Original Article

Urochloa decumbens Has Higher Mycorrhizal Colonization In Degraded Than In Pristine Areas In The Brazilian Cerrado

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ABSTRACT

Brazil has extensive degraded areas, where vegetation fails to establish due to harsh soil conditions. However, some invasive species such as *Urochloa decumbens* are successful pioneers in such areas, but the reasons deserve investigation. Mycorrhizal fungi are abundant in Cerrado soils, and their association with plants are beneficial for their establishment in natural and degraded areas. This study investigated whether arbuscular mycorrhizal colonization of native and exotic plants in the Cerrado differs between pristine and degraded areas. We collected 135 plants from four functional groups in 68 areas and determined the percentage of mycorrhizal colonization. The invasive grass *Urochloa decumbens* had significantly higher colonization rates in degraded than in native areas, and higher colonization than the native species. These results are important for soil management since *Urochloa decumbens* is widely used in early soil restoration efforts, and for nature conservation concerning the management of invasive plants in restoration areas.

Keywords: mycorrhiza, grassland, restoration, native plants, exotic plants.

1. INTRODUCTION

Soils contain a diverse array of microorganisms, with arbuscular mycorrhizal fungi (AMF) amongst them. These form symbiotic associations with roots of approximately 80% of all vascular terrestrial plants (Berruti et al., 2015). In this association, the plant gets nutrients and water from areas inaccessible to the roots, whereas the heterotrophic microbe receives organic carbon compounds produced by the plant through photosynthesis. In this realm, AMF creates a positive effect on life of higher plants, particularly in sites with low water and mineral nutrient availability (Blume et al., 2016).

In natural, non-cultivated areas, AMF are especially diverse and abundant in areas with nutrient-poor soils and plant species-rich communities (Smith & Read, 2008). The Brazilian Cerrado has these characteristics, being considered a biodiversity hotspot for conservation priorities. These natural communities undergo several human pressures, such as the expansion agro-industry and the construction of hydroelectric power stations. The construction of dams in such areas caused the removal of the upper soil layers, having created areas where the total vegetation and part of the soil surface as well as the seed bank were mechanically removed (Carvalho et al., 2015). In the region of Ilha Solteira/SP, the removal of approximately 9 meters of the original soil profile for the construction of the dam foundation of the hydropower plant of Ilha Solteira – Usina Hidrelétrica de Ilha Solteira – effectively eliminated the vegetation of a massive area (Carvalho et al., 2015). The process of natural re-establishment of vegetation in this area is slow. In some regions there is progress by using the exotic grass *Urochoa decumbens*. This grass acts protecting the soil and inducing the accumulation of organic matter (Rosa et al., 2014) and phosphorus in the soil (Vendruscolo et al., 2016), which can further facilitate the development of higher vegetation composed by shrubs and trees. Kitamura et al. (2008) recommends the use of *Urochloa decumbens* in the process of restoration of degraded areas because it also prevents the physical impacts of rain on the soils, enhances the soil organic matter content and helps the formation of soil aggregates, facilitating root penetration and microbial life.

Urochoa decumbens is an African grass that was introduced in Brazil as a forage species. It is very well adapted to Cerrado conditions, constituting one of the main invasive plants in this biome. In fertilization experiments, it responded to phosphorus additions and was able to dominate areas and displace other grasses and herbs (Lannes et al., 2016). Whether mycorrhizal colonization can help explain alien plant invasion success is still controversial. Some studies show that AMF can influence the success of invasion by alien plants (Grilli et al., 2014) and that invaders have a higher overall AMF colonization than native grasses (Lekberg et al., 2013; Menzel et al., 2017), while others demonstrate that plant invasion is not dependent on mycorrhizal colonization for their success (Bunn et al., 2015; Busby et al., 2011; Reinhart et al., 2017).

In temperate areas, recent efforts aiming at restoring eroded soils for vegetation recovery have been using mycorrhizal inoculation, showing positive results in terms of improving soil properties and facilitating the re-establishment of the original flora (Asmelash et al., 2016). Comparable studies in tropical areas are still scarce, but see Faria et al. (2013) and Santos et al. (2018). In natural habitats, plant diversity and productivity are positively correlated with mycorrhiza diversity (Hu et al., 2018), and different plant functional groups have specific and different mycorrhizal colonization rates, with forbs being generally more colonized than grasses (Bunn et al., 2015).

Because different functional groups in the Cerrado can have diverse responses to nutrient deficiency (Lannes et al. 2016) and mycorrhizal associations can ammeliorate nutrient limitation, this study aims at investigating, under field conditions, how plants from these different functional groups (non-invasive grasses, invasive grasses, legume forbs and other forbs) differ in relation to mycorrhizal colonization rates. As there are several degraded areas in the Cerrado that are prone to restoration, we assessed whether mycorrhizal colonization of these functional groups are affected by different land uses (pristine vs. degraded areas). This information will be important to understand whether Cerrado functional groups differ in relation to mycorrhizal colonization and are affected by land use, which can effectively contribute to the management of plants in degraded areas in this biome.

2. MATERIAL AND METHODS

This research was conducted at the municipalities of Ilha Solteira/SP and Selvíria/MS. In these areas, there are ecosystems in various successional stages, including grassland, forest, *Urochloa* fields and degraded exposed subsoil. This region has a humid tropical climate with a pronounced dry season from May to September and a rainy season that concentrates more than 70% of the rainfall from October to April. Soils are mostly composed by strongly acid Latosols (US classification: Oxisols). They are well-drained, deep, red, clay-rich, structurally strong but poor in mineral nutrients (EMBRAPA, 2013).

Within this region, sampling was performed during the peak of the growing seasons in 2016 and 2017 (February-March), which coincides with the rainy period in the area. Root fragments from one single specimen of each of four species of different functional groups were collected in 68 different areas (Figure 1): the naturalized grass *Melinis repens*, the invasive grass *Urochloa decumbens*, a legume forb *Stylosanthes guianensis* and the non-legume herb *Waltheria indica* (Malvaceae). For means of comparison, these species were collected in degraded areas, i.e., areas with a long erosion process with extremely poor soils and exposed subsoils, and in pristine areas, i.e., areas with no apparent human disturbance. Samples were kept in a sterilized bag and transported to the laboratory.

To assess mycorrhizal infection, roots were first cleared and then stained for analysis. Within 12 hours after harvest, fine roots were cut and placed in glass tubes with chloridric acid (HCl) 1 M, and kept in 90 °C water bath during one hour. After this first hour, the HCl solution was renewed and roots were kept in water bath for two more hours. This two-step clearing process was needed due to the intense natural colour of the roots, especially on those of *Waltheria indica* and *Stylosanthes guianensis*. The cleared samples were rinsed with tap water, rinsed with 5% HCl and rinsed with water again. The roots were then stained for 20 minutes in a solution 5% ink (Parker Qink Black, Newell Rubbermaid, Saint Herblain, France) in 5% acetic acid solution, also at 90 °C, following the protocol by Vierheilig et al. (1998). To quantify mycorrhizal colonization rate, root dissections were analysed under magnification and the presence of arbuscles, vesicles or hyphae was recorded.

The effect of the land use on total and functional group mycorrhizal colonization was tested through Student t-tests. The effect of functional type on mycorrhizal colonization was tested using ANOVA followed by Tukey test. All statistical analyses were done using R version 2.10.1 (R Core Team, 2014) and the significance level employed was $p<0.10$.
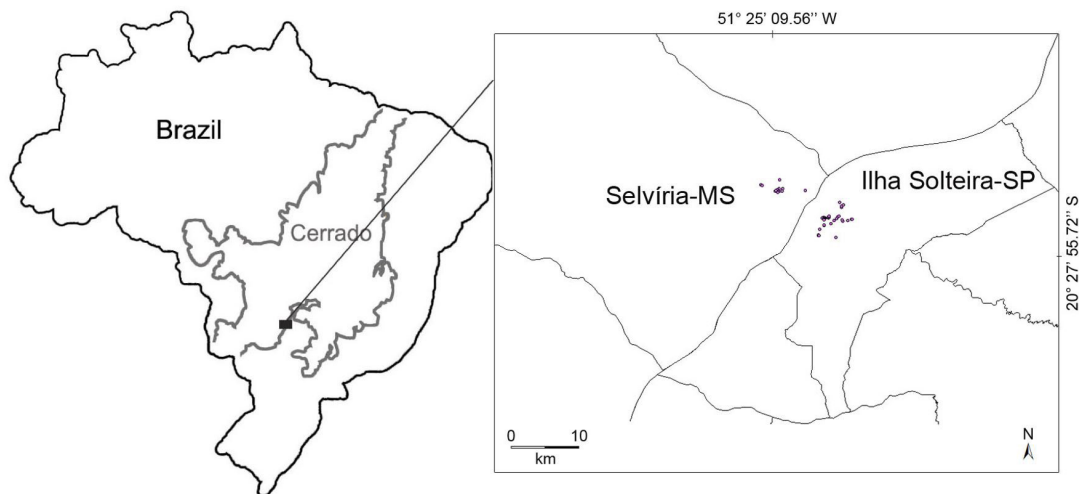
Figure 1. Locations of field sampling sites in the Cerrado indicating the 68 areas where the 135 specimens of *Melinis repens*, *Urochloa decumbens*, *Stylosanthes guianensis* and *Waltheria indica* were collected in the rainy seasons of 2016 and 2017.

3. RESULTS

The results comprise 135 plant samples distributed in 68 areas. Comparing different functional groups across all sites, no significant difference in mycorrhizal colonization was detected (p=0.241) (Figure 2).

Across species, soil use type had a significant effect on fungal colonization (p=0.076), with higher values in the degraded area than in the native area (Table 1). Species did not differ in colonization in the native area (p=0.436) (Table 1), but the invasive grass *Urochloa decumbens* had higher colonization in the degraded area than the other studied species (Table 1). The alien invasive grass *Urochloa decumbens* had significantly higher mycorrhizal colonization in the degraded area in relation to the native area (P=0.023), which was not observed for the other species (Table 1).

4. DISCUSSION

Mycorrhizal colonization rates measured in these areas and plants were comparable to data from others, as reported in Table 2.

Human influence in the environment tends to reduce mycorrhizal establishment, colonization and diversity worldwide (Grilli et al., 2014) and specifically in Brazil (Carrenho & Gomes-Da-Costa, 2011). In this study, although intuitively one could think that the removal of approximately nine meters of topsoil in the degraded area could have eliminated potential root colonizers fungi spores from the soil system, plants living in degraded showed a higher AMF colonization than the ones living in more natural, pristine, non-degraded areas. This can be explained by the fact that mycorrhizae living in harsher environments tend to produce more
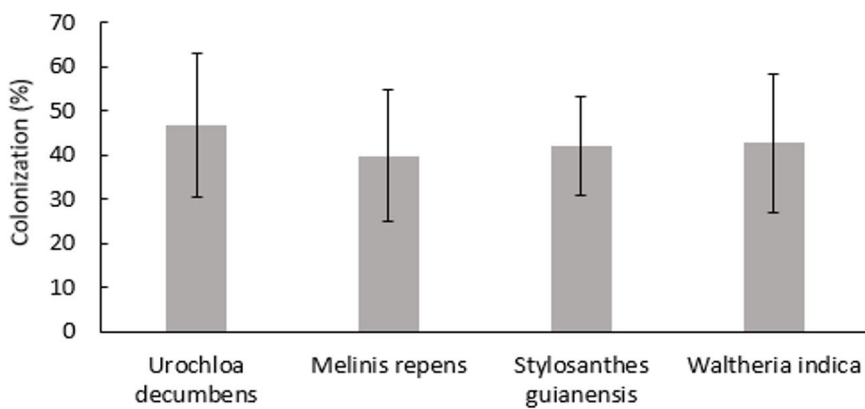
Figure 2. Arbuscular mycorrhizal colonization rates in four common grassland species from the Cerrado: the exotic invasive grass *Urochloa decumbens* (n=39), the naturalized grass *Melinis repens* (n=38), the legume forb *Stylosanthes guianensis* (n=20) and the non-legume forb *Walteria indica* (n=38). P (ANOVA)=0.241.

Table 1. Average values of mycorrhizal (AMF) colonization rates (percentage) for species of four functional groups in degraded and native areas in the Cerrado.

Land use type	Degraded area			Native area			F	P
Plant species	Average	sd	N	Average	sd	N		
Urochloa decumbens	53.0aA	16.6	18	41.4aB	14.1	21	5.6	0.023
Melinis repens	43.1bA	14.8	20	36.0aA	14.4	18	2.2	0.144
Waltheria indica	41.5bA	15.0	19	43.8aA	16.5	19	0.2	0.661
Stylosanthes guianensis	42.8bA	11.7	18	36.4aA	0	2	0.5	0.457
All species	45.0A	15.1	75	40.4B	14.9	60	3.2	0.076
F	2.384			0.923				
p	0.076			0.436				

ANOVA F and p values are shown for the effect of the land use type (horizontal comparisons) and for the effect of plant type (vertical comparisons) on colonization. Standard deviations (sd) and number of replicates (N) are shown. Different lowercase letters indicate significant differences in the columns (species effect – Tukey results), and different uppercase letters indicate significant differences in the lines (land use effect – t-test results).

Table 2. Arbuscular mycorrhizal (AMF) colonization in plant species from different families and ecosystems.

Species	Family	AMF (%)	Ecosystem	Ref.
Hedychium coronarium	Zingiberaceae	66	SPF	1
Eupatorium adenophorum	Asteraceae	69	SPF	1
Crotalaria anagyroides	Fabaceae	71	SPF	1
Musa sp.	Musaceae	38	AA	2
Panicum maximum	Poaceae	51	AG	3
Zea mays	Poaceae	74	AG	3
Urochloa humidicola	Poaceae	60	AG	3
Glycine max	Fabaceae	33	AG	3

SPF – Subtropical pine forest; AA – Agricultural area; AG – Agricultural grassland. 1 – Songachan & Kayang (2011); 2 – Oliveira & Oliveira (2005); 3 – Ramos et al. (2012).

propagules aiming to guarantee long-term survival (Sylvia & Jarstfer, 1992), which indirectly influences root colonization rates. In natural areas, the lower colonization rates can be attributed to the stability of such ecosystems, mainly due to the presence of the most superficial soil horizons that are still protected (Caproni et al., 2003). These observations are in accordance with Silva et al. (2006), who measured more spores in degraded areas due to high human activity than in non-degraded areas.

Under experimental conditions, *Urochloa decumbens* shows a good response to AMF inoculation, as reported by Carneiro et al. (1995). Our study has revealed that this exotic invasive grass had significantly higher colonization rates in the degraded areas than in the native areas. In the native area, *Urochloa decumbens* was more colonized than the other functional groups. This pattern can help explain why this species is so successful and widely used for restoration of degraded areas. Once associated with mycorrhiza, these exotic species living in such harsh environments can grow and thus make the soil conditions more appropriate for the next plants in the succession process, which will allow the vegetation to develop and grow, facilitating the success of the restoration process.

It is important to note, however, that alien plant invasions are an environmental problem of increasing concern, once it may cause severe ecological and economic consequences where they occur (Simberloff et al., 2013). As mycorrhizal associations can help them to become even more aggressive in colonization terms (Menzel et al., 2017), it is important to understand whether the African invasive plants living in the Cerrado benefit from associations with AMF to increase its invasion potential. This might be the case in the degraded Cerrado area studied here, since *Urochloa decumbens* is an abundant and widespread species that generally arrives first and dominates large patches of the environment in this region (Teixeira et al., 2019). Whether this ability is specifically due to its association with AMF or to other characteristics still deserves investigation.

As one of the main invasive grasses causing species extinction in the Cerrado (Pivello et al., 1999), it is important to evaluate whether the use of *Urochloa decumbens* for recuperating degraded areas is beneficial or not for nature conservation. Due to its ability to better associate with fungi and expand in such areas, it could be seen as an potential colonizer for landslide surface areas or other highly degraded regions located distantly from natural areas of high biodiversity. We recommend, however, that other studies of this type using different Cerrado grass species can be conducted to search for native species that are as effective as *Urochloa decumbens* for the recuperation of degraded areas.

5. CONCLUSIONS

Plants from different functional groups differed in arbuscular mycorrhizal colonization in the degraded areas, but not in the native areas. In the degraded area, the exotic invasive grass *Urochloa decumbens* had significantly higher colonization rates than native plants. These results are important for nature conservation, both for restoration ecology and for the management of alien invasive plants in highly biodiverse areas as the Brazilian Cerrado.

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