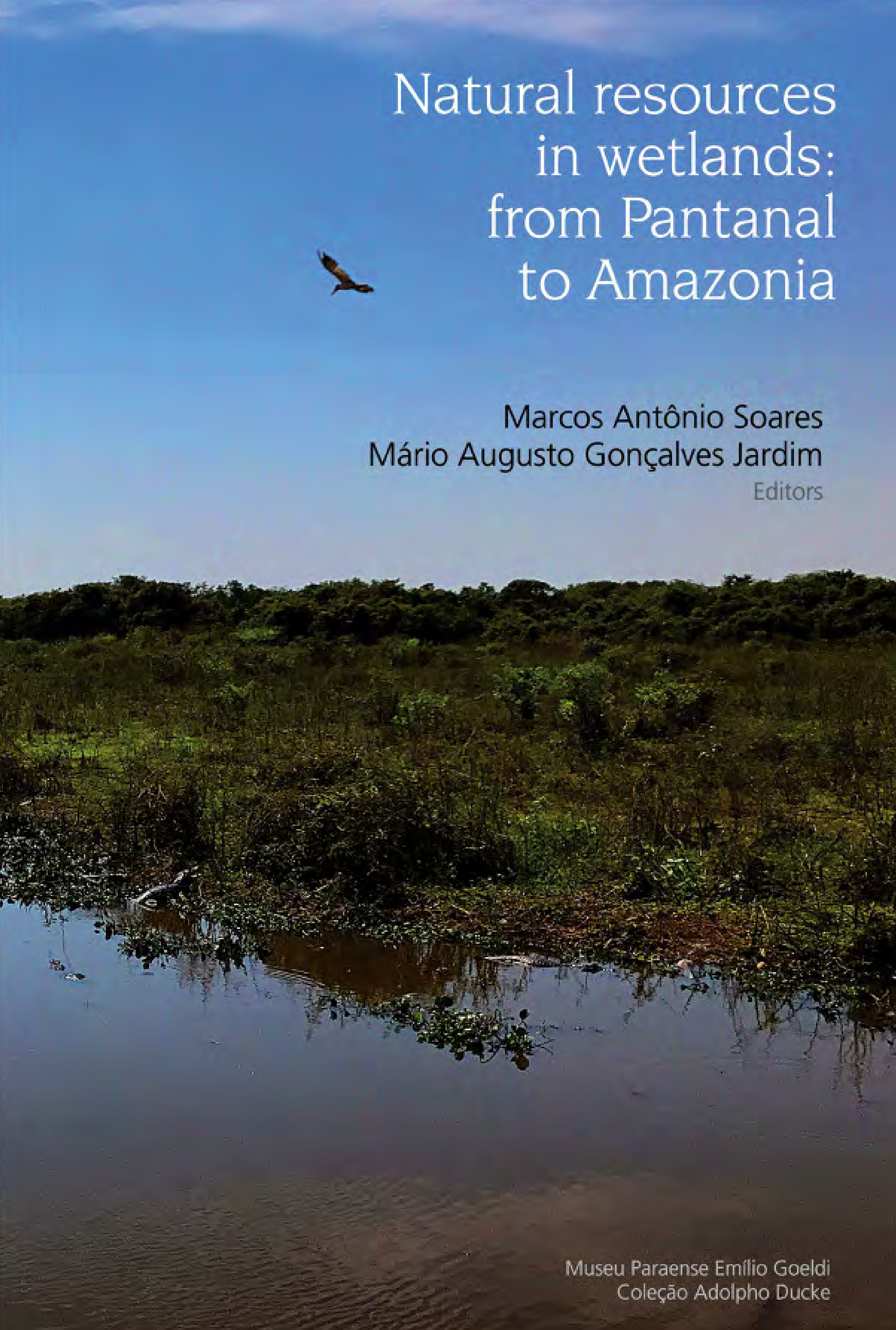

Natural resources in wetlands: from Pantanal to Amazonia

Marcos Antônio Soares
Mário Augusto Gonçalves Jardim
Editors

Museu Paraense Emílio Goeldi
Coleção Adolpho Ducke

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Presentation

The National Pantanal Research Institute (INPP) was created by Law Nº. 12,954, of February 5, 2014 and aims to integrate and articulate actions in the Pantanal region, promote new initiatives and provide the development of models and databases To integrate the transfer of knowledge generated in the region. It is based in the Campus of the University of Mato Grosso, in Cuiabá, because it is not yet fully regulated, it functions as an Advanced Campus of the Museu Paraense Emilio Goeldi, Research Unit directly to the Ministry of Science, Technology, Innovation and Communications.

The Amazon and Pantanal have similar biotic and abiotic characteristics in several aspects such as biodiversity, land use pressure, water scarcity and urban explosion. The gathering of articles from researchers from both regions working on a theme as important as the conservation and sustainable use of resources in wetlands will provide new academic and scientific knowledge on the Amazon and the Pantanal.

The Natural Resources Book in Wetlands: From Pantanal to Amazonia edited by the Museu Paraense Emilio Goeldi / National Pantanal Research Institute aims to promote the publication, dissemination and socialization in the national and international scope of the intellectual production on Biodiversity, Biotechnology and Innovation that deals exclusively with the Pantanal Biome and Amazon Biome through information on the biological diversity of populations and communities of animals, plants and microorganisms and their ecological patterns and processes; Development of technologies, patents and innovations, as well as biotechnological products and processes based on biodiversity. It contemplated researches carried out by teachers and Students of the Doctoral Program in Biodiversity and Biotechnology in Network/Regional North/Pará; Doctors and Students of the Doctoral Program in Network/Regional Center West (MT, MS, GO and DF) and Researchers of the National Institute of Science and Technology in Wetlands (INAU).

Maria de Lourdes Pinheiro Ruivo
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Stakeholders in community
management of turtle in Brazilian Amazon

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Introduction

The Amazon is a biome of rich biodiversity, represented by high species indexes, biomass values, ecological, economic and cultural importance. Studies have revealed that at least 50,000 tree species occur in the region (Stege et al. 2013), mammals (425 species), birds (1,300 species), reptiles (371 species), amphibians (427 species) (Hubert and Renno 2006) and freshwater fish (about 3,500 species) (Junk et al. 2007; Roma et al. 2010). The turtle have been around since the Jurassic (250 million years) to this day. In Brazil, 36 species are recorded, including 29 freshwater, five marine species and two terrestrial species (Van Dijk et al. 2014). These species are distributed in 5 families: four belonging to the order Cryptodira (Geomydidae, Kinosternidae, and Testudinidae) And two belonging to the order Pleurodira (Podocnemididae and Chelidae) (Van Dijk et al. 2014). Historically the Amazonian riverside communities depended on the turtles in their life strategies. The consumption of adult animals and their eggs are still a significant food resource for riverine populations, and with some economic importance in the market of small inland towns (Fachín-Terán, 2005), at different times of the year. They are also used as pets and traditional medicines; eggs, juveniles, adults, body parts, all are exploited indiscriminately, without regard to sustainability (Pritchard and Trebbau 1984).

Community management studies of turtles have shown that, due to a multiplicity of problems, both turtle populations (*Podonemis expansa*) and tracajás (*Podonemis unifilis*) populations have been drastically reduced. Due to the dependence of these resources, some riverside communities of the Middle Amazon, with the support of environmental community associations, took the initiative to protect the nesting areas through participatory local management projects (Pinto and Pereira 2004; Andrade 2015; 2008).

According to Cantarrelli et al. (2014), the development of such programs requires the participation of educational and research institutions and nongovernmental organizations that can represent various related sectors of society, rather than through centralized government agencies. The author also suggests that such joint action requires a multidisciplinary approach, along with the active participation of local communities.

The Social participation of stakeholders in the conservation of turtles is considered essential to ensure the sustainability of this resource. Understand their interests and incorporate the perceptions into organizational management is essential for the integration of management systems for sustainability (Lauriano 2012). Stakeholder analysis recognizes the different interest groups involved in the use and conservation of natural resources and provides tools to help identify and resolve compensation (Grimble 1998).

For this author stakeholder analysis is useful for researchers in two main ways: improve the selection and design of research projects: the explicit consideration of potential trade-offs between different stakeholders helps prevent unexpected, makes good design, and improves the probability of successful implementation; better address the distributional impacts, social and political of research projects: explicit analysis of the interests and impacts of interventions in different stakeholders help to ensure that the results of the investigation of effectively designed to meet the needs of those it is intended.

The stakeholder analysis is a tool used to identify and describe the key actors and their respective roles, inter-relationship and interest in relation to a problem. This methodology aims to understand the system from the identification of key actors (stakeholders) (Grimble and Chan 1995; Pomeroy and Douvere, 2008), and recognize the different interest groups involved in the use of conservation of natural resources. According to Grimble and Chan (1995), interest groups can be individuals, communities, social groups and/or institutions of any size, class or social level. The term therefore, includes those who depend on the system to survive, those who make policy - planners and managers from government and other institutions, as well as other social groups also benefit from the system.

Community turtle management studies have shown that, due to a multitude of problems, both populations of bigger turtles (*Podonemis expansa*) such as smaller turtles (*Podonemis unifilis*) were drastically reduced. Due to the dependency on these resources, some river dweller communities of the Middle Amazon, supported by environmentalists community associations, took the initiative to protect nesting areas through participatory local management projects (Pinto and Pereira 2004; Andrade 2015; 2008).

According to Cantarrelli et al. (2014), the development of these programs requires the participation of educational and research institutions and non-governmental organizations that can represent several related sectors of society, rather than rely on the means of centralized government agencies. The author also suggests that such joint action requires a multidisciplinary approach, with the active participation of local communities.

The participation expressed primarily as the idea of general equality of all members of the adult community – has become the fundamental principle for the creation of property and thus to achieve sustanaibility and development success, concluding that in this way participation has been changing local social structures (Schnegg and Linke 2016).

Point out that by providing alternative avenues for participation, such acts may safe social and physical space for community engagement at the local level and, in this way, may engage a range of people – watershed group members and non-members alike – in actively improving the state of their community and their environment (Lukacs et al. 2016).

Since 1999, in the Eastern Amazon region, River dweller communities of 15 municipalities in the states of Amazon and Pará have been developing a Community turtle management project. This is the Pé-de-Pincha Project, institutional program of extension of the Federal University of the Amazon (UFAM), whose mission is to preserve, through participatory management, natural resources of communities and act on environmental awareness processes and the search strategies for local development.

Participatory management of the project involves different social groups with different interests, resulting in alliances and conflicts in their development. Thus, the identification of stakeholders in the community management of turtles is of paramount importance to ensure the permanence of long-term activity.

In this study we identify the key players and their characteristics, the type of the interests, involvement of stakeholders and their roles played in the management, their degree of importance and influence and identification of conflicts and alliances. This article makes a significant contribution to understanding the way stakeholders perceive and perform community management of turtles, in order to strengthen the participation of Amazonian riverine communities.

Materials and Methods

The survey was conducted in the physiographic area of the Middle Amazonas, in the municipalities of Parintins and Barreirinha / AM, Oriximiná and Terra Santa / PA (Figure 1).

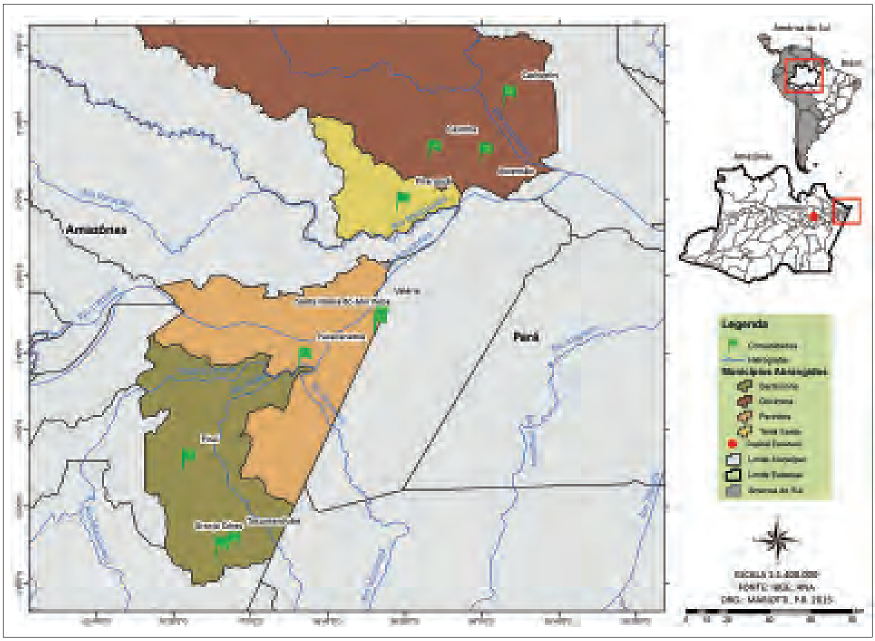

Figure 1. Area of study in the municipalities of Barreirinha and Parintins in the state of Amazonas and Holy Land and Oriximina in the state of Pará.

The research subjects were the stakeholders in community conservation of Turtles included in Pé-de-Pincha Project from the Federal University of the Amazon consisting of funding agencies and sponsors, government, NGOs, media, community surrounding and study area. This research was developed through participatory observation with field expeditions from 1999 to 2015.

Participatory observation is understood as the method of collecting detailed information in which the researcher becomes a participant in the event or social group studied (Geertz, 1989). This technique provides conditions for understanding the habits, attitudes, interests, personal relationships and characteristics of daily community life rather than the non-participant (Richardson 1999).Beside methods was, used documentary analysis involving the technical-scientific reports of the pé-de-pincha project. The participation and interation among stakeholders were analysis according to Grimble and Chan (1995). It each field expedition took two weeks the follows activities: A - participatory planning: meetings to define people, areas, educational strategies and preparation of material; B - training and training: teachers, volunteers and community stakeholders; C - execution of chelonian management: construction of nurseries and nurseries, collection and transfer of nests, monitoring the hatching or birth of pups; care of puppies; D - monitoring: training of young scientists to capture and recapture animals in the wild; E- evaluation: evaluation meetings and reporting.

To obtain data, we used a procedure according to the methodology proposed by stakeholders analysis Grimble and Chan (1995). For identification of stakeholders and interests was organized a list according to (Souza and Irigaray2008; Silveira et al. 2012; Silva et al. 2015). The variables used in the analysis were detached as follows:

For the identification of the groups was grouped in to represent 10 categories table: category I) agencies: This category was sought by agencies that are committed to environmental compensation agreement and companies sponsoring environmental projects; category II) government (federal, state and local): are government agencies that fund or facilitate the execution of the project; category III) media: they are organizations that make the disclosure; Category IV) NGOs: are organizations promoting local, regional and international organizations that are interested in developing community management; Category V) University: made up of professors, academics and technicians; VI) local rural entrepreneurs: are entrepreneurs who own ranches or farms in the communities; Class VII) schools: composed of teachers, students and managers of local schools; Category VIII) Community associations: are organized groups in communities along the river to facilitate the implementation of Community programs; Class IX) river dweller communities: are residents of the communities involved directly in the project to make themselves available to help in the management; Category X) Catholic and Evangelical churches are religious leaders to community service.

To identify the characteristics, they were classified at different levels: national, state, local, offsite and indoors. The assignments were associated according to the stages of implementation of the Community as management oversight, education, dissemination and implementation or operation. The degree of importance and influence in the community management of turtles, was classified according to Le Tissier (2011). The identification of conflicts and alliances were obtained by participatory observatory during meeting and assemblies.

Results

Identification and characterization of stakeholders

Identification and characterization of stakeholders in community conservation of turtles in communities along the river - Pé-de-Pincha Project/UFAM (Table 1) shows the results of the identification of stakeholders in community conservation of turtles in the Middle Amazon region, in the municipalities of Parintins and Barreirinha / AM, Oriximiná and Terra Santa / PA. We identified 68 parts grouped by similarity of features: funding agencies, funding agencies, government (federal, state and local), media, NGOs, universities, local rural entrepreneurs, community associations, schools, community and churches.

Types of stakeholders involved in the community conservation of turtles

They were listed five levels of approaches in the typology of stakeholders in the community conservation of turtles. The common interests between parts of the national and state levels were the supervision, control and policy proposition. Among the parts of local, it was to ensure chelonian feature, protect lakes and spawning areas and still ensure access to knowledge to conserve the resource (Table 2).

In practice, all stakeholders are positioned at any of these levels according to the interest in the action. Among the funding agencies has been identified, BASA (public bank) and the Foundation for Research in the State of Amazonas (FAPEAM) have interest in financing social and environmental projects in the Amazon region in compliance with responsibility policy, and Mineração Rio do Norte (MRN) is the environmental compensation interest. The sponsoring agencies Dantech, Honda, Multibrás, Petrobras have interest in disclosure of its brands.

At the national level, there is an interest in proposing public policies, promotion, retention, supervision and control and training. At this level are the following Federal Government agencies: ICMBIO/RAN (Chico

Table 1. Identification and characterization of stakeholders in community conservation of turtles in the Lower Amazon - Pé-de-Pincha Project/UFAM.

Categories	Characteristics
I - Agency	National level: Financier/: BASA (public bank), Pro Várzea / DFID-GTZ-KFW (Pilot Program for the Protection of the Basin), Mineração Rio do Norte) (bauxite exploration company), Sponsors: PETROBRAS (oil exploration company).Estadual level: FAPEAM (agency of development) and Moto Honda, Rio Limpo / Multibras (industrial companies), local level: Dantech
II - Government	National level: UFAM, IFAM (educational institution), CNPq, ICMBIO, IBAMA (government agency), MEC / SESu (secretariat of higher education).State Level: SDS, CEUC (secretariat of environment and sustainable development).Local Level: environment municipalities secretary secretariat, education municipalities secretary
III - Media	National level: Cultural TV / Brazil, TV Time / Record, Rede Globo with the programs Globo Rural, How will it be? Jornal Hoje, Jornal NacionalState level: Newspapers: The Critic, Amazon in Time, Jornal do Comércio; Television Network: Amazon Network (Amazon TV and Amazon Sat), TV A Critica, Site: UFAM, Pé-de-Pincha Project; Blogs; FacebookLocal level: Radio: Alvorada and Clube de Parintins, Rádio Paraíso de Terra Santa, Rádio Atalaia de Óbidos and Local Magazine.
IV - Non Profit Organization – NGOS	Local level: GRANAV, Vinte Quilos Project, UNIDAS.
V - Local Rural Entrepreneurs	Local level: Farm holders and local entrepreneurs.
VI - Community Associations	Local level: ATAAV, ASASE-3, MAPEP, ASCOM, ACPLASA, ASCOMINHA, ARQUMO, ACORJUVE, ACORQAT, PQI.
VII - Comunitaries	Local level: Field coordenators, community leadership, Young scientists, proprietor/ rural producer, local population.
VIII - Churches	National Level: Catholic, Protestant and evangelic.

Adapted from Grimble and Chan (1995). Source: Annual Technical Reports Pé-de-Pincha Project/UFAM (1999-2014).

Mendes Institute for Biodiversity/RAN), MEC/SESU (Ministry of Education), Pro Várzea/IBAMA (Lowland of Resources Management Project), UFAM (Federal University of the Amazon), IFAM (Federal Institute of Education, Science and Technology of the Amazon) and CNPq (National Council for Scientific and technological).

Table 2. Types of stakeholders in community conservation of turtles in the Lower Amazon region - Pé-de-PinchaProject, adapted from Grimble and Chan (1995).

Stakeholders Group	Interest
National level: BASA, MRN, Pro Várzea/DFID-GTZ-KFW	Compliance with responsibility and environmental compensation policy.
PETROBRAS, Dantech, Moto Honda, Rio Limpo/Multibras	Disclosure of its brands.
CNPq	Promotion
ICMBIO/RAN IBAMA	Conservation, monitoring, Control and proposition of Public Policy.
MEC/SESU	Promote university extension.
UFAM	Capacit
IFAM	Capacit
State level: SDS/CEUC	Supervision and control Public policies proposition. Development.
FAPEAM	Promotion
Media National: Cultural TV/Brazil, TV Time/ Record, Rede Globo with the programs Globo Rural, How will it be? Jornal Hoje, Jornal Nacional	Disclosure
Media state: Newspapers: TV the Critic, Amazon in Time, Jornal do Comércio; Television Network: Amazon Network (Amazon TV and Amazon Sat), TV the Critic, Website: Pé-de-Pincha Project; Blogs; Facebook	Disclosure
Media Local: Radios: Alvorada and Clube de Parintins, Rádio Paraíso de Terra Santa, Rádio Atalaia de Óbidos and Local Magazine	Disclosure
Local level: NGOs: (ATAAV, GRANAV, ASASE-3, MAPEP, Vinte Quilos project), ASCOM, ACPLASA, ASCOMINHA, ARQUMO, ACORJUVE, ARCOQAT, PQI.	Ensure Turtles feature. Protect lakes and lakes complex. Keeping lakes and spawning areas.Access to knowledge to conserve the resource.
Local level: Farm holders and local entrepreneurs.Local level: Field coordenators, community leadership, Young scientists, proprietor/rural producer, local population.	Reciprocity Ensure Turtles feature. Protect lakes and spawning areas. Rational use of consumption. Access to knowledge to conserve the resource.
National level: catholic church, evangelical church.	Social interests.

Adapted from Grimble and Chan, (1995). Source: This research and Annual Technical Reports Pé-de-Pincha Project / UFAM (1999-2014).

At the state level, there is an interest in monitoring, control and public policy and dissemination. Among the stakeholders in the community management of turtles, is IBAMA-AM / NUFAS (Brazilian Institute of Environment and Renewable Resources), SDS / CEUC (State Secretariat of Environment and Sustainable Development), media (radio, newspapers, television, website, blog, social networks and local dissemination of magazines).

At the local level are positioned stakeholders with direct participation and are the ones that actually make the management of turtles in the communities along the river. At this level, are non-governmental organizations, local municipalities, community association, local rural businesses, schools and churches. In the study area, residents were located, community leaders, young scientists, owners and/or local producers. These are responsible for the use and maintenance of the system, and at this level are all selected with direct action. All are positioned in continuous according to their common interest: ensure Turtles feature, keep lakes and spawning areas and ensure access to knowledge and resources.

Associations of stakeholders with the roles performed in community conservation of turtles

To operationalize the community conservation of turtles was carried out in different stages, among these: participatory planning that aims to evaluate the results of previous years and organize new proposals (Figure 2).

Training for the technical staff (community and academics).In the communities that were not yet participating, training was done in the preparation of areas for egg transplants, transfer techniques and nursery construction. In this period, the final decisions were also made to carry out the project and meetings with teachers of the municipal and state education network to plan project activities in schools, as well as training in environmental education (Andrade, 2008).

These procedures are in line with Freire (1979) thinking, because for the author it is necessary to foster the potential to think the world critically through action-reflexion-action on its practices and social systems. Stage 2 consisted of the training and training of people who promoted or contributed to the mobilization / sensitization of the community members involved in the municipalities. According to Andrade (2008) in the Amazon region, due to the high level of degradation of the populations of turtle and threat to the nests, each community constructed its "brooder" (place where the collected nests were transferred) (Figure 3).

Collection or transfer areas nests threatened to areas protected by the communities: the time that permanent staff of the project and the students / volunteers move to the communities to work over 20 days, track, monitor and perform with local community to transfer nests, lectures and ecological-cultural events. During this period, they were also carried out courses or workshops on ecotourism, handicrafts, food processing, community gardens, fish, poultry and other topics of interest.

Figure 3. Training of volunteers in the management of turtles.

Hatching: Birth of turtle's puppies. The UFAM team returns for the construction of nurseries, training of local staff in the handling of the puppies, the collection of biometric data and the marking of puppies (Figure 4).

Loosening: time the community has the opportunity to show the fruit of their work to the local population and fraternize for their work (Figure 5).

Figure 4.Transfer of eggs to brooder.

Figure 5. Release of turtles in communities.

The association between the steps and stakeholders, were found four levels of operationalization of community management: enforcement, education, monitoring and dissemination. Among these levels, the role in all stages of the management was represented by UFAM (3) and local community (21), these groups have the assignment the development of community management, as they are positioned at the local level and appear in all management phases. Therefore, at the local level, and take direct decisions and establish standards to perform the work (Figure 6).

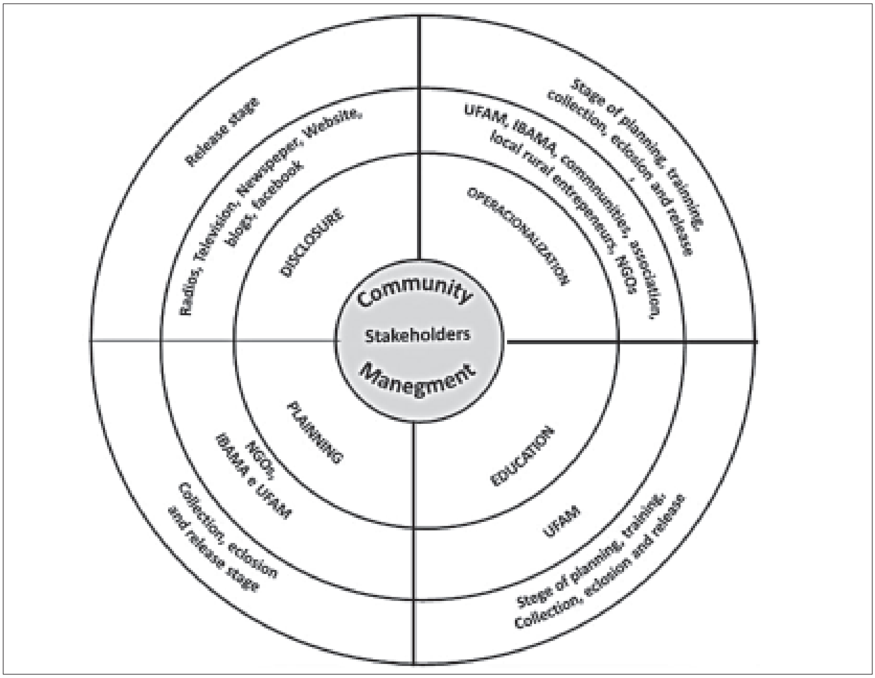

Figure 6. Association of stakeholders and their respective roles played in the community conservation of turtles.

Stakeholders with importance and influence in the community conservation of turtles

To implement the conservation of turtles were identified 36 social agents with direct involvement. When they were asked about the importance and influence, the answers were: a majority (30) said that there are stakeholders more important and influential than others. Among the stakeholders most cited was school, work groups and UFAM. And among the cities of Barreirinha, Parintins and Oriximiná school it was the most cited (Table 4).

Table 4. Stakeholders most important and influential in the community conservation of turtles in the study area, adapted from Le Tissier (2011).

Categories	Stakeholders	Barreirinha		Parintins		Oriximiná		Terra Santa	
		IMP	INF	IMP	INF	IMP	INF	IMP	INF
I	Residents	6	6	6	6	6	6	6	5
II	UFAM	5	4	5	4	4	4	5	4
III	IBAMA	4	5	4	5	5	5	5	6
IV	Volunteer Agents	3	3	3	3	3	3	3	3
V	Church	2	2	1	1	2	2	2	2

Source: Interviews IMP: importance; INF: Influence.

With this result the school and who have more influence to ensure this feature. This actor was important because within the community schools can be object of investment community, aimed at preparation of active subjects futures, opinions trainers, able to reflect their own attitudes and actions, or better, form critical subjects and questioning of the facts and their position in society. However, schools can view the conservation of turtles a form of local development, seeking together public agencies, new organizational formal awakening, to meet the needs of communities.

Identification of conflicts between stakeholders in the community management

To perform community conservation of turtles, there is the need for the involvement of different social groups, causing conflicts and alliances, which varied according to the studied municipalities. Among the conflicts highlighted by the Community in the reports were:

Conflict competition for resource use: this conflict was observed between the community and local community support fromTerra Santa / PA, Barreirinha / PA and Oriximiná / PA. In Terra Santa, in the area of Piraruacá Lake, only the community volunteers environmental agents make the protection of habitats, while most of the local community residents continues removing the eggs. In Barreirinha, community say they are protecting the nests, although consumed elsewhere. Finally, in Oriximiná, only a small local group prevents and hinders the realization of the management.

Conflicts of interest: occur between large local farmers, voluntary environmental agents and project coordinators. This conflict was observed in Parintins due to some land owners not to release the beaches for collecting the nests, causing the interruption of the protection of managed areas and encouraging intrigue among the local community, in addition to making possible the invasion of external users.

Neglect of conflict: is understood as the procedure that it does not care about the case. In this case, local governments, depending on the administration paid no attention to the environmental organizations and the project coordinators when they sought support from financial and / or logistical required to perform community management.

Inspection: conflict that existed between IBAMA and local community, where residents triggered to support IBAMA and the limited financial resources and / or personnel often were not met, which compromised the areas of control action.

Discussion

The sponsoring or funding stakeholders were found to agencies that have committed to finance the project due care to environmental compensation and companies that sponsor projects or environmental programs. The BASA is a financial institution promoting the federal government in the region. And MRN which is a private company linked to a multinational program in the mining industry that exploits resources such as bauxite, and a potentially capable activity causing environmental impacts, and resources for environmental compensation.

The interests of the environmental compensation are being discussed by Fonseca (2015) suggesting that it is necessary to reflect on how this instrument is established, as it may contribute to approval of environmental interventions that are in the opposite direction to their preservationist or conservation objectives, and which contributed to serious consequences in the future. It warns though, you need to be evaluated and fired effectively, because there must be compensation fact, not only in administrative and legal sphere. In parallel to these recommendations, discusses the company’s support to Pé-de-Pincha project that consisted of material consumption, equipment, shirts and fuel as a way to run the user-pays principle laid down in Law N° 6.938 / 1981 determining the user of environmental resources for economic purposes the duty to pay for their use.

Among these, the Pro Várzea that was a program with the objective of establishing a technical basis, scientific and resource conservation policy in the region. Through Pro Várzea/IBAMA were effected 31 demonstration units of community creating turtles, with about 12,300 animals in captivity. The idea of this experience was that, communities that protect their turtle breeding beaches could reserve a percentage of young turtles for breeding. This system would allow a source of income generation that encourage communities to protect the turtles (Andrade, 2008; 2015).

The sponsoring agencies are made by companies established in the region that sponsor projects or environmental programs. At this level we have founded Dantech, Multibrás, Pro Varzea, Petrobras that made the possibility of disclosure of their brands and / or implementation of the project on local interests.

The support for Pé-de-Pincha Project from 1999 to 2001was sponsored enterprises Dant-Tech, Honda and Multibrás. In 2012-2014 and 2014-2016, was sponsored by Petrobras. The sponsorship of these companies allowed to distribute promotional material (such as shirts, hats, brochures, newsletters), purchasing equipment and vehicles (cars, boats, outboard motors), conduct research (marking of animals with microchips, monitoring animal migration using radio satellites), as well as promoting courses and workshops.

As the national level, we standing out institutions that promote and achieve results in the research on conservation of turtle sit as: the federal government (National Research Council for Scientific and Technological Development (CNPq), Ministry of Education through the Department of Education Superior (MEC/SESU) and Federal University of the Amazon (UFAM) and ICMBIO and IBAMA. Its interest are the cientifc development, conservation, monitoring and proposing public policies, promote university extension and training.

IBAMA, is the environmental agency officially responsible for wildlife management in the wild. Since the beginning of the project, IBAMA has been the main partner of UFAM with logistical support and human and material resources through. In addition to the commitment of community management actions of the turtle, and delegates UFAM the ability to coordinate and implement management actions with communities through SISBIO License No. 19232 that the project has and the current Term Technical Cooperation, which provides in its clauses / task items UFAM the Pe de Pincha Project. Among the results from this partnership, we obtained:

1) Signing of the Joint Decree No. 001/2000 of IBAMA, Pará and Amazonas, setting the Piraruacá as the Lake Community Conservation, being vetoed commercial fishing;

2) The implementation of the Environmental Agents courses Volunteers (AAV). Today, IBAMA no longer makes this accreditation, leaving only the Institute of Environmental Protection of Amazonas (IPAAM) conduct these courses in the state;

3) Training of volunteer environmental agents of the communities to organize monitoring and control, and to pass on information to the supervisory sector of environmental agencies. The role play within communities is an educator / sensitizer for environmental issues and organization of environmental task forces.

As pointed Pezzutti (2003), community management of turtles has several advantages:

As the satisfaction of direct users through their participation, enabling reliable data collection; the amelioration or elimination of constant conflicts between coastal and IBAMA; training conducted throughout the process; and the strengthening of social institutions with the development of participatory models, creating better balance of power among public institutions.

While the MEC/SESU convenes Federal Institutions to submit development proposals for programs and projects within the university extension, in accordance with the provisions of Law No. 12,155 of 23 December 2009. Through the edicts of the Department of Education Superior (MEC/SESu) was obtained funding for the extension program (extension activities) in 2010, 2012, 2013 and 2014.

UFAM is the overall executing agency of Pé-de-Pincha Project, consisting of professors, students and technicians, mainly taking interest in Community training activities, associating the research and teaching activities, participating with:

a) Own resources for realization of part of the shares, in addition to providing the integration of scientific knowledge to know venue for the participatory monitoring activities and development of a turtle management plan and lakes in the region;

b) Community empowerment in the work of environmental monitoring and turtle management techniques. According to Andrade et al., (2011), the expectation is to allow, in the future, they can manage and conduct the work through their own organizations, with only the support of other institutions, without them interfering directly.

c) Application of voluntary academic training and the insertion part of this outreach program in the pedagogical content of courses of Animal

Science undergraduate, Agronomy, Fishing Engineering, Forestry, Natural Sciences and Biological Sciences (Andrade, 2012).

As the state level is positioned SDS / CEUC and FAPEAM, means of communication that has the interest in monitoring, maintenance and control, proposing public policies and disclosure. The State Secretariat for the Environment and Sustainable Development of Amazonas (SDS/ CEUC), the body responsible for promoting public policies environmental Amazonas, in compliance with this objective, was formed in 2011 on Turtle working group, through Ordinance SDS No. 128 of 27.01.2011, with the duties to formulate guidelines for the management of turtles in protected areas, proposed awareness program and training technicians and community involved in the management of turtles and propose standards for fundraising aimed at implementation of participatory management of turtles (DOE No. 32123 of 03/08/2011) .

The project has been working with the SDS, in monitoring the populations of turtles, seeking to effect the ProBUC (Biodiversity Monitoring Program UC) with a focus on participatory monitoring of turtles in state conservation units. In 2013, this group presented the draft of the priority areas for turtle conservation in the Amazon by the State Council for the Amazon Environment (CEMAAM). The created legal instruments are essential to support research with information about new public policies generated for the protection and conservation of this resource.

Among these also the FAPEAM (government stakeholders level) which provided scholarships for students and tutors to carry out the work of monitoring, growth and survival of turtles puppies in Barreirinha, Parintins and Nhamundá. Among the results obtained with such support, made it possible for young scientists discover some konowledge as story:

> “The species that occur in the local area, the small and big turtles known by the locals as pitiú, headstrong, *aperem*a” yellow and red tortoise mong the most frequently cited species was the small turtles possibly to live in rivers, lakes and creeks. They noted further that there was little consumption of turtles and eggs, among the most consumed was the tortoise, and lard used as medicine (student, 14, from the Oriximiná)”.

The importance of FAPEAM participation was also highlighted by the local coordinators and teachers who influenced young scientists to participate in the turtles community management in the municipality of Barreirinha.

> "The formation of new generations of environmentalists and researchers, of the Amazon Youth Scientist program (JCA) of the Foundation of the Amazonas State Research (FAPEAM) today act as project leaders in their communities" (teacher, 51 from of Barreirinha)".

This result shows the reflection of the contribution of this foundation, providing the initiative of these young people in the conservation of turtles, and enables them to take responsibility to lead the action in their local environment.

The media were essential to the social and environmental impact were publicly known. The project, we highlight the newspapers, local circulation magazines and regional, local and national television networks. In addition, the Internet media such as the website, facebook and blogs, that have interest in disclosure, strengthening the project and contribute to the involvement of partners, opinion leaders, the government, communities and society in general considering the local reality.

As for the local level are made up of NGOs, local governments, community associations, local rural businesses, schools, churches and residents of Barreirinha, Parintins, Oriximiná and Terra Santa.

NGOs were organized in cities and communities along the river to take action to control and environmental monitoring. As pointed Pignatti (2005), environmental NGOs are entities with some institutions are private organizations with public purposes, non-profit and some voluntary participation. In Pé-de-Pincha Project, NGOs, were formed by voluntary environmental agents who had the role of an educator / sensitizer for environmental and organization of environmental joint efforts in their communities, as the speech of an environmental agent at Terra Santa:

> The control work is done when we receive the complaint, and when we have free time, because our work match of not having the necessary resources, such as fuel, because unfortunately we do not have a fuel quota to our work makes it difficult to our action often (volunteer environmental agent, 46 from the Terra Santa / PA).
>
> The seized resulting from infractions material is donated to charitable institutions, especially perishables (meat, fish, etc.) and perishable noes (utensils in general) are the competent bodies (volunteer environmental agent, 52 from the Terra Santa / PA).

The Terrasantense Association of Voluntary Environmental Agents (ATAAV) participates in the coordination of the project with logistical support, hosting and human resources. The Environmentalist of Andirá River Movement (MAPEP) participates in Barreirinha county region of protection of turtles by the coordinators in the communities. The environmental group Natureza Viva (GRANAV) is based organization that, according Pantoja (2006), arose from the need to organize and legitimize the River Dweller movement for protection of lakes and management of fishery resources in the region of Parintins, taking part in the project through the support of voluntary environmental agents. The Environmentalist Association of Three Communities of Lake Macurani (ASSASE 3) is an association that participates in the project with voluntary environmental agents that develop the management of turtles in these locations.

Local rural entrepreneurs are exploring the site and/or that have properties in communities. His interests are focused on reciprocity, allowing Pe de Pincha Personnel to do the research on their private beach of their properties so that the handling of the turtles is carried out, and in return receive the inspection by environmental agencies and the monitoring and control by the community. Thus, as the area is freely accessible, it is protected.

Regarding the association between key stakeholders in the community management of turtles were UFAM and groups directly involved in community management chelonian, including environmental agents, NGOs, community associations, community leaders, residents, field engineers and farmers. These parties play important roles from planning, enabling each location to be developed management of turtles, which although it seems divided, execution is held annually continuously.

The UFAM's mission is to cultivate knowledge in all areas of studies through teaching, research and extension, contributing to the formation of citizens and development in the Amazon. Therefore, there is at all stages, thus fulfills its role of teaching, research and extension through Professors, technicians and students involved in the project, in addition to encouraging the riparian conserve their resources in many places is only food source.

In the collecting stage, IBAMA stands out for being the body that authorizes the handling of animals, as well as participation in the formation of voluntary environmental agents and in monitoring, particularly in the trays. According to Pereira and Pinto (2001), IBAMA legitimizes the agreement only in the context of a system of free access. Agreements can define how the fishery resources will be used, but do not define who can fish, either prohibit foreign fishermen have access. This situation causes a greater disincentive to management, not to allow the social group that invests spontaneously in monitoring and controlling and reducing access to the resource to receive the benefits of this effort.

As for conflicts generated directly affected the local support groups, causing many, discouraged, give up their activities. Also caused dissatisfaction and distrust, allowing the formation of smaller groups, thus generating conflicts of influence, giving bonds a few communities, which often benefit from the opportunities that arise.

To Pereira and Pinto (2011), social conflicts must suffer intervention of society itself, which, in an organized manner, must create some kind of social institution to correct this problem. This understanding becomes necessary because the space is the same as among local rural farmers to access to the beaches, especially on land use.

These conflicts therefore operate at the local level, with fishermen from other communities and municipalities to prevent fishing and access to nesting sites and the community; this conflict more complex, since the legislation does not prevent the entry of outsiders for fishing.

Conclusion

This research has found that stakeholders in community management to better ensure the sustainability of management of turtles were the residents of communities of focal area and directly affected by the population of turtles. This balance is challenged by people from the outside community, possibly because they have a major economic and cultural dependence on this resource.

The stakeholders are fundamental for the Turtles community management, as they possess the empirical knowledge of where they live, and so may establish rules to ensure the use, as for their daily survival, depends on the use of natural resources.

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Endophytic fungal diversity
associated with the roots of cohabiting plants in the Pantanal wetland

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Introduction

Wetlands are defined by their climatic, hydrological, soil physical-chemical, and botanical parameters (Junk et al. 2013; Junk et al. 2016), and they offer important environmental services, including water storage, sediment retention, microclimate regulation, recreation, ecotourism, organic carbon storage, wood production, medicinal plants, fish, agricultural products, potable water for humans and animals, and pastures for livestock (Junk et al. 2013). The Pantanal is one of the largest world wetlands and covers an area of 160,000 km^2 (Junk et al. 2011a). Compared with other permanently flooded wetlands, the Pantanal is an intermittent and seasonally flooded plain (Nunes da Cunha and Junk 2011; Junk et al. 2016). There is an inversely proportional relationship between the water-depth height (flooding) and species distribution (Nogueira et al. 2011), and this height can act as a stressor on the plant community and promoter of habitat and species diversity (Junk et al. 2011b; Johnson et al. 2016).

A limited number of plant species has adapted to seasonal flooding (Junk et al. 2013; Wittmann et al. 2017). *Vochysia divergens* Pohl, popularly known as “Cambará”, is one of the few tree species adapted to these conditions; it is traditionally used in the Brazilian folk medicine to treat the flu, asthma, and infections (Hess and Delle Monache 1999; Almeida et al. 2014; Hokama et al. 2017). Another species adapted to wet soils is *Axonopus leptostachyus* (Flüggé) Hitchc., which is a dry-season grass popularly known as “Rabo de burro” (Schessl 1999; Rebellato and Nunes da Cunha 2005; Bueno et al. 2014). *A. leptostachyus* is considered a key species in controlling the plant community when *V. divergens* is the dominant species (Santos et al. 2012).

Consistent with the fact that wetlands promote diversity, a huge variety of endophytic fungi has been isolated from plants that grow in such environments (Kandalepas et al. 2010; Chen et al. 2011; Xing and Guo 2011; Silvani et al. 2013; Hokama et al. 2017). Endophytic fungi that inhabit the interior of plant tissues, resulting in asymptomatic colonization, can be isolated from different organs or tissues (Schulz and Boyle 2005; Peay et al. 2016). The host plant geographic location impacts on the endophytic fungal community diversity, which is greater in tropical regions (Kharwar and Strobel 2011), where the plant biodiversity is also higher (Junk et al. 2011a). Endophytes can offer several benefits to the host plant, particularly growth promotion (Soares et al. 2015; Soares et al. 2016a) and protection from pathogens (Soares et al. 2016b).

Dark septate endophytes (DSEs) constitute an important group of endophytic fungi that belong to the phylum Ascomycota (Jumpponen and Trappe 1998) and are characterized by the presence of septate melanized hyphae and microsclerotia (Mandyam and Jumpponem 2015). These fungi are associated with the roots of diverse plant families and occur in various environments, especially in those predisposed to stress, such as: habitats with low water availability and high soil compaction; deserts and pastures (Yuan et al. 2010b; Knapp et al. 2012; Kivlin et al. 2013; Cagigal 2017; Petipas et al. 2017); nutritionally poor environments; alpine regions; high-salinity environments; and polar regions (Newsham et al. 2009; Porcel et al. 2012; Mandyam and Jumpponen 2005, 2015; Knapp et al. 2015, Knapp and Kovács 2016). DSEs occur more frequently in these stressful environments because they mitigate adverse stress effects on the host plant (Knap and Kovács 2016; Cagigal 2017).

Colonization of roots of different plant species by the same fungal isolate interconnects these plants via fungal mycelia, which in turn mediate the plant-plant transfer of substances that provide nutrition (Porcel et al. 2012; Babikova et al. 2013; Surono and Narisawa 2017) and defense against herbivory (Barto et al. 2012; Jung et al. 2012; Babikova et al. 2013). There are very few studies on the occurrence and distribution of endophytes in the Pantanal wetland (De Siqueira et al. 2017; Souza et al. 2015) and the compounds isolated from these microorganisms (Silva et al. 2017; Brissow et al. 2017). The present study reports the endophytic fungal diversity associated with the roots of *A. leptostachyus* and *V. divergens*, which are two plant species that cohabitate in the Pantanal.

Materials and Methods

A. leptostachyus and *V. divergens* samples were collected during the dry season in the city of Poconé, State of Mato Grosso, Brazil. The mean annual rainfall of the region is 1,250 mm, and the temperature ranges from 27.4 ºC to 21.4 ºC (Girard et al. 2011). Three samplings were performed for each plant. *A. leptostachyus* (1AL) and *V. divergens* (1VD) are cohabiting species, and they were collected at site 1 (16º21’15.9”S and 56º28’70.7”W). *A. leptostachyus* (2AL) was also collected at site 2 (16º18’97.8” and 56º32’24.3”W), approximately 7 km far from the first sampling site. Site 1 is a seasonally flooded clean field, and the initial *V. divergens* invasion contained several young individuals with approximately 1.2 m tall. Site 2 is a natural clean field or field with “Rabo de burro” featuring dominant *A. leptostachyus* (Nunes da Cunha and Junk 2011).

The plants and soil adhered to their roots were collected and transported under refrigeration until processing. The voucher specimens were prepared and deposited in the Herbarium of Federal University of Mato Grosso (UMFT), Cuiabá, MT, Brazil, under the codes 40.492 (2AL) and 40.493 (1AL). Composite samples of the rhizospheric soil (soil attached to the roots) and non-rhizospheric soil (0-10 cm depth) were collected for physicochemical analysis and mycorrhizal arbuscular fungal spore counting.

Soil analyses

The soil samples were air-dried, homogenized, sieved through a 2-mm mesh, and analyzed with respect to their pH (in H_2O and a saline solution of 0.01 $CaCl_2$) and concentration of Ca, Mg (extraction with KCl 1 mol L^{-1}), K, P (Mehlinch I) and Al (extraction with KCl 1 mol L^{-1}). The chemical content was determined by titration with NaOH 0.025 mol L^{-1}.

To perform fungal spore counting, the spores were extracted from 50-g samples of non-rhizospheric and rhizospheric soil by wet sieving (Gerdemann and Nicolson 1963) followed by centrifugation in 60% sucrose solution. Briefly, the soil dispersed in water was sieved (710-, 500-, and 38-µm mesh). The spores and remaining soil were collected in 38-µm mesh sieves and centrifuged in 60% sucrose (2:1 sucrose solution: water with spores) at 3000 rpm for three minutes. Next, the supernatant was collected using a 38-µm mesh sieve, and the spores were transferred to gridded Petri dishes (Giovannetti and Mosse 1980) and counted under a stereoscopic microscope.

Isolation of endophytic fungi and assessment of root-fungus interaction

V. divergens seeds were surface disinfested by immersion in 70% ethanol for 1 minute and 2.5% sodium hypochlorite for 6 minutes, and rinsed three times in autoclaved distilled water. The seeds were placed into Petri dishes (two seeds per dish) containing mineral medium (MM) – 1900 mg L^{-1} KNO_3, 441 mg L^{-1} $CaCl_2 \bullet H_2O$, 370 mg L^{-1} $MgSO_4 \bullet 7H_2O$, 170 mg L^{-1} KH_2PO_4, and 1362.4 mg L^{-1} $(NH_4)_2SO_4$ – solidified with 15 g L^{-1} agar, and further incubated at room temperature for 3 to 7 days. Nine surface-sterilized root fragments were then used for endophytic fungal isolation.

To visualize and count the fungal structures typical of DSE and arbuscular mycorrhizal fungus (AMF), a portion of the collected roots was fixed in a solution of formaldehyde, acetic acid, and ethanol at 01:01:18 (v/v), and stained according to the method reported by Phillips and Hayman (1970), with modifications, under the conditions described in Table 1. The slides were mounted with polyvinyl lacto glycerol (16.6 g of polyvinyl alcohol, 100 mL of lactic acid, 10 mL of glycerin, 100 mL of distilled water) containing 10 root fragments of approximately 1 cm each. A total of 100 fields were counted to determine the percentage of the following fungal structures: non-septate hyphae, coil hyphae, arbuscule, vesicle, brown septate hyphae, and microsclerotia.

The roots were surface disinfested by immersing them in tap water with neutral detergent (1%) for 5 minutes, under shaking (150 rpm), followed by rinsing with distilled water, and sequential immersion in 70% ethanol for 1 minute, 2.5% sodium hypochlorite for 3 minutes, 70% ethanol for 30 seconds, and autoclaved distilled water for three times. Then, isolation of endophytic fungi was stimulated by the presence of the host plant under *in vitro* conditions (Bardgett and Putten 2014). Root fragments of approximately 1 cm were placed near the roots of *V. divergens* seedlings. Fungal growth was monitored for 15 days, and the emerging mycelia were transferred to a new Petri dish containing potato-dextrose agar (PDA). The strains were preserved in PDA under refrigeration.

Identification of the isolated fungal strains

The endophytic fungi were grouped into morphotypes following the method used by Szilagyi-Zecchin et al (2016). The grouping was confirmed by analyzing microscopic traits in permanent slides of fungi microcultures (Kern and Blevins 1999) and by detecting genetic markers capable of differentiating strains of the same species (Santos et al. 2012). DNA was extracted from a representative specimen of each morphological group using the Axygen® kit (Corning, USA), according to the manufacturer's recommendations.

Table 1. Root processing and staining according to the method reported by Phillips and Hayman (1970), with modifications.

Solution	Plant species			Seedlings *in vitro*[2]
	1VD[1]	1AL[1]	2AL[1]	
10% KOH	-	Autoclaved for 5 minutes	-	60 °C for 1 hour
2.5% KOH	Autoclaved for 5 minutes. Solution replaced and incubated for 8 hours	-	90 °C for 2 hours	-
KOH was drained	+	+	+	+
Rinsing in distilled H_2O	+	+	+	+
1% HCl	Room temperature for 8 hours	Room temperature for 24 hours	Room temperature for 2 hours	60 °C for 5 minutes
HCl was drained	+	+	+	+
0.05% trypan blue	70 °C for 15 minutes	70 °C for 15 minutes	70 °C for 15 minutes	60 °C for 5 minutes

[1] Field-collected roots.

[2] Roots of *Vochysia divergens* and *Combretum lanceolatum* seedlings inoculated with the fungal strains isolated from the roots of 1AL, 1VD, and 2AL. *Abbreviations*: 1AL = *Axonopus leptostachyus* collected at site 1; 2AL = *Axonopus leptostachyus* collected at site 2; 1VD = *Vochysia divergens* collected at site 1.

The genetic variability of the strains belonging to the same species was confirmed by analyzing the ISSR (*inter-simple sequence repeats*) (Longato and Bonfante 1997) and IRAP (*inter-retrotransposon amplified polymorphism*) (Santos et al. 2012) molecular markers. The polymerase chain reaction (PCR) was performed with the following reaction solution: 1 µL BHI (10 pM) for ISSR – or 1 µL CLIRAP1 (10 pM) and 1 µL CLIRAP4 (10 pM) for IRAP –, 4 µL DNA, 2.5 µL 10x buffer, 0.75 µL $MgCl_2$, 1 µL dNTP (2.5 mM), 0.3 µL Taq DNA polymerase (Invitrogen®, Thermo Fischer Scientific, USA), and water to a volume of 25 µL. The amplification conditions are presented in Table 2. The amplified fragments were separated by electrophoresis in agarose gel (1.4%) with ethidium bromide (0.05%) staining, and further visualized and photographed in the photodocumentation apparatus under ultraviolet light.

Table 2. Amplification conditions for identifying the endophytic fungal strains isolated from *Axonopus leptostachyus* and *Vochysia divergens*.

PCR step	Molecular marker			
	ITS	26S rDNA	β-tubulin	ISSR and IRAP
Initial denaturation	94 °C for 2 minutes	94 °C for 2 minutes	94 °C for 2 minutes	94 °C for 2 minutes
Denaturation	94 °C for 45 seconds	94 °C for 45 seconds	94 °C for 45 seconds	94 °C for 1 minute
Annealing	50 °C for 45 seconds	50 °C for 45 seconds	56 °C for 45 seconds	50 °C for 2 minutes
Extension	72 °C for 1 minute	72 °C for 1 minute	72 °C for 1 minute	72 °C for 2 minute
Number of cycles	35	35	35	35
Final extension	72 °C for 10 minutes	72 °C for 10 minutes	72 °C for 10 minutes	72 °C for 10 minutes

Abbreviations: IRAP = inter-retrotransposon amplified polymorphism; ISSR = inter-simple sequence repeats; ITS = internal transcribed spacer; PCR = polymerase chain reaction.

Molecular identification was performed by partial sequencing of 26S rDNA (D1/D2 domain), ITS (*internal transcribed spacer*), and â-tubulin. The 26S rDNA was amplified using the NL 1 and NL 4 primers (Rodrigues and Fonseca 2003); the ITS region of the rDNA, including the 5.8S gene, was amplified using the ITS 4 and ITS 5 primers (White 1990); and â-tubulin was amplified using the BT2A and BT2B primers (Glass and Donaldson 1995). The PCR reaction was performed under the conditions reported in Table 2, with the following reaction solution: 1 µL DNA, 2.5 µL 10x buffer, 0.75 µL $MgCl_2$, 1 µL dNTP (2.5 mM), 1 µL ITS 4 (10 pM), 1 µL ITS 5 (10 pM), 0.3 µL Taq DNA polymerase (Invitrogen®), and water to a volume of 25 µL. The amplified fragments were purified and sequenced, and the obtained sequence was compared with the GenBank database using the nBLAST tool (http://www.ncbi.nlm.nih.gov/). The sequences of the fungal morphotypes isolated from *A. leptostachyus* were deposited in GenBank under the accession numbers KJ439067-KJ439180 and KJ192202, while the sequences of the fungal morphotypes isolated from *V. divergens* were deposited under the accession numbers KJ439181-KJ439216, KJ192201, and KJ522782-KJ522784.

Detection of possible DSE strains

The infective potential of the fungal strains and differentiated microsclerotia (endophytism) was tested by inoculating the mycelial fragment of each morphotype in *V. divergens* seedlings maintained in MM medium. The control Petri dish did not receive any inoculum. After inoculation, the Petri dishes were incubated at room temperature under natural luminosity for 15 days. The seedlings' roots were stained *in vitro* according to the method reported by Phillips and Hayman (1970), with modifications (Table 1), and observed under an optical microscope. The presence of hyphae and microsclerotia, which are typical structures of DSE, was evaluated. To determine the degree of infection specificity, the fungi that exhibited microsclerotia were inoculated *in vitro* in *Combretum lanceolatum* Pohl seedlings, using the same method used to inoculate *V. divergens* seedlings.

Statistical analysis

The number of isolates was used to assemble the rarefaction curve to test whether the number of collected fragments was sufficient to sample the fungal diversity in the roots. Colonization frequency was expressed as percentage (Petrini et al. 1982) and analyzed using analysis of variance and the Scott-Knot test at 5% probability. Each Petri dish containing nine root fragments was considered as one sampling unit.

The following indexes were calculated: (i) Shannon-Weaver (Odum 1983; Pielou 1966) and Fisher and Simpson (Yuan et al. 2010b) diversity indexes; (ii) Pielou evenness index (Pielou 1966) for species and genera; (iii) species richness index (Gotelli and Colwell 2001); (iv) Margalef (Yuan et al. 2010b), Menhinick (Menhinick 1964), and Chao 2 (non-parametric) (Chao 1987) richness indexes; (v) Morisita index to analyze the species composition and determine the commonality of taxa among two or more communities.

Five treatments with three replicates each were performed to extract spores from the rhizospheric and non-rhizospheric soils of 1VD, 1AL, and 2AL. A composite sample was used, and the three sampling points were treated as one sampling unit. The occurrence frequency of fungal structures in the field-collected roots was analyzed via three treatments (plants) and three replicates in a 7x3x3 factorial scheme. Each mounted slide of an individual plant was considered as one sampling unit. The colonization frequency of fungal structures in the field-collected roots and spore counts of the soil were analyzed using a normality test, analysis of variance, and Scott-Knott test at 5% probability.

Results

Isolation and identification of endophytic fungi

A total of 391 endophytic fungal strains were isolated and identified from 648 root fragments (3 plant species x 3 individuals per species x 9 fragments x 8 replicates), i.e. 216 fragments per plant species. The endophytic fungal colonization frequencies in the roots of 1VD, 1AL, and 2AL were 68.0%, 58.7%, and 85.5%, respectively, which were not significantly different from each other.

The fungal strains were distributed among the three host plant species: 134, 95, and 162 strains were isolated from 1VD, 1AL, and 2AL, respectively (Table 3), and initially grouped into 47, 65, and 91 morphotypes, respectively, according to their micro and macromorphological traits. After molecular identification, the morphotypes from 1VD, 1AL, and 2AL were grouped into 32, 38, and 60 morphospecies, respectively, according to their morphological and molecular traits (Table 3).

The predominant phylum was Ascomycota (98.13%); 1.07% of the strains were classified as Basidiomycota (orders Agaricales and Tremellales), and 0.80% as Zygomycota (order Mucorales). The fungi belonging to the phylum Ascomycota were distributed into 11 orders: Capnodiales, Chaetosphaeriales, Diaporthales, Eurotiales, Glomerellales, Helotiales, Hypocreales, Magnaporthales, Mycroascales, Pleosporales, and Sordariales. Eurotiales was the most abundant order, corresponding to 34.99% of the total Ascomycota strains isolated (Figure 1); all of the isolates from this order belonged to the class Eurotiomycetes.

In *A. leptostachyus*, the phylum Ascomycota occurred at a frequency of 98%, among which the most common class was Sordariomycetes (46.1%), followed by Eurotiomycetes (40.7%), Dothideomycetes (11.1%), and Letiomycetes (2.1%), and the less common orders were Eurotiales (39.9%), Hypocreales (18.2%), and Magnaporthales (11.3%).

Fungal strains belonging to the phyla Zygomycota (1.6%), Basidiomycota (0.8%), and Ascomycota (97.6%) were isolated from *V. divergens*, and the following classes were identified: Leotiomycetes (32%), Dothideomycetes (24.6%), Eurotiomycetes (24.6%), Sordariomycetes (16.4%), Tremellomycetes, and Zygomycetes. Helotiales was the most common order, accounting for 31.2% of the total Ascomycota strains isolated from 1VD.

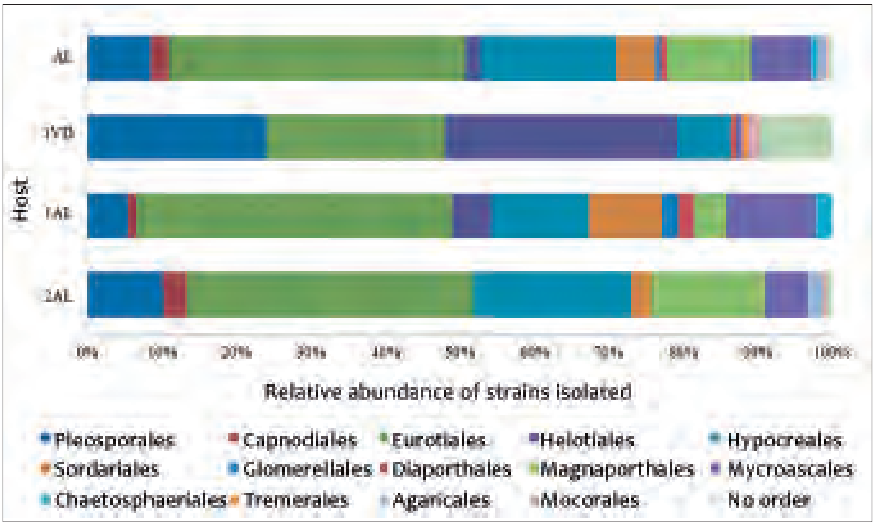

Figure 1. Classification according to the fungal orders isolated from the roots of *Axonopus leptostachyus* and *Vochysia divergens*. *Abbreviations*: AL = *Axonopus leptostachyus* collected at sites 1 and 2 (1AL + 2AL); 1AL = *Axonopus leptostachyus* collected at site 1; 2AL = *Axonopus leptostachyus* collected at site 2; 1VD = *Vochysia divergens* collected at site 1.

Species accumulation was estimated from a rarefaction curve. The curves for the three plant species remained within the 95% confidence interval, showing that the sampling method used was effective in recovering species within the root endophytic fungal community. A total of 53 fungal species were found specifically in *A. leptostachyus* roots, and another 12 species were identified only in *V. divergens* roots. Analysis of *A. leptostachyus* collected at different sites revealed that 17 and 9 fungal species colonized 2AL and 1AL, respectively. After comparing the host plants of different cohabiting species (1AL and 1VD), the number of shared fungal species decreased to 9, and after the genetic variability analysis, the following 7 species were considered to belong to the same strain: *Fusarium oxysporum, Fusarium proliferatum, Diaporthe phaseolorum, Penicillium javanicum, Periconia macrospinosa, Talaromyces aculeatus,* and *Talaromyces verruculosus.*

The diversity indexes are reported in Table 4. The isolated fungal genera and species followed an even distribution, as indicated by the Pielou index: 0.81 and 0.83 for genera, and 0.81 and 0.90 for species isolated from *V. divergens* and *A. leptostachyus*, respectively. In addition, 70 genera and 30 species were identified in *A. leptostachyus* (38 genera and 22 species in 1AL; 60 genera and 35 species in 2AL) and 32 genera and 25 species were identified in *V. divergens*.

Table 3. Identification, occurrence frequency, and endophytism of the fungal strains isolated from the roots of *Axonopus leptostachyus* and *Vochysia divergens*.

Fungal species isolated	Total found[1]	*V. divergens* (1VD)[1]	*A. leptostachyus*			Putative DSEs[2]
			AL[1]	**1AL[1]**	**2AL[1]**	
Acremonium sp.	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Aspergillus aculeatus	2 (0.005)	-	2 (0.008)	-	2 (0.01)	-
Aspergillus japonicus	4 (0.010)	2 (0.01)	2 (0.008)	2 (0.02)	-	-
Aspergillus niger	7 (0.018)	4 (0.03)	3 (0.012)	-	3 (0.02)	-
Aspergillus sp.	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Aspergillus terreus	10 (0.026)	-	10 (0.039)	1 (0.01)	9 (0.06)	-
Aspergillus viridinutans	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Byssothecium circinans	3 (0.008)	3 (0.02)	-	-	-	-
Chaetomium bostrychodes	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Chaetomium cupreum	2 (0.005)	-	2 (0.008)	2 (0.02)	-	-
Chaetomium subaffine	2 (0.005)	-	2 (0.008)	2 (0.02)	-	-
Cladosporium flabelliforme	5 (0.013)	-	5 (0.019)	1 (0.01)	4 (0.02)	-
Cladosporium perangustum	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Cochliobolus geniculatus	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Colletotrichum dematium	2 (0.005)	-	2 (0.008)	2 (0.02)	-	-
Corynespora olivacea	3 (0.008)	2 (0.01)	1 (0.004)	-	1 (0.01)	-
Corynespora smithii	1 (0.003)	1 (0.01)	-	-	-	-
Corynespora sp.	3 (0.008)		3 (0.012)	-	3 (0.02)	-
Cryptococcus sp.	1 (0.003)	1 (0.01)	-	-	-	-
Curvularia lunata	1 (0.003)	1 (0.01)	-	-	-	++
Diaporthe phaseolorum var. meridionalis	3 (0.008)	1 (0.01)	2 (0.008)	2 (0.02)	-	-
Fusarium oxysporum	17 (0.043)	5 (0.04)	12 (0.047)	2 (0.02)	10 (0.06)	++$^{\text{1VD22}}$
Fusarium proliferatum	8 (0.020)	2 (0.01)	6 (0.023)	3 (0.03)	3 (0.02)	-
Fusarium solani	2 (0.005)	-	2 (0.008)	1 (0.01)	1 (0.01)	++$^{\text{2AL33}}$
Fusarium succisae	6 (0.015)	-	6 (0.023)	3 (0.03)	3 (0.02)	-
Gongronella butleri	3 (0.008)	2 (0.01)	1 (0.004)	-	1 (0.01)	-

Table 3 (cont.). Identification, occurrence frequency, and endophytism of the fungal strains isolated from the roots of *Axonopus leptostachyus* and *Vochysia divergens*.

Fungal species isolated	Total found[1]	*V. divergens* (1VD)[1]	*A. leptostachyus*			Putative DSEs[2]
			AL[1]	**1AL[1]**	**2AL[1]**	
Helotiales sp.	1 (0.003)	-	1 (0.004)	1 (0.01)	-	-
Lentithecium arundinaceum	3 (0.008)	-	3 (0.012)	-	3 (0.02)	-
Lepiotaceae sp.	3 (0.008)	-	3 (0.012)	-	3 (0.02)	-
Leptosphaeria senegalensis	10 (0.026)	10 (0.07)	-	-	-	-
Leptosphaerulina chartarum	3 (0.008)	-	3 (0.012)	3 (0.03)	-	-
Lophiostoma cynaroidis	3 (0.008)	-	3 (0.012)	2 (0.02)	1 (0.01)	-
Magnaporthales sp.1	2 (0.005)	-	2 (0.008)	1 (0.01)	1 (0.01)	-
Magnaporthales sp.2	1 (0.003)	-	1 (0.004)		1 (0.01)	-
Magnaporthales sp.3	1 (0.003)	-	1 (0.004)	-	1 (0.01)	++
Magnaporthales sp.4	3 (0.008)	-	3 (0.012)	1 (0.01)	2 (0.01)	-
Magnaporthales sp.5	5 (0.013)	-	5 (0.019)	-	5 (0.03)	-
Magnaporthales sp.6	1 (0.003)	-	1 (0.004)	1 (0.01)	-	-
Magnaporthales sp.7	4 (0.010)	-	4 (0.016)	-	4 (0.02)	-
Magnaporthales sp.8	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Magnaporthales sp.9	1 (0.003)	-	1 (0.004)	-	1 (0.01)	++
Magnaporthales sp.10	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Magnaporthales sp.11	2 (0.005)	-	2 (0.008)	-	2 (0.01)	++
Magnaporthales sp.12	4 (0.010)	-	4 (0.016)	-	4 (0.02)	-
Magnaporthales sp.13	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Magnaporthales sp.14	1 (0.003)	-	1 (0.004)	1 (0.01)	-	-
Microsphaeropsis arundinis	5 (0.013)	-	5 (0.019)	-	5 (0.03)	-
Nectria sp.	10 (0.026)	2 (0.01)	8 (0.031)	-	8 (0.05)	++^{1VD11}
Neocosmospora striata	3 (0.008)	-	3 (0.012)	2 (0.02)	1 (0.01)	-
Neosartorya pseudofischeri	20 (0.051)	3 (0.02)	17 (0.066)	-	17 (0.10)	+^{2AL1}
Paecilomyces sp.	3 (0.008)	-	3 (0.012)	1 (0.01)	2 (0.01)	++^{2AL11}
Papulosa amerospora	2 (0.005)	2 (0.01)	-	-	-	++

Table 3 (cont.). Identification, occurrence frequency, and endophytism of the fungal strains isolated from the roots of *Axonopus leptostachyus* and *Vochysia divergens*.

Fungal species isolated	Total found[1]	*V. divergens* (1VD)[1]	*A. leptostachyus*			Putative DSEs[2]
			AL[1]	1AL[1]	2AL[1]	
Penicillium brefeldianum	3 (0.008)	-	3 (0.012)	3 (0.03)	-	-
Penicillium chermesinum	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Penicillium citrinum	7 (0.018)	1 (0.01)	6 (0.023)	-	6 (0.04)	-
Penicillium griseofulvum	2 (0.005)	2 (0.01)	-	-	-	-
Penicillium janthinellum	5 (0.013)	-	5 (0.019)	2 (0.02)	3 (0.02)	-
Penicillium javanicum	20 (0.051)	7 (0.05)	13 (0.051)	11 (0.12)	2 (0.01)	-
Penicillium ochrochloron	3 (0.008)	-	3 (0.012)	2 (0.02)	1 (0.01)	-
Penicillium shearii	5 (0.013)	5 (0.04)	-	-	-	-
Penicillium simplicissimum	5 (0.013)	-	5 (0.019)	1 (0.01)	4 (0.02)	-
Penicillium solitum	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Penicillium sp.1	2 (0.005)	-	2 (0.008)	2 (0.02)	-	-
Penicillium sp.2	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Penicillium vasconiae	1 (0.003)	-	1 (0.004)	1 (0.01)	-	-
Periconia macrospinosa	21 (0.054)	1 (0.01)	20 (0.078)	11 (0.12)	9 (0.06)	-
Phialocephala sp.	40 (0.102)	39 (0.29)	1 (0.004)	1 (0.01)	-	-
Pleosporales sp.	6 (0.015)	4 (0.03)	2 (0.008)	-	2 (0.01)	++^{2AL37}
Pyriculariopsis parasitica	1 (0.003)	1 (0.01)	-	-	-	-
Scytalidium cuboideum	3 (0.008)	-	3 (0.012)	3 (0.03)	-	-
Sordariomycetes sp.	7 (0.018)	7 (0.05)	-	-	-	-
Stagonosporopsis cucurbitacearum	9 (0.023)	9 (0.07)	-	-	-	++^{1VD12}
Staphylotrichum coccosporum	2 (0.005)	2 (0.01)	-	-	-	-
Talaromyces aculeatus	5 (0.013)	3 (0.02)	2 (0.008)	2 (0.02)	-	-
Talaromyces purpurogenus	7 (0.018)	-	7 (0.027)	7 (0.07)	-	-
Talaromyces verruculosus	16 (0.041)	3 (0.02)	13 (0.051)	5 (0.05)	8 (0.05)	-
Thielavia terrestris	5 (0.013)	-	5 (0.019)	4 (0.04)	1 (0.01)	-
Thozetella sp.	2 (0.005)	-	2 (0.008)	2 (0.02)	-	-

Table 3 (cont.). Identification, occurrence frequency, and endophytism of the fungal strains isolated from the roots of *Axonopus leptostachyus* and *Vochysia divergens*.

Fungal species isolated	Total found[1]	*V. divergens* (1VD)[1]	*A. leptostachyus*			Putative DSEs[2]
			AL[1]	**1AL[1]**	**2AL[1]**	
Trichoderma erinaceum	3 (0.008)	-	3 (0.012)	-	3 (0.02)	-
Trichoderma gamsii	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Trichoderma harzianum	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Trichoderma strigosellum	2 (0.005)	-	2 (0.008)	1 (0.01)	1 (0.01)	-
uncultured fungus	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
1 VD3[3]	3 (0.008)	3 (0.02)	-	-	-	-
1 VD5[3]	4 (0.010)	4 (0.03)	-	-	-	++
1 VD20[3]	1 (0.003)	1 (0.01)	-	-	-	-
1 VD44[3]	1 (0.003)	1 (0.01)	-	-	-	++
1AL16[3]	2 (0.005)	-	2 (0.008)	2 (0.02)	-	-
1AL63[3]	1 (0.003)	-	1 (0.004)	1 (0.01)	-	-
2AL8[3]	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
2AL12[3]	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
2AL29[3]	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
2AL38[3]	1 (0.003)	-	1 (0.004)	-	1 (0.01)	++
2AL62[3]	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
2AL74[3]	1 (0.003)	-	1 (0.004)	-	1 (0.01)	-
Total	391 (1.00)	134 (1.00)	257 (1.00)	95 (1.00)	162 (1.00)	15

[1] Values outside parentheses represent the number of strains of each fungal species, and numbers within parentheses represent the occurrence frequency of the fungal species. [2] One + or – sign indicates the presence or absence, respectively, of microsclerotia produced by inoculation of the fungal species in *V. divergens* plants. Two ++ signs indicate the presence of microsclerotia in *Vochysia divergens* and *Combretum lanceolatum*. The superscript indicates that only one morphotype in the species exhibits microsclerotia. [3] Unidentified morphotypes. *Abbreviations*: AL = *Axonopus leptostachyus* collected at sites 1 and 2; 1AL = *Axonopus leptostachyus* collected at site 1; 2AL = *Axonopus leptostachyus* collected at site 2; 1VD = *Vochysia divergens* collected at site 1; DSE = dark septate endophyte.

Table 4. Diversity and richness indexes of the endophytic fungi isolated from roots of *Axonopus leptostachyus* and *Vochysia divergens*.

Index	*V. divergens* (1VD)	*A. leptostachyus*		
		AL	1AL	2AL
Species richness (*S*)	32	70	38	60
Shannon-Weaver (*H'*)	2.7	3.9	3.3	3.6
Simpson (D_s)	0.87	0.97	0.95	0.96
Fisher (á)	11.2	38.9	21.8	29.3
Margalef (Dmg)	5.6	14.1	7.7	10.5
Chao 2	40.1	123.2	76.8	124.5
Menhinick (Dmn)	2.5	4.9	3.9	4.3

Abbreviations: AL = *Axonopus leptostachyus* collected at sites 1 and 2; 1AL = *Axonopus leptostachyus* collected at site 1; 2AL = *Axonopus leptostachyus* collected at site 2; 1VD = *Vochysia divergens* collected at site 1.

According to the Morisita index, the endophytic fungal communities isolated from the 1AL and 2AL roots are more similar to each other (0.366) than they are to the community isolated from 1VD, which in turn is more similar to the community isolated from the host plants collected at the same site (0.167).

Microscopic analyses of the roots of three host plant species evidenced their interaction with DSEs and AMF, as indicated by the fungal structures observed (Table 5). The most common structures detected in *A. leptostachyus* roots (1AL and 2AL) were non-septate hyphae of AMF and brown septate hyphae (most likely from DSE). *V. divergens* (1VD) roots were extensively colonized by non-septate hyphae.

Table 5. Occurrence frequency of fungal structures in *Axonopus leptostachyus* and *Vochysia divergens* roots.

Fungal structures	Plant species				
	A.L	V.D	1AL	1VD	2AL
Non-septate hyphae	81.17 aA	78.67 aA	87.33 aA*	78.67 aA	75.00 aA
Arbuscule	45.33 bA	25.67 cB	59.67 bA	25.67 cB	31.00 bB
Vesicle	28.83 bA	42.00 bA	36.00 cA	42.00 bA	21.67 bB
Coil hyphae	34.50 bA	23.00 cA	45.33 bA	23.00 cB	23.67 bB
Resting spore	12.00 cA	0.67 dA	19.67 cA	0.67 dB	4.33 cB
Septate hyphae	75.33 aA	50.33 bB	85.33 aA	50.33 bB	65.33 aB
Microsclerotia	42.83 bA	50.67 bA	56.33 bA	50.67 bA	29.33 bB
CV (%)	22.81		22.92		

A. leptostachyus presented a more expressive number of arbuscule and septate hyphae, as compared with *V. divergens*. The 1AL and 2AL roots were similar only with respect to their number of non-septate hyphae; the other fungal structures were more abundant in 1AL. The roots of the cohabitant plants 1VD and 1AL exhibited similar frequencies of non-septate hyphae, vesicle, and microsclerotia; the other fungal structures predominated in 1AL.

Fungal strains with potential DSE

V. divergens seedlings inoculated with 98.72% of the isolated endophytic fungal strains did not exhibit visible signs of disease, when compared with the non-inoculated control. Approximately 10.8% of the fungal morphotypes were considered as DSEs due to the presence of microsclerotia (Fig. 2) and melanized septate hyphae. The morphotypes considered as DSEs in *V. divergens* preserved these traits when inoculated in *C. lanceolatum* (Table 3). The morphotypes isolated from 1AL were not considered as DSE because they lacked microsclerotia when inoculated in *V. divergens*.

Pathogenic fungal strains were isolated from 1VD and 2AL roots at low frequencies – 0.75% and 2.47%, respectively – and identified as *F. oxysporum* 1VD21, *Lepiotaceae* sp. 2AL53, and *Magnaporthales* sp.1 2AL66.

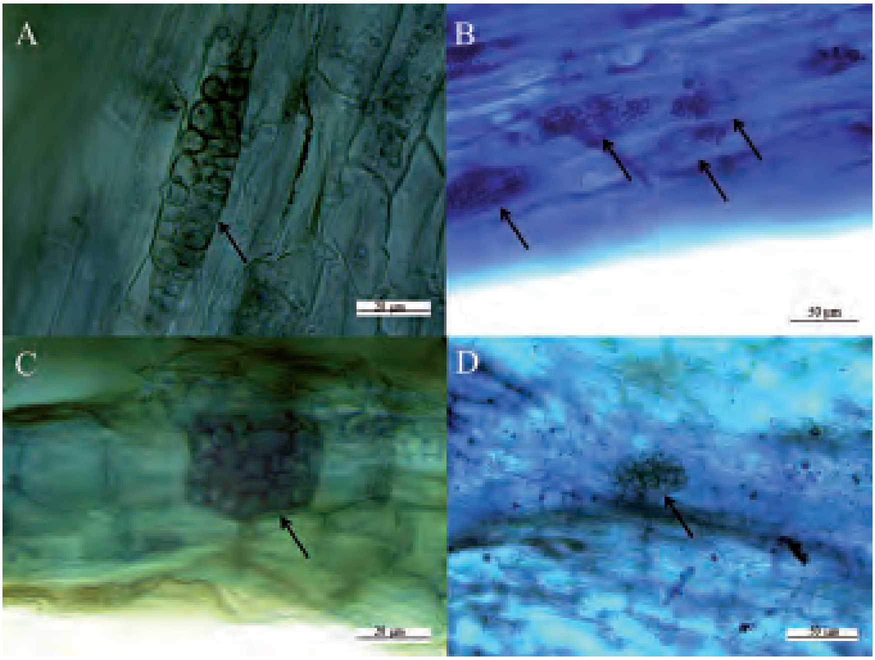

Figure 2. Microsclerotia present in aseptic *Vochysia divergens* seedlings. Microsclerotia are present in roots inoculated with the fungi, as indicated by the arrow.

Soil analysis

The soil samples collected at both sites were slightly acidic, with pH values near to 5.0. Soil from site 2 exhibited a higher organic matter and mineral content than soil from site 1. The concentration of P, K, and Al in soil from site 2 was respectively 2.56-, 1.99-, and 1.92-fold greater than that detected in soil from site 1 (Table 6). The rhizospheric soils of *A. leptostachyus* (1AL and 2AL) presented the highest endomycorrhizal fungal spore density per gram of dry soil (Table 7).

Table 6. Chemical analysis of soil samples collected at sites 1 and 2, in the city of Poconé, State of Mato Grosso, Brazil.

Site	pH		P	K	K	Ca+Mg	Ca	Mg	Al	H	H+Al	O.M
	H_2O	$CaCl_2$	mg/dm^3		cmolc/dm^3							g/dm^3
1	5.03	3.93	3.83	38.00	0.10	0.67	0.37	0.30	0.50	2.16	2.67	16.00
2	5.07	4.20	9.80	75.67	0.19	2.17	1.29	0.88	0.96	4.34	5.29	27.00

O.M: organic matter. Site 1: 16°21’15.9”S and 56°28’70.7”W. Site 2: 16°18’97.8” and 56°32’24.3”W.

Table 7. Spore count of endomycorrhizal fungi in the rhizospheric and non-rhizospheric soil of *Axonopus leptostachyus* and *Vochysia divergens*.

Soil	Spores/g dry soil*
Rhizospheric soil	
1VD	178.25 a
1AL	529.07 b
2AL	703.34 b
Non-rhizospheric soil	
Site 1	256.09 a
Site 2	344.31 a
CV (%)	34.64

*Means followed by the same letter do not differ significantly (Scott-Knott test at 5% significance level). *Abbreviations*: 1AL = *Axonopus leptostachyus* collected at site 1; 2AL = *Axonopus leptostachyus* collected at site 2; 1VD = *Vochysia divergens* collected at site 1. CV (%): coefficient of variation.

Discussion

In natural environments, plant species are associated with different fungal species (Partida-Martínez and Heil 2011; Suryanarayanan 2013; Kia et al. 2017). In this study, *A. leptostachyus* and *V. divergens* hosted a great richness and relative diversity of endophytic fungi, which resemble the flora and fauna diversity present in wetlands (Junk et al. 2013). The endophytic fungal community richness is higher under stressful conditions (Yuan et al. 2010a; Hokama et al. 2017), and correlates positively with variations in humidity (Peay et al. 2016). For instance, 41 morphotypes were isolated from 12 host plants growing in an environment with low temperature and rainfall (Knapp et al. 2012); 82 morphospecies were obtained from 48 plants inhabiting a semi-arid region (Loro et al. 2012); 33 species of endophytic fungi were isolated from *P. autralis* growing in environments with different levels of salinity (Soares et al. 2016); 16 morphospecies were isolated from 48 individuals of two plant species inhabiting a boreal forest (Tejesvi et al. 2013); and 30 morphospecies were detected in *Phoenix dactylifera* growing in coastal dunes (Mohamed et al. 2017). These reports indicate that there is a higher diversity of morphospecies in tropical and temperate environments (Junk et al. 2011b; Kharwar and Strobel 2011; Loro et al. 2012; Vaz et al. 2014; Peay et al. 2016).

In the present study, we collected *A. leptostachyus* and *V. divergens* root samples during the dry period. Dryness is a stressful condition for *V. divergens* (Novais et al. 2013) because this plant species is adapted to flooding conditions (Nunes da Cunha and Junk 2004). During dry periods, its deep roots can access deep sources of water (Dalmolin et al. 2013), which is favored by their association with DSEs and AMFs (Petipas et al. 2017); these microorganisms increase the contact surface with the soil (East 2013) and the plant resistance to stressful environments (Peay et al. 2016). Studies performed under controlled conditions have demonstrated the ecological importance of the presence of endophytic fungi to improve the adaptability of plant species to environments with water scarcity (Nagabhyru et al. 2013; Vázquez-De-Aldana et al. 2013; Santos et al. 2015; Fesel and Zuccaro 2016; Peay et al. 2016), suppress diseases, and increase pest resistance via root-associated fungi (Berendsen et al. 2012; Jung et al. 2012; Cameron et al. 2013; Bardgett and Putten 2014).

Despite the numerous studies on the endophytic fungal diversity, only a small proportion of fungi was identified up to now, suggesting that such diversity is underestimated (Kharwar and Strobel 2011; Deng and Cao 2017). The diversity index values that we found for the endophytic fungal communities isolated from *A. leptostachyus* and *V. divergens* roots were similar to those reported for the roots, leaves, and stem of *Zanthoxylum bungeanun* (Li et al. 2016a), roots of *Cynodon dactylon* and *Phragmites australis* (Kim et al. 2014), leaves of *Oryza granulate* (Yuan et al. 2010a) and *Paullinia cupana* (Sia et al. 2013), petioles of *Rhizophora apiculata* and *Ceriops tagal*, and petioles and roots of *Bruguiera sexangula var. rhynchopetala* (Xing and Guo 2011).

The Shannon-Weaver diversity index (*H'*) values often range from 1.5 to 4.5 (Li et al. 2016), and can be affected by the host plant species (Sun et al., 2012). The endophytic fungal diversity evaluated by this index exhibits relatively low values (<0.9) in a boreal forest (Tejesvi et al. 2013) and Caatinga (Santos et al. 2015), and higher values in grasses than in tree or shrubby species (Nalini et al. 2014). In this study, the Chao-2 and Margalef richness index values were high and similar to those reported for *H. brasiliensis* (Gazis and Chaverri 2010) and *O. granulate* (Yuan et al. 2010a). We found a high richness of endophytic fungi species on *A. leptostachyus* roots, when compared with literature data (Knapp et al. 2012; Loro et al. 2012; Tejesvi et al. 2013; Li et al. 2016a; Li et al. 2016b; Martins et al. 2016; You et al. 2016; Potshangbam et al. 2017).

Molecular identification evidenced that morphological separation was inaccurate because, in certain cases, different morphotypes constituted the same species. Identification of the morphological groups revealed the presence of three phyla divided into seven classes, with the predominance of Ascomycota. This phylum is dominant in root endophyte communities of different hosts and environments (Kim et al. 2014; Vaz et al. 2014; Wehner et al. 2014; Santos et al. 2015; Aguilar-Trigueros and Rillig 2016; Li et al. 2016b; Peay et al. 2016; Szilagyi-Zecchin et al. 2016; Mohamed et al. 2017; Toghueo et al. 2017). The class Sordariomycetes predominated in *A. leptostachyus*, while the class Leotiomycetes predominated in *V. divergens*. The results obtained for the former, which is a tree, are in line with those reported for *Huperzia serrata* (Chen et al. 2013) – a bryophyte found in wetlands –, *Indigofera suffruticosa* (Santos et al. 2015), and *Panicum virgatum* (Kleczewski et al. 2012) – a grass native to the North America. These reports demonstrate that the class Sordariomycetes has a cosmopolitan distribution in savannas.

The fungal species isolated from 2AL roots – *A. leptostachyus* collected in the pasture environment – were previously reported as endophytes of plants that inhabit different environments, such as mangroves (Xing and Guo 2011), agricultural areas (Shetty et al. 2016), rainforests (Chen et al. 2011), mountainous forests (Martins et al. 2016), and the Amazon rainforest (Sia et al. 2013). Hence, these endophytic fungal species are not exclusive to a particular site or biome, despite being unique to this host plant species. The presence of fungal species specific to 1AL roots – *A. leptostachyus* collected in seasonally flooded clean field – was reported in the roots (Huang et al. 2015; Bonfim et al. 2016), leaves (Santos et al. 2015), petioles (Clay et al. 2016), and flowers (Selvanathan et al. 2011) of different host plants. Some of the fungal species and genera found only in grasses were also identified in different organs of various host plants (Herrera et al. 2013; Sia et al. 2013; Toju et al. 2013; Nalini et al. 2014; Bonfim et al. 2016; Martins et al. 2016; Górzyñska et al. 2017), demonstrating a generalist character in terms of both the host plant type (not specific to grasses) and organ infected. The fungal genera isolate from *V. divergens* were also identified in other host plants (Herrera et al., 2013; Sia et al. 2013; An et al. 2015; Santos et al. 2015; Clay et al. 2016; Cosoveano et al. 2016; Faria et al. 2016; Mohamed et al. 2017; Górzyñska et al. 2017).

The capacity of endophytic species to overcome taxonomic barriers of the hosts and colonize phylogenetically distant species, even in disconnected geographic locations, cause the widespread occurrence of endophytic fungi in different host plant species (Kivlin et al. 2013; Wehner et al. 2014; Aguilar-Trigueros and Rillig 2016; Bokati et al. 2016; Khiralla et al. 2017; Potshangbam et al. 2017). In spite of the lack of studies on the adaptations that enable these endophytic generalists to colonize a wide range of species, this phenomenon indicates that they are capable of overcoming a variety of host defense mechanisms (Suryanarayanan 2013; Fesel and Zuccaro 2016; Deng and Cao 2017; Khiralla et al. 2017).

Periconia macrospinosa was the dominant endophytic fungal species in *A. leptostachyus*, with an occurrence frequency of 0.08 (0.12 in 1AL and 0.06 in 2AL); it is one of the main species present in *Anacardium othonianum* (Faria et al. 2016). *Phialocephala* sp. was the dominant endophytic fungal species in *V. divergens*, with an occurrence frequency of 0.29. The host plant species greatly interferes in the fungal species that colonize it (Wehner et al. 2014; Bonfim et al. 2016; Raghavendra et al. 2017). Although certain endophytic fungi prefer grass as a host, they are still considered generalists because they inhabit various plants in different environments (Herrera et al. 2013; Higgins et al. 2014; Huang et al. 2015; Santos et al. 2015; Clay et al. 2016; Martins et al. 2016; Shetty et al. 2016; Górzyñska et al. 2017).

The inner plant tissue is a favorable shelter for endophytic fungi due to the lower competition for nutrients (Schulz and Boyle 2005). Nevertheless, certain fungi produce antifungal molecules that inhibit the growth of other species present in the same environment (Rai et al. 2013; Kumar and Kaushik 2013). The fact that *Colletotrichurn demantium* inhibits the growth of *Curvularia lunata* by producing antifungal molecules (Gond et al. 2012) explains, at least in part, why we did not isolate both species from the same host: the former was exclusively identified in *A. leptostachyus* collected at both sites, while the latter was exclusively identified in *V. divergens* collected at site 1.

The endophytic fungal community present at a given time varies according to location, seasonality, and vegetation surrounding the host plant (Schulz and Boyle 2005; You et a. 2016; Raghavendra et al. 2017). The cohabiting species 1VD and 1AL shared a larger number of endophytic fungal species than the same host plant species located at different sites (1AL and 2AL). The genetic variability among the strains belonging to the same species isolated from the cohabitants 1VD and 1AL indicated that seven of the nine fungal species connected the roots of both host plants at the sampling time. Such fungi-mediated root connections provide nutrient exchange that benefits both host plants (Babikova et al. 2013; Porcel et al. 2012).

It is estimated that one gram of soil contains approximately 100 mg of fungal hyphae (Whitfield 2007). The presence of hyphae connecting roots of different host plants, forming a hyphal network, is extremely important for the maintenance of plant species in a given environment because it may transfer disease resistance signals (Song et al. 2010; Witzany 2010; Yang et al. 2014). Endophytic fungi favored the maintenance and dispersal of the invasive forb *Centaurea stoebe* in a given area of North America (Aschehoug et al. 2014). *V. divergens* is also considered an invasive species in the Pantanal due to its ability to disperse and develop rapidly (Pot and Pot, 1994; Nunes da Cunha and Junk 2004; Dalmolin et al. 2013).

We detected a low index of endophytism of the fungal strains isolated from the roots of *A. leptostachyus* and *V. divergens* towards *C. lanceolatum* and *V. divergens* seedlings; only certain strains behaved pathogenically in the *V. divergens* seedlings. However, we can not conclude that the other isolated strains are unable to infect the host, because the dying technique used may not have allowed accurate staining and visualization of the roots, and/or the laboratory environment may not have successfully imitated the natural environment to which the fungi are adapted (Mandyan et al. 2010). Fungal isolates may behave endophytically or pathogenically depending on the conditions provided to the host and fungus (Junker et al. 2012); the conditions provided here were stressful for the host plant and favorable for the fungi growth (Mandyam et al. 2013). This finding evidences the delicate balance that exists between fungal pathogenicity and host defense, which determines whether an interaction is asymptomatic or not.

Microsclerotia may be absent *in vitro* due to many reasons, such as the host plant species, incubation conditions, and length of interaction (Mandyan et al. 2010). During the early developmental stage, the formation of microsclerotia is apparent by detection of dark hyphal fragments with Sudan IV staining (Yuan et al. 2010a), but colonization is not evident with Trypan blue staining (Barrow and Aaltonen 2001). All of the DSEs isolated from *V. divergens* and *A. leptostachyus* belonged to the classes Sordariomycetes and Dothideomycetes, and were distributed in the orders Pleosporales (three morphotypes), Hypocreales (three morphotypes), Magnaporthales (three morphotypes), and Sordariales (one morphotype), and the family Papulosaceae (one morphotype). The orders to which the DSEs identified in this study belonged are commonly reported in the literature, such as Pleosporales (Xu et al 2015; Bokati et al. 2016; Bonfim et al. 2016; González-Teuber et al. 2017; Spagnoletti et al. 2017), Hypocreales (Mandyam et al. 2013; An et al. 2015; Bokati et al. 2016; González-Teuber et al. 2017), Magnaporthlaes (Ban et al., 2012; Knapp et al. 2012), and Sordariales (Sieber and Grüning, 2013; Bonfim et al. 2016).

The morphotypes isolated from *A. leptostachyus* that we considered as DSEs exhibit the same behavior in different hosts, as reported by other authors (Ban et al. 2012; Jumpponen and Mandyam 2013; Knapp et al. 2012; Mandyam et al. 2013; Spagnoletti et al. 2017). *Fusarium solani* is known as an important plant and human pathogen (Maroua et al. 2017; Bissan et al. 2017) that acts as DSE in *Salix variegata* (An et al. 2015). However, a fungus belonging to a genus or species that is frequently cited as pathogenic may not express the same behavioral interactions with a particular host plant species (Ek-Ramos et al. 2013; Selim et al. 2017).

The DSE species isolated from *V. divergens* that exhibited microsclerotia (*C. lunata* and *F. oxysporum*) were previously reported as pathogenic (Kanhed et al. 2014; Tobih et al. 2015). The genus *Nectria*, considered as DSE in this study, also displays the endophytic (Banhos et al. 2014; Toghueo et al. 2017) and saprophytic (Hirooka et al. 2011) behaviors. These reports evidence the fine line between pathogenicity and symbiosis among fungi and their hosts (Junker et al. 2012; Fesel and Zuccaro 2016). The genera *Fusarium* and *Curvularia* act as DSEs in different hosts (Jumpponen and Mandyam 2013; Mandyam et al. 2013; Priyadharsini and Muthukumar 2017; Spagnoletti et al. 2017), demonstrating their ability to colonize the roots of a range of hosts in different stressful environments.

One morphotype (1VD22) of *F. oxysporum* isolated from *V. divergens* behaved as DSE, while another morphotype (1VD21) behaved pathogenically; the morphotypes 2AL11 and 2AL66 of *Magnaporthales* sp.1 isolated from *A. leptostachyus* also behaved as DSE and pathogen, respectively. Despite the molecular identity, small genetic differences between strains may confer to a mutualistic isolate the ability to manifest disease symptoms (Delaye et al. 2013; Fesel and Zuccaro 2016; Selim et al. 2017). The genetic control and chemical signaling underlying this conversion remain to be unraveled (Suryanarayanan 2013; Mandyam and Jumpponen 2015; Selim et al. 2017), although it is not surprising that isolates belonging to the same fungal species establish different relationships with their hosts. The DSEs identified here exhibited a low degree of host specificity, and differentiated microsclerotia when inoculated in *V. divergens* and *C. lanceolatum.* To date, the scientific literature has suggested that DSEs exhibit little or no degree of host specificity (Rodriguez et al. 2009; Mandyam and Jumpponen 2015; Potshangbam et al. 2017).

Regarding the soil analyses, the high P level at site 2 was probably associated with the elevated occurrence frequency of fungi of the genera *Aspergillus* and *Penicillium* in *A. leptostachyus* and *V. divergens* roots. Due to their high efficiency in solubilizing phosphate, these fungi can double P concentration in an environment deficient of this element (Li et al. 2016c; Nelofer et al. 2016; Priyadharsini and Muthukumar 2016). A total of 35, 25, and 21 strains belonging to these genera were isolated from 2AL, 1AL, and 1VD, respectively.

The soil spore counting resulted in a density of 178-529 spores/g dry soil, which is greater than the range reported by the scientific literature (2-745 spores per 25 g of soil) (Muthukumar and Vediyappan 2010; Oliveira and Oliveira 2010; Priyadharsini et al. 2012; Uma et al. 2012; Birhane et al. 2017). The higher spore density in the rhizospheric soil of 1AL and 2AL indicates that the host plant species is an important determinant of the quantity of AMF spores (Yang et al. 2014; Birhane et al. 2017), and that soils containing grasses have higher spore density, as compared with soils where dicotyledonous trees grow (Muthukumar and Vediyappan 2010).

The soil nutrients, plant species and age, root density, AMF propagule counts and colonization efficiency are some of the factors that may affect mycorrhizal colonization (Smith et al. 2011; Birhane et al. 2017). All of the analyzed fragments of *A. leptostachyus* and *V. divergens* roots that were colonized by AMF and DSE presented structures typical of such fungi. In particular, resting spores were much more abundant in 1AL than in 2AL and 1VD.

The fungal structures non-septate hyphae, arbuscule, and vesicle have been identified in the root system of 12 plant species that inhabit pasture and dry soil (Birhane et al. 2017). As arbuscules are key sites in the movement of carbon and organic and inorganic nutrients (Porcel et al. 2012), the higher occurrence frequency of these fungal structures in 1AL root samples indicates that the associated partners often use them to exchange nutrients. On the other hand, the lower quantity of arbuscules in 2AL root samples may be related to the high soil P concentration, which is a limiting factor for the formation of these structures (Smith et al. 2011). The number of vesicles detected in the cohabiting species 1AL and 1VD were statistically equal in quantity. These lipid-rich structures play storage function and can act as propagules (Biermann and Linderman 1983; Olsson et al. 2011). Comparison among the septate hyphae colonization of roots of cohabiting plants has evidenced a higher number of isolates in *Deschampsia flexuosa* than in *Trientalis europaea* (Tejesvi et al. 2013).

In summary, a total of 391 endophytic fungal strains were isolated from three host plant species present in wetlands – 1VD, 1AL, and 2 AL. The fungi were classified into three phyla distributed in 36 genera and 81 species. Ascomycota was the dominant phylum in the three host plant species; Sordariomycetes and Leotiomycetes were the most common classes in *A. leptostachyus* and *V. divergens*, respectively; and *P. macrospinosa* and *Phialocephala* sp. were the dominant species in *A. leptostachyus* and *V. divergens*, respectively. *P. macrospinosa* and *P. javanicum* were the dominant species in 1AL, and *Neosartorya pseudofischeri* was the dominant species in 2AL. The host plant species influenced the endophytic fungal community composition more strongly than the sampling site, and cohabiting plant species shared hyphae of fungi belonging to the same species and most likely the same strain. *A. leptostachyus* established more interactions with endophytic fungi, as indicated by the high occurrence frequency of fungal structures in its roots. Finally, the DSEs identified belonged to the orders Pleosporales, Hypocreales, Magnaporthales, and Sordariales. Together, the data here reported provide basic knowledge on the symbiosis of plants inhabiting wetlands and their associated fungi.

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Terra Preta Nova:
Environmental Biotechnology, Green Patent

Antônio Pinheiro

Introduction

To analyse the production and use of research results of the Museu Paraense Emilio Goeldi (The Emilio Goeldi Museum of Parana - MPEG), in the period from 2003 to 2012, we identified three patent applications and two trademark applications made directly by the institution, and another three patents in joint-ownership with other institutions. We opted for one of the patent applications to establish its relevance as a green patent, Terra Preta Nova (TPN). The technology is symbolic to the discussion about the production of the knowledge and use in product, process and service (biobusiness); the application of the results in the form of technology can contribute to the process of solid waste management.

Studies on the process of terra preta (black earth), terra mulata (mulatto earth), involve from the microfauna process in the soil, which involves fungi and bacteria that accelerate the decomposition process of organic matter. In the case of Terra Preta Nova, these elements are present, but what stands out in its process is the incidence of the different substrates that the process can transform, and the presence of charcoal (biochar), which prevents gas emissions and expands the use of the technology to different areas and levels of application – from an artisanal to industrial scale, and with capacity for carbon capture due to the charcoal. And therefore its application is focussed in the field of municipal solid waste.

According to a World Bank study conducted by Hoornweg and Bhada-Tatav, the world generated 1.3 billion tons of solid waste, and the amount generated *per capita* almost doubled in the last decade. The quantity of solid waste generated per person in the planet's cities increased from 0.64 kg per day to more than 1.2 kg per day between 2003 and 2013. There are projections that indicate that, by 2025, 4.3 billion urban inhabitants will generate about 1 kg of solid waste each per day, totalling 2.2 billion tonnes per year (Hoornweg and Bhada-Tata 2012).

In Brazil, solid waste collection in the different regions consumes resources to the amount of R$ 1.9 billion (North); R$ 5.9 billion (Northeast); R$ 1.15 billion (Centre-West); R$ 14.6 billion (Southeast) and R$ 3 billion (South) (Abrelpe 2014). Management and appropriate technology can transform solid waste into economic resources, according to reverse logistics, that involves a process from selective collection, through to packaging collection points to landfill site. Throughout the world, this has created a market to satisfy the adequate disposal of organic solid waste and its processing into products, motivated by national solid waste policy -NSWP (Abramovay et al. 2013; Abrelpe 2013; Iswa/Abrelpe 2010; CNI 2014), converging with World Bank actions (Hoornweg and Bhada-Tata 2012) that support public policies for managing solid waste and for facing the increase in pollutants in the world and pollution of the planet.

According to Cammarota (2013), pollutants are waste generated by human activities and that cause negative environmental impact, an undesirable change: pollution. Pollution is linked to the concentration or quantity of residues present in the air, water, ground. The control of which is through the establishment of air quality standards and indicators in the quality of air (concentration of CO, NO_x, SO_x, Pb), of water (concentration of the O_2, phenols, Hg, Ph and temperature) and soil (erosion rate) that we want to respect in a given environment (Cammarota 2013; EPA 2015; 2014).

Pollution, though localised, while environmental damage is diffused in nature, it affects the whole planet and its effects are global; this has forced a greater awareness, which ranges from the media with editorial offices in environmental fields, issues on political agendas, environmental groups and in scientific and technological actions, that describe and seek solutions to the problems. Research findings into biochar (terra preta) can contribute to solutions to the problem of the accumulation of organic solid waste, which includes the fight against greenhouse gas emissions, the pollution of water bodies by slurry and the recovery of areas degraded by erosion, with the transformation of organic solid waste into resources with market and social value.

The Museu Paraense Emilio Goeldi research results utilisation were analysed and the research results with biochar, Terra Preta Archaeological (TPA) were identified, the experiment that generated Terra Preta Nova (TPN) – a composting process developed from the replication of terra preta. Terra preta or terra preta de índio are soils that were formed from prehistoric human occupation. They are highly fertile and stable - true composting deposits of plant and animal material

(Kämpf and Kern 2005; Kern et al. 2009; D'Aquino et al. 2003; Kern et al. 2007; Lehmann 2007; Monteiro et al. 2009; Winklerprins 2014). They are given different nomenclatures as a basis for biochar technology. Balee (2010) introduces several nomenclatures given to terra preta (Table 1)

The potential of Terra Preta Nova, the possibilities as environmental biotechnology, product generation, green technology, with a market (innovation) led us to search on the Internet for "biochar + pyrogenic + carbon + charcoal", and we identified research and development initiatives for similar products to the "composting process of Terra Preta Nova - TPA". This allowed us the possibility of relating TPA to biochar technology and add it to the use of two trademarks: Terra Preta Nova (TPN) and Terra Preta Arqueológica (TPA), and to realise that, like all biochar technologies, it relates to the improvement of solid waste mitigation, pollution and its effects. And this highlights its relevance to reducing climate change, by being able to sequester carbon from the ground and not emit methane gas, reducing greenhouse gas effects.

The three actions selected (Table 2) serve as a reference to demonstrate the relevance of Terra Preta Nova as a technology that can contribute to solid waste management processes, as a product and with a value (price and market).

We related these three initiatives to World Bank research (Scholz et al. 2014) that identifies technology initiatives that are similar to terra preta (biochar) in different parts of the world, having as reference small producers' associations, highlighting the extent to which TPN can be applied, from small to large scale with socio-environmental effects, since it can generate jobs and income for waste collectors and can be applied on an industrial scale in landfill sites, complimenting selective collection and the transformation of solid waste into biochar.

Terra Preta Nova (Tpn), Composting Process, Environmental Biotechnology - Green Technology

The terra preta experiment by the Museu Paraense Emilio Goeldi in the town of Tailândia - Pará, which began in 2002, involves waste from sawmills, butchers, and others; its results led to the composting process, subject of the green patent - environmental biotechnology, submitted by MPEG. The FOS 2007 areas of knowledge (OECD), item 2.8, establishes the scope of the application of environmental biotechnology, *biotreatment,* and environmental management attainment.

Table 1. Terra Preta Nomenclatures (Dark Earth).

ACRONYM	TERM	SYNONYMS	COMMENT
ADE	Amazon Dark Earth, Terra Preta Amazônica	Relic anthrosols (Lehman et al., 2.8)	Includes all anthrosols of Amazonia
ABE	Archaeological Black Earth (e.g., Woods and Glaser, 2.1; Ruivo et al., 2.7)	Same as above	Same
TP	Terra preta or terra preta de índio	"Kitchen midden" (Sombroek, 1966); cultic archaeoanthrosol (Neves et al., 1.3)	Tierranegra in Colombia (Mora 1.11) charcoal black A horizon soils with potsherds and lots of soil organic matter
TM	Terra mulata grey-brown garden soil (Sombroek, 1966); also archaeoanthrosol (Neves et al., 1.3; TropischePlaggenböden (Kämpf et al., 1.5), tierraparda in Colombia (Kern et al., 1.4) "transitional" soils (Kern et al., 1.4); i.e., some authors see these as transitional between TP and TC (terra common) [not an anthrosol]	TM terra mulata soil grey-brown garden soil (Sombroek, 1966); also archaeoanthrosol (Neves et al., 1.3; TropischePlaggenböden (Kämpf et al., 1.5), tierraparda in Colombia (Kern et al., 1.4) "transitional" soils (Kern et al., 1.4);	"Transitional" soils (Kern et al., 1.4); i.e., some authors see these as transitional between TP and TC (terra comum) [not an anthrosol]
TPN	Terra Preta Nova	"New Black Earth" experimental anthrosol	Biochar = pyrogenic carbon = charcoal Source: BALEE (2010).

Source: BALEE (2010).

Table 2. Synthesis of Initiatives for Product Development from Terra Preta (Biochar).

International Biochar Initiative (IBI) - USA/World	Terra Preta Ek/Terra Preta GmbH (Germany)	NEWAPP - Project European Union
It emerged in 2006 at the World Congress Soil Science (WSSC) in Philadelphia - USA. Involving academic institutions, entrepreneurs, investment banks, NGOs, federal agencies. It is dedicated to demonstration actions, PD&I implementation, R&D and commercialisation of biochar technology. Its action includes partners in different parts of the world.	Terra Preta Ek and Terra Preta GmbH (Germany) Terra Preta Ek has been researching and producing terra preta products for over 10 years, and has given rise to Terra Preta GmbH in 2012. Terra Preta GmbH develops and manufactures products from so-called terra preta, terra preta soils with charcoal (biochar) and other ingredients, but also the production method. The company has accumulated the commercial experience of Terra Preta Ek and has product patents, which made it attractive as a partner to the NEWAPP Project	NEWAPP is a research project focusing on hydrothermal carbonisation (HTC) of biomass residues. The project involves the complete selection of wet biomass streams and their sources at European level and the preparation of a market study for potential HTC carbon products.

Source: Author's own from www.iniciativebiochar, www.terra-preta.de and http://www.newapp-project.eu/en/.

Environmental biotechnology is the application of biological techniques to solve and/or prevent problems related to environmental depletion (decontamination, soil recomposition, etc.), involving knowledge and multidisciplinary research, attaining normative and legal aspects (environmental standards, bioethics), scientific and technological aspects, and it has important economic and social impacts (Cammarota 2013; OECD, 2007).

Environmental biotechnology processes generally consider five basic elements: (i) the toxic compound (or mixture) to be disposed of or reduced in concentration; ii) the medium in which the compound is present (air, liquid, solid); (iii) the characteristics of the place or chain that contains it; iv) the biological agent that will lead to biodegradation (micro-organisms, enzymes, plants, microbial consortia); and v) the conditions of the process (temperature, pH, humidity, aerobic or anaerobic conditions) (CAMMAROTA, 2013, p.5). According to Adjiman ([S.d]), it's possible to differentiate environmental biotechnology from industrial biotechnology (Table 3).

Table 3. Differentiation table between environmental biotechnology and industrial biotechnology.

Variable	Environmental Biotechnology	Industrial Biotechnology
Objectives	Nutrient Minimisation	Productivity Maximisation
Biomass	Mixture (consortia)	Specific strains
Process types	Continuous	Batch - Automated Task.
Substrates	Mixture (waste)	Pure and well defined
Optimisation	Ecological selection	Genetic engineering

Source: ADJIMAN, [S.d].

Terra Preta Nova is inserted as - environmental biotechnology due to generating the minimisation of nutrients, and involving the action of different agents - the biological agent that will lead to biodegradation, the mixture of residues and the conditions such as: temperature, pH, humidity, aerobic or anaerobic conditions, in which the presence of charcoal, that reduces gas emissions and enhances carbon capture, is emphasised, and in another aspect the process is continuous where the characterisation of the product is not homogeneous, due to there being no selection of the biological agent which leads to biodegradation, the selection is natural. There are international initiatives aimed at product certification in order to obtain a certain safety level regarding the product generated and its use, where the focus is not the biological agent (bacteria, fungi, enzymes) but the concentration of biochar.

There are two biochar certification programs, the *Biochar Certification Program* from the IBI and the *European Biochar Certificate* (EBC) (IBI, 2014, EBC, 2014). They have established partnerships with where there is convergence, although there are small differences in the definitions of biochar, and in the *standard* elements for certification (Tables 4 and 5).

Table 4. IBI and EBC - Different Definitions for Biochar: Product/Product and Process Characteristics.

	IBI	EBC
Definition of Biochar	Biocarbon - solid material obtained from thermochemical conversion of biomass in an oxygen limited environment.	Biocarbon, a coal-like substance obtained from a sustainable pyrolysed biomass process under controlled conditions and which is used for any purpose that does not involve the rapid mineralisation of CO_2.
Minimum Concentration of carbon	The organic carbon content of the biocarbon should be greater than 10% of the dry mass (DM). The biochar is divided into 3 classes based	The carbon content of the biochar should be greater than 50% of the dry mass (DM). Organic pyrolysed material with a carbon content of less than 50% is classified as Bio-Carbon-Minerals (BCM).

Table 5. Standard Assessment Components for IBI and EBC Certification.

Parameters of the Biochar products or process	IBI	EBC
Restrictions on raw material (clean biomass)	X	X
Sustainability of the raw material (production and transportation)	X	X
Certification of the pyrolysis process (energy efficiency, fossil fuel use, energy generation, emissions).		
Verification occurs with inspection of pyrolysis facilities.		X
Toxic Analysis: • Thermal production of polycyclic aromatic hydrocarbons (PAH) and dioxins • Origin of raw materials - mainly metals	X	X
Stable carbon content in biochar for carbon accounting	X	X
The biochar properties include: pH, liming, particle size, particle density, humidity, surface area, nutrients, formation of volatile matter, ash formation ...	X	X
Division of properties into the basic and advanced optional tests required	X	X
Grade 3 - Biocarbon, base analysis and carbon content	X	X
Grade 2 - Biocarbon evaluated on the basis of emission limit levels of polycyclic aromatic hydrocarbons (PAH) and metals		X

Wilson Biochar Associates; IBI - Biochar Stands 2.0 (2014); EBC-guidelines 4.1 (2014).

The Composting Process and the trademarks Terra Preta Nova (TPN) and Archaeological Black Terra (TPA), related to the new technology

The experiment that generated Terra Preta Nova (TPA) supports the establishment of 17 different types of organic compounds, from different organic residues discarded in small or large quantities (MPEG, 2012). The application of the technique, which satisfies both rural and urban areas, is a complementary technology to the selective collection and separation of waste (CNI, 2014; UNEP, 2013; ISWA; ABRELPE, 2010). It reproduces the biological process occuring in the natural environment to waste produced by traditional populations, identified on archaeological sites, without selecting the biological degradation agent (fungus, bacteria, enzymes), and inserting charcoal.

MPEG enabled the economic use of the technology by protecting the search results with patent application and with the registration of two trademarks. The patent is considered to be green technology. According to INPI, green patents involve environmentally friendly technologies or so-called green technologies, and these technologies are prepared and presented in an inventory published by the World Intellectual Property Organization (WIPO), which in Brazil is regulated by Resolution INPI PR Nº. 83/2013, article 2, items 4 and 5.

The composting process, in replicating terra preta, terra preta do índio – biochar/charcoal follows a pattern of carbon concentration close to that found in processes established by prehistoric humans. The distribution of organic matter in the soil is variable, both in depth (along the soil profile) and horizontally. The distribution of carbon in the soil depends on several factors, such as soil type, relief, ground cover or soil use, climatic conditions, predominant natural vegetation in the area, land use and management practices, among others (Madari et al. 2009). And this influences the establishment of the product profile of Terra Preta Nova, which includes carbon concentration.

Technological profiles of the composting process according to the claims of the patent deposit and trademark registration - mixed and nominative

i. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised by soil management with the inclusion of organic residues of vegetable origin (coal, sawdust and crushed blade residues) and animal (butchery waste) - Figure 1;

ii. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised by providing at least 17 possibilities coming from different organic substrates for the production process of organic substrates and a final product (organic compound) called Terra Preta Nova (TPN);

iii. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised by the non-generation of methane gas and carbon capture effect;

iv. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised as green technology applicable to post-consumption reverse engineering, regarding the implementation of reverse logistics systems;

v. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised by allowing for small and large scale, coming from domestic actions through to industrial scale, as well as the use of this technology in areas near to urban centers;

vi. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND, characterised by the use of ashes (derived from incineration) and waste from burning (carbon - charcoal);

vii. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised by the layouts of industrial landfill models;

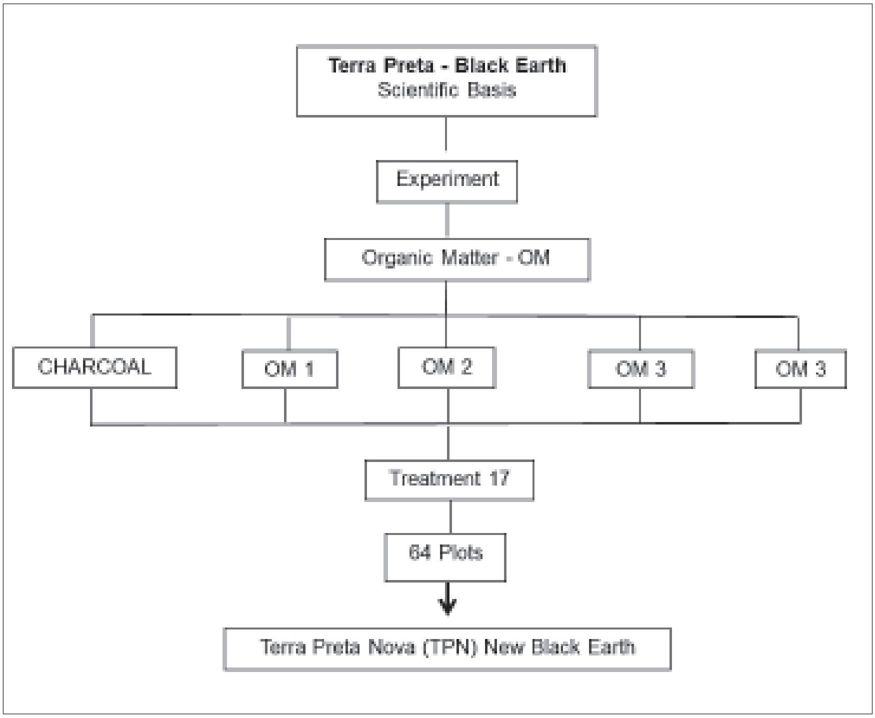
viii. “PROCESS OF TRANSFORMATION OF SOLID WASTE INTO ORGANIC COMPOUND”, characterised by the process of soil recomposition;

ix. “TRANSFORMATION PROCESS of SOLID WASTE INTO ORGANIC COMPOUND”, characterised by the production of organic compound used as fertilizer, low carbon green technology called Terra Preta Nova-(TPN - mixed trademark; nominative trademark - Archaeological Terra Preta) Figure 2; Table 6;

From the TPN experiment the profile, describing the chemical and physical properties of the final product, is identified, and must be determined from each installed process. The biological aspect comes from the types of substrates, which define the product profile generated, and this can be understood from Figure 2, which illustrates the composting process. As for the characterisation of Terra Preta (biochar) Rezende, Angelo and Mangrich (2011), from Matovich (2011), estimate that the optimum amount of biochar in soils for agriculture varies between 1 and 5%, and report that Matovich took as a value for the study that developed the average of 3% of biochar in the first 30 cm of soil column, which leads to a dosage of 13.5 t/ha (1 ha = 10000 m^2).

Terra Preta Nova, while being biochar (pyrogenic carbon/charcoal) has special properties of not emitting methane, and even of capturing carbon, in its process of transformation of the substrates, as one of the substrates used is charcoal, so it fits with green technology. The sum of filing of the patent and registration of the mixed trademark Terra Preta Nova (TPN, Figure 1) and the nominative trademark Terra Preta Arqueológica (TPA) (INPI, 2014) enables its social and economic use in products applied to processes and technological services, as a reference of quality and environmental appeal. The trademarks related to the composting process allow the valorisation of the scientific and technological know-how services that the Museu Paraense Emilio Goeldi (MPEG) owns and can offer.

Figure 1. Process of generation of Terra Preta Nova.



Source: Goeldi Museum/INPI - Patent application process.

Figure 2. Mixed trademark - Terra Preta Nova.

Source: MPEG (2012) Application for registration of Mixed Trademark - MPEG.

Table 6. Trademarks: Terra Preta Nova/Terra Preta and Terra Preta Arqueológica. **Terra Preta Nova -** Process N°. 907743501, application for registration of the Mixed Trademark - MPEG**Terra Preta Arqueológica -** Process N°. 907744575, registration request of the Nominative Trademark - MPEG

OBJECTIVE	Soil restoration and/or generation of organic compounds/ Reverse Engineering.
CONTACT	NIT Amazônia Oriental
AREA OF PRACTICE	Green Technology / Reverse engineering of organic waste.
DESCRIBE THE PROJECT AS BUSINESS APPLICATION	The concession for Use of the Trademark connects to the licensing of the Patent Process involving the production of organic substrate which serves both to restore degraded areas, landfills and smaller applications such as generation of organic substrates used in agriculture (organic fertilizer) and or gardening - condominiums, shopping centers, technology parks.
TRANSFER VALUE*	To be defined from the business model process and the activities to be developed by Museu Goeldi in completing the the technology transfer.
UTILITY TO THE MARKET	The use of the trademarks and of the process applied to waste management and reverse engineering (organic waste). It can be applied in condominiums as a way of processing their organic waste, in landfills, in business segments (supermarket chains and other companies that generate organic waste) and non-governmental organisations and cooperatives of waste collectors.
EXPECTED RETURN	Technology transfer as a way for the economic exploitation of research results to be made feasible and contribute to sustainable development by generating income and occupation.

Source: NIT Amazônia Oriental/SEBRAE Model of Prospecting Projects for Technological Showcase Presentation.

Terra Preta Nova - market asset: product with market and value

Terra Preta Nova (TPN), as it is environmental biotechnology for green patent, it can meet the most artisanal scales (involving waste collectors cooperatives) as well as large scale industrial use; the claims made in the patent application give an idea on the scope, however only the market, the acceptance of the technology by the consumer and their willingness to pay the price can attest to its share value as a product.

The identification of a possible market value for Terra Preta Nova products is the best way to identify one of the main elements of its economic viability: the price.

Pricing is part of a broader process, which in order to reach the effective market requires a business plan; in this process, three applications represent the final objectives of a valuation: (i) technology commercialisation and licensing; (ii) risk analysis in R&D investments; and (iii) prioritisation of R&D projects. The three international actions we have selected, relating to the generation of products from terra preta (biochar), have their particularities and give us the scope of the economic-technological possibilities of the Goeldi Museum technology. The International Biochar Initiative (IBI) has affiliates worldwide and has established a certification seal for biochar products; it maintains a relationship with The European Biochar Certificate (EBC), from 2012 (EBC, 2014), and does not publish product prices, only the certification process.

From the best known methods for general asset pricing and, in particular, for new technologies, we have identified four approaches: (i) valuation based on development cost; (ii) valuation using multiples; (iii) discounted cash flow; and (iv) theory of real options. We opted for the valuation using multiples method approach. The method has its limitations and in spite of them is often used because of its quick response and simplicity of approach, which means even a non-expert can use it to show a lay person what has been practiced, such as price and possibilities of technology markets (Baek et al. 2007; Wipo 2005; Reilly et al. 2009; Santos and Santiago 2008; Bioempreede, 2011). For our aims, the simplicity of approach of the multiples method is sufficient for us, because he demonstrates that Terra Preta Nova generates a similar product on the market and with an established retail price and market.

The multiples method is based on surveying the technological profile, to identify similar products and prices charged. This provides access to the price of similar products to those generated by its technology and have a parameter with which to relate the cost of their process and the possibility of a price to internalise it and make a profit.

Terra Preta GmbH (Terra Preta Ek) in Germany has two aspects: an individual initiative (Terra Preta Ek), which generates another one, which is a collective action, the NEWAPP Project, which aims to use biochar technology as a solution for solid waste problems in the European Union. Of all the experiments, only Terra Preta Ek reveals the price charged for terra preta products, and it shows us that this price has relevance, since we understand that its expansion, through Terra Preta GmbH, in addition to the NEWAPP Project, satisfies two interests: company expansion and adding experiences to NEWAPP, by already having a market and an established price for the product. Pricing is one of the most difficult processes in relation to generating an innovation – a product made available to the market.

The market and price aspects for the products generated were related to Terra Preta Ek's commercial initiatives and its participation in a consortium to consolidate a technology (biochar), for large scale application; its similarity with Terra Preta Nova serves to identify the real possibilities of this technology on the market, globally. Using this reference, due to the similarity of the product sold with Terra Preta Nova (TPA), we realised the commercial potential of the new technology and its price. The price charged by Terra Preta Ek, for the sale of terra preta can be seen in Table 7.

Table 7. Products and values of Terra Preta (Terra Preta Ek).

Product	quantity	Price
Terra preta	5 litres	•49.00
Terra preta	10 Litres	•89.50

Source based on the prospectus of the company Terra Preta (2017). Available at http://terra-preta.de/terra-preta-5-liter-bestellen/ acesso 28/04/2017

The experience of Terra Preta Ek and their results led to the creation of Terra Preta GmbH, that joined NEWAPP, an activity supported by the European Commission that aims to consolidate a new recovery path for wet biomass waste streams, such as the organic fraction of municipal solid waste, sewage sludge and other biodegradable waste, such as wastes from the food industry, vegetable or agricultural waste, sludges from waste or wastewater treatment with the application of HTC technology , which allows the generation of charcoal through pyrolysis. Part of this initiative (consortium):

- The Spanish National Research Council (*Agencia Estatal Consejo Superior de Investigaciones Científicas* -CSIC) – Institute of Chemical Technology - Research center founded in 1990 by the CSIC and the Politecnic University of Valencia - UPV (itq.upv-csic.es);
- The Associação das Cidades e Regiões para a Reciclagem e Gestão Sustentável dos Recursos (*Association of Cities and Regions for Recycling and Sustainable Resource Management* - ACR+ (www.acrplus.org);

- Associação Europeia das Indústrias de Biomassa (*European Industrial Biomass Association* - EUBIA). International non-profit association with headquarters in Brussels, Belgium. Its main objective is to support European biomass industries at all levels by promoting the use of biomass as a source of energy, the development of innovative bioenergy concepts and the encouragement of international cooperation in the field of bioenergy (www.eubia.org);

- Federação dos Produtores de Matérias Primas Secundárias e Gestão de Resíduos (*Federal Association for Secondary Raw Materials and Waste Management* - BVSE). It represents more than 670 small and medium enterprises that seek to adapt and perfect their shares and performance to meet the demands of the market and the environment;

- Ingelia - A technology based company located in Valencia - Spain, owner of an innovative process for hydrothermal carbonization of biomass (HTC process). Its objective is to develop sustainable projects and profit from biomass processing produced by the local population (www.ingeliahtc.com);

- |||UNTRANSLATED_CONTENT_START||| Universidade Técnica da Dinamarca (*Technical University Of Denmark* – DTU) (www.dtu.dk); |||UNTRANSLATED_CONTENT_END|||

- TTZ (*Technology Transfer Center Bremerhaven* - TTZ). Non-profit organization based in Bremerhaven, Germany. It provides technology transfer services and conducts customer-oriented research, development and training (www.ttz-bremerhaven.de);

- TERRA PRETA GMBH - Company located in Berlin, Germany; joined the consortium in September 2012. The Company arose from the Terra Preta Ek company, which has been researching and producing black earth products for more than 10 years (www.terra-preta.de).

The importance of Terra Preta Ek within the consortium lies in its experience in producing and selling products made from from Terra Preta over the last ten years. The company is developer and manufacturer of products based on the so-called black earth principle, black earth soils (biochar). It maintains a development sector to generate and protect innovative products and its terra preta products, which are already on the market,are used from greenhouses, small and medium-sized areas of gardens, outdoor spaces, for sowing, and it's expansion with the use in the production of seedlings for gardening and agriculture. The products were tested for their effectiveness, and the company Terra Preta obtained official permission from the German agricultural authorities to market and sell their products (Terra Preta 2015).

Black Earth Ek/Terra Preta GmbH holds a significant portfolio of trademarks and domain names, which gives it a unique position in the European market, especially in *e-commerce* activities (www.terra-preta.de). We consulted the Internet, through Google, on *Patents* using "Thermo - Terra Preta GmbH" and we identifiedapproximately 281 results, relating to "Patents and Trademarks", associated with Terra Preta GmbH; the company sells its products via the Internet (*e-commerce*) through direct purchase with pre-established quantities and values. Its importance for the NEWAPP Consortium reinforces its reference for the identification of the feasibility of a price reference for Terra Preta Nova, which allows a parameter so that, faced with a cost table as part of a business plan, if it reaches the value to be practiced, taking into account the local reality where the product will be produced and distributed.

Terra Preta Nova reaches other economic sectors and its socioeconomic relevance for the job creation and income stands out. A study led by Scholz et al. (2014) certified by the World Bank, identified promising biochar systems in different countries, with an emphasis on collective actions (cooperatives) as a basis for financing projects to develop them. The World Bank study demonstrates that biochar development is particularly relevant to development in national contexts to address the global challenges associated with food production and coping with climate change.

Technologies based on biochar range from rural spaces to urban spaces, its production process generates jobs and income for excluded groups (solid waste collectors); its use in degraded areas restores soil's capacity for use and agricultural production. The presence of charcoal (biochar) captures carbon, which is good in the combat against global warming, and avoids generating methane, allowing it to be produced near to major urban centres, whether on an industrial scale, or by cooperatives of waste collectors. The policies that have been deployed in different parts of the world favor a mixed form, which allows a vision of production on a large scale, and the participation of collectors cooperatives, and it is part of the National Solid Waste Policy (Brazil 2010), as well as in other countries.

Final considerations about the new technology: Terra Preta Nova

The Museu Paraense Emílio Goeldi owns an environmental biotechnology, Terra Preta Nova, biochar technology/green technology. It can assist national and global responses to some of the issues present in urban and rural areas: 1) How to manage problems related to waste production (solid organic waste) in urban centers, rural areas and industrial parks?; 2) How to equate the environmental issue without disregarding the social need for development? These issues involve a complexity of problems and actors involved.

It involves social agents (city halls, communities, businesses and researchers) and, on the other hand, the environment (vegetation, soil, climate and water). The solution requires: a) an interdisciplinary and systemic approach as a fundamental research tool; b) identification and articulation of management techniques that complement and enable the best direction and generation of satisfactory results for the transformation of the residues into products; c) interaction between the multiple actors that act in the production and processing of the waste; and d) identification/establishment of new users for the generated resources.

This scenario finds the answers in biochar technology, which Terra Preta Nova comes in. It can contribute to the process of solving these problems, as an element of environmental management, with applications in reverse logistics. It has technological, social and economic relevance (market value), and its application can be used at small and large scale, such as social innovation (social technology, TS) or of the market (industrial technology).

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Methodological baseline
for dendrochronological studies in the Pantanal

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Introduction

The Pantanal is a large wetland covering approximately 150,000 km^2 and is located in the center of the South America (Junk and Nunes da Cunha 2005, 2016). This complex wetland has a large macrohabitat diversity, that results into a high biodiversity, but with few endemic species (Junk et al. 2006). The Pantanal has the status of a National Patrimony by the Brazilian Federal Constitution and hosts also three of the 113 Ramsar sites in South America. However, its diversity of habitats has been threatened by deforestation transforming them into exotic pastures (Seidl et al. 2001; Junk et al. 2006; Ioris 2012), resulting in biodiversity loss (Harris et al. 2005; Alho 2011). In this context it is of fundamental importance to understand how tropical wetland forests respond to global change which requires information on their long-term dynamics.

Retrospective analyses of tree rings provide information on growth rates over the entire lifespan and can be applied in a large multi- and interdisciplinary field studying the ecology of tree species, population dynamics and long-term dynamics of tropical forests related to disturbance regimes, biomass productivity and climate-growth relationships. Furthermore, tree-ring analyses also allow reliable estimates of felling cycles, minimum logging diameters, and yield projections for timber resources that are the necessary empirical foundation of sustainable and adaptive forest management practices. These applications contribute to the conservation of tropical forest ecosystems and their multiple environmental services (Worbes 2002; Rozendaal and Zuidema 2011; Brienen et al. 2016; Schöngart et al. in press).

The existence of annual tree-ring in the tropics was taken into consideration at the end of the 19th century (Worbes 2002), but started in the tropical regions of America only about 50 years ago (Schöngart et al. in press). To test periodicity of tree-ring formation, Worbes (1995) suggests several independent dendrochronological methods to evidence annual tree-ring formation such as ring count of trees with known age, cambial wounding, dendrometers, radiocarbon dating, regression analysis of ring width and climatic data, densitometry and stable isotope analysis. Tropical trees form annual tree rings as a consequence of unfavorable growth conditions during a regularly occurring period of the year resulting into cambial dormancy. This can be either triggered by seasonal variation in precipitation, anoxic conditions in the rhizosphere induced by annual flood-pulses and seasonal variation in the salinity (mangroves) (Brienen et al. 2016; Schöngart et al. in press).

For the tropical regions of America more than 220 tree species comprising 46 botanical families form annual tree rings (Schöngart et al. in press). Annual tree-ring formation is evidenced in all Brazilian biomes, such as the Amazonian non-flooded forests and floodplain forests (Worbes 1989; Worbes et al. 1992; Schöngart et al. 2002, 2004, 2005, 2015; Dünisch et al. 2003; Brienen and Zuidema 2005, Assahira et al. 2017), the Atlantic forest (Callado et al. 2001, 2004; Lisi et al. 2001, 2008; Tomazello-Filho et al. 2004; Estrada et al. 2008; Marcati et al. 2008), the Cerrado (savannas) (Luchi et al. 2005; Locosselli et al. 2013, 2016), the Pampas (Seitz and Kanninen 1989; Oliveira et al. 2009; Silva et al. 2010), and the Caatinga (Locosselli et al. 2015; Mattos et al. 2015; Pagotta et al. 2015).Ishii (1998), Mattos (1999), Fortes (2006), Leite (2012), Machado et al. (2015) and Sallo et al. (2017) evidenced annual tree rings also for some species in the Pantanal and first dendroecological applications have been performed (Schöngart et al. 2011; Rosa et al. 2017). However, there is a demand for more information about growth periodicity and rates for tree species in this large wetland complex characterized by different macrohabitats (Nunes da Cunha and Junk 2014) to identify promising tree species for dendroecological applications providing relevant data for sustainable management and conservation of these endangered ecosystems.

The aim of the current study was (1) to assess the growth of some Pantanal tree species through cambium wounding (Windows of Mariaux) evidencing annual tree-ring formation, (2) to typify tree rings by wood anatomical features and their distinctiveness as a basis for future dendroecological studies and (3) to provide data on diameter growth rates and tree ages based on tree-ring analyses for characteristic tree species of the Pantanal.

Materials and Methods

Study Area

The study area is located in the Pantanal characterized by an aquatic phase occurring from December to April (Nunes da Cunha and Junk 2014) (Figure 1). In addition to the regular and predictable annual flood pulse, pluriannual episodes of the extreme flood and drought occurs in the region such as in the 1960/70s often accompanied by wild fire with dramatic impact on the biota (Junk et al. 2006) (Figure 1). The soil in the study area is hydromorphic, of sandy clay texture depending on the macrohabitat with Neosol, Gleysol and Plinthosol soil types prevailing at the sites (Couto and Oliveira 2010).

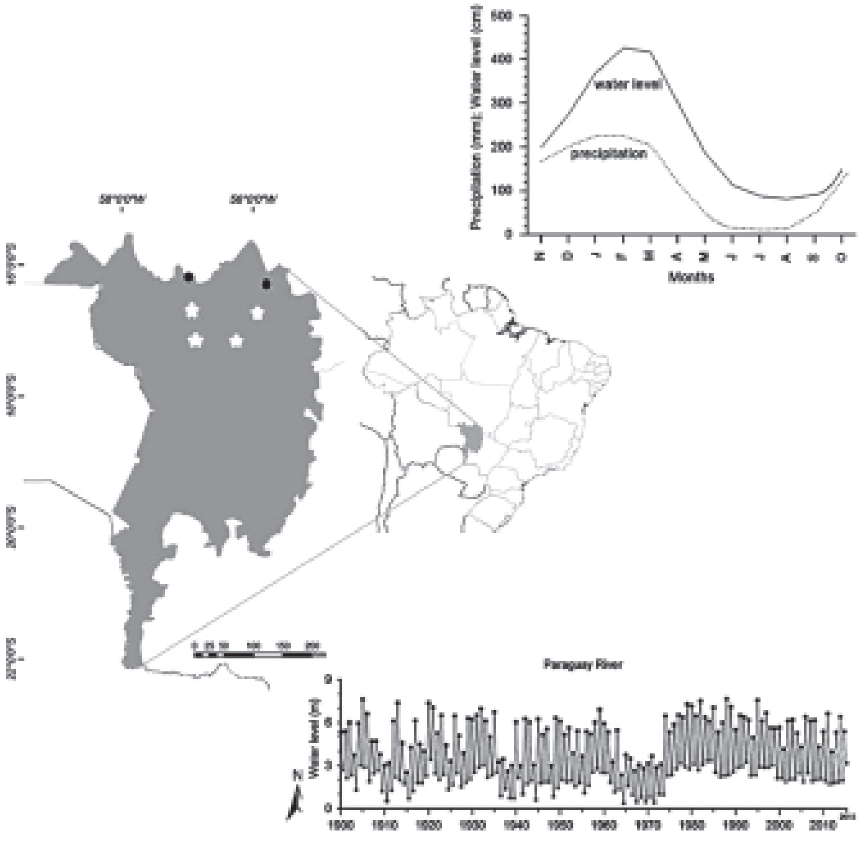

Figure 1. The study site in Pantanal (SESC Pantanal Natural Reserve) of Brazil. The inset figures indicate the seasonality in rainfall (city of Cuiabá) and annual water level fluctuations in Paraguay River, Ladário Country – MS, between 1900 and 2015 show multi-annual flood and drought episodes (data provided by the National Water Agency-ANA).

Sample Collection and Data Analysis

Wood samples were collected in different forested macrohabitats along the highways MT-456, in Pantanal of Barão de Melgaço, and MT-060, in Pantanal of Poconé. Samples of 67 species belonging to the wood collection from the Herbarium of Federal University of Mato Grosso were analyzed. Cambial wounding (Mariaux 1967, 1969, 1970) was applied at *Trichilia catigua* (in 1993), *Inga* sp. (in 1994) and *Albizia inundata* (in 1995) specimens. This method is based on injuries caused by mechanical wounding of the secondary cambium of few square centimeter, known as 'Mariaux Windows' printing a datable scar in the wood. After one year the samples were obtained to compare the number of formed tree rings after the scar and the duration of the monitoring period (Worbes 1995).

The samples were analyzed in the Dendroecological Laboratory of INPA in Manaus-AM and the Dendroecological Laboratory of Brazilian Central-West Biomes of the National Institute of Science and Technology in Wetland (Instituto Nacional de Ciência e Tecnologia em Áreas Úmidas - INCT-INAU/UFMT). The disc wood samples were gradually polished to improve the visual contrasts of the growth rings. The basis for dendrochronological studies in the tropics are the wood anatomical features of tree rings which can be typified by intra-annual variations of wood density (type 1), marginal parenchyma bands (type 2), alterations of fiber and parenchyma bands (type 3) or variations in the size and/or distribution of vessels (ring-porous; type 4) (Worbes 1989, 2002). However, these wood anatomical characteristics can considerably change within a single species depending on tree growth conditions (Worbes 1989; Schöngart et al. 2004). Furthermore we observed the distinctiveness of tree rings in different parts of the cross section (external, internal and central part), growth ring anomalies such as false rings (intra-annual wood density variations), and wedging rings (only formed in part of the cross-section) (Worbes 1989, 2002). All the samples were subjected to anatomical macro- and microphotography. The collected information were inserted in a database and evaluated to indicate the potential of each tree species for future dendroecological studies.

The growth rings were counted to estimate tree ages. Dividing the measured diameter at breast height (DBH) by the number of growth rings was applied to obtain mean diameter increment (MDI) rates for the trees (Worbes et al. 2003). For specimen with cores not including the pith age was estimated dividing the DBH by the obtained MDI (Worbes and Junk 1999; Schöngart et al. 2005). Wood density was estimated relating dry mass to fresh volume. Therefore the wood samples were dried at 105°C for 72 hours and dry mass was determined (Schöngart et al. 2005).

Results

Growth Periodicity of Pantanal Tree Species

The application of Mariaux´s windows showed that all three analyzed Pantanal tree species (*Trichilia catigua*, *Inga* sp. and *Albizia inundata*) formed one growth ring per year (Figure 2).

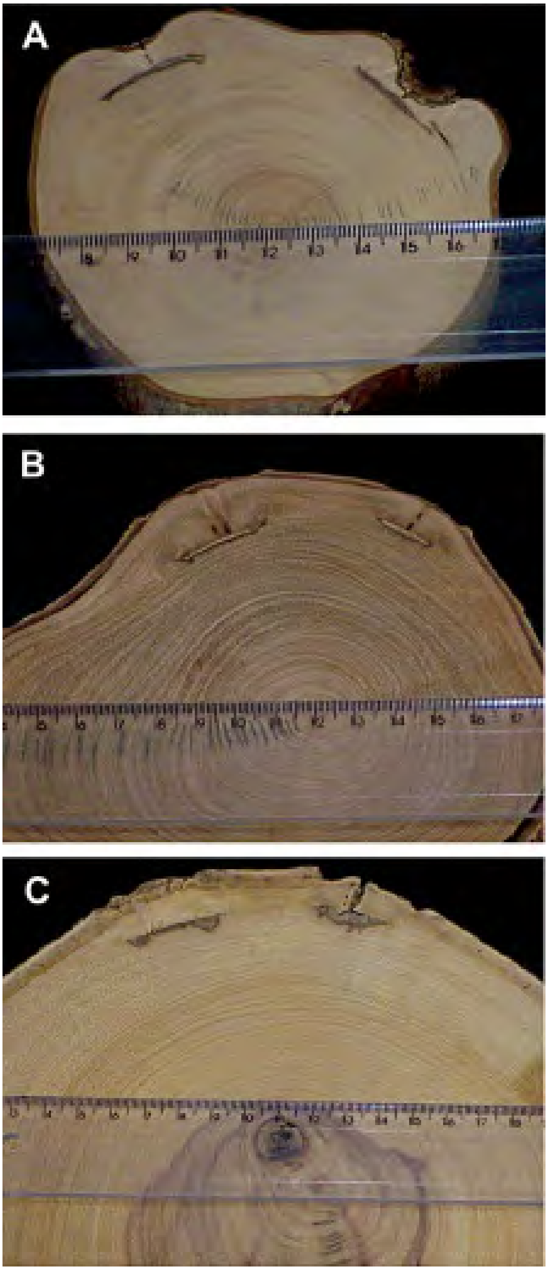

Figure 2. Cambial scars caused by mechanical incisions "Mariaux Windows" clearly showing a growth layer, in A) *Trichilia catigua* (in 1993), B) *Inga* sp. (in 1994) and C) *Albizia inundata* (in 1995).

Wood anatomical Structure of Tree Rings

The majority of analyzed tree species (34%) presented tree rings formed by intra-annual variation of wood density (type 1) (Table 1), commonly observed in species of the families Urticaceae, Euphorbiaceae, Lauraceae, Myrtaceae, Polygonaceae and Rubiaceae (Figure 3A). Species with tree rings limited by marginal parenchyma bands (type 2) belonged to the families Bignoniaceae, Fabaceae, Meliaceae and Sapindaceae (Table 1, Figure 3B) and comprised a third of the analyzed species. The families Chrysobalanaceae, Moraceae and Vochysicaeae have dominantly species presenting patterns of altered fiber and parenchyma bands (type 3) (Table 1, Figure 3C), while ring-porous species (type 4) were always associated with the other types, as in the case of some species of the families Bignoniaceae, Fabaceae and Vochysiaceae (Figure 3D).

Species showing distinctive tree rings were *Astronium fraxinifolium* and *Spondias mombin* (both Anacardiaceae), *Jacaranda mimosifolia*, *Handroanthus impetiginosus*, *Tabebuia aurea* and *Tabebuia* sp. (Bignoniaceae), *Albizia inundata*, *Cassia grandis*, *Copaifera langsdorffii* and *Hymenaea stigonocarpa* (Fabaceae) (Table 1). The species *Alchornea castaneifolia* (Euphorbiacecae), *Anadenanthera colubrina* (Fabaceae) and *Garcinia brasiliensis* (Clusiaceae) showed indistinct rings. The species *Sapium obovatum* (Euphorbiaceae), *Mouriri guianensisi* (Melastomataceae) and *Banara arguta* (Salicaceae) presented relatively indistinct rings. In the inner part of the cross section, we observed distinct rings at 63% of the total samples, 31% with relatively indistinct rings, and 6% presented indistinct rings. At the outer part of the transversal cut, 70% of all species showed distinct rings, 25% indicated relatively indistinct rings, and 5% presented indistinct rings. The analyzed cores indicated distinct rings in 67% of the total analyzed samples, 23% presented relatively indistinct rings, and 10% showed indistinct rings.

Only 44% of the analyzed species did not show any false rings, and 64% of them did not provide wedging rings (Table 1). The species presenting the largest number of false and wedging rings were *Copaifera langsdorfii* (Fabaceae), *Banara arguta* (Flacourtiaceae) and *Trichilia stellatotomentosa* (Meliaceae). Species of the families Bignoniaceae and Moraceae showed no false rings, whereas Chrysobalanaceae, Euphorbiaceae, Lauraceae, Moraceae and Fabaceae did not present any wedging rings (Table 1).

Table 1. Tree species distributed in Pantanal and some dendrochronological features: Growth distinctiveness zones in the inner (in), outer (out) part of the stem disc and at a core sample (core) expressed in (+) distinct, (+-) relatively indistinct, (-) indistinct. Wood structure based on growth zone delimitation: 1 (intra-annual density variation), 2 (marginal parenchyma bands), 3 (patterns of alternating parenchyma and fiber bands), and 4 (vessel distribution and/or size variations). Combinations often happen (indicated by the parentheses). False rings and wedging rings: (+) abundant, (+ -) moderate, (-) few, (0) absent.

Family/Species	Distinctiveness		Wood	False	Wedging	
	In	out	core	structure	rings	rings
Anacardiaceae						
Astronium fraxinifolium Schott	+	+	+	1(4)	+ -	-
Spondias mombin L.	+	+	+	3	0	0
Apocynaceae						
Aspidosperma cylindrocarpon Müll. Arg.	+	+	+	1	0	0
Bignoniaceae						
*Handroanthus heptaphyllu*s (Vell.) Mattos	+ -	+	+ -	2(4)	0	0
Handroanthus impetiginosus (Mart. ex DC.) Mattos	+	+	+	2	0	0
Jacaranda mimosifolia D. Don	+	+	+	2(3,4)	0	0
Tabebuia sp.	+	+	+	2(4)	0	-
Tabebuia aurea (Silva Manso) Benth. & Hook. f. ex S. Moore	+	+	+	2	0	0
Boraginaceae						
Cordia glabrata (Mart.) A. DC.	+	+	+	2	0	0
Burseraceae						
Protium heptaphyllum (Aubl.) Marchand	+ -	-	+ -	1(4)	-	-
Calophyllaceae						
Calophyllum brasiliense Cambess.	+ -	+	+	2(3)	-	0

Table 1 (cont.). Tree species distributed in Pantanal and some dendrochronological features: Growth distinctiveness zones in the inner (in), outer (out) part of the stem disc and at a core sample (core) expressed in (+) distinct, (+-) relatively indistinct, (-) indistinct. Wood structure based on growth zone delimitation: 1 (intra-annual density variation), 2 (marginal parenchyma bands), 3 (patterns of alternating parenchyma and fiber bands), and 4 (vessel distribution and/or size variations). Combinations often happen (indicated by the parentheses). False rings and wedging rings: (+) abundant, (+ -) moderate, (-) few, (0) absent.

Family/Species	Distinctiveness		Wood	False	Wedging	
	In	out	core	structure	rings	rings
Capparaceae						
Crateva tapia L.	+	+	+	1	0	0
Celastraceae						
Peritassa dulcis (Benth.) Miers	+ -	+ -	+ -	1	+	-
Chrysobalanaceae						
Couepia uiti (Mart. & Zucc.) Benth. ex Hook. f.	+ / + -	+ / + -	+ / + -	1 / 3 / 3(1)	- / 0	0
Licania parviflora Benth.	+	+	+ / + -	3/1 (3)	- / 0	0
Clusiaceae						
Garcinia brasiliensis Mart.	-	-	-	2	-	0
Combretaceae						
Buchenavia tomentosa Eichler	+	+	+	2	0	0
Dilleniaceae						
Curatella americana L.	+	+	+	(4)	0	0
Euphorbiaceae						
Alchornea castaneifolia (Humb. & Bonpl. ex Willd.) A. Juss.	-	-	-	1	0	0
Alchornea discolor Poepp.	+ / + -	+ / + -	+ / + -	1/2	+ / -	- / 0
Mabea paniculata Spruce ex Benth.	+ / + -	+	+	1	- / 0	0
Sapium obovatum Klotzsch ex Müll. Arg.	+ / + - / -	+ / + - / -	+ / + - / -	1/1(2)	- / 0	- / 0
Sebastiania brasiliensis Spreng.	+	+	+	1	0	0

Table 1 (cont.). Tree species distributed in Pantanal and some dendrochronological features: Growth distinctiveness zones in the inner (in), outer (out) part of the stem disc and at a core sample (core) expressed in (+) distinct, (+-) relatively indistinct, (-) indistinct. Wood structure based on growth zone delimitation: 1 (intra-annual density variation), 2 (marginal parenchyma bands), 3 (patterns of alternating parenchyma and fiber bands), and 4 (vessel distribution and/or size variations). Combinations often happen (indicated by the parentheses). False rings and wedging rings: (+) abundant, (+ -) moderate, (-) few, (0) absent.

Family/Species	Distinctiveness			Wood	False	Wedging
	In	out	core	structure	rings	rings
Fabaceae						
Albizia inundata (Mart.) Barneby & J.W. Grimes	+	+	+	2	0	0
Albizia saman (Jacq.) F. Muell.	+	+	+	2(1)	-	0
Albizia sp.	+	+	+	2	0	0
Anadenanthera colubrina (Vell.) Brenan	-	-	-	2(1)	0	0
Cassia grandis L. f.	+	+	+	2(3)	0	0
Chloroleucon sp.	-	+ -	+	2	+	+
Copaifera langsdorffii Desf.	+	+	+	1	+	+
Dalbergia riedelii (Benth.) Sandwith	+ / + -	+ / + -	+ / + -	2/2(1)	-	- / 0
Dipteryx alata Vogel	+	+	+	2	0	0
Enterolobium contortisiliquum (Vell.) Morong	+	+	+	2	- / 0	- / 0
Erythrina fusca Lour.	+	+	+	3	0	0
Hymenaea stigonocarpa Mart. ex Hayne	+	+	+	2/2(1)	+ - / - / 0	0
Inga sp. 1	+ / + -	+ / + -	+ / + -	2	+ / - / 0	- / 0
Inga sp. 2	+ -	+ -	+ -	1(2)	0	0
Inga uruguensis Hook. & Arn.	+	+ / + -	+	2(1)	-	0
Machaerium sp.	+ -	+ -	+ -	3	-	0
Lamiaceae						
Vitex cymosa Bertero ex Spreng.	+	+	+	1	0	0
Lauraceae						
Ocotea diospyrifolia (Meisn.) Mez	+ / + -	+ / + -	+ -	1	+ / + -	0
Ocotea sp.	+ / + -	+ / + -	+ / + - / -	1/1(2)	+ - / -	- / 0

Table 1 (cont.). Tree species distributed in Pantanal and some dendrochronological features: Growth distinctiveness zones in the inner (in), outer (out) part of the stem disc and at a core sample (core) expressed in (+) distinct, (+-) relatively indistinct, (-) indistinct. Wood structure based on growth zone delimitation: 1 (intra-annual density variation), 2 (marginal parenchyma bands), 3 (patterns of alternating parenchyma and fiber bands), and 4 (vessel distribution and/or size variations). Combinations often happen (indicated by the parentheses). False rings and wedging rings: (+) abundant, (+ -) moderate, (-) few, (0) absent.

Family/Species	Distinctiveness			Wood	False	Wedging
	In	out	core	structure	rings	rings
Malpighiaceae						
Byrsonima crassifolia (L.) Kunth	+ / + -	+	+ / + -	1	+ / -	- / 0
Melastomataceae						
Mouriri guianensis Aubl.	+ / + - / -	+ / + - / -	+ / + - / -	1/1(2)	- / 0	0
Meliaceae						
Trichilia catigua A. Juss.	+	+	+	1(2)/1(4)	+ - / -	+ - / 0
Trichilia elegans A. Juss.	+	+	+	2(3)	0	-
Trichilia stellatotomentosa Kuntze	+	+	+ / + -	2(1)	+	+
Moraceae						
Brosimum lactescens (S. Moore) C.C. Berg	+	+	+	3	0	0
Ficus sp.	+	+	+ / + -	3	0	- / 0
Myrtaceae						
Calyptranthes sp.	+ -	+	+	1(3)	+	+ -
Campomanesia eugenioides (Cambess.) D.Legrand ex Landrum	+ / + -	+	+	1(2,4)	+ - / -	- / 0
Eugenia florida DC.	+	+	+	3	0	0
Psidium striatulum DC.	+ / + -	+ / + -	+ / + -	1(3)/1(2,4)	- / 0	+ / 0
Nyctaginaceae						
Neea hermaphrodita S. Moore	+ -	+	+	1	0	0

Table 1 (cont.). Tree species distributed in Pantanal and some dendrochronological features: Growth distinctiveness zones in the inner (in), outer (out) part of the stem disc and at a core sample (core) expressed in (+) distinct, (+-) relatively indistinct, (-) indistinct. Wood structure based on growth zone delimitation: 1 (intra-annual density variation), 2 (marginal parenchyma bands), 3 (patterns of alternating parenchyma and fiber bands), and 4 (vessel distribution and/or size variations). Combinations often happen (indicated by the parentheses). False rings and wedging rings: (+) abundant, (+ -) moderate, (-) few, (0) absent.

Family/Species	Distinctiveness			Wood	False	Wedging
	In	out	core	structure	rings	rings
Polygonaceae						
Coccoloba mollis Casar.	+ / + -	+ / + -	+ / + - / -	1/1(2)/ 1(4)	+ - / - / 0	+ - / - / 0
Triplaris americana L.	+	+	+ / + -	1	+ - / -	0
Rubiaceae						
Duroia duckei Huber	+ / + -	+ / + -	+ / + -	1/ 1(2)	+ - / - / 0	- / 0
Genipa americana L.	+	+	+ / + -	1 (2,4)	+ / + -	0
Rutaceae						
Zanthoxylum rigidum Humb. & Bonpl. ex Willd.	+	+	+	3 / 2 (3)	- / 0	0
Salicaceae						
Banara arguta Briq.	+ - / -	+ / -	+ / -	1	+ / + -	+ / 0
Casearia aculeata Jacq.	+ -	+ -	+ -	1	0	0
Sapindaceae						
Cupania vernalis Cambess.	+	+	+	2	0	0
Magonia pubescens A. St.-Hil.	+	+	+	2	0	0
Talisia esculenta (A. St.-Hil.) Radlk.	+	+	+	3	0	0
Urticaceae						
Cecropia pachystachya Trécul	+	+	+	1/ 1(2)	+ / - / 0	0
Vochysiaceae						
Callisthene fasciculata Mart.	+ -	+	+	3	-	+ -
Vochysia divergens Pohl	+ / + - / -	+ / + -	+ / + - / -	3/3(4,1)	- / 0	0

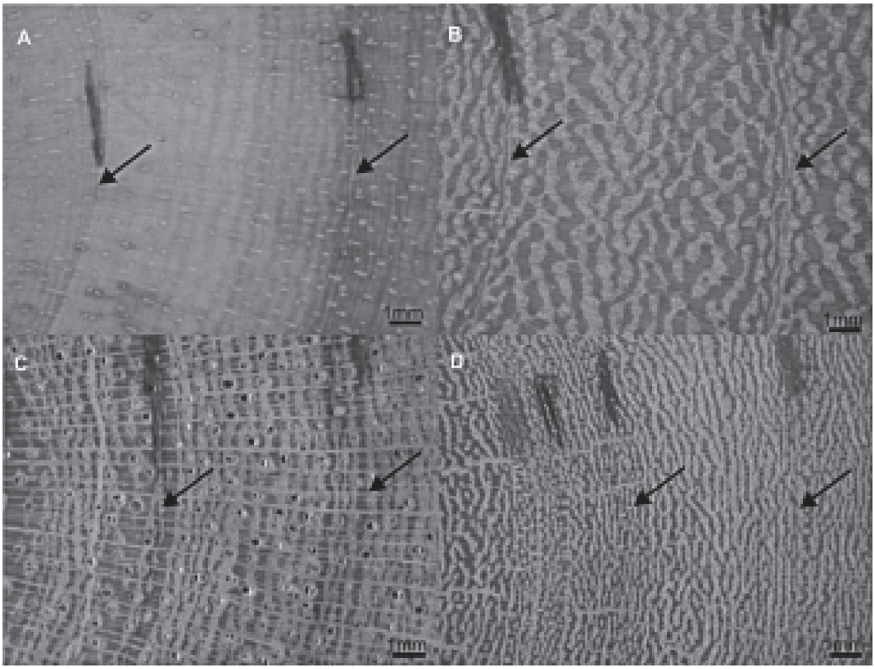

Figure 3. Wood anatomical features of tree rings for some tree species of the Pantanal, A) *Triplaris americana* (Polygonaceae) – wood density variation. B) *Cassia grandis* (Fabaceae) –marginal parenchyma bands. C) *Vochysia divergens* (Vochysiaceae) – repeated patterns of alternating parenchyma and fiber bands. D) *Handroanthus impetiginosus* (Bignoniaceae) – variation in vessel size and/or distribution variations. Tree rings are indicated by arrows.

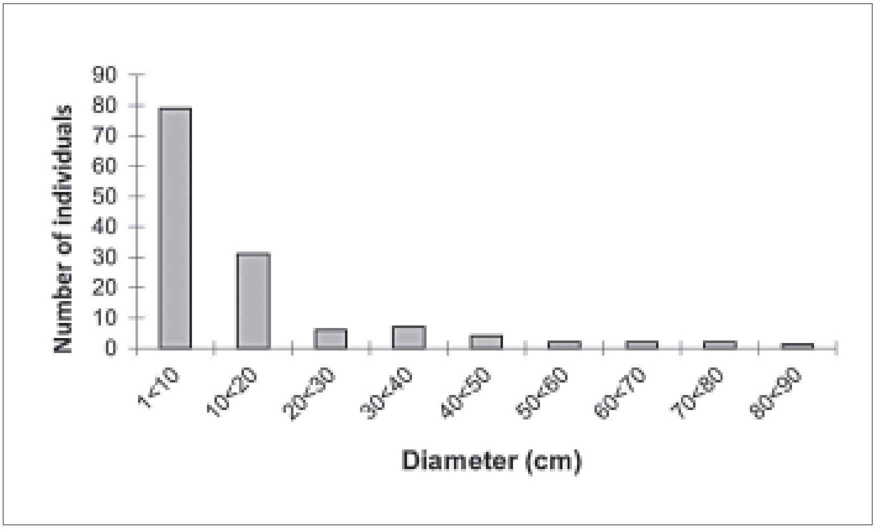

Figure 4. Age class distribution of all the sampled individuals.

Age, Mean Diameter Increment (MDI) and Wood Density of Pantanal Tree Species

Licania parvifolia was the species presenting the oldest ages (137 years); it was followed by *Vochysia divergens* (106 years) and *Anadenanthera colubrina* both (104 years). Figure 4 illustartes the distribution of all individuals sampled according to 10-cm diameter classes. Most of the studied trees presented small diameter (1 to 20 cm DBH) while only few analyzed trees (*Vochysia divergens*, *Buchenavia tomentosa*, *Tabebuia impetiginosa* and *Anadenanthera colubrina*) represented the large diameters. The mean DBH recorded for all the analyzed samples was 13.40 ± 9.68 cm.

Mean diameter increment rates of the assessed species are listed in Table 2 indicating the highest rates of MDI for *Cecropia pachystachya* (14.0 ± 6.5 mm), *Curatella americana* (9.2 ± 1.3 mm) and *Couepia uiti* (8.5 ± 4.3 mm), whie the lowest MDI was observed for *Trichilia stellatotomentosa* (1.8 ± 0.2 mm) (Table 2). The sampled species showed wood density varying from 0.29 to 0.81 g/cm^{-3} (*Sapium obovatum* and *Licania parviflora,* respectively) (Table 2). Most trees presenting high wood densities belong to canopy species.

Table 2. Growth patterns of 34 characteristic tree species in the Pantanal indicating sample size (N), diameter, wood density, mean diameter increment (MDI) and maximum observed tree age based on tree-ring analyses.

Species	N	Diameter (cm)	Wood density (g cm^{-3})	MDI (mm)	Max. tree age (years)
Alchornea discolor	3	3-15	0.34 ± 0.04	7.7 ± 6.5	15
Anadenanthera colubrina	1	57	0.75	5.5	104
Banara arguta	2	17-31	0.75*	8.2 ± 0.3	40
Byrsonima crassifolia	3	2-12	0.52*	5.0 ± 1.7	31
Callisthene fasciculata	1	4	0.68	3.2	7
Campomanesia eugenioides	1	6	0.84*	2.91	21
Cecropia pachystachya	3	7-19	0.30 ± 0.07	14.0 ± 6.5	11
Chloroleucon sp.	1	9	0.53	2.7	35
Coccoloba mollis	7	3-11	0.59 ± 0.04	4.4 ± 1.2	35
Cordia glabrata	1	43	0.77*	5.9	74
Couepia uiti	3	3-12	0.44 ± 0.02	8.5 ± 4.3	9
Curatella americana	2	14-17	0.65*	9.2 ± 1.3	17
Dipteryx alata	1	31	0.88*	9.2	35
Duroia duckei	4	3-13	0.52 ± 0.01	5.2 ± 2.8	14
Garcinia brasiliensis	2	6-11	0.64 ± 0.05	2.6 ± 0.1	42
Genipa americana	2	4-20	0.65 ± 0.00	7.3 ± 1.9	23
Hymenaea stigonocarpa	1	33	0.75	6.1	55
Inga uruguensis	2	3-4	0.58*	2.9 ± 1.0	17
Inga sp.	2	7-15	0.36 ± 0.00	5.3 ± 1.2	18

Table 2 (cont.). Growth patterns of 34 characteristic tree species in the Pantanal indicating sample size (N), diameter, wood density, mean diameter increment (MDI) and maximum observed tree age based on tree-ring analyses.

Species	N	Diameter (cm)	Wood density (g cm^{-3})	MDI (mm)	Max. tree age (years)
Licania parviflora	3	3-23	0.81 ± 0.09	2.8 ± 1.1	137
Mabea paniculata	3	3-4	0.56 ± 0.10	6.1 ± 0.7	8
Mouriri guianensis	5	2-12	0.60 ± 0.06	4.5 ± 2.0	32
Neea hermaphrodita	1	8	0.36	7.5	11
Ocotea sp.	5	2-9	0.47 ± 0.05	5.7 ± 1.9	16
Ocotea diospyrifolia	1	3	0.55	5.3	6
Sapium obovatum	5	5-11	0.29 ± 0.09	7.1 ± 3.2	17
Spondias mombin	1	38	0.41	8.4	46
Handroanthus heptaphyllus	1	11	0.67	7.2	20
Handroanthus impetiginosus	1	63	0.80	5.9	89
Talisia esculenta	1	9	0.70	6.6	14
Trichilia stellatotomentosa	1	5	0.68	1.8 ± 0.2	42
Triplaris americana	2	5-12	0.42 ± 0.16	5.3 ± 1.2	11
Vitex cymosa	1	5	0.52*	7.3	7
Vochysia divergens	26	1-80	0.40 ± 0.08	7.9 ± 2.8	106

*Reference used for wood density values: Chaves et al. (2009)

Discussion

Growth periodicity

Applying cambial wounding we provide evidence of annual tree ring formation for further three tree species in the Pantanal. Earlier studies already indicated for *Vochysia divergens* (Mattos 1998; Fortes 2006; Machado et al. 2015; Sallo et al. 2017), *Handroanthus heptaphyllus* and *Tabebuia aurea* (Ishii 1998; Leite 2012) as well as for *Sterculia apetala* (Santos Junior et al. 2006) annual tree ring formation applying different dendrochronological techniques such as radiocarbon-dating, cambial wounding, dendrometer bands and climate-growth relationships. These studies indicate varying triggering factors of growth rhythms, either triggered by the anoxic conditions during the aquatic phase (Ishii 1998) or dry conditions leading to cambial dormancy during the terrestrial phase (Machado et al. 2015; Sallo et al. 2017) Trees in Central Amazonian floodplains (Schöngart et al. 2002) evidenced that cambial dormancy and ring formation were induced by the anoxic conditions induced by the predictable annual flood with high amplitudes. However, differences in the climate-growth relationship among different ecotypes were observed, such as evergreen, brevi-deciduous, deciduous and stem succulent tree species. On non-flooded tropical sites many studies evidenced annual tree-ring formation triggered by rainfall seasonality Worbes (1999); Dünisch et al. (2003); Menezes et al. (2003); Dünisch (2005); Lisi et al. (2008); Brandes et al. (2016).

It is important to develop to perform studies monitoring diameter growth by dendroemeters and leaf phenology and developing tree-ring chronologies from species of different ecotypes (evergreen, brevi-deciduous, deciduous and stem-succulent) analyzing climate-growth relationships for a better understanding of triggering factors for tree growth in this complex wetland system.

Anatomical Structure of Tree-Ring Growth

The presented wood anatomical features and characteristics of tree rings are a basis for future dendroecological applications. The wood anatomical features of tree rings for the families in the Pantanal correspond to those described for other tropical regions (Worbes 2002). Interaction between genetically defined wood anatomy and abiotic factors such as rainfall, temperature and flooding influencing the tree physiology and leaf phenology (Borchert 1999; Brienen et al. 2016) result to differences in the distinctiveness of growth rings. Alves & Angyalossy-Alfonso (2000) demonstrated in a review of 491 species from 133 genera and 22 families across different Brazilian biomes, that 48% of the analyzed species possessed distinct tree rings. Worbes (2002), analyzing the suitability of 139 species for tree-ring analysis across South America, showed that the distinctiveness of annual rings often varies between life stages with tree rings clear in the adult phases and absent or vague in the juvenile phases, or vice versa.

In the Brazilian Coastal Atlantic forest, 53-59% of the examined 117 tree species formed distinct tree rings (Silva et al. 2016). In the rainforests and savannahs of southeast Brazil Tomazello Filho et al. (2004) observed distinct tree rings for 46% of 41 analyzed species. Roig et al. (2005) indicate for the dry forests of Mexico a lower percentage with 35% of 52 studied tree species indicating potential for dendrochronological applications. Similar findings were highlighted by Gutiérrez and Ramos (2013) for the Peruvian rainforest reporting 30% of 80 tree species with distinct annual growth rings. Other studies demonstrate that the formation of distinct tree rings depends also on the seasonality of the environmental conditions (Brienen et al. 2016). Trees of a species growing at sites with a distinct seasonality form clearly visible tree rings, which may be vague or even absent in the same species growing at sites with a weak or irregular seasonality (Geiger 1915). For tree-ring studies it is therefore important to carefully examine the wood anatomy of species explored for the first time as well as previously studied species at new sites with different environmental conditions.

Tropical species frequently present tree-ring anomalies (e.g., Worbes 2002; Roig et al. 2005, Brienen et al. 2016) such as wedging rings or partially missing rings, which trees form during one or consecutive years in just one section of the circumference. These anomalies are commonly found in trees growing under poor light conditions with increased interspecific competition resulting in differences of the local supply of phytohormones, water, minerals and carbohydrates (Dünisch et al. 1999). Some taxa such as Annonaceae (Worbes 2002) or *Araucaria angustifolia* (Oliveira et al. 2010) tend to form wedging tree rings quite frequently. Another commonly reported problem in tree-ring analysis of tropical species is the occurrence of false rings and double rings (Worbes 2002; Brienen et al. 2016), which might result from occasional rainfall events during the dry season (Worbes 1995) or due to exceptional, but short-lived, drought events during the growing season (Schöngart et al. 2002). The present study evidenced that species from the families Anacardiaceae, Apocynaceae, Bignoniaceae, Boraginaceae, Combretaceae, Dilleniaceae, Fabaceae, Meliaceae, Moraceae, Rutaceae, Sapindaceae and Verbenaceae present potential for future dendroecological studies due to the distinctiveness of growth rings and low frequencies of ring anomalies.

According to Ishii (1998), native Pantanal species such as *Genipa americana* (Rubiaceae) and *Handroanthus heptaphyllus* (Bignoniaceae) also have distinctive rings, but Ishii noticed that *Handroanthus heptaphyllus* presented in the Southern Pantanal false or wedging rings which might be a consequence of a temporal shift of about 2-3 months in the periodicity in the flood-pulse patterns compared to the rainfall seasonality. Our current research evidenced that tre species in the Pantanal form annual growth rings, and it corroborates results reported for many tropical tree species (Zuidema et al. 2012; Brienen et al. 2016; Schöngart et al. in press).

Age, Mean Diameter increment (IDM) and Wood Density

One of the main age determination results recorded for tropical trees is the annual increment rate (Worbes 1989). The potential of the tree ring analysis to set the age and long-term reliable growth rates is very high. Information about tree age and growth rates is essential, since it helps understanding tree population dynamics and the development of sustainable management systems applicable to tropical timber species (Worbes et al. 2003; Brienen and Zuidema 2005). The analysis conducted with individuals belonging to the dendrochronological collection of the Dendroecological Laboratory of Brazilian Mid-Western Biomes mostly comprised young individuals, and it evidences that the Pantanal species collection should invest in gathering older individuals in order to support future studies.

The increased mean diameter and tree wood density may change depending on different abiotic and biotic factors, among which one finds the successional stage, soil type and flood duration (Schöngart 2003). The mean diameter increment rates (MDI) and wood density (WD) varied among the species (MDI-3.0 to 14 mm and WD-0.29 to 0.8 g/cm^{-3}) in the current study. Wood density and mean diameter increment changed depending on age (Table 2); younger species grew faster and recorded low wood density. This behavior is typical of pioneering species. Similar results were found by Worbes (1997), Schöngart et al. (2003) and by Worbes et al. (1992), who conducted studies in flooded Amazonian forests. The age gradient in their study increased during the successional young stage, and dramatically decreased until reaching its stage climax. However, there was wood density increase during the successional development.

Wood density and increment differences were found between the species *Tabebuia barbata* (1.71 ± 0.51 increment and 2.88 ± 1.00 mm, and WD 0.83 ± 0.08 and 0.80 ± 0.08 g/cm^{-3}, in igapó and várzea, respectively) and *Vatairea guianensis* (2.29 ± 0.70 increment and 3.59 ± 1.53 mm, and WD 0.65 ± 0.04 and 0.66 ± 0.03 g/cm^{-3}, in igapó and várzea, respectively), both distributed in Amazonian *várzea* and *Igapó* flooded forests. This result shows that the higher the wood density, the lower the increase rates. These increment differences are associated with nutritional differences between plants in *igapó* (nutrient-poor) and in *várzea* (nutrient-rich) sites (Fonseca et al. 2009). The trees distributed in the flooded forest of Mapire, Venezuela, studied by Dezzeo et al. (2003) presented high wood density values and low growth rates. The low nutrient supply to the soil in Mapire forest caused by river flooding was reported by Dezzeo et al. (2003) as a possible cause for slow growth. Similar behavior was observed by Worbes et al. (2003) in upland forests in Cameroon (Africa) and by Shimamoto et al. (2016) in the tropical Atlantic forest; it shows that species presenting higher wood density have low annual increment. Based on wood density, age and growth rate data of the assessed species, it is possible classifying these species as, pioneering, long-lived pioneer and climax species (Swaine and Whitmore 1988; Worbes et al. 1992).

Wittmann et al. (2008) assessed the low wood density species *Cecropia pachystachia* (WD-0.39 g/cm^{-3}) and their results corroborated those in the current study (*Cecropia pachystachia* WD -0.30 ± 0.07 g/cm^{-3}). According to Worbes et al. (2003), species wood density indicates its life strategy; pioneer trees usually have lighter wood, whereas mature forest trees show high density often combined with low increment rates (Worbes 1989), as it was evidenced in the present study. Genus *Cecropia* includes pioneering species that trigger secondary forest successions (Franco-Rosselli and Berg 1997). *Cecropia pachystachia* is the species of major occurrence in Pantanal, and it indicates the presence of disturbed areas showing clearings (Arruda and Nunes da Cunha 2012). There was variation in the density values of the species assessed by Wittmann et al. (2008), namely: *Banara argunta, Handroanthus heptaphyllus*, *Mouriri guianensis, Neea hermaphrodita* and *Vitex cymosa,* and it may suggest that the environment is the factor influencing the changes in these values.

Conclusions

Data collection performed through the destructive method is an important reference to dendrochronological studies, since these data help finding false and wedging rings in the Pantanal biome, as well as in other biomes. The analysis applied to the collection of the Dendroecological Laboratory of Brazilian Central-West Biomes mostly comprised young individuals, fact that shown the need of investing in the collection of older individuals in order to subsidize further studies.

The Mariaux window method proved the existence of annual rings in Pantanal tree species; therefore, this technique must be extended to dubious species such as *Curatella americana* and to other species that have indistinct rings. It is worth paying closer attention to these species in studies focused on further clarifications. Dendrochronological methods should be combined whenever there is doubt about growth-rings annuity.

It is worth investing in more wood density analysis, because the environmental characteristics in the same biome seem to influence the results. Wood density is a good variable to help understanding the life strategies of tropical trees. Young species often present low wood density and fast growth, whereas mature species show high densities and slow growth. It was observed that the mean diameter increment decreases as wood density increases. Wood density reflects the tolerance trees have to shading. Wood density, age and growth rate data of the herein assessed tree species allowed classifying them as pioneering, long-lived pioneer and climax species.

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Antimicrobial and antioxidant activity of endophytic fungi isolated from H*yptis suaveolens* roots

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Introduction

Endophytic fungi colonize the internal tissues of plants without causing visible damage to their host plant, and they can be transmitted vertically through seeds (White et al. 2017) or horizontally between individuals (Rodriguez et al. 2009). Endophyte-host interactions vary from parasitism to symbiotic mutualism under the effect of biotic and abiotic factors (Newton et al. 2010; Siqueira et al. 2016). Different factors, including the host plant species, age (De Errasti et al. 2010), and organ analyzed (De Abreu et al. 2010), salinity (Soares et al. 2016), as well as climatic factors (Naik Et al. 2009) and geographic distribution (Göre and Bucak 2007) influence the endophytic fungi community.

The endophytes' ability to synthesize bioactive secondary metabolites that share similarity with the host plant metabolites has prompted researchers to design biotechnological processes using endophytic microorganisms (Kusari et al. 2013; Gond et al. 2012). Endophytes, especially those isolated from medicinal plants, stand out as important sources for prospecting secondary metabolites with different bioactivities, such as antimicrobial, insecticide, antitumor, antidiabetic, and immunosuppressant (Strobel and Strobel 2007). Endophytes promote plant growth and can enhance the resistance of their host to pathogens (Soares et al. 2015; Soares et al. 2016b). These microorganisms have aided in the development of biotechnological processes in agriculture, medicine, and industry (Verma et al. 2009, Zhou et al. 2011, Zhao et al. 2011).

The increasing number of human pathogens and the availability of drug-resistant animals have driven the search for new antibiotics (Yu et al. 2010). Antimicrobial metabolites produced by endophytes are promising molecules for the treatment of tuberculosis, cutaneous infections, gastrointestinal infections, pneumonia, meningitis, septicemia, and other diseases caused by pathogenic microorganisms (Kelly and LaMont 2008; Davies 2010). Endophytes also produce some secondary metabolites with antioxidant activity, which represent a frontier in the treatment of chronic degenerative diseases associated with oxidative stress in cellular metabolism, such as Alzheimer's disease, cardiovascular diseases, and cancer (Prior and Wu 2013).

There are few reports on endophytic microorganisms isolated from Pantanal plant species (De Siqueira et al. 2017; Souza et al. 2015). Some of these microorganisms produce bioactive secondary metabolites (Silva et al. 2017; Brissow et al. 2017) that may be involved in a host-endophyte relationship (Vitorino et al. 2013; Parpinelli et al. 2017).

Hyptis suaveolens (L.) Poit (Lamiaceae), popularly known as "field mint", "Lavender - brava", "bambural" or "old tapara", is a medicinal plant used to treat a variety of diseases (Prasanna and Koppula 2012). Species of the Lamiaceae family provide bioactive compounds with insecticidal properties and several pharmacological activities, such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer (Ghaffari et al. 2011; Edeoga et al. 2005). Although the medicinal properties of *H. suaveolens* are well-known, there are no reports on either the endophytic community that colonize it or the potential of these microorganisms to produce bioactive metabolites. In this sense, this study aimed to (i) identify the endophytic fungal community colonizing the *H. suaveolen* roots and (ii) analyze the endophytic species' ability to produce metabolites with antimicrobial and antioxidant activities.

Materials and Methods

Collection of plant material

Plant material was collected in the Pantanal wetland (S16° 18'95.6" and W56° 32'26.6"), in the region of Poconé, State of Mato Grosso, Brazil, during the wet period (February). Three adult individuals of *Hyptis suaveolens* were collected randomly, and their root system was kept intact. The plants were stored in plastic bags and transported under refrigeration until processing. A voucher specimen was deposited in the Herbarium of Federal University of Mato Grosso (UFMT), Cuiabá, MT, Brazil, under the number 40.003.

Isolation and identification of endophytic fungi

The *H. suaveolens* roots were separated into two parts: one was used for the isolation of endophytic microorganisms and the other was maintained in a 5:5:90 formaldehyde, glacial acetic acid, and 50% ethyl alcohol solution for observation of fungal structures.

The root surface was disinfested using the Petrini and Muller (1986) methodology. Briefly, the roots were sequentially immersed in 70% ethanol for 2 min, 2.5% sodium hypochlorite for 3 min, 70% ethanol for 30 sec, and finally rinsed three times with autoclaved distilled water. Root fragments of approximately 1 cm were inoculated into Petri dishes containing Potato Dextrose Agar (PDA) culture medium, and incubated for 15 days, at 28 °C. The fungal strains were purified and stored at 4 °C in PDA medium. The root colonization rate (CR) by endophytic fungi was calculated by the formula: CR = (number of fragments with endophytes / total number of fragments analyzed) x 100.

The strains were activated in PDA medium for 7 days, at 28 °C, and grouped into morphotypes according to the macromorphological aspects of the cultures, such as surface and background color of the culture medium, border, shape, relief, and color of the mycelium (Pimentel et al. 2006). Permanent slides obtained from microcultures (Kern and Blevins 1999) were used to analyze the micromorphological and auxiliary morphotypical characteristics. The structures observed under the microscope were used in different fungi identification keys (Barron 1968; Sutton 1973; Sutton 1980; Carmichael et al. 1980; Alexopoulos et al. 1996).

Part of the collected roots were bleached following the procedure reported by Koske and Gemma (1989). Fragments of the bleached roots were stained with 0.05% trypan blue for 15 min, in a water bath at 70 °C (Brundrett et al. 1996). Slides containing 10 stained fragments of approximately 1 cm each were mounted with polyvinyl lacto glycerol (16.6 g of polyvinyl alcohol, 100 mL of lactic acid, 10 mL of glycerin, and 100 mL of distilled water). The fungal structures associated with the roots were identified and quantified using a microscope (Koske and Tessier 1983). Ten root fragments were analyzed for each individual of *H. suaveolens*.

Analysis of morphotypes based on molecular markers and molecular identification of selected lineages

Total DNA was extracted from the selected lineages using the AxyPrep™ kit (AXYGEN Biosciences), according to the manufacturer's instructions. The genetic variability of the morphotypes was determined using the molecular markers ISSR (*inter-simple sequence repeats*) (Longato and Bonfante 1997) and IRAP (*inter-retrotransposon amplified polymorphism*) (Santos et al. 2012). The ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC GCT TAT TGA TAT GC) primers were used for the amplification of the ITS region (White et al. 1990).

Polymerase chain amplification reactions (PCR) (total volume = 25 μL) were performed in Eppendorf vials, and were composed of 3 μL of genomic DNA; 2.5 μL of 10x buffer without $MgCl_2$; 0.75 μL of $MgCl_2$; 1 μL of 2.5 mM dNTPs; 1 unit of Taq polymerase (Invitrogen, Brazil), and 10 pmol of each primer. The molecular sieves ISSR and IRAP were amplified under the following conditions: initial denaturation (94 ºC, 2 min); 35 denaturation cycles (94 ºC, 1 min); annealing (50 ºC, 2 min); synthesis (72 °C, 2 min); final synthesis (72 °C, 10 min). ITS was amplified under the following conditions: initial denaturation (94 °C, 2 min); 35 cycles of 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min; elongation (72 °C, 10 min).

PCR products were purified (Dunn and Blattner 1987) and quantified by 0.8% agarose gel electrophoresis (Xu et al. 2008). Sequencing was performed by the Sanger method, using the Big Dye Kit and the ABI3100 Applied Biosystem sequencer. Next, ITS rDNA sequences were compared to sequences deposited in the GenBank database using BLASTn (http://www.ncbi.nlm.nih.gov).

Assessment of the infective potential of the fungal strains

The *H. suaveolens* seeds were surface disinfested by immersion in 70% ethanol (1 min), 2.5% sodium hypochlorite (6 min), and three rinses in autoclaved distilled water. The seeds were inoculated in mineral medium (MM) – 0.22 g $CaCl_2$, 0.950 g KNO_3, 0.68 g $(NH_4)_2SO_4$, 0.186 g $MgSO_4.7H_2O$, 0.084 g KH_2PO_4, and 9 g agar to 1000 mL of distilled water – and incubated for 7 days, at 28 °C, with photoperiod of 12 hours of light for germination. Thereafter, the seedlings were transferred in pairs to new plates containing MM. Disks containing mycelial fragments of endophytic strains were inoculated near the host plant roots. Each plate contained two seedlings and one fungal strain. The control treatment consisted of uninoculated seedlings considered as endophyte-free. After 7 days of interaction, the *in vitro* infected radicles were immersed in 10% KOH for 30 min, at 30 °C, rinsed in distilled water, and neutralized with 1% HCl for 10 min. Finally, the root fragments were stained with 0.05% trypan blue in a water bath at 60 °C for 5 min, mounted on glass slides with polyvinyl lactoglycerol, and analyzed by light microscopy (Koske and Tessier 1983).

Antagonistic activity of endophytic fungi against bacteria

The antimicrobial activity was assessed using the agar block test (Ichikawa et al. 1971). Qualitative analysis of antibiotics was carried out using all the endophytic fungal strains isolated from *H. suaveolens* roots. Two bacterial strains, *Staphylococcus aureus* (ATCC 43867) and *Escherichia coli* (ATCC 25922), were activated in Brain Heart Infusion (BHI) culture medium for 24 h, under shaking at 100 rpm. The optical density of the suspension was adjusted to 0.1, and an aliquot was seeded in nutrient agar medium. Mycelial discs of endophytic fungi with approximately 5 mm of diameter were deposited on the culture dishes previously inoculated with bacteria. After incubation for 4 h, at 4 ºC, and for 24 h at 28 °C, the diameter of the inhibition halo was measured. The quantitative test was repeated three times for the qualitatively selected strains.

Preparation of crude extract of endophytic fungi

The extracts were prepared as described by Rosa et al. (2012). The fungi were grown in Petri dishes for 15 days, at 28 ± 2 °C. The culture medium containing the mycelium was triturated and macerated in ethyl acetate (EtOAc), at a 1:1 ratio (mycelium weight:EtOAc volume), for 24 h, under shaking at 100 rpm. After sonication in an ultrasonic bath for 1 h, the samples were vacuum filtered and the solvent was removed using a rotary evaporator at 37 °C. The resulting solid was solubilized in EtOAc to a concentration of 20 mg/mL to obtain the crude extract.

Assessment of antimicrobial activity by disk diffusion and determination of minimum inhibitory concentration (MIC) and minimum concentration of death (MDC)

The disk diffusion assay was performed as reported by Bauer et al. (1966), with modifications. The bacterial strains *Staphylococcus aureus* (ATCC 43867), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), and *Escherichia coli* ESBL+ were activated in BHI broth for 24 h, under shaking at 100 rpm. The optical density was adjusted to DO 0.08 and the suspension was seeded in Petri dishes containing Mueller-Hinton agar. Paper discs containing 10 μL of the crude extract (20 mg/mL) or 5 μL of tetracycline (5 mg/mL; positive control) were deposited on the seeded bacteria and the plates were incubated for 4 h at 4 °C, and for 24 h at 37 ± 2 °C. The diameter of the inhibition halos was measured.

The minimum inhibitory concentration was determined using the resazurin microplate assay, according to Palomino et al. (2002). Different concentrations of the crude extract or tetracycline (positive control) were tested against the four bacterial strains. After 24 h and prior to addition of resazurin, bacterial growth was evaluated by inoculating aliquots from each well of the microplates into Petri dishes containing Mueller-Hinton agar.

Antioxidant activity

The antioxidant activity of the extracts was determined by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay, adapted from Cheung et al. (2003). Briefly, 1 mL of DPPH solution (50 μg/mL in methanol (MeOH)) were mixed with 1.4 mL of MeOH and 0.1 mL of each sample – crude extract (0.04-1.6 mg/mL), ascorbic acid (2 mg/mL, positive control), or MeOH (negative control). After a 30-min incubation at 28 ± 2 °C, in the dark, the absorbance was recorded at 517 nm. The cation activity of the DPPH radical was calculated using the equation: $\mathrm{AAo\%} = [(\mathrm{AbsN}-\mathrm{AbsT})/\mathrm{AbsN}]\mathrm{x}100$, where: *AAo%*= Antioxidant activity (in percent), AbsN= absorbance of the negative control, AbsT= absorbance of the fungal extract and/or positive control.

Quantification of total phenolic compounds

The total phenolic compounds were quantified by the Folin-Ciocalteu method, as reported by Gao et al. (2000), and expressed in mg of gallic acid equivalents (EAG) per mL of extract.

Statistical analysis

The data from the antibiosis and antioxidant assays, and quantification of total phenolic compounds were transformed into arcosene (“ x / 100) and subjected to analysis of variance. The means were compared by the Scott-Knott test at 5% probability, using the Assitat 7.6 beta software (Silva, 2013). The diversity indices of Shannon (H ‘) and Simpson (D) were determined using the Past 3.14 software (Hammer et al. 2001).

Results and Discussion

Endophytic fungi colonize several species of medicinal plants (De Carvalho et al. 2012), including those belonging to the family Lamiaceae (Huang et al. 2008, Debbab et al. 2010, Vitorino et al. 2012). We isolated and identified sixty-nine strains of endophytic fungi from the *H. suaveolens* roots. The root CR by cultivable fungi was 30.28%. The Shannon (H') and Simpson (D) diversity indexes were H' = 2.13 and D = 0.82, respectively. The strains were grouped into 16 morphotypes according to their macro and micro-morphological characteristics. Nine strains did not differentiate reproductive structures when cultured in PDA medium; these structures are important for taxonomic distinction and classification at the genus and species levels (Larone 2002). The molecular markers ISSR and IRAP help to evaluate the inter- and intraspecific genetic variability of filamentous fungi (Dos Santos et al. 2012, Abadio et al. 2012). The use of these markers confirmed that the 16 morphotypes belonged to genetically distinct groups with the number of amplicons varying from 1 to 10.

Comparison between the sequences of the rDNA ITS region of the different morphotypes with the sequences deposited in GenBank using BLASTn revealed identity indexes of 95 to 99%. The identity of the strains was determined by identity indexes e" 95% between the sequences obtained in this study and those deposited in GenBank (O'Brien et al. 2005). We identified 12 taxa at the species level: *Aspergillus terreus, Cladosporium flabelliforme, Fusarium oxysporum, Macrophomina phaseolina, Mycoleptodiscus indicus, Neosartorya pseudofischeri, Penicillium chermesinum, Taifanglania biformis, Taifanglania curticatenata, Taifanglania hechuanensis, Thanatephorus cucumeris, Trichoderma koningiopsis*. Four strains were identified at the level of the class Dothideomycetes (1), order Pleosporales (2), and genus Phanerochaete (1) (Table 1).

The endophytic fungal strains isolated from the *H. suaveolens* roots were distributed in the phyla Ascomycota (94.2%) and Basidiomycota (5.8%), and belonged to the classes Dothideomycetes (66.7%), Eurotiomycetes (13%), and Sordariomycetes (14.5%). A small number of strains were pooled as Agaricomycetes (5.8%). The dominance of the classes Dothideomycetes and Sordariomycetes corroborates reports on the endophytic fungi isolation from *Nyctanthes arbor-tristis* (Gond et al. 2012), *Lippia sidoides* (De Siqueira et al. 2011), and *Sapindus saponaria* (Garcia et al. 2012).

Pleosporales sp. - Neg.2 was the most frequent group (36.23%), followed by Dothideomycetes sp - Neg.1 (20.29%) and *A. terreus* - F6 (10.14%). The other species occurred at a relative frequency of 1.45%: *F. oxysporum* - F3, *P. chermesinum* - F99, M. *indicus* - F21, *N. pseudofischeri* - F36, *Phanerochaete sp* - F11, *T. biformis* - F19, *T. curticatenata* - F27, and *T. koningiopsis* - F4 (Table 1).

Endophytic fungi of the root system are highly diverse (Vandenkoornhuyse et al. 2002). The endophyte community of terrestrial plants is often composed of fungi of the classes Sordariomycetes and Dothidiomycetes (Suryanarayanan et al. 2011), while plants from aquatic or humid environments are most frequently colonized by endophytic fungi of the class Eurotiomycetes (Sandberg et al. 2014). A high variety of fungi colonize host plant roots possibly due to their direct contact with the soil, where a great diversity of organisms exist.

The Dark Septate Endophytes (DSE) are soil fungi that can colonize the roots and establish a wide range of symbiotic interactions with the host plants (Jumpponen and Trape 1998; Sieber and Grünig 2006). The functional role of this endophytic fungus group is still controversial, despite its wide distribution in different biomes and climatic regions (Mandyam and Jumpponen 2005). DSE are characterized by their typical intercellular dark hyphae growing parallel to the longitudinal axis of the root and microsclerotia (Peterson et al. 2008, Upson et al. 2009a). The presence of melanin in DSE hyphae confers some selective advantages to live in diverse environments and under extreme conditions, such as heavy metal contaminated soils (Ban et al. 2012, Zhao et al. 2015), drought stress (Knapp et al. 2012, Redford et al. 2002), acidic conditions (Postma et al. 2007) or geothermal soils. Several septa-brown hyphae were observed in *H. suaveolens* internal tissues (Figure 1), with a percentage of infection of 65%. In the treated roots, no microsclerotic structure was observed.

Some factors influence the plant-endophyte relationship, such as: adaptation to a given host or organ, development stage of the colonizing microorganism, pathogenic capacity of the microorganism, host defense response, and environmental conditions (Schulz and Boyle 2005). We determined the endophytes' infection capacity by assessing the interaction between the isolated fungal species and *H. suaveolens* seedlings *in vitro*. The strains *T. hechuanensis* - F17, *T. biformis* - F19, *M. indicus* - F21, *T. curticatenata* - F27, Dothideomycetes sp. - Neg. 1, and Pleosporales sp. - Neg. 2 colonized the internal tissues of the seedlings (Figure 2) without clear evidences of symptoms or signs of disease; i.e. the seedlings inoculated with them remained healthy and

Table 1. Molecular identification of the 16 morphotypes of cultivable endophytic fungi isolated from *Hyptis suaveolens* roots, compared with the sequences deposited in GenBank, and endophytism data.

Morphotypes	Frequency (%)	Isolated species	N° of GenBank Accession	Species with sequence identity deposited in Genbank (*)	Similarity %	*In vitro* infection	Symptoms
Morp.1	2.9	*Macrophomina phaseolina*-F1	KF554487	*Macrophomina phaseolina* (JX945170)	99	-	M
Morp.2	1.45	*Fusarium oxysporum*-F3	KF554489	*Fusarium oxysporum* (JN232163)	98	-	M
Morp.3	1.45	*Trichoderma koningiopsis*-F4	KF554490	*Trichoderma koningiopsis* (AB568478)	99	-	M
Morp.4	10.15	*Aspergillus terreus*-F7	KF554491	*Aspergillus terreus* (JQ697547)	99	-	M
Morp.5	7.25	*Cladosporium flabelliforme*-F24	KF554492	*Cladosporium flabelliforme* (HM148092)	99	-	M
Morp.6	1.45	*Phanerochaete* sp.-F11	KR818853	*Phanerochaete* sp. (HQ607891)	99	-	M
Morp.7	8.7	*Pleosporales* sp. -F16	KR818854	*Pleosporales* sp. (KJ188726)	98	+	M
Morp.8	7.25	*Taifanglania hechuanensis*-F17	KF554494	*Taifanglania hechuanensis* (DQ185070)	97	+	NS
Morp.9	1.45	*Taifanglania biformis*-F19	KF554495	*Taifanglania biformis* (DQ191963)	97	+	NS
Morp.10	1.45	*Mycoleptodiscus indicus*-F21	KF554496	*Mycoleptodiscus indicus* (GU980694)	98	+	NS
Morp.11	1.45	*Taifanglania curticatenata*-F27	KF554498	*Taifanglania curticatenata* (EU004811)	95	+	NS
Morp.12	1.45	*Neosartorya pseudofischeri*-F36	KF554502	*Neosartorya pseudofischeri* (EF669937)	99	-	M
Morp.13	4.35	*Thanatephorus cucumeris*-F40	KF554503	*Thanatephorus cucumeris* (DQ223780)	98	-	M
Morp.14	1.45	*Penicillium chermesinum*-F99	KF554504	*Penicillium chermesinum* (AY742693)	98	-	M
Morp.15	20,29	*Dothideomycetes sp* -Neg.1	KR818855	*Dothideomycetes* sp. (AB847071)	97	+	NS
Morp.16	36.23	*Pleosporales* sp. -Neg.2	KR818856	*Pleosporales* sp. (KJ188727)	98	+	NS

+ colonized radicle seedling; – not colonized radicle seedling; NS: no apparent symptoms; M: caused the death of the seedling.

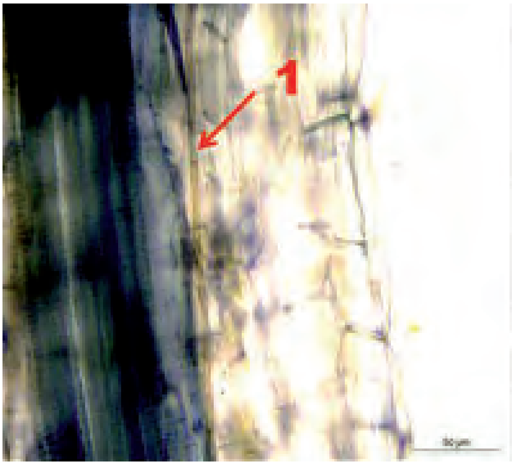

Figure 1. Structures of dark septate endophytes (DSE) in internal tissues of *Hyptis suaveolens* roots. Root fragments bleached and further stained with 0.05% trypan blue. (1) Brown septate hyphae.

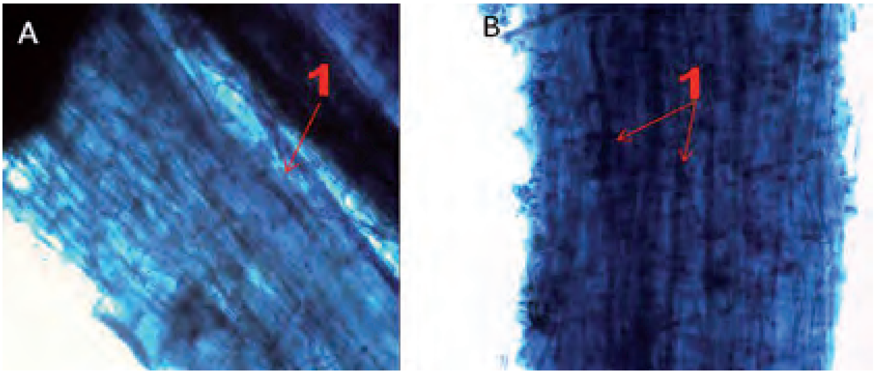

Figure 2. Endophytic fungus structure in the internal tissues of *Hyptis suaveolens* radicles. The radicles were cleared with 10% KOH and stained with 0.05% trypan blue (1: 1: 1 lactic acid, glycerol, water). Representative images of the *in vitro* infection of *H. suaveolens* rootlets with endophytic fungi. (A) Radicle infected with the endosteal fungus *Pleosporales* sp. - Neg.2. (B) Radicle infected with the endophytic fungus *T. curticatenata* - F27. (1) - Brown septated hyphae.

indistinguishable from the control seedlings. On the other hand, inoculation with the other endophytic species (62.5%) resulted in the host plant death under the conditions tested (Table 1). The interaction *C. lanceolatum*-endophytic fungi, obtained from host colleted in Pantanal, demonstrated the pattern of similar interaction under *in vitro* conditions (de Siqueira et al. 2016).

Our findings corroborate the report on the *in vitro* pathogenic behavior of endophytic fungi isolated from *Arabidopsis thaliana* (Junker et al. 2012). The fungal-endophyte-plant interaction is characterized by a balance between fungal virulence and plant defense under environmental conditions. The imbalance of these variables may result in the shift from endophytic to pathogenic behavior (Schulz et al. 2005). Endophytic fungi can establish phytopathogenic relationships and cause disease symptoms or even death of seedlings *in vitro*, probably due to the artificial culture conditions that may favor the fungus growth in relation to the host growth and/or defense. The absence of apparent symptoms of disease indicates endophytic compatibility in the fungus-host association (Wu and Guo 2008).

The antagonistic behavior of endophytes against pathogens can occur through several mechanisms, such as competition for nutrients and ecological niches, predation and parasitism, production of antimicrobial molecules like antibiotics and toxic volatile compounds, and production of enzymes, siderophores, and hydrocyanic acid (Romeiro 2007). We found that 12 of the 69 endophytic fungal strains isolated from *H. suaveolens* roots inhibited the growth of both pathogenic bacteria, *S. aureus* (ATCC 43867) and *E. coli* (ATCC 25922). The species *A. terreus* (strains: F6, F7, F10, F12, F33, and F39) effectively suppressed the pathogens growth, followed by *M. indicus* - F21, and *C. flabelliforme* - F24 species. *T. biformis* - F19 suppressed the growth of both pathogens the least strongly. There was intra-specific variation in the endophytes' potential to inhibit pathogenic bacteria growth (Table 2).

Table 2. Antimicrobial activity of endophytic fungi isolated from *Hyptis suaveolens* roots.

	Inhibition halo (mm)[1]	
Endophyte	*E. coli* (ATCC*25922*)	*S. aureus* (ATCC 43867)
Aspergillus terreus - F6	32 ± 0.15 eB	24 ± 0.12 dA
Aspergillus terreus - F7	28 ± 0.00 dB	18 ± 0.12 cA
Aspergillus terreus - F10	28 ± 0.06 dA	29 ± 0.10 fA
Aspergillus terreus - F12	28 ± 0.10 dA	34 ± 0.06 hB
Taifanglania biformis - F19	12 ± 0.00 aA	12 ± 0.00 aA
Mycoleptodiscus indicus - F21	24 ± 0.12 cB	20 ± 0.00 cA
Cladosporium flabelliforme - F24	18 ± 0.00 bA	16 ± 0.26 bA
Taifanglania curticatenata - F27	16 ± 0.06 bB	12 ± 0.15 aA
Cladosporium flabelliforme - F32	22 ± 0.25 cA	20 ± 0.25 cA
Aspergillus terreus - F33	24 ± 0.12 cA	26 ± 0.21 eA
Neosartorya pseudofischeri - F36	17 ± 0.06 bA	23 ± 0.17 dB
Aspergillus terreus - F39	25 ± 0.31 cA	31 ± 0.12 gB

[1] The results are expressed as mean ± standard deviation of the inhibition halos (mm) of three replicates. Lowercase letters (a - h) represent significant differences in a column. Uppercase letters (A - B) represent significant differences in a row. $p < 0.05$, Scott – Knott test.

Endophytic fungi associated with medicinal plants produce metabolites with not only antimicrobial activity, but also with other pharmacological activities such as anti-inflammatory, anti-angiogenic, antitumor, and immunomodulatory (Strobel 2002; Strobel and Daisy 2003, Pompeng et al 2013, Katoch et al, 2014). In addition to this study, several studies have examined the plant-fungus interaction and production of bioactive compounds by endophytic fungi with biotechnological potential (Artanti et al. 2011, Chen et al. 2011).

The results of the disk diffusion test evidenced that the *T. curticatenata* - F27 extract suppressed the *E. coli* (ATCC 25922) and *S. aureus* (ATCC 43867) growth more effectively than it suppressed the growth of the other bacterial strains tested. The *T. biformis* - F19 extract strongly inhibited *S. aureus* (ATCC 43867) growth, while *C. flabelliforme* - 24 extract efficiently inhibited *E. coli* (ATCC 25922) growth (Table 3). All the tested endophytic fungal extracts were less efficient than tetracycline in suppressing bacterial growth. The literature reports that EtOAc extracts of endophytic fungi usually exhibit antimicrobial activity. The EtOAc extract of the endophytic *Aspergillus* sp. isolated from *Justicia adathoda* displayed antimicrobial activity against *Candida albicans* (ATCC 24433), *E. coli* (ATCC 35218), *S. Aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603) (Prabavathy and Nachiyar 2012).

Table 3. Antimicrobial activity of the ethyl acetate extract of endophytic fungi isolated from *Hyptis suaveolens* roots, as assessed by the disk diffusion method.

Extract[1]	Inhibition halo[4]			
	E. coli (ATCC 25922)	*E. coli* ESBL+	*P. aeruginosa* (ATCC 9027)	*S. aureus* (ATCC 25923)
Aspergillus terreus - F7	++	+	++	++
Cladosporium flabelliforme - F24	+++	+	+	++
Mycoleptodiscus indicus - F21	++	+	+	++
Neosartorya pseudofischeri - F36	+	+	+	++
Taifanglania biformis - F19	+++	+	+	++++
Taifanglania curticatenata - F27	+++	++	++	++++
Tetracycline[2]	++++	+	+	++++
Ethyl acetate[3]	NA	NA	NA	NA

[1] The extracts were tested at 20 mg/mL.

[2] Tetracycline (positive control) was tested at 5 mg/mL.

[3] Vehicle control.

[4] Inhibition halos: NA, no activity; 5 to 10 mm = +; 11 to 15 mm = ++; 16 to 20 mm = +++; > 20 mm = ++++.

The MIC of the *A. terreus* - F7 extract against *E. coli* (ATCC 25922) was 5000 μg/mL, while the MIC of the *T. curticatenata* - F27, *N. pseudofischeri* - F36, and *T. biformis* - F19 extracts against *S. aureus* (ATCC 43867) was 30, 80, and 160 μg/mL, respectively. For crude extracts, MIC values lower than 100 μg/mL are considered potentially interesting for the exploration of bioactive compounds (Rios et al. 1998, Rabanal et al. 2002).

Table 4. Antimicrobial activity of the ethyl acetate extract of endophytic fungi isolated from *Hyptis suaveolens* roots, expressed as Minimum Inhibitory Concentration (MIC).

Extract	MIC (μg/mL)[1]			
	E. coli (ATCC 25922)	*E. coli* ESBL+	*P. aeruginosa* (ATCC 9027)	*S. aureus* (ATCC 25923)
Aspergillus terreus - F7	5000	NA	NA	2500
Cladosporium flabelliforme - F24	NA	NA	NA	2500
Mycoleptodiscus indicus - F21	NA	NA	NA	1250
Neosartorya pseudofischeri - F36	NA	NA	NA	80
Taifanglania biformis - F19	NA	NA	NA	160
Taifanglania curticatenata -F27	NA	NA	NA	30
Tetracycline	0.8	NA	NA	0.8

[1] Results represent the mean of three replicates. NA, no activity.

All the endophytic fungal extracts exerted bactericidal activity against *S. aureus* (ATCC 43867). Endophytic species of the genus *Neosartorya* also produce antimicrobial compounds (Rosa et al. 2012, Jayasuriya et al. 2009).

The *A. terreus* - F7 and *T. curticatenata* - F27 extracts presented the strongest antioxidant activity, reaching values of 61.73 ± 2.27% and 59.44 ± 3.01%, respectively. Other extracts that exhibited moderate antioxidant activity were: *T. koningiopsis* - F4 (37.09 ± 5.31%), Pleosporales sp. - Neg. 2 (36.38 ± 1.59%), Dothideomycetes sp - Neg.1, (31.66 ± 6.59%), *F. oxysporum* - F3 (30.46 ± 1.09%), and *C. flabelliforme* - F24 (29.75 ± 1.02%).

All the extracts presented total phenolic compouds in their composition. The highest levels were detected in the *M. indicus* - F21, *T. koningiopsis* - F4, and Dothideomycetes sp. - Neg. 1 extracts (0.50, 0.49, and 0.48 mg/mL, respectively), and the lowest levels were detected in the *T. curticatenata* - F27 and *T. hechuanensis* - F34 extracts (0.04 and 0.05 mg/mL, respectively).

Although the *A. terreus* - F7 and *T. curticatenata* - F27 extracts displayed the highest percentages of antioxidant activity, they did not have the greatest levels of total phenolic compounds, indicating that these two variables do not always correlate directly. The antioxidant efficacy of phenolic compounds varies greatly (Sengl et al. 2009). The antioxidant activity of a given extract does not depend only on the concentration of the phenolic compounds, but also on their structure and synergistic and/or antagonistic interaction (Liu et al. 2007). Other groups of biomolecules, such as polysaccharides (Li et al. 2011), carotenoids (Artanti et al. 2011), and ascorbic acid (Prasad et al. 2013) also exert this bioactivity. The EtOAc extract of *A. terreus* - *F7* and its compounds terrein, butyrolactone I, and butyrolactone V1 display *in vitro* schistosomicidal and leishmanicidal activities, and antitumor activity against MDA-MB-231 and MCF-7 cells (da Silva et al. 2017).

Conclusion

The *H. suaveolens* roots are colonized by an assemblage of endophytic fungi of the classes Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Agaricomycetes. The species *T. hechuanensis* - F17, *T. biformis* - F19, *M. indicus* - F21, *T. curticatenata* - F27, Dothideomycetes sp. - Neg. 1, and Pleosporales sp. - Neg. 2 demonstrate endophytic potential to colonize internal tissues of the *H. suaveolens* root system without resulting in apparent symptoms of disease when co-cultivated. These endophytes represent an important source for the prospection of bioactive molecules, since extracts obtained from the cultivated fungal species exert antimicrobial and antioxidant activity. Chemical characterization and structural elucidation of the components are necessary to better understand the actions of the bioactive extracts produced.

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Table 5. DPPH radical scavenging by and concentration of total phenolic compounds in the ethyl acetate extract of endophytic fungi isolated from *Hyptis suaveolens* roots.

Extract	DPPH scavenging (%)				Total Phenols (mg GAE / mL extract)
	0.04 mg/mL	0.08 mg/mL	0.16 mg/mL	1.6 mg/mL	
Aspergillus terreus - F7	1.72±0.27 bA	5.36±0.74 dB	16.02±0.88 fC	61.73±2.27 gD	0.29±0.01 c
Cladosporium flabelliforme - F24	1.61±2.27 bA	2.50±0.83 cB	5.67±1.06 dC	29.75±1.02 eD	0.40±0.02 d
Dothideomycetes sp. - Neg. 1	2.24±0.35 bA	4.70±0.44 dB	8.22±0.73 eC	31.66±6.59 eD	0.48±0.01 e
Fusarium oxysporum - F3	1.18±0.09 bA	1.79±0.01 bA	3.17±0.10 cB	30.46±1.09 eC	0.30±0.03 c
Macrophomina phaseolina - F1	0.48±0.10 aA	1.79±0.18 bB	5.38±0.78 dC	17.80±3.73 dD	0.30±0.03 c
Mycoleptodiscus indicus - F21	0.19±0.33 aA	1.13±0.19 aB	3.63±1.03 cC	20.99±0.93 dD	0.50±0.05 e
Neosartorya pseudofischeri - F36	0	0	1.63±0.57 bA	19.36±0.38 dB	0.25±0.04 b
Penicillium chermesinum - F99	0.49±0.8 aA	1.51±0.10 bB	5.23±0.52 dC	18.33±2.23 dD	0.25±0.03 b
Pleosporales sp. – Neg.2	1.55±0.63 bA	2.75±0.37 cB	5.08±0.21 dC	36.38±1.59 fD	0.38±0.03 d
Taifanglania biformis - F19	0	0	0	6.24±0.62 bA	0.24±0.06 b
Taifanglania curticatenata -F27	0	1.46±0.48 bA	3.43±0.41 cB	59.44±3.01 gC	0.04±0.00 a
Taifanglania hechuanensis - F34	0	0	0	1.94±0.57 aA	0.05±0.01 a
Thanatephorus cucumeris - F40	0	0	0.63±0.29 aA	12.72±0.76 cB	0.24±0.02 b
Trichoderma koningiopsis - F4	2.26±1.42 bA	4.38±0.06 dB	7.04±0.23 eC	37.09±5.31 fD	0.49±0.03 e
Ascorbic acid	16.02±1.13 cA	49.50±1.95 eB	91.37±0.18 gC	97.45±0.09 hC	

The results are expressed as mean ± standard deviation of three replicates. GAE: gallic acid equivalents. Lowercase letters (a - h) represent significant differences in a column. Uppercase letters (A - B) represent significant differences in a row. $p < 0.05$, Scott – Knott test.

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Pedological relationships
with the macrohabitats of the Pantanal wetland

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Introduction

One of the factors causing great negative environmental impact on soil and water resources lies on the conversion of vast natural ecosystem areas into agricultural sites (FAO 2015, Scanlon et al. 2007, Lal 2001). Soybean crops drive the land use changes in Brazil, especially in the savanna Biome, which has more than 50% of its 2 million km^2 converted into agricultural land and pasture (Kling and Machado 2005, Machado et al. 2004). Deforestation in the Amazon, besides compromising the water cycle, worsens global warming (Fearnside 2005). Pantanal is the largest floodplain in the world (Ponce and Cunha 1993), but 18% of its original vegetation was already removed (Haris et al. 2006, 2005); moreover, its savanna (Adámoli 1982) area, which is distributed on the plateau, just has 63% of its total extent preserved (Haris et al. 2006, 2005). The intense agriculture in the Upper Paraguay Basin causes erosion and sediment entry into the Pantanal area, fact that leads the region to major social, economic and ecological issues (FAO 2015, Galdino, Vieira and Pellegrin 2006).

Such issues express the relevance of better understanding Mato Grosso Pantanal floodplain in order to present mitigating measures to minimize the effects from negative environmental impacts. This impact mitigation actions become the priority for this biome, which was granted the status of National Heritage by the 1988 Federal Constitution (Art. 225, § 4), since it is a wetland site of international importance for RAMSAR conservation. In addition, it was categorized as Biosphere Reserve by UNESCO (Natural Heritage of Humanity), due to its unique opportunity to protect the local biodiversity (Haris et al. 2005) and the conditions necessary to boost new Research focused on biodiversity preservation.

According to Junk et al. (1982), surveys carried out at the Pantanal flood plain, before the area was acknowledged as World Biosphere

Reserve back in the early 1980s, were scarce. These studies aimed to help better understanding the abiotic component and its performance within different environments subjected to the annual flood pulse recorded in this complex and humid region. However, in the last few years, these research have been presenting significant advances in terms of environment featuring, mainly in soil-related features; in hydrosedimenlogical and biodiversity processes (Cardoso et al. 2016; Dalmago et al. 2016; Chwerdtfeger et al. 2014a; Chwerdtfeger et al. 2014b; Junk and Nunes da Cunha 2012; Corradini 2011; Fernandes et al. 2010; Assine and Silva 2009; Corradini et al. 2009; Zani et al. 2009; Zani 2008; Silva et al. 2006; Zani et al. 2006; Assine and Soares 2004; Assine 2003) and in carbon biogeochemistry (Dalmagro et al. 2017; Melo et al. 2015; Johnson et al. 2013; Messias et al. 2013).

According to some research, most soil types composing these flood plains are subject to hydromorphism processes, which are also subject to local relief, and it leads to permanent or temporary environment saturation (Nascimento et al. 2013; Coringa et al. 2012, Assine and Soares 2004) that results in physicochemical changes of soils. Despite being an environment that accumulates different categories of organic and/or inorganic sediments, it depends on the rainfall regime, or mainly on lateral flows (Bertsch and Seaman 1999). These conditions define the pedogenetic processes that have acted and/or that still act in the local soil types; therefore, they change the profile of the soil basic geochemical composition (Bini et al. 2011).

The main pedogenetic processes recorded in Pantanal soils are redoximorphic (Chig et al. 2016; Beirigo et al. 2011b) and glezation (Buol et al. 2003; Fanning and Fanning 1989) and plintization (Beirigo et al. 2011a; Couto and Oliveira 2010) are the most common processes among them. These processes are responsible for mottles found in the entire soil profile, or in some parts of it. The color of these mottles, range from red to Yellow and greyish (Chig et al. 2016; Couto and Oliveira 2010; USDA 2010).

If the aforementioned assumptions are taken into account it is possible understanding that the pedogeomorphological aspects are differentiation factors of already established Pantanal macrohabitats (Nunes da Cunha and Junk 2014). It is worth knowing the role soil plays in the occurrence of units such as paleolevees, floodplain, landis, corixos and mounds fields, among others. According to some authors (Nunes da Cunha and Junk 2011; Zeilhofer and Schessl 1999), diverse macrohabitats compose the Pantanal landscape, and form a mosaic which is rarely found in other wetlands. The understanding about the importance of pedogeomorphological aspects to the development of different Macrohabitats is the factor encouraging the sustainable use and conservation of Pantanal biodiversity.

The classification published by Nunes da Cunha and Junk (2011) for Pantanal habitats does not take into consideration the soil types. Therefore, the hypothesis that the physicochemical attributes of wetland soils are valuable tools to characterize the habitats already established in Pantanal was addressed in order to fill this gap in the literature. Thus, the present study was carried out to define the soil association with key macrohabitats in the large Pantanal wetland.

Information about all the classified soil types found in the Northern wetland were collected during the first year of the project called "Identification and classification of Pantanal soils" (Coringa et al. 2012; Mello et al. 2012; Beirigo et al. 2011a; Couto and Oliveira 2010; Beirigo 2008; Sousa 2003; Couto et al. 2002). These data were then related to the already identified phytophysiognomies.

Macrohabitats located in Pirizal region, which is a paleoleque of Cuiabá River (Assine et al. 2006), were floristically characterized by Nunes da Cunha et al. (2016) and Nunes da Cunha and Junk (2014, 2011) in the second year of the project. One soil profile from each macrohabitat was described according to the Manual of Description and Soil Collection in the Field (Santos et al. 2005), as well as five georeferenced samples of pedological horizons A and B.

The soil samples were air-dried and sieved in a 2-mm mesh (fine dry earth in the air) and the chemical and physical analyses were carried out in laboratory environment for survey purposes. Nodes bigger than 2-mm, were separated and quantified as gravel soil fraction. A subsample was used to test water stability, according to the method by Daniels et al. (1978).

The following soil chemical attributes were determined: pH in water and in 1 N KCl solution, electrical conductivity (EC) at the ratio 1:2.5 (v/v); exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+), extracted through KCl 1 mol/L and quantified through atomic absorption spectrophotometry (AAS); Al^{3+} concentrations through titration by using NaOH 0.025 mol/L, K^+, Na^+; available and exchangeable P extracted by using HCl 0.05 mol/L H_2SO_4 + 0.0125 mol/L (Mehlich-1), K^+, and Na^+ quantified through flame photometry, and available P determined through colorimetry; the potential acidity (H + Al) was extracted by using calcium acetate solution 0.5 mol/L at $pH_{7,}$ and determined by applying a titration solution containing NaOH 0.025 mol/L; organic C was determined through the Walkley-Black method and by wet oxidation using potassium dichromate 0.1667 mol/L, which was subsequently unheated through titration by using 0.1 mol/L ammonium ferrous sulfate; and conductivity (Embrapa 2011).

The Sum of Bases (SB), Cation Exchange Capacity (CEC), Saturation of Bases (V%), Saturation of Al^{3+}, and the Saturation of Na^+ (m%) and (PST%) were calculated based on the results.

Particle size analyzes were performed according to Embrapa (2011). The clay fraction (<0.002 mm) and water-dispersible clay by the hydrometer method, sand fraction to the total per-went through stretching and silt (0.05 – 0.002 mm) by difference. The total sand fraction was fractionated into very coarse sand (2 – 1 mm) thick (1 – 0.5 mm), medium (0.5 – 0.25 mm), thin (0.25 to 0.10 mm) very thin (0.10 – 0.05 mm), according to the scale atterberg scale modified through sieving. We calculated the degree of flocculation (GF), the relationship between silt and clay texture.

Classification of Soils

Based on interpretation of the chemical, physical and morphological descriptions, the described profiles were classified up to the fourth categorical level (subgroups), according to the Brazilian Soil Classification System (SiBCS) (Santos et al. 2013), for survey purposes.

Statistical analysis

The analysis was based on univariate frequency tables and contingency tables, which were used to analyze the similarity between soil classes (up to the second level, according to criteria set by Santos et al. 2013) and Pantanal macrohabitat classes, according to the classification by Nunes da Cunha et al. (2016) and Nunes da Cunha and Junk (2014, 2011).

Description of the pedological relationship with the Northern Pantanal macrohatitats

According to Nunes da Cunha et al. (2016), Nunes da Cunha and Junk (2014, 2011) and Coutinho (2006), Pantanal macrohabitats are included in a biodiversity-rich ecosystem, which can be identified as permanently land areas, namely: forest macrohabitats (semi-deciduous forests, deciduous forests and forests) located in areas locally known as "paleolevees". In addition, they include polyspecific forest areas, such as riparian forests, and monospecific forest areas (carandazais, cambarazais and paratudais). Savanna formations like the open Low-tree Savanna (savanna field and dirty field) are found in periodically terrestrial areas, as well as camp formations that show different flood levels and amplitudes such as the clean fields and ebb. Moreover, there are periodically aquatic areas comprising brackish or sweet water.

The main information about the current distribution of available soils are found in different mappings and soil-related studies (Couto 2002; Jacomine et al. 1997; Brazil 1997, 1982a, Embrapa 1982; Brazil 1979, 1971), and in elaborated syntheses (Oliveira et al. 2004; Amaral Filho 1986), besides Zeilhofer (2006) attempt to estimate the distribution of Pantanal soils through digital mapping.

According to Couto and Oliveira (2010), based on the distribution of these soils and on a macroscopic view, regardless of the details needed to understand the multiple processes and relations between aquatic and terrestrial ecosystems, as well as on the flood pulse of this huge wetland (Melfi et al. 2004) (Figure 1) one can measure their morphological characteristics (Junk et al. 2014). Basically, the pedogenetic gleization and plintization processes give the soils a gray shade, which is observed in the entire, or in some parts, of the soil profile. Such processes give rise to Gleysols and Plinthosols, which are often found in Pantanal Matogrossense.

The ancient lobes of Cuiabá River show a large variety of soil classes such as Fluvic Cambisols, Gleysols, Luvisols, Neosols, Planosols, Plinthosols and Vertisols (Embrapa 2009, Couto et al. 2002), which are equivalent to the WRB/FAO system (Cambisols, Gleysols, Luvisols, Arenosols, Fluvisols, Solonetzs, Plinthosols and Vertisols). These classes are distributed in typical pedological features of redoximorphic surroundings (Beirigo et al. 2011b; Beirigo 2008). According to Zeilhofer and Schessl (1999), the occurrences of some soils depend on certain relations to the distribution of different phytophysiognomies in the Pantanal landscape.

The exploratory analysis of physicochemical analysis results from diagnostic horizons of soils associated with macrohabitats in this Pantanal part shows that only three of them (*Espinheiral, Tabocal* and pond) prevail in a single soil class. The other macrohabitats are found in two or more soil classes (Figura 1A).

Macrohabites associated with a single soil class:

1 – Macrohabitat called flooded field with shrubs – Predominant of shrub vegetation, which is characterized by the species *Mimosa pellita* H. et B. (*Leguminosae-Mimosoideae*), locally called **Espinheiral** (Mello et al. 2015). The predominant soil layer under this tree is the Gleysol (Figure 2A) which is distributed along the floodplain of Cuiabá River (Novaes Filho 2012; Beirigo et al. 2011a). *Espinheiral* is a native and invasive species, which easily suppresses C_4 plants (grass) (Yule et al. 2009), it is tolerant to flooding processes occurring in ciliary vegetation, in capon edges, wetlands and dry lagoons. *Espinheiral* occurs in other soil classes in other Pantanal parts, but always with great fertility (Pott and Pott 1994).

Gleysols are poor or poorly drained hydromorphic mineral soils that present gleyzed horizon, which is characterized by iron reduction caused by the presence of stagnant water. This horizon is evidenced by neutral colors and/or live-colored mottles (Santos et al. 2013). According to RadamBrasil (1982) Gleysols are associated with the alluvial plains found in Pantanal as a whole.

2 – *Tabocal* is a macrohabitat observed in Luvisols, according to Beirigo et al. (2011b). Tabocal is a local name given to sparse emergent vegetation and monodominant *taquaras Guadua* sp. (Taboca/Poaceae) sub-forest (Nascimento 2012; Beirigo et al. 2011a; Hofmann et al. 2010). This species is not very demanding for soil fertility; however, it can naturally present better responses in fertile soils (Pereira and Beraldo 2007). Luvisols are shallow to shallow, non-hydromorphic (Embrapa 2005), moderately acid to almost neutral, moderately imperfectly drained soils found in smooth or undulating areas, which are quite susceptible to erosion. It is common finding pebbles and quartz boulders on the surface of these soils, which are called desert pavements (Santos et al. 2013). In addition, according to Beirigo et al. (2011a), they are eutrophic soils with high magnesium saturation (PMgT) and clay fraction activity (Ta) (Tr e”27 cmol_c kg of clay^{-1}).

3 – Pond (Figure 1) macrohabitats, locally known as *Lagoinhas,* are observed in Planosols. According to Nascimento (2012), they are the lowest sites in paleoplains, besides being often found under graminous formation and surrounded by small elevations. These elevations, wherein the flood goes from 80 to 100 cm high, prevent the rainwater drainage or the rise of groundwater; therefore, they help keeping the water confined in these sites for longer periods of time, which is often observed in Planosols.

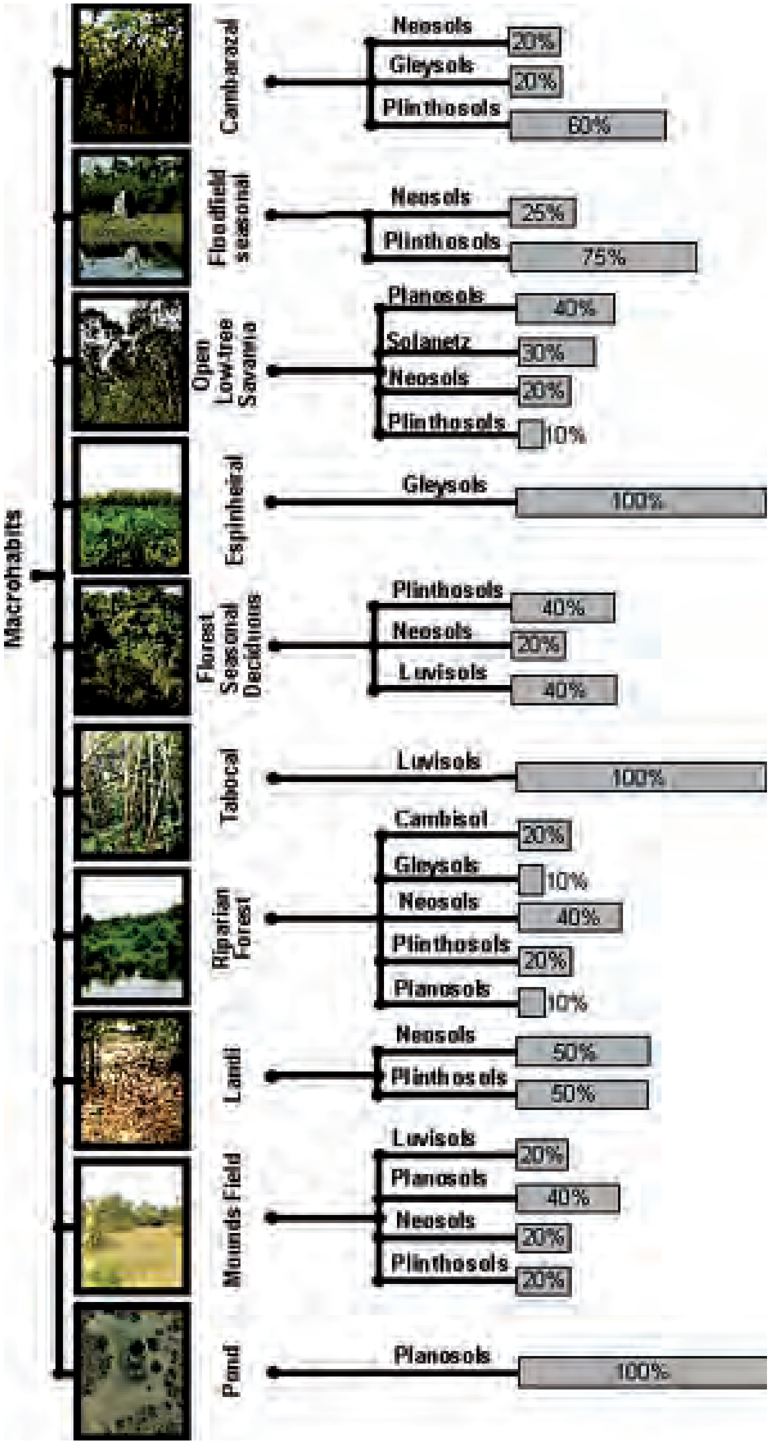

Figure 1. Relation between macrohabitats and soil classes in Northern Pantanal according to the univariate frequency table.

Superficial or subsurface

Planosols are imperfectly or poorly-drained mineral soils presenting superficial or subsurface eluvial horizon, which contrasts the B horizon. The B horizon presents expressive clay concentration, and slow or very slow permeability. The well-marked difference between horizons A or E and B lies on the characteristic abrupt transition, which has a strong structure set on blocks, prismatic or columnar structures (Santos et al. 2013). It often presents a sodium ($100Na^+/T$, e”15%) or solodic ($100Na^+/T$, <15% and e”6%) character (Beirigo 2011b; Couto and Oliveira 2010). According to Nascimento (2012), Sodium, in this region, comes from hydrology and from a current evapotranspiration that promotes the seasonal accumulation of bases on horizons located in the highest portions of the landscape, or from the precipitation of salts at the edges of saline lagoons. This macrohabitat suggests the occurrence of the planic B horizon, which effectively works as a barrier to normal root development and to vertical water flow.

Macrohabites associated to two or more soil classes:

1 – Landi: Plinthosols and Neosols (Figure 2D) are found in this macrohabitat through observations in the field. It was noticed that the predominant habitats in their surroundings presented the same soil classes, because this macrohabitat is characterized by forest formation locally known as “guanandi” (*Calophyllum brasiliense* Camb.). These formations occur in corridors presenting small level differences; they work as channels to drain water during flood and ebb periods in Pantanal (Nogueira et al. 1989). This environment is very erosion conducive, mainly because they are always close to “cambarazais”, “paleolevees” and “mounds”. According to Couto et al. (2002), Landis cause great effects in ecological terms, due to the elevation gradient, which determines its flood and drought periods.

According to Santos et al. (2013), Plinthosols concern soils composed of mineral material, which is formed under redoxorphic conditions caused by drainage restrictions and water percolation. These soils are subjected to the temporary effect of excessive moisture, which favors the consequent plinthic and/or other horizon’s appearance: pale or variegated colors or moderate to abundant mottling amounts. Neosols are often deep, imperfectly and poorly drained soils of sandy or open sand texture; moreover, they are poorly developed and composed of a horizon A superimposed on C.

Yet, Neosols can be classified according to the lower or greater influence of hydromorphic conditions. They are differentiated as Ortic Quartzarenic Neosol and Hydromorphic Quartzarenic Neosols at a subsequent level. Hydromorphic Quartzarenic Neosols are located in the lower positions, wherein the groundwater is closer to the surface (Cardoso et al. 2016). According to Nascimento (2012), Neosols in Pantanal are more often found in ancient lobes.

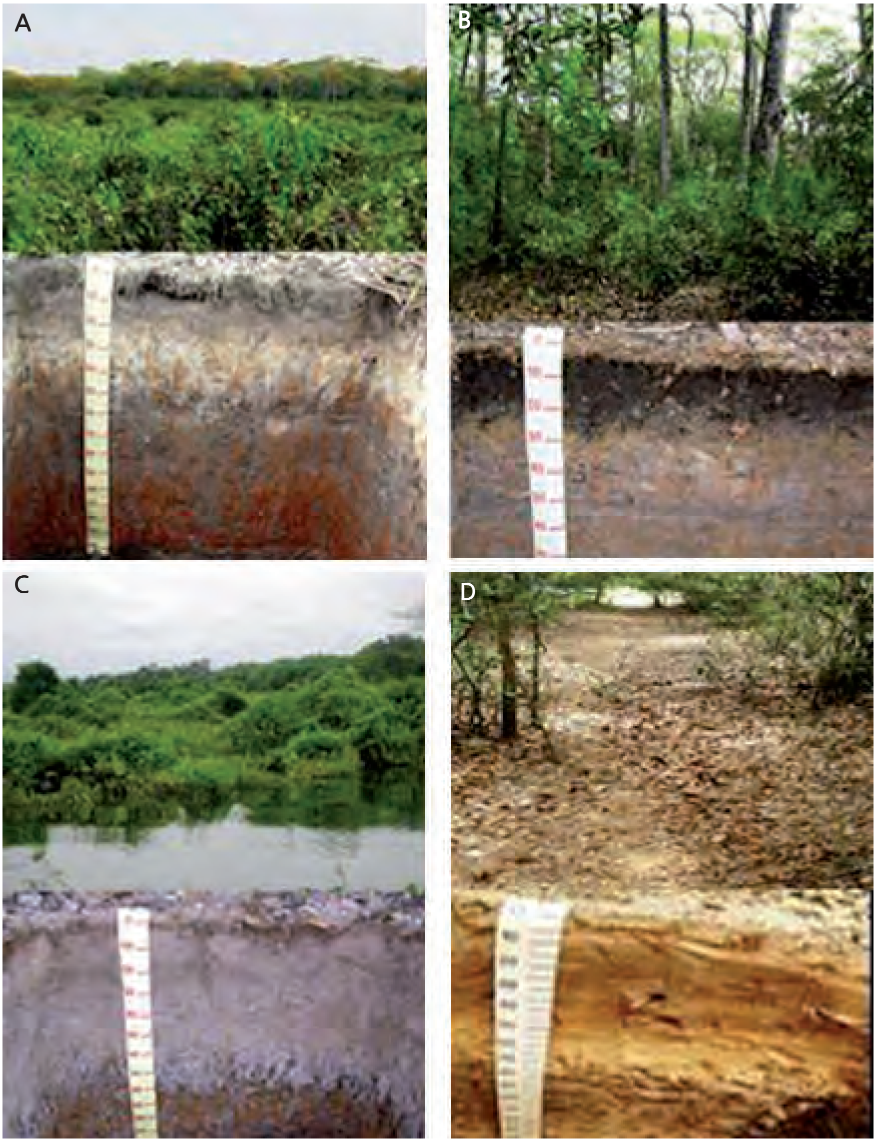

Figure 2. Details about vegetation types and soil profiles: *Espinheiral* (A) and *Cambarazal* (B) under Gleysol, Riparian Forest (C) under Planosols and Landi (D) under Plinthosol (Adapted: Mello et al. 2015).

2 – A Seasonally Flooded Semi Evergreen Forests dominated by *Vochysia divergens* Pohl (*Vochysiaceaee*) (regionally known as **Cambarazal**) (Arieira and Nunes da Cunha 2006) is a species tolerant to prolonged periods of flooding (Prance and Schaller 1982, Stafleu 1948), and to high levels of aluminum in the soil (Nascimento and José 1986). In the northern pantanal the Cambarazais were found in three types of soil classes (Figure 1): Plinthosols, Gleysols (Figure 2B) and Neosols. Ferreira Júnior (2009) observed the occurrence of Cambará on Plintossolo. Already, Mello et al. (2015), Novaes Filho (2012), Biudes et al. (2009) observed Cambarazal on Gleysol.

3 – Flooded polyspecific forest (**riparian forest**) (Figure 1) is a macrohabitat where the emergent arboreal stratum is sparse; the undeveloped understory is located in riparian zones (riverbank). Species in this macrohabitat are tolerant to flood (Junk 1989, White 1979) and such tolerance increases when trees become adults (Junk 1989). The riparian forests were more often observed in Neosols, and more rarely found in Gleysols, Cambisols and Planosols (Figure 2C). Cambisols are soils composed of mineral material and B horizon; their thickness ranges from 50 to 100 cm and without restriction of drainage, in relief slightly altered, and with fertility varying from eutrophic or dystrophic (Santos et al. 2013). When these soils are located in floodplains, they are subject to floods, which are frequent and of medium to long duration. These are limiting factors to the full agricultural use of these soils.

4 – The **Open Low-tree Savanna** (*Cerrado sensu stricto*) are found on mountain ranges (paleolevees) and non-flooded areas (Figure 1); they are more often observed in Planosols and Neosols, and more rarely in Plinthosols. They are mainly sandy in almost their entire profile; at least in their more superficial portion (Couto and Oliveira 2010), fact that gives them sandy cord appearance (Cunha 1980). According to Cunha (1980), savanna vegetation growth, composition and density in paleolevees change according to the soil water regime. These soils are characterized as non-hydromorphic at the highest points of mountain ranges, but they present hydromorphic soil features in the lower middle third, because they are subject to periodic flooding. Such condition, according to Beirigo (2008), impels the new typical pedological features of redoximorphic environments.

Beirigo (2008) found paleolevees in four Ortic *Natric* Planosols, an Argiluvic Plinthosol and a Haplic Plinthosol and in their surroundings, as well as in flooded fields over dystrophic Argiluvic Plinthosol. Barbiero et al. (2008) recorded Quartzarenic Neosols dominance along mountain ranges and even in ebbs. Zeilhofer and Schessl (1999), studied the relation between vegetation and environmental conditions, they found mountain range in Planosols. Coringa et al. (2014), Couto and Oliveira (2010), Ferreira et al. (2010), Oliveira et al. (2006), Sousa (2003) recorded Luvisols and Planosols occurrence in the higher portions of reliefs (mountain ranges). Such references corroborate with the ones in the current study when they describe paleolevees as being very common in Planosols.

5 – The **Mounds fields** (Figure 1) according to Eiten (1994b, 1983) Oliveira-Filho (1992a, 1992b), are savanna formations characterized by woody vegetations called seasonally flooded savanna (Nunes da Cunha et al. 2007). They are found in small terrain elevations; according to Marimon et al. (2012) and Ribeiro and Walter (2008), they are surrounded by grassy fields, which are a phyto-physiognomic savanna type. These soils are subjected to the hipersalonic climate, which is marked by different floods (Cianciaruso and Battle 2009), as well as by intense fire regimes (Marimon et al. 2012, 2008).

According to RadamBrasil (1981) mound fields prevail in Dystrophic Cambisols and dystrophic Plinthosols hills, whereas Dystrophic Argiluvic Plinthosols prevail in flood fields where the ferruginous concretions stand out.

According to Marimon et al. (2012) the hydromorphic Plinthosols found in mound fields at Araguaia State Park are deep and poorly drained soils, despite being dystrophic and strongly to moderately acidic.

It is necessary to understand the systemic relation in this environment to better understand the relation between wetland soils and different phytophysiognomies. This environment can accommodate different categories of coexisting phenomena conditioned in different forms; moreover, they can interrelate their different parts. Therefore, the Landscape, topography and flood time influence habitat features and the soil genesis of these plains. Settlements of transported sediments located far from the river differ from the current ones; they are carried by floods found close to current perennial rivers, which present intense and constant water flow (Assine et al. 2014, Birth 2012, Corradini and Assine 2012). These flood conditions also help forming the diversity of habitats found in Northern Pantanal.

According to Nascimento (2012) and Coringa et al. (2005), areas not directly influenced by floods are very likely to present sodium accumulation, besides presenting nodules in some horizons; as well as in areas directly influenced by the flood, which are subject to strong redoximophic features: mottles and nodules. According to Lima et al.

(2005), they alter the equilibrium of elements in the soil and cause electrochemical potential (Eh) decrease, fact that leads to changes in Fe and Mn oxide reduction and oxidation conditions (van Bodegom et al. 2003, Vepraskas and Faulkner 2001). They show brown, gray, blue, black and yellow mottles that characterize the frequently observed hydromorphic soils (Fanning and Fanning 1989).

The macrohabitats identified by Nunes da Cunha and Junk (2014, 2011) was used in the present study, which was conducted the Pirizal region, and enabled observing a heterogeneous pedological constitution. The phytophysiognomy Landis and Mounds fields were identified in Plinthosols, the Pond in Planosols, and the mountain ranges in Neosols. There were floodplains in Neosols, Gleysols and Espodosols. With respect to the identified soil classes, abrupt textural change was observed in Mound fields, pond and Landi (which presented abrupt character), only (Figure 3). A textural B horizon of high clay content was underlying horizon A or E (Santos et al. 2013).

The presence of plinthite and petroplinthite is a relevant factor since these elements are directly responsible for plinthosol classifications according to the Brazilian Soil Classification System (BSCS) (Santos et al. 2013). Both elements were recorded when the Landis and Mounds fields in Plinthosols were classified. It is worth emphasizing that this condition was also observed by Ferreira Junior (2009) in Mounds fields.

There was gley horizon and illuvial accumulation of organic matter on the soil of some common flood fields found in areas covered by stagnant water for short and/or long periods. Mountain ranges were observed in Neosols, they presented a small textural difference, which changes from sand-franc to sandy-loam. According to Souza and Souza (2010), they are not subjected to flooding, except for exceptional flood cases, because of their higher position.

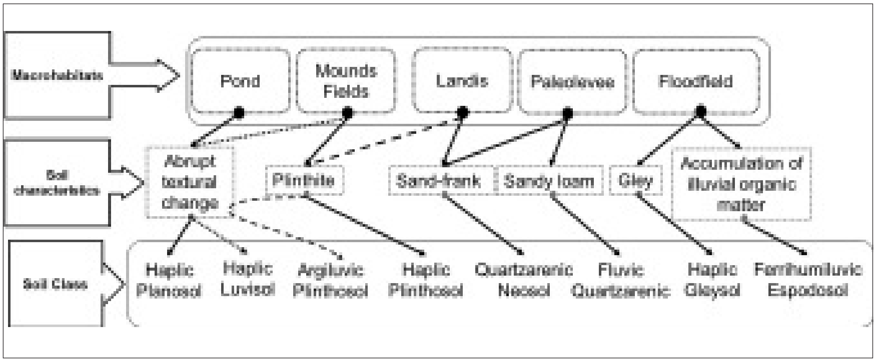

Figure 3. Characteristics of each soil class within the macrohabitats.

The Fluvic character is evidenced by the uneven distribution of organic carbon content in depth (Santos et al. 2013), and is the most obvious morphological feature in the Neosols found in humid areas, since they are inherited from alluvial sedimentation processes (Beirigo et al. 2011b).

The similarity of macrohabitats associated with soil classes based on physical and chemical attributes of soil samples collected 0 to 20 cm on the soil can be observed in the dendrogram (Figure 4). The relative dendrogram analysis applied to phytophysiognomy similarities associated with soil classes (Figure 4) allowed the record of three groups. The first group was composed of the following macrohabitats: Paleolevee upon Fluvic Quartizarenic, Landi upon Argiluvic Plinthosol, Paleolevee upon Quartzarenic Neosol, Quartzarenic Neosol, floodfield upon Quartzarenic Neosol, Paleolevee sand upon Quartzarenic Neosol; only Landi was not upon Quartzarenic Neosol. The second group was composed of macrohabitats Floodfield upon Ferrihumiluvic Espedosol, pond upon Haplic Planosol, Floodfield upon Haplic Gleysol and Landi upon Haplic Plinthosol. The third group was composed of phytophysiognomy Mounds Field upon Haplic Plinthosol, only.

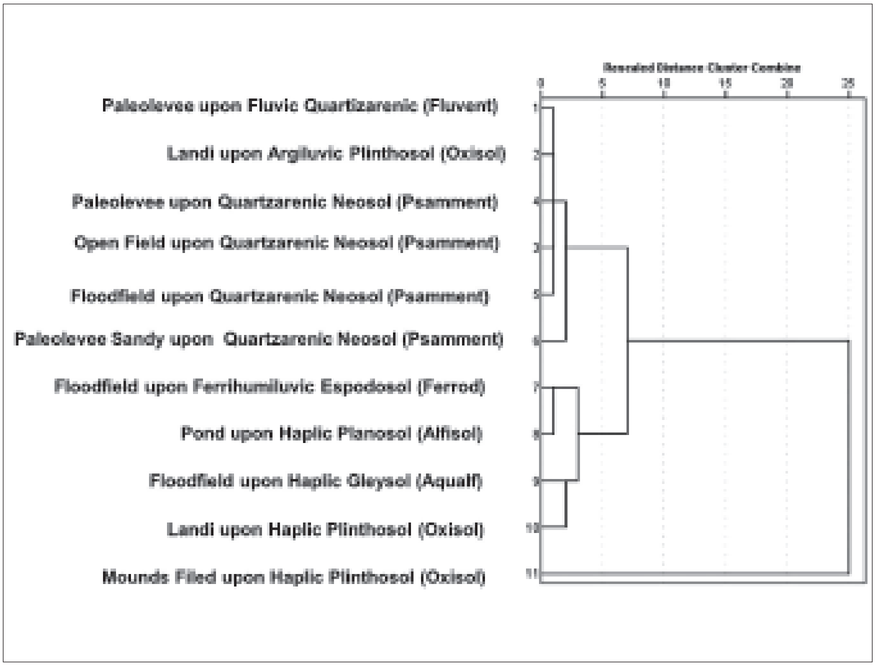

Figure 4. Dendrogram from the hierarchical cluster analysis based on the physicochemical soil attributes from samples collected 0 to 20 cm down the soil.

The similarity of macrohabitats associated with soil classes based on the physicochemical attributes of soil samples collected 60 to 80 cm down the soil can be observed in the dendrogram shown in Figure 5. This soil depth refers to the subsurface horizon; assumingly, it will have less influence on changes caused by intemperic processes and on soil physicochemical characteristics than the surface horizon (0 to 20 cm), which is more strongly affected by the speed and intensity of changes caused by these processes. Such results can be confirmed by the dendogram analysis, which only distinguished two distinct groups wherein the first group presents the greatest similarities among Paleolevee upon Quartzarenic Neosol, Paleolevee upon Fluvic Quartizarenic, Open Field upon Quartzarenic Neosol, Paleolevee upon Quartzarenic Neosol, Landi upon Argiluvic Plinthosol, Paleolevee upon Quartzarenic Neosol, floodfield upon Haplic Gleysol and floodfield upon Espodosol Ferrihumiluvic.

The second group presents similarities between Landi upon Haplic Plinthosol, Pond upon Haplic Planosol and Mounds Field upon Haplic Plinthosol. The Landis belong to two different groups: mountain ranges and flood areas; therefore, they differ from each other, mainly in clay content (Figure 6). Such difference reinforces the observations made in the field, where Landis were in the same soil classes and prevailed in the macrohabitats found in the environment.

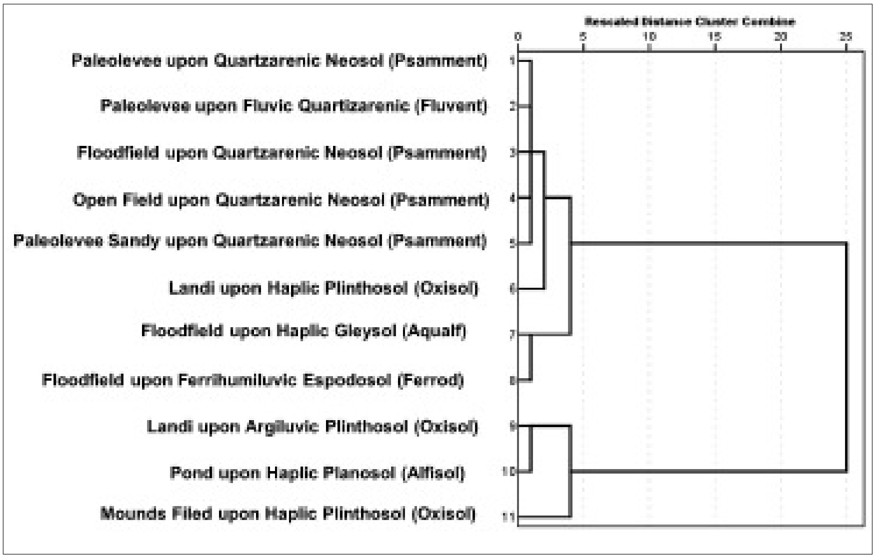

Figure 5. Dendrogram resulting from the hierarchical cluster analysis based on the physicochemical attributes of soil samples collected 60 to 80 cm down the ground.
Figure 6. Box-Plot of the physicochemical attributes based on the different macrohabitats studied in two different soil depths.

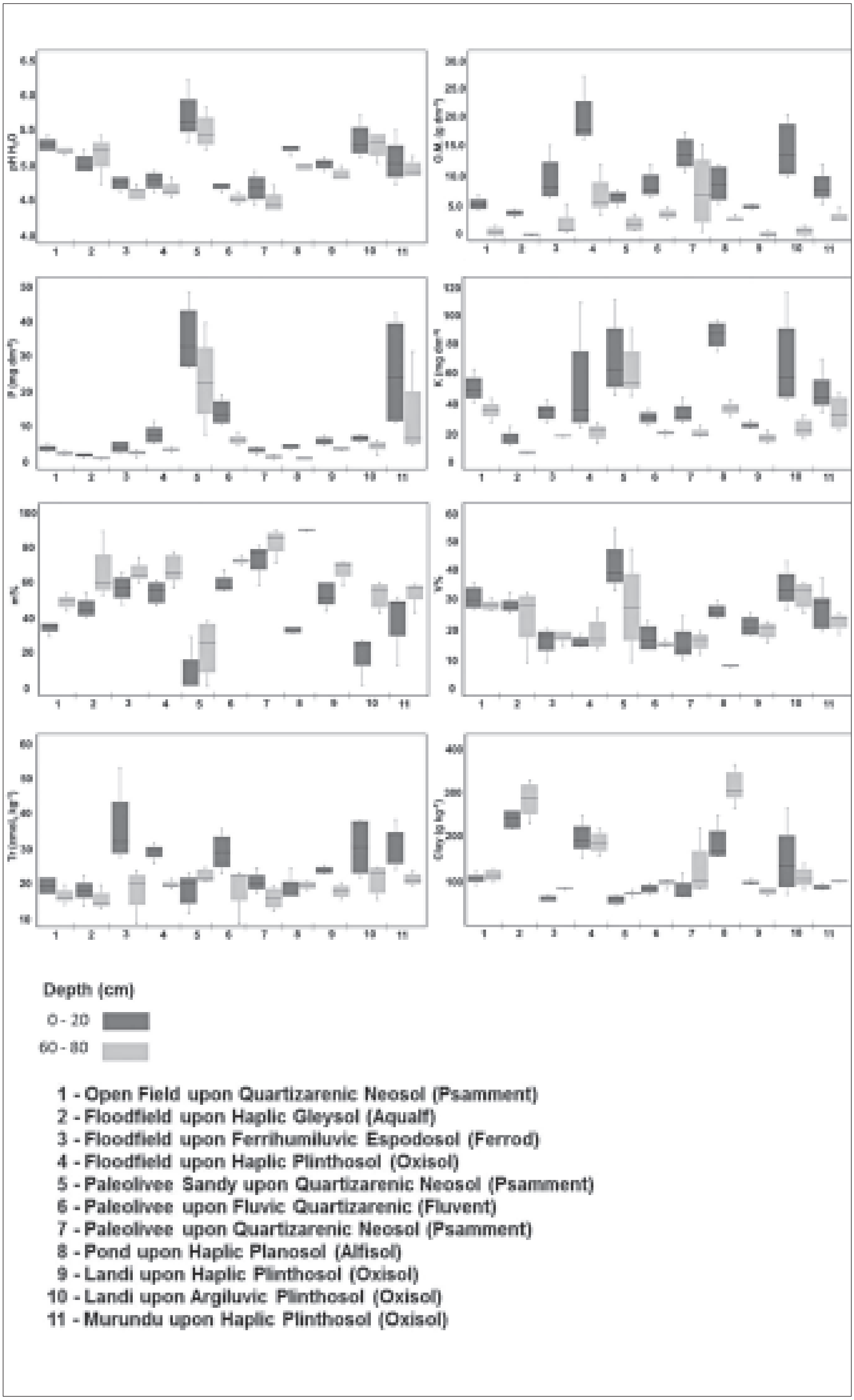

Figure 6. Box-Plot of the physicochemical attributes based on the different macrohabitats studied in two different soil depths.

Barbiero et al. (2008) recorded similar condition in paleolevees, as well as an intermittent channel locally known as "ebbing". It can be said that Landis present themselves as very recent phytophysiognomies within or near other macrohabitats.

In general, soils in different macrohabitats (Figure 6) have low fertility (V <50%) and can be described as dystrophic (Santos et al. 2013). Most macrohabitats show low clay fraction activity (Tb) (Tr <27 $cmol_c$ kg of $clay^{-1}$), except for those in the areas most susceptible to flooding and for the macrohabitats presenting high aluminum saturation (m%> 50). Arieira et al. (2016) found low fertility in Cambarazais. Beirigo et al. (2011b) recorded that most of the Pantanal soils present high Al^{3+} (m%) saturation and high levels of exchangeable Al^{3+}. According to Nunes da Cunha et al. (2015) and Assine et al. (2014) low soil fertility usually occurs in paleo-alluvial deposits, and low clay activity prevalence of Pantanal soils was also observed by Coringa et al. (2012).

The main differences between soils in these landscape units are based on the natural fertility conditions, which is a condition that was also observed by Cardoso et al. (2016). These conditions comply with the topographic gradient, which tend to exert strong influence on the floristic composition and on abundance of species distributed in Pantanal landscape units. Mello et al. (2015) showed that Cambarazal and Espinheiral presented higher natural fertility level than mountain ranges and riparian forests, due to their higher Organic Carbon concentrations.

Among the soil classes by Coringa et al. (2012), there are the Planosols and Gleysols, which have greater natural fertility. Their natural fertility derives from the material sources. With regard to Pantanal soils, they come from sandy sediments, silt-sand, sand clay and semi-consolidated areno-conglomerates flowing from Pantanal plateaus and environs (Couto and Oliveira 2010). Soil fertility in wetlands is mostly linked to the organic material produced by the flora (non-decomposed biomass) and/or by the local fauna (bird feces), as well as by water flow in periodic floods. According to Assine (2016) in the regions of the old lobes, the loss process occurs and not more that of addition. Nascimento (2012) states that soils that do not receive flood water tend to be eutrophic; such profile is often associated with higher pH (> 6) and sodium and/or solodic character.

There was similarity between depths 0 to 20 cm and 60 to 80 cm of different phytophysiognomies, when it comes to the soil texture expressed in the textural triangle (Figure 7). The texture relations

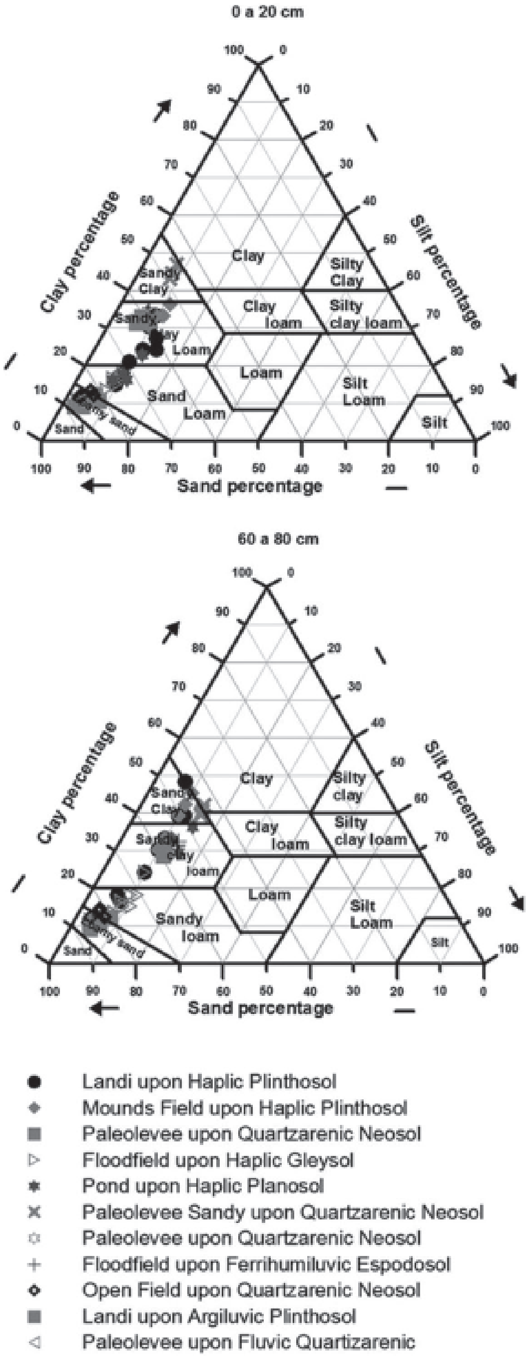

Figure 7. Textural classes of the different studied macrohabitats in two different depths.

observed in different macrohabitats in ponds showed clay loss from the superficial horizon (Elluviation) to the subsurface horizon (Podzolization process) (Figure 3). There was little or no loss in other areas and it characterizes greater textural homogeneity, which complies with the results recorded by Couto and Oliveira (2010) in the same region. It is worth highlighting that the Neosol soil type, which is characterized by low clay increment in depth, prevails in the region.

However, it is possible observing that there is a small textural difference (Figure 6) among phytophysiognomies, fact that is evidenced by the textural soil classification (Figure 7), which changes from free sand to clayey sand. This prior stratification features more homogeneous units within a natural system, which rates high variability in its attributes and complex pedogenesis processes. This is fundamental information when soil units are delimited for different purposes (McBratney et al. 2003, Webster 2000, Wilding and Drees 1983, McBratney and Webster 1981).

Final Considerations

Results obtained by means of the elaboration of a data bank that characterize the soil classes already related to its macrohabitat, reinforce the hypothesis of this work that the physical-chemical attributes of the soils of the tropical wetlands (Wetland soils) is a valuable tool to define management forms for the different macrohabitats already established in the wetland.

The physicochemical soil attributes in the different assessed macrohabitats evidenced acidic natural conditions, high aluminum saturation, sandy texture prevalence, low organic matter content, and low phosphorus content.

Information available about “Pantanal” soils show close relation between the physicochemical attributes of the subsurface horizons in these soils and the vegetation cover. These conditions are associated with groundwater elevation and, consequently, with flood level, which influences the multivariate nature of the deposited sediments and the processes and forms of deposition/sedimentation. It is a determining factor to define the pedogenetic processes in the region. Moreover, it promotes soil formation with great soil texture and fertility variability.

The occurrence of soil units in different macrohabitats allows concluding that the species belonging to the same flora are distributed according to their adaptation to flood and drought.

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Floristic, Phytosociology and Spatial Distribution of a monodominant *Mauritia flexuosa* L.f. forest in an Southern Amazon in the Arc of Deforestation

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Introduction

The mid-north region of the State of Mato Grosso goes through colonization processes encouraged by the Federal and State Government since the 1940s (Irigaray et al. 2013). Driven by the initial formation of small lots for the extractive activity of syringe, later to become latifundia with the structuring of access routes that enable production flow, expelling the indigenous and traditional communities that initially lived in harmony with nature, and also ending animal and plant richness in the region (Simonian 2010 p. 25). Laurance et al. (2001) states that this access opening (roads and highways), which were created in a disorderly way, propitiated land grabbing, this being one of the most determinant actions for increasing deforestation in the region.

Fearnside (2005) states that since the 1940s there was a relation between deforestation and the economy; rural credit, interest rates, inflation index and land price also were, and still are determining factors for the increase or reduction of deforestation. Nobre (2008) states that the loss of biodiversity, increase of greenhouse gas emission and changes in the hydrological regime are directly derived from cattle breeding and the expansion of the agricultural frontier. Changes in the hydrological regime are one of the most aggressive consequences for the Amazon Biome, since only the Amazon Forest is responsible for the evaporation of approximately 8 trillion liters of water annually, interfering, among other things, with global atmospheric circulation and heat flow (IPCC 2007). These factors cause the imbalance of forest ecosystem services and develop serious socioeconomic problems.

The mid-north region of the State of Mato Grosso has vegetation typical of the Cerrado and Amazon biome, and Ecological Tension Zone (ETZ), formed by the contact between Cerrado and Amazon biomes (Radambrasil 1982). These transition formations were shaped after several phases of climate change at the end of the Quaternary geological period (Prance 1979; Ab'Saber 1982; Mayle et al. 2000). ETZ areas are large and unstable extensions resulting from a dynamic transition state attributable to rainfall fluctuations (Ratter, 1992). Although they are generally more floristically diversified (Ratter 1992), forest patches with a predominance of a single species, also called monodominant or oligarchic areas, can be found (Peters et al. 1898).

The municipality of Alta Floresta (AF), in the northern region of the State of Mato Grosso, has the following vegetal formations: open ombrophylous tropical forest, dense tropical forest, savanna and ETZ (Loureiro et al. 1980). Alta Floresta is one of the municipalities that make up the arc of deforestation zone, responsible for the highest rate of deforestation in the Amazon Forest between 1998 and 2006 (De Mello and Artaxo 2017). According to Fearnside (2005), the arc of deforestation is the area where deforestation of the Brazilian rainforests is occurring at a faster rate. A very important and sensitive physiognomy of the Amazon, present in the municipality of Alta Floresta, which has been suffering from deforestation and more recently with the change of the forest code making it unprotected (Feistauer et al. 2014), are the *Mauritia flexuosa* L.f. palm swamp, areas of fertile soils, rich in phosphorus and organic matter.

M. flexuosa is distributed from the northern Orinoco - Venezuela and Colombia, to Southeast Brazil (Ratter 1992; Rull and Montoya 2014; Virapongse et al. 2017). This species is generally found in oligarchic formations known as buritizais, morichales, among others (Rull and Montoya 2014), and can be found from few individuals to more than 600 adult individuals per hectare (Sampaio 2008). Martins et al (2012) report more than 200 different uses of the species by Brazilian traditional communities, With emphasis on food and beverages, construction, medicinal, ritual, handcrafts and household items (Nascimento 2009; Santos end Coelho-Ferreira, 2012), making it one of the species with the highest number of utilities described, besides being considered as the tree of life by some indigenous groups (Fernandes 2011).

Despite this wide distribution and density, few studies have been developed regarding the structure and distribution of *M. flexuosa* in Amazon and Cerrado areas, and only one in an area of Ecological Tension Zone, however, the study was developed in the southwest region of Mato Grosso, with different environmental conditions, once the vegetation is located along the banks of a large river (Guaporé), whose long-term flood pulse is a marking characteristic (Sander 2014), and the sample plots of this research are linked to areas of intermittent streams. Therefore, the aim of this study was to survey the floristic and phytosociology and analyze the spatial structure of an area of *M. flexuosa* dominance in the municipality of Alta Floresta, ecological tension zone in the arc of deforestation.

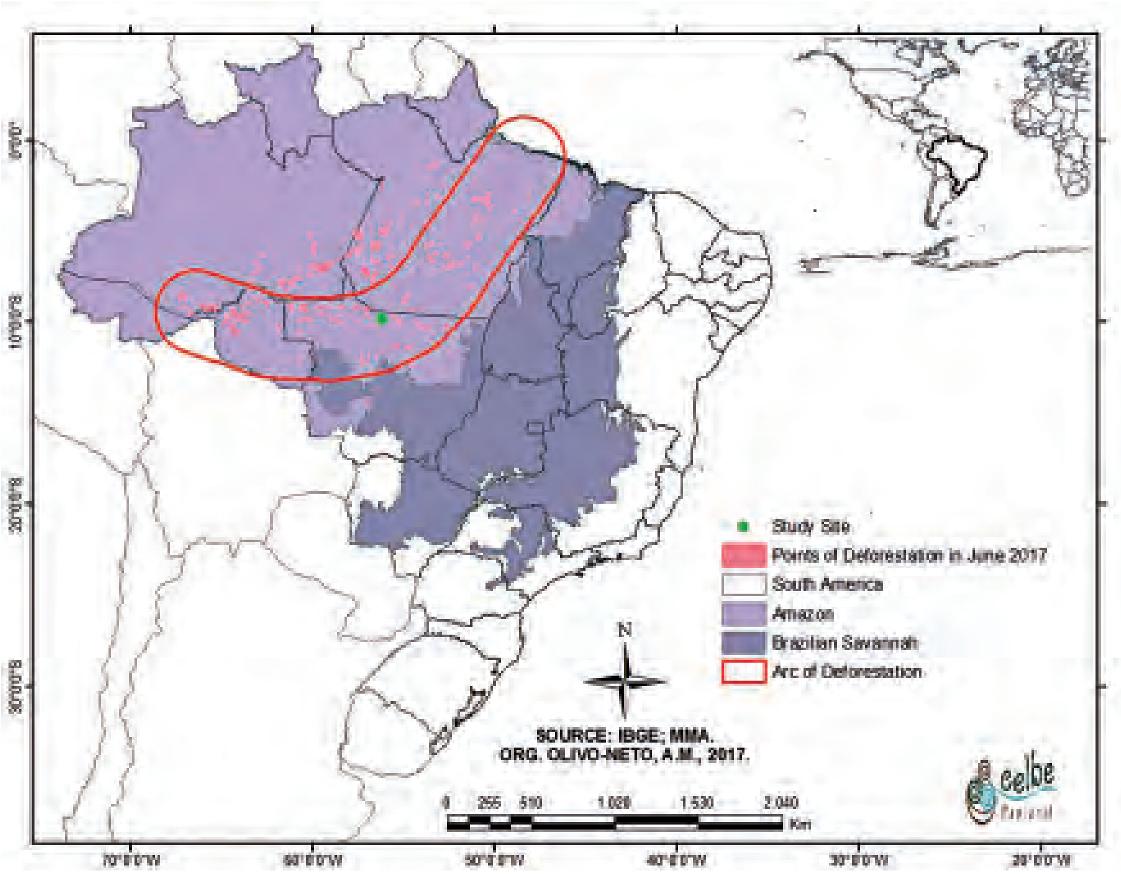
Material and Methods

Study area

The modules (Figure1) were settled in the municipality of Alta Floresta, located in northern Mato Grosso, within the Amazon Biome in an Ecological Tension Zone (ETZ), with low flood levels and great richness. It has a warm humid tropical climate with high average temperatures (23 and 26 °C) during the year, with daily maximums of 34 to 37 °C. Not infrequently, temperatures around 40 °C are recorded. Alta Floresta is set in an area called arc of deforestation. The dominant vegetation type is open ombrophilous tropical forest, which is associated with palm trees and lianas. This type of forest is characterized by large, well-spaced trees and by frequent grouping of palm trees (Oliveira 2006). According to the soil map developed by the “Departamento de Planejamento do Estado de Mato Grosso” (Department of Planning of the State of Mato Grosso), the study area soil classis a typic hapludult. This type of soil presents good drainage, low rate of base saturation and low clay activity, average texture and mildly undulating relief (Seplan - MT, 2000). The region relief compartments are South Southern Amazon Plateau. The morphostructural domains comprise the Phanerozoic Sedimentary Basins and Coverings (IBGE 2006).

Methodology

To evaluate the tree community structure, 5 transects of 20x100m were subdivided into 5 plots of 20x20m, totaling 1 ha (Figure 2). In the plots, all living and dead individuals with DBH (diameter at breast height measured at 1.30 m from soil) greater than or equal to 5 cm were sampled, except for buritis trees (*Mauritia flexuosa* L. f.), whose individuals were sampled in their entirety. The height of each individual was estimated visually, the stems of the turf-form individuals were measured separately and the sum of their basal areas was considered the basal area of this individuals.



SOURCE: IBGE; MMA.
ORG. OLIVO-NETO, A.M., 2017.

Figure 1. Location area municipality of Alta Floresta, located in northern Mato Grosso.

Figure 2. *Mauritia flexuosa* L.f. palm swamp in Alta Floresta.

All individuals sampled were numbered and georeferenced, and botanical samples were collected from them, and subsequently deposited in the HERBAM (Herbarium of Southern Amazonia/UNEMAT/Alta Floresta). The taxonomic identification of the species was carried out with the help of parataxonomists following the IV version of the APG (2016). The taxonomic identification and/or its confirmation occurred with the help of specialized literature and in comparison with exsicates available in HERBAM and in virtual herbarium sites.

The analysis of phytosociological data and species composition was performed by calculating Absolute and Relative Density (AD, RD); Absolute and Relative Frequency (AF, RF); Absolute and Relative Dominance (ADo, RDo); Import Value Index (IVI) (Ellenberg end Mueller-Dombois 1974), calculated using Mata Nativa 2.0 software (Cientec 2006).

The Shannon's diversity index was obtained in the equation: $H' = - \Sigma ((pi) (\ln pi))$, where $pi = (ni / N)$ is the probability that a sampled individual belongs to species i; Ni= total number of individuals of species i; N= total number of individuals sampled in the area. The uniformity was calculated through Pielou evenness (J) (Magurran 1988).

To analyze the abundance, density and structure of *M. flexuosa*, the individuals were divided into three height categories: Infantile (0-3 m height plants with no apparent stipe), Juvenile (3-6 m heights plants with stipe covered with leaf sheath and not yet reproductive), Adults (with apparent stipe and not covered by leaf sheaths, and reproduction signs). The total full height was considered for all infantile and juvenile individuals, and the height of the stipe was considered for adult individuals. Regarding to CCH (circumference at chest height), the individuals were divided into 3 main classes: 1 - Infantile - no apparent stipe, 2 - Juvenile - stipe covered by leaf sheath and not yet reproductive, 3 - Adults - (> 15 cm) with stem above ground (apparent stipe and not covered by leaf sheaths) and reproduction signs. Population structure between plots was compared using X^2 statistics. The Haberman test (1973) allowed to identify categories with excess (marked with +) or absent (marked with -).

The location of all individuals was noted based on Cartesian coordinates (X, Y) for spatial distribution analysis. The spatial distribution analysis was performed using the *a posteriori* test (adjusted residual analysis), described by Haberman (1973), used for the Ripley function K (t), This function is useful for summarizing patterns of punctuated points, testing hypotheses about patterns and estimates parameter. The bivariate or multivariate function K (12) is used to describe the relation between two or more points patterns (Dixon 2002).

The spatial distribution pattern of the univariate individuals of *M. flexuosa* was carried out for each category using the transformation G (t) of the function K (t) (Besag and Diggle 1977; Ripley 1977, 1979, 1981). The significance (P d” 0.01) of L (T) function was determined based on Monte Carlo simulations (Besag and Diggle 1977; Marriott 1979). For the bivariate function K (12), point type “1” and point type “2” were added to test the hypothesis of spatial dependence between two data groups, also using the transformation G (t), maintaining the significance and simulations of Monte Carlo. The function simulations K (t), K (12) and Monte Carlo were obtained using the TOOLBOX software (Fisher 2000).

K (t) Ripley’s was used to analyze the spatial distribution of all classes of individuals. Regarding to K (t) 12, a comparison was made concerning mature (adult) X immature (Infantile + Juvenile) individuals and a comparison between *M. flexuosa* and the other more abundant species.

Results and Discussion

Sampling resulted in 578 individuals belonging to 54 species, 41 genera and 26 botanical families (Table 1). The number of species was relatively high since it was an area with a monodominant species, according to Connell and Lowman (1989), the dominance of a single species can reduce diversity. These circumstances might be explained by the location of the area being in an Ecological Tension Zone, closer to the Amazon, which according to Ratter (1992) are, in general, a floristically more diverse region. Marimon and Felfili (2000) in their study in a monodominant *Brosimum rubescens* Taub forest recorded 57 species. Sander et al. (2017) recorded only 25 species in a monodominant *M. flexuosa* forest. Arieira and Nunes da Cunha (2006) sampled in five plots of a monodominant *Vochysia divergens* Poul. flooded forest in North Pantanal and recorded 83 species. Ferreira and Almeida (2005) recorded 30 species in a “igapó” (flooded area), and 19 species in a forest flooded by white water rivers.

Table 1. Floristic list of the tree species sampled in the monodominant forest divided by family according to the APG IV classification.

Família	Nome Científico	N
Achariaceae		1
	Lindackeria paludosa (Benth.) Gilg	1
Anacardiaceae		5
	Anacardium sp.	1
	Tapirira guianensis Aubl.	2
	Tapirira retusa Ducke	2
Annonaceae		11
	Anaxagorea dolichocarpa Sprague & Sandwith	1
	Anaxagorea sp.	1
	Duguetia echinophora R.E.Fr.	1
	Fusaea sp.	7
	Guatteria sp.	1
Araliaceae		21
	Dendropanax arboreus (L.) Decne. & Planch.	21
Arecaceae		224
	Desmoncus polyacanthos Mart.	1
	Mauritia flexuosa L.f.	222
	Socratea exorrhiza (Mart.) H.Wendl.	1
Burseraceae		15
	Protium heptaphyllum (Aubl.) Marchand	4
	Protium spruceanum (Benth.) Engl.	7
	Trattinnickia burserifolia Mart.	4
Chrysobalanaceae		1
	Licania hypoleuca Benth.	1
Elaeocarpaceae		7
	Sloanea nitida G.Don	7
Euphorbiaceae		2
	Croton matourensis Aubl.	1
	Hevea benthamiana Müll.Arg.	1
Hypericaceae		11
	Vismia sandwithii Ewan	11
Lauraceae		2
	Ocotea sp.	1
	Ocotea sp. 2	1
Lecythidaceae		4
	Cariniana micrantha Ducke	4
Malpighiaceae		1
	Byrsonima sp.	1

Table 1. Floristic list of the tree species sampled in the monodominant forest divided by family according to the APG IV classification.

Família	Nome Científico	N
Malvaceae		**42**
	Apeiba membranacea Spruce ex Benth.	1
	Luehea sp.	39
	Luehea sp2.	1
	Theobroma speciosum Willd. ex Spreng.	1
Melastomataceae		**23**
	Bellucia sp.	4
	Miconia hypoleuca (Benth.) Triana	1
	Miconia poeppigii Triana	11
	Miconia sp.	2
	Miconia sp2	3
	Mouriri collocarpa Ducke	1
	Tococa sp.	1
Meliaceae		**56**
	Trichilia cipo (A.Juss.) C.DC.	43
	Trichilia sp.	13
Moraceae		**9**
	Ficus americana Aubl.	5
	Ficus sp.	2
	Naucleopsis sp.	2
Myristicaceae		**4**
	Virola sp.	4
Nyctaginaceae		**1**
	Neea sp.	1
Primulaceae		**3**
	Cybianthus guyanensis (A.DC.) Miq.	1
	Cybianthus sp.	2
Salicaceae		**42**
	Casearia javitensis Kunth	2
	Casearia pitumba Sleumer	32
	Casearia sylvestris Sw.	4
	Laetia procera (Poepp.) Eichler	4
Siparunaceae		**7**
	Siparuna cuspidata (Tul.) A.DC.	7
Styracaceae		**1**
	Styrax guyanensis A.DC.	1
Urticaceae		**2**
	Cecropia sp.	2
Violaceae		**1**
	Rinoreocarpus ulei (Melch.) Ducke	1
Vochysiaceae		**82**
	Qualea acuminata Spruce ex Warm.	82

In a study conducted by Roucoux et al. (2013) in Amazon forest, a total of 14 species were recorded in an area of 0.5 ha, being the most representative: *M. flexuosa*, *Mauritiella armata* (Mart) and *Tabebuia insignis* (Miq.) Sandwith. Endress et al. (2013), in a *M. flexuosa* swamps in an Amazon forest on Peru, identified 138 tree species in 12 plots with 0.1 ha each where *M. flexuosa* was also the most representative. In the *Mauritia flexuosa* palm swamps in the Brazilian savanna (veredas), the floristic richness is generally high (Araújo et al. 2002), however, most species are herbaceous, when analyzed only with DBH> 10cm, these areas are also shows low richness.

Of 54 species sampled, 24 species had a single individual, classifying it as "rare" or "locally rare" species, an expressive number, representing almost 50% of the species. Sander et al. (2017) obtained a smaller percentage, 9 of 25 species were considered rare in a monodominant *M. flexuosa* forest in southwest Mato Grosso. In this case, the high concentration of rare species can be explained by the fact that this community is under the influence of the flood pulse. According to Junk and Da Silva (1999) e Junk and Wantzen (2004), floods are a preponderant factor for the distribution of plants and favors species that present ecological adaptation to floods. The plots of this study are not located in areas of floods with great amplitude duration, not presenting this driver as regulator or conditioning factor.

The families with greatest species richness were: Melastomataceae with seven species; Annonaceae, Malvaceae and Salicaceae with four species each, Anacardiaceae, Arecaceae, Burseraceae, Moraceae with three species. The families with the greatest number of individuals were Arecaceae (224), Vochysiaceae (82), Meliaceae (56), Malvaceae (42), Salicaceae (42) and Melastomataceae (23), which combined comprised 94.6% of the total of individuals recorded.

The greater number of species of the family Arecaceae is expected, considering that the area has a monodominant species. *M. flexuosa* was the most abundant species with 222 individuals, having more than twice as many individuals as the second most abundant species *Qualea acuminata* Spruce ex Warm., with 82 individuals and five times more than the third most abundant *Trichilia cipo* with 43 individuals (Table 1).

M. flexuosa had the highest IVI (46.19%) and was the dominant specie at the study area. *M. flexuosa* combined with *Q. acuminata* (9.21%) and *T. cipo* (4.69%), composed more than 60.0% of the 55 species IVI (Table 2). *M. flexuosa* also showed the highest basal area (26.81 m2 / ha), relative density (37.88%), relative dominance (87.53%) and coverage (70.25%) (Table 2). Endress et al. (2013), recorded 27.8m^2/ha of basal area in the Peruvian Amazon. Despite having a lower IVI when compared to Sander et al. (2017), the BA and RDo were higher, showing that individuals in this area have larger diameters, which can be explained by the characteristics of the Alta Floresta soil (present study area), such good drainage, low base saturation and low clay activity, medium texture and gently undulating relief (Seplan – MT 2000). While in Vila Bela da Santíssima Trindade (Sander et al. 2017) gleysol is the predominant soil type. This hydromorphic soil is permanently or periodically saturated by water and presents low clay activity and flat relief (Seplan – MT 2000).

Shannon’s diversity index (H ‘) was 1.92; And Pielou evenness value (J) was 0.61. Values considered low when compared to studies carried out in the Amazon or in Ecological tension zones, but higher than other studies carried out in monodominant areas, as Vochysia divergens Pohl monodominance in Northern Pantanal, State of Mato Grosso (Nascimento and Nunes da Cunha 1989). Similar values were also found by Martins et al. (2013) in a study conducted in a swamp forest (H = 2.12; J = 0.65)

Table 2. Tree species sampled in the monodominant floodable *M. flexuosa* forest and their respective number of individuals (N), basal area (BA), relative density (RD), relative frequency (RF), relative dominance (RDo), Coverage value %(CV) and specific importance value (IVI) in decreasing order of IVI.

Nome Científico	N	U	BA	RD	RF	RDo	CV (%)	IVI (%)
Mauritia flexuosa	222	25	26.813	37.88	13.16	87.53	62.71	46.19
Qualea acuminata	82	21	0.787	13.99	11.05	2.57	8.28	9.21
Trichilia cipo	43	10	0.447	7.34	5.26	1.46	4.4	4.69
Luehea sp.	39	11	0.352	6.66	5.79	1.15	3.9	4.53
Casearia pitumba	32	10	0.321	5.46	5.26	1.05	3.25	3.92
Dendropanax arboreus	21	11	0.101	3.58	5.79	0.33	1.96	3.23
Miconia poeppigii	11	6	0.089	1.88	3.16	0.29	1.08	1.78
Trichilia sp.	13	4	0.16	2.22	2.11	0.52	1.37	1.62
Vismia sandwithiana	10	4	0.07	1.71	2.11	0.23	0.97	1.35
Siparuna cuspidata	7	5	0.032	1.19	2.63	0.1	0.65	1.31
Sloanea nitida	7	4	0.041	1.19	2.11	0.13	0.66	1.14
Protium spruceanum	7	4	0.031	1.19	2.11	0.1	0.65	1.13
Fusaea sp.	7	3	0.018	1.19	1.58	0.06	0.63	0.94
Trattinnickia burserifolia	4	3	0.116	0.68	1.58	0.38	0.53	0.88
Ficus americana	5	3	0.053	0.85	1.58	0.17	0.51	0.87
Protium heptaphyllum	4	3	0.035	0.68	1.58	0.11	0.4	0.79
Laetia procera	4	3	0.021	0.68	1.58	0.07	0.38	0.78

Table 2 (cont.). Tree species sampled in the monodominant floodable *M. flexuosa* forest and their respective number of individuals (N), basal area (BA), relative density (RD), relative frequency (RF), relative dominance (RDo), Coverage value %(CV) and specific importance value (IVI) in decreasing order of IVI.

Nome Científico	N	U	BA	RD	RF	RDo	CV (%)	IVI (%)
Bellucia sp.	4	3	0.021	0.68	1.58	0.07	0.37	0.78
Virola sp.	4	3	0.02	0.68	1.58	0.07	0.37	0.78
Casearia sylvestris	4	3	0.018	0.68	1.58	0.06	0.37	0.77
Cariniana micrantha	4	2	0.022	0.68	1.05	0.07	0.38	0.6
Cecropia sp.	2	2	0.045	0.34	1.05	0.15	0.24	0.51
Ficus sp.	2	2	0.034	0.34	1.05	0.11	0.23	0.5
Miconia sp.	2	2	0.022	0.34	1.05	0.07	0.21	0.49
Tapirira guianensis	2	2	0.018	0.34	1.05	0.06	0.2	0.48
Casearia javitensis	2	2	0.013	0.34	1.05	0.04	0.19	0.48
Tapirira retusa	2	2	0.008	0.34	1.05	0.03	0.18	0.47
Cybianthus sp.	2	2	0.005	0.34	1.05	0.02	0.18	0.47
Miconia sp2	3	1	0.018	0.51	0.53	0.06	0.29	0.37
Croton matourensis	1	1	0.1	0.17	0.53	0.33	0.25	0.34
Naucleopsis sp.	2	1	0.033	0.34	0.53	0.11	0.23	0.33
Cybianthus guyanensis	1	1	0.052	0.17	0.53	0.17	0.17	0.29
Duguetia echinophora	1	1	0.032	0.17	0.53	0.1	0.14	0.27
Hevea benthamiana	1	1	0.018	0.17	0.53	0.06	0.12	0.25
Styrax guyanensis	1	1	0.013	0.17	0.53	0.04	0.11	0.25
Ocotea sp. 2	1	1	0.01	0.17	0.53	0.03	0.1	0.24
Apeiba membranacea	1	1	0.007	0.17	0.53	0.02	0.1	0.24
Miconia hypoleuca	1	1	0.006	0.17	0.53	0.02	0.1	0.24
Guatteria sp.	1	1	0.006	0.17	0.53	0.02	0.09	0.24
Anacardium sp.	1	1	0.006	0.17	0.53	0.02	0.09	0.24
Anaxagorea sp.	1	1	0.005	0.17	0.53	0.02	0.09	0.24
Licania hypoleuca	1	1	0.004	0.17	0.53	0.01	0.09	0.24
Ocotea sp.	1	1	0.004	0.17	0.53	0.01	0.09	0.24
Mouriri collocarpa	1	1	0.003	0.17	0.53	0.01	0.09	0.24
Lindackeria paludosa	1	1	0.003	0.17	0.53	0.01	0.09	0.24
Neea sp.	1	1	0.003	0.17	0.53	0.01	0.09	0.24
Rinoreocarpus ulen	1	1	0.003	0.17	0.53	0.01	0.09	0.24
Byrsonima sp.	1	1	0.003	0.17	0.53	0.01	0.09	0.24
Luehea sp2.	1	1	0.002	0.17	0.53	0.01	0.09	0.23
Tococa sp.	1	1	0.002	0.17	0.53	0.01	0.09	0.23
Anaxagorea dolichocarpa	1	1	0.002	0.17	0.53	0.01	0.09	0.23
Theobroma speciosum	1	1	0.002	0.17	0.53	0.01	0.09	0.23
Socratea exorrhiza	1	1	0.002	0.17	0.53	0.01	0.09	0.23
Desmoncus polyacanthos	1	1	0.001	0.17	0.53	0	0.09	0.23

For general comparisons, we divided these results into two large groups: those without apparent stipe, in which the infantile and juvenile individuals are inserted; And those with apparent stipe, which are the adult individuals. The density of *M. flexuosa* evaluated in this study was 222 individuals per hectare, 51 being without apparent stipe and 171 with apparent stipe (table 3). Rivadeneyra et al. (2016) in their survey in the Peruvian Amazon, in the municipality Tingo María, recorded 297 individuals with no apparent stipe and 351 individuals with apparent stipes.

Endress et al. (2013), only sampled individuals with apparent stipes and recorded 127 individuals. Roucoux et al. (2013), in similar sampling, registered 69 individuals. Sampaio et al. (2008), studying different areas of Cerrado, recorded from 220 to 347 individuals without apparent stipe and from 540 to 800 individuals with apparent stipe (Table 3). This large difference in abundance of individuals between sites of the same biome and between different biomes tells us that the determinants of their density are more closely linked to local environmental factors

The population structure of *M. flexuosa* was statistically different among the transects sampled (421.7444866, P <0.05, d.f. = 21). None of the transects showed an inverted J trend. This does not, in fact, demonstrate that the population is declining, but that a greater number of infantile and juvenile individuals are not found in these areas (Figure 3 and 4). In the initial categories of height and CCH was found a significant number of individuals, which shows that even without the inverted J trend, a regeneration is happening in the area.

The spatial distribution pattern obtained through K (t) analysis varies between the life stages. In Alta Floresta, immature individuals (Infantile and Juvenile) tended to present a uniform pattern, however, in some transects of the infantile category, a tendency of aggregation was observed. The adult trees were very varied in their spatial distribution form, so it was possible to notice a randomness trend (Table 4). No dependence was observed between the distribution of mature (adult) individuals with immature individuals (Infantile and Juvenile) and the association between *M. flexuosa* and *Qualea acuminata*, through K (t) 12 analysis. Only two transects showed a tendency of immature and mature dependence.

Table 3. Survey of the abundance of M. flexuosa in South America

Country	Locality	Biome	Level (m)	Study area (ha)	Number of Plots	Method (CAP/PAP/DAP)	Without aprent estip. (ha-1)	Aparent Stip (ha-1)	Density of Mauritia (ha-1)	Reference
Brasil	Alta Floresta	Amazon	200	1	5 (50x20)	All - *M. flexuosa,* without seedling	51	171	222	This Work
Brasil	Santarém Novo	Amazon	50	1.5	15 (10x100)	Indivíduos com DAP >10	-	69	69	Roucoux, K.H., et al (2013)
Peru	Tingo Marìa	Amazon	230	1.0	20 (1/20)	DAP > 8	297	351	648	Rivadeneyra, M.G. (2016)
Peru	Iquitos	Amazon	200	0.5	1 (50x100)	DAP > 10	-	186	186	Roucoux, K. H., et al (2013)
Peru	Maijuna	Amazon		0.1	3 (10.3 radio)	DAP > 5	0	127	127	Endress, B. A. et al. (2013)
Brasil	Vazante	Savanna	450	0.1	15 - 23 (5x5)	All - *M. flexuosa*	320	580	900	Sampaio, M.B. et al. (2008)
Brasil	Angelin	Savanna	450	0.1	15 - 23 (5x5)	All - *M. flexuosa*	253	653	906	Sampaio, M.B. et al. (2008)
Brasil	Brejo do Antônio	Savanna	450	0.1	15 - 23 (5x5)	All - *M. flexuosa*	347	800	1147	Sampaio, M.B. et al. (2008)
Brasil	Brejo da Vaca	Savanna	450	0.1	15 - 23 (5x5)	All - *M. flexuosa*	220	540	760	Sampaio, M.B. et al. (2008)

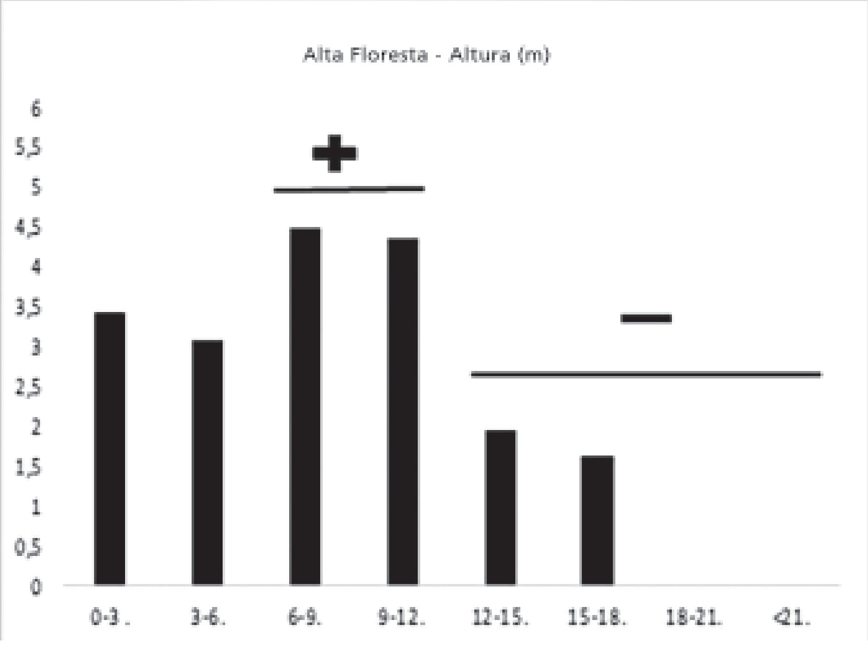


Figure 3. Population structure (log10 number of individuals) of height of *Mauritia flexuosa* in Alta Floresta.

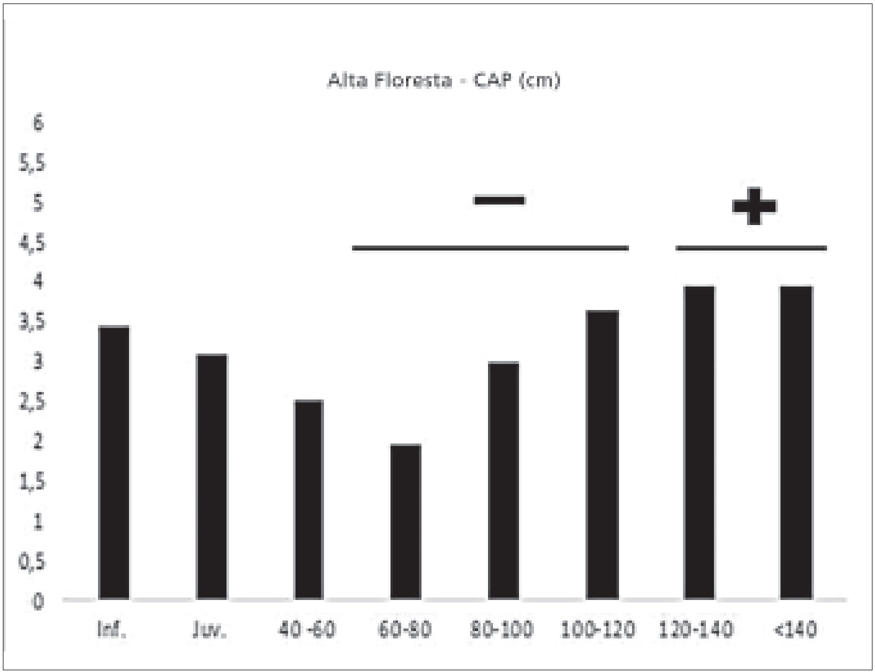


Figure 4. Population structure (log10 number of individuals) CAP classes of *Mauritia flexuosa* in Alta Floresta.

Conclusion

Sampling resulted in 578 individuals belonging to 54 species, 41 genera and 26 botanical families. This number of species is equivalent to other studies carried out in monodominant areas in the Pantanal (*Vochysia divergens* - Arieira and Nunes da Cunha 2006), in the Cerrado (*Brosimum rubescens* - Marimon and Felfili 2000); Higher than others studies in areas with *M. flexuosa* monodominance in the Cerrado, Amazon and ETZ (Araújo et al 2002; Roucoux et al. 2013; Sander 2014); and smaller than a study carried out in the Amazon (Endress et al. 2013), demonstrating the heterogeneity of these areas and that the local environmental conditions influence in the species composition. The number of rare or locally rare species sampled shows the fragility of these environments, and that changes can dramatically alter their species composition and, consequently, the structure.

The density of the *Mauritia flexuosa* palm swamps may vary within the biomes and between biomes, from 69 individuals to over than 1,000 per ha (Sampaio 2008; Schmidt and Figueiredo, 2008; Endress et al. 2013; Roucoux et al. 2013). The flood pulse may be the most important factor for regulating species richness in these areas, as already described for other forests in the Pantanal and Amazon (Junk and Da Silva 1999; Junk and Wantze 2004; Parolin 2010).

Even if the study does not show an inverted J trend, it does not effectively mean that the population is declining, but that a greater number of infantile and juvenile are not found there. This fact may have been due to non-sampling of seedlings, which can be found in large numbers in these areas, as demonstrated by Sampaio et al (2008).

The spatial distribution pattern of K (t) varied between the life stages, ranging from the Uniform and Aggregate pattern. No dependence was observed between the distribution of mature individuals (adults) with immature individuals (Infantile and Juveniles) and the association between *M. flexuosa* and *Qualea acuminata*, through the K (t) 12 analysis.

Studies of spatial structure such as the one developed in this study have not yet been performed in any other population of *M. flexuosa*, and may help us to understand the relation of dependence or not dependence of the species with itself and with other species in the environment which it lives. This relation of dependence can guide us in works of forest restoration, indicating the species and possible positions of planting.

Table 4. Spatial distribution analysis K Ripley: K (t) for the different categories and K12 (t) to M. flexuosa X *Qualea acuminata* (ind 93 h-1.); Immature individuals (+ infant Juvenile) X mature (adult) for the Alta Floresta site.

	Categorias	Alta Floresta				
		T1	T2	T3	T4	T5
K(t)	Infantil	Random 0-0.66. Uniforme 0.66-0.99. Agregado 0.99-6.6	Random 0-0.99. Agregado 0.99-6.6	Random 0-0.66. Uniforme 0.66-6.6	Random 0-0.66. Uniforme 0.66-6.6	Todos Random
	Juvenil	Random 0-0.66. Uniforme 0.66-6.6	Random 0-0.66. Uniforme 0.66-6.6	Random 0-0.66. Uniforme 0.66-6.6	Random 0-0.66. Uniforme 0.66-6.6	Random 0-0.66. Uniforme 0.66-6.6
	Adulto	Random 0-0.66, 1.32-6.6. Uniforme 0.66-1.32	Random 0-0.66; 1.32-2.31. Uniforme 0.66-1.32; 2.32-6.6	Random 0-0.66; 4.29-4.62. Uniforme 0.66-4.29; 4.62-6.6	Random 0-4.62; 6.27-6.6. Agregado 4.62-6.27	Random 0-0.66; 1.32-1.65; 6.27-6-6. Uniforme 0.66-1.32; 1.65-6.27
	Geral	Random 0-1.32. Agregado 1.32-6.6	Random 0-1.32; 2.64-5.28. Agregado 1.32-2.64. Uniforme 5.28-6.6	Random 0-0.66; 1.98-3.3; 3.96-6.6. Uniforme 0.66-1.32. Agregado 1.32-1.98; 3.3-3.96	Random 0-0.66. Uniforme 0.66-1.32. Agregado 1.32-6.6.	Random 0-0.66; 1.32-6.6. Uniforme 0.66-1.32
K12(t)	ImaXMat	Independente 0-0.99, Dependente 0.99-6.6	Independente	Independente	Independente 0-0.99, 2.97 - 6.6. Dependente 0.99-2.97	Independente
	Mf X *Qualea acuminata*	Independente	Independente	Independente	Independente	Independente

This work comes to aggregate information at population level and its relation with the community, in an area of ecological tension zone, in the arc of deforestation, with few researches developed in such scale, and that has been deforested since the 40s by the colonization processes and land grabbing.

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Bioprospecting *Serjania marginata* Casar.: chemical and larvicidal evaluation and agricultural productivity

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Introduction

Brazil is often mentioned as the nation with the greatest biodiversity on the planet, but there is no official data on the size of the domestic market for natural products (Kinnup and Barros 2012). Although increasing demand has been noted in the market, this has mostly been met with non-standardized raw materials or even through extractive activities with no criteria. Technology to produce the raw plant material and sustainable extractive procedures are extremely important, especially considering the need to meet the standards imposed by legislation in the areas of chemistry, pharmaceutical and food industries, and to enter the national and international market (Bara et al. 2006).

Among the biomes in Brazil, Mato-Grossense Pantanal is a flood plain with about 150,000 km^2 located in the Central-West region. It is estimated that there are about 2,000 plant species in Pantanal, 13% of which are aquatic macrophytes and 87% are terrestrial species. The flora of the region is composed of elements from morphoclimatic and phytogeographic areas like Cerrado, Amazon Forest and Chaco. In Pantanal there are macrohabitats, which are landscape units subjected to similar hydrological conditions and particular vegetation. Mato-Grossense Pantanal is declared national patrimony by the Federal Constitution of 1988, and its use must be made according to the law, under conditions that ensure environmental preservation, including the use of natural resources (Cunha et al. 2016).

The Cerrado occupies 22% of the national territory and possesses unique characteristics (such as soil type and climate) which directly affect plant development. Approximately 6,500 species of plants are native to the Cerrado; of these, only about 200 have some identified economic use due to their composition with important active ingredients, which are useful for multiple purposes. However, Cerrado is on the list of the 17 most degraded ecosystems in the world. It is considered a "hot spot" for global biodiversity and was included among the priority biomes for conservation at a global level (Santos et al. 2010; Conservation International 2011; Fernandes et al. 2016).

The extractive exploitation of wild material results in continuous prospecting of native plants, which in turn leads to ecological deterioration. Market demand requires alternative routes of extraction to be found, since meeting this demand will drastically reduce natural sources. For these reasons, genetic richness must be preserved and growing practices to domesticate the plants should be studied, along with chemical composition and biological activities in order to offer the consumer a high-quality, standardized product. Conserving a natural resource does not imply only protecting its genetic biodiversity, but also defending the cultural biodiversity that integrates and uses the resource (Bandoni and Czepak 2008).

In order to ensure the effectiveness and safety of natural products, it is necessary to align studies related to cultivation and chemical composition of the plant with the biological and toxicological activities observed. The importance of studies on physiological behavior consists of creating knowledge that permits the identification of optimal cultivation conditions. This is because the influence of numerous environmental factors leads to variations in content, chemical composition, and biomass accumulation in plants (Vimolmangkang et al. 2010).

Dengue is an infection of public health significance whose vector is the mosquito *A. aegypti* L. (1762) (Diptera: Culicidae) (Braga and Valle 2007; Barreto et al. 2008; Miyazaki 2009). The problem was aggravated by the entry and transmission of two new infections in Brazil, Chikungunya fever and Zika virus. Official data from Brazil, dating from 2016 to the fourth epidemiological week of 2017, indicate the Central-West region with the highest incidence rates of dengue cases, including severe dengue. On the other hand, the incidence rate of cases of Zika virus and Chikungunya fever is low (Ministério da Saúde 2017).

The main form of mosquito control is by chemical insecticides, especially in periods of heavy infestation. However, large-scale use has contributed to the emergence of significant problems such as toxicity to the environment and humans; and the emergence of resistance (Braga et al. 2004; Porto et al. 2008). The occurrence of this resistance hinders control and action strategies leading to the need to investigate and monitor such control actions (Lima et al. 2006).

Insecticidal products of herbal sources appear as an option to control insects due to the complex composition of their active principles which decrease the risks of resistance development. This condition is attributed to the mixing of several components with different modes of action (Barreto 2005, Rocha et al. 2008; Porto et al. 2013). Plant species of the genus *Serjania* (Sapindaceae), called timbó, are known by their insecticidal potential, but many of them have not had their use scientifically proven (Shaito and Lucchini 1998). In the United States, an insecticide from *Serjania* spp., based on rotenone, is already commercialized with insecticidal and acaricidal action, being effective against caterpillars, beetles, fleas, aphids, ants, leafhoppers, flies, scale insects and mites (Moreira et al. 2005).

Tannins present in the bark of *Magonia pubescens* stems, Sapindaceae, were correlated with the insecticidal potential of the plant (Silva et al. 2004), since using the ethanolic extract the authors determined toxic activity against third-instar larvae of *A. aegypti*.

Species of Sapindaceae family are widely distributed, participating with their specific representatives of the two major geographic formations that characterize it, Cerrado and Pantanal, setting in these particular physionomies a variation of the two characteristic vegetation types (Radlkofer 1900; Ferruci 2004, Souza and Lorenzi 2012). Specifically, those of the genus *Serjania* have large occurrence in tropical regions, including Brazil (Mato Grosso do Sul, São Paulo and Pernambuco), Paraguay, Bolivia and Argentina (Guarim Neto and Santana 2000; Rodal and Nascimento 2002; Arruda 2008; Sprengel-Lima and Rezende 2013; Moreira et al. 2013).

Ex situ adaptations are important in the search for quality products that ensure safety and efficacy of the active substances of these plants. To this end, different cultivation practices should be studied that target greater production of biomass and secondary metabolites, as well as preserve the environment (Leite et al. 2010). In an agronomic study performed by Tabaldi et al. (2012) in Dourados, MS, it was observed that *S. marginata* plants developed well when grown *ex situ*. It is known that inappropriate processes of *ex situ* cultivation have caused severe changes in physical, chemical and biological attributes of the soil. As a result, systems that include and contribute to reaching greater biodiversity, diversifying production, balancing use/recycling/recovery of nutrients, maintenance and/or recovery of soil attributes, and decreasing risks and losses due to pests and disease should be sought. Cover crops, also known as green manure, are being used as an alternative for diversifying and improving the productive potential of agricultural areas in various regions around the world (Calegari 2014), since they contribute to the maintenance and improvement of the physical, chemical, and biological attributes of the soil (Bolliger et al. 2006; Calegari et al. 2008). Use of green manure is a fundamental cultivation practice in the Cerrado region, because it protects, improves, and maintains soil quality, in addition to providing considerable increase in levels of organic matter and nutrients, thus benefiting agroecosystems (Leite et al. 2010). Therefore, studies on agricultural production are needed in order to obtain results for the sustained management of medicinal plants and the chemical profile of the species, as well as to standardize the extracts that are obtained.

The objectives of this study were to compare extracts of native and cultivated *S. marginata* Casar plants, evaluate the insecticidal potential and document the biomass production of the cultivated plant.

Material and Methods

Plant material - Leaves of *S. marginata* were harvested from native plants and plants grown *ex situ*. The native plants occur in a Cerrado fragment located in the Lagoa Grande Settlement in Dourados, MS, Brazil (21°59’41.8"S and 55°19’24.9"W, at an altitude of 429 m). The species was identified by Arnildo Pott and specimens voucher were deposited in the herbarium CGMS (41054) (Campo Grande-MS) and herbarium DDMS (n° 5000) (Dourados-MS). The cultivated plants were the result of an experiment developed at the Medicinal Plant Garden (Horto de Plantas Medicinais - HPM) (22°11’43.7''S and 054°56’08.5''W, at an altitude of 430 m), at the Universidade Federal da Grande Dourados (UFGD), in Dourados, MS, Brazil, where they were subjected to different cultivation practices. The climate of the region, according to Köppen classification, is tropical with a dry winter season (Aw) (Peel et al., 2007), with mean annual precipitation of 1500 mm and mean annual temperature of 23.6°C. Temperatures and rainfall during harvest periods in Dourados, MS can be found in Table 1. The soil, which originally contained Cerrado vegetation, is a Red Oxisol (typical Haplortox), with a heavy clay texture; the topography was flat.

Prior to the experiment, the soil properties (0-20 cm layer) were as follows: water pH = 4.07; exchangeable in $cmol_c\ dm^{-3}$: Al = 0.66; and K = 0.04; Mg = 0.2; Ca = 0.3; available P = 4.00 mg dm^{-3}; organic matter = 11.9 g dm^{-3}; and CEC = 4.61 $cmol_c\ dm^{-3}$.

Table 1. Mean minimum and maximum temperatures and monthly precipitation in the months when leaves were harvested from *S. marginata* plants.

Month	Minimum temperature (°C)	Maximum temperature (°C)	Precipitation (mm/month)
February 2011	21.6	31.5	197.2
October 2011	18.6	31.0	127.7
December 2011	19.4	32.4	77.6
February 2012	20.7	32.7	118.6

The *S. marginata* plants were cultivated in plots where plant residues from three types of green manure plants were added to the soil, with or without addition of nitrogen in coverage application. The plants were velvet bean, also known as mucuna-preta (*Stizolobium aterrimum* Piper & Tracy) with and without nitrogen (N); rattlebox, also known as crotalária (*Crotalaria spectabilis* Roth) with and without N; and jack bean, also known as feijão-de-porco (*Canavalia ensiformes* L.) with and without N. There were also two control plots (soil without addition of green manures), one with N and one without N, thereby comprising eight treatments. The experimental arrangement was split-plots, with green manures in the whole plots and nitrogen in the subplots in randomized blocks, with four replicates; each experimental unit was composed of five plants. The N used was urea at a dose of 80.0 kg ha^{-1} in a cover application, with fractions of 25%, 25% and 50%, respectively, at the age of 30, 45, and 60 days after transplanting.

The cultivation practices in the field included sprinkler irrigation whenever soil moisture was below 70% of field capacity, which was measured with a tensiometer, as well as manual weeding when invasive plants were present. No diseases or pests were noted.

Leaves were harvested in three periods during the plant's life cycle, for both native and cultivated plants. The first was before flowering (February 2011), the second during flowering (October 2011), and the third after flowering (December 2011), corresponding to days 445, 685, and 745 of the cultivated plants' life cycle, respectively. The leaves were dried in a circulating air oven at 37 ± 2°C and ground in a Wiley type mill with a 10 mesh sieve.

Extraction - The powdered leaves were successively extracted using percolation (Prista et al. 1995) at room temperature with EtOH-H_2O 70%. The 70% ethanol extract was filtered, concentrated under vacuum at approximately 40 °C and lyophilized, producing the powdered extract, after which the yields were calculated. The extracts were obtained in 3 periods, before (HDA-BF) during (HDA-DF) and after (HDA-AF) flowering in Dourados. All the samples were performed in triplicate.

Chromatographic analysis **-** The extracts obtained from the samples were analyzed in an analytical LC (Varian 210) system with the ternary solvent delivery system equipped with an auto-sampler, a photodiode array detector (PDA) monitored at *ë* = 200-800 nm. The LC column was C-18 (25 cm x 4.6 mm; particle size, 5 ìm; Luna, Phenomenex, Torrance, CA, USA), with a small pre-column (2.5 cm x 3 mm) containing the same packing used to protect the analytical column. In each analysis, the flow rate and the injected volume were set as 1.0 mL min^{-1} and 10 ìL, respectively. All chromatographic analyses were performed at 22 °C. Elution of extracts was conducted using methanol (solvent A) with acetic acid 0.1% (solvent B). The solvent gradient program was as follows: 0 min, 75% B; 40 min, 25% B; returning to the initial condition in 45 min.

Quantitative determination of phenolic compounds content **-** Total soluble phenolic compounds content was determined for HDA-BF, HDA-DF and HDA-AF. All samples (10 mg) were dissolved in MeOH:H_2O (8:2 v/v) (10 mL). The phenolic content was determined by the Folin-Ciocalteu reagent colorimetric method with some modifications (Sousa et al. 2007). An aliquot of the crude extract solutions (0.2 mL) was diluted in MeOH: H_2O (8: 2 v/v) (0.8 mL) (200 µg mL^{-1}). Then, 0.05 mL of the solutions was mixed with 0.15 mL of the Folin-Cicauteau reagent (1/10 dilution, Merck) and after 3 min, 0.05 mL of a saturated Na_2CO_3 solution was added. After 120 min at room temperature, the absorbance values were measured at 750 nm (ELx800 spectrophotometer). The blanks were prepared with all the reagents except the sample. Quantifications were expressed by reporting the absorbance in the gallic acid calibration curve (6.25, 12.5, 50, 100, and 200 µg mL^{-1}), used as the phenol standard. All the samples were performed in triplicate.

Biomass production and composition of macro and micronutrients - Biomass production and composition of macro and micronutrients were assessed for the *ex situ* cultivated *S. marginata* plants which were harvested in February 2012 at 120 days after flowering, day 810 of the plant's life cycle. Leaves and stems were separated to obtain fresh and dry mass as well as leaf area. The fresh mass was obtained after weighing on a digital scale accurate to 0.1 g; to obtain the dry mass, the materials were placed in paper bags and into a forced air circulation oven at 38 + 2 °C until weight was constant, then weighed on a digital scale. Leaf area was determined using Windias 3 software (Windias, Delta-T Devices, Cambridge, UK). After weighing, leaf samples were ground in a Wiley type mill and subjected to chemical analysis. Phosphorus (P) concentrations of dried leaves were determined using the colorimetric molybdovanadate method; potassium (K) concentrations were determined by flame photometry, and calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) concentrations were analyzed by atomic absorption spectrometry. To determine total nitrogen (N) concentrations, samples were subjected to sulfuric digestion, and the N concentrations were measured using Kjeldahl distillation (Malavolta 2006).

Bioassay with *A. aegypti* L. - The acute test was performed with *A. aegypti* eggs from a stock colony after collection in Campo Grande, MS, together with the team of the Zoonoses Control Center of Campo Grande. The F1 generation eggs obtained under laboratory conditions were allowed to mature for one week and after that were subjected to hatching in dechlorinated water, corrected with sodium bicarbonate and pH between 6.5 and 7.0.

Third-instar larvae were used totaling 25 individuals for each 25 mL of solution after prior toxicity assessment at concentrations that killed 100% and zero larval mortality ranging from 1.0 g L^{-1} to 0.1 g L^{-1} of the *S. marginata* extracts (HDA-BF, HDA-DF and HDA-AF), in quadruplicate, during the observation period of the test. The doses that caused higher mortalities were then resized to establish lethal concentrations by extrapolating the highest initial dose. At the same time, the negative control (blank) was carried out in medium containing only dechlorinated water, following the methodology adapted from Laranja et al. (2006).

Chronic assays were performed with concentrations of 0.062 g L^{-1}; 0.125 g L^{-1}; 0.250 g L^{-1} and 0.50 g L^{-1} to observe interference from concentrations below the LC_{50} of the acute tests on lethality or other deleterious effects on larvae and pupae. The larvae were separated into groups of 25 individuals, in the same ratio larva / mL of the acute test, with four replicates per treatment. The experimental design was completely randomized, containing, in each treatment, 100 larvae.

Bioassay with *A. salina*- To evaluate the toxicity towards *A. salina* Leach (1819) (Anostraca: Artemiidae) (Asem et al. 2010) of the different crude extracts (HDA-BF, HDA-DF and HDA-AF), assays were conducted with naupilus for 48 h, obtained from cysts that had been hatched in artificial seawater (saline solution), under partial illumination, according to the adaptations of Montanher et al. (2003) and Motti et al. (2015). The tests were previously performed with concentrations of 1.0 g L^{-1}; 0.5 g L^{-1}; 0.25 g L^{-1}; 0.125 g L^{-1} and 0.062 g L^{-1} prepared from a stock solution by adding 0.015 g of the crude extract sample to a final volume of 30 mL of 1% seawater DMSO.

For each concentration, 10 larvae were used per replicate, in quadruplicate, with a blank control (saline solution) and a negative control (rotenone). Samples were placed in 10 mL capacity test tubes containing a final volume of 5 mL. In all tests the naupilus were left in contact with the extracts solutions for 24 h.

After the previous test, concentrations capable of causing 100% to zero mortality were established in the exposed population. The number of deaths and survivors was then determined. Data were expressed as a percentage of mortality and subsequent Probit analysis.

Statistical analyses - Peaks obtained by HPLC-PDA were subjected to multivariate analysis of variance (MANOVA) to obtain the values for P and F, using SYSTAT 12 software. The production data were subjected to analysis of variance (ANOVA) and, when significance was detected using the F-test, the means were tested by Tukey test, to 5% probability. For the evaluation of insecticidal activity and toxicity of the crude extracts, the mortality rates for *A. aegypti* and *A. salina* were defined for 10%, 50% and 90% of the exposed population, and analyzed by the Probit method using the software Leora® POLO 97355947870655352. The larval duration and young viability data, in hours, for *A. aegypti*, were evaluated by one-way ANOVA and Tukey's test at 5% significance. The mortality observed in each phase and the total of winged formed were evaluated by percentage.

Results and Discussion

Extraction - Leaves of *S. marginata* plants yielded significant amounts of extracts, with the native plants standing out as more productive. Among the cultivated plants, the highest mean yield was for the control without N, which surpassed the mean for the control with N by 3%, and the mean for the treatment with *C. ensiformes* with N, which presented the lowest value, by 6.5% (Table 2). It should be emphasized that considering the harvest periods, native plants collected before and during flowering presented higher and similar yields (32.6 and 32.3%, respectively), and the mean for the three harvest periods surpassed the mean for the cultivated plants without green manure and without N by 1.6%.

Table 2. Yields of EtOH 70% extracts from native *S. marginata* and the same species cultivated in soil covered with three green manures, with or without nitrogen, harvested before, during and after flowering, in Dourados, MS.

Native plant/green manures		Before %	During %	After %	Mean %
Native plant	—	32.6	32.3	23.3	29.4
Stizolobium aterrimum	With N	26.4	21.1	17.9	21.8
	Without N	22.0	21.8	25.3	23.0
Crotalaria spectabilis	With N	18.1	23.0	24.9	22.0
	Without N	21.4	22.5	28.6	24.2
Canavalia ensiformes	With N	13.5	26.7	23.2	21.1
	Without N	17.0	28.2	28.2	24.5
Without green manures	With N	18.8	27.9	27.0	24.6
	Without N	27.2	27.3	28.4	27.6
Mean	21.9	25.6	25.2	24.2	

In general, the extract yields from leaves of *S. marginata* can be considered high when compared to other species of the same genus, such as *Serjania erecta* Radlk, in which yields for chloroformic and methanolic extracts from leaves were 5.55% and 7.78%, respectively (Arruda 2008). Studying medicinal species of the genus *Mikania* (a climbing vine, similar to *S. marginata*), Carollo (2008) also obtained a higher extract yield for another Cerrado native, *Mikania cordifolia*, and determined a yield of 11% for EtOH 95% extract; however, for cultivated *M. glomerata* the yield was 8.6%.

The higher yields from the native plants were probably due to the environmental stress conditions to which they were subjected, including possibility of attacks from pathogenic microorganisms and animals, high temperatures and water deficit, which according to Taiz and Zeiger (2017) stimulate greater production of secondary metabolites that carry out important ecological functions in plants. They protect the plants against herbivores (grazing) and infection by pathogenic microorganisms. Interestingly, the same compounds that increase plant adaptation can also make them undesirable as food for humans. Many cultivated plants have been artificially selected to produce relatively low levels of these compounds, which consequently can make them more susceptible to insects and diseases.

Although the yields for native *S. marginata* were greater, it was observed that the cultivated plants in some of the treatments presented yields close to those of the native plants, which may be considered relevant, as this fact eliminates the need to harvest native plants which consequently increases their risk of extinction. Cultivated *S. marginata* plants, when grown in soil with the green manure species *C. spectabilis* without N and harvested after flowering, or grown in soil with *C. ensiformes* without N and harvested during and after flowering presented yields of 28.6%, 28.2%, and 28.2%, respectively, compared with a mean of 29.4% for the native plants.

It is known that the effects of cultivation and several practices including fertilizing are contradictory in medicinal plants. Several studies have reported the existence of antagonistic effects in production of secondary metabolites relative to organic and chemical fertilizing (Reis et al. 2004). Carollo (2008) assessed the effect of organic fertilizers (with broiler litter in semi-decomposed state) and chemical fertilizers on the species *Mikania glomerata, M. cordifolia,* and *M. micrantha*, collected in two seasons, in August and December. The author observed that *M. cordifolia* plants cultivated with broiler litter in semi-decomposed state had increased concentrations of fertaric acid, 3.5-DCQ acid, and isoquercetin in both periods. The author emphasized the fact that the accumulated fertaric acid was up to 20 times greater in plants collected in December, which is why the species was more affected by the harvest season than by the type of fertilizer used. In *M. glomerata*, the effects were similar, with the main substances being the phenylpropanoid derivatives chlorogenic acid, fertaric acid, 3.5-DCQ acid, and the flavonoid 3-O-sulfate.

Consequently, the importance of seeking appropriate cultivation practices for plant production should be considered; it is necessary to find a way to avoid the continuous use of native species (and risk of extinction for the plants). As a result, we recommend that new cultivation practices should be tested, including the use of chemical and organic fertilizers, in order to obtain greater production of mass and secondary metabolites.

Chromatographic analysis - All leaf extracts (70% ethanol) of *S. marginata* (Figure 1) harvested in different periods presented phenolic compounds, data analyzed considering chromatograms and UV-visible spectra by HPLC-PDA. The absorption bands estimated from the spectra suggest the presence of flavonoids (ë$_{max}$ 240-290 nm and 300-390 nm) (Merken and Beecher, 2000) and tannins (ë$_{max}$ 279 nm; ë$_{max}$ 203 and 282 nm) (Baldé et al. 1991; Idowu et al. 2010; Zhang et al. 2013).

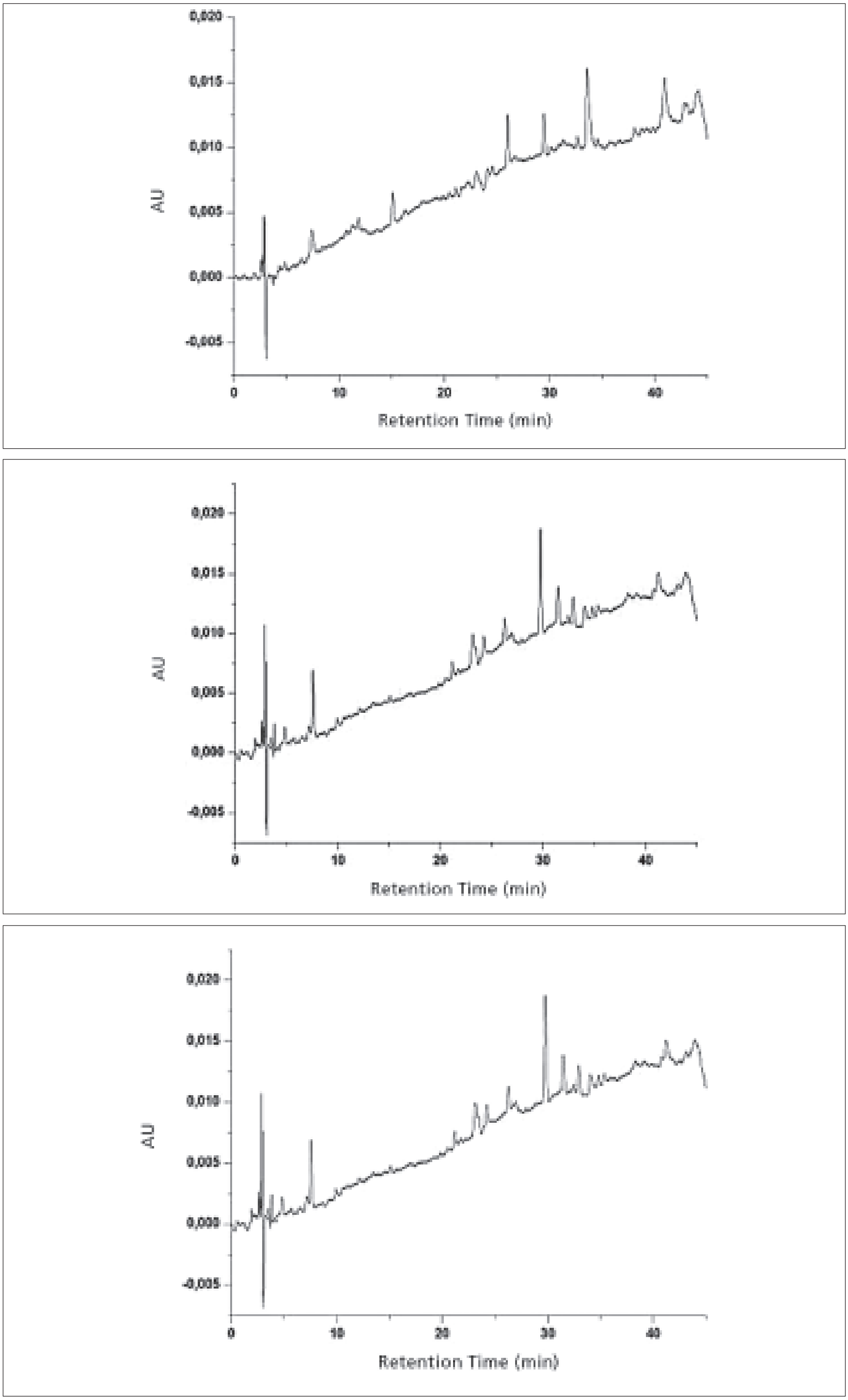

Figure 1. Chromatographic profiles (254 nm) representing the three harvest periods for cultivated *S. marginata* plants. Before (A), during (B), and after flowering (C).

There were nine distinct groupings regarding the different treatments, with Wilk's Lambda = 0.000, $F = 100,203$ and $P < 0.001$ according to the retention time and abundance of the peaks (Figure 2). The first and second canonical roots explained 79% and 21% of the results, respectively. These data show that the separation occurred according to the three periods during the plant's life cycle, and not as a function of the different green manures or nitrogen.

Quantitative determination of phenolic content **-** To determine the content of total phenolics, the absorbance obtained for the extracts HDA-BF, HDA-DF, and HDA-AF (0.301, 0.249, and 0.403 nm, respectively) were used to perform the regression equation for quantifying gallic acid ($w = 0.\ 047x + 0.1176$, $R^2 = 0.98$). We decided to determine the content of total phenolics only for the plant harvested at three different periods. The HDA-BF extract showed the highest amount of phenolic compounds (60.72 ± 0.09 mg of gallic acid per mg of extract), followed by HDA-AF (40.94 ± 0.03 mg of gallic acid per mg of extract) and HDA-DF (28.06 ± 0.10 mg of gallic acid per mg of extract). The content of phenolic compounds decreased during the flowering period indicating that the best period for obtaining high contents of these compounds is after flowering.

The quantification of these secondary metabolites conducted for other species of *Serjania;* with the ethanolic extract from *S. erecta* leaves, showed that there was 298.4 mg g^{-1} of phenolic compounds. On the other hand, a lower amount was found in the roots of this species (213.4 mg $g^{-1}$of total phenolics) (Cardoso et al. 2013). In addition, it was determined that in 500 mg of aerial portions of *S. lethalis,* there

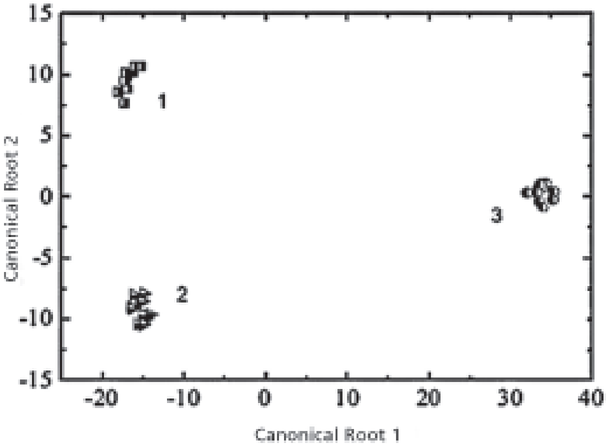

Figure 2. Discriminant analysis of the treatments (1, 2 and 3) by HPLC-PDA.

were 23.27 mg of tannins and 1.12 mg of flavonoids (Araújo et al. 2008). The study by Heredia-Vieira et al. (2015) reports the presence of tannins and flavonoids in 70% ethanol extract of *S. marginata*.

Biomass production and composition of macro and micronutrients - The dry mass of leaves and fresh and dry mass of stems from cultivated *S. marginata* harvested at 810 days were significantly influenced by the interaction between the green manures used in coverage treatment of the soil before planting, and by the nitrogen applied to soil at the beginning of the cultivation cycle (Table 3). The greatest dry mass of leaves occurred in cultivated plants after the use of *C. spectabilis* with N or *C. ensiformes* without N. Similar results were observed for fresh and dry mass of stems.

Table 3. Dry mass of leaves and fresh and dry mass of stems of cultivated *S. marginata* plants treated with green manures and nitrogen.

Green manures	Dry mass, leaves		Fresh mass, stems		Dry mass, stems	
	Nitrogen (80 kg ha^{-1})					
	Without	With	Without	With	Without	With
Without green manures	583.15aA	581.36aA	2344.55aA	1429.92bA	745.19aA	468.22bA
Stizolobium aterrimum	442.84aA	466.81aA	1879.45aA	1904.24abA	700.05aA	751.26abA
Crotalaria spectabilis	369.07aB	643.49aA	1377.03aB	3922.42aA	435.11bB	1337.82aA
Canavalia ensiformes	629.37aA	385.63aB	2204.11aA	1118.57 bA	767.99aA	396.74 bA
C.V. (%)	29.47		51.10		49.74	

Means followed by different lowercase letters in columns and capitals in lines are significantly different according to the Tukey and F tests, respectively ($P < 0.05$).

Among the green manures used, it was only necessary to use additional nitrogen to obtain greater production with *C. spectabilis,* corroborating the data obtained by Tabaldi et al. (2012), showing that among the three green manures used this species produced the lowest aerial mass. That is because studying the production of cultivated *S. marginata* treated with the same green manures and nitrogen, and harvesting the plants at day 240, the authors noted that the green manures *S. aterrimum* and *C. ensiformes* were the most promising cover crops for cultivating medicinal plants, due to the increased biomass production and N savings they offer. The beneficial effects of soil coverage resulted from the low C/N ratio (18), greater N fixation per year, less loss of nutrients to leaching, and better maintenance of soil structure, allowing better root distribution and greater nutrient absorption (Fageria 2007; Wutke et al. 2014). The beneficial effects of green manures have been demonstrated in other crops such as sugarcane (Ambrosano et al. 2011), corn (Zanatta et al. 2007), rice-wheat succession cropping (Mandal et al. 2003; Singh et al. 2007), lettuce and cabbage (Fontanétti et al. 2006) and in corn and tomatoes (Tosti et al. 2012).

The fresh mass of leaves and leaf area were not influenced by the green manures or nitrogen; the means were 1862.63 g/plant and 48604.53 cm^2/plant, respectively. This lack of significant influence may be related to the fact that since *S. marginata* is a native plant adapted to its conditions, even the addition of green manures as a cover treatment was not sufficient to cause changes in physical, chemical, and biological characteristics of the soil, which would in turn impact the production of leaves in the plants. It is likely that foliar expansion is less influenced by the environment than dry mass, which in contrast, varied with the use of green manures and nitrogen. Furthermore, since *S. marginata* is a climbing species with large number of leaves, the plants have a plasticity of development to adapt to a range of light regimes, so that leaves exposed to different light regimes can be found on the same plant, with some leaves exposed to the sun and others in the shade (Taiz and Zeiger 2017). As a consequence, the mean leaf area remained similar in the different treatments. Green manure can be considered a promising practice for production of *S. marginata* and soil preservation; the use of *S. aterrimum* and *C. ensiformes* is more recommended.

The macro and micronutrients content assessed in the leaves of *S. marginata* were not influenced by the green manures used as predecessors to cultivation or the nitrogen used as cover treatment in the crop (Table 4).

In general, the levels were low compared with those considered by Marschner (2011) as optimal for plant growth. However, as there are no studies in the literature referencing the chemical composition of *S. marginata*, it can be assumed that the plant, since it is native, is undemanding and/or very efficient in its use of nutrients. Foliar analysis indicates the relationship between growth and nutritional status of the plant; a plant with optimal proportions and levels of nutrients in its leaves can be considered well-nourished. The productivity of most agricultural crops increases linearly with the amount of fertilizers that they absorb to guarantee adequate nutrition, which in turn is evaluated by the nutrient content in specific organs such as leaves (Fontes 2011). The nutrients act as components of organic compounds for energy storage and plant structures such as enzymatic cofactors, and in electron transfer reactions (Taiz and Zeiger 2017).

Table 4. Macro and micronutrients content in leaves of cultivated *S. marginata* plants grown with three green manures (GM), without or with nitrogen (N).

Green manures / Nitrogen	N%	P	K	Ca	Mg	Cu	Fe	Mn	Zn
		g kg^{-1}				mg kg^{-1}			
Without GM / Without N	2.4	2.3	7.4	7.9	4.1	6.9	901.9	168.1	69.8
S. aterrimum / Without N	1.5	2.3	8.3	7.7	4.0	9.3	759.6	166.4	69.0
C. spectabilis / Without N	3.1	2.2	8.2	7.9	4.4	24.5	571.7	170.4	66.8
C. ensiformes / Without N	2.3	2.7	8.4	7.1	4.1	8.1	658.9	143.6	65.5
Without GM / With N	2.4	2.4	8.6	7.4	3.9	30.5	933.9	160.8	29.0
S. aterrimum / With N	2.5	2.1	8.2	6.9	3.9	11.4	981.2	158.2	60.8
C. spectabilis / With N	2.3	2.4	8.7	7.1	3.7	7.8	988.5	148.0	56.9
C. ensiformes / With N	2.5	2.8	10.5	6.1	3.4	7.1	641.5	112.0	69.5
Mean content	2.4	2.4	8.5	7.3	3.9	13.2	804.6	153.4	60.9

We conclude that the extract from *S. marginata* plants harvested after flowering showed higher concentration of phenolic compounds. The HPLC-PDA profile showed that the differences between extracts are due to the different harvest periods of the plants. More dry mass of leaves and fresh and dry mass of stems were obtained from *S. marginata* cultivated and harvested at 810 days with use of the green manure *C. spectabilis* and chemical nitrogen fertilizer.

Bioassay with *A. aegypti* – The different extracts of *S. marginata* evaluated (HDA-BF, HDA-DF and HDA-AF) were able to produce acute effect of mortality in the larvae exposed for 24 h, with concentrations ranging from 0.098 to 1.182 g L^{-1} for 10%, 50% and 90% of the population. From these data, lethal concentrations were established (Table 5).

The crude extract with the highest larvicidal activity was obtained from the plant harvested after flowering, with lethal concentrations for LC_{10} 0.0098 g L^{-1}, LC_{50} 0.070 g L^{-1} and LC_{90} 0.487 g L^{-1} (Figure3).

Table 5. Lethal acute concentration, for the 24 h period, of ethanolic extracts of *S. marginata* collected in the three periods, before flowering [HDA-BF], during flowering [HDA-DF] and after flowering [HDA-AF], with concentrations of 0.001 to 1.0 g L^{-1} on larvae of *A. aegypti* L. (1762) (Diptera: Culicidae).

Sample	Lethal Concentration	g L^{-1}	Lethality range
HDA-BF	LC$_{10}$	0.093	0.063 upon 0.171
	LC$_{50}$	0.346	0.267 upon 0.455
	LC$_{90}$	0.990	0.689 upon 1.981
HDA-DF	LC$_{10}$	0.094	0.258 upon 0.155
	LC$_{50}$	0.333	0.225 upon 0.491
	LC$_{90}$	1.182	0.715 upon 4.289
HDA-AF	LC$_{10}$	0.010	0.002 upon 0.037
	LC$_{50}$	0.070	0.005 upon 0.131
	LC$_{90}$	0.500	0.313 upon 2.060

Doses established by correlation at 95%.

After determination of the acute lethal concentration of the crude extracts, the action capable of causing mortality by chronic lethal exposure was verified. The data revealed that mortality of larvae and pupae occurred with concentrations of 0.25 and 0.12 g L^{-1}. Although the percentage was very low, the delay in the cycle was statistically significant for the young phase (larvae + pupae). The cycle extension for the young phase showed a difference of 107 h when compared to the control, which reinforces the information on the insecticidal action of the 0.5 g L^{-1} dose, which caused total larval mortality in a mean

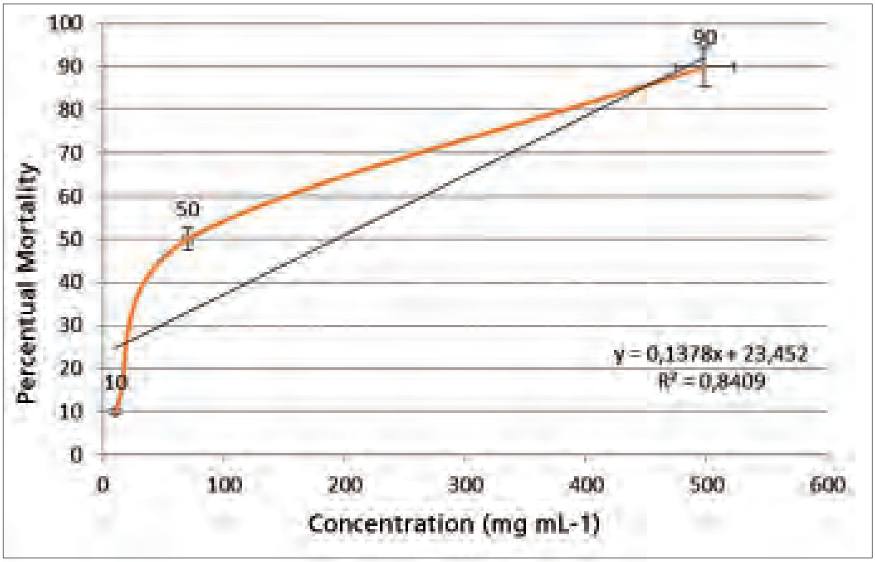

Figure 3. Effective lethal concentration (LC) of *S. marginata* extract after flowering on *A. aegypti* L. larvae after 24 h exposure.

period of 114 h. Thus, the lowest doses, although not effective to cause lethality, were able to affect the biological cycle, which serves as an indication for the use of this plant as a synergistic insecticide to be used in periods of lower infestation (Table 6).

Table 6. Effects of chronic exposure to doses of 70% ethanolic extract of *S. marginata* (larvae + pupae), larval duration, larval and pupal mortality, and emergence of adults were evaluated with concentrations between 0.062 and 0.50 g L $^{-1}$ in the biological cycle of *A. aegypti* L (Culicidae).

Concentration (g L $^{-1}$)	Larvalduration (hours)	Younger (larvae + pupae)	Emergence of adults
0.500	-	-	0
0.250	144b	275.2b	76
0.125	138b	204b	85
0.062	126a	168a	99
Control	126a	174a	100

Means followed by different lowercase letters in columns are significantly different according to the Tukey ($P < 0.05$).

The data obtained in this study can be considered efficient when compared to those observed in the literature. Rodrigues et al. (2006) observed larvicidal effect of *S. lethalis* on *A. aegypti* larvae and determined the LC_{50} 404.16 μg mL $^{-1}$ for the ethanolic extract of the stem bark of this plant. However, the ethanolic extract of the root bark was more active reaching 285.76 μg mL $^{-1}$.

Bioassay with *A. salina* - The toxicity rate towards *A. salina* was established for the crude extracts (Table 7), and it was possible to observe that the studied plant species, according to the basis of the tests (Meyer et al. 1982), presented toxicity with values of LC_{10} 0.05 g L $^{-1}$, LC_{50} 0.245 g L $^{-1}$ and LC_{90} 0.445 g L $^{-1}$. When comparing with the rotenone pattern after the phytotoxic analysis by the Probit method, a more homogeneous concentration profile was observed, with a trend line closer to that calculated (Silva et al. 2009, Figure 4). The results obtained in the present study were similar to those reported by Silva Filho et al. (2009) where concentrations above 0.5 g L $^{-1}$ were effective as larvicide regardless of plant stage.

Table 7. Calculation of the lethal concentrations (LC) mg.mL $^{-1}$ and range of the crude extract of *S. marginata* leaves on the naupilus of *A. salina*.

Lethal Concentration	g L $^{-1}$	Lethality range
LC_{10}	0.093	0.063 upon 0.171
LC_{50}	0.346	0.267 upon 0.455
LC_{90}	0.990	0.689 upon 1.981

Confidence interval established by correlation at 95%.

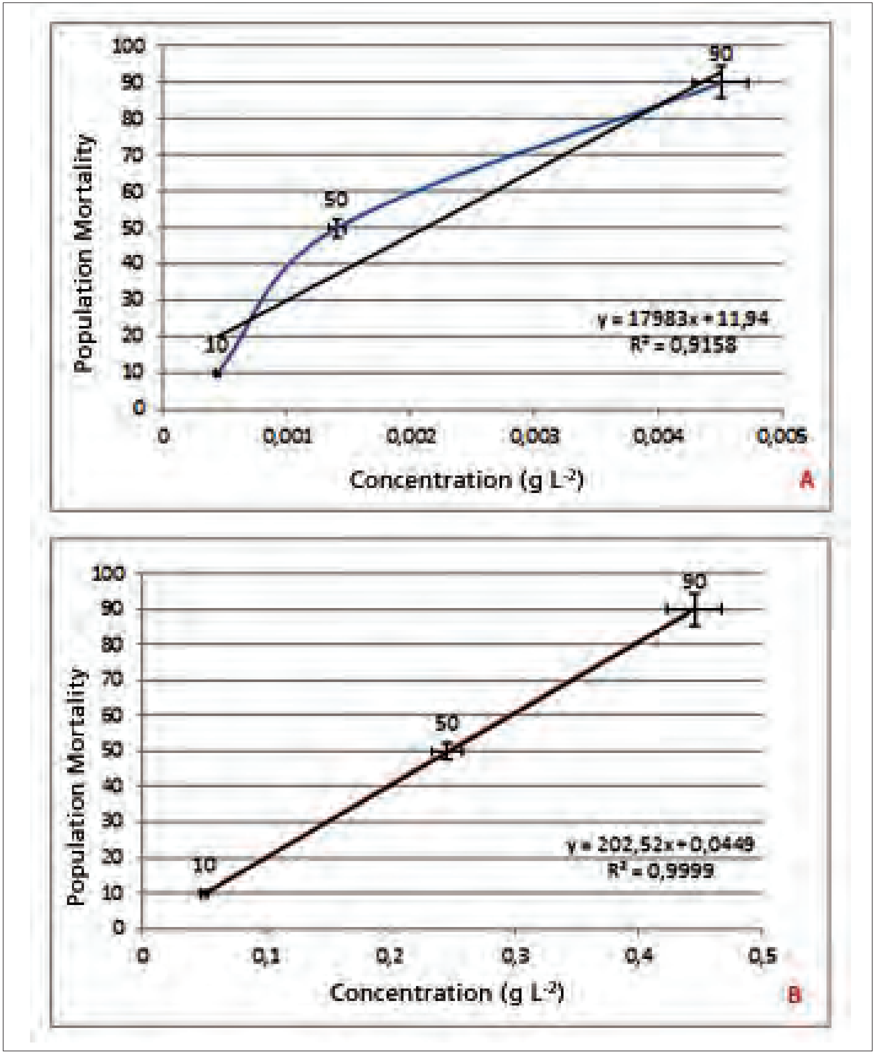

Figure 4. Graphical representation of the toxicity profile of Rotenone [A] and 70% ethanolic extract of *S. maginata* [B] towards *A. saline* L., after 24 h of exposure and determination of lethal concentrations for 10, 50 and 90% of the population.

Conclusions

The extract from *S. marginata* plants harvested after flowering showed higher concentration of phenolic compounds. The HPLC-PDA profile showed that the differences between extracts are due to the different harvest period of the plants. More dry mass of leaves and fresh and dry mass of stems were obtained from *S. marginata* cultivated and harvested at 810 days with use of the green manure *C. spectabilis* and chemical nitrogen fertilizer. The 70% ethanolic extracts of *S. marginata* showed insecticidal activity towards *A. aegypti* larvae, causing total mortality of the population with concentrations above 0.5 g L^{-1} preventing adult formation and extending the cycle in up to 144 h, independently of the harvest period. The larvicidal action was more effective for extracts obtained after flowering, with values of LC_{10} 0.0098 g L^{-1}, LC_{50} 0.070 g L^{-1} and LC_{90} 0.487 g L^{-1}. The species showed toxicity towards *A. salina* when compared with rotenone, the commercial insecticide molecule of the genus, and the toxicity range was less effective in terms of g L^{-1}, evidencing that doses above 0.5 g L^{-1} are effective as larvicide, independently of the harvest period.

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Understory of the seasonally-flooded brevi-semideciduous
Vochysia divergens forest, Pantanal, Brazil

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Introduction

Understanding the floristic diversity of tropical forests is an important tool to help conserving biodiversity, as well as to support public policies (Schmitt et al. 2010; Edwards et al. 2014). Overall, the understory is the major plant biodiversity component in a particular forest ecosystem; thus, it becomes an important component to help maintaining ecological processes and forest biodiversity dynamics (Denslow 1987; Montgomery and Chazdon 2001; Meira Neto and Martins 2003; McIntosh et al. 2016, Mestre et al. 2017). This synusia also affects energy flow, nutrient cycling, and regeneration ability (Gilliam 2007; Lü et al. 2011; Mestre et al. 2017); in addition, it modifies different environments for seedling germination and establishment, and influences canopy composition and structure (Uhl et al. 1988; Harms et al. 2004; Gilliam 2007; Royo and Carlson 2006). Moreover, it contributes to the food chain, provides habitat and forage for many animal species (Lü et al. 2011; Deb et al. 2015), and influences the density and spatial distribution patterns of woody seedlings (Montgomery and Chazdon 2001).

Although most of the published data concerning the floristic composition, structure and dynamics of Brazilian forests are restricted to tree communities, Gentry (1990, 1992) highlights that it is worth investing understory inventories because it represents 75% of the richness in tropical forests, even among vascular species. Although the growing global awareness about understory comprises a high percentage of total diversity, most floristic inventory studies do not approach this component (Duivenvoorden and Lips 1995; Ter Steege 2000; Lü and Tang 2010; Assis and Wittmann 2011; Deb et al. 2015), for example, in Central Amazon (Wittmann et al. 2002, 2004), in

Brazilian cerrado (Marimon et al. 2002; Maracahipes-Santos et al. 2011, 2015); and in the Atlantic forest (Reis et al. 2007; Urbanetz et al. 2010; Landim et al. 2015).

Knowing and understanding the diversity of understory plants is, therefore, essential to help developing proper forest conservation and sustainable management strategies (Montagnini and Jordan 2005; Deb et al. 2015). The understory vegetation changes from forest to forest; this variation may be explained by several factors such as canopy structure and composition (Lü et al. 2011; Deb et al. 2015; Mestre et al. 2017), nutrient availability and soil moisture (Poulsen and Pendry 1995; Newbery et al. 1996; Paixão et al. 2013), succession background (LaFrankie et al. 2006), forest management strategies (Hart and Chen 2008; Ares et al. 2009) and fragmentation (Benitez-Malvido and Martinez-Ramos 2003; Rasingam and Parthasarathy 2009).

The species distribution and composition in understories found in Brazil were addressed in the Amazon by Costa et al. (2009), Drucker et al. (2008), Zuquim et al. (2009), Paixão et al. (2013), Moulatlet et al. (2014), Tuomisto et al. (2014); in the Atlantic forest by Oliveira-Filho et al. (2004); Marques et al. (2014), Polisel et al. (2014), Silva et al. (2017) and in the Brazilian cerrado by Weiser and Godoy (2001), Saporetti et al. (2003); Tannus and Assis (2004), Munhoz and Felfili (2007), Zanelli (2013), Amaral et al. (2017), among others.

There is lack of studies about understory in Pantanal; consequently, the understanding about the total diversity of its forests, which is an important component to support biodiversity restoration and conservation plans, remains poor. *Cambarazal*, which is a seasonally-flooded brevi-semideciduous forest and a monodominant community composed of *Vochysia divergens* Pohl. individuals, has been the subject of studies of management purpose that approach the species structure, composition and relation to floods (Nascimento and Nunes da Cunha 1989; Arieira and Nunes da Cunha 2006, 2012). Nunes da Cunha and Junk (2004) hypothesized that the multiannual water level changes drive *V. divergens* invasion in Pantanal. In addition, Nunes da Cunha and Junk (2004) and Arieira et al. (2011) hypothesized that droughts, along with multi-year fires, control the expansion of these forests. The regeneration dynamics and gap-maker were approached by Arruda and Nunes da Cunha (2012). The global climate effect on the radial growth of *cambará* individuals was investigated by Fortes (2006) and Fortes et al. (in preparation).

However, studies about the understory component in this forest formation were not performed. The current study intends to answer the following questions: (a) what are the species composing the understory of the seasonally-flooded brevi-semideciduous *V. divergens* forest? (b) What is the similarity between this synusia and the forest canopy flora? (c) How does this vegetation contribute to *Cambarazal*?

State Law n. 8.830 - from January 21st, 2008 – addresses the protection and management of the upper Paraguay Basin in Mato Grosso State. However, article 11 sets the minimum diameter conditions that should be respected at the time to cut *Vochysia divergens species* for cleaning and intensive livestock purposes (art. 41, § 2). Thus, studies about this plant species are essential to help standardizing the law.

Materials and Methods

Study Site

The study site is located at Reserva Particular do Patrimônio Natural (RPPN) SESC Pantanal, PELD Site, in Pantanal of Barão de Melgaço, Mato Grosso State (Figure 1). It is the core area of *Cambarazal* forest, which forms a continuous band parallel to Cuiabá River.

The annual rainfall in RPPN SESC Pantanal ranges from 1000 to 1500 mm; the total rainfall may be lower than 40 mm in the dry season (Hasenack et al. 2010). The aquatic phase of the flood pulse takes place from December to April, whereas the earthly phase happens from May to September (Nunes da Cunha and Junk 2004). In addition to the standard flood multi-annual periods, extreme flood and drought events take place on a yearly basis (Junk et al. 2006); the drought events in 2005 were followed by remarkable wild fire events (Couto and Oliveira 2010).

Data Collection and Analysis

Data were collected in three transverse transects to *Cambarazal* forest - Espírito Santo base towards Biguazeiro port (Figure 1). Six/seven rows were set in each transect 500 m from each other. Each row was 250 m long; five plots (10 x 10) were distributed in each row, 50 m apart from each other (Figure 2).

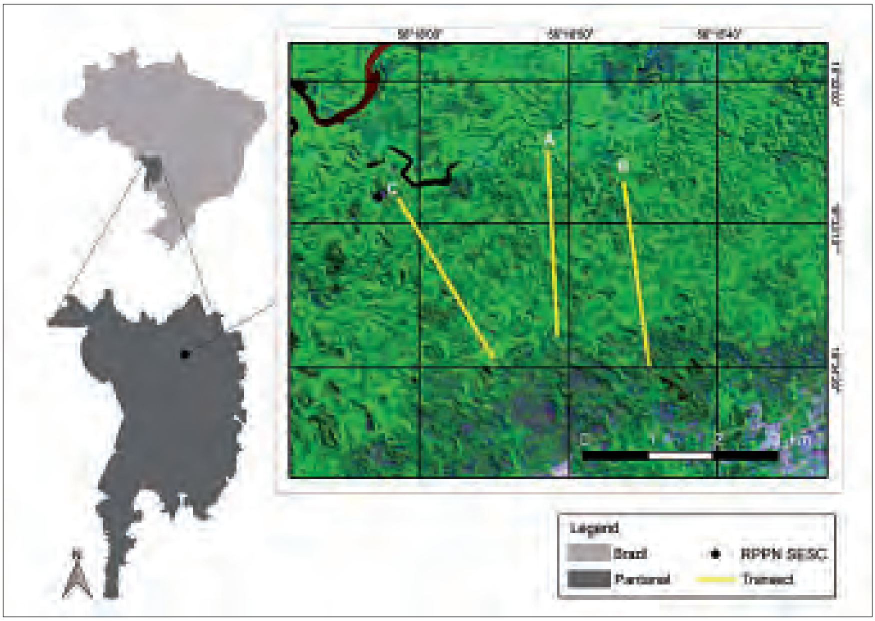

Figure 1. Map showing the study site located at Reserva Particular do Patrimônio Natural, SESC Pantanal, Pantanal of Barão de Melgaço, Mato Grosso State, Brazil.

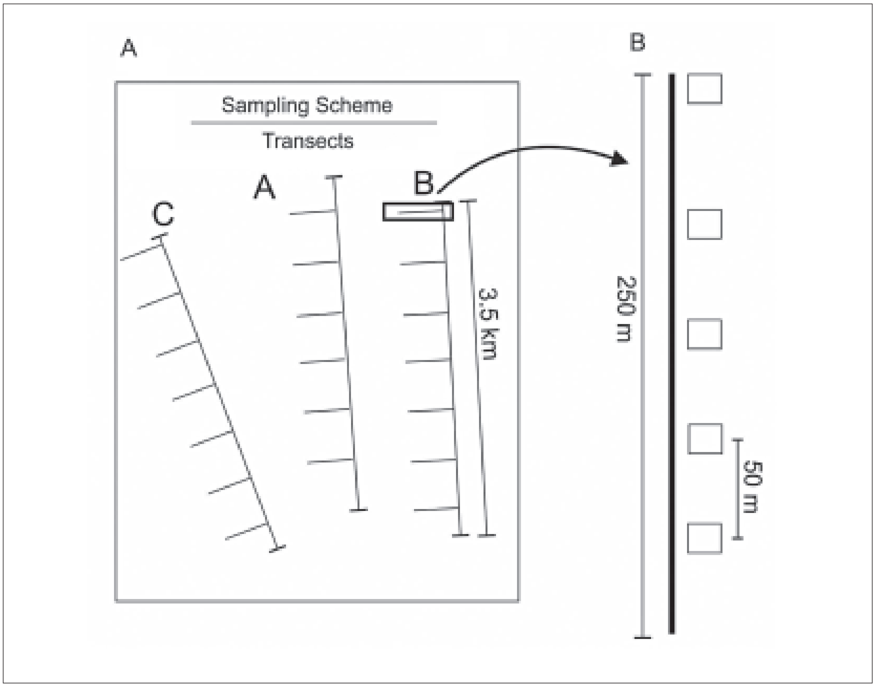

Figure 2. Sampling scheme. A) distribution of three transects in the study site, 6-7 rows were set in each transect. B) five plots (10 x 10 m) were allocated in each 250-m rows, 50m apart from each other.

Individuals showing height between 0.50 and 4 meters were included in the sample by taking into account the understory features. Individuals showing 0.44 cm diameter above ground level (DSH) were included in the sample. Multiple-stem plants were individually measured and the sum of their basal areas was considered to be the basal area of the plant.

The collected botanical material was herborized and identified through the comparison to the material deposited at the herbarium of Federal University of Mato Grosso (UFMT). The botanical families were sorted according to *Angiosperm Phylogeny Group* (APG) III (Stevens 2017). The spelling and authors of the species were defined according to the Tropics (2017). The species were classified according to growth form, through field observations and consultations on Flora do Brasil 2020 website (2017).

Phytosociological parameters were calculated according to Mueller-Dombois and Ellenberg (1974). The importance value of each species was calculated according to Kent and Coker (1994). Species diversity was calculated through the Shannon-Weaver (H'; Natural log) and Evenness (J') indices (Magurran 1988).

A similarity dendrogram was generated by using the Jaccard Similarity Coefficient for presence-absence data along with the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method in order to test how the sampled rows were grouped according to composition. The Venn diagram was generated based on the species composition by taking into consideration the dendrogram result showing similarity between rows(http://bioinformatics.psb.ugent.be/webtools/Venn/).

Results

Floristic Composition and Phytosociological Parameters

The total of 5,662 individuals belonging to 67 species, 60 genera and 33 families (Table 1) were assessed. The arboreal growth form prevailed among the species (56.7%; 38/67); it was followed by shrubs (32.8%; 22/67) and herbs (5.9%; 4/67) (Table 1).

The families showing the largest number of species were Fabaceae (9 spp.), Rubiaceae (7 spp.), Euphorbiaceae and Myrtaceae (5 spp.), and Malvaceae and Polygonaceae (3 spp.) (Figure 3). Other 19 families were represented by a single species (Table 1).

Table 1. List of family, species and growth form in the understory sampled in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest in RPPN SESC Pantanal, Brazil.

FAMILY/SPECIES	GROWTH FORM
APOCYNACEAE	
Tabernaemontana siphilitica (L. f.) Leeuwenb.	Shrub
ARECACEAE	
Bactris glaucescens Drude	Palm tree
BIGNONIACEAE	
Handroanthus heptaphyllus (Vell.) Mattos	Tree
BURSERACEAE	
Protium heptaphyllum (Aubl.) Marchand	Tree
CHRYSOBALANACEAE	
Couepia uiti (Mart. & Zucc.) Benth. ex Hook. f	Tree
Licania parvifolia Huber	Tree
CALOPHYLLACEAE	
Calophyllum brasiliense Cambess.	Tree
CELASTRACEAE	
Peritassa dulcis (Benth.) Miers	Shrub
Salacia elliptica (Mart.) G. Don	Tree
CLUSIACEAE	
Garcinia brasiliensis Mart.	Tree
COMBRETACEAE	
Buchenavia oxycarpa (Mart.) Eichler	Tree
Combretum lanceolatum Pohl ex Eichler	Tree
COSTACEAE	
Costus arabicus L.	Herb
CUCURBITACEAE	
Cayaponia podantha Cogn.	Liana
DIOSCOREACEAE	
Dioscorea trifida L. f.	Herb
ERYTROXYLACEAE	
Erythroxylum anguifugum Mart.	Shrub
EUPHORBIACEAE	
Alchornea discolor Poepp.	Tree
Croton sp.	Tree
Mabea paniculata Spruce ex Benth.	Tree
Sapium obovatum Klotzsch ex Müll. Arg.	Shrub
Sapium sp.	Tree
FABACEAE	
Aeschynomene fluminensis Vell.	Shrub
Albizia inundata (Mart.) Barneby & J.W. Grimes	Tree
Andira cujabensis Benth.	Tree
Bauhinia sp.	Shrub
Inga sp.	Shrub
Mimosa pellita Humb. & Bonpl. ex Willd.	Shrub
Pterocarpus santalinoides L'Hér. ex DC.	Tree

Table 1 (cont.). List of family, species and growth form in the understory sampled in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest in RPPN SESC Pantanal, Brazil.

FAMILY/SPECIES	GROWTH FORM
FABACEAE	
Zygia inaequalis (Humb. & Bonpl. ex Willd.) Pittier	Tree
Not identified	Tree
LAMIACEAE	
Vitex cymosa Bertero ex Spreng.	Tree
LAURACEAE	
Ocotea sp.	Tree
Ocotea diospyrifolia (Meisn.) Mez	Tree
LOGANIACEAE	
Strychnos sp.	Scandent shrub
MALPIGHIACEAE	
Byrsonima laxiflora Griseb.	Tree
MALVACEAE	
Byttneria rhamnifolia Benth.	Herb
Helicteres guazumifolia Kunth	Shrub
Waltheria communis A. St.-Hil.	Herb
MELASTOMATACEAE	
Mouriri guianensis Aubl.	Tree
MELIACEAE	
Trichilia catigua A. Juss.	Tree
MORACEAE	
Brosimum lactescens (S. Moore) C.C. Berg	Tree
Sorocea sprucei (Baill.) J.F. Macbr.	Tree
MYRTACEAE	
Calyptranthes lucida Mart. ex DC.	Tree
Campomanesia eugenioides (Cambess.) D.Legrand ex Landrum	Shrub
Eugenia florida DC.	Tree
Eugenia inundata DC.	Shrub
Eugenia sp.	Shrub
NYCTAGINACEAE	
Neea theifera Oerst.	Shrub
Neea hermaphrodita S. Moore	Shrub
OLACACEAE	
Dulacia egleri (J.O. Rangel) Sleumer	Shrub
POLYGONACEAE	
Coccoloba cujabensis Wedd.	Tree
Coccoloba sp.	Tree
Triplaris americana L.	Tree
RUBIACEAE	
Amaioua guianensis Aubl.	Tree
Duroia duckei Huber	Tree
Palicourea crocea (Sw.) Schult.	Shrub
Psychotria carthagenensis Jacq.	Tree
Rudgea cornifolia (Kunth) Standl.	Tree

Table 1 (cont.). List of family, species and growth form in the understory sampled in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest in RPPN SESC Pantanal, Brazil.

FAMILY/SPECIES	GROWTH FORM
RUBIACEAE	
Sphinctanthus hasslerianus Chodat	Shrub
Tocoyena formosa (Cham. & Schltdl.) K. Schum.	Tree, Shrub
SALICACEAE	
Banara arguta Briq.	Shrub
Casearia rupestris Eichler	Shrub
SAPINDACEAE	
Cupania castaneifolia Mart.	Shrub, Tree
Paullinia pinnata L.	Liana
URTICACEAE	
Cecropia pachystachya Trécul	Tree
VERBENACEAE	
Lippia alba (Mill.) N.E. Br. ex Britton & P. Wilson	Shrub
VOCHYSIACEAE	
Vochysia divergens Pohl	Tree

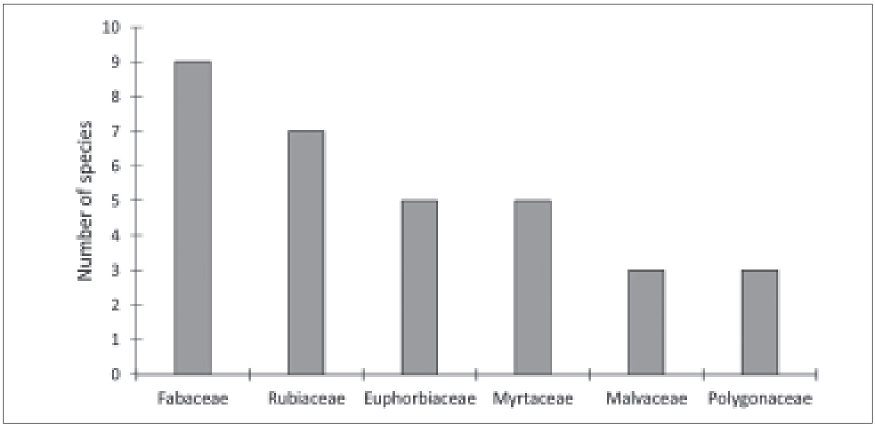

Figure 3. Number of species in the sampled families in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest, Pantanal, Brazil.

The richest genera were *Eugenia* (3 ssp.), *Coccoloba* (2 ssp.), *Neea* (2 ssp.), *Ocotea* (2 ssp.) and *Sapium* (2 ssp.) (Table 1).

The understory in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest was numerically dominated by the species *Peritassa dulcis*, which held 14% (796) of the sampled individuals (Table 2). The five most important species were *Duroia duckei, Peritassa dulcis, Eugenia florida, Palicourea crocea* and *Banara arguta*. These first five species comprised 48% of the sampled individuals (Table 2).

Table 2. List of species, in descending VI order, and their respective phytosociological parameters: AD (absolute density), RD (relative density), AF (absolute frequency), RF (relative frequency), ADo (absolute dominance), RDo (relative dominance), CV (coverage value) and IV (importance value), total and percentage sampled in the seasonally flooded brevi-semideciduous forest of *Vochysia divergens*, Pantanal, Brazil.

Species	AD	RD	AF	RF	ADo ($m^2.ha^{-1}$)	RDo	CV	CV (%)	IV	IV (%)
Duroia duckei	742	13.10	76.10	7.09	0.40	8.30	21.40	10.70	28.50	9.50
Peritassa dulcis	796	14.10	72.70	6.80	0.40	7.40	21.50	10.70	28.30	9.40
Eugenia florida	542	9.60	78.40	7.30	0.30	6.80	16.50	8.20	23.80	7.90
Palicourea crocea	362	6.40	38.60	3.60	0.10	2.80	9.20	4.60	12.80	4.30
Banara arguta	294	5.20	37.50	3.50	0.20	4.10	9.30	4.70	12.80	4.30
Vochysia divergens	148	2.60	18.20	1.70	0.30	6.20	8.80	4.40	10.50	3.50
Byrsonima laxiflora	203	3.60	33.00	3.07	0.20	3.80	7.40	3.70	10.50	3.50
Licania parvifolia	41	0.70	28.40	2.70	0.30	5.60	6.40	3.20	9.03	3.00
Rudgea cornifolia	362	6.40	13.60	1.30	0.07	1.30	7.70	3.90	9.00	3.00
Amaioua guianensis	335	5.90	17.10	1.60	0.07	1.30	7.20	3.60	8.80	2.90
Alchornea discolor	65	1.20	25.00	2.30	0.30	5.10	6.30	3.10	8.60	2.90
Ocotea diospyrifolia	102	1.80	39.80	3.70	0.10	2.29	4.10	2.00	7.80	2.60
Trichilia catigua	78	1.40	26.10	2.40	0.20	3.71	5.10	2.50	7.50	2.50
Tabernaemontana siphilitica	149	2.60	44.30	4.10	0.03	0.67	3.30	1.70	7.40	2.50
Mouriri guianensis	99	1.80	39.80	3.70	0.08	1.50	3.20	1.60	6.90	2.30
Campomanesia eugenioides	113	2.00	26.10	2.40	0.08	1.50	3.50	1.80	5.90	2.00
Mimosa pellita	103	1.80	36.40	3.40	0.04	0.70	2.50	1.30	5.90	2.00
Albizia inundata	48	0.90	20.50	1.90	0.10	2.10	2.90	1.50	4.90	1.60
Coccoloba sp.	64	1.10	25.00	2.30	0.06	1.28	2.40	1.20	4.70	1.60
Mabea paniculata	58	1.00	27.30	2.50	0.05	1.05	2.10	1.04	4.60	1.50
Croton sp.	63	1.10	28.40	2.70	0.01	0.28	1.40	0.70	4.04	1.40
Waltheria communis	82	1.50	19.30	1.80	0.02	0.34	1.80	0.90	3.60	1.20
Eugenia sp.	65	1.20	18.20	1.70	0.03	0.67	1.80	0.90	3.50	1.20

Table 2 (cont.). List of species, in descending VI order, and their respective phytosociological parameters: AD (absolute density), RD (relative density), AF (absolute frequency), RF (relative frequency), ADo (absolute dominance), RDo (relative dominance), CV (coverage value) and IV (importance value), total and percentage sampled in the seasonally flooded brevi-semideciduous forest of *Vochysia divergens*, Pantanal, Brazil.

Species	AD	RD	AF	RF	ADo ($m^2.ha^{-1}$)	RDo	CV	CV (%)	IV	IV (%)
Byttneria rhamnifolia	45	0.80	22.70	2.10	0.030	0.57	1.40	0.70	3.50	1.20
Casearia rupestris	89	1.60	9.10	0.90	0.050	0.97	2.50	1.30	3.40	1.10
Sorocea sprucei	105	1.90	11.40	1.06	0.020	0.40	2.30	1.10	3.30	1.10
Erythroxylum anguifugum	37	0.70	17.10	1.60	0.050	1.03	1.70	0.80	3.30	1.10
Neea theifera	81	1.40	15.90	1.50	0.020	0.29	1.70	0.90	3.20	1.10
Triplaris americana.	50	0.90	14.80	1.40	0.050	0.93	1.80	0.90	3.20	1.10
Zygia inaequalis	29	0.50	18.20	1.70	0.040	0.74	1.30	0.60	2.90	1.00
Psycotria carthaginensis	35	0.60	17.10	1.60	0.030	0.68	1.30	0.70	2.90	1.00
Sapium obovatum	19	0.30	12.50	1.20	0.050	1.08	1.40	0.70	2.60	0.90
Inga sp.	24	0.40	13.60	1.30	0.040	0.79	1.20	0.60	2.50	0.80
Bactris glaucescens	46	0.80	11.40	1.06	0.009	0.19	1.00	0.50	2.10	0.70
Garcinia brasiliensis	18	0.30	14.80	1.40	0.010	0.26	0.60	0.30	2.00	0.70
Strichynos sp.	2	0.00	2.30	0.20	0.060	1.14	1.20	0.60	1.40	0.50
Cecropia pachystachya	9	0.20	5.70	0.50	0.030	0.53	0.70	0.30	1.20	0.40
Andira cujabensis	12	0.20	8.00	0.70	0.010	0.24	0.50	0.20	1.20	0.40
Pterocarpus santalinoides	7	0.10	8.00	0.70	0.010	0.27	0.40	0.20	1.10	0.40
Sapium sp.	10	0.20	6.80	0.60	0.020	0.29	0.50	0.20	1.10	0.40
Sphinctanthus hasslerianus	12	0.20	8.00	0.70	0.002	0.04	0.20	0.10	1.00	0.30
Paullinia pinnata	24	0.40	4.60	0.40	0.006	0.12	0.50	0.30	1.00	0.30
Neea hermaphrodita	11	0.20	5.70	0.50	0.003	0.05	0.20	0.10	0.80	0.30
Ocotea sp.	8	0.10	4.60	0.40	0.007	0.15	0.30	0.10	0.70	0.20
Tocoyena formosa	14	0.30	4.60	0.40	0.003	0.05	0.30	0.20	0.70	0.20
Bauhinia sp.	7	0.10	5.70	0.50	0.002	0.05	0.20	0.08	0.70	0.20

Table 2 (cont.). List of species, in descending VI order, and their respective phytosociological parameters: AD (absolute density), RD (relative density), AF (absolute frequency), RF (relative frequency), ADo (absolute dominance), RDo (relative dominance), CV (coverage value) and IV (importance value), total and percentage sampled in the seasonally flooded brevi-semideciduous forest of *Vochysia divergens*, Pantanal, Brazil.

Species	AD	RD	AF	RF	ADo ($m^2.ha^{-1}$)	RDo	CV	CV (%)	IV	IV (%)
Salacia elliptica	5	0.10	3.40	0.30	0.004	0.08	0.20	0.09	0.50	0.20
Aeschynomene fluminensis	3	0.10	3.40	0.30	0.001	0.02	0.10	0.04	0.40	0.10
Coccoloba cujabensis	7	0.10	2.30	0.20	0.002	0.04	0.20	0.08	0.40	0.10
Dulacia egleri	5	0.09	2.30	0.20	0.004	0.07	0.20	0.08	0.40	0.10
Dioscorea trifida	3	0.05	2.30	0.20	0.001	0.02	0.10	0.04	0.30	0.10
Calophyllum brasiliense	2	0.04	2.30	0.20	0.001	0.02	0.10	0.03	0.30	0.09
Calyptranthes lucida	2	0.04	2.30	0.20	0.001	0.02	0.06	0.03	0.30	0.09
Combretum lanceolatum	5	0.09	1.10	0.10	0.005	0.09	0.20	0.09	0.30	0.09
Couepia uiti	2	0.04	2.30	0.20	0.002	0.03	0.07	0.03	0.30	0.09
Eugenia inundata	2	0.04	2.30	0.20	0.001	0.02	0.05	0.03	0.30	0.09
Lippia alba	2	0.04	2.30	0.20	0.001	0.01	0.05	0.02	0.30	0.09
Protium heptaphyllum	3	0.05	2.30	0.20	0.001	0.02	0.07	0.04	0.30	0.09
Not identified	2	0.04	1.10	0.10	0.002	0.05	0.08	0.04	0.20	0.06
Buchenavia oxycarpa	1	0.02	1.10	0.10	0.002	0.05	0.06	0.03	0.20	0.06
Brosimum lactescens	2	0.04	1.10	0.10	0.001	0.02	0.06	0.03	0.20	0.05
Costus arabicus	2	0.04	1.10	0.10	0.001	0.02	0.06	0.03	0.20	0.05
Cupania castaneifolia	2	0.04	1.10	0.10	0.000	0.01	0.04	0.02	0.10	0.05
Vitex cymosa	1	0.02	1.10	0.10	0.001	0.02	0.04	0.02	0.10	0.05
Cayaponia podantha	1	0.02	1.10	0.10	0.000	0.00	0.02	0.01	0.10	0.04
Helicteres guazumifolia	1	0.02	1.10	0.10	0.000	0.00	0.02	0.01	0.10	0.04
Handroanthus heptaphyllus	1	0.02	1.10	0.10	0.000	0.00	0.02	0.01	0.10	0.04
TOTAL	5,662	100	1074	100	5.038	100	200	100	300	100

If one takes into consideration that, according to the classification by Oliveira and Amaral (2004), locally rare species are those presenting a single individual, 7.5% of the species belonging to this synusia are rare. Among the rare species, it is worth highlighting *Buchenavia oxycarpa, podantha Cayaponia, Helicteres guazumifolia*, *Handroanthus heptaphyllus* and *Vitex cymosa* (Table 2).

Floristic Diversity

Species richness ranged from 14 to 37 species in each row (23.65 species, on average). Shannon diversity indices of the *Cambarazal* forest understory community were compared to other forest types (Table 3).

The cluster analysis applied to the dendrogram (Figure 4) identified three groups. Group 1 comprised rows 1 to 6 (transect A), 10 and 11 (transect B), 17 to 20 (transect C). Group 2 comprised rows 2 (transect A) and 7 to 9 (transect B) in the eastern part of the *Cambarazal* forest burnt in 2005. Group 3 comprised rows 12 and 13 (Transect B) and 14 to 16 (transect C). Group formation may be explained by the geographical proximity between transects and, therefore, by the greater similarity in their floristic composition, flood time and colonization time.

The result generated by the Venn diagram identified the understory species characteristic to each group (Table 4). Group 1 matched the understory

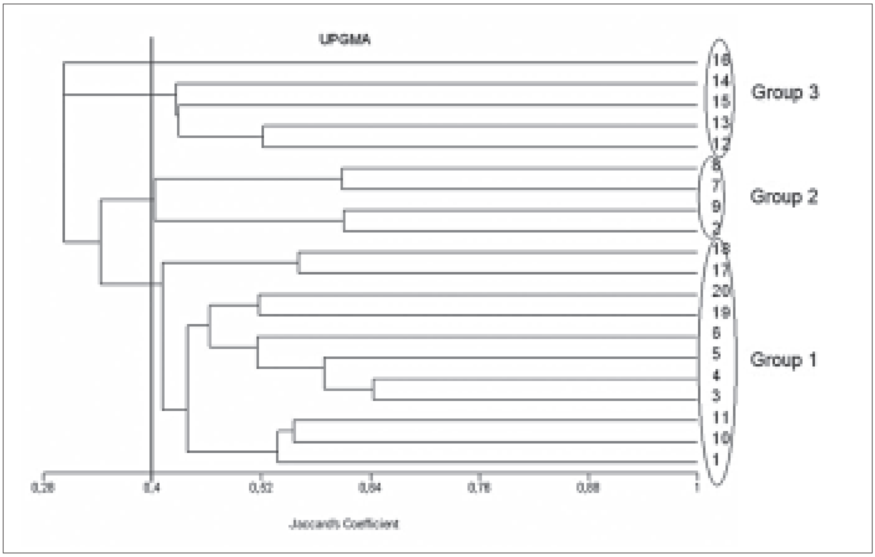

Figure 4. Cluster analysis applied to the sampled rows (1 to 20) in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest in Pantanal.

Table 3. Diversity indices found in understory surveys applied to different forest types and in the understory survey applied to seasonally-flooded brevi-semideciduous *Vochysia divergens* forest, Pantanal, Brazil.

Forest Type	Place	Richness	Shannon's diversity index	Pielou's evenness index	Source
Dry Tropical Forest	Tamil Nadu, India	21	1.74-1.92		Gandhi and Sundarapandian (2014)
Seasonal Tropical Forest	Xishuangbanna, China	214	3.37-4.30	0.80-0.91	Lü et al. (2011)
Subtropical Coastal Forest	Viamão, RS, Brazil	36	2.98	0.84	Mueller and Waechter (2001)
Terra Firme Forest	Amazônia Central, Brazil	355	5.6	0.95	Oliveira and Amaral (2005)
Dry Forest	Areia e Remígio, PB, Brazil	54	2.99	——	Pereira et al. (2002)
Dense Ombrophylous Forest	Camaragibe, PE, Brazil	35	2.6	0.73	Silva (2010)
Dense Ombrophylous Forest	Uma, BA, Brazil	449	5.32	0.87	Lopes et al. (2015)
Dense Ombrophylous Forest	São Pedro da Alcântara, SC, Brazil	24-69	2.09-3.55	0.37-0.61	Siminski et al. (2004)
Mixed Ombrophilous Alluvial Forest	Curitiba, PR, Brazil	39	2.49	0.68	Barddal et al. (2004)
Dense Tropical Forest	Florestas de Araucária, SP, Brazil	226	3.40-3.88	0.84-0.88	Polisel et al. (2014)
Semideciduous Gallery Forest	Reserva Tamanduá, DF, Brazil	69	2.89	0.82	Silva et al. (2004)
Semideciduous Seasonal Forest	Viçosa, MG, Brazil	109	3.38	0.72	Meira-Neto and Martins (2003)
Semideciduous Seasonal Forest	Viçosa, MG, Brazil	80			Meira-Neto et al. (2005)
Semideciduous Seasonal Forest	Chiapetta, RS	88	2.42 - 3.45	0.80 - 0.85	Coelho et al. (2011)
Semideciduous Seasonal Forest	Linhares, ES, Brazil	63	3.73	0.9 - 0.89	Barroso et al. (2014)
Semideciduous Seasonal Forest	Estação Ecológica de Caetetus, SP, Brazil	57			Souza et al. (2014)
Brazilian cerrado	Itumbiara, GO, Brazil	76	2.42 - 3.12	0.44 - 0.50	Soares et al. 2015
Seasonally-flooded brevi-semi-deciduous Vochysia divergens forest	Pantanal, MT, Brazil	67	3.15	0.75	The current study

Table 4. Understory features of groups in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest in Pantanal, Brazil.

	Group 1	Group 2	Group 3
Rows	1; 3; 4; 5; 6; 10; 11; 17; 18; 19; 20	2; 7; 8; 9	12; 13; 14; 15; 16
N of adult *Vochysia divergens*	45	21	70
N of *Vochysia divergens* samples	75	0	71
Cannopy openness (%)	26.9 ± 3.8	25.8 ± 2.4	20.4 ± 6.2
Number of individuals	1714	626	3953
Basal area ($m^2.ha^{-1}$)	0.0017	0.0002	0.0001
Characteristic species	14	1	7
	Costus arabicus	*Buchenavia oxycarpa*	*Aeschynomene fluminensis*
	Vitex cymosa		Not identified
	Coccoloba cujabensis		*Tocoyena formosa*
	Helicteres guazumifolia		*Waltheria communis*
	Handroanthus heptaphyllus		*Cayaponia podantha*
	Triplaris americana		*Sphinctanthus hasslerianus*
	Eugenia inundata		*Combretum lanceolatum*
	Dulacia egleri		
	Salacia elliptica		
	Cupania castaneifolia		
	Calophyllum brasiliense		
	Neea hermaphrodita		
	Dioscorea trifida		
	Brosimum lactescens		

Table 4 (cont.). Understory features of groups in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest in Pantanal, Brazil.

	Group 1	Group 2	Group 3
Common species	Groups 1, 2 and 3	Groups 1 and 2	Groups 1 and 3
	19	8	18
	Zygia inaequalis	*Mabea paniculata*	*Psycotria carthaginensis*
	Duroia duckei	*Sapium obovatum*	*Amaioua guianensis*
	Eugenia florida	*Couepia uiti*	*Ocotea* sp.
	Mimosa pellita	*Lippia alba*	*Erythroxylum anguifugum*
	Trichilia catigua	*Pterocarpus santalinoides*	*Garcinia brasiliensis*
	Byrsonima laxiflora	*Andira cujabensis*	*Vochysia divergens*
	Cecropia pachystachya	*Inga* sp.	*Bauhinia* sp.
	Tabernaemontana siphilitica	*Calyptranthes lucida*	*Palicourea crocea*
	Byttneria rhamnifolia		*Strychnos* sp.
	Mouriri guianensis		*Albizia inundata*
	Campomanesia eugenioides		*Neea theifera*
	Ocotea diospyrifolia		*Bactris glaucescens*
	Coccoloba sp.		*Paullinia pinnata*
	Banara arguta		*Casearia rupestris*
	Peritassa dulcis		*Rudgea cornifolia*
	Croton sp.		*Protium heptaphyllum*
	Eugenia sp.		*Sapium* sp.
	Licania parvifolia		*Sorocea sprucei*
	Alchornea discolor		

of mature *Cambarazal* forests (seasonally-flooded brevi-semideciduous *Vochysia divergens* forest), which hosted *Costus arabicus*, *Vitex cymosa*, *Coccoloba cujabensis*, *Helicteres guazumifolia*, *Handroanthus heptaphyllus*, *Triplaris americana*, *Eugenia inundata*, *Dulacia egleri*, *Salacia elliptica*, *Cupania castaneifolia*, *Calophyllum brasiliense*, *Neea hermafrodita*, *Dioscorea trifida*, and *Brosimum lactescens*. These are the same species found in forests prone to polyspecific associations; in the present case, these species are associated with *Cambarazal* forest. The small-sized species constituted the understory elements. This group shares 19 species with other groups (Figure 5).

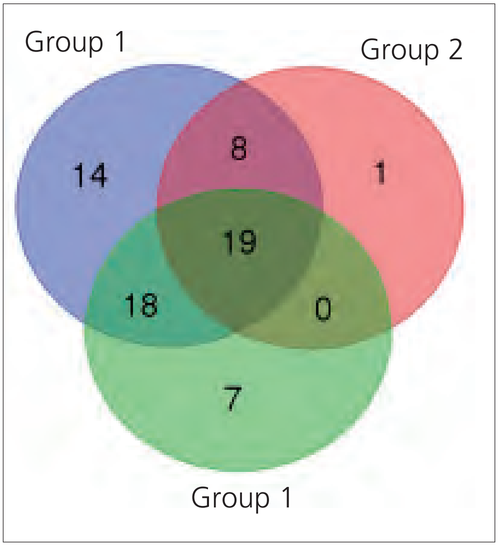

Figure 5. Venn diagram of the understory-characteristic species in the seasonally-flooded brevi-semideciduous *Vochysia divergens* forest, in Pantanal, Brazil.

Buchenavia oxycarpa is the only characteristic species in Group 2 (Table 4). However, this group shares 8 species with Group 1 (Figure 5), namely: *Mabea paniculata*, *Sapium obovatum*, *Couepia uiti*, *Lippia alba*, *Pterocarpus santalinoides*, *Andira cujabensis*, *Inga* sp., and *Calyptranthes lucida*. The fire effect can be seen in the smaller number of adult and juvenile *V. divergens* composing the understory, as well as in the decreased species richness (Table 4). The dense herbaceous stratum is dominated by species *Scleria melaleuca* Rchb. ex Schltdl. & Cham. (*capim-navalha*).

Group 3 comprises understory-characteristic species such as *Aeschynomene fluminensis*, *Tocoyena formosa*, *Waltheria communis*, *Cayaponia podantha*, *Sphinctanthus hasslerianus*, *Combretum lanceolatum* and one non-identified species belonging to family Fabaceae (Table 4). These species are typical of open environments such as the transition from *Cambarazal* forest to flooded *campo sujo* (grassland-woodland savanna).

Discussion

Floristic Composition and Phytosociological Parameters

The comparison applied to the floristics of understory tree elements in *Cambarazal* forest (Arieira and Nunes da Cunha 2006) allowed finding 32 species in common; these species represented 38% (32/83) of the species in this stratum. Arruda and Nunes da Cunha (2012) studied the forest gap dynamics in the *Cambarazal* forest shared 67% (31/46) of the species, with this current study.

The understory provides 28 species to the *Cambarazal* forest. This number of species show the importance of the present study to expand the knowledge of *Cambarazal* forest floristic composition. According to Gentry and Emmons (1987), the understory community is clearly composed of a set of different species sampled in the canopy.

The *Cambarazal* forest understory is open and does not present herbaceous species, with predominance of trees. The same condition was found by Polisel et al. (2014) in an *Araucaria* forest understory community, and by Lopes et al. (2015), in a dense ombrophylous forest.

According to Gilliam et al. (1994, 1995) the species found in the understory can be classified as resident (annual, subshrub, and shrub permanently living in the understory) and transient species (trees and lianas that remain in the understory while they are still young and reach the canopy as they grow). According to the aforementioned authors, the arboreal species in *Cambarazal* forest are of the transient type; they constitute resident understory species along with other growth forms, since the dominant *Vochysia divergens* does not allow other species to develop canopy (Nascimento and Nunes da Cunha 1989; Arieira and Nunes da Cunha 2006).

The species-richest families investigated in the *Cambarazal* forest are also found in the understory of tropical forests; other families such as Fabaceae, Myrtaceae, Rubiaceae and Euphorbiaceae show more representativeness in alluvial ombrophylous dense forests (Barddal et al. 2004; Siminski et al. 2004; Lopes et al. 2005), in *terra firme* forests (Oliveira and Amaral 2005), in gallery forests (Silva et al. 2004) and in semideciduous seasonal forests (Meira Neto and Martins 2003; Coelho et al. 2011). According to Arieira (2005), the richest families in the *V. divergens* forest stratum were Fabaceae, Myrtaceae, Rubiaceae and Vochysiaceae; whereas according to Nascimento and Nunes da Cunha (1989), the richest families were Rubiaceae, Fabaceae and Myrtaceae.

The species *Buchenavia oxycarpa, Cayaponia podantha, Helicteres guazumifolia*, *Handroanthus heptaphyllus* and *Vitex cymosa* are rare in the *Cambarazal* forest probably to the available flood. However, these species are common in other forest types, e.g. polyspecific forests and upland forests in the region (Nunes da Cunha and Junk 2014).

The herein sampled species - *Andira cujabensis*, *Callisthene fasciculata*, *Calophyllum brasiliensis*, *Campomanesia eugenioides*, *Couepia uiti*, *Eugenia inundata*, *Licania parvifolia* and *Tocoyena formosa* - are often found in other Pantanal forest types (Oliveira-Filho et al. 1989; Nascimento and Saddi 1992; Ratter and Dargie 1992; Nunes da Cunha and Junk 2001), fact that highlights these species' ability to widely settle in the region.

Unlike the *Cambarazal* forest canopy, which is dominates by *Vochysia divergens* of high density and frequency, besides importance value (IV) 161.5 (Arieira and Nunes da Cunha 2006); *Duroia duckei* and *Peritassa dulcis* achieved IV 28.5 and 28.3, respectively, in the understory due to their high density (742 and 796 ind/ha^{-1}, respectively), frequency (76.1 and 72.7%, respectively) and dominance values (0.4 m^2 ha^{-1} each). The species *Eugenia florida* and *Banara arguta*, *Duroia duckei* and *Peritassa dulcis* presented the highest IV in the understory, and were clustered in ecological groups by Arruda and Nunes da Cunha (2012). Moreover, they and classified as pioneer and as early secondary species in *Cambarazal* forest gaps.

The 148 *V. divergens* sampled individuals were young and recorded on *Cambarazal* forest edges, mainly towards open areas. According to Longhi et al. (1999), the continuity of the species in the forest community depends on presence of individuals in all forest stratum; however, *V. divergens* showed very low density and frequency in the herein sampled understory.

Floristic Diversity

The understory of the *Cambarazal* forest analyzed in the current study presented 67 species. This species richness was greater than that found in the understory of dry tropical (Gandhi and Sundarapandian 2014), coastal (Mueller and Waechter 2001), dry (Pereira et al. 2002), and semideciduous seasonal forests (Barroso et al. 2014; Souza et al. 2014), as well as in some ombrophylous dense forests (Barddal et al. 2004; Siminski et al. 2004; Silva 2010) (Table 3). However, *terra firme* (Oliveira and Amaral 2005), Seasonal (Lü et al. 2011) and ombrophylous dense forests (Polisel et al. 2014; Lopes et al. 2015) showed more expressive species richness in the understory than *Cambarazal* forest.

According to Table 3, the f diversity index in the *Cambarazal* forest understory was greater than that of Brazilian cerrado (Soares et al. 2015), gallery forests (Silva et al. 2004) and dry forests (Pereira et al. 2002; Gandhi and Sundarapandian 2014); however, it was smaller than that of *terra firme* (Oliveira and Amaral 2004), semideciduous seasonal (Meira Neto and Martins 2003; Coelho et al. 2011; Barroso et al. 2014) and ombrophilous dense forests (Polisel et al. 2014; Lopes et al. 2015).

According to Nascimento and Nunes da Cunha (1989) and Arieira and Nunes da Cunha (2006), the low species diversity in *Cambarazal* forest is linked to flood, which is a limiting factor for the establishment of plant species (Junk et al. 1989).

Arieira and Nunes da Cunha (2006) sampled the *Cambarazal* forest canopy and founded lower diversity (H ʹ = 2.1 to 2.9) than that in the understory in the current study (H ʹ = 3.15); thus, evidencing that the understory provided 28 unique species. Gentry and Emmons (1987) stated that the understory diversity pattern differed from the pattern presented by the canopy species, because it responded to different abiotic factors such as light, nutrient and water availability, wind and temperature (Denslow et al. 1990). The result by Barddal et al. (2004) for the understory in mixed ombrophylous forest corroborates the statement above.

Gentry and Dodson (1987) reinforced the importance of including understory communities in floristic analyses, although these synusiae remain neglected. Monospecific forests are not among the most species-rich communities (Hart et al. 1989; Nascimento and Nunes da Cunha 1989; Nascimento et al. 1997; Marimon and Felfili 2000; Arieira and Nunes da Cunha 2006). In addition, the flooded forests in Pantanal are not species-rich (Nunes da Cunha and Junk 2001); thus, it is necessary conducting understory studies to help better understanding the diversity of these communities.

Pinho et al. (2011) and Pinho and Marini (2014) emphasized that the *Cambarazal* forest is very importance for wetland bird conservation; it is the key habitat of many nesting-bird species. According to these authors, the highest richness and abundance of bird species are recorded in the dry season, because of the food availability in the understory. They selected the mature *Cambarazal* forest to prior protection.

The effect of fire on group 2 evidenced the low species richness, due to the small number of V. divergens in the canopy and understory. There was *Scleria melaleuca* prevalence; which is an aquatic grass growing in supporting emerging shrubs that survives the burn (Pott and Pott 2000). This grass species are distributed in disturbed areas considered or in gaps (Arruda and Nunes da Cunha 2012). Martins et al. (2008) compared the growth rates of understory plants living under different disturbance levels and found a large number of herbaceous vine individuals and a small number of tree species in burned areas. Their result was similar to that found in the present study.

The species in Group 3 were typical of flooded *campo sujo* (grassland-woodland savanna). The species *V. divergens* (Table 4) showed the highest density, and it is typical of *cambará* encroachment (Nascimento and Nunes da Cunha 1989; Nunes da Cunha and Junk 2004). Nunes da Cunha and Junk (2004) hypothesized that wet multi-annual events drive *Cambarazal* forest encroachment (Nunes da Cunha and Junk 2010; Nascimento et al. 2015).

Conclusion

From the perspective of the priority for protection the core *Cambarazal* forest region, that occur along Cuiabá River is area of conservation potential. This study shows that the *cambará* continue to advance, but fire controls this expansion. The core area of *Cambarazal* forest is mature with highest diversity of species and can be used e.g. long-term study about edge effects which include change in the floristic composition and physiognomy.

Therefore, it is essential protecting of *Cambarazal* forest core region in order to help keeping ecological processes and environmental services such as carbon stock, nutrient cycling, biodiversity conservation and preferential habitat for wildlife species.

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Hymenopteran predators,
parasitoids and pollinators caught with malaise traps in Campo Grande, Ms, Brazil

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Introduction

The Cerrado has suffered constant degradation in their natural ecosystems due to human activities such as agriculture and livestock, besides the growth of cities and roads. About half of the 2 million km² of the Cerrado were transformed into annual crops and other types of use, and pastures formed with African grass cover more than 500,000 square kilometers (Klink & Machado, 2005). The dynamic replacement, which includes both, deforestation and forest fires, causes changes in landscape, habitat and species fragmentation, invasion of exotic species (Brazil 2010). Although partially degraded, these areas therefore constitute important sources of natural resources, plants and animals, which can minimize drastic removal of the natural biome.

Even with all the impact and advance of occupancy rates, the Brazilian Cerrado is still highly representative for species richness. The representation of regional fauna on Brazilian biodiversity varies between groups, from less than 20% (bees and ants) to more than 50% for Lepidoptera. In three insect orders Lepidoptera, Hymenoptera and Isoptera, the estimated number of species for the biome is 14,425 and 47% is estimated for the fauna Brazil (MMA 2002).

Members of the Insecta Class occupy the most diverse ecological niches, inhabiting vegetables, soils and water, and are of utmost importance to the planet's vital chains. Besides the innumerable benefits to man, they serve as staple food for several species of birds, fish, amphibians, mammals and arthropods. A large proportion of insect species are beneficial to humans, including those that prey on or parasite other insects, exercising natural control of their hosts

(Nomura 2003). Insects inhabit almost all habitats and play an important role in maintaining the biotic communities and balance of terrestrial ecosystem (Schauff 1986).

The Insecta Class comprises the largest group of known living beings, representing about 70% of the animal species, being the focus of studies on interactions between the organisms and the environment where they live, in the different biomes (Tank 2009). Insects stand out as important organisms in several areas of study, such as feeding (bees), human health (mosquitoes and other insects that can transmit pathogens), veterinary (insect vectors of disease), agriculture (insect pests) and natural control agents (natural enemies of pests) (Costa et al. 2010).

The order Hymenoptera represents the third largest order regarding number of species described within the Insecta Class. The order Hymenoptera presents approximately 115,000 described species distributed among 99 families in different regions of the world (Goulet and Huber 1993). However, it is assumed that the total number is actually five to ten times greater. They rank second among the hyperdiverse insect orders, with over 250,000 estimated species (Hanson and Gauld 2006).

They are abundant in nature and occupy the most diverse types of environment available (Amaral et al. 2005). This order is probably the most beneficial of all classes of insects. Hymenoptera constitute an interesting group regarding their biology, since they exhibit a great diversity of habits and behaviors culminating in the social organization of wasps, bees and ants (Triplehorn and Jonnson 2011).

Parasitoids are insects that live during their larval stages parasites of arthropods (usually other insects), causing the death of their hosts. These insects are important components of terrestrial ecosystems and play a fundamental role as natural enemies, mortality agents and herbivorous insect population regulators (Godfray and Hassel 1994; Cornell and Hawkins 1995); they are also indispensable because of their influence in the dynamics and structure of their communities (Morris et al. 2004).

Parra et al. (2002) believe that this order has a strong potential for applied biological control, especially Chalcidoidea and Ichneumonoidea. These insects are sensitive to environmental disturbances, particularly to pesticides, which make them ideal candidates for conservation studies (Lasalle 1993). According to Roland (1993), the greatest outbreaks of forest pests have been linked to forest fragmentation, probably due to changes in interactions with natural enemies.

The municipality of Campo Grande in the state of Mato Grosso do Sul, Central-West region, Brazil, has anthropized fragments of the Cerrado, especially of the Cerradão, where this Hymenoptera parasitoid fauna is totally unknown. Knowledge about the abundance and richness of these groups should provide useful data for future studies on environmental impacts in the region.

The purpose of this work was to study the occurrence, abundance and richness of hymenopteran parasitoids, predators and pollinators caught with Malaise traps over a 5-week period in two physiognomies, the Cerrado fragment and an area of *Brachiaria decumbens*, pasture in disturbed situation, in the municipality of Campo Grande, Mato Grosso do Sul, Brazil.

Materials and Methods

Thirty samples were obtained, collected in six Malaise traps at São José Farm, an experimental area in Campo Grande, MS, Brazil, from October to November 2010. The experimental area covers 2000m², with 11 hectares of the Cerrado fragment (anthropized Cerradão) and 14 hectares of land devoid of native vegetation due to the invasion of *Brachiaria decumbens* (signal grass).

Six Malaise traps were set up, three outside the Cerrado fragment and three inside the fragment, 100 meters apart from each other. Traps inside the fragment were placed 50 meters away from the border. This type of trap intercepts the insects flight, which tend to go to the upper region containing a collecting flask with liquid (alcohol 90%) where the insects are trapped. The trap consists of a tent of fabric with fine mesh, with collectors in the upper end.

The coordinates of the traps on pastureland area were: 20°24’13.16" S and 54°36’45.69" W, 20°24’09.60" S and 54°36’47.32" W, 20°24’03.62" S and 54°36’44.93"W; and those of the Cerrado fragment were: 20°24’11.87" S and 54°36’42.20" W, 20°24’08.35" S and 54°36’43.83" W, 20°24’06.22" S and 54°36’42.63" W. (Figure 1).

Species commonly found in this area: *Qualea grandiflora* (commonly known in Brazil as Pau-terra-da-folha-grande), *Qualea multiflora* (pau-terra-da-folha-pequena), *Matayba guianensis* Aubl. (pau-pombo), *Myrsine ferruginea* (Capororoca), *Xylopia aromatica* Ruiz et Pav, Spain (pimenta-de-macaco, or Monkey pepper) and *Curatella americana* L (lixeira, or Sandpaper tree). Taken together, these species comprise more than half of the tree species richness. Other common species belong to the fig family (Moraceae) and *Alibertia sessilis* (vell.) K. Schum (marmelinho do campo). Species predominating along the borders include *Cecropia pachystachya* Trécul. (Embaúba) and *Annona crassiflora* Mart. (Marolo), while open areas commonly present *Dipteryx alata* Vogel (Cumbaru) and invasions of exotic grass species.

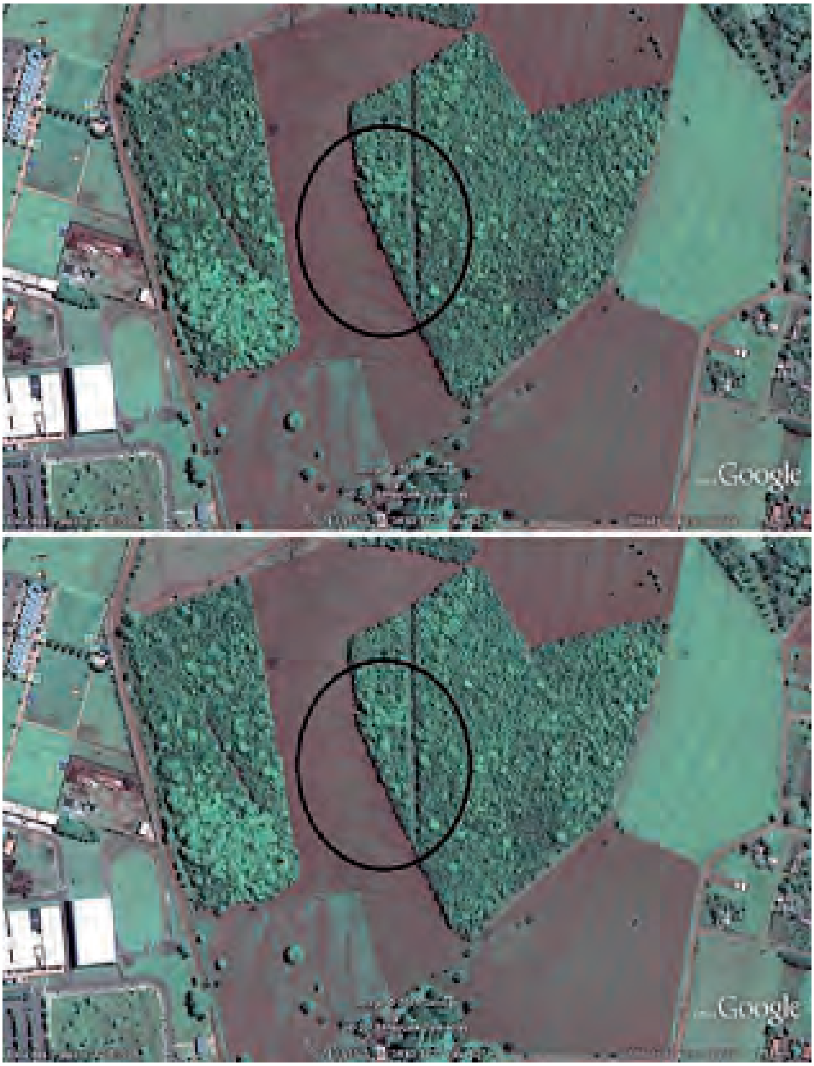

Figure 1. Aerial photo of experimental area, the Cerrado fragment area, and pastureland area (*Brachiaria decumbens* grass) – São José Farm, Universidade Católica Dom Bosco, (UCDB) Campo Grande, MS, Brazil, October to November, 2010.

The meteorological data were provided by the weather station at the Farm School of Dom Bosco Catholic University – UCDB. The temperature varied from 13.3ºC to 34.8ºC; total precipitation varied from 12.4 to 51.6 mm, with higher rainfall in the third and fourth weeks; and wind speed varied only slightly, from 7.0 to 8.83 km/h. The soil type is Neosol and the climate is humid tropical (Embrapa, 1999), characterized by a very marked dry season in winter and rainy season in summer, with an annual average temperature of 23ºC (Table 1).

Table 1. Weekly weather data: average temperature, total precipitation and average wind speed in the experimental area, October to November, 2010, Campo Grande, MS, Brazil.

Sampling Day	Temperature(ºC) (max-min)	Total Precipitation (mm) (max-min)	Average Wind Speed (km/h) (max)
04 Oct 10	21.98 (34.2-13.9)	12.4 (12.2-0.0)	7.01 (40.9)
11 Oct 10	22.88 (33.9-16.6)	3.8 (2.0-0.0)	8.34 (41.8)
18 Oct 10	24.40 (34.8-13.9)	51.6 (54.4-0)	7.0 (38.6)
25 Oct 10	24.58 (34.8-13.9)	51.6 (50.4-0.0)	7.72 (38.6)
01 Nov 10	23.13 (34.3-13.3)	43.4 (26.4-0.0)	8.83 (53.1)

Data analysis

Abundance and relative abundance were evaluated in the three areas of the Cerrado and pastureland over a five week period, with one sampling collection per week from October to November 2010.

The results were compared using richness, diversity, dominance, evenness, and constancy indices, while similarity between the environments was evaluated by calculating the coefficient of similarity.

Shannon-Wiener Diversity Index. The Shannon-Wiener Index (H ‘) was used to determine the diversity of insects collected in the traps. This index increases as the species richness increases in the area and when there is a greater distribution of individuals among all species. Multivariate analysis of Unweighted Pair Group Method Average (UPGMA) was used for this analysis, using Past Program 2.17 version.

Sorensen Index. To calculate fauna similarity, the Sorensen index was used, which is given by the formula Ss = 2a / 2a + b + c, where “a” corresponds to the total of species common to both sites, “b”, to the total of species found only in area “1”, “c”, total of species registered only in area “2”. Multivariate analysis of Unweighted Pair Group Method Average (UPGMA) was used for this analysis, using Past Program 2.17 version.

Relative Frequency or Abundance. This index represented the percentage of the number of superfamilies in relation to the total number of individuals collected, according to the formula below (Silveira Neto et al., 1976):

$F = n / N \times 100$
Where, F = percentage of frequency;
N = number of individuals from each superfamily;
N = total number of individuals collected.
* The relative frequency or abundance of the samples was calculated using Excel 2007 Program.

Margalef Wealth Diversity Index. Morphospecies richness refers to numerical abundance of a given geographic area, region, or community. The measure used to define species richness in the study was Margalef index, given by:

Where S is the number of species recorded and N is the total number of individuals.

Classification

Taxonomy. The material was identified using Hanson and Gauld's (2006) identification keys for Neotropical Hymenoptera. The material under study is deposited in the Parasitoid Hymenoptera collection of Department of Ecology and Evolutionary Biology of Federal University of São Carlos (DCBU), São Carlos, SP, Biological Institute (IB) in Campinas, SP, and Museum of Zoology of University of São Paulo (MZUSP) in São Paulo, SP, Brazil.

Results and Discussion

A total of 3,445 specimens of Hymenoptera were collected, except Formicidae, belonging to nine superfamilies. Vespoidea were the most abundant (929 specimens, 27% of the total), followed by Chrysidoidea (798 specimens, 23.2%), Apoidea Apiformes (618 specimens, 17.9%), Ichneumonoidea (417 specimens, 12.1%), and Chalcidoidea (346 specimens, 10% of the total). The least abundant specimens were Ceraphronoidea, which occurred in only four samples, followed by Cynipoidea, in six samples, and Evanioidea in eight samples (Table 2).

Table 2. Frequency of occurrence of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps inside a fragment of the Cerrado and its surroundings in Campo Grande, MS, Brazil, October to November, 2010.

Superfamily	Number of captured insects		Number of samples in which they were present	
	Frequency	(%)	Frequency	(%)
Apoidea Apiformes	618	17.9	30	100.0
Apoidea Spheciformes	212	6.2	25	83.3
Ceraphronoidea	4	0.1	4	13.3
Chalcidoidea	346	10.0	29	96.7
Chrysidoidea	798	23.2	28	93.3
Cynipoidea	8	0.2	6	20.0
Evanioidea	48	1.4	8	26.7
Ichneumonoidea	417	12.1	30	100.0
Platygastroidea	41	1.2	18	60.0
Proctotrupoidea	24	0.7	12	40.0
Vespoidea	929	27.0	30	100.0
Total of insects	3,445			

A comparison of the groups occurrence in the two physiognomies revealed that a larger number of Hymenoptera (2004) were captured in the Cerrado fragment than in the pastureland (1441) (Table 3).

Table 3. Frequency of specimens of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in two physiognomies – pastureland and the Cerrado – in Campo Grande, MS, Brazil, October to November, 2010.

Superfamily	Past		Cer	
	Frequency	(%)	Frequency	(%)
Apoidea Apiformes	224	15.5	394	19.7
Apoidea Spheciformes	124	8.6	88	4.4
Ceraphronoidea	1	0.1	3	0.1
Chalcidoidea	171	11.9	175	8.7
Chrysidoidea	188	13.0	610	30.4
Cynipoidea	4	0.3	4	0.2
Evanioidea	7	0.5	41	2.0
Ichneumonoidea	209	14.5	208	10.4
Platygastroidea	19	1.3	22	1.1
Proctotrupoidea	17	1.2	7	0.3
Vespoidea	477	33.1	452	22.6
Total insects	1441		2004	
Number of superfamilies	11		11	

*Past = Pastureland area
**Cer = Cerrado area

Almost all species were flowering, especially the arboreal in the border of the Cerrado fragment. It is common to find blooming species at the edge of fragments, because often, due to changes in soil, light and humidity conditions, there is an increase in abundance of pioneer heliophyte species. One of the main characteristics of pioneer species is the great investment in reproduction, either by prolongation of the flowering period or by repeated reproductive investments.

Vespoidea, especially Vespidae, occurred in all samples collected in the two physiognomies (477 in the open area and 452 in the Cerrado fragment). Vespoidea are of special interest for conservation because they are considered predominant predators in terrestrial ecosystems, controlling a variety of insect populations. Their structure, physiology and behavior vary widely (Lasalle 1993).

Vespidae, which are widely diversified and cosmopolitan, comprises both solitary and social species. Food for their larvae generally consists of insects captured by the female, mostly larvae of Lepidoptera and Coleoptera, and sugary substances (nectar and Hemiptera excreta), and even fragments of meat from vertebrates in the case of some social species (Triplehorn and Jonnson 2011). A greater predominance of Vespoidea in a forest fragment was observed by Pereira and Penteado-Dias (2006).

Chrysidoidea were collected mostly within the Cerrado fragment (610 individuals, 30% of the total), while 188 individuals (13% of the total) were captured in the pastureland area (Table 3). Chrysidoidea, comprising 7,000 described species, includes seven families occurring in Brazil (Rafael et al., 2012). Chrysididae are small and easily recognized by their sizes and colors, similar to some chalcids and bees. However, little is known about the biology of these species in Brazil (Kimsey and Bohardt, 1990). The family Bethylidae comprises about 2,400 species, which are known parasitoids of Coleoptera and Lepidoptera. Many species of Bethylidae are potentially useful in biological control programs because they use agricultural pests as hosts. Dryinidae family contains more than 1,600 species which are parasitoids of Auchenorrhyncha (Homoptera). Embolemidae includes 54 described species, of little known biology, some described as parasites of Achilidae (Hemiptera). The family Sclerogibbidae has 23 described species, which are parasitoids of Embioptera. Scolebythidae comprises 12 species, most of them fossils, and biological data suggest that they are parasitoids of Cerambycidae larvae (Azevedo and Olmi 2010).

The apiform Apoidea were mostly native bees, more common in the Cerrado fragment, where 394 individuals (19.7% of the total) were captured, while 224 individuals (15.5% of the total) were caught in the open areas. Unlike the findings of other researches in Brazil, which considered them constant and abundant, no specimens of *Apis mellifera* were collected in this study (Aoki et al. 2012; Krug 2007; D'avila and Marchini 2008).

Ichneumonoidea presented similar species abundance in pastureland and the Cerrado, i.e., 209 (14.5%) and 208 (10.4%), respectively. They include Braconidae and Ichneumonidae, constituting a very large and important group of about 150,000 described species (Belshaw et al. 1998), whose members are parasitoids of other insects or arthropods. In the adult phase they feed on plant fluids (nectar) (Jervis et al. 1993). Braconidae comprises about 14,890 described species distributed across the various regions of the world (Sharkey 1993; Wharton 1997), while Ichneumonidae comprises more than 3,300 described species in North America (Triplehorn and Johnson 2011), and 17,000 in the tropical region, but it is estimated that only 10% of them are known (Hanson and Gauld, 2006). Their hosts include species of Lepidoptera, Hymenoptera, Diptera, Coleoptera, Neuroptera and Mecoptera, as well as adult spiders and eggsacs. Most are solitary, and some are hyperparasitoids (Triplehorn and Johnson 2011). Along with Chrysidoidea, they were also abundant in *Eucalyptus* understory, captured in Malaise traps by Pereira and Penteado-Dias (2006).

However, Fonseca et al. (2005) studying the family Asteraceae, in the Cerrado areas, observed that the abundance of parasitoids and predators was higher in open areas, with more abundant luminosity, favoring the growth and productivity of these plants and thus offering more resources for herbivores, which are hosts of the parasitoids and predators. Not only can climatic factors cause changes in insect abundance, but also variations in the quality and availability of food resources. Also, Townes (1972) noted that because they are larger insects, Ichneumnoidea often require water and only occur efficiently in places where this resource is available.

The Cerrado fragment where the research was carried out was rich in plant species, and almost all of them were in the flowering phase, especially the trees of the border. In this area there are some watercourses near the fragments. According to Lenzi et al. (2003), some plant species are more visited because of their resources, especially during the flowering period and the high number of inflorescences, facilitating access to parasitoid and predatory wasps.

Of the superfamily Chalcidoidea, fifth in abundance, 171 individuals (11.9%) were captured in pastureland and 175 individuals (8.7%) in the Cerrado. Chalcidoidea constitutes a large and important group of insects (Triplehorn and Johnson, 2011). According to Noyes (2009), this group is one of the richest among the Hymenoptera, comprising about 2,200 described species in North America and 7,400 species in the Neotropics (Hanson and Gauld, 2006). They are often overlooked because of their very small size, some less than 0.5 mm in length (Rafael et al. 2012). Most of them are parasitoids of other immature insects, attacking mainly the egg and larval stages of the host, but are rarely predators (Noyes 2009). These hosts belong mostly to the orders Lepidoptera, Diptera, Coleoptera and Hemiptera. There are some phytophagous species whose larvae feed inside galls, seeds or stems (GIBSON 1993). Silva (2009) reported that they were the most abundant in a sample collection carried out in the Caatinga, using Moericke traps.

Spheciform Apoidea constituted the sixth group in number of individuals, although differing in the two physiognomies, i.e., 124 (8.6%) in pastureland and 88 (4.4%) in the Cerrado. These are large solitary predatory wasps that build nests dug in the ground or suspended. Their prey includes larvae of spiders and insects of the orders Blattaria, Mantodea, Orthoptera and Lepidoptera (Rafael et al. 2012).

The fewest individuals captured belonged to the following groups: Ceraphronoidea (four specimens), Cynipoidea (eight specimens), Proctotrupoidea (24 specimens) and Platygastroidea (48 specimens).

Cynipoidea constitutes one of the most abundant groups of Hymenoptera (Ronquist, 1995), including five families whose representatives have phytophagous habits or behave as primary and secondary parasitoids of other insects. According to Buffington (2006), the majority of these insects is small, consisting of gall-inducing species or tenants of galls (in rare cases, both), which mostly attack oaks or Rosaceae.

Platygastroidea comprises two families, Platygastridae and Scelionidae, which are parasitoids of a wide variety of insects and spiders (AUSTIN et al. 2004). Platygastridae are minute black wasps, usually shiny, and most of them are parasitoids of Cecidomyiidae larvae (Diptera) (Triplehorn and Johnson, 2011). Scelionidae are small parasitoid insects that feed on eggs of spiders and insects of the orders Orthoptera, Mantodea, Hemiptera, Coleoptera, Diptera, Lepidoptera and Neuroptera. Some species are used successfully in agricultural pest control (Rafael et al. 2012).

Ceraphronoidea includes two families with cosmopolitan distribution. They are tiny parasitoids from 1 to 3 mm long or smaller. With approximately 800 species around the world, the family Ceraphronidae is a group with little data available regarding its hosts and ecological interactions. However, it has a very large number of hosts of the orders Diptera, Hymenoptera, Thysanoptera, Hemiptera and Neuroptera (Masner 2006a).

Proctotrupoidea are parasitoids of immature stages of insects and other arthropods (Chilopoda, Diplopoda and Arachnida) (Loiácono and Margaría 2002). According to Triplehorn and Johnson (2011), they are approximately 3 to 6 mm long, solitary or gregarious parasitoids of larvae of Coleoptera and Diptera.

Evanioidea, whose metasoma is attached well above the hind coxa, includes species that parasitize cockroach oothecae and are likely to be found in forest soils where there are cockroaches (Triplehorn and Johnson 2011).

These findings confirm the importance of conserving areas of natural vegetation for the preservation of parasitoid and pollinating insects, especially Chrysidoidea (as shown in Table 3). The fragmentation of natural areas can alter the composition of pollinators and consequently the quality of pollination, according to Aizen and Feinsinger (1994).

A comparison of the indices of Margalef richness, Shannon-Wiener diversity, Evenness and Dominance between the two areas of study (Table 4) indicated that pastureland showed higher values, although similar, with richness of 3.17 and 3.03, diversity of 0.71 and 0.69, and dominance of 0.71 and 0.69, respectively, for pastureland and the Cerrado fragment.

Table 4. Indices of richness, diversity, evenness and dominance of insects of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in a Cerrado fragment and pastureland in Campo Grande, MS, Brazil, October to November 2010.

Index	Pastureland	Cerrado
Margalef richness	3.17	3.03
Shannon-Wiener diversity	0.79	0.77
Pielou evenness	0.76	0.74
Dominance	0.19	0.20

The similarity between the environments was relatively high, presenting values consistently higher than 0.71 and up to 0.89, especially among samples from the Cerrado fragment (Table 5).

Table 5. Coefficient of similarity among the Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in three areas in each of the two physiognomies, pastureland and the Cerrado fragment, in Campo Grande, MS, Brazil, October to November, 2010.

	Coefficient of similarity					
	A1 Past	A2 Past	A3 Past	A1 Cer	A2 Cer	A3 Cer
A1 Past	0.71	**0.87**	0.78	0.75	0.82	0.85
A2 Past			0.84	0.71	0.75	0.81
A1 Cer					**0.88**	0.80
A2 Cer						**0.89**

*Past = Pastureland
**Cer = Cerrado

In the comparison among the three areas of each physiognomy (Table 6), it was found that area three presented the largest number of insects captured in the two physiognomies, where specimens of all the superfamilies were found. Larger numbers were captured in area two, followed by area 3 of pastureland.

Table 6. Relative abundance (Frequency) of specimens of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in the Cerrado fragment and pastureland; Campo Grande, MS, Brazil, October to November, 2010.

Superfamilies	Relative abundance (%)					
	A1 Past	A2 Past	A3 Past	A1 Cer	A2 Cer	A3 Cer
Apoidea Apiforms	26.1	6.9	33.3	39.4	8.7	23.1
Apoidea Spheciformes	7.2	8.6	7.5	4.0	6.6	2.9
Ceraphronoidea	0.0	0.0	0.1	0.3	0.1	0.1
Chalcidoidea	9.1	16.0	8.5	6.1	9.0	9.1
Chrysidoidea	17.6	17.5	4.6	28.1	35.5	26.8
Cynipoidea	0.0	0.5	0.3	0.3	0.3	0.1
Evanioidea	1.5	0.0	0.0	0.0	3.1	2.0
Ichneumonoidea	14.8	13.6	11.5	9.5	11.4	9.7
Platygastroidea	0.9	1.4	1.2	0.3	2.2	0.6
Proctotrupoidea	0.4	1.4	1.2	0.9	0.6	0.0
Vespoidea	22.4	34.0	31.7	11.0	22.6	25.6
Total insects	369	518	726	318	871	845

*Past = Pastureland
**Cer = Cerrado

As for the distribution of the most abundant superfamilies in the three areas, Vespoidea and Ichneumonoidea were captured in all traps with minor variations, regardless of the physiognomy. However, in the case of Chrysidoidea, the largest number of specimens was always collected in the Cerrado fragment. As for Chalcidoidea, the fourth group in occurrence, which ranged from six to 16, also showed little variation among the physiognomies and areas.

As for apiform and spheciform Apoidea, the three areas ofpastureland and within the fragment differed, and did not show a pattern of occurrence, although the numbers of spheciform Apoidea were consistently lower.

Among the groups of lower occurrence, it was noted that Evanioidea, Cynipoidea and Ceraphronoidea were not captured in all collection points of pastureland, but the latter two groups were captured in all collection points of the Cerrado fragment. Platygastroidea and Proctotrupoidea, despite the low numbers, were found in all collection points in the two physiognomies.

As for species richness (Table 7), Ichneumonoidea presented the highest species richness in both pastureland and the Cerrado areas, followed by Chrysidoidea, which appeared in most of the sample collections. Braconidae and Ichneumonidae (Ichneumonoidea) are rich in number of species, especially in the Neotropics, where they can parasitize species of agricultural pests (Hanson and Gauld, 2006). The superfamily Chalcidoidea, another important group for agricultural pest control, varied significantly in richness in the Cerrado fragment, i.e., from 1.26 to 9.65, but less so in the pastureland, i.e., 3.4 to 5.2.

Table 7. Margalef richness index of morphospecies of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in the Cerrado fragment and pastureland; Campo Grande, MS, Brazil, October to November 2010.

Superfamily	Area 1		Area 2		Area 3	
	Past*	Cer**	Past	Cer	Past	Cer
Chrysidoidea	4.53	3.37	3.58	5.24	1.47	5.66
Ichneumonoidea	8.31	4.22	3.94	6.64	6.63	5.66
Chalcidoidea	3.40	1.26	5.02	6.29	4.79	9.65
Proctotrupoidea	0.38	0.42	0.71	0.35	1.10	-0.33
Platygastroidea	0.38	0	0.71	2.10	1.10	0.33
Cynipoidea	-0.38	0	0	0.35	0.37	0
Ceraphronoidea	-0.38	0	-0.36	0	0	-0.33
Evanioidea	0.38	-0.42	-0.36	0	-0.37	0.33
Vespoidea	3.02	3.37	4.30	5.60	7.74	6.99
Apoidea Spheciformes	3.40	1.26	3.58	3.50	4.42	2.33
Apoidea Apiformes	3.02	1.26	2.51	2.10	2.21	2.33

*Past = Pastureland
**Cer = Cerrado

Spheciform and apiform Apoidea presented variable richness in pastureland and the Cerrado, although with a tendency for lower values in the Cerrado fragment, especially for Spheciformes. Pollination by bees is particularly important and it is estimated that they constitute 40% of the pollinators (Lima and Rocha, 2012). It is, therefore, of utmost importance to study their species richness in natural, fragmented or agricultural environments.

Vespoidea were always abundant, but presented variable richness in each of the areas of the two physiognomies, particularly in the areas number 3 of pastureland and the Cerrado (7.4 and 6.99, respectively).

The groups with lower occurrences, such as Proctotrupoidea, Platygastroidea, Evanioidea, Ceraphronoidea and Cynipoidea, presented low species richness in most of the sample collections, and were absent in some of the samples from the Cerrado or pastureland.

Vespoidea, Chrysidoidea, Chalcidoidea and Ichneumonoidea were the most abundant and constant superfamilies in the two physiognomies and in all sample collections, which makes them the best indicators of the occurrence of parasitoid insects and predators in environments. The variations in number and richness of insect species collected during the five-week period between the areas and physiognomies confirm the need for repetitions.

Conclusions

The different groups of Hymenoptera (except Formicidae) prefer areas of natural vegetation rather than degraded areas. There are great richness of Hymenoptera parasitoids, predators and pollinators in the Cerrado fragment and its surroundings in Campo Grande, MS. Malaise traps capture mainly species with highly developed flying ability. The most abundant superfamilies were Vespoidea, Chrysidoidea, Ichneumonoidea and Chalcidoidea.

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Effects of insecticide
of Brazilian cashew nuts liquid (CNSL) on *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae)

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Introduction

Aedes aegypti (Linnaeus, 1762) (Diptera: Culicidae) is one of the greatest health issues Brazil. It is the vector for virus infections such as dengue fever, Chikungunya and the Zika Virus (Resendes et al. 2010; Magalhães and Machado 2014, MS 2015).

Failures in control strategies and insecticide resistance make the need for ecological, systematic and integrated strategies relevant. In addition to the lessening the exclusive use of insecticides, social practices should also be associated with environmental and health surveillance.

Among the general population, the elimination of larval breeding is an effective vector-control action, although the predominance is the proposition according to which control should be directed to the adult mosquito. This hinders the success of control strategies. Plants produce secondary substances of defense against herbivores that can be used as insecticides in the form of oils, extracts or their by-products. Plant-based insecticides are still economically viable for they are accessible and renewable resources. Due to the complexity of their molecules, the development of populations resistant to them is a low probability.

This frequently promotes a higher security to the environment and to the health of animals and humans due to photo and thermal characteristics (Isman 2006). This is an environmental advantage, but also a disadvantage because the insecticide loses its efficiency more quickly (Kalaivani et al. 2012). However, studies show that insecticides made from plants exert effects on other development stages, not always exerting an immediate toxic effect (Dourado et al. 2015). Such insecticides may act in many ways, such as repellents, causing mortality at later stages of development, deformities, impaired reproduction or egg viability, decreased sexual encounters or reduced virus transmission (Correa and Salgado 2011).

Although they have some advantages already known and discussed by the literature, it is important to note that plant extracts or their isolated assets, when applied individually, may not reestablish an ecological balance. A stable ecological system involves several aspects, for example, type of active substance and the way it should be applied and incorporated into the environment (Brechelt 2004).

The best way to turn an isolated substance or asset into a technical product is by creating a formulation that will be used suitably. Such process occurs by mixing the target compound with inert products, solid or liquid, and sometimes an adjuvant product. Among materials available in Brazil used as inert agents for solid formulations, there are asbestos, apatite, sand, calcined clay, attapulgite, bentonite, calcite, kaolin, sulfur, talc, among others (Funasa 2001).

Formulations may be liquid or dry. Dry formulations may be dry powder (P), wettable powder (PM) or soluble powder (PS). Dry powder can be used as a ready product direct from the factory by sprinkling. The wettable powder must contain a wetting agent to make it soluble. The soluble powder is the ideal formulation since it forms a perfect water-soluble blend. The preparation of a powder botanical insecticide ensures an effective control and a prolonged use of assets at quantifiable volumes, guaranteeing a dose-response by target organisms and the environment (Feltri and Chorilli 2010).

Solutions based on cashew nut liquid (CNSL) provide efficient insecticides against *A. aegypti* larvae (Porto et al. 2013). The product is obtained during the processing of cashew nuts, *Anacardium occidentale* (Anacardiaceae), an export product rich in anacardic acid, its main compound (Correia et al. 2006). It has a structural similarity to salicylic acid and cardols derived from resorcinol. It is a representative of cardanol, cardol and ethyl-cardol (Agostini-Costa et al. 2000). However, CNSL is caustic and flammable, representing a danger to the applicator. It also presents solubility at varying levels according to extraction method (Stasiuk and Kozubek 2010).

Thus, seeking an easier application method, a chemically stable and a simplified handling for dilution powder formulations were prepared containing different proportions of CNSL related to an inert matrix harmless to the environment.

The product containing 10% of CNSL and its dosages was evaluated as for the development, mortality and reproduction of *A. aegypti*, determining its degradation profiles in wastewater by anacardic acid quantification in laboratory conditions.

Materials and methods

The experiment was developed in the laboratory of Entomology of the Catholic University of Dom Bosco. The liquid commercial product of cashew nut, CNSL (Cashol®), was produced using the specimen cashew *A. occidentale* (cashew) provided by the company Resibras, located in the city of São Paulo, SP.

Formulation of the insecticide

The Cashol® commercial product (Lot A044-11) was dispersed into a water:ethanol solution and added to a solid matrix based on corn starch at proportions of 10, 15 and 20% (w/w) of CNSL. The powder formulated products were sieved using a 150 µm sieve (Tyler®).

Breeding of A. aegypti

Eggs were obtained from a colony kept in laboratory conditions. Egg hatching was carried out in dechlorinated water, stabilized with sodium bicarbonate ($NaHCO_3$) with a pH between 6.5 and 7.0. The establishment of the mass colony of *A. aegypti* larvae and the food supply followed procedures established by the literature (Laranja et al. 2006; Serpa et al. 2008).

Bioassay

Based on the results of previous experiments with powder-formulated products containing 10, 15 and 20% CNSL, we decided to formulate designated product using CNSL 10%, with which a biological cycle of *A. aegypti* could be observed.

The bioassay was performed for each experimental unit containing 25 third instar larvae of *A. aegypti* using an aqueous medium with the proportion 1:1. The solutions of 10% products were diluted in dechlorinated water at concentrations of 1,000, 500 and 250 µg mL^{-1}. There were four experimental units per concentration, followed by a positive control with Rotenona® (Sigma-Aldrich) at concentrations ranging from 0.02 to 20.9 µg mL^{-1}, and a negative control only with dechlorinated water.

After the experimental procedure began in periods of 24 hours, each unit was monitored as for larval duration, larvae and pupae mortality and number of adults emerged. Chronic assays were performed at concentrations of 250, 500 and 1,000 µg mL^{-1} to observe interferences from concentrations below the LC_{50} of acute tests on lethality or other deleterious effects on larvae and pupae. The larvae were separated into groups of 25 individuals in the same larva/mL ratio as the acute test, with four replications per treatment. The experimental design was completely randomized, containing 100 larvae in each treatment.

The mortality data for the 24-hour period, aiming to calculate the lethal concentration (LC), were evaluated using the Probit (McLaughlin 1991) method and the software Leora® (POLO 97355947870655352) to determine the lethal concentrations with a 95% confidence significance interval and the EC_{50} (µg mL^{-1}). The means for larval duration were analyzed by Tukey test ($p<0.05$). Larval and pupal mortality was transformed into percentage. Data of number of adults in real numbers.

Chemical analysis of CNSL components of wastewater after 30 days and LC of A. aegypti

The spectrophotometric UV-VIS (Thermo Spectronic, Aquamate, 320 nm) was used to quantify CNSL and its major component, anacardic acid, by evaluating the stability of the formulated product 30 days after exposure in laboratory conditions.

The analysis was performed by scanning between 200 and 700 nm. The degradation pattern was defined by anacardic acid concentrations ranging from 0.02 to 0.1 mg mL^{-1}, with three replications per concentration to prove the presence of both CNSL and formulations, as shown by the following equations:

1st day: $y= 0.016x - 0.147$, $r^2=0.944$
30th day: $y= 0.0177x - 0.5439$, $r^2=0.993$

Peaks obtained by UV-VIS were submitted to multivariate analysis of variance to obtain the values of P and F using the Asistat 7.7 Beta software. Production data were subjected to analysis of variance (ANOVA) and, when significant, Tukey test at 5% probability.

The product 10% and water solutions were stored at room temperature (Equitherm® thermohydrogen, temperature 23.8 ± 1.04 °C and relative humidity of 43.27 ± 9.76).

The product was analyzed by high performance liquid chromatography (HPLC, Model: Varian 210®) using a diode arrangement detector (DAD) and the software Star WS (Workstation 2.0). The column was the C-18 reverse phase (25 cm x 4.6 mm x 5 µm). Elution was performed using an acetonitrile/water/acetic acid gradient system (62/37/1 v:v:v) (A) and tetrahydrofuran (B), which eluted 5% B in 30 minutes, up to 100% B, remaining up to 35 minutes at this ratio and returning to the initial condition in 40 minutes. The flow rate of the pump was 1 mL min^{-1} and the volume injected was 20 µL. The analysis was conducted at 22ºC, and injections were performed in triplicate.

The comparison curves used to quantify anacardic acid and CNSL at the tested concentrations were prepared using solutions containing commercial anacardic acid and a Cashol® product available from Resibras.

The HPLC analysis used the same products. However, after dilution in the solvent system, the solutions were filtered using a quantitative filter paper with a 20-25 µm particle retention, thickness of 0.19 mm and weight of 80 g/m^2, and later a 0.45 µm membrane, diluted in concentrations between 0.1 and 10 µg mL^{-1} in triplicate using the external standard method.

Results and discussion

CNSL is characterized as a compound rich in phenolic lipids. It presents difficulties of solubilization at concentrations above 1%, which led to the need to transform it into a powder formulation due to its already effective insecticidal action.

The use of solid inert materials to bind insecticidal substances favors the action due to its electrostatic charge, which makes it adhere to the epicuticle, leading to dehydration. When in solutions, it serves as a matrix for a slow dissolution of active substances, suffering less interference from relative air humidity, temperature and radiation (Lorini et al., 2003; Martins and Oliveira, 2008).

LC_{50} values < 1,000 µg mL^{-1} (Oliveira et al. 2011) are regarded as toxic for oils and plant extracts. The effective lethal concentration (LC) for 10%, 15% and 20% formulations containing cashew nut liquid (CNLS) (Table 1).

Table 1. Lethal concentrations (LC) of formulated powdered products containing cashew nut liquid (CNLS) against *Aedes aegypti* larvae after a 24 h exposure and a 12/12 h photoperiod.

Sample	Lethal Concentration(CL)	μg mL^{-1}	Lethality range
	CL_{10}	588.0	495.0 – 658.0
10%	CL_{50}	1,037.0	930.0 – 1,165.0
	CL_{90}	1,769.0	1,468.0 – 2, 435.0
	CL_{10}	205.371	84.2 – 297.2
15%	CL_{50}	1,071.31	800.9 – 1991.4
	CL_{90}	5,615.783	2,670,0 – 8,807.6
	CL_{10}	224.456	148.0 – 275.6
20%	CL_{50}	802.492	681.1–1,008.9
	CL_{90}	2,896.134	1,941.7 – 5,710.7

CL: 95% Confidence level

In this study, it is possible to observe that the established lethality range for CE (50) between 930 and 1,165 μg μL^{-1} meets the criteria of low toxicity. It has not yet been correlated with biodegradation factors only regarding the formulation 10% CNSL. The results for Lethal Concentration (LC_{50}) (Tables 2, 3 and 4) indicated that the product containing 10% CNSL showed a better effectiveness, with mortality rates higher than the others, even at the lowest dose.

Table 2. Insecticidal action of powder based on 10% Brazilian cashew nuts liquid (CNSL) with 10%, CNSL against *Aedes aegypti* (Culicidae).

Powder-based formulation with 10% CNSL			
Instar	Concentration (μg mL^{-1})	Mortality^{1,2} (%)	CE(50)^{3} (μg mL^{-1})
Larvae	1,000	77	1,037.0 (930.0-1165.0)
	500	5	
	250	8	
Pupae	1,000	4	
	500	15	
	250	13	
Adults	1,000	100	
	500	65	
	250	56	

[1] Values are means of quadruplicates.

[2] Percentage of death in total population.

[3] CE_{50} (95% Confidence interval)

Therefore, the product 10% was used for studies on stability and chemical degradation, considering as reference the dose concentration of 1,000 μg L^{-1} as the other formulations. After 24 hours of exposure to 10% CNSL, a lethal concentration has EC_{50} values of 1,037.66 μg mL^{-1}, and limits action between 927 and 1,165 μg mL^{-1} (Figure 1).

Table 3. Insecticidal action of powder based on 10% Brazilian cashew nuts liquid (CNSL) 15% CNSL against *Aedes aegypti* (Culicidae).

Powder-based formulation with 15% CNSL			
Instar	Concentration ($\mu g\ mL^{-1}$)	Mortality^{1,2} (%)	CE(50)^{3} ($\mu g\ mL^{-1}$)
Larvae	1,000	58	1,071.3 (800.9-1,991.4)
	500	51	
	250	6	
Pupae	1,000	0	
	500	8	
	250	6	
Adults	1,000	0	
	500	0	
	250	0	

[1] Values are means of quadruplicates.
[2] Percentage of death in total population.
[3] CE_{50} (95% Confidence interval)

Table 4. Insecticidal action of powder based on 10% Brazilian cashew nuts liquid (CNSL) with 20% CNSL against *Aedes aegypti* (Culicidae).

Powder-based formulation with 20% CNSL L			
Instar	Concentration ($\mu g\ mL^{-1}$)	Mortality^{1,2} (%)	CE(50)^{3} ($\mu g\ mL^{-1}$)
Larvae	1,000	48	802.4 (681.1-1,008.9)
	500	41	
	250	6	
Pupae	1,000	1	
	500	8	
	250	6	
Adults	1,000	0	
	500	0	
	250	0	

[1] Values are means of quadruplicates.
[2] Percentage of death in total population.
[3] CE_{50} (95% Confidence interval)

The effects of the product formulation 10% CNSL was monitored throughout the insect development up to the adult formation at concentrations of 1,000, 500, 250 mg L^{-1} (Table 5). There were effects of these concentrations on the development and mortality of *A. aegypti*.

The stretching larval stage evidences the deleterious effects caused to development. This change is one of the effects of plant insecticides reported by several authors, who report lethal effects on antifeedant, reproduction, metamorphosis and development, morphological abnormalities, among others (Monssini and Kemmelmeier 2005;

Martinez and Meneguim 2009; Roel et al. 2010). There is deleterious effects on development, such as pupal mortality, and effects on the reproductive capacity.

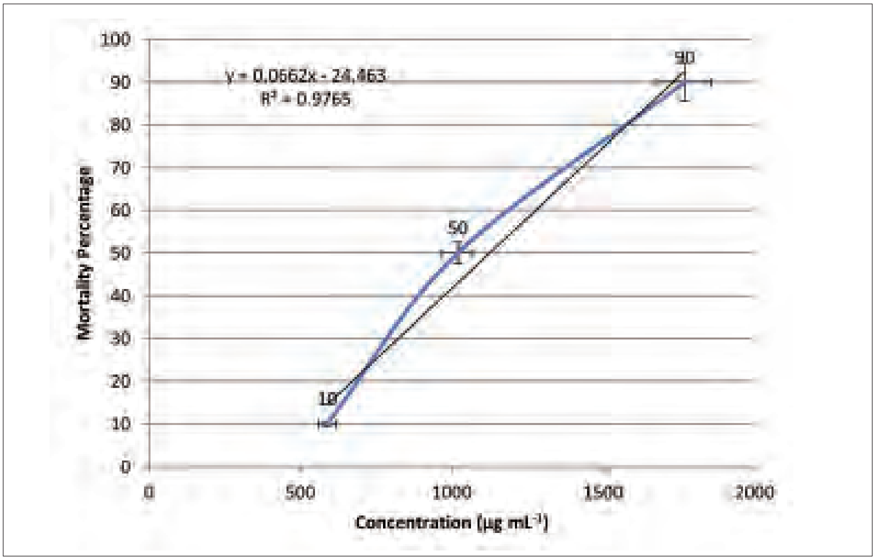

Figure 1. Effective Lethal Concentration (CLE) of formulated powder product containing 10% Cashew Liquid (CNSL) against *Aedes aegypti* larvae after 24 h of exposure.

Table 5. Effects of powder formulation doses containing 10% Brazilian cashew nut liquid (CNSL) on development and larval and pupal mortality of *Aedes aegypti* L. (Dip.: Culicidae).

Concentrations (µg mL^{-1})	Larvae development (Hours)	Mortality (%)	(%)	Adults emerged
		larval	Pupal	
1,000	168.0^{b}± 0.0	77	4,0	19
500	145.2^{a}± 8.84	5	15	65
250	144.9^{a}± 6.54	8	13	56
Control	144^{a}± 0.0	1	4	85
CV%	5.8	119.23	55.5	33.3

Values followed by the same letter do not differ by Tukey test ($p<0.05$)

Regarding larval mortality in the control group, 99 larvae survived and became pupae. Among the pupae formed, only 4% died, and 85 adults emerged at the end of the cycle. The doses 500 and 250 mg L^{-1} caused a low larval and pupal mortality, i.e., 5 and 8% for larvae and 15 and 13% for pupae, respectively. However, the observed larval and pupal mortality caused by the highest dose (1,000 mg L^{-1}) was 4 and 77%, respectively; only 19 adults emerged. The results indicate that the formulation of 10% CNSL containing a 10% dilution of 1,000 mg L^{-1} is considered effective as an insecticide, as less than 20% of larvae formed adults at the end of the cycle, i.e., the efficiency was higher than 80%.

Consoli et al. (1989) reported that, in laboratory conditions, the control of *Aedes fluviatilis* by plant extracts and anacardic acid resulted in a repellent action and inhibited the oviposition using a concentration of 10 ppm. Mukhopadhyaya et al. (2010) reported an insecticidal activity of CNSL against *A. aegypti* larvae using concentrations of 12, 19 and 38.0 ppm. The substance was effective, presenting a mortality rate of 50% for larvae and 84.6% for pupae.

The residual power of an insecticide is an advantage in terms of control, but causes accumulation and harmful effects on the environment. This has led to many cases of resistance by the target insect and to accumulation in the environment. Biodegradability is an important aspect for the evaluation and selection of insecticides in addition to scope of action and source (Viegas Jr. 2003).

The product CNSL 10%, when compared to standard anacardic acid, showed a significant reduction in the initial concentration within 30 days. This indicates that the product, under temperature and relative humidity, degraded. This also occurred with contents evaluated in wastewater (Table 6). The degradation of the product within 30 days makes it suitable for bioaccumulation and avoid undesirable effects on non-target organisms. Although a degradation of product stability was observed, it was sufficient to exert its larvicidal effects effectively and control the biological mosquito cycle.

Table 6. Variation in anacardic acid concentration, in mg mL^{-1}, in the water of the test assays after 30 days, with a mean temperature of 23.8 ± 1.04 °C, relative humidity of 43.27 ± 9.76, and photoperiod of 12/12 h.

Dilution of powder at 10% CNSL	Day	Concentration anacardic acid (mg mL^{-1})	CV%
0.02	0	$3.8^{b} \pm 0.26$	25.62
	30	$1.3^{b} \pm 0.76$	5.58
0.05	0	$5.8^{b} \pm 0.026$	14.53
	30	$2.54^{b} \pm 0.46$	4.8
0.1	0	$9.2^{a} \pm 0.046$	1.86
	30	$5.1^{a} \pm 0.056$	6.9

* Average decrease of 65% in mg mL^{-1} compared to the initial value.
** Average decrease of 56.2% in mg mL^{-1} compared to the initial value.
*** Average decrease of 44.6% in mg mL^{-1} compared to the initial value.
Values followed by the same letter do not differ by Tukey test ($p<0.05$).

The pH of the medium was 6.8 and 7.2 for the initial and final period, respectively. These results suggest the decomposition of anacardic acid. The more acid pH is directly related to a higher content of anacardic acid in the liquid. The total content of CNSL also decreased after 30 days, and the pH ranged from 7.5 to 8.2 (Table 7).

Table 7. Variation of cashew liquid concentration (CNSL), mg mL^{-1}, in test waters after 30 days. Mean temperature of 23.8 ± 1.04 °C, relative humidity of 43.27 ± 9.76 and photoperiod of 12/12 h.

Dilution of powder at 10% CNSL	Day	CNSL Concentration (mg mL^{-1})	CV%
0.02	0	$25.0^{c}\pm0.02$	21.9
	30	$4.0^{c}\pm0.01$	9.3
0.05	0	$11.4^{b}\pm 0.06$	2.01
	30	$7.4^{b}\pm0.01$	7.1
0.1	0	$21.8^{a}\pm 0.02$	3.86
	30	$15.2^{a}\pm 0.02$	$15.2^{a}\pm 0.02$

* Average decrease of 16% in mg mL^{-1} compared to the initial value.
** Average decrease of 70% in mg mL^{-1} compared to the initial value.
Values followed by the same letter do not differ by Tukey test ($p<0.05$).

This profile remained stable even at high concentrations. The pH and dispersion results of the 10% CNSL product in wastewater are in agreement. Phenolic compounds at low concentrations are related to the formation of micelles, whereas the increase in concentration tends to increase surface tension and hinder the dispersion of compounds, slowing their degradation (Risfaheri et al. 2009).

Mazzetto et al. (2009) reported that cardanol is the main product of the degradation of anacardic acid with an average biodegradability of 96% in 28 days in freshwater and ecotoxicity in 96 hours at lethal concentrations (LC_{50}) of < 11 g L^{-1} for fish, < 66 g L^{-1} for daphnia and < 1 g L^{-1} for algae. The initial and residual concentrations of anacardic acid identified in this study were lower than the previously cited studies.

The degradation profile of the 10% formulation shows an average decrease of 78% for the highest concentration after 30 days (Table 8). The inert matrix used as a carrier did not interfere with pH. There was no turbidity of the medium or formation of precipitates during the observation period.

The degradation of the product is desirable as it avoids bioaccumulation and undesired effects on non-target organisms. Although there was degradation, the product stability was enough for larvicidal effectiveness in the biological cycle of the mosquito.

Table 8. Variation of the product concentration 10% Cashew Nut Liquid (CNSL) in wastewater of biological assays after 30 days. Mean temperature of 23.8 ± 1.04 °C, relative humidity of 43.27 ± 9.76, and photoperiod of 12/12 h.

Dilution of powder at 10% CNSL	Day	Concentration of powder at 10% CNSLmg mL^{-1}	CV%
0.125	0	$12.5^{d} \pm 0.04$	7.41
	30	$2.2^{d} \pm 0.003$	8.10
0.250	0	$25.0^{c} \pm 0.012$	9.79
	30	$4.0^{c} \pm 0.013$	17.04
0.5	0	$50.0^{b} \pm 0.035$	1.72
	30	$13.4^{b} \pm 0.002$	0.79
1.0	0	100.0 ± 0.001	0.18
	30	21.6 ± 0.034	8.4

* Average decrease of 17% in mg mL^{-1} compared to the initial value.
** Average decrease of 27% in mg mL^{-1} compared to the initial value.
*** Average decrease of 78% in mg mL^{-1} compared to the initial value.
Values followed by the same letter do not differ by Tukey test ($p<0.05$).

The results of quantification by HPLC indicated a concentration of 8.49% of anacardic acid in the product at 10%. After the 30 days period, the concentration in wastewater decreased to 0.17%. According to the laboratory conditions, not considering ambient temperature, the product degraded the content of test solutions. Figure 2 shows the chromatographic profile for anacardic acid determined by HPLC using the commercial product as the control.

The use of plant-derived products with insecticidal properties meets the objectives proposed by green chemistry for developing biodegradable, target-specific insecticides without resistance (Rajasekaran and Duraikannan 2012).

The intake of phenolic compounds has negative consequences for *A. aegypti*, including damage to epithelial cells, reduction of protein and lipid levels, and directly affects oviposition and growth of insects (Tagliari 2004). This was also observed in this study: the formulated product containing active phenolic compounds also interfered with the insect’s biology.

According Farias et al. (2011), the sodium anacardate obtained from CNSL caused infeasibility of eggs and mortality of larvae and pupae of *A. aegypti*. There were no adverse effects at dosages above 0.3 g/Kg. Guissoni et al. (2013) reinforces the use of LCC as an insecticide and its innocuousness in chronic tests in rats, using even doses of 2 g/kg of body weight. This was defined as the limit for CNSL. Such suggestions for the viability of CNSL-derived products are an alternative to fight the vector.

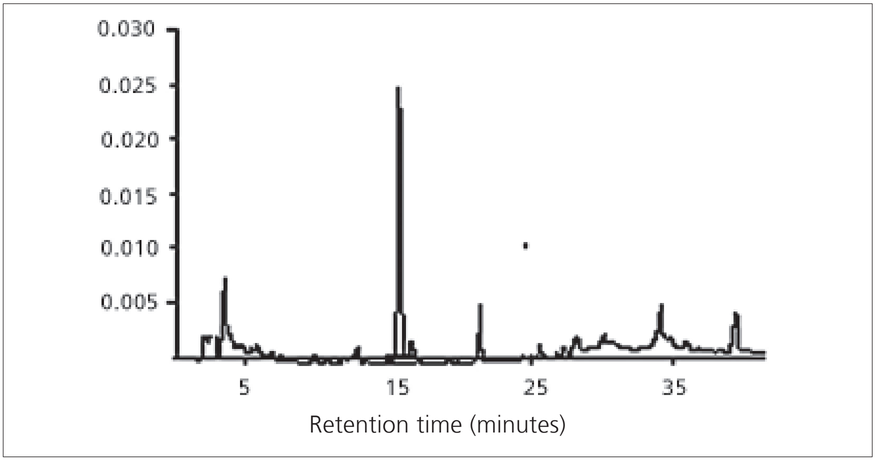

Figure 2. Chromatogram of the powder formulation and characteristic retention peaks to determine anacardic acid by HPLC, diode array detector (DAD) and C-18 reverse phase column (25cm x 4.6mm x 5ìm) using acetonitrile/water/acetic acid gradient system (62/37/1 v:v:v).

Santos et al. (2007), using wettable powder of pyrethroids on target species, reported satisfactory residual effects after three months of application in addition to a high insecticidal efficiency when compared to concentrated suspension.

The products containing 15% and 20% CNSL had the lowest insecticidal effectiveness and presented solubility problems. All solutions prepared with the 15% product showed body formation at the bottom of test containers. The product containing 20% CNSL showed the lowest value for EC_{50}: 802.49 µg L^{-1}. However, the dispersions showed phase separation, indicating inefficiency in solubilizing the embedded active product. Thus, the products formulated with high concentration of CNSL were no longer used in other experiments.

The toxic effect of different concentrations of CNSL on larvae *of Aedes aegypti* after 24 hours of treatment was also described by Dourado et al. (2015), With the objective of evaluate the insecticidal potential of anacardic acid, a CNSL component from *A. occidentalle*, upon *A. aegypti* and verify the histomorphological alterations in the larval midgut. The third instar *A. aegypti* larvae in the control group showed elongated and wormlike external appearance. The histological sections showed a normal morphology of stomodeum stomach and intestinal cecum (Figure 3: 1AB). In the middle region of low cylindrical cells, acidophilic cytoplasm with variable areas, central spherical nucleus and clear nucleolus were seen. The brush border is thicker and marked (previous) and the peritrophic matrix (PM) is evident (Figure 3,C1).

Morphological analysis in the treated group based on different concentrations of CNSL showed concentration of 0.001 mg mL^{-1} in three regions, cell shedding in the midgut, food debris and fragmentation of MP. The changes were apparent in gastric cecum, such as loss of brush border, and the absence of material ingested by the larvae in the light (Figure 3, 2A). In the middle midgut lining cells were rounded, with lots of side crossings and scaling, with a decrease in brush border and profuse secretion in the light (Figure 3, 2B).

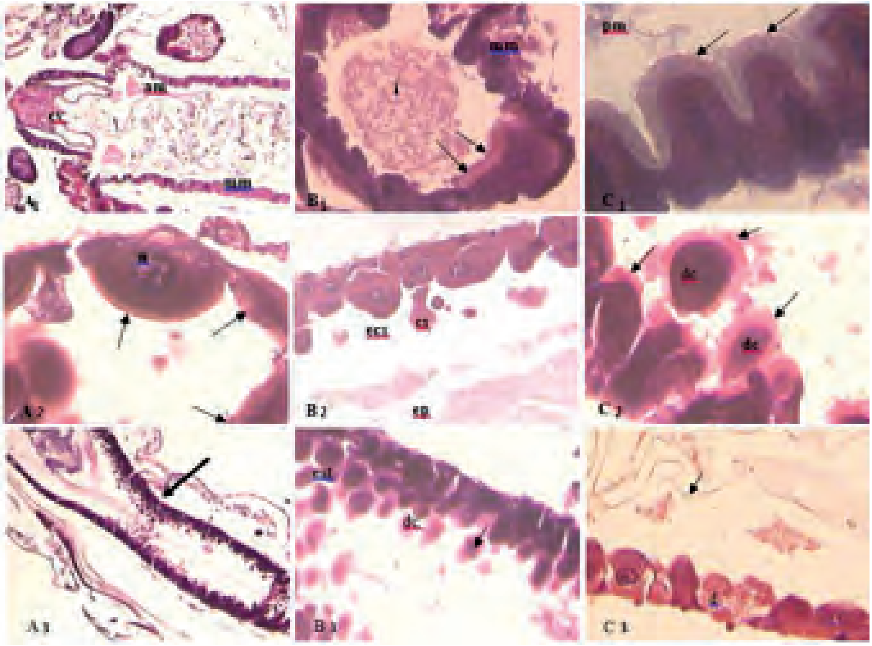

Figure 3. Photomicrography A1, B1 e C1= of 3rd instar larvae *Aedes aegypti* digestive system tissue. Control group. A) longitudinal section of gastric cecum, cardia valve (cv), anterior midgut (am), middle midgut (mm), HE. 00X. B) Transversal section of gastric cecum, ingesta (i), brush border (arrows), HE. 400X. C) longitudinal section of posterior midgut (pm) and its brush borders (arrows), HE. 400X. Photomicrography A2, B2 e C2= of 3rd instar *A. aegypti* larvae digestive system treated with *Anacardium occidentale* - Anacardic acid 0.01 mg mL^{-1}. A) Transversal section of gastric caecum, nucleus (n), brush border (arrows), HE. 400X. B) Longitudinal section of middle midgut, endoperitrophic space (ep), ectoperitrophic space (ecs), cellular secretion (cs), HE. 200x. C) Longitudinal section of posterior midgut, brush border (arrows), desquamation cell (dc), HE. 400x. deleterious changes included total or partial destruction of the lining cells, high vacuolization of the cytoplasm and increased subperitrophic space with accumulation of acidophilic materials (Figure 3BC). Photomicrography A3, B3 e C3= of Longitudinal sections from 3rd instar *A. aegypti* larvae digestive system treated with *A. occidentale* - Anacardic acid 0.1 mg mL^{-1} (A, B) and 1.0 mg mL^{-1} (C). A) Strangulation between the anterior and middle midgut (arrow). HE. 200x. B) Stratification in the median midgut (est) desquamation cell (dc). HE. 400x. C) Degeneration of the median midgut (d), remnants of the peritrophic matrix (small arrow). 400x. HE.

The epithelium hindgut cells completely lost the lateral junction of flooring and base, resulting in detachment of the cells to the light of the hindgut. The brush border was evidenced in these cells (Figure 3: 2C).

The transition zone between the anterior and medial regions were formed by throttling midgut with intestinal obstruction at a concentration of 0.1 mg mL^{-1} (Figure 3: 3A, 3B e 3C); in the posterior region, the cells had elongated brush border. The deleterious changes included total or partial destruction of the lining cells, high vacuolization of the cytoplasm and increased subperitrophic space with accumulation of acidophilic materials (Figure 3BC).

The powder formulation based on 10% CNSL is an efficient insecticide against *A. aegypti*. It is considered as a promising commercial product safe to the applicator and rapidly degradable in the environment.

Conclusions

The insecticide based on 10% CNSL, powdered in a matrix based on corn starch, is effective when applied at the larval stage of *A. aegypti*. The powder formulation containing 10% CNSL presents a significant degradation according to statistical evaluation of results after a period of 30 days, reducing its deposit on the environment.

Acknowledgments

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Vegetation composition
in a Permanent Plots System: an Adaptation of the RAPELD Method for the Neotropical Wet Savanna

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Cátia Nunes da Cunha
Jeannini Maria Felfili

Introduction

Wetlands are among the most fragile and threatened ecosystems in the world, subject to anthropogenic actions of both aquatic and terrestrial forms (Gopal and Junk 2000; Junk et al. 2006). Over the past 50 years, it has been estimated that 50% of natural wetlands have been lost worldwide (WCD 2000). The loss of these wetlands is a concern both for the loss of biodiversity and for environmental functions such as storage and regularization of water flow; wetland loss also has substantial influence on the carbon cycle and other greenhouse gases (Hulme and Sheard 1999; Mitra et al. 2005; Ecosystem Assessment 2005; Junk et al. 2006; Naiman et al. 2010).

The main drivers of biodiversity loss are changes in land use, especially the conversion of wetlands to agricultural areas, resulting in habitat reduction. Release of carbon stocks contained in vegetation and soil to the atmosphere, seems to affect the global climate (Millennium Ecosystem Assessment 2005; Junk et al. 2006; Naiman et al. 2010). In addition, silting and pollution by pesticides and agricultural fertilizers or the discharge of untreated waste or human sewage are also factors that are importante in the loss of biodiversity in wetlands (Junk et al. 2006).

In the Pantanal, the largest continuous wetland in Brazil, natural fields and forest areas have been gradually replaced by artificial pastures (Nunes da Cunha & Junk 2004; 2009a; 2009b). In addition, all anthropogenic activity in the upper parts of the Upper Paraguay Basin (BAP) reflects directly on the floodplain, which changes the hydrological cycle or affects the quantity and quality of the water. Examples include the creation of hydroelectric plants, the application of agrochemicals and agricultural inputs, industrial discharges, and gold mining (Nunes da Cunha and Junk 2009b).

The Pantanal was declared a National Patrimony by the Brazilian Constitution of 1988, it houses sites of relevant international importance by the RAMSAR Wetlands Convention. In 2000, areas of this biome were considered Biosphere Reserve by UNESCO (Harris et al. 2005). However in a recent analysis of biodiversity, Junk et al. (2004, 2006) have shown that knowledge about the distribution pattern of the species for this region is incomplete and scarce. The published studies are concentrated in some groups, sometimes only species lists and thus the conclusions drawn are incomplete on biodiversity (Junk et al. 2006) and are not sufficient to define their protection and / or appropriate forms of management.

To assess biodiversity, one of the premises is the establishment of biological inventories that allow comparisons and generalizations from the occurrence of different taxa at sampling sites (Magnusson et al. 2005). Biodiversity inventories made in common sample units are the only way to obtain comparable data in space and time (Belovsky et al. 2004; Zuchim et al.2007). As a methodological proposal for long-term studies, for the estimation of diversity there is the implantation of permanent plots (Felfili et al. 2005). For the Pantanal, this standardization may be important for adequate comparison between different variables, and can demonstrate spatial heterogeneity. The great variability of vegetation in different landscape units has already been demonstrated for Cerrado vegetation (Silva et al. 2006).

However, attempts to describe plant distribution patterns for this region are still insufficient due to the absence of systematized and standardized sampling; this may have hampered the quantification, separation, and understanding of these patterns. Therefore, information on experimental manipulation, besides ecological associations, is necessary to elucidate the determinants of the distribution of plants in the region (Zuquim et al. 2007)

Thus, the integrated project “Meso-Scale Biodiversity Patterns of the Different Pastoral Systems of the Pantanal of Mato Grosso” (BIOPAN), financed by the Ministry of Science and Technology of Brazil (MCT) through the Pantanal Research Center (CPP) and the Federal University of Mato Grosso (UFMT), offered the opportunity to evaluate the distribution patterns of herbaceous, shrubs and trees species at mesoscale. BIOPAN was designed to evaluate, in an integrated and standardized way, the patterns of alpha and beta diversity in communities within production units with different intensities of use in the Mato Grosso Pantanal and aims to establish management criteria compatible with the maintenance of local biodiversity. For this reason, the methodology used by the Biodiversity Research Program, PPBIO (Magnusson et al. 2005), was adapted to the environmental conditions of the Pantanal, since the biological diversity research initiatives in the in this region were always carried out in isolation or within specific groups.

The RAPELD method has recommended integrated researchs, where the informations about processes and different taxonomic groups are collected and analyzed together, which allows inferences about cause and effect. Since this method has designed to develop "rapid assessments", such as the RAP surveys carried out by Conservation International inside "long-term ecological research" (LTER) sites, the Brazilian acronym for LTER is PELD (PELD), (Magnusson et al. 2005). Thus, a modification of the Gentry method (1982), developed for rapid inventories of biodiversity in long-term ecological research sites in Amazonia (Magnusson et al. 2005), hence the name for the method, RAPELD (Magnusson et al. 2005).

Thus, the objectives of this work were: to describe the protocol used for vegetation studies in Neotropical wet savannas; and to synthesize vegetation characterization data for the different strata (herbaceous, shrub and thre) in permanent plots.

Materials and Methods

Selection of the study area

In 2005, through CNPq and FAPEMAT funding, we began the construction and implementation of the permanent plot system (Pirizal Grade). We stressed that all stages of its development, this was the effort of a multidisciplinary team. The Pirizal grid received this denomination because it was installed in three private farms in the District of Pirizal, municipality of Nossa Senhora do Livramento (16°15'S and 56°22'W), sub-region of the Poconé Pantanal (Figure 1).

The sampling site included three landscape units covered by forest formations: Landizal, Cambarazal and Cordilheira, where the first two are evergreen forests seasonally flooded t and remaining dry during the dry season (Nunes da Cunha et al 2010). Only the Cordilheiras sites, formed by tree savannas, are free of flooding (Nunes da Cunha et al. 2010). In addition to the forests, there are also native fields and areas with cultivated pasture (Nunes da Cunha et al., 2010; Fantin-Cruz et al. 2010b).

The local climate is AW type according to Köppen's classification system, with two well-defined seasons: the dry season, from May to September and the rainy season, from October to April (Nunes da Cunha and Junk 2004; Costa et al. 2010). The maximum annual precipitation is 1,384 mm in January (Figure 2), and minimum precipitation occurs in July. The annual average temperature is 25.8 °C, with October being the warmest month and July the coldest (Rebellato and Nunes da Cunha 2005; Costa et al. 2010). The seasonal flooding process is divided into four phases (Rebellato and Nunes da Cunha 2005; Costa et al. 2010).

According to Fantin-Cruz et al. (2010a; 2010b), the sampling site has an inclined surface NE/SO, where the lowest measured topographic dimension was 112.52 m and the highest 116.56 m, with a topographic variation of 4.04 m. Most of the area (43%) is at altitudes higher than 114.0 m (high), followed by 113.5 to 114.0 m in 40% of the area (averages) and 17% at altitudes below 113.5 m (low, Figure 2). The average annual depth of the flooding in the grid is 20 to 40 cm (Figure 3), and the average duration is between 65 and 130 days (Fantin-Cruz et al., 2010a; 2010b).

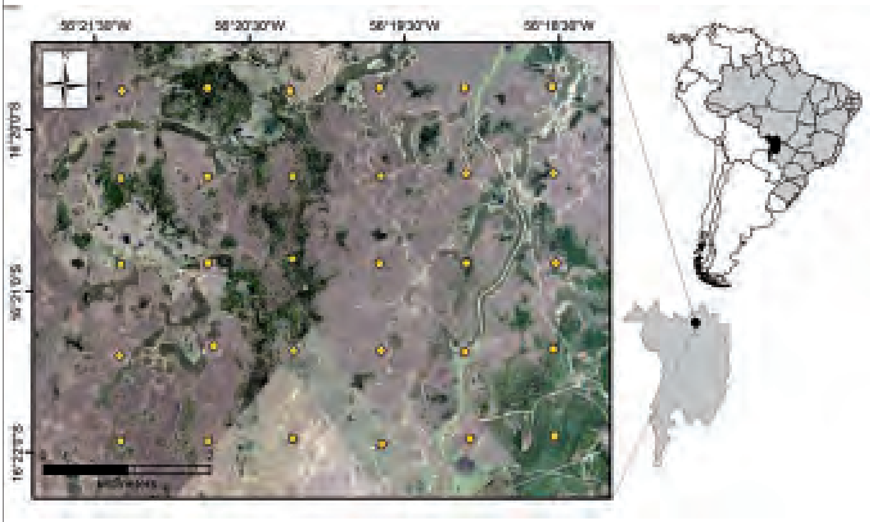

Figure 1. Selected area for implantation of the Pirizal grid, municipality of Nossa Senhora do Livramento, Pantanal sub-region of Poconé-MT (Pinho 2005). Yellow circles represent the 30 plots selected for this study.

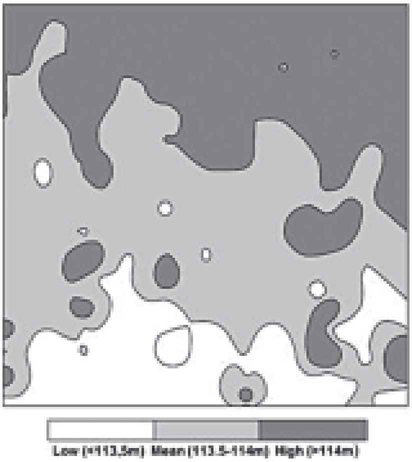

Figure 2. Topographic surface of the Pirizal grid sampling site, North Pantanal, MT. Source: Fantin-Cruz et al. (2010a).

Figure 3. Mapping of the a) intensity and b) duration of the flooding at the sampling site of Pirizal, North Pantanal, MT. Source: Fantin-Cruz et al. (2010a).

The Pirizal grid occupies a total area of 25 km², consisting of 30 plots systematically allocated, and each one was designed to follow the topographic contour (plots) of the soil, in order to minimize the internal topographic variation, according to the sampling design proposed by Magnusson et al. (2005). The plots are 250 m long and have a variable width, adjustable to the sampled life form. To access the plots, there are six parallel trails of 5 km, each one with an identification letter (A, B, C, D, E and F) where 5 plots (Figure 4) were installed, oriented east-west. The first part of each trail begins from 500 meters from the beginning of the respective plot. Thus, for example, the plot at A1 is located 500 meters from the beginning of track A. The plots are 1 km from each other. The marking of the trails was done with fixed iron pickets in the soil every 10 m, and the altimetry of the starting point maintained throughout the 250 meters.

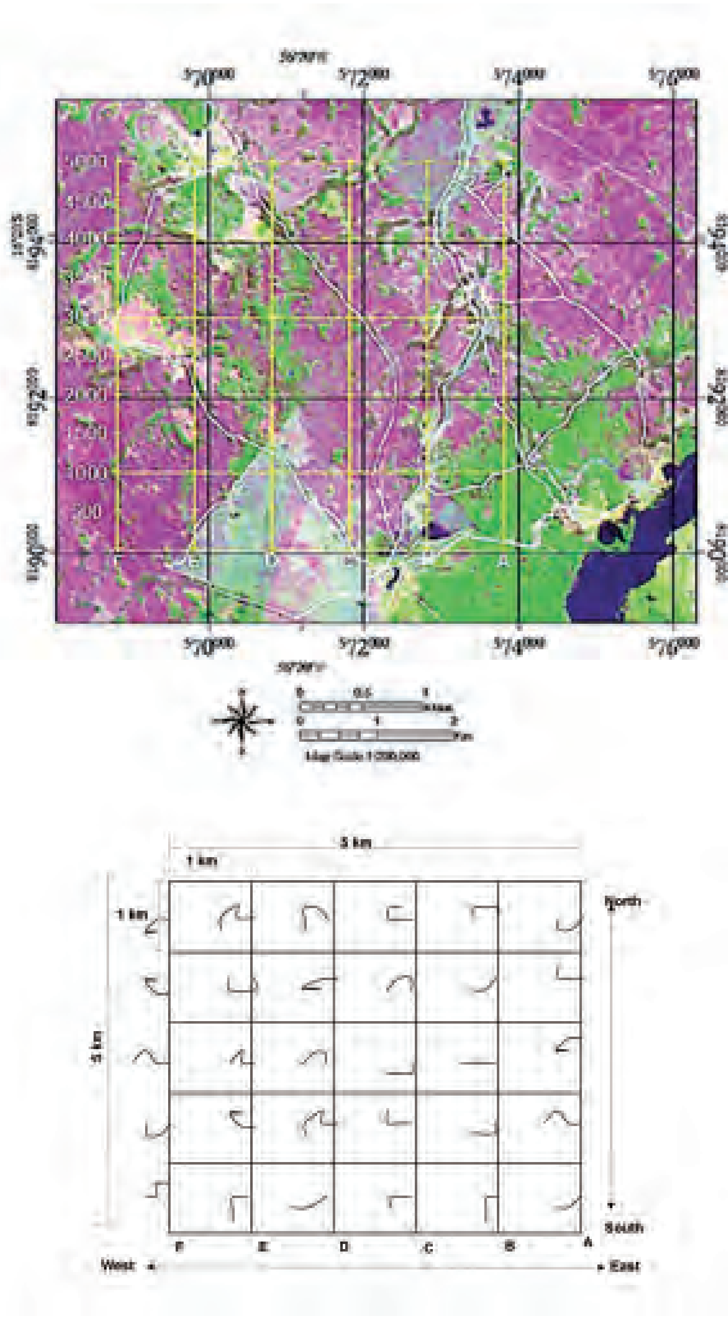

Figure 4. Satellite image showing the Pirizal grid (5 km x 5 km) with 6-track systems (north-south only) and scheme with distribution of 250-meter plots on the tracks (A, B, C, D, E and F), North Pantanal- MT, Brazil.

The system of permanent plots was randomly allocated in the landscape and the plots were distributed among different vegetation types and land use types in the habitat mosaic. In addition, the grid was implemented in farm areas that have extensive cattle raising, which allowed us to evaluate the effect of landscape changes caused by the replacement of native vegetation by introduced pasture.

Data collection Vegetation sampling

Herbaceous plants: herbaceous samples were collected during months representing the hydrological phases of the Pantanal: in the dry season, between September and October 2006, when the soils were dry; in the flooding season, between February and March 2007, when the 27 floodplain plots had maximum water depth; and in the ebb season, when the water table had already fallen, with some plots flooded only to water saturation. The method for sampling herbaceous plants was that of the point plot (Bullock 1996). In this method, punctual parcels 1meter from each other were distributed along the 250 meters of each plot. The punctual portion was a thin metal rod positioned vertically, so that any herbaceous morphospecies that appear were recorded. The sampling intensity was of 250 points along each plot. In all, there were 750 one-off parcels per plot in the three sampled periods. The number of point plots where each species occurred was used to calculate its coverage in the plot (number of point plots in which the species occurred / 750).

The vegetation types occurring in the implanted plots were categorized in the field according to their physiognomy, present plant species, and flood regime and with the aid of the descriptions found in Nunes da Cunha et al. (2006; 2010).

Tree and shrub plants: 25 plots from the Pirizal grid were collected from May to December 2007, as the remaining five plots consisted only of the herbaceous stratum. Standardized samplings were carried out in the woody vegetation, adapting the RAPELD protocol (Magnusson et al 2005) to savanna vegetation, according to the manual for the monitoring of permanent plots in Cerrado and Pantanal biomes (Felfili et al 2005). Thus, in each plot, trees and shrubs were sampled in 2 bands of different widths (Figure 5). Plants with diameter at breast height ³ 1 cm <5 cm werein the 4 m wide range, with 2 m to each side of the central line. In this range, we sampled shrubs and tree regeneration. For individuals with DBH ³ 5 cm, a 20 m wide band was used, with 10 m on each side of the central line, this included individuals with tree aspect.

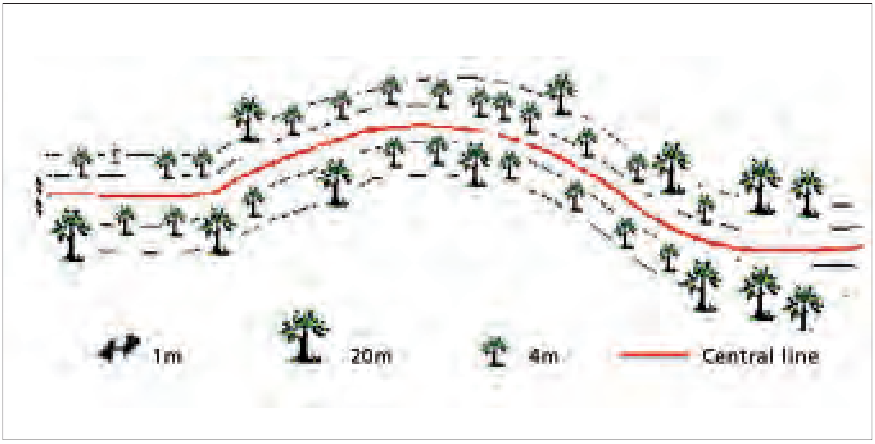

Figure 5. Scheme of the distribution of sampling strips to vegetation with the 1 m buffer strip (Pirizal grid, North Pantanal, MT).

To facilitate standardization in relation to the size of the sample areas and to cause the least possible trampling in the herb sampling area, the bands for sampling the trees were established after the 3 m buffer strip on both sides of the center line had been set.

All individuals with diameters within the limits determined above were mapped with x and y coordinates, and marked with numbered platelets. In the mapping, the x coordinate referred to the distance along the major axis of the plot. The y coordinate was the individual's distance perpendicular to the center line. This measurement was made with the strap stretched to remain horizontal. This was important so that the inclusion of individuals referred to distances measured in the horizontal plane, such as on a map. The distance x was measured to the middle of the tree.

For trees smaller than 5 cm of DBH, the numbered labels were tied with wrapped wire to avoid damage to the plants. For larger trees, the labels were nailed with galvanized iron nails. Each nail was placed at a distance of exactly 30 cm from the point of diameter measurement to avoid modifications in the remeasurement site, and the nail was stuck in the tree perpendicular to its surface, to avoid the entry of microorganisms.

It is noteworthy that the aluminum labels were tied towards the center line, to facilitate their reencounter in the recensus, as well as, in the reunion of the trees, since these were measured and marked in sequence in the plot.

The diameter measurement was performed at 1.30 m from the soil, for trees with large buttresses (*sapopemas*) the diameter was measured approximately 50 cm above the end of this irregularity. In the presence of other types of irregularities in the trunk the measuring point was moved to more cylindrical sections of the stem. For the measurements of diameter a sewing tape was used with precision of ± 1 mm. The height was measured with a 15 m graduated rod made by the team responsible for sampling woody plants.

Identification of species

For the herbaceous stratum a specimen of each morphospecies was collected, herborized, and deposited in the Central Herbarium of the Federal University of Mato Grosso (UFMT) and the Federal University of Minas Gerais (UFMG). The woody material was also collected herborized, and later deposited in the Central Herbarium of the Federal University of Mato Grosso-HCUFMT. The species were classified into families according to the *Angiosperm Phylogeny Group III* (2009) system. Through the material collected during the floristic surveys the listing was elaborated with families, genera and species. The names of the species and their authors were checked through consultations with the Missouri Botanical Garden (http: www.mobot.org.).

Data entry

All worksheets have been standardized so that the field annotation is the same for numbers, symbols, and letters. Subsequently, they were digitalized separately for each plot and made available in the form of metadata (https://ppbiodata.inpa.gov.br/metacatui/#view/menger.108.3), that is, a set of generated data that help the researchers responsible for research or other researchers to find, understand and efficiently use the data collected.

In addition, for herbaceous species, the number of punctual plots where each species occurred was used to calculate its coverage in the plot (number of punctual plots in which the species occurred / 250), and was calculated for the 30 plots in the three hydrological phases. The calculations of the cover of the species in the different types of vegetation were done as follows:[(sum of the number of punctual parcels in which the species occurred / total number of punctual parcels occurring in that vegetation type x 250) = total occurrences / plots in that type of vegetation x 250)].

For the woody species, CBH data were converted to diameter (DBH = CBH /p) and basal area (BA = CBH^2 / 4p). For individuals with multiple stems, DBH was calculated from the total basal area [DBH = Ö (4 TBA /p)].

Data analysis

Herbaceous: the species richness was considered as the total number of species in each plot sampled in the three hydrological phases of the Pantanal. A multidimensional non-metric acaling (NMDS) (Cox and Cox 2001) was used to order the plots in a gradient determined by the similarity in the cover of the herbaceous species. NMDS has been one of the most employed sorting techniques in community structure analyses since it does not require a multivariate normal distribution and does not presuppose linear relationships between the variables (McCune and Grace 2002).

The ordering was done with data covering the total species (species cover) applied to an association matrix calculated with the Bray-Curtis index (Legendre and Legendre 1998), a measure widely used in community analysis (McCune and Grace 2002). Subsequently, the relationship between the first two axes of the NMDS (dependent variables) and maximum flood depth data of the plots (independent variable) were analyzed through a multiple linear regression. To evaluate the relationship between floristic similarity and geographic distance or spatial autocorrelation, the Mantel test (1000 randomizations) was performed using the species cover matrix and the geodetic coordinate matrix of the initial points of each plot .

Trees and shrubs: to describe the distribution patterns of the woody species, two matrices were constructed, the first one based on the abundance of the species (absolute density) and the second on the floristic composition (qualitative data, presence/absence). The quantitative and qualitative matrices were constructed with all 25 plots sampled, having as objects the species found as attributes. To summarize each data matrix, the non-metric multidimensional scaling (NMDS) analysis (Cox and Cox 2001) was used, where the distance measure used in the association matrix was the Bray-Curtis index. To test the significance of the NMDS axes, a Monte Carlo permutation test was performed (Cox and Cox 2001).

For both herbaceous and woody plants, sorting analyses were performed using the statistical package PCOrd 6.01 (McCune and Mefford 1999).

Results and Discussion

HerbaceousCommunity

Sampling of the plots in the three hydrological phases of the Pantanal gathered a total of 209 species distributed in 43 families. Among the most species rich families were Poaceae (59), Cyperaceae (30) and Fabaceae (14). The complete listing of species can be accessed in Rebellato et al. (2012). Of the total species, 93 occurred in non-flooded plots and 150 in flooded plots, of which 34 were common. Weeds ranged from 22 (in an exotic seasonally flooded field) to 56 (in semi-deciduous seasonal forest on dry soil) species.

The ordering (Figure 6) performed by the NMDS method separated the floodplain from the three non-flooded plots (B2, E2 and F2) on the first axis. The two axes captured much of the variation of the original distances ($r^2= 0.69$), and the stress value of 9.56 indicated a satisfactory ordering (McCune and Grace 2002).

It was observed that the largest parts of the plots were grouped according to the type of vegetation (Figure 6), due to their similarities in terms of abundance and composition of herbaceous species, which are arranged negatively along the first axis of the NMDS, *i.e.*, in the depth gradient of the flood, starting from highly flooded communities to those not flooded. The different depth ranges in which vegetation types occur produce a certain spread of the plots of each type of vegetation along the first and second axis of the NMDS, probably due to the effects of the flood depth on the occurrence and cover of the species which characterize them.

Woody Community

In the studied plots, 99 tree-shrub species were identified, distributed in 79 genera, 40 families and one indeterminate species (the species list is available in Nunes da Cunha et al. 2010). Regarding the habit of growth of the species sampled, 36 (36.40%) species were shrubs, 63 (63.60%) species were trees. The 30 most important tree species represented 97% (6926 ind.) of the total sample (7152 ind.), while for shrubs and regenerants the 30 most importante species represented 97.14% (7299 ind.) of the total sample (7514 ind.).

The families that contributed most to the richness of species were Fabaceae (21 species), Rubiaceae (seven species), Myrtaceae (five species), Malvaceae, Melastomataceae, Bignoniaceae, Euphorbiaceae,

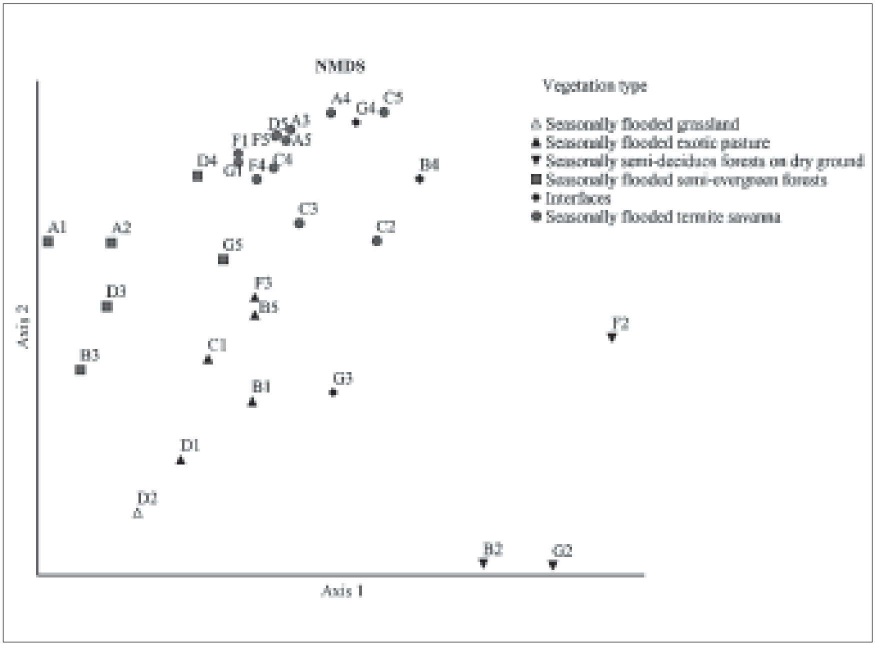

Figure 6. NMDS (non-metric multidimensional scaling) of the 30 plots sampled and classified *a priori* in relation to the type of vegetation, North Pantanal, MT.

Malpighiaceae, Moraceae, Annonaceae and Sapindaceae (three species). These families comprised 61 species, constituting 62% of the species sampled (Figure 7). The remaining 38 species are distributed in 29 families, of which 23 were represented by one species (Nunes da Cunha et al. 2010). The genera with the greatest species richness were *Eugenia* (four species), followed by *Alibertia* and *Byrsonima* (three species).

The most species rich plot in the tree category was E2, with 28 species (13.24 ± 6.10 SD) (Figure 8). The plots with the lowest values of richness were C2, C3 and F5, with five species each. For abundance values, plot B3 presented the highest value, 477 ind.ha^{-1} (176.48 ± 126.61 SD) (Figure 9). The F5 plot, on the other hand, also presented the lowest value of abundance (20 ind.ha^{-1}), as well as for richeness. For the shrubs and regenerants category, the most species were in E2, with 33 species (15.26 ± 6.23 SD) (Figure 8). For D2 plot, only four species were recorded. For the values of abundance, plot D3 presented the highest value, 906 ind.ha^{-1} (329.04 ± 251.94), while the F4 and F5 plots had the lowest abundances, 37 and 38 ind.ha^{-1}, respectively (Figure 9).

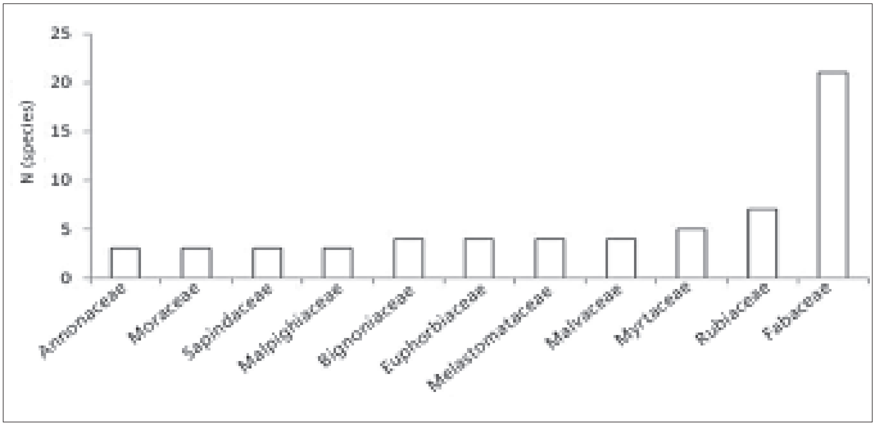

Figure 7. Distribution of families according to the number of species (N) sampled in the Pirizal grid, North Pantanal, MT.

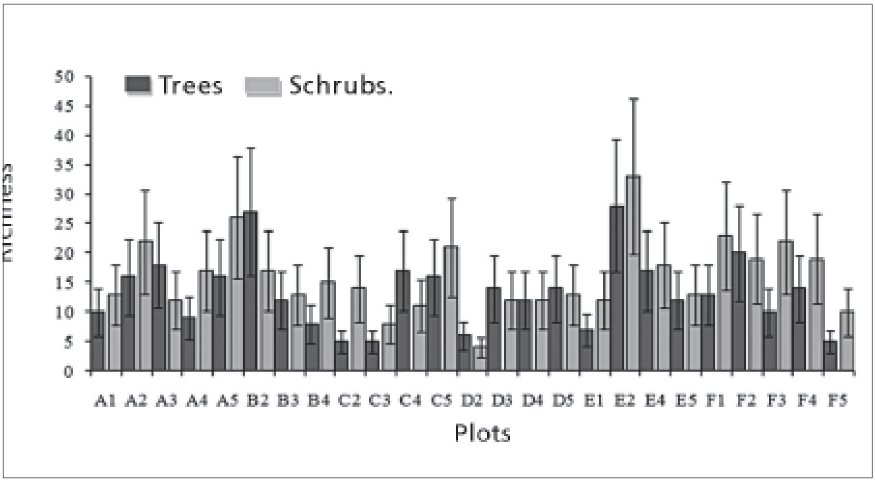

Figure 8. Averages and standard deviations of the richness of the woody species sampled in 25 plots in the North Pantanal, MT.

The NMDS axes for the quantitative data of the tree category explained a large part of the original distances, since the Monte Carlo test was significant for both axes (p = 0.032). In addition, the stress value (11.32) indicated that the ordering can be considered satisfactory and that the greatest correlation between the distance matrix of random scores and the distance matrix of the original data was not found at random. The ordering of the NMDS for qualitative data was also considered satisfactory for both axes (p = 0.004), but presented higher high stress value (15,47).

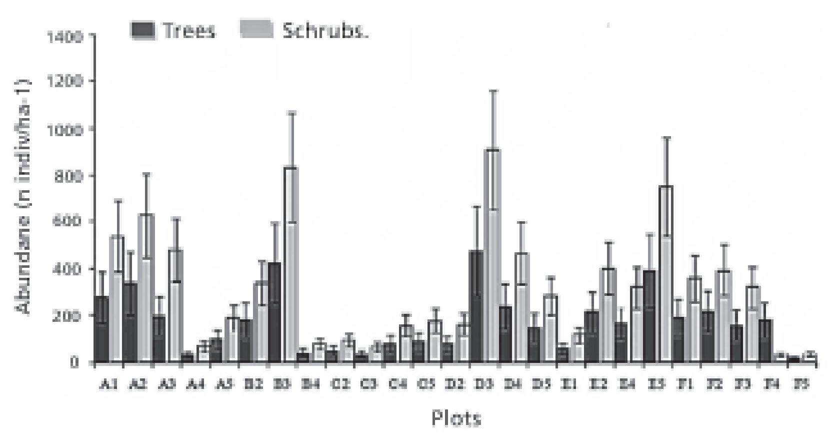

Figure 9. Averages and standard deviations of the abundance of the woody species sampled in 25 plots in the North Pantanal-MT

Although both analyses explain the species ordering, it can be observed that in the quantitative NMDS (Figure 10A), there is a clear structural and phytophysionomic gradient, where the plots formed by forest typologies and with greater abundance of individuals are very close, separated from those formed by savanna typologies *(murundus fields* and *cerradões*) with lower values of abundance. In the qualitative NMDS (Figure 10A1), the analized plots form distinct groups, separating flooded savannas (*murundus fields*) and semi-evergreen seasonally flooded forests (B2, E2 and F2).

The NMDS analyses for shrub and regenerating species showed that the quantitative data, as well as for the tree category, explained most of the original distances, since the Monte Carlo test was significant for both axes (p = 0.004). The stress value (12.65) also indicated for the shrub and regenerating category that the ordering was satisfactory, and a higher correlation between the distance matrix of random scores and the distance matrix of the original data was also not found by chance. The ordering of the NMDS for qualitative data was also considered satisfactory for both axes (p = 0.004), with a small increase in the stress value (13.06). For both quantitative and qualitative NMDS different groups can be discriminated, evidencing that the species are distributed according to environmental gradients and not to chance. This can be seen in the ordering diagrams (Figures 11B and 11B1) that the plots formed by cerradões (B2, E2 and F2) distance themselves from the others probably because they are free areas of the flood (ground ranges), whereas the othres are flood áreas (seasonal forests and *murundus field*)

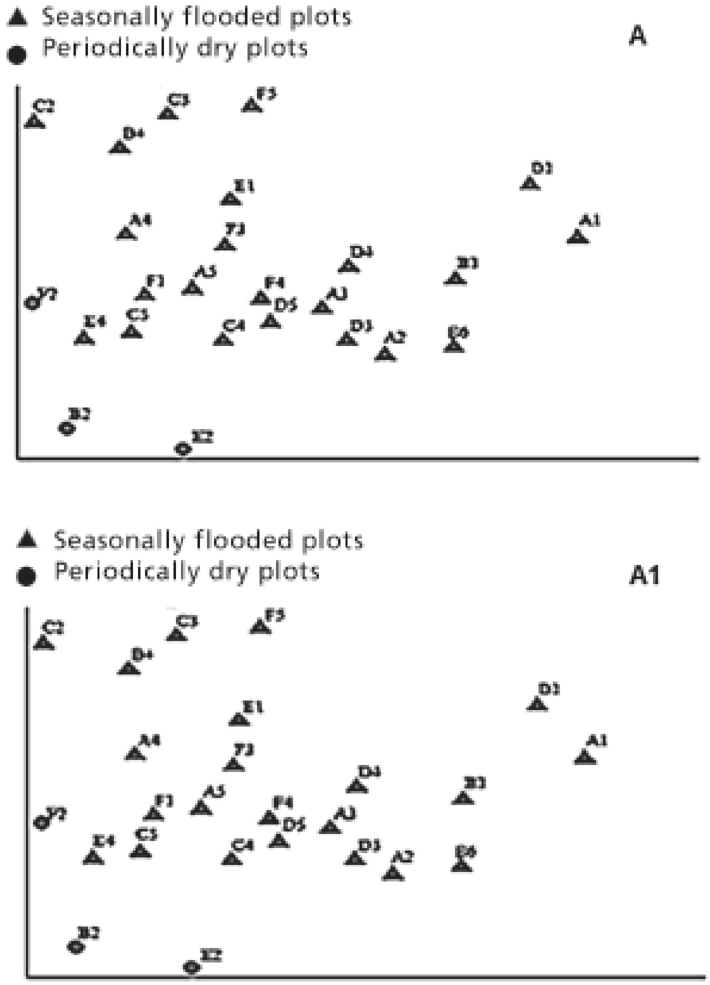

Figure 10. NMDS ordination by the 25 plots sampled for trees species in the North Pantanal, MT. A= quantitative data and A1= qualitative data.

In the analysis of tree, shrubs and regenerating groups, the results indicated the presence of environmental gradients, where most of the species occupied preferential sectors. Species from non-flooded areas occurred at one end and flooded areas, which accounted for the majority of the sampling, occurred at the other end.

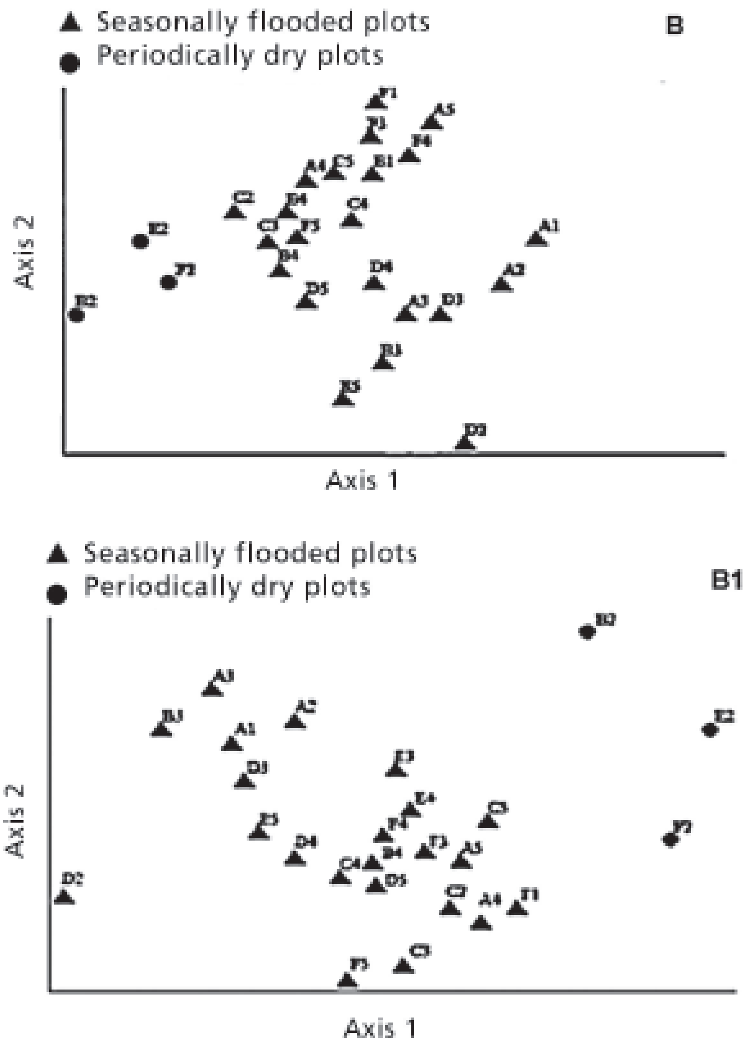

Figure 11. NMDS ordination by the 25 plots sampled for shrubs species in the North Pantanal, MT. B= quantitative data and B1= qualitative data.

It was also observed that the sharing of species between neighboring plots did not result in floristic overlap, *i.e.*, it was possible to distinguish three groups (Forests, Fields and *Cerradão*, i.e., a woodland savanna) according to their typology. For the qualitative and quantitative data of the tree species the groupings were arranged in the direction Forest-Field-*Cerradão*.

For shrublands, the qualitative data showed the same pattern,as for trees, while for the quantitative the grouping was in the direction *Cerradão*-Field-Forest. As observed in the results, it is possible that environmental factors acting in the distribution of woody species are influencing the mesoscale in the region, similar to the results found for the Pantanal (Haase 1992, Prado et al., 1994; Adamoli and Pott 1999; Damasceno-Júnior et al. 1999; 2004; Nunes da Cunha and Junk 1999; 2001; Arieira and Nunes da Cunha 2006; Junk et al. 2006).

In all analyses, the environmental factors were shown to be stronger predictors of the floristic patterns and of abundance between the plots. This confirms results of previous work performed in the same site for woody species (Nunes da Cunha and Junk 2001; Nunes da Cunha et al.; 2010; Fantin-Cruz et al., 2010a). In the analysis of tree, shrub and regenerating groups, it was possible to observe the presence of environmental gradients, where most of the species occupied preferential sectors. Species from non-flooded areas occurred at one end and flooded areas, which accounted for the majority of the sampling, occurred at the other end.

It was also observed that the sharing of species between neighboring plots did not result in floristic overlap, *i.e.*, it was possible to distinguish three groups (Forest, Field and *Cerradão*) according to their typology. For the qualitative and quantitative data of the tree species the groupings were arranged in the direction Forest-Field-*Cerradão*. As for shrublands, the qualitative data showed the same pattern of trees, while for the quantitative the grouping was in the direction *Cerradão*-Field-Forest.

For the distribution of woody species, the environmental factors possibly act to limit the mesoscale dispersion process in the region, *i.e.*, there is environmental control in the distribution of the species, similar to the results found for the Pantanal (Haase 1992; Prado et al. 1994; Adámoli and Pott 1999; Damasceno-Júnior et al. 1999, 2004; Nunes da Cunha and Junk 1999; 2001, Arieira and Nunes da Cunha 2006; Junk et al. 2006).

The results showed that there were no statistically significant implication for the analyses performed since there was no spatial dependence on the residuals of the regression models, which also suggests that there were no pseudo-replications in the sample (Diniz-Filho et al., 2003) . These are facts that meet both the assumptions for estimating the association between the data series analyzed (Peres-Neto et al. 2006) and the RAPELD sampling method (Magnusson et al., 2005). In RAPELD, the plots are delineated to minimize the spatial structure effect in the study that relates the composition of species with environmental variables.

Thus, the system of permanent plots generated very informative data regarding general patterns of vegetation composition. The adaptations carried out for this study, such as the reduction of the level of inclusion of the woody species, as well as the width of its plots, can be used in future studies to detect the main factors that affect the distribution of woody species at the mesoscale. In addition, the results of this study may guide management measures for the maintenance of regional biodiversity in the Pantanal, as well as in the classification of wetland environments.

Conclusions

In the analysis of the results it was observed that the system of permanent plots (Pirizal Grid) is feasible for the study of vegetation classification. In this study system it is possible to evaluate not only the composition and structure of different typologies, but also to relate these attributes to abiotic factors.

Within this context, we observed the data set generated by the BIOPAN Project - Pirizal Grid strongly suggest that the structure and floristic composition of the herbaceous and woody components are affected by abiotic factors, mainly the seasonality of the flood. Since, the results indicated the presence of environmental gradients, where most of the species occupied preferential sectors. Species from non-flooded areas occurred at one end and flood areas, which accounted for the majority of the sampling, occurred at the other end.

Thus, it is believed that the use of permanent plots is essential to monitor changes in the composition, structure and functioning of vegetation typologies in the long term, seeking a better understanding of the processes and mechanisms that generate or maintain the regional landscape.

Finally, this database, in addition to elucidating information on species diversity and ecosystem functions, can also demonstrate man-made changes in the landscape over time and, thus, generate actions that support the management and conservation of the Pantanal.

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