UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

LETÍCIA KOUTCHIN DOS REIS

RESTAURAÇÃO ECOLÓGICA POR TRANSPLANTE DE PLÂNTULAS PROVENIENTES DA REGENERAÇÃO NATURAL

Campo Grande
2019

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"Dissertação apresentada ao Programa de Pós-Graduação em Biologia Vegetal como um dos requisitos para obtenção do grau de Mestre em Biologia Vegetal, ênfase em Ecologia da Restauração pela Universidade Federal de Mato Grosso do Sul"

Orientadora: Profa. Dra. Letícia Couto Garcia

Co-orientador: Prof. Dr. Geraldo Alves Damasceno Junior

Campo Grande
2019

Agradecimentos

Gostaria de agradecer aqui todos os colaboradores em qualquer etapa da elaboração desta pesquisa de dissertação.

Agradeço aos meus pais, principalmente, por todo apoio, amor, atenção e compreensão sobre minha ausência durante a fase do mestrado. Obrigada mãe por todo apoio em todos os momentos mais difíceis em minha vida, por sempre ser uma mulher de garra enfrentando a depressão diária, estarei com você sempre! Obrigada pai por toda ajuda que precisei, e por toda compreensão em não conseguir sempre ir ficar com vocês. Agradeço a vocês, por tudo e todos os esforços não medidos para que eu pudesse voar para fora do nosso canto, com uma boa educação e que me permitiu chegar aonde cheguei.

Agradeço ao meu namorado, já Mestre Breno Emanuel, pelo companheirismo e por ter paciência e me tranquilizar nessas fases mais difíceis, obrigada por sempre estar ao meu lado, torcendo e me apoiando, obrigada por todo amor e carinho, te admiro muito, como pesquisador e como namorado. Agradeço as minhas amigas de graduação, Nicolle, Keren, Luana e meus amigos de mestrado, Diego Rezende e Giovani Andrella, por toda ajuda, apoio e parceria em todas as fases, com certeza o mestrado foi mais feliz com a companhia de vocês! Agradeço ao Juliano, Clara, Felipe, Karen, Evaldo, Daniel, Vinicius, Tiago, Francine, Vanielle, pelo auxílio em campo durante as coletas e plantios do experimento, sem vocês, nada disso teria acontecido! Agradeço também ao companheiro de laboratório, Evaldo, pelas ajudas, dúvidas e parceria com a tal e tão temida estatística, obrigada

Agradeço a UFMS-PROPP, principalmente Antônio e Willian pela parceria em auxílio financeiro em minhas viagens para a Base de Estudos do Pantanal-BEP. Agradeço também a todos os funcionários da BEP, a cozinheira Cida pela alimentação, a Graciane pela limpeza, ao chefe da BEP Wagner por ter me recebido com carinho, ao Pernambuco pelas ajudas, auxílios com os materiais e cuidado com as mudas desde a coleta ao plantio, a Cleide, Tiana e José, e agradeço aos motoristas (Almir, Jorge e João) pela locomoção até a base e pelas aventuras vividas durante elas.

Agradeço a todos os professores por quem já passei durante o mestrado, todos os professores de disciplinas, aqueles que nos deram frutos após as disciplinas. Obrigada Prof. Maria Rosângela pela sua energia contagiante e aulas didáticas que nos renderam um grande manuscrito a ser publicado, obrigada Prof. Rafael Arruda pela disciplina de bioestatística, obrigada Prof Eric pela oportunidade em fazer uma disciplina de comunicação cientifica, extremamente essencial para um pesquisador e obrigada Prof. Ieda pela oportunidade em aprender sobre a vida docente universitária em estágio docência. Agradeço a todos os professores do curso de botânica de campo, tudo foi maravilhoso e nunca será esquecido!

Por fim, não menos importante, agradeço aos meus orientadores, minha orientadora Prof. Dra. Letícia Garcia, uma mulher pesquisadora e guerreira, obrigada por ter me acolhido com carinho desde a minha defesa de TCC, pela disciplina maravilhosa e reuniões que nos permitem ver a vida acadêmica, profissional e científica, obrigada pelas oportunidades que me fazem crescer profissionalmente como pesquisadora. Ao meu coorientador Prof. Dr. Geraldo Damasceno pela orientação, pelos colegas de laboratório apresentados, pela disciplina lindíssima de ecologia, por todo o conhecimento adquirido e obrigada pela confiança. Vocês foram essenciais para minha formação de pesquisadora novata neste país. Agradeço ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Mato Grosso do Sul pela oportunidade em adquirir tanto conhecimento.

"O desejo profundo da humanidade pelo conhecimento é justificativa suficiente para nossa busca contínua."

Stephen Hawking

SUMÁRIO

RESUMO GERAL

Um dos principais gargalos dos programas de restauração ecológica é a aquisição de mudas de diversas espécies do ambiente local ou regional em quantidade suficiente. Para superar este desafio, o transplante de plântulas provenientes da regeneração natural, como sendo um método que permite a conservação do material genético que seria suprimido ou que está disponível dentro de fragmentos conservados, passa a ser de grande importância para a restauração ecológica. Objetivamos com esta dissertação apresentar resultados sobre a prática da restauração por transplante de plântulas jovens provenientes de espécies dominantes da regeneração natural, apresentando dois capítulos. O primeiro trata-se de uma análise bibliométrica sobre estudos realizados no mundo sobre esta prática, na qual encontramos que a mesma foi mais implantada em áreas úmidas, seguida de regiões de florestas tropicais (concentradas no Brasil, onde foram detectados os estudos com maiores riquezas de espécies utilizadas). No segundo capítulo, abordamos a implantação experimental, em ambiente sazonalmente inundável, considerando o uso de proteção anti-herbivoria no qual encontramos diferentes índices de sobrevivência, altamente dependentes da espécie escolhida, tamanho da plântula e a posição topográfica em que essas plântulas são coletadas e implantadas. Ademais, espécies quando cercadas apresentaram significativo aumento dos índices de sobrevivência e menores taxas de herbivoria foliar. As taxas de sobrevivência atingidas foram consideráveis levando em conta que o estudo ocorreu em um período de cheia excepcional (sete meses). Apesar dos custos de implantação de cercas serem maiores, o custo-efetividade individual que considera o número de sobreviventes pelo custo da implantação, foi maior comparado ao de plantio de mudas tradicional. Vale ressaltar que a prática de transplante de plântulas é, em sua maioria, indicada em casos extremos, em que há necessidade de aumentar riqueza e diversidade de formas de vida, em locais desprovidos de rede de coleta de sementes e viveiros, bem como em áreas remotas, enfatizando o seu uso a partir de indivíduos de espécies com alta densidade a fim de não impactar suas populações em ambientes naturais. A

fim de corroborar a sua viabilidade em maior diversidade, estudos futuros poderão testar, com maior número de espécies, o que aqui encontramos como o melhor resultado desta prática em áreas inundáveis (transplante de plântulas da menor classe de altura, coletadas da topografia mais sujeita à inundação, plantadas no relevo mais alto e com o uso de cercas anti-herbivoria).

PALAVRAS-CHAVE: Resgate de plântulas, insumos para restauração ecológica, áreas úmidas, Pantanal Wetland.

INTRODUÇÃO GERAL

Dentre os ecossistemas mundiais, as florestas tropicais têm elevada biodiversidade e endemismo, sendo evidenciadas pelas necessidades de ações voltadas a proteção e conservação desses ambientes (Myers et al., 2000). Essas florestas estão sendo intensamente supridas pela ação antrópica, o que tem levado muitos desses ecossistemas a grande ameaça pela exploração intensiva e extensiva (Ribeiro et al., 2009). Alguns ramos da ciência visam à conservação e à recuperação de ambientes degradados e devastados, um deles é a Ecologia da Restauração, que embasa a melhoria das práticas de restauração ecológica e que pode ser considerada uma disciplina recente (Roberts et al. 2009). A restauração ecológica é definida como "o processo de auxílio ao restabelecimento de um ecossistema que foi degradado, danificado ou destruído" (SER 2004), ou seja, a restauração ecológica visa criar comunidades ou populações viáveis, que protegem e aumentam a capacidade natural de mudança e regeneração dos ecossistemas (Engel e Parrotta 2003). Na prática, a maioria dos projetos de restauração ecológica tem focado no restabelecimento de comunidades ricas em espécies nativas, tendo como objetivo favorecer a dinâmica e os processos ecológicos que envolvem a sustentabilidade da área restaurada (Brancalion et al. 2013).

No entanto, um dos principais gargalos da prática em programas de restauração ecológica é a aquisição de mudas de diversas espécies do ambiente local ou regional em quantidade suficiente (Fonseca et al. 2001; Santarelli 2004). A limitação do número de espécies disponíveis nos viveiros de mudas florestais nativas é decorrência, dentre outros fatores, das dificuldades para obtenção de sementes e da ausência de tecnologia para produção de mudas por sementes de muitas espécies nativas (Zamith e Scarano 2004; Silva et al. 2011). Técnicas como plantio de mudas, nucleação (como o uso de chuva e banco de sementes (Tres et al. 2007), poleiros artificiais (Vogel et al. 2018), transposição de solo e transposição de galharia (Marcuzzo et al 2013) e semeadura direta (Ceccon 2016; Palma et al.. 2015) são as mais c utilizadas em projetos de restauração ecológica.

Outro método atual de grande importância dentro da restauração, pela conservação do material genético, é o transplante de plântulas (Calegari 2011). O transplante de plântulas é a retirada de plântulas nativas de diversas formas de vidas que se regeneram em fragmentos, sendo transplantadas e desenvolvidas posteriormente em viveiros para implantação em campo visando restauração ecológica (Silva et al. 2011). Em comparação aos outros métodos citados, o transplante dispensa a coleta ou remoção de sementes do banco de sementes, e parte do desenvolvimento inicial que aconteceria nos viveiros até o momento de plantio (Calegari 2011). Portanto, o objetivo do transplante de plântulas é aumentar a diversidade de formas de vida e o número de espécies em viveiros (Nave 2005; Viani e Rodrigues 2008). Considerando que a obtenção por mudas em viveiro acaba sendo limitada, viveiros não dispõem de muita diversidade de espécies de mudas nativas e a sua localização frequentemente está distante das áreas remotas a serem restauradas, portanto, o transplante pode facilitar esse processo.

No entanto, a remoção das plântulas ou mudas pode comprometer a regeneração natural das espécies, uma vez que a retirada de indivíduos de espécies de baixa densidade pode impactar no desenvolvimento ecológico e funcional do ambiente (Viani e Rodrigues 2008). Apesar da produção de mudas, através do transplante ser uma prática antiga, existem poucos estudos publicados que relatam o uso dessa técnica para restauração ecológica no

Brasil (Nave 2005). Os estudos escassos apresentam conclusões distintas, expondo restrições como a implantação de poucas espécies de hábito arbóreo, principalmente com distribuição dentro do domínio do Cerrado (Ferreira et al. 2015; Turchetto et al. 2016) e da Mata Atlântica (Vidal 2008; Calegari et al. 2011). Portanto, são necessários estudos para corroborar a sua viabilidade para a restauração ecológica. Assim, esta dissertação visa responder questões em aberto sobre a prática de transplante de plântulas. No capítulo um, apresentamos uma revisão bibliométrica sobre a prática em uma visão global do seu uso. No capítulo dois, avaliamos seu uso experimental em uma área úmida como alternativa para restauração de Áreas de Preservação Permanente no Pantanal.

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CAPÍTULO I

Transplantation of seedlings from natural regeneration for ecological restoration: a bibliometric analysis

Letícia Koutchin dos Reis[1], Geraldo Alves Damasceno Junior[2] and Letícia Couto Garcia[2]

[1] Programa de Pós-Graduação em Biologia Vegetal, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS.

[2] Laboratório de Botânica, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS.

Abstract

The use of the wildlings collected from forest areas followed by transplant them in the field has been used in restoration projects intending to reduce costs, increase conservation of the local genetic material, and the adaptability in the field. However, there are many other questions about this practice that have to be explored in more depth. Therefore, we sought through a bibliometric analysis to review the published knowledge on a global scale. We searched the Web of Science platform for keywords (seedling transplanting; seedling wildling; seedling rescue; seedling transfer AND restoration) covering published peer-reviewed articles from 1988-2018. We examined 428 articles but only 31 (8.44%) were included in the analysis, because they actually used the method. From our chosen articles, we summarized the number of citations, number of species used and its growth forms, survival rate, time spent, costs, and range of ecosystems. The vast majority of the articles (86%) included the variables that we sought (survival rate, number of species, growth habit). Cost variables and time transplanting of a species after collection were found in only two included articles (6.45%). Wetlands were the most researched ecosystem and, among these, the most studied were coastal regions. The largest number of articles was in tropical forests and in the southeastern region of Brazil. Most of the studies used tree species and only one species. The highest survival rates were recorded in tropical forests (75%), while the lowest was for swamps (27%). Data about transplanting time after collecting are still rare, but few available data show better survival after 24h of outplanting. This analysis showed that published papers regarding the use of the collecting and transplanting of seedlings is scarce but it presents great potential to supplement or replace the use of other practices aiming to increase the diversity of growth forms and decrease costs at once.

Keywords: Scientific production, Ecological restoration, Planting methods, Wildling seedlings

Introduction

The anthropization is the main cause of the deforestation, and the most extensive global direct driver of land degradation is expansion and unsustainable management of agriculture (Foley et al. 2005; IPBES 2018). One of the alternatives to mitigate these growing problems is restoration (Pausas et al. 2006; Roberts et al. 2009; Aronson and Alexander 2013). Restoration is an ancient practice, being performed around the world (Rodrigues and Gandolfi 2001). Currently, most of the forest restoration methods involve planting of seedlings or direct seeding of tree species (Rodrigues and Gandolfi 2001; Wortley 2013; Palma and Laurence 2015). There are many challenges in relation to the trajectories of restoration practices, such as the inclusion of native species and the high cost associated with the most common techniques (Rodrigues et al. 2012). Several restoration techniques have been improved aiming to overcome restoration bottlenecks (Cole et al., 2010).

One of the practices used is the collection and transplantation of the wildling flora, from young individuals collected in the forest floor and transplanted to new sites aiming them to become adults (Viani et al. 2007). This practice has been promising for the implementation of restoration projects owing to elimination of conventional steps that depend on seed collection and/or seedling production and allows conservation of local genetic material. This results in plants with greater climatic adaptability (Nave 2005; Viani & Rodrigues, 2007; Rodrigues et al. 2009; Calegari et al. 2011). In addition, it can be used in remote areas, difficult to access, where it would be challenging for the arrival of nursery seedlings or where there is a lack of native seed supply and community-based seed networks. Compared to the methods such as direct seeding or seedling planting/container stock (Palmerlee and Young 2010, Palma and Laurence 2015), directly transplanted wildling that comes from regeneration, eliminating some steps such as seed collection, germination, and development to the ideal size in nurseries (Calegari et al., 2011). These stages are mostly complicated, owing to factors such as seed dormancy, low germination rates, and predation of fleshy fruits (Brito and Martins 2007). On the other hand, we need to better understand the constraints of this practice, such as damage to the root system, accompanying diseases, and even the impact on the natural environment (Viani and Rodrigues 2007, Viani and Rodrigues2008). A compilation of results already obtained around the world on this practice certainly will be very helpful for this promising method in ecological restoration (Viani et al. 2007; Nave 2005, Calegari et al. 2011).

Therefore, we examined, through a bibliometric analysis, the studies already carried out on transplanting seedlings from natural regeneration, organizing information on the environments in which they were used and costs on a global scale in order to assist future ecological restoration programs. In this way, we aim to obtain details of available studies and answer which ecosystems worldwide have been used this practice, the number of species used, and if the survival index is related to the studied environments and growth forms. Moreover, the compilation of these results could help overcome bottlenecks and future challenges of restoration and enhancing species diversity.

Methods

Bibliometric analysis

We did an advanced search through the platform *Web of Science.* The database *Web of Science©* was chosen because it is considered the most extensive and multidisciplinary basis, being widely used by academics worldwide (Azevedo et al. 2005). On the platform, we have the following databases: Main collection of Web of Science, Derwent Innovations Index, KCI - Database of Korean journals, Russian Science Citation Index and SciELO Citation Index. In the Web of Science, we used the advanced search with the following combination of terms: seedling transplanting; seedling wildling; seedling rescue; seedling transfer accompanied by the term "AND restoration". After the individual search of each word, we combine the searches by the term OR. We refine the search to only scientific articles and reviews. We do not include abstracts of conferences, dissertations, and theses, being considered gray literature (Wortley et al., 2013). The period of the search was from 1988 to April 2019. After screening generally for these keywords, we generated a pdf list of all papers. From these generated lists, we read the abstracts and discarded the articles that did not correspond to the evaluated practice or that were not of related areas.

Data analysis

We read the selected articles and analyzed it according to the following variables: (1) publication data: year of publication and number of citations per article; (2) field and restoration information: available coordinates, ecosystem/ biome/ geographical region, number of species used to implement the technique, growth form of species (tree, shrub, and herbaceous (terrestrial and aquatic)), survival rate, time of restoration, time needed for the collection period of the individuals, duration of monitoring time, and the costs of this practice. We divided the regions according to the type of environment. Firstly, by ecosystems: tropical forests (rainforest), wetlands (areas of permanent or temporary swamps, including Amazonia and Brazilian Pantanal biome), and prairies. Afterwards, we subdivided the wetlands according to Finlayson and van der Valk (2012) in: estuarine, marine, swamps, mangroves, lakes, and coastal. For cost survey, we averaged by region in order to compare the cost of the practice in different environments. We also did a simple linear regression to explain the relation between the number of species / life-forms used and percentage of survival withdrawn from the articles. Statistical analysis was done using the free software R (R Core Team 2018).

Results

Publication data

After searching in the *Web of Science* platform, we initially found 428 articles, of which only 31 were included in the analysis after screening (tab. supl. 1). Among these, the vast majority of selected articles (86%) presented the variables we were investigating. The first article found using this technique was published in 1998 (Balestri et al., 1998) (Figure 1). This pioneering article discusses the survival and growth of *Posidonia oceanica* (L.) Delile seedlings from natural regeneration after been transplanted in degraded coastal areas (Balestri et al. 1998). From years 2001 to 2003 and 2005 to 2006 we did not find publications on this subject (Fig. 1). The highest number of articles found per year was five, in 2015. Considering the citations per article and year of publication, the first article of 1998, above mentioned had 79 citations. The article with the highest number of citations was published in 2011, with almost 200 citations (Godefroid et al. 2011). This article mentions the use of the technique as a way of reintroduction of species in places that presented low density and or are in extinction process, with the title "*How successful are plant species reintroductions*?." Overall, the mean citations were 30.9 in the total of 31 article surveyed and an average of two published articles per year.

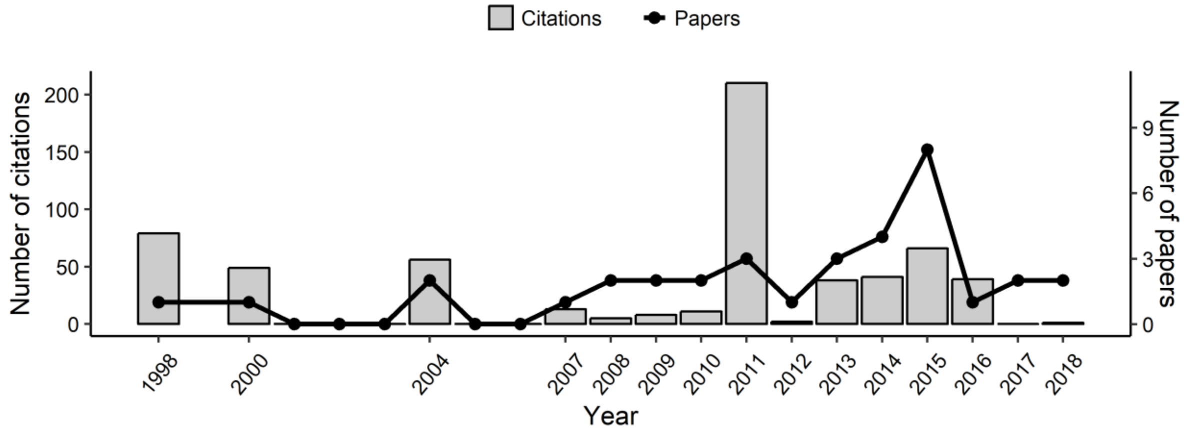

Figure 1. Number of published papers per year and their total of citations in the period from 1998 to 2018 on seedling transplantation.

Field and restoration information

Geographical distribution of data

Most of the research was done in the tropical belt of the world (Fig. 2). The largest number of studies was in South America (17), mainly in Brazil (9). We also found studies conducted in Africa (5 studies), Australasia (2 studies), Europe (2 studies), and North America (4 studies). Some studies have also been conducted in freshwater or marine environments.

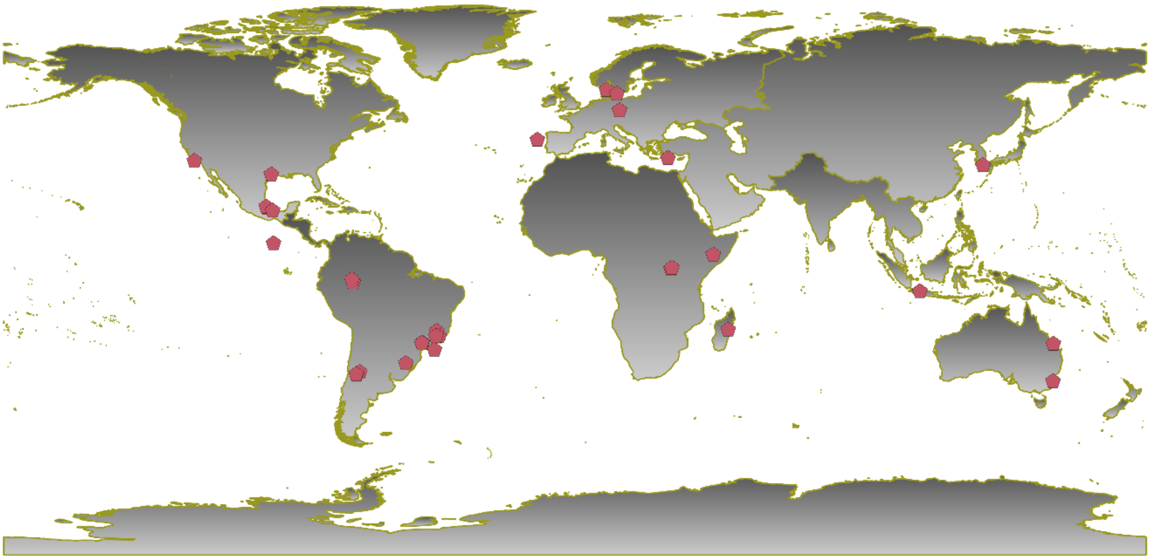

Figure 2. Global map of sites that have been implemented seedling transplant practice for using in restoration.

Considering the ecosystem types, wetlands (54%) were the most studied, followed by tropical forests (38%), and prairies (8%) of included studies. The studied wetlands ranged from coastal areas (27%) to mangroves and lakes (4%) (Figure 3).

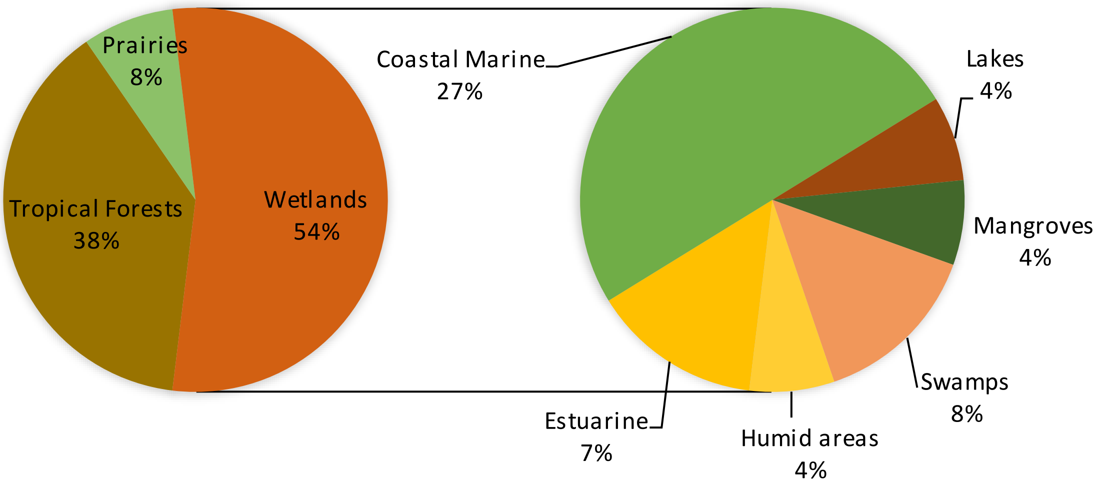

Figure 3. Published papers (%) by ecosystem type that have been implemented seedling transplant techniques for restoration.

Number of species and survival rate

The number of species used in the articles was low (average of none species). Most of the articles used only one species for the transplant (87%), while only 12.9% of the articles included more than ten species (Figure 4). Tropical forests were the type of ecosystem in which they transplanted the largest number of species (from 60 to 119 species), more specifically in Brazil (Atlantic Forest). Approximately 32% of the studies used an intermediate stage after collection, such as rooting development period in greenhouse or nursery/shading net (Otsamo and Vesa 1998) and rustification of the seedlings (i.e., period with few watering and sun exposure before planting) (Viani et al., 2007; Ribeiro et al. 2011; Meli and Dirzo 2013), resulting on average of 68.5% of survival. Others (29%), transplanted immediately between 2h and 24h after removal of the seedlings from the soil (Gruezmacher and Duivenvoorden 2008; Li et al. 2010; Turchetto et al., 2016), which presented, on average, a higher survival rate (76.9%),

In terms of growth forms, most of the studies used tree species (48.3%), followed by herbs (35.4%), aquatic weeds (8.2%), and shrub species (12.9%) (Figure 4). Most of the studies in the wetlands were with aquatic herbs for transplantation (74.6%), mainly in mangrove and marshland regions (87.4%). Most of the tree species used occurred in tropical forests (83.4%), mangroves, and coastal areas (16.5%) (Figure 4).

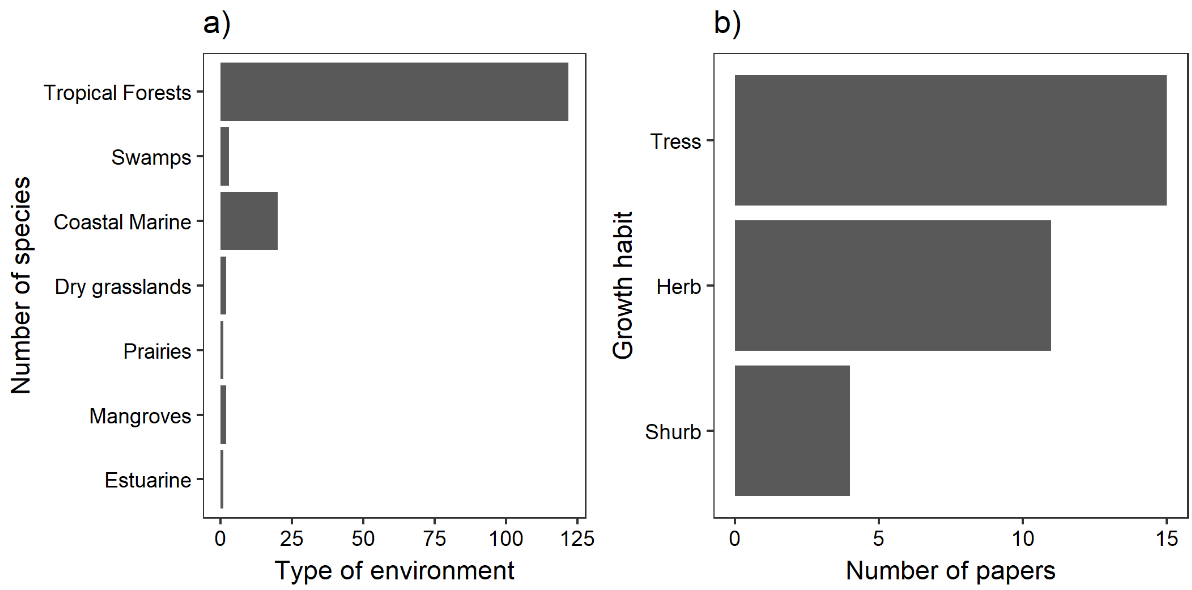

Figure 4. Number of papers on seedling transplantation published between 1998 and 2018 regarding: (a) number of species used by ecosystem types; (b) number of papers by growth habit (trees, herbs/ marine grasses, and shrubs).

Almost all articles included the survival index information, from experiments in nurseries to field implantation for restoration. The highest average survival rates were recorded in tropical forests, followed by prairies, estuarine, and coastal areas. The lowest survival rate was recorded in swamps areas, with approximately 27% survival (Figure 5).

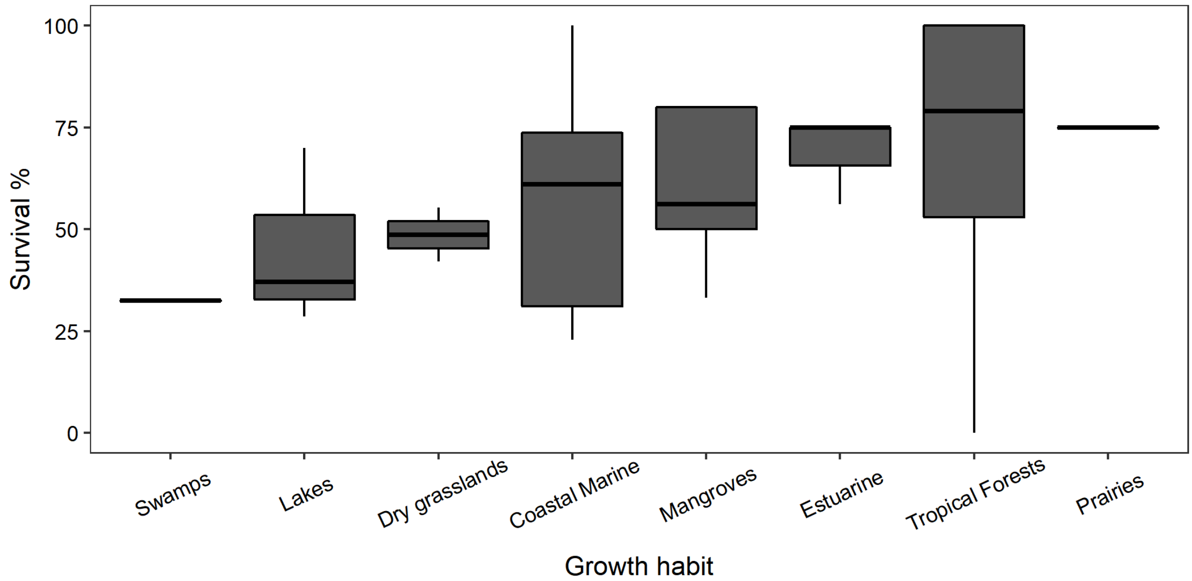

Figure 5. Seedling survival (%) after transplantation according to type of ecosystem in articles published from 1998 to 2018.

Tropical forests had the highest survival rates and studies that used more than eight species. The study with the highest survival rate was published in 2010 (Park and Lee 2010). The authors of this study carried out a research using an herbaceous species in coastal areas of Korea with 100% survival. The lowest index was found in Australia, in marine areas, including an herbaceous species, with 22.3% survival (Evans et al. 2018). We did not see relation of the survival index with the number of used species (Figure 5), or the growth form of the transplanted species (*F* value= 3.25, *p* value =0.74).

Monitoring and planting time, and costs

The sampling time used for the restoration / transplant was recorded in only two articles. A study that monitored for 17-month and that was carried out on mangroves that took six months to transplant, for a species that reached a 50% survival after planting (Abbot and Marohasy 2014). The other article monitoring along 13-month and that the transplanting of individuals was performed after 24h reached 75% survival after planting (Li et al. 2010). The monitoring time after planting was 16.5 months on average, while the shortest monitoring time after planting was 70 days, in tropical forest for the transplantation of two tree species (Gruezmacher and Duivenvoorden 2008).

From the 28 analyzed studies, seven stated the importance of developing restoration with economic perspectives, but only two provided real cost of this practice. The average cost of this practice for environments degraded by mining is U$ 2.100-3.400. ha^{-1} in tropical forest regions (Román-Dañobeytia et al. 2015). The other study that calculated costs, estimated a value of U$ 500,000/ thousand mature plants that were removed from soil with excavator and were cultivated over three years (U$ 50.00/mature plant) in mangroves (Abbot and Marohasy 2014).

Discussion

Number of articles, citations per year

The number of restoration studies (31) that used seedling transplantation as a practice is still low on a period of 15 years, proving itself to still be a new field for studying and practice in the restoration ecology. Another fact that could have influenced our findings, would be the non-inclusion of gray literature, as dissertations, theses, and reports that involved the practice. In addition, many articles were discarded for using the collection of the seed bank and then transplanting to the soil for restoration (Nguyen et al. 2017; Zhao et al. 2017; Le Stradic et al. 2015), therefore, being considered as transplantation of propagules and not of seedlings. The average of two articles published per year is, however, higher than other techniques found for restoration, such as nucleation use, mean of 0.7 articles per year (Boanares and Azevedo 2014). We highlight that from 2008 at least one article has been published per year, demonstrating a growing branch of research.

The number of citations recorded by the *Web of Science* platform was relatively low, possibly because there are few publications on the subject, which also reflects the few quotes.

The relation between a few articles with many citations is generally common in science. As articles become older, citations can increase such as Balestri et al. (1998) with 79 citations. To corroborate this, the most recently published articles presented few citations compared to the oldest articles. Although the article by Balestri et al. 1998 is the oldest of this research, cited by them, other previous articles have also mentioned the transplantation of seedlings or seeds as alternatives to restoration (Meinesz et al. 1991). Therefore, the use of seedling transplants may have been boosted in the period 1991 to 1998, pointing out the lack of technical attention to restoration processes in the world (Meinesz et al. 1991). Even so, we do not currently find great attention for the use of this practice for ecological restoration. (Harrison and Gregorio 2010). One of the reasons is the better knowledge of other restoration techniques commonly and more consolidated used worldwide, as planting trees and sowing seeds. Seedling transplanting is still being investigating for survival improvement. For instance, we observed that only part (9%) of the included articles presented innovations in the seedling transplantation technique, as the use of containers for translocation of large numbers of seedlings (Abbot and Marohasy 2016).

Geographical distribution of data

We found studies done in several geographic regions / ecosystems. Some of them were performed in close places (i.e., nurseries), which justifies some geographical points overlapping on the map (Figure 2), as well as owing to different published studies made in the same geographical area for experimentation by same authors (Viani and Rodrigues 2009, Viani and Rodrigues 2008, Rodrigues et al. 2010). Brazil was the country with more studies, mainly in the southeastern region of the country. The southeastern region of the country covers the most degraded Brazilian biome (Atlantic forest), where it is also the most developed region and, consequently, there are more research groups studying and publishing about restoration (Brancalion et al. 2010). The first report of the use of this practice was in

1862, in the Tijuca National Forest, in a restoration of degraded area, with young individuals removed from surrounding forests (Cézar and Oliveira, 1992). From that initial moment, other experiments were carried out with the transplant technique of individuals in regeneration (Viani 2005). In Brazil, the use of this practice was due to its practicality and low cost (Corvello, 1983, Thoma 1998, Pareja 1998).

Wetlands were the most researched ecosystem for the use of the seedling transplant (Figure 3). Within wetlands, the great majority of the studies occurred in coastal regions, which justifies the position of the great majority of the points in the global map (Figure 2). This large number is associated with the first published articles, such as de Balestri et al. (1998), covering large questions and surveys about wetlands from the 2000s. In addition, the following articles were conducted in Colorado wetlands (Cooper and MacDonald 2000). Among a quarter of studies were made in tropical forests, as in Brazil in the Amazon and in the Atlantic Forest biomes (Viani and Rodrigues 2007; Viani and Rodrigues 2008; Gruezmacher and Duivenvoorden 2008). Motivation for using these practices in all these studies were justified for limitations of species in nurseries, regarding mainly other life-forms availability, such as herbaceous. In addition, seed accessibility is also indicated as an important factor in the use of this practice.

Number of species, growth forms, and survival rate

Most studies used a low number of species (average of four species) compared to the value found for the most commonly restoration techniques (average of ten species per study with direct seeding and five with seedling planting (Palma and Laurence 2015). The pioneer study of 1998 (Balestri et al., 1998) investigated only one herbaceous species to test the practice of seedling transplantation. Considering that the practice of collecting and transplanting from natural regeneration is capable of using many numbers of species (Nave et al 2007), our results shows very few studies with large numbers of species. Studies with more than eight species were represented by the seedling collection and their adaptation in nurseries (Viani and Rodrigues 2008; Calegari et al. 2011). It does not present a posterior test of field implantation for restoration purposes, but they only suggest the use of previously rustified and adapted species. In addition, studies with large numbers of species are restricted to one type of environment, tropical forests such as Atlantic Rainforest and Amazon. These studies used seedlings from places where native vegetation clearing were authorized for agricultural or hydroelectric purposes, where a rescue of high number of species and life forms is possible without affecting population from natural remnants. On the other hand, studies that used low richness in regions such as savannas and wetlands, focused on using key species with a high dominance.

Almost half of the studies used tree species confirming world trend that tree species were the first to be used for forest restoration in most parts of the world (i.e., tropics), and only in recent years the need for using other growth forms have been highlighted (Garcia et al. 2016; Mayfield 2016). Conversely, we found a great number of studies with herbaceous species (35.4%) because most of them included researches in wetlands, where there is a great abundance of aquatic and herbaceous species.

Regarding the survival rate, the high values come from studies in regions of tropical forests with high species richness, being these mainly trees. However, as previously mentioned, survival data are available for studies that have been made in nurseries after soil removal (Viani and Rodrigues 2008; Calegari et al. 2011) and there is no available data about survival rate after transplanted seedling from regeneration planted directly to the field. The large number of surveys in wetlands shows that the lowest survival rates recorded were in marine areas (Figure 5). Hence, these experiments in marine areas may have had low survival rates because they are more unstable environments, exposed to tides and floods, being more difficult to reach restoration success (Park and Lee 2010). Moreover, another factor that can influence all restoration practices, and is independent of the region, is that the survival depend on the species adaptability (Viani and Rodrigues 2007). For example, some coastal areas have 100% survival for a given species, so this success may depend exclusively on the choice of the correct species to be used (Park and Lee 2010). There is great natural variation in survival between species and botanical families. Most studies in tropical forests, with a large number of species in nurseries, obtained a variation of survival according to the species. This large variation in species survival may reflect the field results depending on the species (Viani et al. 2007).

Planting and monitoring time, and costs

Post-planting monitoring time (16.5 months) is equivalent to the average time found for studies of other techniques. This value can be justified by the short duration of studies and projects usually coincidentally until the availability for monitoring are exhausted. Regarding time of collection between the withdrawal and planting of the seedlings is indicated by some authors to be in 24 h aiming to reduce the water stress and, consequently, to decrease the mortality of the seedlings (Nave 2005, Li et al. 2010; Calegari et al. 2011).

To compare with other techniques such as direct seeding and tradition seedling planting, in which by the way a review showed that cost varies from U$ 700 to 900 ha-1 (Palma and Laurence 2015), we do not believe our data is comparable because we found transplant costs information only in two studies. One study in mangroves found a high cost of U$ 50.00/mature plant (Abbot and Marohasy 2014). However, the method used for the transplant was quite different; they used excavator to remove big trees and put them into polyethylene containers for transportation and monitored the development of the plants over three years, which may highly increase the costs. The other study averaged U$ 2,500 ha-1 and used seedling transplant for restoring a degraded mining in the tropics (Román-

Dañobeytia et al. 2015). These costs included the use of substrates and fertilizers, for the production of seedlings in nursery, planting, and monitoring.

Conclusions

The practice of seedling collection from natural regeneration followed by transplantation to the field is rarely used or few results have been published worldwide. Nevertheless, our data have shown that this practice can be efficient in terms of using high diversity of plants for ecological restoration purposes from places where vegetation clearing for development projects takes place and are authorized. Moreover, seedling transplanting can increase nurseries richness and be complementary to other common planting techniques. However, our data show that the number of species used is still low, conversely from the main goal usually attributed to this practice, which is enhancing species richness. Most of the research used tree species. Nevertheless, the current concept of inclusion and complementation of other growth forms besides trees is still needed for studies around the world. Wetlands and tropical forests have more studies and knowledge for the use of this practice compared to dry environments and savannas, where this technique is sporadically used, but it may still have potential for these regions. Data about costs were also extremely rare, and the articles did not provide detailed data. Considering that cost is the most important information used to decide about viability of using the practice on large-scale, future studies should inform and detail it deeply, especially regarding cost-effective methods. Similarly, information about transplanting time after collecting are still rare, but few available data show better survival after 24h; therefore, further studies are necessary to confirm this information. Moreover, we highlight need of attention on local environmental impact over natural regeneration, because it can occur in cases of mismanagement. Hence, it is indicated to focus on collecting only species with high regeneration density. Finally, considering its potential to be used in sites without nurseries, collecting wildling seedling stock is also an option for remote areas with difficult access or with scarcity of local seed collection.

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Annex I - Tab. 1 Suplementary

Table 1. List of articles included for bibliometric analysis with authors, journals, title, location, and number of citations.

Author	Journal	Title	Country	nº of citations
Evans et al. 2018	Restoration Ecology	Assessing the effect of genetic diversity on the early establishment of the threatened seagrass *Posidonia australis* using a reciprocal-transplant experiment.	Australia	0

Author	Journal	Title	Country	nº of citations
Phong et al. 2017	Ocean & Coastal Management	Mangrove transplantation in Brebes Regency, Indonesia: Lessons and recommendations.	Indonesia	0
Henry et al. 2017	Mycorrhiza	Transfer to forest nurseries significantly affects mycorrhizal community composition of *Asteropeia mcphersonii* wildlings	Madagascar	0
Turchetto et al. 2016	Forest Ecology And Management	Can transplantation of forest seedlings be a strategy to enrich seedling production in plant nurseries?	Brazil	1
Roman-Danobeytia et al. 2015	Ecological Engineering	Reforestation with four native tree species after abandoned gold mining in the Peruvian Amazon.	Peru	10
Buisson et al. 2015	Restoration Ecology	Limiting processes for perennial plant reintroduction to restore dry grasslands.	France	3
Abbot e Marohasy 2014	Wetlands Ecology and Management	The excavation and cultivation in containers of mature grey mangroves, *Avicennia marina*.	Australia	4
Castanho e Prado 2014	Plos One	Benefit of shading by nurse plant does not change along a stress gradient in a coastal dune	Brazil	4
Fuentealba Martinez-Ramos 2014	Agroforestry Systems	Transplanting native tree seedlings to enrich tropical live fences: an ecological and socio-economic analysis	Mexico	9
Duclos et al. 2013	Biotropica	Shrub cover influence on seedling growth and survival following logging of a tropical forest.	Uganda	16

Author	Journal	Title	Country	nº of citations
McAdoo et al. 2013	Rangeland Ecology & Management	Site, competition, and plant stock influence transplant success of Wyoming big sagebrush.	EUA	15
Meli e Dirzo 2013	Applied Vegetation Science	Effects of grasses on sapling establishment and the role of transplanted saplings on the light environment of pastures: implications for tropical forest restoration.	Mexico	7
Viani et al. 2012	Revista Arvore	Leaf area reduction and transplant timing for the use of seedlings from understorey in forest restoration.	Brazil	2
Ribeiro et al. 2011	Revista Árvore	Survival and initial growth of Euterpe edulis Mart. seedlings transplanted to gaps and understory in a Semideciduous Forest, Viçosa, MG.	Brazil	8
Godefroid et al. 2011	Biological Conservation	How successful are plant species reintroductions?		200
Calegari et al. 2011	Revista Árvore	Seedling production of native tree species in nurseries via rescue of young plants.	Brazil	2
Li et al. 2010	Estuarine Coastal And Shelf Science	Assessing establishment success of Zostera marina transplants through measurements of shoot morphology and growth.	China	
Park e Lee 2010	Ecological Engineering	Development of transplantation method for the restoration of surfgrass, *Phyllospadix japonicus*, in an exposed rocky shore using an artificial underwater structure.	Coreia	11

Author	Journal	Title	Country	nº of citations
Dostalek et al. 2009	Central European Journal Of Biology	Planting of different-sized tree transplants on arable soil.	Czech republic	2
Viani e Rodrigues 2009	Scientia Agricola	Potential of the seedling community of a forest fragment for tropical forest restoration.	Brazil	6
Gruezmacher e Duivenvoorden 2008	Colombia Forestal	Growth of transplanted timber species seedlings in the south of the Colombian Amazon: a preliminary study.	Colombia	1
Gruezmacher e Duivenvoorden 2008	Colombia Forestal	Growth of transplanted timber species seedlings in the south of the Colombian Amazon: a preliminary study.	Colombia	1
Viani e Rodrigues 2008	Acta Botanica Brasilica	Impact of seedling removal on regenerating community structure of a seasonal semideciduous forest.	Brazil	4
Viani e Rodrigues 2007	Pesquisa Agropecuaria Brasileira	Survival in nursery of native species saplings obtained from natural regeneration of forest fragments.	Brazil	13
Bull et al. 2004	Restoration Ecology	An experimental evaluation of different methods of restoring *Phyllospadix torreyi* (surfgrass).	EUA	24
Lof et al. 2004	Forest Ecology And Management	Sowing and transplanting of broadleaves (*Fagus sylvatica* L., *Quercus robur* L., *Prunus avium* L. and *Crataegus monogyna* Jacq.) for afforestation of farmland	Denmark and southern sweden	32

Author	Journal	Title	Country	nº of citations
Cooper e MacDonald 2000	Restoration Ecology	Restoring the vegetation of mined peatlands in the southern Rocky Mountains of Colorado, USA.	EUA	49
Balestri et al. 1998	Journal Of Experimental Marine Biology And Ecology	Survival and growth of transplanted and natural seedlings of *Posidonia oceanica* (L.) Delile in a damaged coastal area.	Italy	79

CAPÍTULO II

Can transplanting seedlings with protection against herbivory be a restoration strategy for seasonally flooded environments?

Letícia Koutchin Reis[1]; Geraldo Alves Damasceno Junior[2] and Letícia Couto Garcia[2]

[1] Programa de Pós-Graduação em Biologia Vegetal, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS.

[2] Laboratório de Botânica, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS.

Abstract

The transplantation of wildlings/seedlings from natural regeneration allows us to use acclimatized individuals increasing species diversity, growth forms, preserving local and endemic genotypes. Besides it, reduces the ecological restoration costs. Considering flooding as an abiotic filter for restoration success, we tested the method of transplanting seedlings from natural regeneration in periodically flooded areas. We tested the effect of height range of wildlings, topographic position (where them were collected and outplanted), and protection against herbivory on survival, growth, and herbivory rate to set up an ideal arrangement for transplanting. Hence, we tested experimental arrangements involving two collection and planting areas (high and low topography), two height classes of seedlings and the use of protection against herbivory of mammals. We also evaluate involved costs. The best experimental arrangement for four studied species considering the evaluated factors was found for small seedlings (10-39 cm) collected in low topography, planted in high topography, and with protection against herbivory. *Inga vera* was the species with the highest survival rate. Besides it, we stress that even under unusual extreme flood event (high flooding over 7 months) it was possible to reach a significant survival rate. The costs of implantation are lower (U$ 739ha-1) than those found in previous studies and the use of protection against herbivory was also considerably low (US$ 2.484.ha-1). However, the estimated costs are for experimental scale and do not represent the use for large-scale, which could reduce and improve cost-benefits significantly. Besides it, in remote places, such as Pantanal wetland, the difficulty of obtaining seedlings is due to the lack of nursery infrastructure, high associated costs of seedling production, and vulnerability to herbivory resulting of high large-mammals density found are the main challenges for restoration. Hence, wildling collection can be an interesting strategy to overcome these bottlenecks.

Keywords: Ecological restoration, Control of herbivores, Pantanal Wetlands, Tree shelter.

1. Introduction

Restoring periodically flooded areas faces major ecological challenges, ranging from abiotic and biotic filters (Hobbs and Norton 2004, Nuttle 2007). The flood pulse is one of the elements governing the establishment of several plant species in seasonally flooded areas (Pott et al. 2009). Depending on the topography of the environment, the length of pulses may vary and the aquatic phase may be more pronounced in lower topography positions (Damasceno- Junior et al. 2005, Junk et al. 2006). Hence, during the beginning of restoration project, the ideal size of the seedlings is a challenge to escape from water depth considering periodic floods. Besides it, hypoxia occurs in these flooded environments and affects physiology of tolerant species that develop different survival strategies (Medri et al. 2002, Kreuzwiese et al. 2004, Colmer et al. 2009). Some responses to flooding may include increasing of vegetative reproduction such as sprouting, decreasing of leaf growth, or even growth pauses to save energy, and activation of anaerobic routes owing to low oxygenation (Medri et al. 2002; Parolin and Wittmann 2010). Therefore, restoration in wetlands must take into account the effects of flooding restricting the species selection to previously adapted and resistant species (Damasceno-Junior et al. 2005; Arruda et al. 2016). This makes restoration a challenge because reduces the time for planting because of the flooding.

Optimal planting time should be well planned, since extremely wet and soaked periods may be highly unviable for shorting the time of outplanting. In addition, the choice of species is one of the main challenges in restoration (Rodrigues et al. 2009). Limited knowledge of native species, phenology, physiology, and mechanisms of dormancy, as well as high rates of predation are some bottlenecks (Zamith and Scarano, 2004; Fenner and Thompson, 2005). Other challenges for wetland restoration in remote locations is the availability of nurseries with regional native species and difficulty in collecting seeds owing to flooding periods. Nowadays, great difficulty has been reported in obtaining regional seedlings available in nurseries with high diversity (Rodrigues e Gandolfi, 2001; Laudonceur et al. 2018) and belonging to functional groups such as zoochoric, recalcitrant seed species, and from other growth forms beside trees. As an alternative, the use of seedling transplantation from natural regeneration have been considered for restoring vegetation (Viani et al. 2007; Calegari et al. 2011) and decreasing costs. This technique eliminates some steps such as phenological monitoring, seed collection, and seedling production or direct sowing. The collection of seedlings from natural regeneration goes through the acclimatization stage, which is shorter than other methods (Turchetto 2016) using individuals already adapted to local conditions (e.g., soil, moisture, natural enemies, etc.). In addition, the practice favors the inclusion of different growth forms (Viani et al. 2007) enhancing diversity of functional groups.

However, many adjustments need to be made to maximize survival in order to obtain greater species richness, mainly in relation to tolerant species behavior, seedling size, and biotic filters such as predation by herbivorous ants and mammals (Turchetto et al. 2016). Herbivory is one of the main biotic filters of ecological restoration success (Keeton 2006). Herbivores such as arthropods and mammals can act as pre-existing filters, impairing plant development from its establishment to adults (Ferreira, 2005) (e.g., changes in the quantity of photosynthetic tissues) (Montti, 2016), and may even cause mortality. Hence, in order to overcome this filter in restoration, protection anti-herbivory has been used, increasing the survival and performance of implanted seedlings (Conner et al. 2000; Keeton 2006; Dick et al. 2016). Recently, other methods of protection against animals are even indicated for sowing, as protective capsules that prevent rodent predation, maximizing the survival and success of ecological restoration (Castro et al., 2015).

However, costs are among the biggest bottlenecks to scaling up ecological restoration (Brancalion et al. 2012). In Brazil, the average cost of conventional seedling planting is among U$ 1.500 to ~4.000 ha-1 (Antoniazi et al. 2016). Considering cost of protection shelter against herbivory, this value goes up even more, although its use has been crucial to increase seedling survival and growth in wetlands (Keeton 2008). In view of high cost of planting in difficult-to-access places, restoration in flooded ecosystems may become more complicated. As an alternative to overcome these challenges, we want to reply the following questions: (1) Is the use of wildlings in seasonally flooded environments feasible for restoration regarding survival, growth, foliar investments and costs? (2) Does the topographic position of wildling collection and outplanting, seedling size and use of protection against herbivory have an effect on survival, growth, and rate of herbivory? Our hypotheses are: (1) transplanting of taller seedlings (30-60 cm) may cause greater survival, resulting in more rapid growth in height owing to leaf escape from water blade; (2) the best arrangement will be to collect seedlings in low topographic position, since they would be prior exposed to longer periods under flooding, and transplanted to less exposed flooding areas (high topographic position), decreasing flood stress of individuals previously acclimated to longer period of inundation; (3) the use of protection against herbivory favors survival, resulting on a greater number of leaves and leaf area, since this environment has a high diversity of herbivores. (4) Finally, for large-scale purposes, we investigated if the cost involved in this practice will be lower than that found for other conventional restoration techniques, even with the inclusion of protection against herbivory.

2. Methods

2.1 Study area

The Pantanal is an extensive wetland savanna in tropical South America, covering about 137.000 km² in the Paraguay river basin (Hamilton 1996). We conducted the study in an area of riparian forest on the right bank of Miranda river, “Passo do Lontra,” municipality of Corumbá, Mato Grosso do Sul. This area belongs to the Biologic field station of Pantanal (*Base de Estudos do Pantanal* – BEP) of the Federal University of Mato Grosso do Sul (19º34'37″S e 57º00'42″ W).

The climate is seasonal Awa in Köppen classification characterized by dry season from May to September and rainy season from October to April. The annual precipitation is approximately 1,090 mm, with an average annual temperature range between 21°C in December and 27°C in July (Hamilton et al., 1996). The Pantanal has several subregions that are characterized by their seasonal flood regime, soils, and cultural aspects (Silva & Abdon 1998). In the region of Miranda/Abobral, municipality of Corumbá, the flood can vary from 2 to 6 months, and can reach up to 4.000 km² with annual cycle between the months of December and June (Hamilton 1996). The vegetation is defined according to its diverse topographies (Damasceno-Junior et al. 2005; Junk et al. 2006), where there is a mosaic composed of monodominat stants such as “Paratudal” composed mainly of *Tabebuia aurea* Benth. & Hook. f ex S. Moore (Bueno et al 2014), palmland: *Mauritia flexuosa* L., *Attalea phalerata* Mart. ex Spreng and *Copernicia alba* Morong ex Morong &. Britton (Pott et al. 2011), riparian forests and riparian capons: *Rhamnidium elaeocarpum* Reissek, *Sapium longifolium*. (Müll.Arg.) Huber., *Inga vera* Willd, and *Tocoyena formosa* (Cham. & Schltdl.) K.Schum. (Damasceno-Junior et al. 1996).

2.2 Collection of seedlings of natural regeneration

Owing to recommendations in previous studies when the collection of seedlings is made in natural environments (Viani et al. 2007), we chose four species focusing on the most abundant ones found in surrounding remnants aiming not to impact natural population dynamics. These species are of different growth forms: *Psychotria carthagenensis* Jacq. (Rubiaceae) is a shrub, *Ocotea diospyrifolia* (Meisn.) Mez (Lauraceae) and *Inga vera* Willd. (Fabaceae) are trees, and *Attalea phalerata* Mart. ex Spreng (Arecaceae) is a palm tree. All these are animal-dispersed species, which may, in the future, provide food resources, being able to attract the fauna to the restoration site (Garcia et al. 2009; Garcia et al. 2014).

In April and May of 2018, we selected two stands of 1,082 ha of riparian forest, included two topography position to collect seedlings, defined according to the last watermark left by the flood on trunks of trees and consequently the time of flooding. We considered low topographic position areas with watermark on trunks >50 cm and larger flooding time and the higher topographic position areas with watermark on trunks <50 cm and consequently with shorter flood time (figure S1a). We collected two height classes for all species: seedlings from 10-39 cm and seedlings from 40-69 cm.

Four people collected from the forest floor 416 seedlings of the four selected species (ca. 100 individuals per species). We collected more seedlings than necessary for the acclimatization process in case of replanting (figure S1b). On average we dug about 10-15cm, but that depended on the species, the *A. phalerata* for example was deeper. We gently pulled individuals up focusing on those that appear to be healthy and without injury. All the seedlings were removed with clod and uprooted until it was bare root. After extraction, we manually clean the roots and place it into containers with water (figure S1a). We cut off the older and more abundant leaves of the seedlings in order to reduce the water imbalance after the root removal of the soil (Calegari et al. 2011).

We transported the seedlings to the nursery (70% shade net), where they were placed in individual bags of polyethylene (20 cm x 30 cm) for acclimatization for 30 days, with regular watering. We used the substrate composed of clay soil and fine litter from the collection site soil. We dampen these substrates before to reduce root dryness (Cury et al. 2013). In the last seven days we stopped watering and exposed seedling to the sun for rustification (i.e., period with few watering and sun exposure before planting).

2.3 Implantation of the experiment

Thirty days after collection and acclimatization, we transplanted the seedlings to six experimental areas. Time frame of outplanting was four months before the flood (September 2017). Initially (i.e., before planting) we applied ant baits based on sulfluramide $3g.kg^{-1}$ (0.3%), for control of leaf-cutting ants of the genus *Atta* (saúvas) and *Acromyrmex* (quenquen), since bait distribution is part of silvicultural tracts for restoration purposes. We do not apply chemical or organic fertilizers for planting. Moreover, considering that areas were not subjected to invasive plants, no grass weed control has been made before planting. Conversely, these areas have been previously subjected to native weed control, because they were sites where people move frequently.

To control herbivory of large mammals, for protection against herbivory treatment, we evaluated the use of wire mesh seedling cages, hereafter called as wire mesh seedling protection. The implanted shelters of galvanized welded seedling guard mesh had 70cm of diameter, mesh one, wire 50cm x100cm, two wooden posts attached by plastic clam ties on the wire mesh (figure 2 and S1e). Aiming to protect mainly against the mostly common mammals (e.g., capybara and tapir that are smaller than 1m) shelters were one-meter height.

For outplanting the collected seedlings, we considered the same topographic definition used during collecting time. The factors tested were: topographic level (plants collected in lower topographic position and planted in higher / lower topographic position and contrariwise); seedling height classes for each collection topography; protection against herbivory (arranged in all height classes and collection topography positions) and species (four species of different growth forms). We installed all these combinations ensuring that all species were subjected to all possible factors (figure 2a). Inter-row spacing was 2mx2m. We inserted 24 plots of 6 m x 8 m, 12 plots implanted in spacing of in high topographic position and 12 plots in low topographic position, containing 12 individuals of all species per plot. Within the space destined to the planting, we transplanted 288 seedlings, being 72 seedlings for each species, of which 36 seedlings in each height class and protection against herbivory, totalizing nine individuals of each species in each combination of treatments. After the initial drought (one month of planting) we lost 92 seedlings, where all were planted again in the second data sample.

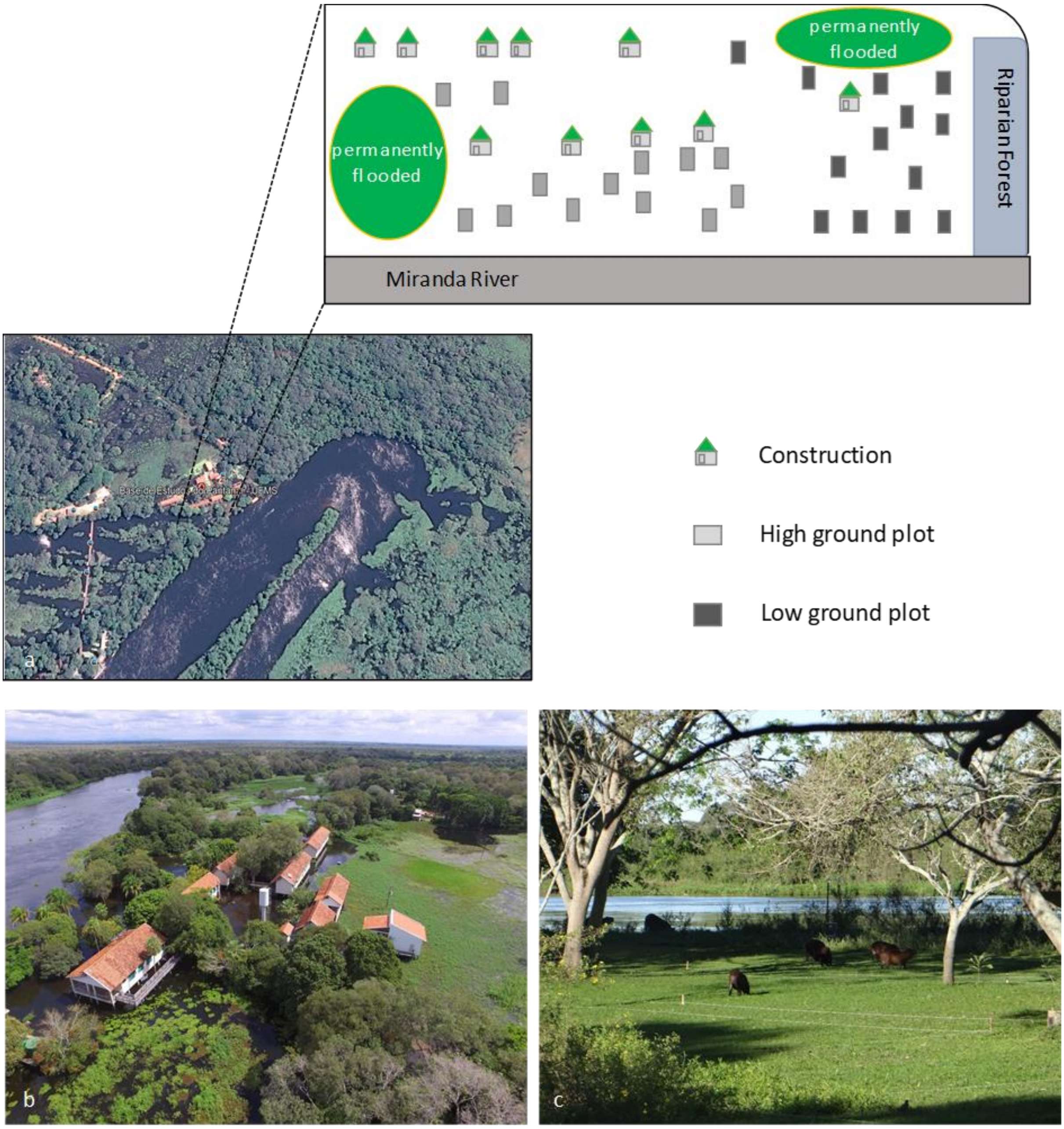

Figure 2. (a) Design of the restoration experiment testing the effect of the collection and planting topography position, height classes of the seedlings, and protection against herbivory treatment (see grazing capybaras in the (c)). Green circles represent permanently flooded areas. Experiment implemented in September 2017 and monitored until September 2018 at the (b) Biologic field station of Pantanal (*Base de Estudos do Pantanal* - BEP - UFMS), along the Miranda River (a).

2.4 Monitoring and data collection

To understand the early outplanting performance of the treatments (during the wet season), we monthly evaluated survival, growth, and herbivory of all individuals in the pre-flood (four months) time and post-flood (three months) time. The flooding period during the survey was exceptional, the lowest topographic position lasted 7 months, and the highest topographic position 6 months (figure 2b). Approximately 70% of the wire of shelters have been lost after flooding period, when we put them back and keep monitoring during post-flood. After 14 months of planting, no seedlings were higher than the wire of shelter, keeping the protection against herbivory over all study period. We consider as a dead seedling the ones that presented a dry stem from top to the base, without leaves. For survival of each species we consider the survival rate of the individuals in each implanted arrangement (n 36). We measured growth in total height (cm) with tape measure and with the digital caliper the diameter at the height of the soil (DHS), number of leaves, and regrowth of each seedling. For the palm species *A. phalerata* we measured height until the leaf apex and diameter at the base closest to the soil. As regrow we consider those individuals that had new branches coming from the roots or those with stem and absence of the shoot and the presence of new branches. For herbivory, we quantified the percentages of defoliation of all individuals. The apparently predated leaves were photographed and the percentage of lost area and leaf area (cm^2) was estimated by using AFSoft Software (Jorge et al. 2009).

2.5 Data analysis

For the herbivory analyzes, we transformed the percentages in herbivory index (IH), categorizing the percentages of herbivory into six categories (Dirzo and Domínguez 1995) (table S1). In order to test the best arrangement of this practice in a seasonally flooded environment, we evaluated the effect of flooding (pre and post-flood), collection and planting topographic level, height classes, and protection against herbivory, comparing survival, herbivory rate, leaf area, regrowth, and species implanted by using generalized linear models (GLM). We verified the initial growth of seedlings in diameter and height, by linear allometric regression for each species. We also evaluated the Relative Growth Rate (RGR) in height and DHS for each species in two sampled periods (pre and post flood).

Finally, we evaluated the quotation of this practice including the cost calculation of the protection against herbivory. We carry out budget research on the cost of all materials used in the collection, transplanting, and protection stages. We included the quotation of three different prices for the same input in commercial stores and averaged the value of each material. For the calculation of yield, we considered the total of 12 days of transplantation of 416 seedlings carried out by four people in half period (in the morning). For direct remuneration, we considered daily labor in the average value of U$15.42 in the region of Mato Grosso do Sul, Brazil. We calculate the value per seedling and per hectare. This calculation involved a considerable loss of 30% of seedlings, with replanting. For the seedling costs, we divided the total cost by the amount of seedlings in one hectare (inter-row-spacing of 2 x 2m: 2500 seedlings / ha). For cost-effectiveness purposes, we also calculated the value per seedling considering the number of surviving individuals of the four species and protection against herbivory in each sampling period (pre and post flood). We emphasize that did not consider administration and indirect labor costs (e.g., non-wage labor costs), or profits, since it was an experimental study. Moreover, the costs of site preparation for planting were not included, since the areas were not subjected to invasive grasses, nutrient depletion, or soil compaction.

3. Results

3.1 Survival

We found values from 0% to 80% of survival depending on the species or the experimental arrangement (tab 2). Flooding was the main factor affecting species survival. We stress that we made a 30% replanting to replace dead individual after initial drought period. From the first month of drought to the first month of flood (pre-flood period), survival was high (70%, 83% respectively). However, after an exceptional 6-month flood, general survival was lower (30%) (post-flood period). Most of the species found alive were *I. vera* and *O. diospyrifolia* (figure S1ad). Contrary to expected, these surviving individuals were more abundant in the shorter height class (10- 39 cm) (tab 2). Regarding the topography position, the seedlings collected at the low and transplanted at the high showed higher survival rate (42%) (tab 2). With respect to the protection against herbivory treatment evaluated, we found a positive effect on the survival of the seedlings. After the flood, the effect of the shelter still stood out over ($p < 0.05$) (table 2).

Table 2. Final survival percentage by period for each collection and planting position topography, height class, and protection against herbivory treatment by species. Obs: Survival value (%) on the left indicates pre-flood period (measured four months after planting) and value on the right indicates post-flood period (measured three months after finishing the flood). * indicates significant differences between treatments (p <0.05)

				Survival (%)			
Specie	**Botany Family**	**Growth habit**	**Succession group**	**planting position photography**	**Collecting position photography**	**Height class (cm)**	**Protection against herbivory**
Attalea phalerata	Arecaceae	Palm tree	Pioneer	high (55.8/34.7)* low (64.3/5.5)*	high (64.8/19.4) low (80.4/19.4)	10-39 (85/9.4)* 40-69 (41.9/0)*	control (61.2/13.1)* shelter (75.2/26.3)*
Inga vera	Fabaceae	Tree	Pioneer	high (70.3/50)* low (96.2/19.4)*	high (93.1/12.5) low (84.3/15.2)	10-39 (96.4/82.6)* 40-69 (63.5/56.8)*	control (63.5/9.7)* shelter (97.2/80.5)*
Psychotria carthagenensis	Rubiaceae	Shurb	Secondary initial	high (77.3/40.2)* low (80.6/20.8)*	high (87.6/26.3)* low(62.4/34.7)*	10-39 (71.3/60.1)* 40-69 (60.5/29.8)*	control (52/21.3)* shelter (89.6/44.4)*
Ocotea diospyrifolia	Lauraceae	Tree	Secondary initial	high (81.1/30.5)* low (91.2/8.3)*	high (92.3/7.8)* low (66.1/29.7)*	10-39 (88.4/80.5)* 40-69 (76.9/11.3)*	control (68.6/41.2)* shelter (80.2/56.1)*

3.2 Growth

In general, most monitored seedlings invested more in diameter than height (figure 3). The height class of the seedlings had no effect on the absolute growth of the species ($p>0.05$), .in addition, the periods sampled, pre and post flooding also had no effect on seedling growth ($p>0.05$).

Regarding species, *O. diospyrifolia* and *P. carthagenensis* invested more in height (figure 3). *P. carthagenensis* seedlings of 40-69 cm height class invested more in height than in diameter, which was different from all other species evaluated. There was no effect between collection and planting topography position on growth, nor between the periods monitored ($p> 0.05$).

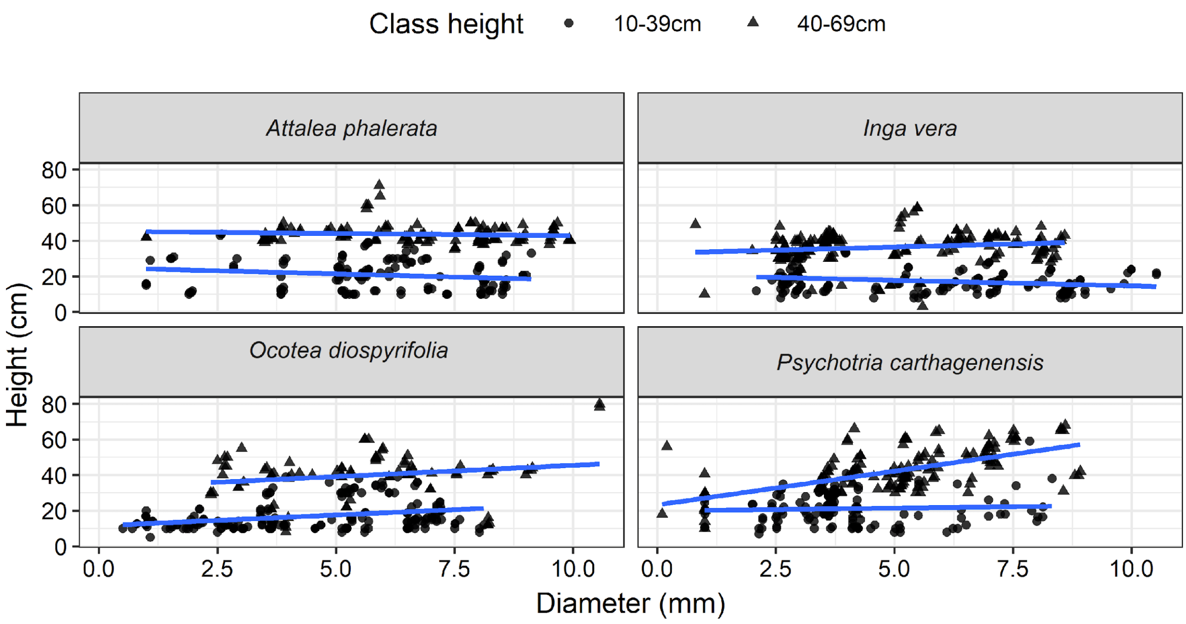

Figure 3. Final allometric relationship (i.e., measured three months after finishing the flood) between stem diameter at the soil level and height of the evaluated species (*Attalea phalerata, Inga vera, Ocotea diospyrifolia*, and *Psychotria carthagenensis*) in the two height classes collected in both collection and planting topography position under all monitored periods (eight months) in Riparian Forest of Miranda River.

Growth of smaller seedlings (10-39 cm) was higher in both height and diameter ($p < 0.05$). The species that most grew in height were *A. phalerata* and *P. carthagenensis* ($p < 0.05$), followed by *O. diospyrifolia* and *I. vera*. Regarding the diameter, *I. vera* stood out with rapid growth when lower height ($p < 0.05$), followed by *O. diospyrifolia*, *A. phalertata*, and *P. carthagenensis*. Seedlings of *O. diospyrifolia* and *I. vera* reduced their growth during flooding; however, in the month after the flooding, with their high relative survivals, these species were able to recover rapidly.

We found a negative effect after the flooding on the growth in height of the species studied regarding relative growth rate (RGR). This effect was more pronounced in the seedlings of height class of 40-69 cm (figure 4). We also observed this effect on small seedlings (10-39 cm) and in seedlings of *O. diospyrifolia* and *P. carthagenensis* (figure 4). On the other hand, we found a positive increase of diameter in almost all species. However, in smaller seedlings of *A. phalerata* the effect was lower than for *I. vera* (figure 4).

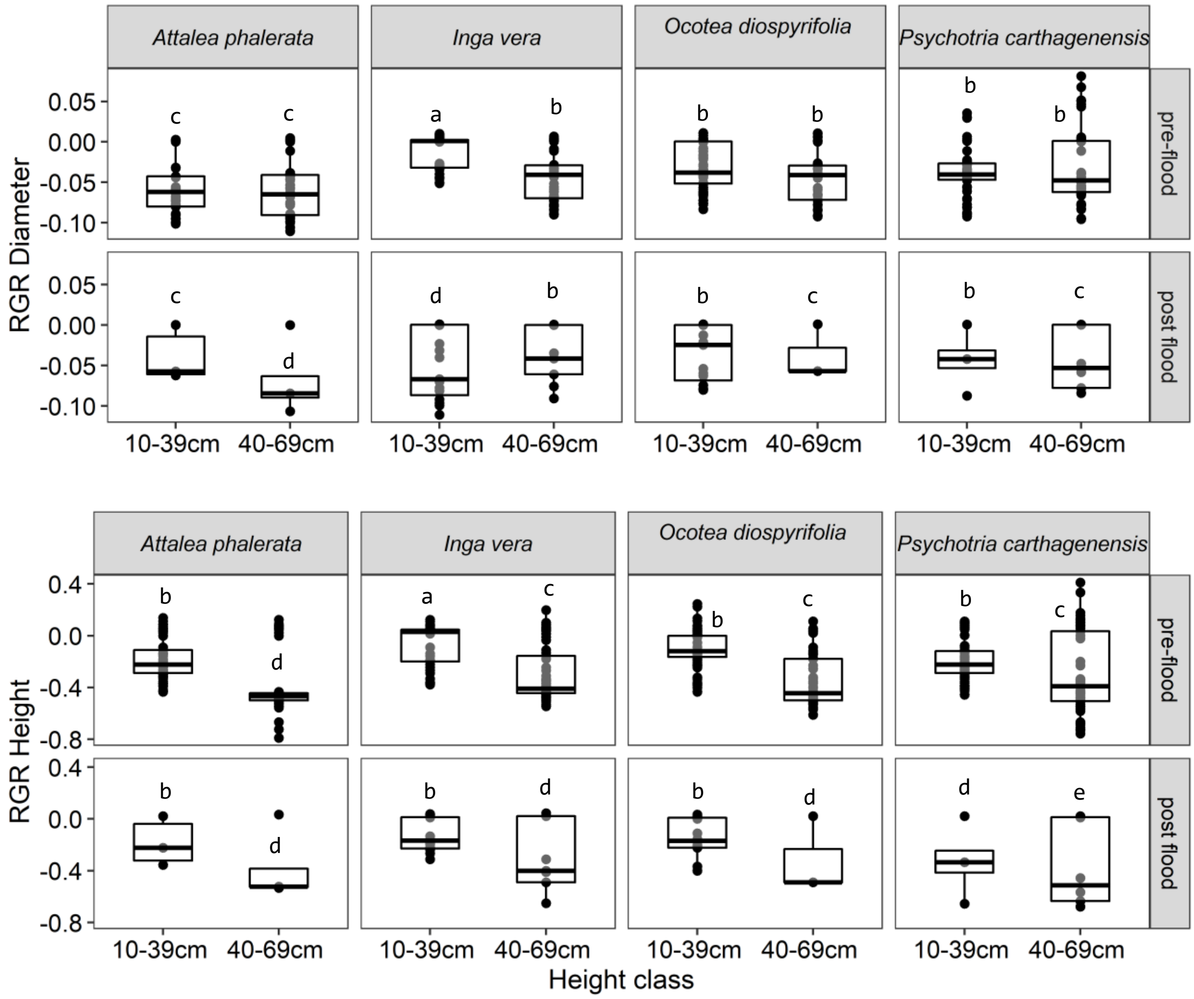

Figure 4. Relative growth rate in height and diameter according to the pre and post-flood periods evaluated for each species (*Attalea phalerata*, *Inga vera*, *Ocotea diospyrifolia*, and *Psychotria carthagenensis*) in the two height classes (10-39 cm and 40-69 cm high).

We verified this difference in seedlings of *A. phalerata* and *P. carthagenensis*, which had higher growth in height and diameter when sheltered ($p<0.01$). The development growth of I. vera was the same for all applied treatments ($p> 0.05$), while transplanted seedlings collected from lower topographic positions ones grew more ($p <0.01$) (figure 5). All seedlings invested more in height and diameter when collected in lower topographic position and planted in higher ($p<0.05$). There was no difference in sapling growth between the sampled periods (pre and post flood) in both collect and planting topographic positions ($p>0.05$).

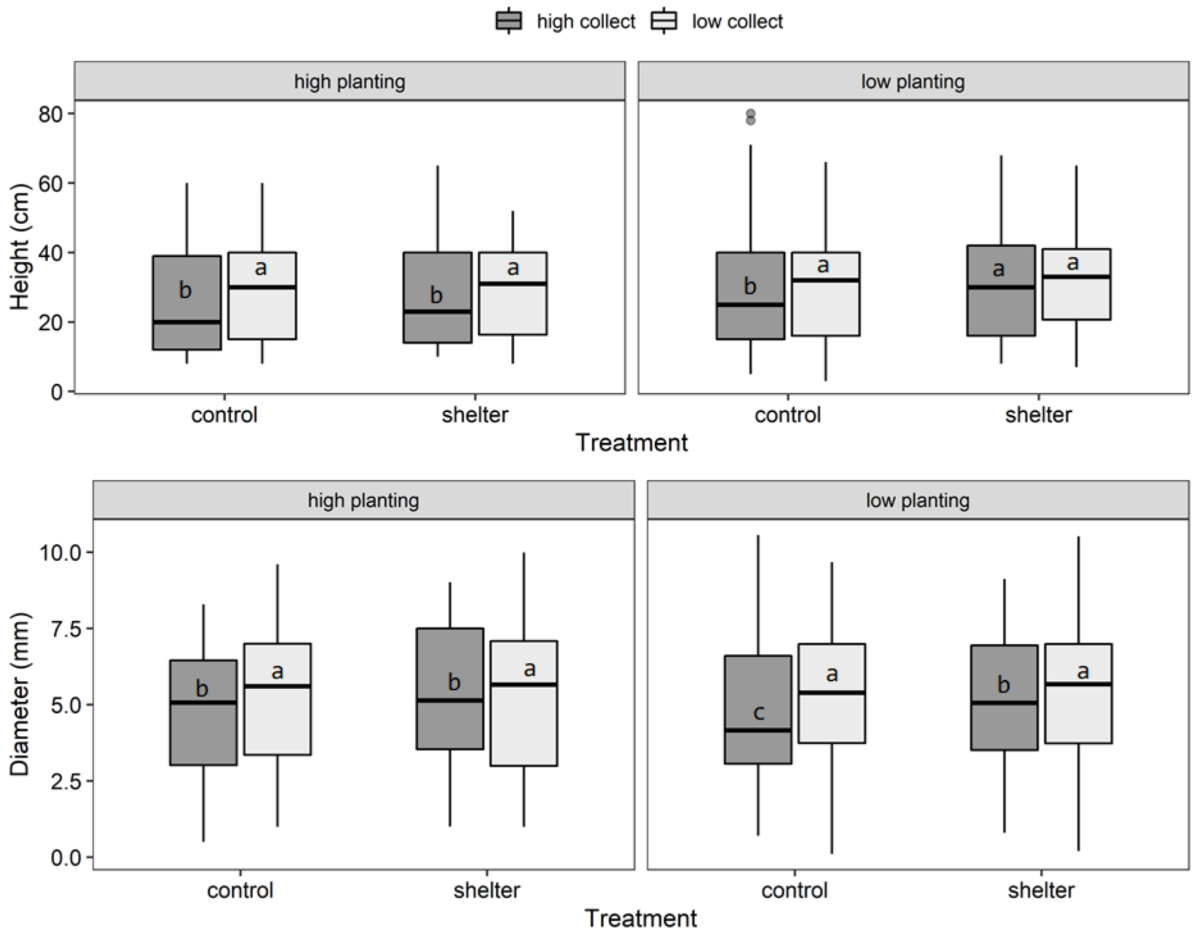

Figure 5. Relation between height and diameter comparing control and application of shelter regarding inversions between the collections of topographic position with topographic planting.

3.3 Herbivory and leaf area

The use of protection against herbivory had a great effect on growth. Sheltered seedlings had 8.2% superior growth in diameter than the control ($p<0.05$). Herbivory effect on seedlings without any type of protection was higher (> 10% of leaf area consumed) ($p<0.01$). Seedlings of *A. phalerata* were the most consumed ($p<0.01$) than seedlings of *I. vera*, *O. diospyrifolia* and *P. carthagenensis* ($p>0.05$), but all species have less herbivory rate when individuals are fenced (Figure 6). Flooding reduced herbivory rates in all evaluated conditions (p <0.001; Figure 7). Before flooding, herbivory rate was lower in shelters, but post-flooding this trend continued only for *I. vera*and *O. diospyrifolia*. (p <0.05; Figure 7).

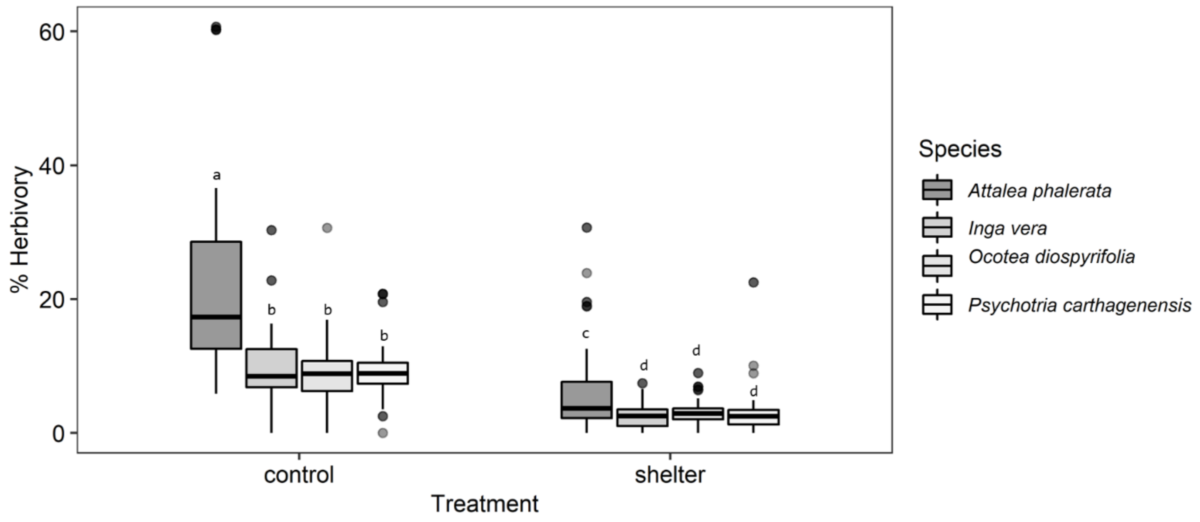

Figure 6. Percentage of herbivory in sheltered and control individuals in the studied species (*Attalea phalerata, Inga vera, Ocotea diospyrifolia*, and *Psychotria carthagenensis*) on riparian forest of Miranda River.

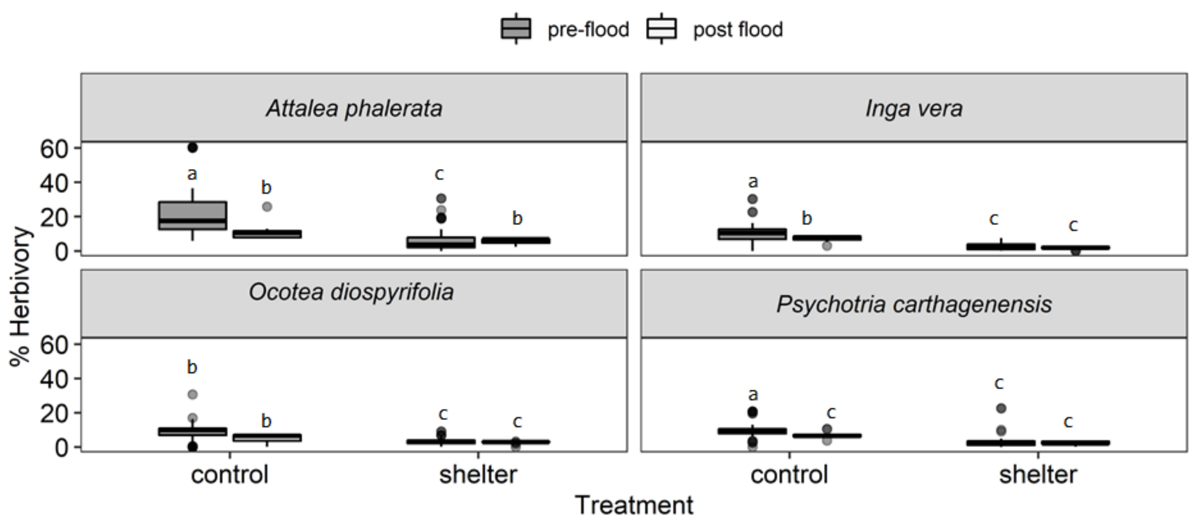

Figure 7. Effect of the protection against herbivory treatment (control and shelter) in two sampled periods (pre and post flood) in *Attalea phalerata, Inga vera, Ocotea diospyrifolia*, and *Psychotria carthagenensis*.

There was no effect of the collection and planting topography over herbivory rates, both being lower for the shelter treatment (figure 8).

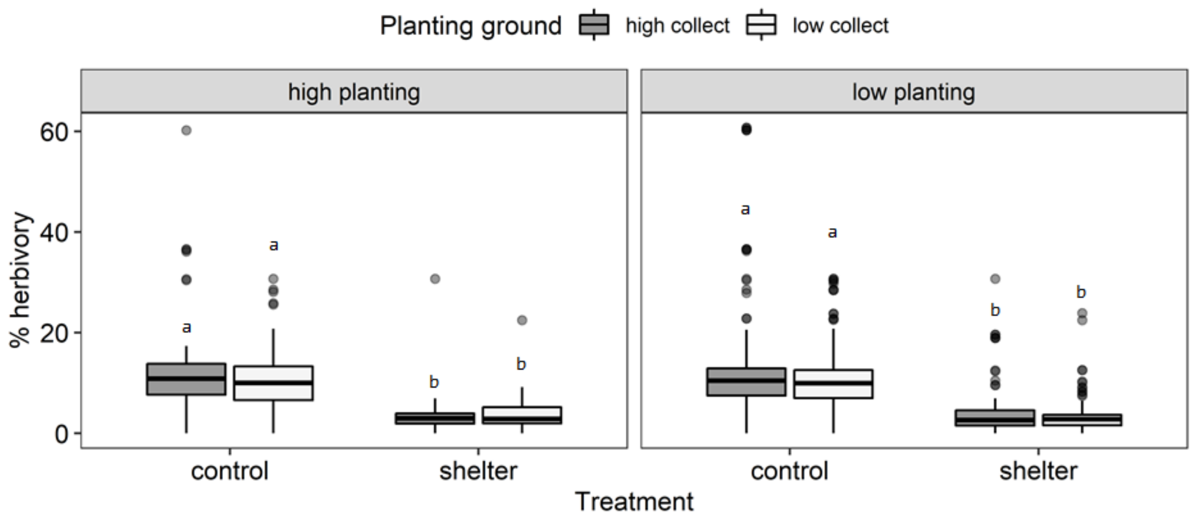

Figure 8. Effect of treatments applied on herbivory (control and shelter) between the collection and planting topographic arrangements

Smaller plants (10-39 cm height) were up to 6% more consumed by herbivory than the tallest ($p < 0.01$), with no difference between species. Regardless of height, *A. phalerata* seedlings were more consumed, having as less predated *P. carthagenensis* and *O. diospyrifoli*a. Seedlings of *P. carthagenensis* and *A. phalerata* presented higher leaf growth, having a greater effect when sheltered ($p < 0.01$) (figure 9). In general , the leaf area was larger for the seedlings found in the shelter protection treatment than the control and also was reduced after the flood period for control, but it increased for fenced seedlings(figure 9).

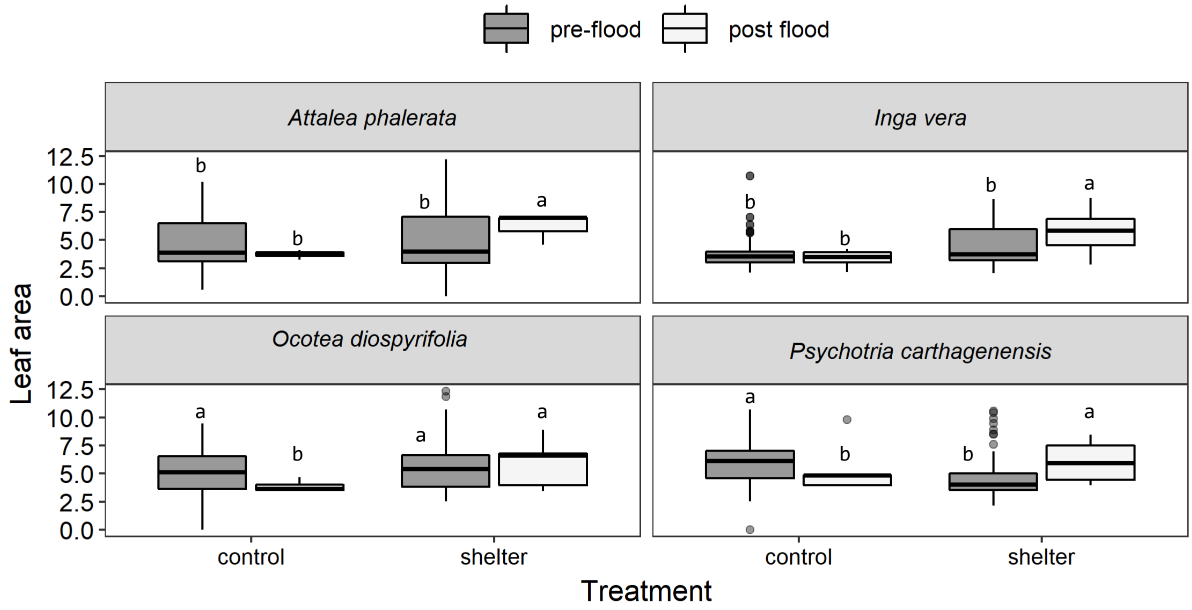

Figure 9. Leaf area index (mm²) in the protection against herbivory (shelter) and control among the evaluated species (*Attalea phalerata*, *Inga vera*, *Ocotea diospyrifolia*, and *Psychotria carthagenensis*) in two periods (pre-flood and post flood). Pre-flooding was measured four months after planting and post-flooding measured three months after finishing the flood.

3.4 Number of leaves, leaf flushing

After transplanting, the seedlings reduced the number of leaves (69.3%), after, they produced new leaves (figure S1d). There was no effect of the collecting or planting topography on the production of leaves. Smaller seedlings invested more in new leaves than larger seedlings ($p<0.05$). The species with the highest number of leaves was *P. carthagenensis* (6-8 leaves). The species that most sprout new leaves were *P. carthagenensis* and *I. vera* (2 -3 leaves).

Sheltered seedlings resulted greater number of leaves and leaf area ($p <0.05$). Sprouting during the pre-flood periods was low; however, the presence of regrowth increased by 12% in *I. vera* (figure S1c) and *P. carthagenensis* seedlings after flooding ($p <0.03$).

3.5 Costs

The yield was 8.5 seedlings/person/morning during the period of seedling collection in the neighboring remnants (figure 10). Costs of inputs used to collection and conditioning of seedlings (shovels, mackerel, bark gloves, buckets, handcarts, and bags for seedling planting) were U$ 0.19/seedling. Material for protection against herbivory (including materials for individual shelter of seedlings such as wooden stakes, plastic clamp ties, and wires) and direct labor cost to shelter implant were U$ 10.57/ seedling (Figure 10). The cost-effectiveness considering the number of surviving individuals was lower in periods of pre-flooding (table S2). Seedlings of *I. vera* had lower cost per seedling (U$ 0.82), followed by *P. carthagenesis* (U$ 0.92) (table S2). In general, seedlings that were protected by shelters were cheapest ones considering the number of surviving individuals, specially pre-flooding period (U$0.65; table S2). In addition, the magnitude of survival on the shelter effect was 8x higher for *I. vera* species (Table S3).

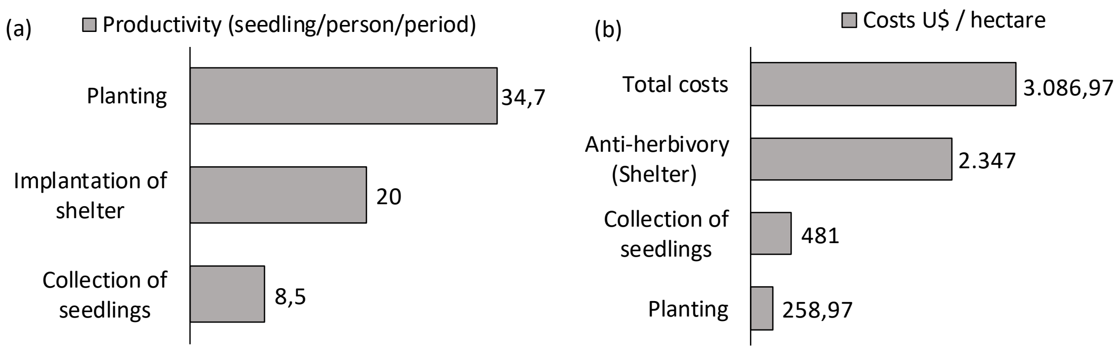

Figure 10. (a) Operating yield (number of seedlings per person per period (i.e., in the morning) and (b) costs (US$) accounted for stages of (1) seedling collection, (2) seedlings planting, and (3) protection against herbivory (shelter), and (4) the total cost of the technique of transplanting seedlings with protection against herbivory (i.e., all steps together: from 1 to 3). Considering daily labor of US $ 15.42 and a 2mx2m inter-row spacing of planting (i.e., value of 2.500 seedlings per hectare). Individual cost of shelter is U$1.00 /seedling.

4. Discussion

4.1 Survival and growth

Despite the success of the first phase, after the exceptional flood period the survival dropped to low rates for some species. The applied arrangement had a positive effect on the survival of the low seedlings (10-39 cm), collected in lower topographic positions and transplanted to the high and shelter. The initial mortality rate of 30% is expected in natural regeneration transplant practices (Viani and Rodrigues 2007, Calegari et al., 2011). However, it is noteworthy that most of the survival rates described in the literature are mostly for tree species and under nursery conditions and not all data from these studies are from field-planted individuals for restoration purposes (Calegari 2009, Viani et al. 2007, Nave 2005). Another factor that we should take into account was the extreme flood in the year of planting, prolonging by six to seven months of flood, twice that normally registered for this environment.

Our first hypothesis that higher seedlings may have higher survival resulting in faster growth was refuted. The lower survival for the larger seedlings (40-69 cm) can be explained by the fact that the extraction of larger roots would result in greater root damage during soil removal and also suffer from water stress causing damage to the absorption tissues (Luttge et al. al. 2008). Other studies indicate that seedlings up to 40 cm in height would have the best survival rates in nursery owing to these factors (Viani et al. 2007; Turchetto 2016).

Smaller seedlings may grow and develop faster, with larger seedlings taking longer to grow after recovery from soil withdrawal (Vidal 2008). In addition, flooding may pause the height growth of plants (Kozlowski 2002) and it was what we found in some species after the extreme flood period resulting in very low growth, compared to the preceding period. After the flooding, species may invest in shoot growth, resulting in an increase in diameter, owing to development of aerenchyma as a strategy of flood tolerance and over a long period of cortical cells and larger intercellular spaces (Sairam et al., 2008; Oliveira and Joly, 2010). *S*pecies such as *I. vera* have characteristics of resource reduction for stem growth (Medri et al., 2007). *I. vera* is abundant in all Pantanal topographies, being extremely adapted to flooded environments (Damasceno-Junior et al., 2005). Adventitious root induction was one of the most important strategies for the survival of genus *Inga* species under short floods (Bogarín *In Prep*). When exposed to long periods of flooding, *I. vera* seedlings can maintain their reproduction, considering it as a favorable environment for their germination and development under water (Lieberg e Joly, 1993). Seedlings of the *Ocotea diospyrifolia* are also abundant in lower Pantanal topographies (Damasceno-Junior et al 2005). However, this species did not tolerate exceptional flooding, as occurred in the study year. Nevertheless, *A. pharelata*, is a species of great abundance in the monodominant stands called acurizais, located in higher reliefs and presents low tolerance to floods (Damasceno-Junior et al. 2005).

Our results confirm the second hypothesis, demonstrating that the seedlings collected in the lower topographic positions presented greater tolerance to this flood disturbance. Seedlings previously exposed to flood may be more easily adaptable than seedlings collected in higher topographic positions, which historically suffer less from hypoxia.

4.2 Herbivory and leaf area

As hypothesized, the use of the wire mesh seedling guard considerably reduced herbivory rates and increased survival of the seedlings. These results are an indication that great pressure by herbivores was performed by mammals (figure 2c). When considering areas subject to potential herbivory filters, studies using shelters potentiate up to 100% of survival (Conner et al., 2000, Keeton 2008).

The study area is moreover, of easy access to herbivores and consequently to the planted seedlings. Considering, open environments, such as the studied site, unprotected seedling individuals induces the increase of herbivory by demand and supply of food (Urbas et al., 2007). In addition, we believe that herbivory was mainly caused by semi-aquatic mammals such as tapirs (*Tapirus terrestris*), capybaras *Hydrochoerus hydrochaeris*), wetland deer (*Blastocerus dichotomus*) which are frequently observed in the area. Hence, our data showed that sheltered seedlings are more protected from these animals. In addition, considering the high large-mammals density in Pantanal in some regions (Tomas et al. 2010) and that in this study we did not evaluate the dry season, when there is greater pressure of herbivorous mammals over vegetation , we can expect even higher rates of mortality and herbivory in unprotected seedlings in the dry season. *A. phalerata* seedlings were the most predated seedling among studied species, probably owing to the fact of palms are mostly palatable, being a potential food for local herbivores (Vehviläinen et al. 2007). Most of the species presented strategies to reduce herbivory, such as the presence of essential oils in leaves of *Ocotea diospyrifolia* (Araujo et al., 2001), alkaloids and crystals in *Psychotria carthagenensis* (Nakata et al., 2012), and flavonoids, or strategies such as leaves with lower leaf area in *Inga* spp. (Heerdt & Melo Jr 2017).

Flooding is one of the factors that can influence leaf area of the species, owing to the pause of foliar oxygenation, preventing and reducing the leaf growth of the seedlings. Therefore, adequate selection of protection devices for seedlings is given to optimize the success of the restoration (Sweeney et al., 2002, Sweeney and Czapka 2004, La and Wong 2005, Keeton 2008). Reduction of herbivory through control by seedling protection allows resource reallocation since, instead of plants investing on defense, they would invest on attributes related to their fitness, such as the increase of leaf area after the flood (Zorn-Arnold et al., 2006).

4.3 Number of leaves, leaf flushing

We believe that the seedlings of highest size class would have leaf escape from the water table during the flood period, which would facilitate leaf respiration. However, owing to exceptional flooding period all were under flood all size classes were a log period under flooding without foliar scape. Despite that, smaller seedlings (up to 39 cm) were able to establish with new leaves and regrowth, especially after the extensive flood period. The presence of regrowth in the initial restoration process indicates a high capacity of these species to regenerate in these periodically flooded environments (Durigan et al. 1998, Shoo et al. 2015). In addition, species that had greater survival to the flood also regrowth, which may present as an effective strategy in the survival of large floods as occurred in the study. Long period submerged in the water, lack of oxygenation in leaf tissues, and roots resulted in leaf chlorosis (Pezeshki 2001). In addition, carbon assimilation may change during this period, limiting photosynthesis, causing changes in leaf pigments and Rubisco's action on seedlings subject to flooding (Pezeshki 2001; Koslowski 2002). Another factor that the flood causes in the seedlings is the fall of the leaves (Viani et al. 2007). Leaf deciduousness is more pronounced in less tolerant to flooding species (Calbo et al. 1998, Reis et al. 2007), and leaf fall usually occur when seedlings are transplanted after nursery development (Viani and Rodrigues 2007; Calegari 2011).

4.4 Costs

The total cost of planting transplanted seedling form natural regeneration without the use of shelter, in a 2x2 m inter-row spacing (our experiment U$739,97/ha-1), was 75% cheaper when compared to conventional sapling planting (i.e., in the state of Mato Grosso do Sul, Brazil, it range from US$ 3.531 in average/ha-1 in regions that require manual (non-mechanizable) activities with 3mx2m (Antoniazzi et al., 2016)). Considering 100% of survival (maximum cost-effectiveness value) in a conventional sapling planting of 3 m x 2 m inter-row spacing (1.666 saplings, 100% survival, resulting on U$2.1 per planted sapling (Antoniazzi et al., 2016)), the cost-effectiveness found in our study for the seedling transplanted that have survived in pre-flooding period was 40% cheaper (U$0.84 in control treatment; table S2). In addition, these general values for the region correspond to the value without the use of protection against herbivory, and there are no studies that present exact values of these seedling protection uses. Our data shows that suing shelters could increase (i.e., only by adding the fencings) to U$739,97/ha-1 for US$ 2.534 / ha of the total value. If we compare cost-effectiveness of conventional sapling planting (U$2.1; Antoniazzi et al., 2016) with cost-effectiveness of seedling transplanted with protection against herbivory found in our study (U$0.65), our data is 61% cheaper. We must highlight that dealing with an exceptional flood may cause maximum damage to tested species. Probably in another situations (e.g., like smaller flood periods) the costs would be smaller since the mortality would be reduced (Damasceno-Junior et al. 2004). Moreover, considering that species like *I. vera* presents 8 times more survival with the use of shelter (Table S3), high level of protection should be encouraged as recommended for sensitive species to herbivore damage (Parsons et al. 2007). For the other species studied, even considering the high cost of the shelter, it would also be indicated to increase the survival of species with great palatability and / or great functional interest (e.g., species belonging to key functional groups for restoration (e.g., animal dispersed, etc.). In addition, the investment in protection against herbivory should be strengthening in remote access places, such as Pantanal, where present a high population density of potential herbivores. Hence, by investing in protection against herbivory more than getting again this difficult access region for replanting would save money, since assess area costs should also take into account. Besides, early restoration failure by lack of maintenance in the first years after planting is notorious and there are several examples in Brazil (Rodrigues et al. 2009), enhancing the need to invest in steps whereby decrease maintenance demand. Likewise, planting in higher density aiming accept possible losses to herbivorous may also not be effective since it would also increase permanent time of herbivorous in the site owing to food availability resulting on possible higher herbivory rates and seedling losses.

It is foreseen by legal instruments in Brazil (Law No. 12,651 / 2012, Chap. X, article 41, II, f) that products used for reconstitution and maintenance of Areas of Permanent Protection may be tax exempt. These products as wires, treated wood poles, boring soil boring, could facilitate the use of protection against herbivory for increasing survival purposes in large-scale. It is also worth noting that our data suggest the need of seedling shelter use, but size may change as seedling develop, and we stress that these protection against herbivory may not always meet the cost-benefit criteria. In addition, our results can serve as a basis for this material use during the restoration process.

5. Conclusion

Seedling transplantation from natural regeneration in periodic flood areas show better survival, growth, and herbivory reduction for small seedlings (10-39 cm) collected in low topography position and protected by wire mash shelter against herbivory. It is worth mentioning that survival varies according to the species and the conditions in which the transplant is done. We recommend collection of individuals already previously exposed to disturbances, as they have better overcome the extensive flood. Seedlings of *Inga vera* and *Psychotria carthagenensis* showed greater survival after extensive flooding. Seedlings of *Ocotea diospyrifolia* and *Attalea phalerata*, although presenting better development, are intolerant to the great floods. In addition, it is necessary alternatives that seek improvements in the rustification of these species, such as pre-exposure to periods of flooding. Although the costs of shelter implantation were more expensive than initially planting in greater density or later replanting the lost seedling, considering the increasing of survival, its cost-effectiveness would be very interesting (61% cheaper than traditional sapling planting) reducing total costs.

Besides, it can reach a rate of survival 8 times higher than no protection uses for some species (e.g., for *Inga vera*). Hence, considering saving money by suggesting an initial planting in greater density, needs subsequently tests since a seedling community in greater density may also increase the time of foraging of the herbivores, owing to greater resource supply, resulting on possibly greater damages by herbivory. On the other hand, suggestions for later replanting in the case of losses would also entail costs not dimensioned here related to the logistics access to remote areas such as the studied ones, which imply large transportation expenses, for example, and need further cost-effective studies.

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Table S1**.** Category for indices of herbivory (IH) according to foliar are consumed for samples in large quantities proposed by Dirzo & Domínguez (1995)

Foliar area consumed (%)	**Category**
0	0
0.1-6.0	1
6.0-12.0	2
12.1-25.0	3
25.0-50.0	4
>50.0	5

Figure S1. Stages of the experiment, where a) red arrow corresponds to the watermark located in the trunk of the tree, it was used to characterized two topographic positions (high: less than 50cm and low: greater than 50cm); b) newly collected seedlings with bare roots and evident xilopodium; c) nursery adapted to the experiment site for collected seedling rustification; d) seedlings presenting new leaves after 7 months of planting (post flooding), and e) 1 meter height individual under shelters that were implanted in the experimental plots for protection against herbivory. We buried wire and wooden posts 70cm to ensure fixation over flooding period.

Table S2. Cost-effectiveness per survival seedling (U$) among evaluated species and treatments (i.e., considering the total cost and the number of survival individuals).

Species	**Pre-flooding**	**Post flooding**
Attalea phalerata	U$1.81	U$6.79
Ocotea diospyrifolia	U$1.18	U$5.43
Inga vera	U$0.82	U$0.92
Psychotria carthagenensis	U$0.92	U$2.41
Treatments		
Crontol	U$0.84	U$2.45
Shelter	U$0.65	U$1.95

Table S3. Magnitude of survival increasing based on the implantation of protection against herbivory among evaluated species and treatments (i.e., considering the total cost and the number of survival individuals).

Species	**% survival without shelter**	**% survival with shelter**	**Magnitude of survival increasing**
Attalea phalerata	13,1	26,3	2,00x↑
Ocotea diospyrifolia	9,7	80,5	8,29x↑
Inga vera	21,3	44,4	2,08x↑
Psychotria carthagenensis	41,2	56,1	1,36x↑

Conclusões gerais

Apesar das grandes vantagens associadas, a prática de transplante de plântulas ainda é raramente utilizada no mundo. Áreas úmidas e florestas tropicais são as regiões com mais pesquisas sobre o uso desta prática. Destacamos alguns gargalos encontrados em nossa pesquisa como a baixa riqueza de espécies na maioria dos artigos avaliados. Além disso, a inclusão de outras formas de vidas também foi baixa, sendo a maioria com espécies utilizadas são de hábito arbóreo. Outro fator de extrema importância para fins de implantação para restauração ecológica é o custo, porém, dados sobre isso foram encontrados apenas em dois estudos analisados. Em áreas úmidas como Pantanal, a implantação da prática foi eficiente ponderando algumas condições como espécie utilizada, tamanho e a posição topográfica em que essas plântulas são coletadas. Ressaltamos que o estudo ocorreu em um período de cheia excepcional e que mesmo assim as taxas de sobrevivência atingidas foram significativas, levando em conta este contexto extremo. Considerando um ambiente sazonalmente inundável, é aconselhável que plântulas sejam coletadas em regiões que já passaram por um período de estresse, como cheias e pequenas inundações. Os custos do uso de coleta de plântulas e posterior transplante foi 75% mais barato comparado ao plantio de mudas convencional. Porém, ressaltamos que as condições foram de ausência de necessidade de controle de plantas invasoras, situação em que estes custos poderiam ampliar. Quando inclui o custo do uso da proteção com cercas individuais, inicialmente os valores parecem altos, porém seu uso resulta em um expressivo aumento na sobrevivência das plântulas, reduzindo drasticamente a herbivoria por grandes mamíferos e aumentando a sobrevivência em até 8 vezes para algumas espécies. Assim, mesmo com a inclusão das despesas com esta proteção, o custo-efetividade da técnica de transplante de plântulas da regeneração natural deste com uso de proteção é 61% menor comparado ao plantio de mudas convencional. Novos estudos são necessários para testar a viabilidade desta prática e em outros tipos de ambientes considerando poucos registros de seu uso em regiões mais secas, como savanas.