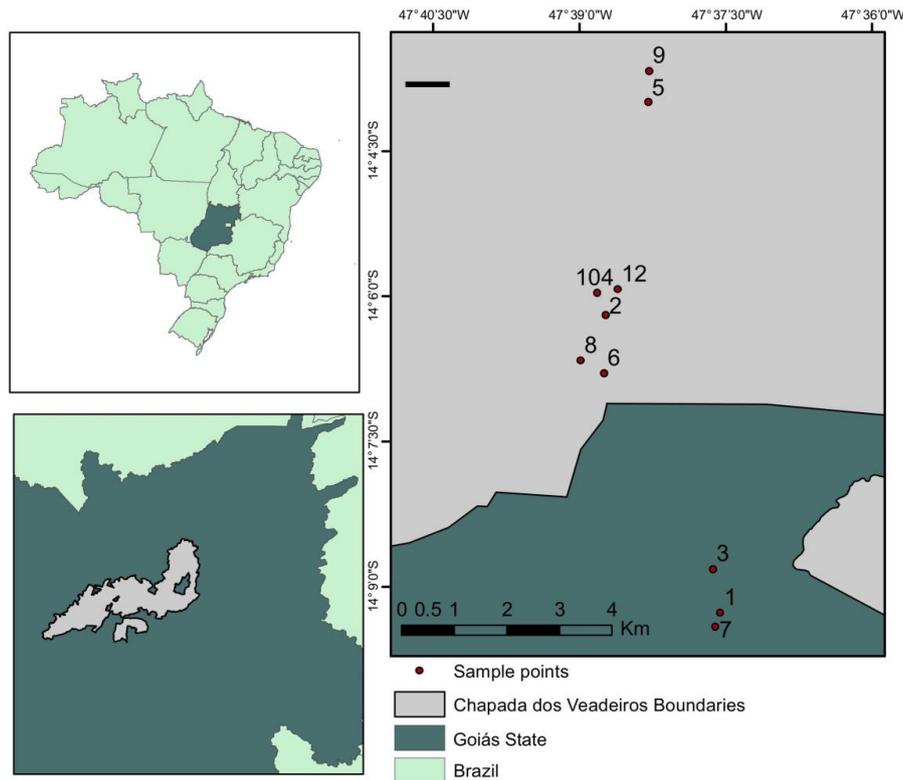


Figure 1. Chapada dos Veadeiros National Park, in Goiás State, Brazil, including phytophysiognomies with and without burning: 1, Campo Limpo without burning; 104, Campo Limpo with burning; 3, Campo Sujo without burning; 12, Campo Sujo with burning; 5, Cerrado *sensu stricto* without burning; 6, Cerrado *sensu stricto* with burning; 7, Cerradão without burning; 8, Cerradão with burning; 9, Veredas without burning; 2, Veredas with burning.

wood), a total of six soil samples (rhizospheric soil and roots of the A horizon; 0-10 cm soil depth) were analyzed (which in turn, consisted of 6 randomly collected subsamples). Thus, a total of 240 soil samples were analyzed in this study (10 plots \times 4 sampling periods \times 6 samples per plot). The samples were collected in the same sampling area in all periods.

Microbiological analyses

Microbiological analyses were performed at the Agricultural Microbiology Laboratory at the Faculdade Evangélica de Goianésia, Goiás, Brazil. Spores of AMF were extracted from a volume of 50 cm³ of rhizospheric soil using the wet-sieving technique (Gerdemann & Nicolson, 1963), including centrifugation at 2000 rpm for 2 minutes in water and a 50% sucrose solution. The spores were separated under a microscope (400-fold magnification), and were taxonomically analyzed according to their phenotypic characteristics as color, size, and shape (Oehl *et al.*, 2011), composing the different AMF genera. In order to identify the genera of AMF from the morphological characteristics, the spores were separated according to their morphotypes (Oehl *et al.*, 2011), and mounted on slides

with pure polyvinyl lactoglycerol (PVLG) and PVLG mixed with the Melzer solution (1:1 v/v).

To determine the percentage of root colonization by AMF, the roots were clarified and stained with 0.05% Trypan-Blue in lactoglycerol (Phillips & Hayman, 1970), and the root colonization was evaluated under a stereoscopic microscope, following the technique of quadrants' intersection (Giovannetti & Mosse, 1980). The extraction of EEG was done by adding 1 g of dried soil in 8 mL of 20 mM sodium citrate (pH 7.0), followed by autoclave for 30 min at 121 °C; then, centrifugation was carried out for 20 min at 5000 rpm (Wright & Upadhyaya, 1996; Nichols & Wright, 2006). For EEG extraction, we followed the Bradford (1976) method modified by Wright & Upadhyaya (1996), where bovine serum albumin was used as the standard protein, using a spectrophotometer at a 595 nm reading. Caution should be taken when interpreting the EEG results, as currently there are no specific analytical tools to measure glomalin (*sensu* Rillig, 2004) without significant interference from other substances (Holátko *et al.*, 2021). The evidence provided so far for glomalin detection, even when measuring antibodies (Gadkar & Rillig, 2006) in soil as a direct metabolic product of AMF is still just correlative (Bedini *et al.*, 2007; Bolliger *et al.*, 2008; Wilson *et al.*, 2009).

Statistical analyses

Each phytophysiognomy was evaluated separately, resulting in a completely randomized design with the plots arranged in a 2×4 factorial scheme with six replicates. The first factor consisted on burning (or not), and the second factor corresponds to the four sampling periods. Before the analyses, the homogeneity of variances and the normality of the residuals were checked using the Bartlett test and graphical checks, respectively. Analyses of variance (ANOVAs with two factors), T-tests (at 5% means discrimination), and graphs of the obtained data were made using the GraphPad Prism 8 software (SWIFT, 2020). We also performed stepwise regressions (in both directions) of the redundancy analysis (RDA) for each measured mycorrhizal parameter (root colonization, spore density, and glomalin), assessing the effect of each tested factor (phytophysiognomy, fire effect, sampling time) on them. This was done using the *ordisep* function of the *vegan* package (Oksanen *et al.*, 2020) in RStudio 2021.09.0+351 (R Core Team, 2020).

Results

Fire had varied effects in the AMF parameters (spore density, root colonization, and EEG) evaluated in the five phytophysiognomies assessed in this study (Fig. 2; the specific values are given in Table S1 [suppl]); such effects were not very strong regarding AMF genera (Table 1). AMF spore density in soil was at its lowest and similar in the first sampling in the burned and unburned areas in all phytophysiognomies (58.667-173 spores/50 cm³ of soil). In the second sampling, five months after the fire event, the spore density values were higher in the burned than in the unburned areas in the Campo Limpo (356.833 vs. 170.667 spores/50 cm³ of soil, respectively) (Fig. 2a) and Cerrado *sensu stricto* (224.667 vs. 90.333 spores/50 cm³ of soil, respectively) (Fig. 2j) phytophysiognomies, not differing in the other phytophysiognomies. In the third sampling, the burned areas had higher spore density than the unburned areas in all phytophysiognomies; and in the last sampling, 13 months after the fire event, spore density was not significantly different in burned and unburned areas.

Roots' mycorrhizal colonization rate was lower in the burned than in the unburned areas in the first three samplings only in the Campo Limpo phytophysiognomy (burned: 0.226, 0.403, and 0.462%; unburned: 0.544, 0.882, and 0.806%), being only relatively similar in the last sampling (burn: 0.64%; unburned: 0.681%) (Fig. 2b). The other four phytophysiognomies did not show significant differences regarding roots' mycorrhizal colonization rate between the burned and unburned areas, but values ten-

ded to increase towards the third sampling period, eight months after the megafire (Fig. 2). EEG soil content was overall not significantly different between burned and unburned plots, in all the investigated phytophysiognomies, and was somewhat similar across the sampling periods, with slightly higher values in the third sampling period (5.718-7.085 mg/g of soil).

Stepwise regressions of RDAs (in both directions) of the mycorrhizal parameters of this study, show that the three factors tested (phytophysiognomy, fire effect, and sampling period) significantly affected AMF spore density and root colonization (Table 2). Specifically, the effect of phytophysiognomy in spore density was quite large, while root colonization was mostly affected by the sampling period, factor that also highly affected EEG content -but this parameter was not affected by fire, and only slightly affected by phytophysiognomy (Table 2).

The AMF genera *Acaulospora*, *Claroideglomus*, *Diversispora*, *Glomus*, *Funneliformis*, *Sclerocystis*, and *Gigaspora* were identified in the rhizospheric soil of the investigated phytophysiognomies (Table 1). Overall, the presence of AMF genera did not differ between burned and unburned areas (Table 1). The genera *Acaulospora*, *Claroideglomus*, *Diversispora*, *Funneliformis*, and *Gigaspora* were identified in all phytophysiognomies, and the genus *Glomus* was found in all phytophysiognomies, regardless of fire action. The genera *Sclerocystis* was not found in the rhizospheric soil of the Campo Limpo phytophysiognomy. In the Campo Limpo phytophysiognomy, with the exception of the genus *Diversispora*, which in the first sampling was only identified in unburned areas, the other genera were found both in burned and unburned plots (Table 1).

Discussion

Arbuscular mycorrhizal fungi (AMF), as well as any soil microorganism, need specific environmental conditions for their proliferation and colonization (de Moura & Cabral, 2019). Soil health and biodiversity recovery after fire will depend on many factors, among them fire intensity, the amount of subsequent rain, preventive actions after the fire, among others. Cerrado soils have mostly low natural fertility, condition that encourage the spread of AMF and their colonization in crop vegetables (de Moura *et al.*, 2019). Climatic conditions, soil movement, the use of chemical fertilizers, wildfires, among other factors, directly influence the amount of AMF spores in soil (de Moura *et al.*, 2019). All of these factors can affect microbial abundance, richness, and ecosystem functions (van der Heyde *et al.*, 2017).

The AMF community and parameters investigated in our study mostly recovered after fire at the end of our sampling, a relatively short period (one year). Furthermore,

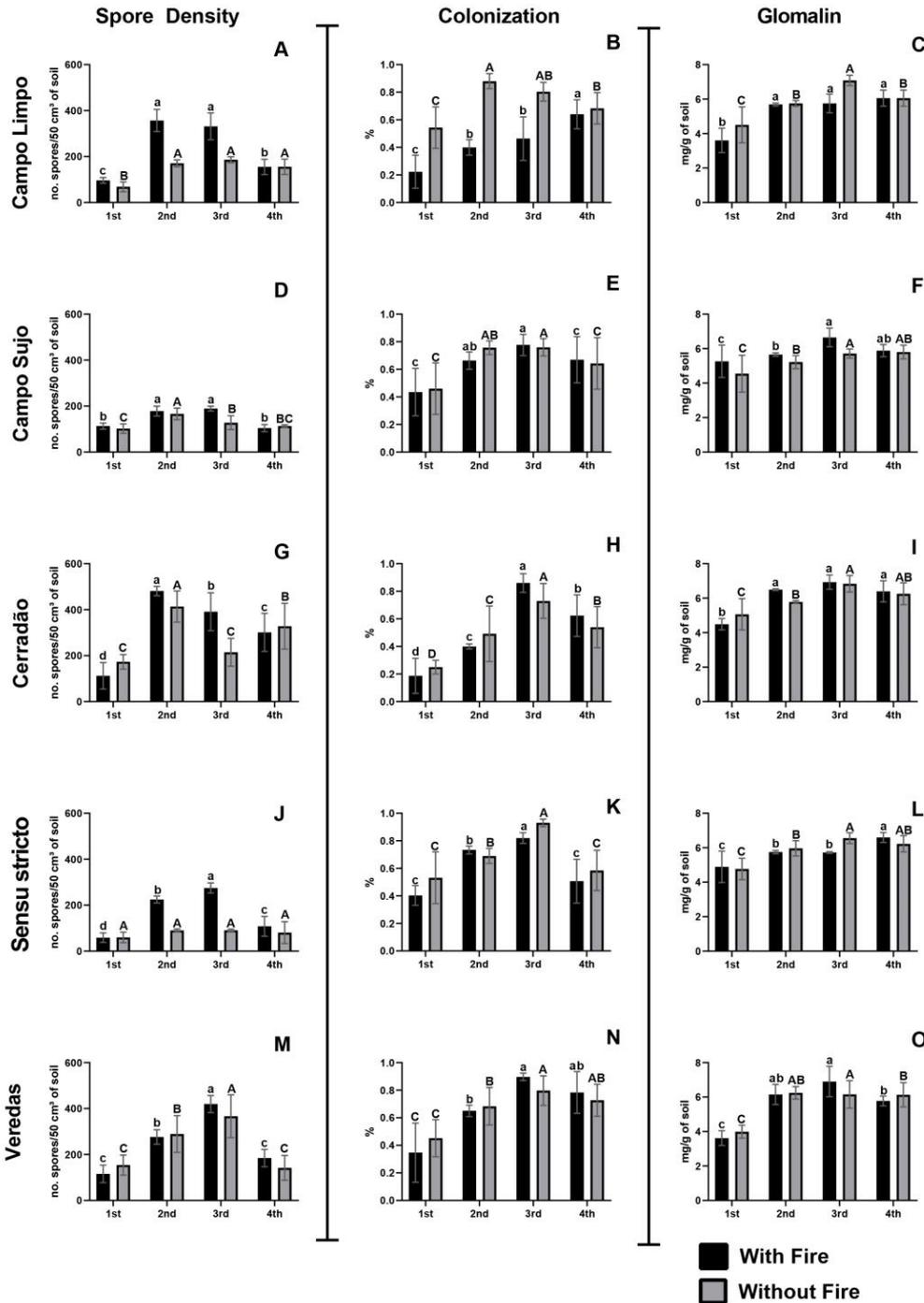


Figure 2. Arbuscular mycorrhizal fungi spore density (A, D, G, J, M), roots' mycorrhizal colonization (B, E, H, K, N), and easily extractable glomalin content (C, F, I, L, O) in Cerrado soils in burned and unburned plots during four samplings times (1, 5, 8 and 13 months after megafire). Small letters indicate significant differences for sites without fire, capital letters for sites with fire.

by the second or third sampling periods, the three AMF parameters investigated (spore density, roots colonization, and EEG content) seemed to have fully recovered. Longo *et al.* (2014) found that after 8 months, fire decreased the richness of AMF spores, but -and as in our study- spore density was not affected. Similarly, Dove & Hart (2017) found in a meta-analysis that mycorrhizal colonization of roots was not affected by fire, also a similar finding to our

results, at least after certain time. Several other studies in South America (Martínez *et al.*, 2005, 2018; Paulino *et al.*, 2009; Rivas *et al.*, 2016) have found a similar pattern: a decrease in AMF biodiversity but no effect (or even an increase) in different community parameters related to their ecosystem functions. In our case, further morphological and molecular characterizations are required to identify spores at a species level, to truly evaluate changes

Table 1. Genera of arbuscular mycorrhizal fungi associated with rhizospheric soil under the five phytophysiognomies evaluated at four sampling periods (1st, one month after fire; 2nd, five months after fire; 3rd, eight months after fire, 4th, 13 months after fire) in the Cerrado biome, without (control) and with the presence of fire (Chapada dos Veadeiros National Park megafire, 2017).

	1st		2nd		3rd		4th	
	Without	With	Without	With	Without	With	Without	With
Campo Limpo								
<i>Acaulospora</i>	+	+	+	+	+	+		
<i>Claroideglomus</i>	+	+	+	+	+	+		
<i>Diversispora</i>		+	+	+	+	+	+	+
<i>Glomus</i>	+	+	+	+	+	+	+	+
<i>Funneliformis</i>	+	+					+	+
<i>Gigaspora</i>	+	+	+	+	+	+		
Campo Sujo								
<i>Acaulospora</i>	+	+						+
<i>Claroideglomus</i>	+			+		+		
<i>Diversispora</i>	+	+	+	+	+	+	+	+
<i>Sclerocystis</i>							+	+
<i>Glomus</i>	+	+	+	+	+	+	+	+
<i>Funneliformis</i>		+					+	+
<i>Gigaspora</i>	+	+					+	
Cerradão								
<i>Acaulospora</i>	+	+	+	+	+	+		
<i>Claroideglomus</i>	+	+	+	+	+	+	+	+
<i>Diversispora</i>	+	+	+	+	+	+	+	+
<i>Sclerocystis</i>							+	+
<i>Glomus</i>	+	+	+	+	+	+	+	+
<i>Funneliformis</i>	+	+	+			+	+	+
<i>Gigaspora</i>	+	+	+	+	+	+	+	
Cerrado sensu stricto								
<i>Acaulospora</i>	+						+	+
<i>Claroideglomus</i>							+	+
<i>Diversispora</i>	+	+	+		+		+	+
<i>Sclerocystis</i>								+
<i>Glomus</i>	+	+	+	+	+	+	+	+
<i>Funneliformis</i>	+						+	
<i>Gigaspora</i>	+	+	+	+	+	+		
Veredas								
<i>Acaulospora</i>	+	+	+	+	+	+	+	+
<i>Claroideglomus</i>		+		+		+	+	
<i>Diversispora</i>	+	+	+	+	+	+	+	+
<i>Sclerocystis</i>							+	+
<i>Glomus</i>	+	+	+	+	+	+	+	+
<i>Funneliformis</i>	+	+						
<i>Gigaspora</i>	+	+	+	+		+	+	

Table 2. Stepwise models (in both directions) of redundancy analyses, RDA (Akaike criterion (AIC) and F-values are shown, with *p*-values as asterisks) testing the effects of the different factors of this study on the mycorrhizal parameters measured. *p*<0.01: **; *p*<0.05: *

Factor	Spore density		Colonization		EGG	
	AIC	F	AIC	F	AIC	F
Phytophysiognomy	2286.1	29.004**	-790.11	5.890**	-107.151	3.046*
Fire effect	2210.8	15.651**	-797.84	9.288**	--	--
Sampling period	2204.8	9.567**	-744.03	70.188**	0.983	142.770**

in AMF biodiversity. This seems to be common feature of AMF in most types of disturbances, which is not necessarily the case for other fungal guilds or even mycorrhizal types (Marín *et al.*, 2017; Steidinger *et al.*, 2020; Marín & Kohout, 2021; Rodríguez-Ramos *et al.*, 2021). AMF niches are relatively broad, driven mainly by temperature and soil pH, and correlated by phylogeny (Davison *et al.*, 2021), which together with their broad distribution and low worldwide endemism (Davison *et al.*, 2015), might explain their high recovery capacity. These broad niches and biogeographic distributions could in part explain our results: related taxa, which arrives relatively quickly after the fire, given their ubiquity, ends up having similar performances (*e.g.* in EGG or roots' colonization) after disturbances.

The AMF spore density results obtained here are similar to those obtained by Cordeiro *et al.* (2005), who found similar patterns when evaluating areas of the native Cerrado biome, more specifically the Cerrado *sensu stricto* phytophysiognomy, where they found a spore density of 368 spores per 50 cm³ of soil. The symbiotic association between plants and AMF is especially stimulated when the plant is in an stressful environment (de Souza *et al.*, 2016). Fungi help the plant in its nutrition and water absorption -besides pathogen and drought resistance (among other disturbances), allowing it to survive several and severe environmental changes (van der Heyde *et al.*, 2017). The highest values of spore density were found in areas under fire in all phytophysiognomies, in the second and third sampling periods, demonstrating the importance of the AMF response to a megafire event. In the fourth sampling period, when the Cerrado areas had partially recovered from fire, the spore density values of the burned and unburned areas were similar.

Fire promotes significant changes in vegetation and plays key roles in plant physiology and community structure (Veenendaal *et al.*, 2018). The post-fire plant regeneration process can take decades. Even if some plants are fire-adapted, variation in fire severity and intensity can lead to irreversible damage to some plants (Klink *et al.*, 2020). Low intensity fires mainly affect undergrowth, which consists of the understory, grasses, and lower vegetation (van der Werf *et al.*, 2017; Klink *et al.*, 2020). In such case, trees are mostly not affected due to the rapid passage of fire. However, in atypical environmental con-

ditions, where flames can reach up to 2.5 m in height, tree crowns can be burned (Kauffman *et al.*, 1994; Miranda *et al.*, 2009). In the Cerrado ecosystem, plant recovery after the incidence of fire occurs gradually, and some species may start flowering a few days after the fire event, while other species take weeks or months. In our study, the first species to be recomposed are the constituents of the herbaceous layer, mainly grasses and other graminoids. Klink *et al.* (2020) determined four patterns of post-fire re-sprouting in the Cerrado ecosystem: 1) at the beginning of the rainy season; 2) in the middle of the rainy season; 3) in the second half of the rainy season; and 4) at the end of the dry season. At least 70% of plant biomass is recovered by the mid-next rainy season (Klink *et al.*, 2020).

The roots' mycorrhizal colonization rate in four out of five of the investigated phytophysiognomies presented a similar temporal trend to that of spore density: in the first sampling, the values were lower in the burned than in the unburned plots. Nutrient bioavailability is an important factor that directly influences the rate of roots' arbuscular mycorrhizal colonization in plants (van der Heyde *et al.*, 2017). An stressful, nutrient-depleted condition might provide an environment conducive to the establishment of this symbiosis, because the plant needs the fungus to acquire nutrients otherwise unavailable, or else, colonization might be impaired in soils where the plant has at its disposal a greater nutritional support (Ezawa & Saito, 2018). This could explain why in our study, burned areas had lower colonization rates than unburned areas. Ash supply temporarily increases nutrient availability, impairing root colonization (Longo *et al.*, 2014). Similar trends regarding mycorrhizal colonization and soil nutrients were found by Martínez *et al.* (2005), de Oliveira *et al.* (2006), and Rivas *et al.* (2016).

The amount of roots analyzed was the same for all treatments, not influencing the results. The passage of fire was fast and, in a way, does not affect the integrity of the roots in the short term. In our study, the first samples were collected soon after the fire was contained. Thus, root sampling was possible even in phytophysiognomies that had their vegetation consumed by fire. The phytophysiognomies Campo Limpo, Campo Sujo, and Cerrado *sensu stricto* are mostly constituted by plants that have a rapid recovery, which does not affect the amount of roots of subsequent collections (Klink *et al.*, 2020). In the case

of the Cerradão phytophysiology, the woody layer is not consumed by fire, not affecting plant survival (Walter, 2006). In the case of the Veredas phytophysiology, its soil is hydromorphic (Walter, 2006), which allows the rapid recovery of plants that constitute this phytophysiology. Other plants, such as *Mauritia flexuosa*, as well as Cerradão trees, are not consumed by fire due to their woody layer.

Very few studies have examined AMF biodiversity and community parameters immediately after the fire—usually some weeks, months or years have passed in most studies. Bellgard *et al.* (1994)—one of those few studies—found reduced AMF colonization immediately after fire compared to pre-fire conditions, but no difference between burn and unburned sites—a similar result to ours. Similarly, after one year, other studied parameters like AMF spore numbers and diversity recovered, which could be related to the sprouting organs of the vegetation present (Bellgard *et al.*, 1994). Very similar results were obtained by Aguilar-Fernández *et al.* (2009) in a tropical dry forest, with very few changes in the AMF community after forest conversion by slash-and-burn followed by cultivation. Studies where the first sampling was conducted some few months after the fire (Rashid *et al.*, 1997; Longo *et al.*, 2014; Xiang *et al.*, 2015) seem to agree with the above-described pattern, overall showing low AMF resistance but high resilience to fire (Xiang *et al.*, 2015). Longo *et al.* (2014) showed that such pattern can be explained by fire having direct effects on the AMF community, not necessarily mediated by the effects of fire in soil physical and chemical properties.

In our study we found that spore density and AMF colonization of roots immediately decreased after the fire, but the effect was not so drastic in EGG soil content. Thus, a reduced amount of AMF spores, colonizing less roots, possibly are over-producing EGG in order to immediately stabilize the post-fire soil conditions. Afterwards, EGG production probably stabilizes. Though, fire did not significantly increase EEG soil content in almost all the phytophysiological systems investigated. Although, and as previously mentioned, EGG measurements usually have interference from other (non-AMF) substances (Holátko *et al.*, 2021), so these results should be taken as a proxy. Moya *et al.* (2019) found higher levels of glomalin in soil after fire, both in treatments with high and low severity fire—an aspect we should test in further studies, as the fire in our system had a very high severity. Similar results regarding glomalin content after fires in Chile have been reported by Rivas *et al.* (2016), but in a very different ecosystem such as the temperate rainforests. The mycorrhizal activity in these forests intensified due to the demand for vegetation in the stressed areas, which were reflected in a greater protein production. The EEG values reported here were similar to those found by Santos (2016) in

Cerrado soils, which ranged from 2.1 to 4.4 mg g⁻¹ of soil. Fokom *et al.* (2012), when evaluating agricultural areas of intercropping with peanut, corn, banana, and cassava crops, found values of 6.51 mg g⁻¹ of soil, also similar to our results; in forested areas, though, they found higher values reaching up to 10.56 mg g⁻¹ of soil.

Glomalin soil content varies according to the species of mycorrhizal fungi found colonizing roots. Wright & Upadhyaya (1999) found a difference in glomalin production according to the studied AMF species: *Gigaspora rosea* and *Gigaspora gigantea* had higher glomalin productivity than *Glomus intaradices* and *Glomus etunicatum* (Wright & Upadhyaya, 1996), when evaluated under cultivation in a culture medium. As in their study, AMF genera as *Glomus* and *Gigaspora* were ubiquitous in our study. Similar to our study, where sampling time highly affected EGG, Rivas *et al.* (2016), when carrying out soil sampling one day and four years after fires on soils in the Andes, found that the difference in glomalin-related protein content was significant, but limited to the first 10 cm of the soil horizon. In contrast to our results, Sharifi *et al.* (2018), when carrying out work in the Zagros forest in western Iran, found that glomalin soil content was greater in burned areas than in native unburned forests; they found that the greater the fire intensity the greater the glomalin soil content, and that these values tended to increase in follow-up samplings. Sharifi *et al.* (2018) strongly recommend the use of glomalin (or proxies) as an indicator of soil degradation by fire, albeit the current difficulties to correctly measure this protein in soil (Holátko *et al.*, 2021).

In summary, the presence of fire influenced the AMF community and its parameters in Cerrado soils, especially during the first sampling period, right after the containment of the flames. The Cerrado's responses to fire can also be seen in the community parameters of AMF, in which the values of spore density, roots' colonization rate, and EGG soil content tended to match up in the burned and unburned areas. Similarly, with few exceptions, the genera of these fungi were similar in burned and unburned areas.

References

- Aguilar-Fernández M, Jaramillo VJ, Varela-Fregoso L, Gavito ME, 2009. Short-term consequences of slash-and-burn practices on the arbuscular mycorrhizal fungi of a tropical dry forest. *Mycorrhiza* 19: 179-186. <https://doi.org/10.1007/s00572-009-0229-2>
- Bedini S, Avio L, Argese E, Giovannetti M, 2007. Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein. *Agric Ecosyst Environ* 120: 463-466. <https://doi.org/10.1016/j.agee.2006.09.010>

- Bellgard SE, Whelan RJ, Muston RM, 1994. The impact of wildfire on vesicular-arbuscular mycorrhizal fungi and their potential to influence the re-establishment of post-fire plant communities. *Mycorrhiza* 4: 139-146. <https://doi.org/10.1007/BF00203532>
- Bolliger A, Nalla A, Magid J, de Neergaard A, Nalla AD, Bøg-Hansen TC, 2008. Re-examining the glomalin-purity of glomalin-related soil protein fractions through immunochemical, lectin-affinity and soil labelling experiments. *Soil Biol Biochem* 40: 887-893. <https://doi.org/10.1016/j.soilbio.2007.10.019>
- Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cordeiro MAS, Carneiro MAC, Paulino HB, Junior OJS, 2005. Colonização e densidade de esporos de fungos micorrízicos em dois solos do cerrado sob diferentes sistemas de manejo. *Pesqu Agropecu Trop* 35: 147-153.
- Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A *et al.*, 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* 349: 970-973. <https://doi.org/10.1126/science.aab1161>
- Davison J, Moora M, Semchenko M, Adenan SB, Ahmed T, Akhmetzhanova AA *et al.*, 2021. Temperature and pH define the realized niche space of arbuscular mycorrhizal fungi. *New Phytol* 231: 763-776. <https://doi.org/10.1111/nph.17240>
- De Moura JB, Cabral JS, 2019. Mycorrhizas in Central Savannahs: Cerrado and Caatinga. In: *Mycorrhizal Fungi in South America*; Pagano MC, Lugo MA (eds). pp: 193-202. Springer, Cham, Germany. https://doi.org/10.1007/978-3-030-15228-4_10
- De Moura JB, de Souza RF, Junior WGV, Lima IR, Brito GHM, Marín C, 2019. Arbuscular mycorrhizal fungi associated with bamboo under Cerrado Brazilian vegetation. *J Soil Sci Plant Nutr* 19: 954-962. <https://doi.org/10.1007/s42729-019-00093-0>
- De Oliveira ALM, de Canuto EL, Urquiaga S, Reis VM, Baldani JJ, 2006. Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. *Plant Soil* 284: 23-32. <https://doi.org/10.1007/s11104-006-0025-0>
- de Santana Leite CCS, dos Santos SMB, Rocha WDJFS, de Barros Silva A, de Mello Baptista GM, 2017. Análise dos incêndios ocorridos no parque nacional da chapada diamantina-bahia em 2008 e 2015 com suporte em índices espectrais de vegetação. *Revista Brasileira de Cartografia* 69: 1127-1141.
- De Souza BR, de Moura JB, Oliveira TC, Ramos MLG, Lopes Filho LC, 2016. Arbuscular mycorrhizal fungi as indicative of soil quality in conservation systems in the region of Vale do São Patrício, Goiás. *Int J Curr Res* 8: 43307-43311.
- Dove NC, Hart SC, 2017. Fire reduces fungal species richness and in situ mycorrhizal colonization: a meta-analysis. *Fire Ecol* 13: 37-65. <https://doi.org/10.4996/fireecology.130237746>
- Ezawa T, Saito K, 2018. How do arbuscular mycorrhizal fungi handle phosphate? New insight into fine-tuning of phosphate metabolism. *New Phytol* 220: 1116-1121. <https://doi.org/10.1111/nph.15187>
- Fokom R, Adamou S, Teugwa MC, Boyogueno AB, Nana WL, Ngonkeu MEL *et al.*, 2012. Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameroon. *Soil Till Res* 120: 69-75. <https://doi.org/10.1016/j.still.2011.11.004>
- Gadkar V, Rillig MC, 2006. The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. *FEMS Microbiol Lett* 263: 93-101. <https://doi.org/10.1111/j.1574-6968.2006.00412.x>
- Gerdemann JW, Nicolson TH, 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Trans Brit Mycol Soc* 46: 235-244. [https://doi.org/10.1016/S0007-1536\(63\)80079-0](https://doi.org/10.1016/S0007-1536(63)80079-0)
- Giovannetti M, Mosse B, 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol* 84: 489-500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>
- Holátko J, Brtnický M, Kučerík J, Kotianová M, Elbl J, Kintl A, *et al.*, 2021. Glomalin-truths, myths, and the future of this elusive soil glycoprotein. *Soil Biol Biochem* 153: 108-116. <https://doi.org/10.1016/j.soilbio.2020.108116>
- Hunke P, Mueller EN, Schröder B, Zeilhofer P, 2015. The Brazilian Cerrado: assessment of water and soil degradation in catchments under intensive agricultural use. *Ecohydrology* 8: 1154-1180. <https://doi.org/10.1002/eco.1573>
- Kauffman JB, Cummings DL, Ward DE, 1994. Relationships of fire, biomass and nutrient dynamics along a vegetation gradient in the Brazilian cerrado. *J Ecol* 82: 519-531. <https://doi.org/10.2307/2261261>
- Klink CA, Sato MN, Cordeiro GG, Ramos MIM, 2020. The role of vegetation on the dynamics of water and fire in the Cerrado ecosystems: implications for management and conservation. *Plants* 9: 1803. <https://doi.org/10.3390/plants9121803>
- Longo S, Nouhra E, Goto BT, Barbara RL, Urcelay C, 2014. Effects of fire on arbuscular mycorrhizal fungi in the Mountain Chaco Forest. *Forest Ecol Manag* 315: 86-94. <https://doi.org/10.1016/j.foreco.2013.12.027>
- Marín C, Kohout P, 2021. Response of soil fungal ecological guilds to global changes. *New Phytol* 229: 656-658. <https://doi.org/10.1111/nph.17054>

- Marín C, Godoy R, Valenzuela E, Schloter M, Wubet T, Boy J, Gschwendtner S, 2017. Functional land-use change effects on soil fungal communities on Chilean temperate rainforests. *J Soil Sci Plant Nutr* 17: 985-1002. <https://doi.org/10.4067/S0718-95162017000400011>
- Martínez O, Valenzuela E, Godoy R, 2005. Hongos aislados desde suelos de bosques de Araucaria-Nothofagus después de un incendio en el Parque Nacional Tolhuaca. *Bol Micol* 20: 35-39. <https://doi.org/10.22370/bol-micol.2005.20.0.268>
- Martínez O, Cabeza R, Paulino L, Godoy R, Valenzuela E, 2018. Evaluation of soil enzymes activities in an Araucaria-Nothofagus forest after a wildfire. *Agro Sur* 46: 17-26.
- Mataix-Solera J, Guerrero C, García-Orenes F, Bárcenas GM, Torres MP, Barcenas M, 2009. Forest fire effects on soil microbiology. In: *Fire effects on soils and restoration strategies*; Cerdá A, Robichaud PR (eds). pp: 133-175. CRC Press, Boca Raton, USA. <https://doi.org/10.1201/9781439843338-c5>
- Miranda HS, Sato MN, Neto WN, Aires FS, 2009. Fires in the cerrado, the Brazilian savanna. In: *Tropical fire ecology*; Cochrane MA (ed). pp: 427-450. Springer, Heidelberg, Germany. https://doi.org/10.1007/978-3-540-77381-8_15
- Moya D, Madrigal J, Fontúrbel T, Marino E, Hernando C, Guijarro M, *et al.*, 2019. Fire severity assessments in both the laboratory and the field. In: *Fire effects on soil properties*; Pereira P *et al.* (eds). pp: 241-266. CSIRO, Clayton South, Australia.
- Nichols KA, Wright SF, 2006. Carbon and nitrogen in operationally defined soil organic matter pools. *Biol Fert Soils* 43: 215-220. <https://doi.org/10.1007/s00374-006-0097-2>
- Oehl F, Sieverding E, Palenzuela J, Ineichen K, da Silva GA, 2011. Advances in Glomeromycota taxonomy and classification. *IMA Fungus* 2: 191-199. <https://doi.org/10.5598/ima fungus.2011.02.02.10>
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, *et al.*, 2020. *Vegan: Community Ecology Package*. R package vers 2.5-7. <https://CRAN.R-project.org/package=vegan>
- Paulino L, Godoy R, Boeckx P, 2009. Ecosystem responses of Andean Araucaria-Nothofagus communities after a wildfire. In: *Ecological advances on Chilean temperate rainforests*; Oyarzún C, *et al.* (eds). pp: 117-132. Academia Press, Ghent, Belgium.
- Phillips JM, Hayman DS, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Brit Mycol Soc* 55: 158-161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Rashid A, Ahmed T, Ayub N, Khan AG, 1997. Effect of forest fire on number, viability and post-fire re-establishment of arbuscular mycorrhizae. *Mycorrhiza* 7: 217-220. <https://doi.org/10.1007/s005720050183>
- R Core Team, 2020. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Ratter JA, Bridgewater S, Ribeiro F, 2006. Biodiversity patterns of the woody vegetation of the Brazilian Cerrado. In: *Neotropical savannas and seasonally dry forests: plant diversity, biogeography and conservation*; Pennington RT *et al.* (eds). pp: 31-66. CRC Press, Boca Raton, USA. <https://doi.org/10.1201/9781420004496-2>
- Rillig MC, 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can J Soil Sci* 84: 355-363. <https://doi.org/10.4141/S04-003>
- Rivas Y, Canseco MI, Knicker H, Etcheverría P, Godoy R, Matus F, *et al.*, 2016. Variación en el contenido de glomalina relacionada a las proteínas del suelo, después de un incendio forestal en un Andisol en bosques de Araucaria araucana del centro-sur de Chile. *Bosque* 37: 409-417. <https://doi.org/10.4067/S0717-92002016000200019>
- Rodríguez-Ramos JC, Cale JA, Cahill JF Jr, Simard SW, Karst J, Erbilgin N, 2021. Changes in soil fungal community composition depend on functional group and forest disturbance type. *New Phytol* 229: 1105-1117. <https://doi.org/10.1111/nph.16749>
- Santos GC, 2016. *Comunidade de fungos micorrízicos arbusculares em campos rupestres quartzíticos e ferruginosos da Serra do Espinhaço*. Master Thesis, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brazil.
- Sharifi Z, Azadi N, Rahimi S, Certini G, 2018. The response of glomalin-related soil proteins to fire or tillage. *Geoderma* 329: 65-72. <https://doi.org/10.1016/j.geoderma.2018.05.008>
- Simon MF, Pennington T, 2012. Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado. *Int J Plant Sci* 173: 711-723. <https://doi.org/10.1086/665973>
- Simon MF, Grether R, Queiroz LPD, Skema C, Pennington RT, Hughes CE, 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proc Natl Acad Sci USA* 106: 20359-20364. <https://doi.org/10.1073/pnas.0903410106>
- Steidinger BS, Bhatnagar JM, Vilgalys R, Taylor JW, Qin C, Zhu K, *et al.*, 2020. Ectomycorrhizal fungal diversity predicted to substantially decline due to climate changes in North American Pinaceae forests. *J Biogeogr* 47: 772-782. <https://doi.org/10.1111/jbi.13802>
- Van der Heyde M, Ohsowski B, Abbott LK, Hart M, 2017. Arbuscular mycorrhizal fungus responses to

- disturbance are context-dependent. *Mycorrhiza* 27: 431-440. <https://doi.org/10.1007/s00572-016-0759-3>
- Van der Werf GR, Randerson JT, Giglio L, van Leeuwen TT, Chen Y, Rogers BM, *et al.*, 2017. Global fire emissions estimates during 1997-2016. *Earth Syst Sci Data* 9: 697-720. <https://doi.org/10.5194/essd-9-697-2017>
- Veenendaal EM, Torello-Raventos M, Miranda HS, Sato NM, Oliveras I, van Langevelde F, *et al.*, 2018. On the relationship between fire regime and vegetation structure in the tropics. *New Phytol* 218: 153-166. <https://doi.org/10.1111/nph.14940>
- Walter BMT, 2006. Fitofisionomias do bioma Cerrado: síntese terminológica e relações florísticas. Doctoral Thesis, Universidade de Brasília, Brazil.
- Wilson GW, Rice CW, Rillig MC, Springer A, Hartnett DC, 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. *Ecol Lett* 12: 452-461. <https://doi.org/10.1111/j.1461-0248.2009.01303.x>
- Wright SF, Upadhyaya A, 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Sci* 161: 575-586. <https://doi.org/10.1097/00010694-199609000-00003>
- Wright SF, Upadhyaya A, 1999. Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. *Mycorrhiza* 8: 283-285. <https://doi.org/10.1007/s005720050247>
- Xiang X, Gibbons SM, Yang J, Kong J, Sun R, Chu H, 2015. Arbuscular mycorrhizal fungal communities show low resistance and high resilience to wildfire disturbance. *Plant Soil* 397: 347-356. <https://doi.org/10.1007/s11104-015-2633-z>

Table S1. Averages and SDs of the mycorrhizal parameters evaluated across sampling times and phytophysiognomies.

Phytophysiognomy	Fire	Spore density (no. spores/50cm ³ soil)				Colonization (%)				EGG (mg/g of soil)			
		1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th
Campo Limpo	With	96.333	356.833	331.333	155	0.226	0.403	0.462	0.64	3.603	5.687	5.749	6.054
		±11.499	±44.073	±53.225	±30.469	±0.109	±0.05	±0.144	±0.094	±0.645	±0.076	±0.495	±0.427
	Without	68.667	170.667	186	155.5	0.544	0.882	0.806	0.681	4.504	5.749	7.085	6.054
		±19.431	±13.96	±12.028	±30.489	±0.138	±0.047	±0.061	±0.102	±0.953	±0.158	±0.274	±0.427
Campo Sujo	With	113	178.333	189.333	104.167	0.435	0.661	0.777	0.669	5.265	5.656	6.65	5.884
		±12.097	±19.754	±9.463	±14.088	±0.156	±0.059	±0.068	±0.155	±0.854	±0.088	±0.495	±0.32
	Without	102	166	127.833	113.333	0.461	0.756	0.76	0.642	4.551	5.221	5.718	5.806
		±18.708	±23.195	±27.552	±4.989	±0.17	±0.042	±0.056	±0.171	±0.972	±0.349	±0.245	±0.359
Cerradão	With	112.333	481	391	301.167	0.187	0.403	0.862	0.624	4.489	6.496	6.931	6.396
		±52.509	±18.403	±75.173	±75.891	±0.115	±0.016	±0.063	±0.14	±0.297	±0.044	±0.376	±0.561
	Without	173	413.5	214.667	328	0.25	0.491	0.731	0.542	5.064	5.78	6.837	6.256
		±29.098	±61.784	±55.211	±90.745	±0.048	±0.185	±0.117	±0.138	±0.829	±0.076	±0.439	±0.58
Cerrado sensu stricto	With	58.667	224.667	274.667	108	0.403	0.736	0.822	0.506	4.893	5.749	5.723	6.598
		±18.83	±13.597	±19.939	±39.467	±0.065	±0.025	±0.035	±0.144	±0.831	±0.088	±0.044	±0.26
	Without	59.833	90.333	90.667	80.833	0.531	0.69	0.93	0.586	4.768	5.967	6.557	6.225
		±20.948	±5.793	±4.643	±43.341	±0.172	±0.048	±0.023	±0.132	±0.57	±0.403	±0.288	±0.428
Veredas	With	115.667	276.333	419.667	184.5	0.346	0.652	0.896	0.781	3.619	6.153	6.899	5.775
		±34.697	±28.802	±33.954	±34.292	±0.195	±0.04	±0.027	±0.138	±0.393	±0.533	±0.806	±0.258
	Without	154	289.333	366.667	141.833	0.451	0.682	0.797	0.726	3.992	6.246	6.154	6.132
		±39.737	±72.91	±85.168	±49.083	±0.124	±0.124	±0.094	±0.107	±0.342	±0.332	±0.727	±0.642

EGG: easily extractable glomalin