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Sustainable Cultivation of Urochloa Grasses and Associated Mycorrhizae

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Abstract: Perennial grasses have multiple important uses such as forage, cover crops, and intercrops, utilized in pest management and supporting scientific experiments. Among those grasses, *Urochloa brizantha* is a tropical forage, having vigorous deep roots, commonly used for arbuscular mycorrhizae (AMF) multiplication in glasshouse and nursery, developing infective propagules to be conserved in germplasm banks. Other grass species, such as *Urochloa decumbens*, is also used for arbuscular mycorrhizae multiplication in addition to its cultivation for guaranteeing the sustainability of livestock systems. In this study, the soil chemical characterization, microcharcoal content and AMF spore identification showed high amount of potassium (K), high microcharcoal content and the occurrence of AMF, most of Glomeraceae. Due to its economic importance for sustainable agricultural production and other uses besides its environmental services, more detailed research is needed on the biotic interactions and inoculant production in these studied grasses.

Keywords: Grasses; Urochloa; mycorrhizae; grassland; soil

1. Introduction

Due to the increasing interest deposited on grasslands for forage cultivation (source of meat and milk) to obtain better food production besides reducing environmental unfavorable emissions, researchers look for decrease nutrient and fertilizers leaching and employ of pesticides, enhancing carbon sequestration, thus improving soil sustainability [1]. However, the deleterious effects of chemical inputs on the soil of monocultures are not totally studied [2]. Fortunately, the effect of manures, wastes, residues, compost, and biochar amendments to the soil systems is increasingly studied worldwide, as organic agroecosystems are more procured. Thus, experiments on the implementation of natural soil conditioners by agriculture are rapidly increasing, and organic or regenerative agriculture adopted the addition of natural residues on horticultural plants and crops. Furthermore, the compatibility among biofertilizers and soil conditioners is also investigated to support sustainable agricultural systems and to deal with the effects of global and especially climatic change. Thus, the mycorrhizal symbiosis, application of compost of selected waste [3], phosphate solubilizing microorganisms, microbial inoculants, and biochar are increasingly explored worldwide. Of particular interest for research are perennial grassland plots of *Urochloa brizantha* (Figure 1), a tropical forage (source of meat and milk), commonly used for Arbuscular mycorrhizae fungi (AMF) multiplication as this plant species associate with this type of fungi, which highly colonize its roots [4]. developing infective propagules [5]. *Urochloa decumbens* is also used for arbuscular mycorrhizae multiplication, in addition to its cultivation for ensuring the sustainability of livestock systems. Moreover, these grass species are increasingly used in no-tillage as *cover crops* (Alves Teixeira et al.2014). [6], such as in coffee trees (Baptistella, 2022). [7]. Some grass species actively propagate

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benefic microsymbionts after their roots are colonized by the soil microbiota. This is the case of *Uroclhoa (Brachiaria*) species, which are largely cultivated in pastures and cover crops. *Urochloa* spp. are frequently selected for intercropping to improve land use and agricultural yields due to their high residue production, and slow decomposition. (Crusciol et al.2023). [8]. The occurrence and life cycle of relevant symbionts such as the AMF in grasslands was previously investigated, especially in Brazil [4]. Reports indicated differences between symbionts AMF species in *U. brizantha*, However, due to the economic importance of grass species, and due to the lack of detailed data in this topic, more detailed research is needed to better understand the plant interactions with the microbiota. In the present study, with the purpose of examining the AMF associated to grass in experimental farms, the AMF community structure was studied in a plot of *U. brizantha* grassland. Regarding spore numbers in the soil/ substrate, high values were observed in *U. brizantha* inoculated with *A. longula* (850 spores in 50 ml substrate), followed by plants inoculated with *Acaulospora colombiana* (755 spores) and *Acaulospora morrowiae* (228 spores, after 120 days of cultivation). It was highlighted the high multiplication potential of *Acaulospora longula* in *Urochloa* [4], thus indicating potential prolific species to be included as inoculants.

Figure 1. grassland plot of *Urochloa brizantha.*

2. Materials and Methods

2.1. Study Site

The *U. brizantha* grassland plot at the experimental farm of EPAMIG (19°42'51.5"S 44°53'37.0"W) in Brazil, South America, (estimated age of cultivation ~25 years, chopped up once a year, (Figure 1) was used to study the AMF community structure. A site of 100 m^2 in the farm was selected to take soil samples in a plain area, with *U. brizantha* pasture, and the samples were collected between February (rainy period) and November (after the dry period) 2015.

2.2. Soil Sampling and Chemical Characterization

Soil samples (three replicate plots (plot size, 5 by 20 m) obtained in February (rainy period) and November 2015 (after the dry period) at the site, were used for determination of chemical soil parameters (Embrapa, 1997) and for AMF spore extraction.

2.3. AMF Spore Isolation and Identification

AMF spores occurring in the soil samples (100g soil) were extracted by wet sieving (Gerdemann and Nicolson, 1963) [9] and were identified using specific descriptions of AMF species. The old and decaying spores with missing clear features were also counted as an approach for viability [10], and the results expressed as percentage of unviable AMF spores.

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2.4. Microcharcoal Content

The number of microscopic (>500µm) charcoal fragments (adapted from [11] was measured on the same Petri dishes used for spore isolation at 400 × magnification using a grid, and results were expressed as number of fragments in the subsamples (25g soil).

3. Results

The *U. brizantha* grass presented a Typic Acrustox soil (Soil taxonomy- USDA, 2022), (Table 1). The region's soil type was classified as sandy clay loam. The occurrence of AMF, most of Glomeraceae (Table 2), was detected. Compared to other reports, *Claroideoglomus* was dominant among the isolated species of Glomeromycota (Table 2). The grass soil studied showed exclusive characteristics: high amount of K and higher charcoal content than samples of cultivated sites in the region (Pagano et al, 2020) [12]. This study showed the AMF community associated with grass, as well as potential for AMF inoculant formulation. Different AMF species (*Acaulospora longula* and *Acaulospora colombiana*) were previously associated with *U. brizantha* in greenhouse, Minas Gerais, Brazil, *by* Barbosa et al. [5].

Table 1. Reports on *Urochloa species* in Brazil.

Vegetation / Field/ greenhouse	Plant species	Geographical coordinates	Altitude (m asl)	Estimated age of cultivation (year)	AMF species/Glomalin	State	Reference
	U. brizantha *U. decumbens*			Experimental Station of The Cerrado	*Diversispora* sp., *Scutellospora* sp. *Glomus* sp. *Gigaspora* sp.	GO	[4]
Greenhouse	*U. brizantha*	19°42'51.5"S 44°53'37.0"W	628,36	>10	*A. colombiana*, *A. longula*/ +	MG	[5]
							[6]
	U. brizantha				NA	SP	[7]
	U. brizantha	48°26'W; 22°51' S	740		NA	SP	[8]

NA: Not available. GO = Goiás; MG= Minas Gerais; SP = São Paulo; + Glomalin content evaluated.

Table 2. Physical-Chemical properties of soil at *U. brizantha*, in the experimental farm, Minas Gerais state, Brazil.

pH (H_2O)	5.4
OC (dag kg^{-1})	NA
Ca (cmolc kg^{-1})	1.5
Mg (cmolc kg^{-1})	0.9
Al (cmolc kg^{-1})	0.7

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K mg·kg-1	43
P mg·kg-1	1.8
Soil texture	Clayey- loam
Charcoal content#	19.66

Means of three replicates. NA: Not available. # Mean number of microcharcoal fragments in soil.

Table 3. AMF species found in the *U. brizantha* grassland soil in the experimental farm, Minas Gerais state, Brazil.

AMF Family	AMF species
Ambisporaceae	*Ambispora appendicula*
Claroideoglomeraceae	*Claroideoglomus etunicatum*
Glomeraceae	*Funneliformes geosporus*
Dentiscutataceae	*Dentiscutata heterogama*
Glomeraceae	*Glomus brohultii*

In the present study, the AMF community structure was studied in an *U. brizantha* grassland in Brazil, The AMF community structure in the *U. brizantha* grassland showed dominance of Glomeraceae, compared to other reports where *Acaulospora* and *Gigaspora* occurred (dos Santos et al. 2022) [9]. In the *Urochloa brizantha* grass, five genera of Glomeromycota, five species and four unidentified morphotypes were detected (Table 1). The *U. brizantha* grass showed exclusive characteristics: higher amount of K and higher microcharcoal content (Table 1) than samples of cultivated sites. This study showed the AMF communities associated to the grass at field conditions, as well as potential for AMF inoculant formulation. Different AMF species (*Acaulospora longula* and *Acaulospora colombiana*) were previously associated with *Urochloa brizantha* (Barbosa et al. 2019). [5]. However, to understand the *U. brizantha* ecology it is necessary to analyze the time of symbiosis establishment, and spore multiplication. The hypothesis of this study is that *U. brizantha* associates with diverse AMF species, which have different behaviors when colonizing this host plant. We examined arbuscular mycorrhizae associated with this grass in an experimental farm. The community structure was studied in a *U. brizantha* grassland in Brazil, which showed a greater diversity of AMF compared to previous reports. Moreover, nearly 50 % of unviable AMF spores were estimated. Four genera of Glomeromycota, five species and four unidentified morphotypes were detected (Table 2). The grass showed exclusive characteristics: higher amount of soil organic matter and higher macroaggregates and charcoal content than samples of cultivated sites investigated in the region by Pagano et. al.2020) The present study showed AMF communities associated to *U. brizantha,* as well as the potential for AMF inoculant formulation.

Figure 1.

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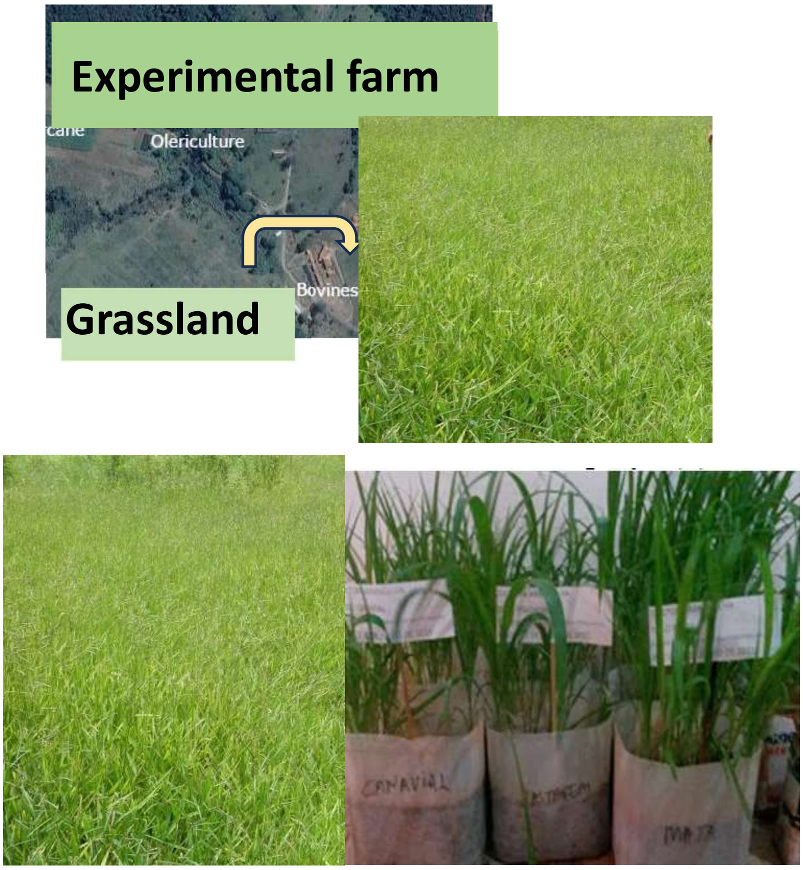

Figure 1. grassland plot of *Urochloa brizantha* in Brazil, (A). *Sorghum* cultivated in pots (B) with *U. brizantha* soil.

Table 2. AMF species associated with *Urochloa brizantha* at the experimental farm, Minas Gerais, Brazil.

AMF species
Dentiscutataceae
Dentiscutata heterogama
Claroideoglomeraceae
Claroideoglomus etunicatum
Racocetraceae
Racocetra fulgida
Glomeraceae
Funneliformes geosporus
Glomus sp. 1
Glomus sp. 2

Data obtained at 0-20 cm soil depth. [a] (n = 3).

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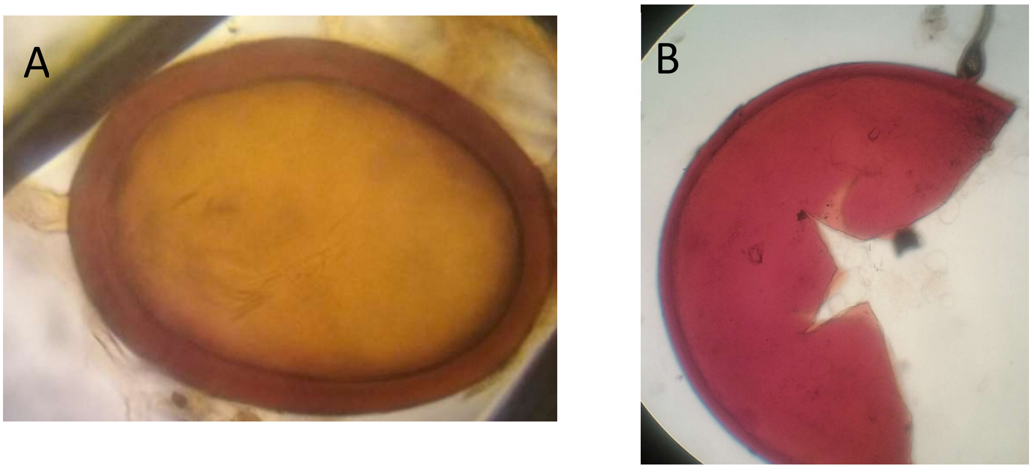

Figure 2. AMF species found in the U. brizantha grassland. Claroideoglomus etunicatum(A).; Dentiscutata heterogama (B).

4. Discussion

Most previous reports on *Urochloa* come from greenhouse experiments, indicating differences between AMF species associated to *U. brizantha*, *A. colombiana*, showing the best AMF root colonization percents in a short cultivation time. (Barbosa et al. 2019) found the highest number of spores of *Acaulospora longula* and *A. colombiana*, exhibiting the highest mycorrhizal root colonization at 76 days, confirming that the inoculation favored the root growth of *U. brizantha* in greenhouse. The need of more information for a better understanding of the symbiotic relationships between different AMF species associated to U. *brizantha* was earlier pointed out.

In the present field study, the results indicated at least five AMF species and other morphotypes, which can be propagated on farm, establishing sustainable *Urochloa* cultures in tropical agroecosystems and also, *Sorghum* cultures cultivated in pots with *U. brizantha grassland* soil. As AMF species vary in their performances for the plant species, it is needed to compile more knowledge to optimize their management. In the present study, the AMF species were mostly from Glomeraceae, which are designated as r-strategists, and are commonly found in anthropized agroecosystems; however, some studies showed the effective potential of *Acaulospora*. Thus, it is necessary to study the propagules during the cultivation time to better understand the symbiosis in *Urochloa* species and other promising tropical forages. The production of biological inputs on farm (on rural properties) is also increasing. The production of biological inputs such as inoculants and products for biological pest management is guided by the Ministry of Agriculture, Brazilian Agropecuary Company (EMBRAPA) for monitoring and regulate the quality and safety of tested products.

5.1. Urochloa Species in Cover Crops.

Perennial grasses have been employed as cover crops, and intercrops, besides their use for forage, and were also utilized in pest management and supporting scientific experiments. Among the *Urochloa* species, *U. humidicola* and *U. decumbens and Brachiaria ruziziensis* were investigated [9]. presenting percents of AMF colonization in their roots of 55.78, and 66, respectively. These high values indicate the relevant role of these plant species benefiting soil health through the fungal network provided by AMF, which benefits plants and protects soil. Furthermore, AMF secrets a protein (glomalin), which favors soil aggregation, improving soil quality. In this sense, only one report showed the glomalin content in *U. brizantha*, which varied from 3.30 to 3.74 mg. g soil $^{-1}$ when inoculated with *Gigaspora margarita* and *A. longula*, respectively [6].

Due to the relevant role of cover crops and intercrops in increasing diversity and sustainability of crops, they are commonly used for several reasons: the residues left after cutting protect the soil and provide nutrients to the next crop or intercropped culture. *Urochloa* species have vigorous, abundant deep roots, opposed to the more superficial and scarce roots of crops. These grass traits aid carbon sequestration, soil organic matter stabilization and nutrient cycling. *Urochloa* roots also improve soil physical characteristics and influence soil nutrient dynamics, reducing nutrient losses and enhancing their cycling, which is benefic for nutrient use efficiency in sustainable agriculture. [6]. Furthermore, these cover crops are commonly employed in coffee trees [7] and in many orchards. Thus, meliorating the *U. brizantha* culture via benefits from AMF and increasing research on the symbionts and related microbiota, will favor sustainable grazy ecosystems destinated to forage and provide sustainable plant systems to reproduce AMF propagules. Therefore, *U. brizantha* has showed high root colonization (35 %) when inoculated with *A. longula*; however, low values (16 %) were reported when inoculated with other species, such as *G. margarita* (16%) or *P. occultum* (27%). The low root colonization when inoculated with *G. margarita* may be related to the characteristic slow root colonizer of this AMF species. The cultivation of *Urochloa* in agroecosystems have multiple benefits, such as reduced risk of soil erosion, better soil structure, higher levels of soil organic matter and biological activity. [6]

5.2. Urochloa Species for AMF Studies

Urochloa brizantha is a tropical forage, having vigorous deep roots, commonly used for arbuscular mycorrhizae (AMF) multiplication in glasshouse and nursery, developing infective propagules for conservation in germplasm banks and, thus, to be used in taxonomy studies of AMF, providing information on the interactions of different AMF for mycorrhizal colonization and spore multiplication to be used in AMF collections and experiments. At the same time, most propagules provide information on the interactions of different AMF for mycorrhizal colonization and spore multiplication to be used in AMF collections. Have unique value for inoculum to be applied in experimental research or in farms. Other grass species, such as *Urochloa decumbens*, is also used for arbuscular mycorrhizae multiplication. A great number of studies have utilized AMF spores multiplied using *Urochloa decumbens* for propagation of spores used as inoculum. Among them, field studies on ecological restoration (Pagano et al., 2022) [7] employed inoculated seedlings of native tree species. Traditionally, a mix of dominant AMF species recruited from the local site can be employed. However, it was common to produce inoculum combining two different AMF species (*Gigaspora margarita* and *Glomus* sp. Other studies in greenhouse, (Pedroso et al. 2018 [13]) applied a mixture of spores (*Glomus macrocarpum*, *Paraglomus occultum*, and *Glomus* sp. to inoculate *U. brizantha* in soil contaminated with heavy metals. They found greater root colonization in inoculated plants, showing beneficial effects of AMF on plant growth and alleviation of soil contaminants.

5.3. Urochloa Species for Carbon Mitigation

Besides the contribution of *U. brizantha* plants fixing atmospheric CO_2, the accumulation of glomalin (a glycoprotein produced by hyphae and spores) in the soil is important due to its role protecting organic carbon, which contributes to the reduction of the CO_2 emissions. Thus, improving the growth of *Urochloa* plants results in a greater photosynthetic activity (increased fixation of atmospheric CO_2), favoring the accumulation of organic carbon by roots and increasing the nutrients cycling in the soil/plant system.

6. Conclusions

This study details the AM fungal community associated with *Urochloa* roots in a grassland ecosystem using morphological methods for spore identification. Moreover, we compile reports on this plant species in tropical South America. *Glomeraceae* was the most prolific fungal family inhabiting the grassland; however, other AM fungal species were found. We confirmed five AMF species and four morphotypes associated to *Urochloa brizantha* in a tropical managed soil This study contributes for the selection of highly-efficient inoculants for the mycorrhizal colonization of plant roots, especially, *Urochloa* species (*U. brizantha*), at field conditions. For instance, *F. geosporus*, *D.*

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heterogama and *C. etunicatum* can be indicated as potential inoculants for *Urochloa brizantha* plots in this tropical region.

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