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ORIGINAL PAPER

Arbuscular Mycorrhizal Fungi Associated with Bamboo Under Cerrado Brazilian Vegetation

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Abstract

Most studies on bamboo have evaluated their commercial use but few have investigated their associated arbuscular mycorrhizal fungi (AMF). These symbiont fungi are fundamental on plant growth, nutrient cycling, biodiversity maintenance, etc., particularly on acidic/dystrophic soils as those of the Brazilian Cerrado. This study aimed to characterize the community composition and ecological interactions of AMF associated with the bamboo species *Actinocladum verticillatum* and *Bambusa vulgaris vittata*, under Cerrado vegetation in central Brazil. Roots and rhizospheric soil samples of *A. verticillatum* and *B. vulgaris vittata* were collected on 12 plots in the Gurupi (Tocantis state) and Porangatu (Goiás state) microregions. The roots' mycorrhizal colonization rate, rhizospheric soil' spore density, and the associated AMF genera were evaluated. There were no differences in the radical mycorrhizal colonization rates among the two bamboo species, although *B. vulgaris vittata* showed higher spore density than *A. verticillatum*. The genera A*caulospora*, *Claroideglomus*, *Diversispora*, *Scutellospora*, *Glomus*, and *Gigaspora* were identified in both bamboo species, while *Sclerocystis* was present only on *A. verticillatum.* The genera *Acaulospora*, *Diversispora*, and *Glomus* were frequently found together. This study may be a first step to future AMF-based bamboo micropropagation efforts in the Cerrado Brazilian vegetation.

Keywords *Actinocladum verticillatum* · Arbuscular mycorrhizal fungi · *Bambusa vulgaris vittata* · Cerrado vegetation · Mycorrhizal colonization · Rhizosphere

1 Introduction

Bamboo species (Poaceae) are naturally found in tropical regions of America, Africa, Asia, and Oceania. Currently, 123 genera and 1675 species of bamboo have been described worldwide (Bystriakova et al. 2004). Bamboo can be classified as an herbaceous and woody plant, having a wood with very high quality and of high commercial interest due to its several uses and applications. Bamboo plants stand out for their great versatility of usages: in the fishing industry, in civil construction and architecture, in the production of furniture and home utensils, as base of high calorific charcoal, in the production of alcohol, in landscaping, in the fabrication of musical instruments, as a cultural and/or traditional plant, as food and medicine, and is also used in the recovery of degraded areas, among many other uses (Maoyi and Banik 1995; van Dam et al. 2018; Zhang et al. 2016). Most of the bamboo cultivation areas in the world are concentrated in Asia, followed by America and Africa (Bystriakova et al. 2004); this distribution coincides with the areas were bamboo is most exploited—although just a few dozen species are commercialized. Human exploitation and anthropogenic effects (land use and climate change) have constituted evolutionary pressures on the distribution and biogeography of bamboo (Bystriakova et al. 2004).

Bamboo is an important element of both the canopy and the understory in Brazilian Cerrado vegetation (Eiten 1972). In fact, recently, new bamboo species have been described on

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this region (Viana et al. 2013a, b). Bamboo in the Cerrado region has also been shown to be adapted to the constant fire events to which this ecosystem is subjected (Soderstrom 1981), thus having a crucial ecosystem engineer role (Marimon et al. 2010). Bamboo is an important crop in the economy of Brazil, particularly in the Cerrado region (Londoño 1998), where several exotic species are commercialized.

Many studies on bamboo have focused on the evaluation of its production potential (França 2011; Shirasuna 2012), its different uses, and its yield productivity (Chen et al. 2017; van Dam et al. 2018), but there are few works dealing in general with the rhizospheric soil and soil biota of bamboo, and in particular, there are fewer studies on the community composition and ecological interactions of the associated arbuscular mycorrhizal fungi (AMF) found in its rhizospheric soil (Jiang et al. 2013; Muthukumar and Udaiyan 2006).

Soil microorganisms, especially AMF, are fundamental in the functioning and output of several ecosystems processes, since they play essential roles in plant development and growth, in the maintenance of plant biodiversity, in the decomposition of organic matter and in nutrient cycling (carbon, nitrogen, phosphorous, among others), in phosphate solubilization, in absorption of water and nutrients, and in soil aggregation, among many other ecosystem processes and services (de Andrade Júnior et al. 2018; Johnson and Pfleger 1992; Marulanda et al. 2003; Silva-Flores et al. 2019; Souza et al. 2016; Van Der Heijden et al. 2015). Despite the reduced number of AMF species (approximately 300 species), most land plant species (82%) form the AMF symbiosis (Brundrett and Tedersoo 2018), which occurs in most plant families, and is widespread across most of the Earth biomes. This symbiosis is believed to have been fundamental on the colonization of land by plants (Brundrett and Tedersoo 2018), and currently is found in most of the plant species of commercial interest.

AMF are particularly important in stressing soil conditions, such as acidic and dystrophic soils, as most of the soils in tropical regions, particularly in the Brazilian Cerrado vegetation (de Moura et al. 2018; Jeffries et al. 2003; Johnson and Pfleger 1992; Ventura et al. 2018). Under this stressing soil conditions, AMF increases the plant tolerance to acidic and very dry soils with high amounts of heavy metals and lower amounts of crucial nutrients as phosphorus (Aguilera et al. 2017; Santander et al. 2019). Despite all these roles, it is surprising that AMF have been barely studied in commercially important species as bamboo (Jiang et al. 2013; Muthukumar and Udaiyan 2006), and also very little in the Cerrado region (Marín and Bueno 2019). In order to understand the ecological interactions, geographical distribution, biogeochemical cycles, and the potential of restoration and micro-propagation of bamboo, it is fundamental to elucidate the composition of the associated AMF community, as these symbiont fungi are crucial in all these processes.

Understanding the symbiotic dynamics between bamboo and its associated AMF community is fundamental for the development of management practices aimed to increase plant productivity and reduce production costs. Therefore, the objective of this work was to identify the established AMF community and their ecological interactions with the bamboo species *Actinocladum verticillatum* (Green Bamboo) and *Bambusa vulgaris vittata* (Yellow Bamboo), in the Cerrado Brazilian vegetation.

2 Materials and Methods

On July 2018, roots and rhizospheric soil samples of two bamboo species commonly found in the Cerrado vegetation in Brazil were collected: *Actinocladum verticillatum*, a native species, and *Bambusa vulgaris vittata*, an exotic species originated from India, hereinafter referred to as Green Bamboo and Yellow Bamboo, respectively. For sampling, five plots were selected in the Gurupi microregion, Tocantins state: three Green Bamboo and two Yellow Bamboo plots; and seven plots in the Porangatu microregion, Goiás state: two Green Bamboo and five Yellow Bamboo plots (Table 1). These sampling sites are among the few sites on which both species occur naturally at the same time in the Cerrado vegetation; these two species are the most common bamboo species in the region. At each sampling plot (100 m^2), six biological replicates were randomly sampled, each replicate consisting of a composed sample of five randomly sampled subsamples that were thoroughly mixed. Overall, 72 root samples and 72 rhizospheric soil samples were analyzed. Analyses of root and soil samples were carried out in the Agricultural Microbiology Laboratory of the Faculdade Evangelica de Goianésia, Brazil.

Spores of arbuscular mycorrhizal fungi (AMF) were extracted from 500 cm^3 of rhizospheric soil by the wet sieving and sucrose density-gradient centrifugation technique (Błaszkowski 2012; Gerdemann and Nicolson 1963). Briefly, 25 g of soil was passed through sieves of 500, 125, and 32 μm, and thoroughly washed with distilled water. The last soil portions on the 500 and 125 μm sieves were collected on the 32 μm sieve, and distributed onto plastic tubes. Twenty-five milliliter of the spore suspensions, that were obtained from the three sieves, was transferred to 50 mL centrifugation tubes. Twenty-five milliliter of a 70% sugar solution was added to the bottom of the tubes, that were centrifuged at 2000 rpm for 2 min. After centrifugation, the samples were decanted, washed, and transferred to Petri dishes. The spores were separated under stereoscopic binocular loupes (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

Table 1 Location of the 12 Bamboo plots evaluated on this study. Green Bamboo: *Actinocladum verticillatum*; Yellow Bamboo: *Bambusa vulgaris vittata*

Plot	Bamboo species	Location	Altitude (m.a.s.l.)
Gurupi microregion, Tocantis state			
A	Green Bamboo	13° 01′ 41.9124″ S 48° 28′ 11.8956″ W	500
B	Yellow Bamboo	12° 53′ 03.4080″ S 48° 33′ 41.1120″ W	439
C	Yellow Bamboo	12° 57′ 28.0404″ S 48° 34′ 26.1408″ W	497
D	Green Bamboo	13° 04′ 10.9920″ S 48° 34′ 59.2320″ W	382
E	Green Bamboo	12° 11′ 01.8204″ S 48° 27′ 20.9232″ W	261
Porangatu microregion, Goiás state			
F	Green Bamboo	13° 16′ 03.6120″ S 48° 40′ 56.8920″ W	409
G	Yellow Bamboo	13° 26′ 28.3920″ S 48° 42′ 41.5080″ W	376
H	Yellow Bamboo	13° 09′ 18.4464″ S 48° 38′ 23.1324″ W	466
I	Yellow Bamboo	14° 06′ 26.5680″ S 49° 06′ 12.4920″ W	559
J	Yellow Bamboo	13° 08′ 52.4508″ S 49° 11′ 41.6112″ W	328
K	Green Bamboo	13° 27′ 44.6292″ S 48° 43′ 17.5548″ W	380
L	Yellow Bamboo	14° 36′ 01.3320″ S 49° 09′ 58.6440″ W	508

morphotypes (Oehl et al. 2011) and mounted on slides with pure polyvinyl lactoglycerol (PVLG) and PVLG mixed with Melzer solution (1:1 *v*/*v*). On each soil sample, just the presence/absence of AMF fungi genera was determined, but not their relative abundances. To support the taxonomic identification, original articles of the species description and species descriptions were provided by the "International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi" website (https://invam.wvu.edu/) were used (INVAM 2018).

In order to determine the percentage of roots' mycorrhizal colonization, the roots were clarified and stained with 0.05% trypan blue in lactoglycerol (Gemma et al. 1989; Phillips and Hayman 1970). Young secondary roots were cut into 1 cm pieces and thoroughly washed with water. To remove the cytoplasm and nuclei from the host roots, the roots were transferred to tubes with KOH (2.5% *w*/*v*) for 72 h. Afterwards, the KOH was removed and the roots were washed with water and the roots were covered with HCl (1% *w*/*w*) for 24 h. Excess HCl was also eliminated by thoroughly washing the roots with water. Then, trypan blue (0.05% *w*/*v*) was added to the roots for 24 h. Roots were finally washed with abundant water. Mycorrhizal colonization of roots was quantified under a stereoscopic microscope, following the technique of quadrants interaction in which 1 cm roots were randomly located on Petri dishes with grid lines and the presence or absence of AMF colonization was registered in at least 100 observations (Giovannetti and Mosse 1980).

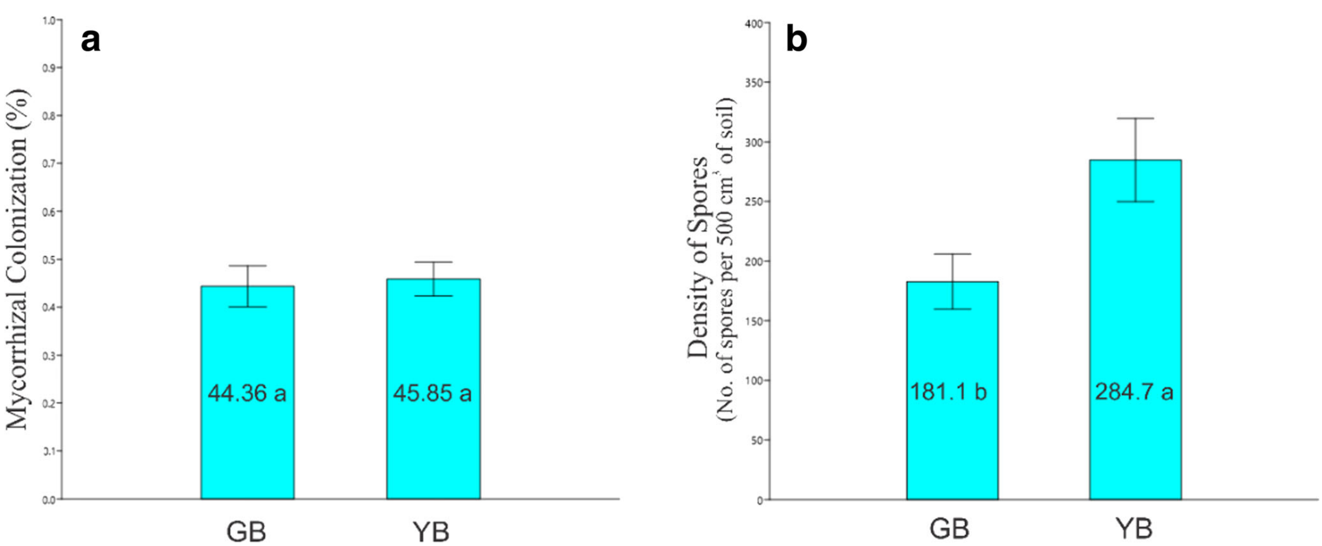

Fig. 1 **a** Roots' mycorrhizal colonization rates. **b**. Density of spores in rhizospheric soil (No. of spores per 500 cm^3 of soil). GB Green Bamboo (*Actinocladum verticillatum*); YB Yellow Bamboo (*Bambusa vulgaris vittata*)

Heatmaps of spore density and roots' mycorrhizal colonization for each bamboo species were generated in the software Quantum Gis 2.18 (Marcuzzo et al. 2011; QGIS Development Team 2013; Santos and Brito 2018). Statistical analyses (i.e., one-way ANOVA and canonical correspondence analyses) were performed on the software Past (Hammer 2018). Data was normally distributed. Canonical correspondence analysis was performed based on the presence/absence data of AMF genera by samples/sites.

3 Results

Mean values of root colonization by arbuscular mycorrhizal fungi (AMF) were 44.36% and 45.85% for the Green Bamboo and Yellow Bamboo, respectively, with no significant difference between them (Fig. 1a). Spore density of AMF was significantly higher in the rhizospheric soil of Yellow Bamboo (average of 285 spores on 500 cm^3 of soil) than in the Green Bamboo (average of 182 spores on 500 cm^3 of soil) (Fig. 1b).

There was a contrasting geographic pattern of root colonization by AMF for both species: Green Bamboo root colonization rates were higher in most of the Porangatu microregion (above 50%), and lower in most of the Gurupi microregion (between 45 and 50%) (Fig. 2). In contrast, Yellow Bamboo showed higher colonization rates in most of the Gurupi microregion (above 40%), while lower colonization rates were registered in most of the Porangatu microregion (between 35 and 40%) (Fig. 2). The density of AMF spores in Yellow Bamboo sites was similar (between 150 and 200 spores on 500 cm^3 of soil) in both microregions (Gurupi and Porangatu) (Fig. 3). Much higher spore density was registered for Green Bamboo, with most of the Gurupi microregion having above 350 spores on 500 cm^3 of soil, and most of the Porangatu microregion having between 250 and 350 spores on 500 cm^3 of soil (Fig. 3).

A low specificity regarding AMF genera on rhizospheric soil was observed between both bamboo species (Table 2). Seven genera of AMF were identified in the rhizospheric soil of *Actinocladum verticillatum* and *Bambusa vulgaris vittata*:

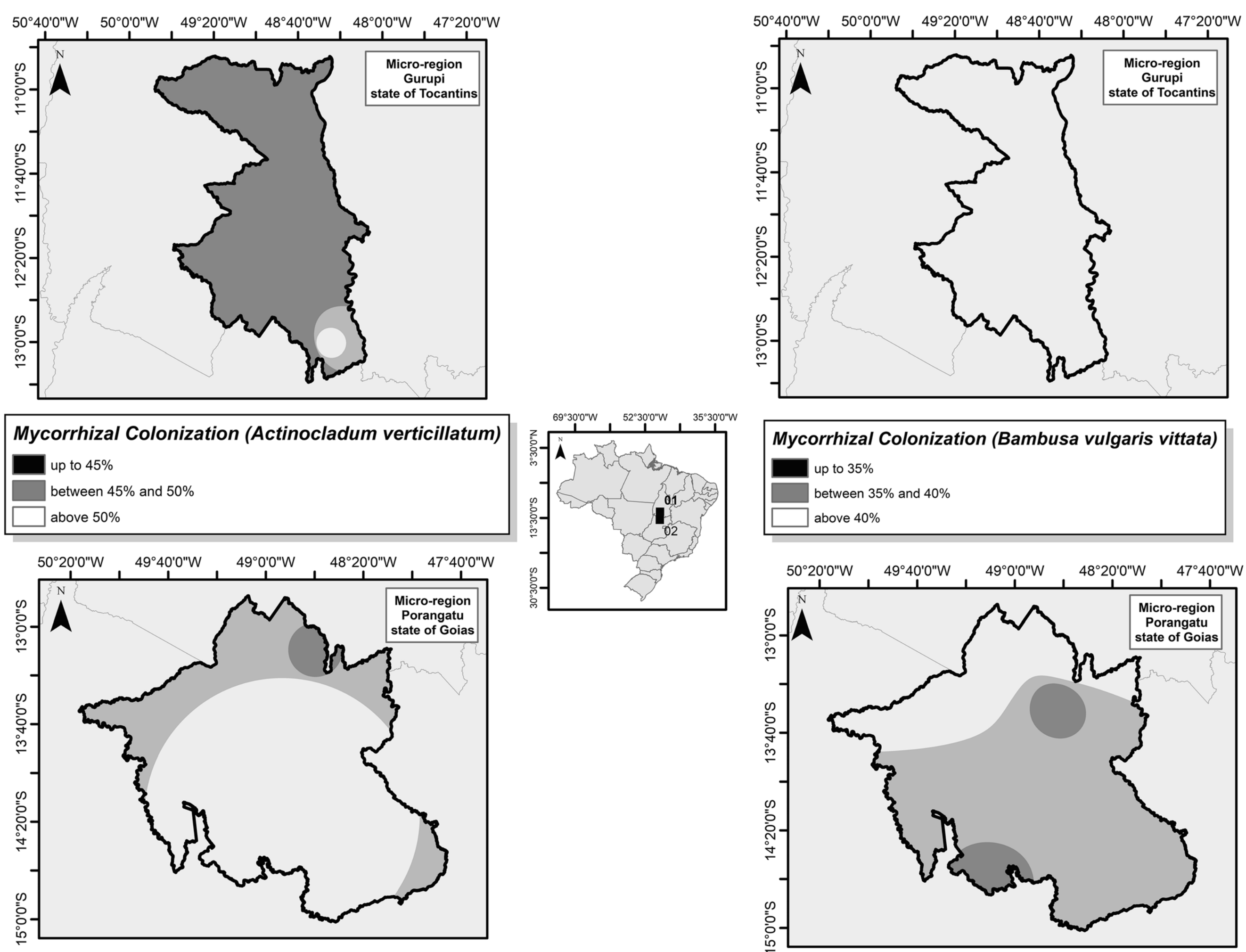

Fig. 2 Heatmaps of roots' mycorrhizal colonization rates in the Gurupi and Porangatu microregions, for both Bamboo species (*Actinocladum verticillatum* and *Bambusa vulgaris vittata*)

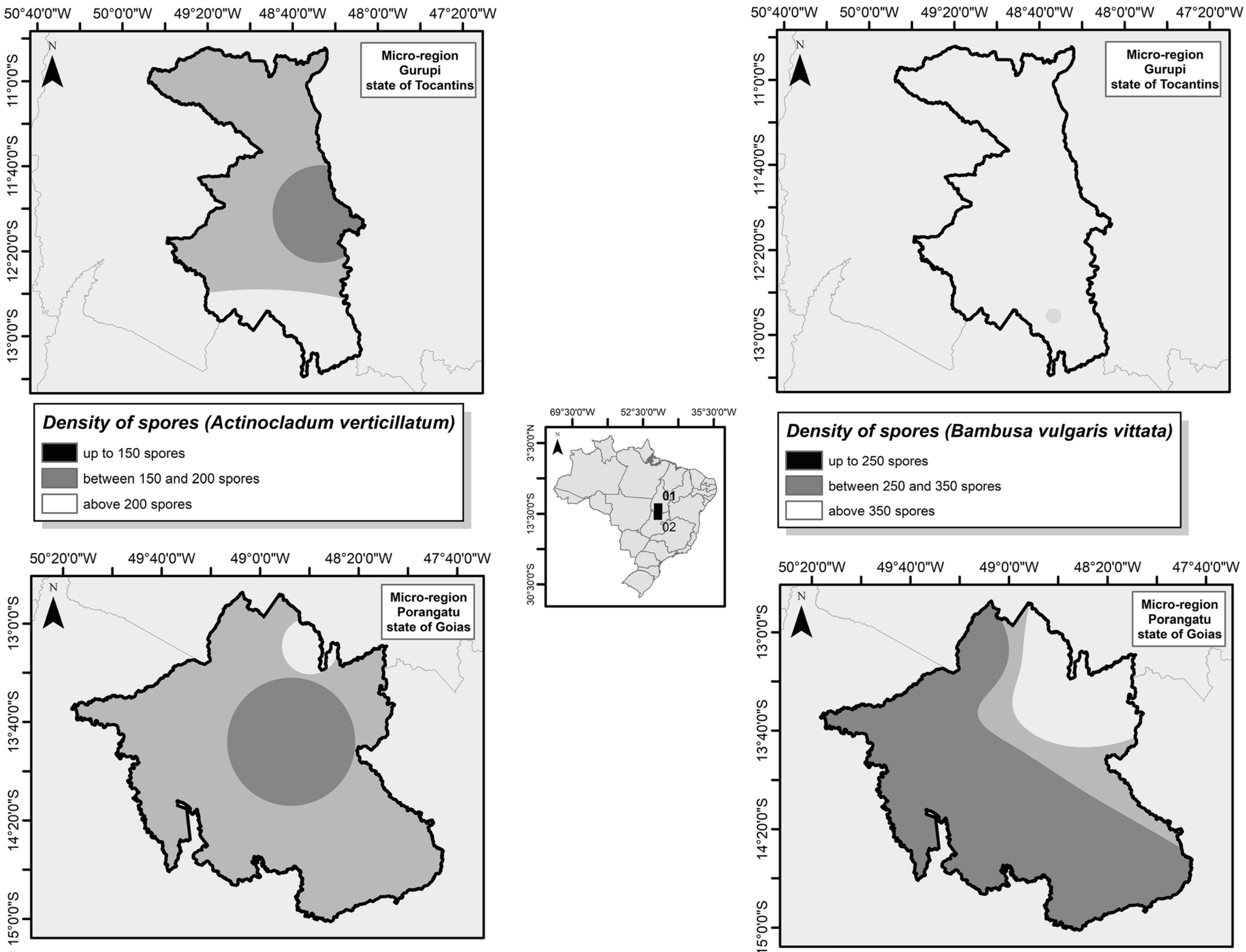

Fig. 3 Heatmaps of spore density in the Gurupi and Porangatu microregions, for both Bamboo species (*Actinocladum verticillatum* and *Bambusa vulgaris vittata*)

Acaulospora, *Claroideglomus*, *Diversispora*, *Scutellospora*, *Sclerocystis*, *Glomus*, and *Gigaspora* (Table 2). The genera *Acaulospora* and *Glomus* were identified in all samples studied, while the genus *Sclerocystis* was identified only in one sample of Green Bamboo; the other genera identified were found to be associated with both bamboo species in both regions (Table 2).

According to the canonical correspondence analysis of the genera associated with the rhizospheric soil, it was observed for both bamboo species that the genera *Acaulospora*, *Diversispora*, and *Glomus* were usually found together, while *Gigaspora*, *Sclerocystis*, *Clareidoglomus*, and *Scutellospora* were very rarely present together in the same sample (Fig. 4).

4 Discussion

To our knowledge, this is the first time that the colonization, genera diversity, and spore density of arbuscular mycorrhizal fungi (AMF) have been reported in *A. verticillatum* and *B. vulgaris vittata* in the literature for comparison purposes. There are also no literature reports of AMF species interaction between *A. verticillatum* and *B. vulgaris vittata*. Das and Kayang (2010) found roots' mycorrhizal colonization rates of 21%, 19%, 21%, and 31% in the bamboo species *Bambusa tulda*, *Dendrocalamus hookerii*, *Dendrocalamus hamiltonii*, and *Phyllostachys manii*, respectively, with no statistical difference among these species, a similar result with this study, where no significant differences were found in the radical mycorrhizal colonization of both bamboo species (Fig. 1a).

Root colonization rates of AMF have been shown to have no effect on AMF spore abundance or species richness in different studies (Aguilera et al. 2017; Mafaziya and Madawala 2015; Marín et al. 2017). Similarly, in our study, there was no relationship between AMF colonization rates and spore density (Fig. 1). Furthermore, contrasting geographic patterns of AMF root colonization (Fig. 2) and spore density

Table 2 Arbuscular mycorrhizal fungi genera present in the rhizospheric soil of *Actinocladum verticillatum* (GB, Green Bamboo) and *Bambusa vulgaris vittata* (YB, Yellow Bamboo)

Plot	*Acaulospora*	*Claroideglomus*	*Diversispora*	*Gigaspora*	*Glomus*	*Sclerocystis*	*Scutellospora*
Gurupi microregion, Tocantis state							
A (GB)	+	+	+	+	+	–	+
B (YB)	+	+	+	+	+	–	–
C (YB)	+	+	+	+	+	–	+
D (GB)	+	+	+	+	+	+	–
E (GB)	+	–	+	+	+	–	+
Porangatu microregion, Goiás state							
F (GB)	+	+	–	+	+	–	+
G (YB)	+	–	+	+	+	–	+
H (YB)	+	–	+	+	+	–	–
I (YB)	+	–	–	+	+	–	–
J (YB)	+	+	+	–	+	–	–
K (GB)	+	+	–	–	+	–	+
L (YB)	+	+	+	–	+	–	+

Geographic location of plots according to Table 1

were observed (Fig. 3), reinforcing the idea that AMF root colonization is not affecting AMF abundance. It is noticeable that Yellow Bamboo, an exotic species originated in India, had higher spore abundance than the native Green Bamboo (Fig. 1). Although this is not a thoroughly studied subject, some studies (Hawkes et al. 2006; Klironomos 2003) have shown that AMF may promote plant invasions, or that the AMF community associated with exotic plants deeply alters the native AMF community. This may explain the higher AMF spore abundance in our study (Fig. 1), but further studies are needed to elucidate the effects of exotic plants and AMF community on local AMF symbionts.

In our study, *Acaulospora* and *Glomus* were the only AMF genera found in all plots (Table 2). Similar results were described by Muthukumar and Udaiyan (2000), identifying the genera *Acaulospora* and *Glomus* in all of their samples when evaluating grass species, including bamboo, in Indian soils. When studying the microbial community associated with the rhizospheric soil of the dwarf bamboo *Sasa kurilensis* in Japan, Kong (2017) also found that *Acaulospora* and *Glomus* were present and abundant in all samples. *Acaulospora* and *Glomus* have been reported associated with roots of *Bambusa tulda*, *Dendrocalamus hookerii*, *Dendrocalamus hamiltonii*, and *Phyllostachys manii* (Das and Kayang 2010). Jiang et al. (2013) confirmed a symbiotic association when inoculating *Glomus* in *Bambusa pervariabilis*, showing an increase in the absorption of phosphorus and potassium in the inoculated plants. Overall, *Acaulospora* and *Glomus* are AMF genera commonly found worldwide in a broad range of ecosystems (Davison et al. 2015), and also are common in agricultural ecosystems (Oehl et al. 2017). In bamboo rhizospheric soils, Wu and Chen (1986) also found the genera *Acaulospora*, *Gigaspora*, *Glomus*, *Archeospora*, and *Entrophospora*, with the last two not being identified in the rhizospheric soil samples investigated in this study.

Diversispora is an increasingly diverse AMF genera (Błaszkowski et al. 2015) that is also distributed worldwide (Davison et al. 2015; Oehl et al. 2017), and in our study—and in contrast with *Gigaspora*—it was frequently found associated with *Acaulospora* and *Glomus* (Fig. 4). In contrast, the AMF genus *Sclerocystis* has a more restricted distribution (Almeida and Schenck 1990; Wu and Chen 1986), and in our study, it was only found in one rhizospheric soil sample of *A. verticillatum* (Table 2). Thus, it seems that our local bamboo study system does reflect the biogeographical patterns of the worldwide distribution of AMF genera (Davison et al. 2015; Oehl et al. 2017).

Among the listed benefits of the arbuscular mycorrhizal association for bamboo, its use in promoting plant growth and establishing seedlings from micro-propagation can be highlighted, although this has been barely studied (Singh 2002). There are no reports in the literature on the establishment of ectomycorrhizal fungi in bamboo in the Cerrado region; however, ectomycorrhizal fungal species were identified associated with *Dendrocalamus strictus* in soils of China (Sharma and Rajak 2010). Muthukumar and Udaiyan (2006) found a positive correlation of the interaction between AMF and other growth-promoting organisms such as nitrogen-fixing bacteria of the genus *Azospirillum*, with the increasing in productivity of bamboo in tropical soils. *Azospirillum* is a bacterial genus commonly found associated with the rhizosphere of bamboo species in Asia, where there are several studies on the interaction of AMF and bamboo species due

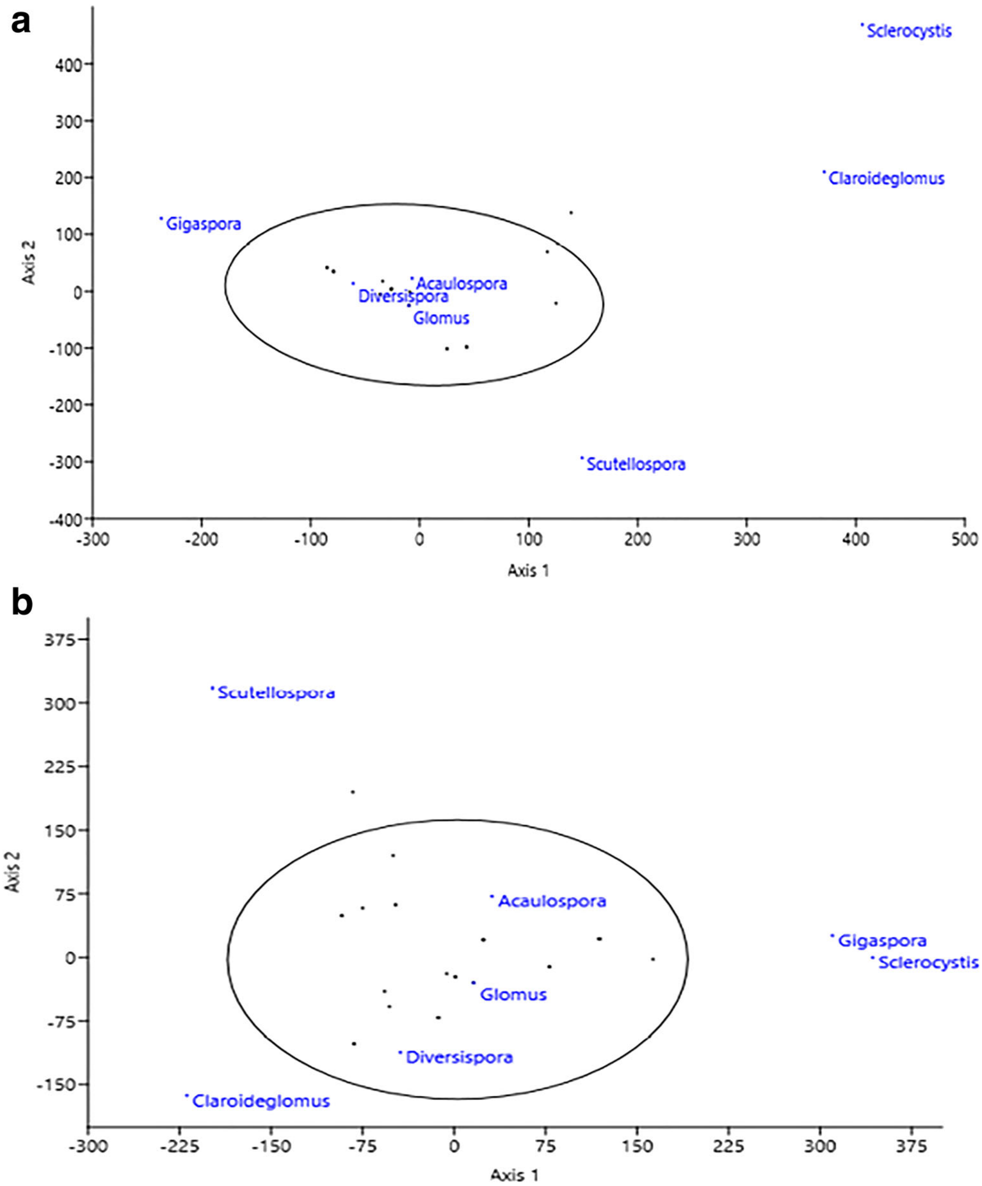

Fig. 4 Canonical correspondence analyses of associated genera found in the rhizospheric soil of *Actinocladum verticillatum* (**a**), and in the rhizospheric soil of *B. vulgaris vittata* Axis 1: first axis of the canonical correspondence analysis. Axis 2: second axis of the canonical correspondence analysis

to its economic importance in the region (Gai et al. 2006). Further studies need to incorporate these rhizospheric interactions when accounting for the AMF community composition and its effects on bamboo plant growth and other ecosystem processes. Also, when prospecting micro-propagation efforts of bamboo, the local AMF community should be taken into account, as local and specific mycorrhizal fungi may have better results on plant growth and survival than non-local and non-specific mycorrhizal fungi (Marín et al. 2018); this specificity may be reflected on this study, as the AMF genus *Sclerocystis* was found in only one rhizospheric soil sample of *A. verticillatum* (Table 2).

The northern and central areas of Brazil have been extremely understudied in terms of mycorrhizal ecology and biodiversity (Bueno et al. 2017), albeit noticeable exceptions (de Assis et al. 2018; Marinho et al. 2019), most research has been concentrated in natural and agricultural ecosystems of the southern part of the country (Bueno et al. 2017; Davison et al. 2015). More studies on mycorrhizal diversity are needed to fill these knowledge gaps in the northern part of Brazil, in particular on species with a very high commercial interest as bamboo, where very little mycorrhizal research has been conducted.

5 Conclusions

This study constitutes one of the few works exploring the arbuscular mycorrhizal symbiosis in an important commercial plant, as bamboo, and the first to do so in the Cerrado vegetation in Brazil. Seven arbuscular mycorrhizal fungi (AMF) genera were described on this study, a high diversity as the explored area was relatively small. Largely distributed AMF genera, as *Acaulospora*, *Diversispora*, and *Glomus*, were frequently found together in the rhizospheric soil of both bamboo species. We found that an exotic bamboo species, *Bambusa vulgaris vittata*, had higher AMF spore density than the native *Actinocladum verticillatum*, although there were no differences on their AMF root colonization rates.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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