RICARDO BERTONCELLO

Restauração ecológica e processos estruturadores de comunidades vegetais

Ecological restoration and structuring processes of plant communities

São Paulo

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Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo para obtenção do título de doutor em Ciências, na área de ecologia de ecossistemas terrestre e aquáticos

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Interações interespecíficas são consideradas importantes forças estruturadoras da vegetação. Enquanto a competição aparentemente prevalece em ambientes com menor estresse abiótico, a facilitação tende a aumentar em importância em ambientes de maior estresse. Consequentemente, é esperado que a facilitação desempenhe um papel importante em áreas tropicais degradadas, nas quais as condições abióticas, envolvendo altos níveis de irradiação, temperatura e evapotranspiração, são muito distintas das condições ótimas para a maioria das espécies florestais. Além disso, face à complexidade dos processos estruturadores de comunidades florestais tropicais altamente diversas, os ecólogos têm percebido a necessidade de simplificação. Um caminho promissor para isso é por meio do uso de características funcionais, que podem ser medidas para qualquer espécie e comparadas em diferentes sistemas e locais. No entanto, em áreas degradadas ainda é incerta a relação entre as características funcionais e o desempenho das espécies. Neste contexto, planejamos um projeto de restauração na planície costeira do sudeste brasileiro para comparar o crescimento e a sobrevivência de mudas de árvores plantadas em diferentes densidades (sistema isolado ou sistema agregado) em um experimento fatorial com adição de nutrientes. No primeiro capítulo analisamos a sobrevivência, altura, diâmetro ao nível do solo e projeção da copa de 4.132 mudas de 19 espécies de diferentes classificações sucessionais, durante um período de 18 meses. Usamos modelos de efeitos mistos para analisar a relação entre o desempenho de espécies e os tratamentos e selecionamos os melhores modelos utilizando o critério de

informação de Akaike (AIC). Verificamos uma maior sobrevivência nos sistemas agregados para as espécies não-pioneiras, indicando o predomínio do processo de facilitação. Em contrapartida, constatamos um menor crescimento em diâmetro nos sistema de plantio agregado, indicando o predomínio de competição. A adição de nutrientes não afetou a sobrevivência nos sistemas agregados, mas, surpreendentemente, teve um efeito negativo sobre as plantas isoladas. Além disso, a adição de nutrientes teve um efeito positivo sobre crescimento, diâmetro e projeção da copa nos sistemas de plantio agregado (indicando a redução da competição), especialmente para espécies pioneiras. Uma vez que o estabelecimento de espécies não-pioneiras em áreas degradadas pode ser bastante difícil, a facilitação para a sobrevivência dessas espécies em sistemas agregados pode ser utilizada como instrumento para melhorar os modelos de restauração. No segundo capítulo utilizamos algumas características funcionais que são facilmente mensuráveis para investigar sua relação com o crescimento e sobrevivência das mudas. Além disso, testamos o efeito das interações interespecíficas na relação das características funcionais com o desempenho das plantas. Para espécies pioneiras, uma maior área foliar específica (SLA - *Specific leaf area*), foi relacionada com uma maior taxa de sobrevivência. Entretanto, ao considerarmos as relações interespecíficas (sistemas agregados), maior SLA foi relacionada com uma menor taxa de sobrevivência. Em geral, as características funcionais explicaram apenas parcialmente o desempenho de mudas no contexto de restauração de áreas degradadas. Nosso trabalho contribuiu para a discussão sobre o potencial da aplicação prática da abordagem funcional na escolha das espécies para a recuperação de áreas degradadas. O caráter manipulativo da restauração ecológica baseada no plantio de mudas amplia as perspectivas para implementar experimentos que visem testar novas hipóteses em ecologia e refinar modelos de restauração.

Palavras-chave em português: Brasil, planície costeira, estrutura de comunidade, caracteres funcionais, nutrientes, interação de plantas, restauração, desempenho de mudas, mudas arbóreas nativas, floresta tropical

Abstract

Bertoncello, Ricardo. Restauração ecológica e processos estruturadores de comunidades vegetais. **2016**. 89p. tese (Doutor) – Departamento de Ecologia, Institutode Biociências, Universidade de São Paulo, São Paulo, 2016.

Ecological restoration and structuring processes of plant communities

Interspecific interactions are considered to be important structuring forces in early successional vegetation. Whereas competition seems to prevail in less severe environments, facilitation tends to increase in importance in harsh environments. Hence, facilitation is expected to play an important role in degraded tropical areas with high irradiance, heat loads, and evapotranspiration, where conditions are far from optimum for most forest species. Moreover, in order to understand complex structuring process of high diverse tropical forests, ecologists have realized the need for simplification. A promising way to do that is through the use of functional traits, which can be measured for any species and compared in different systems and different locations around the world. However, in these conditions, little is known about the role of species interaction on the relation of functional traits and species performance. In this scenario, we planned a restoration project on the southeastern Brazilian coastal plain to compare growth and survival of tree seedlings planted at two densities (isolated or aggregated) in a factorial experiment with nutrient addition. In the first chapter we analyzed survival, height, ground level diameter, and crown projection of 4,132 saplings from 19 species that ranged along a successional gradient, over an 18-month period. We used mixed-effect models to analyze the relationship between species performance and treatments, and Akaike's information criterion (AIC) to select the models. The best model showed higher survival in aggregated plantations (indicating facilitation) for non-pioneer species. In contrast,

we found lower diameter growth in aggregated plantation (indicating competition). Fertilizer addition did not affect survival in clusters, but, surprisingly, it had a negative effect on isolated plants of both pioneer and non-pioneer species. On the other hand, fertilizer addition had a positive effect on diameter and crown projection growth in aggregated plantations (reducing competition), especially for pioneer species. Thus, whether facilitation or competition was the predominant interaction depended on the response analyzed. We concluded that, as establishment of non-pioneer species in disturbed sites can be challenging, restoration designs could take advantage of higher survival rates in clusters and use resource addition to ameliorate growth and decrease competition for limited resources. In the second chapter we used the core simple-measurable traits to investigate whether functional traits were related to growth and survival and whether there was a difference in the effect of the functional traits on plant performance of isolated individuals or of individuals subjected to interaction with neighbors. Our main findings were that for pioneer species, the greater the specific leaf areas (SLA), the greater the survival rates, but, under aggregated conditions, the greater the SLA the lower the survival rates. However, functional traits only partially explained saplings performance in a restoration context and more research is needed to understand its role in predicting seedlings outcomes, especially considering the potential applicability of this methodological approach. The manipulative character of ecological restoration broadens perspectives to use experiments to generate and test new hypotheses in ecology and to refine restoration models.

Brazil, coastal plain, community structure, functional traits, nutrient, plant interaction, restoration, seedling performance, tree saplings, tropical forest

1. Introdução geral

1.1. Contextualização teórica do experimento

1.1.1. Interação entre plantas como processo estruturador de comunidades vegetais

De acordo com a Sociedade de Restauração Ecológica (SER) a restauração de áreas degradadas é uma *"atividade intencional que inicia ou acelera a recuperação de um ecossistema com relação a sua saúde, integridade e sustentabilidade"* (SER, 2004). A Restauração Ecológica pode ser vista como uma disciplina transdisciplinar, sendo a aplicação prática da ciência chamada Ecologia da Restauração. Esta, por sua vez, tem sua formação derivada de conceitos clássicos da Ecologia, como sucessão ecológica (Connell & Slatyer, 1977; Pickett et al. 2008), ecossistemas (Ehrenfeld & Toth 1997), comunidades (Palmer et al. 1997), competição, nicho, limitação de recrutamento, mutualismo, herbivoria, biogeografia de ilhas, distúrbio, ecótipos (Young et al. 2005) "ecosystem engineering" (Hastings et al. 2007), e facilitação (Brooker et al. 2008).

Desde as primeiras tentativas de delinear a Ecologia da Restauração como ramo da ciência ligado à Ecologia, em meados dos anos 80, o caráter manipulativo desta disciplina já abria perspectivas para gerar novas hipóteses e utilizar experimentos para testá-las (Jordan et al. 1987). Na medida em que experimentos planejados são incorporados nas ações de restauração, os projetos de restauração podem ser uma excelente oportunidade para testar as teorias vigentes em Ecologia, pois possibilitam sua manipulação e/ou controle.

Apesar disso, cerca de 30 anos se passaram e ainda são poucos os projetos que incluem réplicas de diferentes sistemas de plantio nos projetos de restauração (Howe & Martínez-

Garza, 2014), desperdiçando, dessa maneira, valiosas oportunidades daquilo que Bradshaw (1987) considerou como um "teste ácido para a ecologia". A restauração ecológica pode ser considerada como uma construção (estruturação) de uma nova comunidade, colocando à prova as principais bases dos processos de estruturação e funcionamento das comunidades (Palmer et *al*. 1997; Howe & Martínez-Garza, 2014; Nunes-Mir et *al*., 2015; Perring et *al*., 2015), tais como as interações entre as espécies.

As interações entre as plantas desempenham um papel essencial na estruturação das comunidades. Embora as interações negativas (i.e. competição) sejam tradicionalmente consideradas como a principal força estruturadora (atual e evolutiva), as interações positivas (facilitação) vêm recentemente ganhando mais força no arcabouço teórico da Ecologia (Bruno et. *al*, 2003; Lortie et *al*., 2004; Perring et *al*., 2015). Todavia, os dois processos podem agir simultaneamente e resultar em interações complexas, afetando os padrões de distribuição e coexistência de espécies, produtividade do ecossistema, diversidade e dinâmica da comunidade (Callaway 1995). Entretanto, estudos que possibilitem a separação clara entre os fatores que atuam no balanço das interações entre plantas são difíceis de serem implantados em condições naturais, especialmente para as espécies arbóreas em florestas tropicais. Parte dessa dificuldade está relacionada à obtenção de réplicas, tanto de situações que possibilitem entender suas complexas interações, quanto de situações que possibilitem separá-los para entendê-los isoladamente.

De acordo com a hipótese do "Gradiente de Estresse" (Bertness & Callaway 1994) a competição tende a se intensificar em situações de menor estresse abiótico (Callaway & Walker 1997), enquanto a importância do processo de facilitação na estruturação das comunidades vegetais tende a aumentar em situações de maior estresse abiótico (Callaway et.

al 2002). Analogamente, em ambientes degradados, que apresentam altas taxas de luminosidade, temperatura, e maior evaporação de água (refletindo condições de maior estresse abiótico), a facilitação pode representar o principal processo estruturador da comunidade. O estabelecimento inicial de uma espécie (Padilla & Pugnaire, 2006), ou de uma ilha de vegetação (Corbin & Holl 2012), pode fornecer melhores condições para que outros indivíduos se estabeleçam. Nesse caso, o processo de facilitação pode atuar por meio de modificações diretas - como a melhora no ambiente físico, através do sombreamento e redução de temperatura, da alteração das características do substrato e do aumento da disponibilidade de recursos - ou indiretas - como a eliminação de competidores potenciais, a introdução de organismos benéficos (micorrizas, microorganismos ou polinizadores) e a proteção contra herbivoria (Callaway 1995).

As relações de competição por recursos e de facilitação entre as plantas estão diretamente relacionadas com o processo de sucessão ecológica (Connell & Slatyer, 1977). Esta, por sua vez, constitui uma das teorias mais importantes para o processo de restauração de uma comunidade, visto que promove sua reestruturação a partir dos elementos disponíveis. Budowski (1965) chamou a atenção para a maneira como o processo de sucessão influencia a estruturação das comunidades em florestas tropicais. Ele aponta uma relação entre as séries sucessionais da floresta e as características das espécies. Ao longo do processo, a presença das espécies iniciais da sucessão acarreta em uma mudança nas condições abióticas, que por sua vez modifica as relações bióticas (Connell & Slatyer, 1977), de maneira que espécies com diferentes características estariam associadas à colonização da área em cada uma dessas fases.

A partir de Budowski (1965), foram propostas diversas classificações sucessionais para as espécies. Porém, Swaine & Whitmore (1988) propuseram uma divisão em que as espécies

arbóreas seriam classificadas em apenas dois grupos: pioneiras e não pioneiras. Aquelas espécies que crescem em áreas abertas ou grandes clareiras seriam as pioneiras, cujas sementes só conseguiriam germinar em clareiras com alta incidência de luminosidade. E no outro grupo estariam aquelas espécies adaptadas às condições de interior da floresta (baixa incidência luminosa) ou à colonização de clareiras pequenas, que seriam as espécies não-pioneiras. Uma das principais características das espécies consideradas pioneiras é o fato de que indivíduos jovens não conseguem se desenvolver na sombra do dossel formada por outras espécies pioneiras, enquanto as espécies não pioneiras têm condições de germinar e de se desenvolver sob a sombra das espécies pioneiras, ou sob a sombra formada pelas espécies não pioneiras (Swaine & Whitmore, 1988). Embora esses dois grupos ecológicos representem extremos de um contínuo, eles devem apresentar respostas diferentes quando submetidos à diferentes condições de interação interespecífica.

As interações interespecíficas são fortemente afetadas pela disponibilidade de recursos, dentre esses, a quantidade de nutrientes no solo (Tilman & Wedin 1991). De acordo com a teoria de competição por recursos, quando as espécies competem por um único recurso, aquela com menor requerimento desse recurso tende a permanecer no sistema, pois o vencedor reduz a disponibilidade do recurso abaixo do nível limite para o perdedor (Dybzinski & Tilman, 2007). Em algumas situações, as espécies melhores competidoras para um único recurso limitante tendem a deslocar as outras espécies do hábitat, independentemente das densidades iniciais das espécies (Wedin & Tilman 1993). Entretanto, se existir um segundo fator que limite o crescimento do vencedor (i.e. luz, temperatura, herbivoria), a coexistência é possível (Dybzinski & Tilman, 2007). Dessa forma, com um fator limitante adicional a coexistência seria possível devido à diferenciação nos eixos (ou dimensões) de seus nichos (Schoener 1974).

Entretanto, estudos que relacionam a disponibilidade de nutrientes com o desenvolvimento das árvores no processo de estruturação de comunidades em florestas tropicais ainda são raros, sendo a maior parte desse tipo de estudo realizada com espécies herbáceas temperadas. Em um trabalho testando de que maneira a adição de nutrientes afeta a estruturação e a diversidade da comunidade durante o processo de sucessão secundária em uma floresta tropical, Siddique et *al*. (2010) concluíram que algumas espécies pioneiras são favorecidas e se tornam dominantes, diminuindo a taxa de acréscimo de espécies e a diversidade de espécies arbóreas coexistentes. Nesse caso, a adição de nutrientes deslocou a relação de competição entre as espécies, pois favoreceu espécies pioneiras que, em geral, são consideradas espécies oportunistas e piores competidoras do que as espécies não pioneiras (Tilman & Wedin 1991). Em condições de menor disponibilidade de nutrientes, as não pioneiras tenderiam a ser melhores competidoras e excluiriam as espécies pioneiras.

Nesse contexto, a aplicação de modelos de restauração que avaliem simultaneamente o papel da distribuição espacial (i.e. interações interespecíficas) e da disponibilidade de recursos sobre espécies de grupos sucessionais diferentes, possibilita uma oportunidade ímpar de responder questões ligadas aos principais processos estruturadores de comunidades vegetais tropicais.

1.1.2. Atributos funcionais na estruturação das comunidades

Atributos funcionais são considerados quaisquer atributos, sejam morfológicos, fisiológicos ou fenológicos, que impactam no crescimento, reprodução ou sobrevivência das espécies (Violle et *al*., 2007). Logo, as características funcionais exercem importante influência sobre a performance das espécies (Mc Gill et *al*., 2006) e devem estar fortemente relacionados aos

processos estruturadores das comunidades vegetais, bem como à dinâmica das espécies nos processos de restauração em áreas degradadas.

A despeito de sua importância para os processos estruturadores, poucos estudos exploraram a relação entre as características funcionais e a interação entre as plantas (Perring et *al.* 2015). Da mesma maneira, até o momento existem poucos estudos que relacionaram a performance das espécies na restauração de comunidades vegetais com seus atributos funcionais, e a maior parte dos trabalhos nessa linha foi desenvolvida com gramíneas em ambientes temperados (Pywell et *al.*, 2006). Todavia, atributos funcionais podem ter aplicabilidade direta em restauração, pois podem simplificar extensas listas de espécies em unidades mais facilmente manejáveis. Além disso, podem ajudar na estruturação e funcionalidade das comunidades vegetais -na medida que os principais grupos funcionais da comunidade estejam representados no plantio-, além de possibilitar a comparação entre sítios diferentes, e o teste de teorias ecológicas (Brown, 2004; Clark et *al.*, 2012).

Nas florestas tropicais, indivíduos de espécies com atributos funcionais mais conservativos (baixa rotatividade e alta eficiência, sensu Wright et al. (2004))
tendem a ter um crescimento menor, porém maior sobrevivência (Kitajima, 1994; Poorter & Bongers, 2006; Gustafsson et *al.*, 2016) Enquanto espécies com atributos funcionais mais aquisitivos (alta rotatividade e baixa eficiência, sensu Wright et al. (2004)) apresentam o padrão contrário, constituindo dessa maneira um trade-off entre crescimento e sobrevivência das plântulas (Kitajima, 1994; Poorter and Bongers, 2006; Gustafsson et *al.*, 2016). Os ambientes tropicais, alvos de restauração ecológica (i.e. pastagens degradadas), apresentam características ambientais completamente distintas das do interior das florestas, portanto, é possível que características funcionais repercutam de maneira diferente no desempenho das

espécies.

Além da compreensão da relação entre características funcionais e desempenho das plantas na restauração das florestas tropicais, outra questão ainda em aberto e de fundamental interesse prático e teórico, seria compreender de que maneira as interações interespecíficas podem alterar essa relação. Em geral, espécies com estratégia aquisitiva são piores competidoras (Kunstler et al. 2016). Por outro lado, as espécies que são facilitadas pela presença de outras espécies tendem a apresentar estratégias funcionais conservativas (Butterfield & Briggs, 2011).

Neste contexto, a aplicação de modelos de restauração que avaliem a relação entre os atributos funcionais e o desempenho das espécies nos projetos de restauração, bem como de que maneira as interações interespecíficas podem modificar essa relação, constituem uma oportunidade ímpar de responder, sob a ótica dos atributos funcionais, questões ligadas aos principais processos estruturadores de comunidades vegetais tropicais.

1.2.   Organização da tese

O primeiro capítulo, intitulado **"Cluster planting facilitates survival but not growth in early development of restored tropical forest"** teve sua publicação aceita na revista *Basic and Applied Ecology*. Esse capítulo analisa o desempenho (crescimento e sobrevivência) de mudas nativas plantadas em um experimento fatorial, considerando as interações interespecíficas (i.e. mudas isoladas e mudas agregadas) e a adição de nutrientes, em uma área de restauração de floresta tropical, situada na planície costeira de Caraguatatuba (SP). O objetivo desse artigo foi verificar de que maneira a proximidade das plantas e a quantidade de

nutrientes disponível afetam a sobrevivência e o crescimento de mudas com diferentes características sucessionais. A performance de indivíduos plantados sem interação (isolados) foi comparada à performance de indivíduos da mesma espécie sujeitos à interações interespecíficas (plantio de mudas agregadas). A melhora na performance da espécie foi interpretada como efeito predominante de facilitação, enquanto a piora na performance indicou o efeito predominante de competição. Além disso, foi avaliada a influência da adição de nutrientes nas relações interespecíficas.

O segundo capítulo, intitulado "Can plant traits predict outcomes of interaction among seedlings in early development of restored tropical forest?" pretende verificar se características funcionais que são universalmente consideradas na literatura e fáceis de medir estão relacionadas ao desempenho (crescimento e mortalidade) da mudas nesse mesmo projeto de restauração na planície costeira de Caraguatatuba. Adicionalmente, avaliamos se as interações interespecíficas modificam a relação entre as características funcionais e o desempenho das mudas na fase inicial da restauração de uma floresta tropical.

1.3.   Uma parceria para inserir pesquisa na obrigação de restaurar

1.3.1. Aproveitando a prática para testar a teoria

A presente tese faz parte do projeto "Ecologia e Restauração dos Ecossistemas Costeiros do Litoral Norte de São Paulo" que constitui uma parceria entre o CENPES - Centro de Pesquisa e Desenvolvimento da PETROBRAS e o Laboratório de Florestas Tropicais do Instituto de Biociências da Universidade de São Paulo, LabTrop-USP (convênio n° 0050.0067886.11.9). Essa parceria teve origem na motivação do LabTrop em compreender os mecanismos de

geração e manutenção da diversidade em florestas tropicais, através de abordagens observacionais e experimentais, como por exemplo a manipulação das plantas em campo. Nesse contexto, aproveitou-se uma demanda legal de restauração florestal por parte da Petrobras para inserção de um projeto de pesquisa.

O projeto "Ecologia e Restauração dos Ecossistemas Costeiros do Litoral Norte de São Paulo" foi composto por quatro linhas de pesquisa:

1- Caracterização de vegetação de referência no entorno da área objeto do projeto de restauração;

2- Desempenho de mudas em diferentes modelos de restauração ambiental na planície de Caraguatatuba (SP);

3- Biomassa e funcionamento do sistema;

4- Chuva de sementes e recrutamento: a regeneração natural em uma área de restauração e em ecossistemas de referência na planície costeira de Caraguatatuba (SP).

A seguir, serão apresentados os detalhes da implantação do plantio de mudas da linha de pesquisa 2, que deu origem à presente tese de doutorado.

1.3.2. Área de estudo e descrição do projeto de restauração

1.3.2.1. Área de estudo

A área experimental do presente trabalho situa-se adjacente à Unidade de Tratamento de Gás Monteiro Lobato (UTGCA), no município de Caraguatatuba (SP), nas coordenadas 23°39'27"S e 45°30'03"W. Trata-se de uma área de 6,5ha situada em faixa de largura de 50m a partir da margem esquerda do Rio Camburu, com cerca de 1.300m de extensão.

O local do plantio era um pasto antes de ser desapropriado pela Petrobras e apresentava predomínio de gramíneas invasoras com alguns poucos indivíduos arbustivos esparsos, em especial *Ricinus communis* (mamona) e *Psidium guajava* (goiaba). O entorno da área do plantio também apresentava predomínio de gramíneas e não havia conectividade direta com a vegetação remanescente que recobre as escarpas da Serra do Mar, tampouco com os remanescentes florestais da própria planície costeira (Figura 1).

**Figura 1: Vista da área a ser restaurada em primeiro plano,
antes do início do plantio em 2012, Caraguatatuba, SP.**

A área está inserida no domínio de vegetação da Mata Atlântica. Segundo o sistema de Köppen, a faixa litorânea recebe a classificação Af**,** caracterizada pelo clima tropical chuvoso, sem estação seca com a precipitação média do mês mais seco superior a 60mm. A precipitação anual média de 1943 até 2004 foi de 1784mm (Santos & Galvani, 2012). Neste trecho da planície, o solo é formado por uma mistura do solo arenoso original com solo argiloso (Ferreira, 2013), oriundo de um evento de escorregamento da Serra do Mar que ocorreu no município de Caraguatatuba em 1967 e encobriu toda a planície costeira com sedimento de origem continental

Por situar-se na planície costeira, próxima à encosta da Serra do Mar, e apresentar topografia plana, a vegetação da área anteriormente era provavelmente constituída por Floresta de Transição Restinga-Encosta.

1.3.2.2. Esquema de plantio

Antes do plantio em fevereiro de 2012, foram removidas as partes áreas das gramíneas e arbustos exóticos invasores. Além disso, foi realizada a calagem de maneira homogênea na área de forma a eliminar a acidez do solo.

Foram plantadas, no total, cerca de 15.690 mudas originárias de ecossistemas da planície costeira do litoral de São Paulo, pertencentes à 48 espécies arbóreas (Tabela 1). A área experimental foi dividida em 62 parcelas contíguas, cujas áreas variaram entre 810m e 1.181m , devido ao formato tortuoso do rio Camburu (área média de 1.000m) (Figura 2). As parcelas foram demarcadas por topógrafos e devidamente numeradas em campo através do uso de estacas, para facilitar a visualização. As 62 parcelas foram distribuídas em sete combinações de tratamentos (descritas abaixo), resultando em oito réplicas (de agora em diante denominadas como blocos) para cada tratamento.

Tabela 1: Espécies arbóreas plantadas no projeto de restauração da área adjacente à Unidade de Tratamento de Gás Monteiro Lobato (UTGCA), Caraguatatuba, SP. As espécies

demarcadas em negrito foram aquelas monitoradas no presente projeto. C.S= Classificação Sucessional; P = espécie pioneira; NP = espécie não pioneira.

Família	Espécie	Autor	Nome popular	C.S
Anacardiaceae	*Schinus terebinthifolius*	**Raddi**	**aroeira**	**P**
Annonaceae	*Annona glabra*	L.	araticum-do-brejo	NP
Annonaceae	*Guatteria australis*	A.St.-Hil.	pindaíba-preta	NP
Annonaceae	*Rollinia sylvatica*	(A. St.-Hil.) Martius	araticum-do-mato	NP
Arecaceae	*Bactris setosa*	Mart.	tucum	NP
Arecaceae	*Euterpe edulis*	**Mart.**	**palmito-juçara**	**NP**
Arecaceae	*Syagrus romanzoffiana*	**(Cham.) Glassman**	**jerivá**	**NP**
Bignoniaceae	*Jacaranda puberula*	**Cham.**	**carobinha**	**NP**
Bignoniaceae	*Tabebuia cassinoides*	(Lam.) DC.	caxeta	NP
Calophyllaceae	*Calophyllum brasiliense*	**Cambess.**	**guanandi**	**NP**
Celastraceae	*Maytenus robusta*	Reissek		NP
Erythroxylaceae	*Erythroxylum amplifolium*	Baill.		NP
Erythroxylaceae	*Erythroxylum argentinum*	O.E.Schulz		NP
Euphorbiaceae	*Alchornea glandulosa*	Poepp.	tapiá	P
Fabaceae	*Erythrina speciosa*	**Andrews**	**mulungu**	**P**
Fabaceae	*Schizolobium parahyba*	(Vell.) S. F. Blake	guapuruvu	P
Lamiaceae	*Aegiphila verticillata*	**Vell.**	**tamanqueira**	**P**
Lauraceae	*Ocotea pulchella*	**(Nees & Mart.) Mez**	**louro**	**NP**
Malvaceae	*Eriotheca pentaphylla*	**(Vell.) A.Robyns**	**embiruçu**	**NP**
Meliaceae	*Cabralea canjerana*	(Vell.) Mart.	cajarana	NP
Moraceae	*Ficus insipida*	Willd.	figueira	NP
Myristicaceae	*Bicuiba oleifera*	**(Schott) J.J.de Wilde**	**bicuíba**	**NP**
Myrtaceae	*Blepharocalyx salicifolius*	(Kunth) O.Berg		NP
Myrtaceae	*Calyptranthes concinna*	DC.		NP
Myrtaceae	*Eugenia astringens*	**Cambess.**		**NP**
Myrtaceae	*Eugenia brasiliensis*	**Lam.**	**grumixama**	**NP**
Myrtaceae	*Eugenia neoglomerata*	Sobral		NP
Myrtaceae	*Myrcia bicarinata*	(O.Berg) D.Legrand		NP
Myrtaceae	*Myrcia ilheosensis*	**Kiaersk.**		**NP**
Myrtaceae	*Myrcia multiflora*	**(Lam.) DC.**		**NP**
Myrtaceae	*Myrcia splendens*	(Sw.) DC.	pau-tinto	NP
Myrtaceae	*Myrciaria glazioviana*	(Kiaersk.) G.M.Barroso ex Sobral	cabeludinha	NP
Myrtaceae	*Plinia edulis*	(Vell.) Sobral	cambucá	NP
Myrtaceae	*Psidium cattleyanum*	Sabine	araçá	P
Myrtaceae	*Siphoneugena guilfoyleiana*	Proença		NP
Nyctaginaceae	*Guapira opposita*	(Vell.) Reitz	maria-mole	P
Pentaphylacaceae	*Ternstroemia brasiliensis*	**Cambess.**		**NP**
Primulaceae	*Myrsine coriacea*	**(Sw.) R.Br. ex Roem. & Schult.**	**capororoca**	**P**
Primulaceae	*Myrsine guianensis*	(Aubl.) Kuntze	capororoca	P
Primulaceae	*Myrsine umbellata*	Mart.	capororoca	P
Primulaceae	*Myrsine venosa*	A. DC.		P

Tab 1. Continuação 23

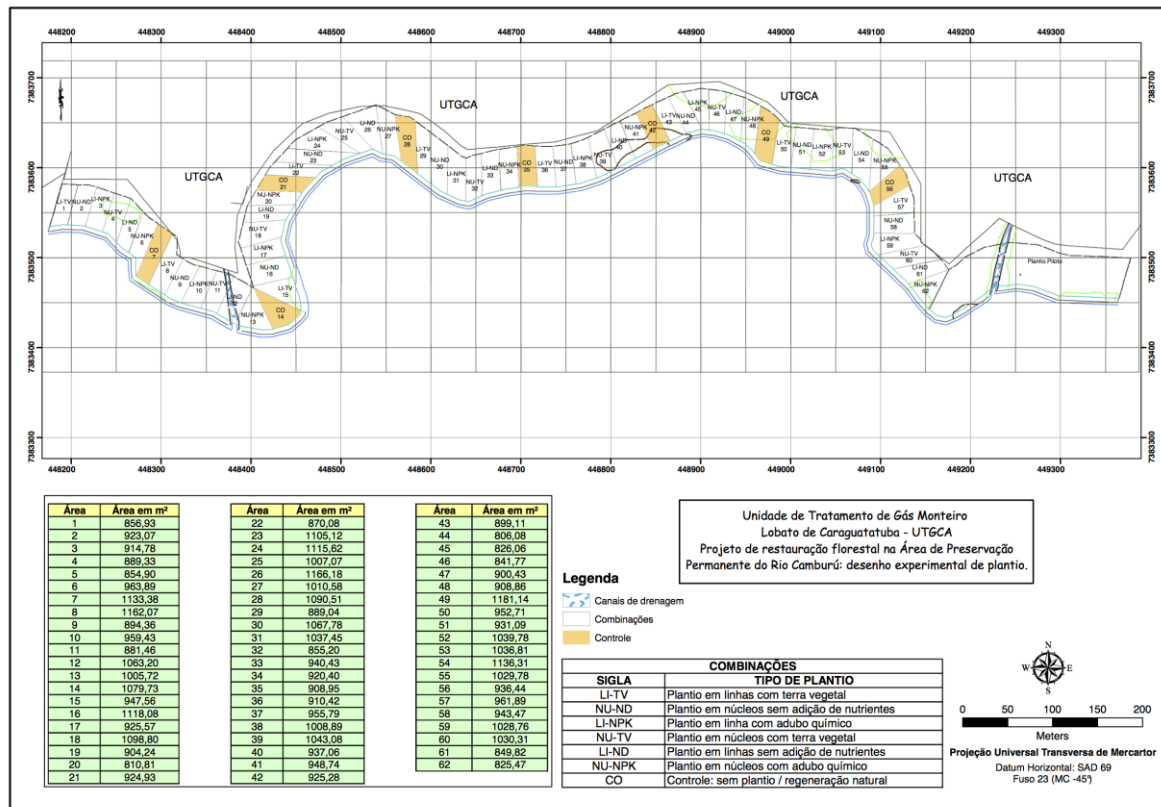| Sapindaceae	*Cupania oblongifolia*	Mart.	cuvatã	NP
Sapindaceae	*Matayba guianensis*	Aubl.		NP
Sapotaceae	*Manilkara subsericea*	(Mart.) Dubard		NP
Sapotaceae	***Pouteria* sp.**			**NP**
Solanaceae	***Solanum cinnamomeum***	**Sendtn.**		**P**
Urticaceae	*Cecropia pachystachya*	Trécul	embaúba	P
Verbenaceae	***Citharexylum myrianthum***	**Cham.**	**pau-viola**	**P**

Figura 2: Esquema de implantação dos blocos e parcelas e quadro de área de cada parcela implantada na área experimental situada na UTGCA, Caraguatatuba, SP.

Tratamentos aplicados no plantio:

A) Adição de nutrientes

Foram aplicadas três condições (níveis):

TV – Adição de 2kg de Terra Vegetal em cada cova no início do plantio;

NPK – Adição de 100g de NPK 20:0:20 por planta a cada 9 meses. Cada adubação foi dividida em três partes iguais e aplicada por três meses consecutivos (a quantidade de adubo químico foi estipulada de acordo com a recomendação da análise de solo).

ND – Sem adição de nutriente ou matéria orgânica.

B) Sistema de plantio

Foram usadas duas condições:

LI – Plantios em Linhas, com espaçamento de 2 x 2 m, sendo o número de linhas variável de acordo com a forma de cada parcela (ver Figura 2). Em média, cada parcela continha 10 linhas, mas o número de linhas variou de 7 a 16, pois o formato triangular de algumas parcelas resultou em linhas mais curtas nas pontas do triângulo. Linhas de espécies pioneiras (P) e não pioneiras (NP) foram alternadas nas parcelas (Figura 3A). Dentro dessas linhas, as espécies foram plantadas aleatoriamente, procurando-se entretanto evitar espécies repetidas na sequência do plantio.

NU – Plantios em Núcleos.foram plantados agrupamentos de 13 mudas, sendo cinco mudas de espécies pioneiras (P) e oito mudas de espécies não-pioneiras (NP).

No centro e nos quatro cantos de cada núcleo, foram plantadas as espécies pioneiras (P). Em posições opostas e a distâncias equivalentes e cruzadas, foram plantadas as oito mudas de espécies não pioneiras (NP) (Figura 3B). A distribuição dos núcleos na área foi realizada com espaçamento de 5m, contando a partir do centro do núcleo. O número de núcleos plantados por parcela variou de 19 a 35. As figuras 4, 5 e 6 mostram exemplos do plantio em núcleos logo após o plantio, após 11 e 20 meses, respectivamente. Duas combinações de espécies pioneiras e não-pioneiras nos núcleos (denominados núcleo A e B) foram repetidas em todas as parcelas com pelo menos 6 réplicas em cada parcela de núcleo. As mesmas espécies plantadas nos núcleos A e B (ao todo 6 espécies pioneiras e 13 espécies não-pioneiras) foram monitoradas nas parcelas de linha (no mínimo 6 indivíduos por espécie em cada parcela). Os demais núcleos foram plantados com combinações diversas, respeitando-se a proporção de 5 pioneiras e 8 não-pioneiras.
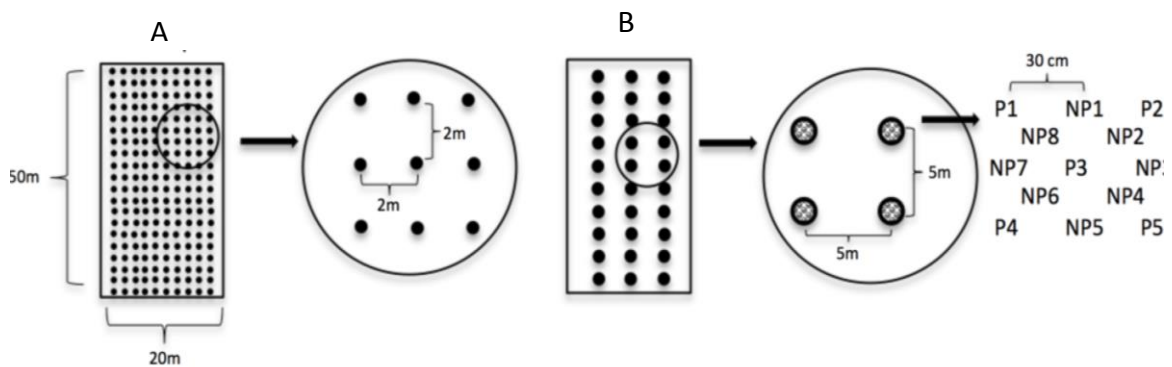

Figura 3: Esquema de distribuição espacial das mudas. A) Plantio das espécies em linha (sistema isolado). B) Plantio das espécies em núcleo (sistema agregado). Cada núcleo foi composto por 13 mudas, sendo 5 pioneiras (P) e 8 não-pioneiras (NP).

A partir dos três tratamentos descritos acima, seis combinações de tratamento e um controle foram implementados em cada bloco:

I - Controle (CO)

II - Plantio em Linhas com adição de Terra Vegetal (LI - TV)

III - Plantio em Linhas com adição de Adubo Químico (LI - NPK)

IV - Plantio em Linhas sem adição de nutrientes (LI - ND)

V - Plantio em Núcleos com adição de Terra vegetal (NU - TV)

VI - Plantio em Núcleos com adição de Adubo Químico (NU - NPK)

VII – Plantio em Núcleo sem adição de nutrientes (NU- ND)

Figura 4: Detalhe do plantio em núcleo logo após o plantio em uma área em restauração ecológica, Caraguatatuba (SP).

Figura 5: Detalhe de núcleo cerca de 11 meses após o plantio.

Figura 6: Detalhe de núcleos cerca de 20 meses após o plantio.

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2. Capítulo 1

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Cluster planting facilitates survival but not growth in early development of restored tropical forest

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Abstract

Interspecific interactions are considered to be important structuring forces in early successional vegetation. Whereas competition seems to prevail in less severe environments, facilitation tends to increase in importance in harsh environments. Hence, facilitation is expected to play an important role in degraded tropical areas with high irradiance, heat loads, and evapotranspiration, where conditions are far from optimum for most forest species. We planned a restoration project on the southeastern Brazilian coastal plain to compare growth and survival of tree seedlings planted at two densities (isolated or aggregated) in a factorial experiment with nutrient addition. We monitored survival, height, ground level diameter, and crown projection of 4,132 saplings from 19 species that ranged along a successional gradient, over an 18-month period. We used mixed-effect models to analyze the relationship between species performance and treatments, and Akaike's information criterion (AIC) to select the models. The best model showed higher survival in aggregated plantations (indicating facilitation) for non-pioneer (slower growing) species. In contrast, we found lower diameter growth in aggregated plantation (indicating competition). Fertilizer addition did not affect survival in clusters, but, surprisingly, it had a negative effect on isolated plants of both pioneer and non-pioneer species. Fertilizer addition had a positive effect on diameter and crown projection growth in aggregated plantations (reducing competition), especially for pioneer species. Thus, whether facilitation or competition was the predominant interaction depended on the effect analyzed. As establishment of non-pioneer species in disturbed sites can be challenging, restoration designs could take advantage of higher survival rates in clusters and use resource addition to ameliorate growth and decrease competition for limited resources.

Zusammenfassung

Interspezifische Interaktionen werden als wichtige strukturierende Kräfte für die frühe Sukzessionsvegetation angesehen. Während Konkurrenz bei weniger strengen Umweltbedingungen vorzuherrschen scheint, tendiert 'facilitation' bei harschen Umweltbedingungen an Bedeutung zuzunehmen. Deshalb wird erwartet, dass 'facilitation' eine wichtige Rolle in degradierten tropischen Gebieten mit hoher Einstrahlung, Wärmebelastung und Evapotranspiration spielt, wo die Bedingungen weit vom Optimum für die meisten Waldarten entfernt sind. Wir planten ein Renaturierungsprojekt in der Küstenebene Südost-Brasiliens, um Wachstum und Überleben von Baumsetzlingen zu vergleichen, die in zwei Dichten (isoliert und aggregiert) in einem faktoriellen Experiment mit Nährstoffzugabe gepflanzt wurden. Wir erfassten über 18 Monate hinweg Überlebensrate, Höhe, basalen Stammdurchmesser und Kronenprojektionsfläche von 4132 Setzlingen aus 19 Arten in einem Sukzessionsgradienten. Wir benutzten gemischte Modelle, um die Beziehung zwischen der Performanz der Arten und den Behandlungen zu analysieren, und wählten die Modelle mit Hilfe des Akaike-Informationskriteriums (AIC) aus. Das beste Modell zeigte höhere Überlebensraten bei aggregierter Pflanzung (Indiz für 'facilitation') für langsam wachsende Nicht-Pionier-Arten. Dagegen fanden wir geringeres Dickenwachstum bei aggregierter Pflanzung (Indiz für Konkurrenz). Düngung beeinflusste die Überlebensrate bei aggregierter Pflanzung nicht, sie hatte aber überraschenderweise einen negativen Effekt auf isolierte Pflanzen von sowohl Pionier-Arten als auch Nicht-Pionier-Arten. Düngung hatte besonders bei Pionierpflanzen einen positiven Effekt auf Dicken- Kronenwachstum bei aggregierter Pflanzung (Indiz für reduzierte Konkurrenz). Ob 'facilitation' oder Konkurrenz die vorherrschende Interaktion war, hing damit vom betrachteten Effekt ab. Da die Etablierung von Nicht-Pionier- Arten auf gestörten Flächen sehr schwierig sein kann, könnten Renaturierungsplanungen sich die höheren Überlebensraten bei aggregierter Pflanzung

zunutze machen und Düngung einsetzen, um das Wachstum zu verbessern und die Konkurrenz um begrenzte Ressourcen zu vermindern.

Introduction

Interspecific interactions play an important role in the establishment of new plant communities. Competition (Dybzinski & Tilman 2007; Weigelt et al. 2007) and facilitation (Bruno, Stachowicz & Bertness 2003; Brooker et al. 2008) can act simultaneously and interact in complex ways in structuring communities, and both mechanisms have been reported to explain species coexistence (Wright 2001; Cavieres & Badano 2009). Whereas competition has been proposed to be a major structuring force in less severe environments, facilitation tends to increase in importance in harsh environments (Bertness & Callaway 1994; Callaway et al. 2002; Maestre et al. 2009), where nurse plants (Padilla & Pugnaire 2006) improve environmental conditions for target plants through shading, ameliorating temperature extremes, changing soil properties, and increasing resource availability (Callaway 1995).

Therefore, facilitation could well play an important role in degraded tropical areas with high irradiance, heat loads, and evapotranspiration, conditions that are far from the optimum for most forest species (Gómez-Aparicio 2009). Some remnant trees and small patches of trees have been shown to facilitate plant establishment in tropical degraded areas (Schlawin & Zahawi 2008; Corbin & Holl 2012; Zwiener et al. 2013). Moreover, in active restoration pioneer trees can also act as nurse plants (Gómez-Aparicio 2009; Prévosto et al. 2012) because they are more tolerant to harsh microclimatic conditions, grow quickly, and ameliorate conditions for non-pioneer species (Connell & Slatyer 1977).

Organisms in close proximity can also compete, and resource competition theory predicts that when species compete for a single resource, those able to cope with the lowest resource levels are likely to win (Dybzinski & Tilman 2007). Moreover, rapidly colonizing, fast-growing species (pioneers) are typically weaker competitors for nutrients (Wedin & Tilman 1993). Therefore, responses to biotic interaction and resource addition may differ according to the

successional status, and it would be expected that pioneer species are most likely to benefit from nutrient enrichment (Lawrence 2003; Siddique et al. 2010), because of subsequent reductions in competition.

In this context, we planned a restoration project in the coastal plain of Brazil to experimentally test how plant proximity and changes in resource availability impact the survival and growth of species from different successional groups. We compared the performance of individuals in multi-species clusters (aggregated plantings) with that of individuals from the same species in less dense (isolated) plantings to determine whether they performed better when planted more densely, which we interpreted as facilitation, or in isolated conditions, indicating competition. We also evaluated the effect of adding resources (fertilizer) on interspecific interactions. Following the framework presented above, we predicted that (1) fast growing plants (pioneers) would provide shade for slow growing species (non-pioneers) and facilitate their survival and growth in degraded areas (Yang et al. 2010); thus non-pioneers would respond more positively in the aggregated planting; and (2) pioneers should be in competitive disadvantage for nutrients and hence would benefit more from nutrient enrichment. Such manipulative experiments in restoration projects represent excellent opportunities to test and refine ecological theories, which in turn may contribute to new insights in developing restoration models (Young, Peterson, & Clary 2005). Despite the theory and empirical bases for those predictions, they have never been tested in a multi-species restoration context in the tropics.

Materials and methods

Study area

We conducted this study adjacent to the Camburu River on the coastal plain, in

Caraguatatuba, São Paulo state, southeastern Brazil (23°39'27"S, 45°30'03"W). The climate is warm (mean annual temperature is 24.5 °C, with mean minimum 18.2 °C, and mean maximum 34.5 °C), characterized by rainy summers and less rainy winters, but there is no well-defined dry season. Mean annual precipitation from 1943 to 2004 was 1784 mm (Santos & Galvani 2012). The site is ~7 m a.s.l., and the topography is almost flat with some areas subject to periodic flooding due to heavy rains or upwelling of groundwater. The soil is a mix between original sandy soil and clay soil originating from a landslide in 1967 (Ferreira 2013). The area was dominated by invasive grasses and was used for grazing prior to the start of this study. The landscape is comprised of a pasture matrix, with a few small fragments of secondary forest on the coastal plains surrounded by a dense tropical forest in the mountain range ca. 1.5 km away.

Experimental Design

The experimental design consists of two main factors: planting system and fertilization set up in an orthogonal fashion with four factorial treatment combinations: isolated plantation fertilized (I-F), isolated non-fertilized (I-NF), aggregated plantation fertilized (A-F), and aggregated non-fertilized (A-NF). There were eight replicate blocks which included ~20 x 50 m plots of each treatment and were separated by an un-manipulated area (Fig. 1A). We selected six pioneer and 13 non-pioneer native tree species to plant using the categorizations of Swaine and Whitmore (1988). Saplings from those species were planted in two planting system: isolated (I) on a 2 x 2 m-grid with alternating lines of pioneer and non-pioneer species (Fig. 1B); and aggregated (A), where mixed-species clusters of 13 saplings were planted with 5-m separation (Fig. 1C). Within each cluster, saplings were separated by 30 cm. Each cluster was composed of five species previously classified as pioneers and eight as non-pioneers species (Fig. 1D). We used two different cluster compositions with a total of 19 species monitored in both the aggregated and isolated plantings.
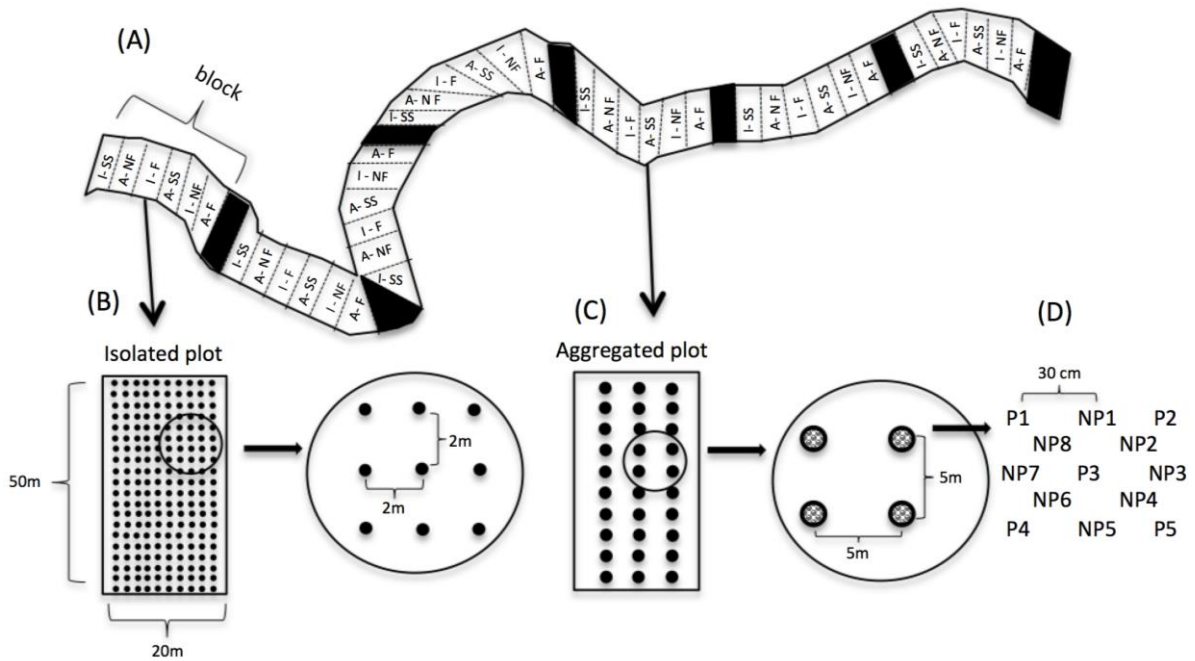
Fig. 1. (A) Experimental design. Each block was composed of six 20 x 50 m plots and was separated by an empty plot (filled in black). I-SS: Isolated plot with standardized soil (2 liters of humus per plant) used to determine the proxy for successional index (data not used in this experiment); A-NF: Aggregated plot non fertilized; I-F: Isolated plot fertilized; A-SS; Aggregated plot with standardized soil (data not used in this experiment); I-NF: Isolated plot non fertilized; A-F: Aggregated plot fertilized. (B) In isolated plots seedlings were planted equidistant (2 m) throughout the plot; (C) In aggregated plots cluster centers were separated by 5 m from each other; (D) In each cluster seedlings were planted 30 cm apart from each other inside clusters, with 5 different pioneer species (P) in the center and on the edge of each plot and 8 different non-pioneer species (NP) around the pioneers.

The soil was prepared by adding lime to the entire study area at a rate of 1500 kg/hectare. The planting holes were dug mechanically to 30 x 30 x 30 cm. Prior to planting, 5 grams of hydrogel diluted in 1 liter of water were added in each planting hole in order to reduce initial mortality due to water stress. Seedlings were planted manually. Seedlings varied between 6 to

18 months age at the time of planting, but most seedlings of the same species were the same age.

The fertilization treatment comprised two additions of 100 g of slow release NPK 20:0:20 per plant separated by 9 months; each addition divided into three equal portions and applied once monthly for three months. We followed soil analysis recommendations for the amount and proportion of nutrients to add, consistent with typical restoration practices in this area. The contrast was a non-fertilized treatment with no nutrient addition. In addition to the treatments described above there was another pair of aggregated and isolated planting plots per block (not presented in our main results) with standardized soil conditions, where 2 liters of humus per plant were incorporated at the time of the outplanting. We used the isolated-planting, standardized-soil plots to establish a proxy for successional index based on plant height growth (see next section).

In each plot, we monitored a minimum of six randomly-selected individuals from each species totaling 4,132 saplings from 19 species. At the time of outplanting, saplings varied in initial size from 2 cm (mostly the palms, since we measured from apical bud) to 140 cm. Although variation among species was high, variation among individuals of the same species was quite low, since they were all from the same cohort. After three months we replanted all dead individuals to eliminate mortality due to outplanting stress, and 30 days after replanting we took initial measurements.

Data collection and analysis

We measured survival, height of tallest stem, ground level diameter, and crown projection of saplings. Crown projection was quantified using the ellipse area calculated from two perpendicular measures. We calculated the Relative Growth Rate (RGR - *sensu* Hoffmann & Poorter 2002) for each growth variable from initial census to the 18-month census for all

surviving saplings. We excluded from our analysis species for which all individuals died in one or more factorial treatments, which resulted in 16 species for data analysis.

Because classification of successional characteristics can be subjective and is certainly better represented as a continuum rather than dichotomous groups (i.e., pioneers and non-pioneers), (Alvarez-Buylla & Martinez-Ramos 1992; Liebermann et al. 1995; Souza & Válio 2003), we decided to establish a continuum proxy for successional classification instead of using the binary pioneer vs. non-pioneer classification. Assuming that pioneer species have higher growth rates than non-pioneers under high light conditions (Baker, Swaine & Burslem 2003), we used the median height RGR of each species from the 18-month period in the isolated system of the standardized soil plots as a proxy. However, in order to assess whether the proxy used for successional index strongly affected the results, we also performed all analyses using the dichotomous groups according to literature (i.e. pioneer and non-pioneer species) and the results were qualitatively similar (see appendix A).

We used mixed-effects models to analyze the relationship between species performance and treatments, because they allow for differences in mean responses across blocks of a field experiment (Crawley, 2013). The response variables were (1) proportion surviving, (2) ground level diameter RGR, and (3) crown projection RGR. We considered successional index (SI), fertilizer (F), planting system (PS) and all possible interactions as fixed effects, and blocks as a random effect.

We used generalized linear mixed effect models (GLMM) to model proportion of surviving seedlings as a linear response, assuming binomial error distribution. For diameter and crown RGR we used linear mixed effect models (LMM). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. To estimate model coefficients we used Maximum Likelihood Estimation using packages lme4 and bbmle in the

R programming environment (Bates et al. 2015). We contrast the most parsimonious model (null model) with all biologically meaningful combinations of fixed variables (Table 1). The overall fit of all models was compared using Akaike's Information Criterion (AIC). We considered every model with ∆AIC < 2.0 to be equally plausible.

Results

Our results showed that height RGRs are continuous for species in the isolated system with standardized soil (Fig. 2), since there was no break point, and 10-90% quantiles of data cleary overlap among species. Species previously categorized as pioneers had the highest RGR for height with two exceptions, *Jacaranda puberula* Cham., which was previously considered as a non-pioneer showed fast growth rates, and the pioneer *Erythrina speciosa* Andr. which grew slowly.
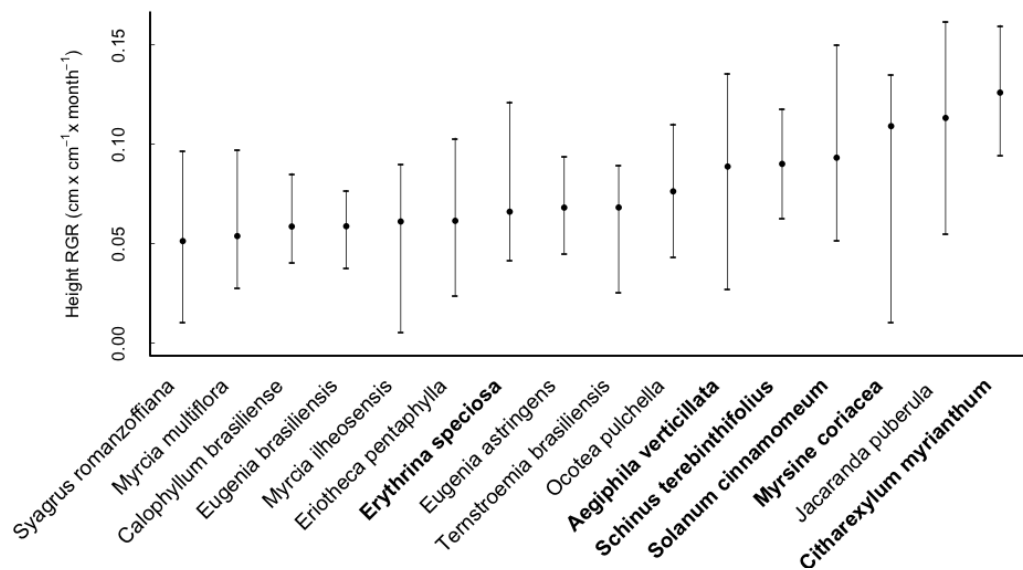
Fig. 2. Median values of height relative growth rate (RGR) over 18-months for 16 species monitored in the isolated, standardized soil treatment. Error bars represent data from quantiles 10% to 90%. Species previously classified as pioneer are marked in bold.

Two models were equally plausible in explaining survival data (Table 1). The estimated coefficients shared between those models are similar and the additional term retained in the second model (SI:F) is weak. The best fit selected model included F, PS, SI, and the PS:SI and PS:F interactions. Survival was higher for pioneer species in isolated systems; fertilizer had a negative effect on plants in isolated systems, but no effect in aggregated systems; and the aggregated system had a stronger positive effect on non-pioneers as compared to pioneers (Fig. 3).

Table 1. Selected models (ΔAIC<2) for the effect of treatments on the survival and growth of 16 species over 18-months. Survival models were binomial generalized mixed models (GLMM) with survival probabilities as response variable, and growth models used Gaussian distribution and had relative growth rate (RGR) as response variable. Successional index (continuous variable), fertilizer (fertilized or non-fertilized), and planting system (aggregate or isolated), and all possible interactions were considered as fixed effects. Every model included block as a random effect. SI: Successional index; F: Fertilizer; PS: Planting system.

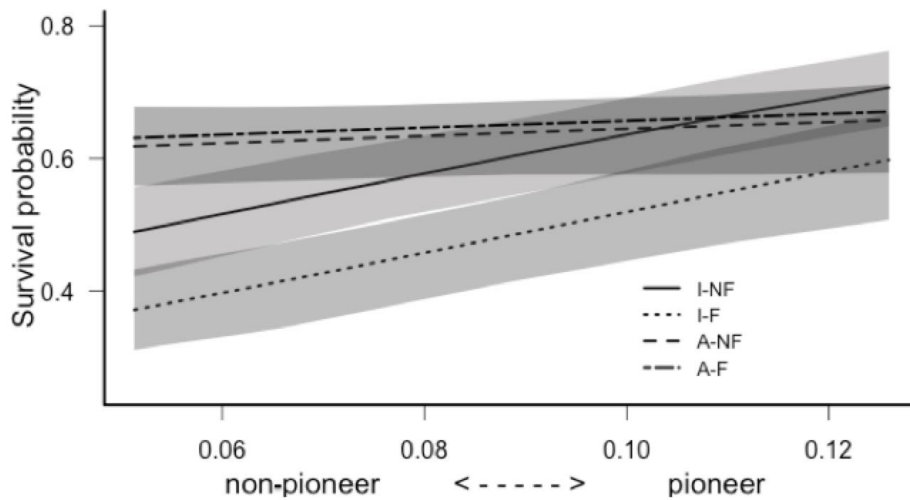| Parameters							Survival	Growth Rate	
								Diameter	Crown
PS x F x SI	PS x SI	SI X F	PS X F	SI	PS	F	Δ AIC	Δ AIC	Δ AIC
	X		X	X	X	X	**0**	-	-
	X	X	X	X	X	X	**1.7**	-	-
	X	X		X	X	X	-	**1.2**	-
	X	X	X	X	X	X	-	**0**	-
				X			-	-	**0**

Fig. 3. Values predicted by model selection using the Akaike Information Criterion for the relationship between survival probability and successional index of species in different factorial treatments for the best fit selected model. A = aggregated, I = isolated, F = fertilized, NF = non-fertilized. Shading represents the 95% confidence interval from 1000 simulations of the predicted values (the confidence interval for A-F is not showed since it overlapped A-NF).

Two different models for ground level diameter RGR were equally plausible (Table 1). The model selected with the lowest AIC included all terms except the triple interaction and all the confidence intervals did not overlap zero (Table 1). Diameter growth was higher in isolated compared to aggregated systems, particularly for non-pioneers, but the pioneer species showed even higher diameter growth in clusters when fertilized (Fig. 4). Fertilizer addition increased growth in the aggregated system, especially for pioneers, and slightly increased growth of pioneers in isolated planting systems.
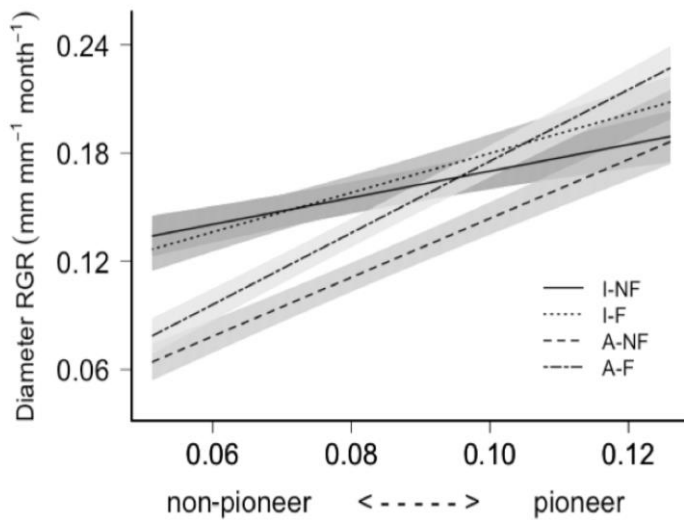
Fig. 4. Values predicted by the best fit model using the Akaike Information Criterion for the relationship between relative diameter growth rate and successional classification of species in different factorial treatments. A = aggregated, I = isolated, F = fertilized, NF = non-fertilized. Shading represents the 95% confidence interval from 1000 simulations of the predicted values.

The model selected for crown projection RGR only included successional index (Table 1). As expected crown projection RGR was greater for pioneers.

Discussion

Although competition and facilitation can occur together, the balance between them will result in net positive or negative interaction. Our results show that this balance varies within the same system (Callaway 1995), depending on the effect analyzed (Hastwell & Facelli 2008; Gómez-Aparicio 2009; Prévosto et al. 2012). In our study, whereas growth was reduced, survival was enhanced in aggregated systems, especially for non-pioneers, which is

consistent with previous studies showing more positive responses of shade-tolerant non-pioneer species to nurse plants (Gomez-Aparicio et al. 2004; Padilla & Pugnaire 2006; Yang et al. 2010).

The results suggest that high irradiance stress and temperature may be major factors limiting seedling survival in open tropical degraded areas. Accordingly, Castanho and Prado (2014) found that shade, either artificial or from an adult plant, was the mechanism responsible for facilitating seedling survival (although not growth) in this system. Since high irradiance and temperatures can lead to photoinhibition (Loik & Holl 1999; Ehleringer & Sandquist 2006), pioneer species able to cope with higher light conditions may act as nurse plants (Padilla & Pugnaire 2006) by reducing irradiance and temperature, improving conditions for more non-pioneer species. In this sense, Pereira (2014), studying in the same area, reported seedlings showing signs of photoinhibition, but with lower levels of photosynthetic stress, soil and foliar temperature in an aggregated system.

On the other hand, surviving seedlings in clusters appeared to compete for nutrients since diameter growth was lower in the aggregated system, and this reduction was counteracted by fertilizer addition, especially for pioneer species. Other studies have reported facilitation for survival but not for growth, in the Brazilian coastal plain (Castanho & Prado 2014), as well as a wide range of ecosystems around the world (Gómez-Aparicio 2009 and references therein). Therefore, ideal environmental conditions for seedling survival are different than for seedling growth (Gómez-Aparicio 2009). Interestingly, however, growth in crown projection was not affected by competition in the aggregated system, which may change over time, as space becomes a limited resource.

Surprisingly, seedling survival was lower in fertilized plots in the isolated system. One possible explanation for this result is that excess of fertilizer might have damaged seedlings in

isolated systems. Species can lose water through their roots due to high osmotic potential resulting from high fertilizer levels (Jacobs & Timmer 2005). In aggregated systems reduction of irradiance intensity may have diminished plant transpiration and some species able to uptake a larger amount of fertilizer may have ameliorated conditions for others. Another possibility is that nutrient addition has been shown to increase herbivory (Andersen et al. 2010; Santiago et al. 2010). Although we did not measure herbivory, we saw no obvious patterns of differential herbivore damage.

In aggregated systems, fertilizer addition seemed to reduce the competition effect for pioneer species much more strongly than non-pioneers. The same patterns have been reported for grass interactions (Wedin & Tilman 1993) and for adult trees in regeneration plots in Amazon (Siddique et al. 2010), where pioneer species were favored by nutrient addition. According to Burslem, Pinard and Hartely (2005), pioneers could outcompete non-pioneers over the short term, as they monopolize and pre-empt resources, yet non-pioneers would prevail in the long term due to competitive success related to low resource requirements. In this study, however, the higher growth rate of pioneers could be leading to individual level asymmetric competition (Freckleton & Watkinson, 2001), as competition for seedling establishment can have a stronger biomass effect on early developmental stages (Weigelt, Steinlein & Beyschlag 2002). During later developmental stages it is likely that pioneer species will stop growing and asymmetric competition may diminish.

Over time, the pioneer species will likely die earlier than non-pioneers, freeing up space and resources for non-pioneers to thrive after the juvenile stage, which is critical to survival. It is likely, however, that the pioneers will release seeds in the soil before they die, since several pioneer species have already fruited in the restoration area. These seeds in turn, may show a relatively longer ecological longevity in the soil seed bank (Vázquez-Yanes & Orozco-

Segovia, 1993) and can germinate when there are natural clearings or in areas that remains open due to the mortality of planted seedlings.

Despite competing for height growth with non-pioneer species in early restoration phase, pioneer species had higher initial survival rates, which makes them suitable for restoration projects (Elliott et al. 2003). However, these species usually have short life cycles and many restoration projects in Brazil have failed after they died (Barbosa et al. 2003; Souza & Batista 2004; Rodrigues et al. 2009a), highlighting the importance of introducing non-pioneer, long-lived species at some point in the restoration process (Rodrigues et al. 2009b). Since establishment of non-pioneer species in degraded areas can be challenging (Gómez-Aparicio 2009), restoration designs could take advantage of higher survival rates of slow growing species in cluster (i.e. they should be outplanted close to pioneer trees). Furthermore, outplanting clusters may enhance spatial heterogeneity in the system (Holl et al. 2013), which may promote an increase in diversity in late successional stages.

Understanding structuring forces in tropical plant communities is important to develop efficient restoration model based on scientific results, since theoretical basis of restoration ecology derives from basic concepts of community ecology (Young, Petersen & Clary 2005). Evaluating performance of tropical trees requires a longer time to observe consistent patterns, and results presented here may show just the first step of the undergoing processes. However, our study shows that cluster planting facilitates survival of non-pioneers species at the beginning of restoration when abiotic conditions are harsh. At the same time, the non-pioneer species are subject to greater competition for diameter growth at this stage, possibly due to competitive asymmetry. The observed shifts in the balance of facilitation and competition as well as the interaction with successional species index can be used to adjust the management regime for restoration projects. Besides practical implications, the results from experimental

restoration projects may improve our ecological knowledge of the successional dynamic and community structuring in these systems.

Acknowledgements

Appendix A. Supplementary data

In order to assess whether the proxy used for successional index strongly affected the results, we also performed all analyses using the dichotomous groups according to literature (i.e. pioneer and non-pioneer species) and the results were qualitatively similar. For survival (Fig. 1A) and crown projection, exactly the same models were selected (Table 1). For growth in diameter, the selected model did not include two interactions (SG:F and PS:F), but results still matched, since pioneer grew faster than non-pioneer and non-pioneer showed lower growth in aggregated systems (Fig. 1B).
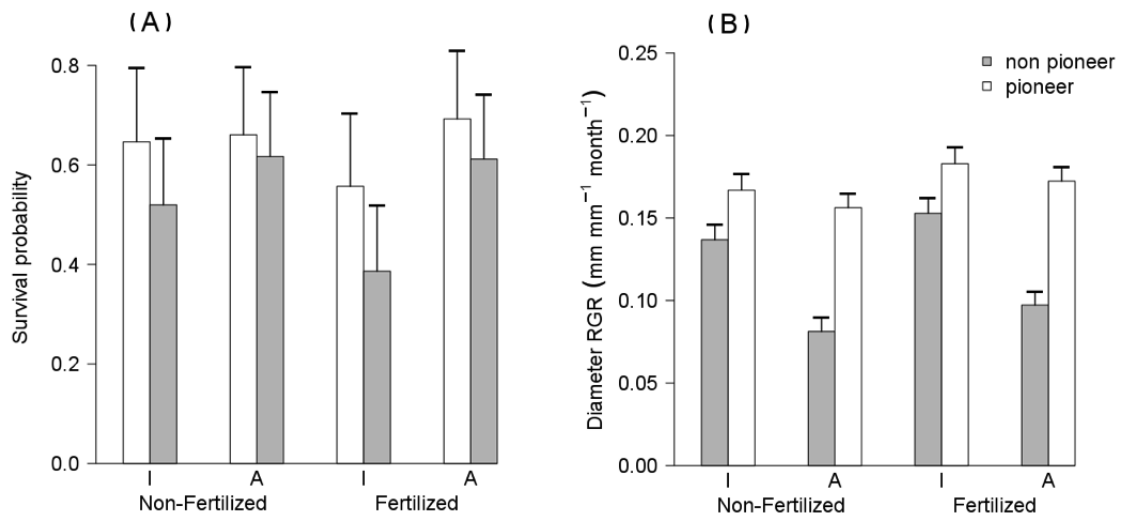
Fig. 1: Values predicted by model selection using the Akaike Information Criterion for the relationship between survival probability and successional group (A), and diameter growth rate and successional group (B) in different factorial treatments for the best fit selected model. A= aggregated system, I= isolated system. Bars represent the 95% confidence interval

Table 1: Selected models (ΔAIC<2) for the effect of treatments on the survival and growth of 16 species over 18-months. Survival models were binomial generalized mixed models (GLMM) with survival probabilities as response variable, and growth models used Gaussian distribution and had relative growth rate (RGR) as response variable. Successional group (discrete variable), fertilizer (fertilized or non-fertilized), and planting system (aggregate or isolated), and all possible interactions were considered as fixed effects. Every model included block as a random effect. SG: Successional group; F: Fertilizer; PS: Planting system.

Parameters							Survival	Growth Rate	
								Diameter	Crown
PS x F x SG	PS x SG	SG X F	PS X F	SG	PS	F	Δ AIC	Δ AIC	Δ AIC
				X			-	-	**0**
	X			X	X	X	-	**0**	-
	X		X	X	X	X	**0**	-	-
	X	X	X	X	X	X	**0.6**	-	-

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3. Capítulo 2

Title: Can plant traits predict outcomes of interaction among seedlings in tropical forest restoration planting?

Abstract

In order to understand complex structuring process of tropical forests ecologists have realized the need for simplification. A promising way to face this question is through the lens of functional traits, which can be measured for any species and compared in different systems, and different locations around the world. However, whether this approach can actually be applied in the restoration of tropical forest, where initial conditions are far from the optimum for most forest species, remains uncertain. Moreover, little is know about the role of species interaction on the relation of functional traits and species performance. In this paper we intended to use core simple-measurable traits to answer the following questions: Are functional traits related to growth and survival? Is there a difference in the effect of the functional traits on plant performance of isolated individuals or of individuals subjected to interaction with neighbors? Our main findings were that for pioneer species, the greater the specific leaf areas (SLA), the greater the survival rates, but, under aggregated conditions, the greater the SLA the lower the survival rates. However, functional traits only partially explained saplings performance in a restoration context and more research is needed to understand its role in predicting seedlings outcomes, especially considering the important applicability of the subject.

Keywords: Brazil, functional traits, restoration, seedling performance, tropical forest.

Introduction

Plant communities often present extremely complex structure, dynamic, and an apparently idiosyncratic species composition (i.e. site-dependent), which turns out to be a huge challenge to understand how communities are structured and what are the major forces driving biodiversity patterns. In the current biodiversity crisis, this knowledge is essential to the success of conservation and restoration actions. To overcome this challenge, ecologists have realized the need for simplification in order to transform complex species lists in a few common attributes that could be potentially related to mechanisms underpinning community structure. Therefore, a promising way to face this question is through the lens of functional traits (Adler et al., 2013), that are morpho-physio-phenological traits, which impact fitness indirectly via their repercussion on growth, reproduction and survival (Violle et al. 2007). Functional traits can be measured for any species and compared in different systems and different locations around the world. They can potentially represent a common "currency" applied to any community, regardless its specific species composition and idiosyncratic processes (Clark et al., 2012).

However, despite all research effort that has been invested in this approach, it is still not clear whether functional traits can truly undertake species identity to explain major communities patterns. Even with some clear relationships among functional traits and community structuring (Kunstler et al., 2016), there is still a lot of criticism regarding the use of this approach. For example, the common focus on a single trait rather than multiple trait combinations could imply in weak links between functional traits and species responses to environmental changes (Laughlin & Messier, 2015), and different trait combinations could result in the same fitness in the same environment (Marks & Lechowicz, 2006). Consequently, there is still a discussion whether functional traits could in fact be used as a proxy for different species strategies in different environments.

In degraded tropical areas like pastures, for example, species are subjected to high irradiance, heat loads, and evapotranspiration, conditions that are far from the optimum of most tropical forest species. In these conditions, there is a current lack of knowledge regarding the relationship between functional traits and species performance (but see Mártinez-Garza et al., 2013), which precludes the prospective use of functional traits for management and restoration actions in a more efficient way. This is particularly important because under different environmental conditions the same traits could express different outcomes (Sandell et al., 2011).

In forested sites, specific leaf area (SLA) has been positively related to growth, since it is associated with high photosynthesis rates, nutrient concentration and respiration rates (Gustafsson et al., 2016; Poorter & Bongers, 2006; Wright et al., 2004). However, in degraded tropical areas, the relationship between SLA and performance is still not clear (Mártinez-Garza et al., 2013). While in tropical forests only small fraction of the total irradiance reaches the soil, in pastures plants are under high irradiance and temperature and often show signs of photoinhibition (Loik & Holl 1999; Ehleringer & Sandquist 2006). Therefore, high photosynthesis and respiration rates associated with high SLA may lead to photosynthetic stress. Probably because of that, when light gradient is manipulated, most tree species reach maximal relative growth rate (RGR) at intermediate light conditions (Veenendaal et al., 1996; Poorter, 1999; Dalling et al., 2004), especially in situations where plants growing at full light also suffered from water limitation or high soil temperature (Poorter, 1999).

To avoid irreversible damage to the photosynthetic system, plants may use others strategies, such as stomatal closure, or direct investment to roots for water uptake to compensate for transpirational losses (Poorter, 1999). Concerning leaf structures, a high leaf dry matter content (LDMC) could be advantageous, due to its capacity to avoid desiccation (Shipley et al. 2006). High LDMC has been related to a higher survival rate of non-pioneer species in

high irradiance and temperature conditions (Martinez-Garza et. al 2013). However, traits responses could be different among successional species groups due to different physiologic strategies, such as lower leaf temperatures in pioneer species (Pereira, 2014) and other associated characteristics, such as differences in life-span, leaf turnover rates, wood density, and different strategies for resource acquisition (Swaine & Whitmore, 1988).

Resource availability is directly related to functional traits, since relevant traits may depend largely on environmental context (Butterfield & Callaway, 2013). For example, in forested areas, where light is a limiting resource, tree maximum potential height correlates positively with survival (Poorter et al., 2008). However in high light conditions higher maximum potential height may not be an advantage, since taller species may perform worse in competition for water (Butterfield & Callaway, 2013). Additionally, since interactions among plants can modify resource availability through competition and facilitation, plant ecological interactions could also play a major role in the determination of successful traits (Adler et al. 2013). For example, species with high SLA require large nutrient amount (acquisitive strategy) and would be favoured in high nutrient environments, while species with low SLA would be favoured in low nutrient environments due to their conservative strategy (Adler et. al 2013). Thus, in situations under low competition, species with acquisitive strategy should have advantage, but in a high competitive environment, species with conservative strategy should have advantage due to lowest resource availability. In this sense, low SLA has been related to species with a stronger competitive effect, while high SLA has been correlated to species with weaker competitive effect (Kunstler et al. 2016). However, despite its importance to ecosystem process, just a few studies have explored the link between functional traits and plant interactions (Perring et al. 2015)

We set up and monitored a restoration project in a factorial design in order to evaluate the effects of plant interaction on seedlings/saplings performance. In a previous paper

(Bertoncello et al. 2016) we analysed the importance of plant interaction and nutrient availability. We found lower diameter growth rate in multi-species clusters (aggregated plantation) comparing to species without interaction (indicating competition). Also, nutrient addition had a positive effect on diameter growth, apparently reducing competition for nutrients. On the other hand, species in clusters had survival facilitated, probably due to a minimization of excess of radiation.

In this paper we intended to use core simple-measurable traits to answer the following questions: Are functional traits related to growth and survival? Is there a difference in the effect of the functional traits on plant performance between individuals in isolated planting and individuals in aggregated planting (subjected to competition and/or facilitation interaction) with neighbours? Given our restoration planting was in early phase and the seedlings were exposed to a high light conditions in a degraded sandy soil, our predictions are: (1) intermediate SLA species would have a better performance in isolated planting, since very high SLA species should be more sensitive to high irradiance; (2) low SLA species would have a better performance in cluster when comparing to its performance isolated, because they would cope better with competition; (3) species with high LDMC would have high survival rates when isolated, but this trait might not be too important in clusters, since there would be an attenuation of desiccation; and (4) maximum potential height should be positively related to performance in aggregated system, as species could be competing for light in this situation, but this relationship should not appear in isolated systems.

Materials and methods

Study area

Data used in this work came from a research project conducted on the coastal plain, in

Caraguatatuba, São Paulo state, southeastern Brazil (23°39'27"S, 45°30'03"W). The climate is warm (mean annual temperature is 24.5 °C, with mean minimum 18.2 °C, and mean maximum 34.5 °C), characterized by rainy summers and less rainy winters, but there is no well-defined dry season. Mean annual precipitation from 1943 to 2004 was 1784 mm (Santos & Galvani 2012). The site is ~7 m a.s.l., and the topography is almost flat with some areas subject to periodic flooding due to heavy rains or upwelling of groundwater. The soil is a mix between original sandy soil and clay soil originating from a landslide in 1967 (Ferreira 2013). The area was used as pasture prior to the start of this study and invasive grasses dominated it just before planting. The landscape is comprised of a pasture matrix, with a few small fragments of secondary forests on the coastal plains surrounded by a dense tropical forest in the mountain range ca. 1.5 km away.

Experimental Design

The experimental design consists of a restoration project, divided in eight replicate blocks, each including one plot of aggregated planting system and one plot with isolated planting system (each plot size of about 20m × 50m). In the isolated system saplings were planted on a 2m x 2m-grid with alternating lines of pioneer and non-pioneer species. In the aggregated system, we used mixed-species clusters of 13 saplings. Within cluster, saplings were planted 30 cm apart each other. Each cluster was composed of five species classified as pioneers and eight species as non-pioneers (*sensu* Swaine and Whitmore, 1988).

The soil was prepared by adding lime to the entire study area at a rate of 1500 kg/hectare. The planting holes were dug mechanically to 30 x 30 x 30 cm. Prior to planting, 5 g of hydrogel diluted in 1 litre of water were added in each planting hole in order to reduce initial mortality due to water stress. Seedlings varied between 6 to 18 months age at the time of planting, but most saplings of the same species were equal-ages. At the time of planting, seedlings varied in

initial size from 2 cm (mostly for palms, since we measured from apical bud) to 140 cm height. Although variation among species was large, variation among individuals of the same species was quite small, since they were all from the same cohort. Seedlings were manually planted. After three months we replanted all dead individuals in order to counterbalance mortality due to planting stress, and 30 days after replanting we took initial measurements.

Data collection

We measured ground level diameter 30 days after replanting and 18 months after that first measurement. In each of the eight blocks we monitored one aggregated system plot and one isolated system plot, with a minimum of six randomly selected individuals from each species per plot.

In order to measure the leaf traits we selected one plant per species per plot and collected the highest branch, preferably sun exposed when that was possible. From each branch we chose three leaves in good conditions to measure the traits. Leaves were placed in buckets with water and maintained at around 5ºC in the fridge overnight to produce a standard degree of turgor. The sample of leaf lamina was dried and weighed on a precision scale. After that, we scanned with 300dpi and processed the data at ImageJ software (Rasband, 2007) in order to measure the leaf area. If there was any hole or missing part in the lamina, we excluded it to calculate the lamina area. The sample was dried in a paper envelope for 72 hours in the oven at 66 ºC and reweighed to produce a value for dry weight.

The functional traits used were specific leaf area (SLA), calculated as the ratio of leaf (only the lamina) area to dry mass (Pérez-Harguindeguy 2013), leaf dry matter content (LDMC) calculated as the oven-dry mass of a leaf (only the lamina), divided by its water-saturated fresh mass (Pérez-Harguindeguy 2013), and potential canopy height (PotH), determined according to Oliveira-Filho (2014). We used the mean values for the leaves collected from the

same individual of each species to determine individual functional traits.

Analysis

In order to evaluate if there was colinearity among the variables to be used in the analysis, we transformed SLA and LDMC in log scale and performed Pearson correlation between paired samples of SLA, LDMC and PotH, for both pioneer and non-pioneer species groups.

We calculated the Relative Growth Rate (RGR – sensu Hoffmann & Poorter 2002) from initial census to the 18-month census for all surviving saplings.

We used mixed-effects models to analyse the relationship between species performance and functional traits, because these models allow for differences in mean responses across blocks of a field experiment (Crawley, 2013). The response variables were survival probability, and relative growth rate (RGR) for ground level diameter.

In order to model diameter RGR, we used linear mixed effect models (LMM), assuming Gaussian distribution. To estimate model coefficients we used Maximum Likelihood Estimation using packages lme4 and bbmle in the R programming environment (Bates et al. 2015). Response variable, diameter RGR, was obtained for each monitored individual in the plot. The predictive variables (fixed effect) were specific leaf area (SLA), leaf dry matter content (LDMC), potential canopy height (PotH), and planting system (AS - aggregated system as base level). The values of potH were attributed to all individuals of each species, and the values of SLA and LDMC were attributed to all individuals of each species in each plot. The random variables were blocks and species.

We rescaled the fixed effect variables (SLA, LDMC and PotH) subtracting the average from each value and dividing by the standard deviation (Standardized score). Therefore the resulting value represents the distance to the population mean in units of standard deviation,

being negative below mean and positive above mean. This procedure allows standardizing different units and scales, in order to compare the coefficients estimated in the models.

We first contrasted the full model with and without squared SLA, in order to test our prediction that species with intermediate SLA would have better performance. If the model with squared SLA was selected, we would keep squared SLA in all other models. If not, we used all other models without squared SLA. After that, we contrasted the null model (i.e. only blocks and species random effects) with all possible models combining each and every fixed effect variables and interactions of two fixed effect variables.

Survival models followed essentially the same procedures, but we used generalized linear mixed effect models (GLMM) assuming binomial error distribution to model proportion of surviving seedlings as a linear response. Survival probability was inferred considering the total of individuals alive and dead of each species in each plot. We also contrasted the null model (i.e. only blocks and species random effects) with all possible models combining each and every fixed effect variables and interactions of two fixed effect variables. However, due to issues related to convergence and degree of freedom, some complex models could not be analysed.

We performed all analysis to pioneers and non-pioneers species group separately. The overall fit of all models was compared using Akaike's Information Criterion (AIC). We considered all model with ΔAIC < 2.0 to be equally plausible.

Results

For pioneer species there was a moderate negative correlation between specific leaf area (SLA) and leaf dry matter content (LDMC) (Pearson coefficient= -0.65), but SLA and

potential canopy height (PotH) and LDMC and PotH did not show meaningful correlation. For non-pioneer species there was a weak negative correlation between LDMC and PotH (Pearson coefficient= -0.40) and no meaningful correlation between SLA and PotH and SLA and LDMC (see figure in Appendix A).

Survival

The raw data for survival proportion of each species in aggregated and isolated system shows a great variation among species, especially for the pioneers (Appendix A)

For survival of pioneer species, the full model with squared SLA did not converge and we decided to continue model selecting without squared SLA. Three models were equally plausible in explaining survival data for pioneer species (Table 1). The best-fitted model (Δ AIC=0) selected only SLA, aggregated system (AS), and their interaction. The interaction showed a strong (confidence interval did not overlap zero) negative value, which means that pioneer species with lowest values of SLA had relatively higher survival rates in aggregated system (Fig.1) than in isolated system. However, the SLA effect without interaction had a positive effect on survival (confidence interval did not overlap zero), and aggregated system also had a weak positive effect on survival (confidence interval overlap zero). The other two selected models (Table 1) included those variables and interaction, but one added a weak positive effect of LDMC on survival (Δ AIC=0.7) and the other added a weak negative effect of PotH on survival (Δ AIC=1.9).

Table 1: Estimated coefficients and standard error of fixed effects for the selected models (ΔAIC<2) concerning the effect of functional traits on survival of 6 pioneer and 7 non pioneer species over 18-months in different planting systems. When confidence interval does not overlap zero, numbers are bolded. SLA: Specific leaf area, LDMC: leaf dry matter content, PotH: Potential canopy high, AS: aggregated planting system.

Parameters selected for pioneer species						Δ AIC
SLA x AS	LDMC x AS	SLA	LDMC	AS	PotH	
-0.66479 (0.24727)		**0.49073 (0.25035)**		0.01607 (0.26442)		0
-0.66059 (0.24713)		**0.47602 (0.24996)**		0.01688 (0.26421)	-1.24434 (1.01834)	0.7
-0.67104 (0.24825)		**0.51575 (0.26125)**	0.06695 (0.19743)	0.02017 (0.26488)		1.9

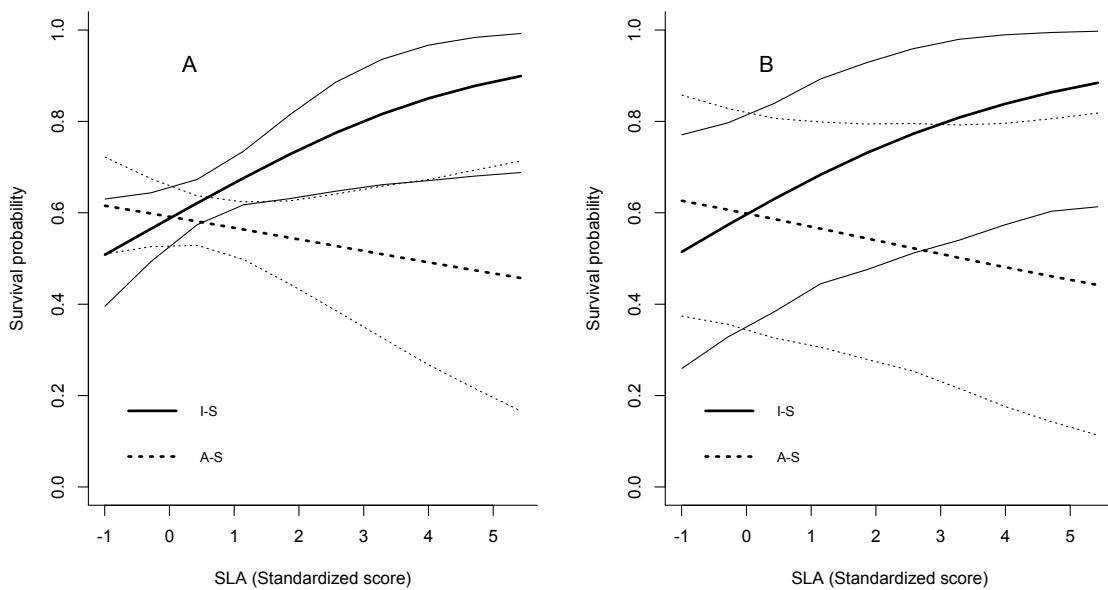| Parameters selected for non pioneer species						Δ AIC
SLA x AS	LDMC x AS	SLA	LDMC	AS	PotH	
		-0.06231 (0.22213)		**0.43029 (0.21277)**		0
0.62778 (0.43391)		-0.06906 (0.23124)	0.42786 (0.28989)	**0.43961 (0.21325)**		1.6
	0.47598 (0.33077)	-0.02426 (0.23109)	0.03387 (0.11095)	**0.46901 (0.21551)**		1.7
		-0.04647 (0.22628)	0.04352 (0.10973)	**0.43230 (0.21300)**		1.8

Fig.1. Values predicted by model selection for the relationship between survival probability of pioneer species and specific leaf area (SLA) of species in different planting systems. I -S = isolated system (solid lines), A-S = aggregated system (dotted lines). Thinner lines represent the 95% confidence interval from 1000 simulations of the predicted values. A) Random effects (blocks and species) not considered in the confidence interval. B) Random effects considered in the confidence interval.

For survival of non-pioneer species, we kept model selecting without squared SLA, for the same reason explained above for pioneer species. Four models were selected (Table 1). The best-fitted model (Δ AIC=0) selected only SLA and AS. SLA had a weak negative effect, but A had a strong positive effect (confidence interval did not overlap zero), indicating that non-pioneer species had higher survival in aggregated system (Fig. 2).
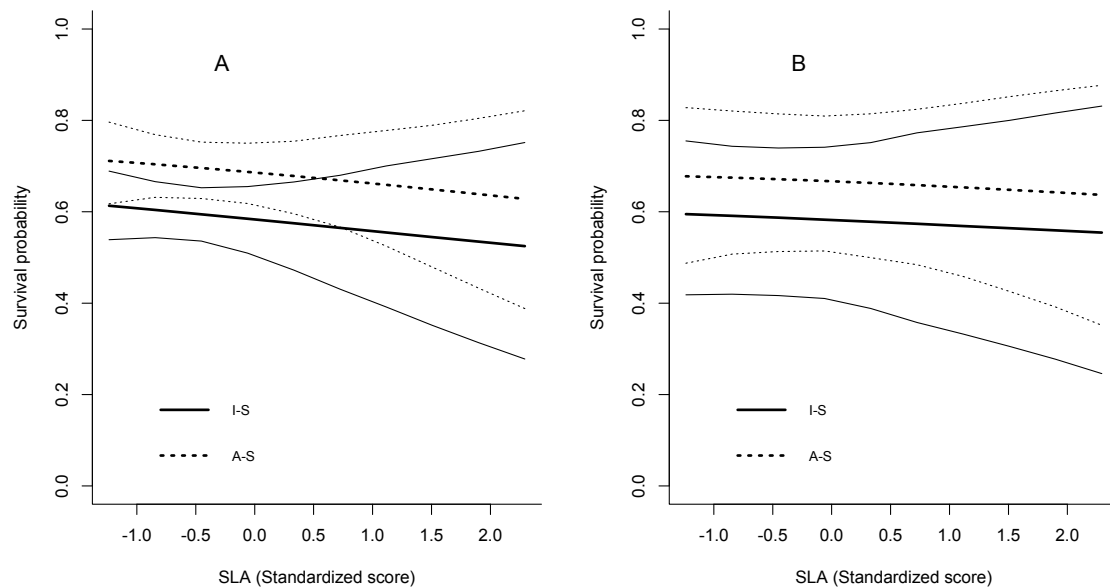
Fig. 2. Values predicted by model selection for the relationship between survival probability of non-pioneer species and specific leaf area (SLA) of species in different planting systems. I -S = isolated system (solid lines), A-S = aggregated system (dotted lines). Thinner lines represent the 95% confidence interval from 1000 simulations of the predicted values. A) Random effects (blocks and species) not considered in the confidence interval. B) Random effects considered in the confidence interval.

All other models selected for non-pioneer species included a weak positive effect of LDMC on survival probability, but one included a weak positive interaction between SLA x AS (Δ AIC=1.6), other included instead a weak positive interaction between LDMC x AS (Δ AIC=1.7), and the last did not include interactions (Δ AIC=1.8).

<u>Growth</u>

The raw data for growth shows that species had lower RGR in aggregated system, especially for non-pioneer species (Appendix A).

For growth of pioneer species, both the full model and the full model with squared SLA were selected. We decided to continue model selecting without squared SLA, because the model containing squared SLA added another term and had higher Δ AIC. Four different models were selected (Table 2). The best-fitted model (Δ AIC =0) showed only a negative effect of aggregated system on growth (confidence interval did not overlap zero). The second model showed also a weak positive effect of PotH on growth (Δ AIC=1.1). The third model added yet a weak negative interaction between PotH and APS (Δ AIC=1.1). Moreover, the null model was marginally selected (Δ AIC=1.9), which means that only the variations within species and within blocks would be sufficient to explain differences in growth.

Table 2: Estimated coefficients and standard error of fixed effects for the selected models (ΔAIC<2) concerning the effect of functional traits on the growth of 6 pioneer and 7 non pioneer species over 18-months in different planting systems. When confidence interval does not overlap zero, numbers are bolded. SLA: Specific leaf area, LDMC: leaf dry matter content, PotH: Potential canopy high, AS: aggregated planting system.

Parameters selected for pioneer species			Δ AIC
PotH x AS	AS	PotH	
-	**-0.014848 (0.007511)**	-	0
-	**-0.01490 (0.00751)**	0.02809 (0.02335)	0.1
-0.012886 (0.012925)	**-0.020848 (0.009591)**	0.035331 (0.024359)	1.1
null model	null model	null model	1.9

Parameters selected for non-pioneer species			Δ AIC
PotH x AS	AS	PotH	
-	**-0.056376 (0.004818)**	-	0
-0.006479 (0.004269)	**-0.053235 (0.005233)**	0.006086 (0.008053)	1.5
-	**-0.056360 (0.004820)**	0.002300 (0.007813)	1.9

For growth of non-pioneer species, both the full model and the full model with squared SLA were selected (Δ AIC < 2). We kept model selecting without squared SLA, for the same reason explained above for pioneer species. Three different models were selected (Table 2). The best-fitted model (Δ AIC=0) showed only a negative effect of the aggregated planting system (confidence interval did not overlap zero). The second model included a weak positive effect of PotH, and a weak positive effect of the interaction between PotH and AS (Δ AIC=1.5). The third model kept only the weak positive effect of PotH, but did not include the interaction between PotH and AS (Δ AIC=1.9).

Discussion

In this work we investigated whether functional traits were related to growth and survival of tree saplings in early development of a restoration planting, and whether plant interactions would change this relationship. Functional traits only partially explained saplings performance. Our main findings were that for pioneer species, the greater the specific leaf areas (SLA), the greater the survival rates, but, under aggregated conditions, the greater the SLA the lower the survival rates. A positive effect of aggregated system in survival of non-pioneer species (facilitation) and a negative effect in growth of both pioneer and non-pioneer species (competition) was observed. However, the effects of functional traits were weakly related to survival and growth of non-pioneer species. This influence of the aggregated system was expected and has already been discussed in Bertoncello et al. (2016). Some trends related to the effect of functional traits on the performance of the species will be also discussed below.

Contrary to our first prediction, we did not observe a better performance in species with intermediate SLA. Rather, for pioneer species there was a strong positive effect in survival in

isolated planting and a strong negative effect in aggregated planting, for non-pioneer there was a weak negative effect in survival, and there was no effect of SLA in growth. The only similar work we are aware did not find a clear relationship between SLA and performance in a tropical forest restoration (Martinez-Garza et al., 2013). Even for forested areas, where SLA has been positively related to growth and negatively related to survival (Kunstler et al., 2016; Poorter & Bongers, 2006), there are still uncertainties. Poorter et al. (2008) reported SLA to be only weakly related to performance of large trees across five Neotropical forests, with no effect on growth and a positive effect on survival, such as we observed for pioneer species in isolated planting.

When considering plant interaction, pioneer species with low SLA had higher survival rates in aggregated system, which may be related to the fact that low SLA species have a high competitive ability (Kunstler et al., 2016), corroborating our second prediction. Species with resource-conservative strategies (low SLA) may have lower requirements for mineral resources, thus may be favoured in low resource environments (Adler et al., 2013).

Pioneer species with high leaf dry matter content (LDMC) had a weak and positive correlation with survival. For non-pioneer species there was also a trend of a positive effect of LDMC in survival, since three out of four models have selected this parameter. This trend corroborates our third prediction, although the importance of LDMC did not change when there was plant interaction. Martinez-Garza et al. (2013) also found higher survival rates for non-pioneers with high LDMC growing on restored pastures, probably because they cope better with desiccation (Shipley et al. 2006). However, contrary to literature (Pérez-Harguindeguy et al., 2013) LDMC was not negatively related to growth rate, for both pioneer and non-pioneer species.

Pioneer species with higher potential canopy height (PotH) had a weak negative relationship to survival, and both pioneer and non-pioneer species showed a trend of a positive effect in growth. In other restored pasture, Martinez-Garza et al. (2013) also found a negative relationship of PotH and survival. They attributed that to the fact that saplings of taller species at this ontogenetic stage probably cannot cope with stressful conditions of pastures that they do withstand as adults (Martinez-Garza et al., 2013), such as high light level, temperature, strong winds and low water availability. In aggregated system PotH had a weak negative relation to growth, probably because in early development of restored pastures there is still no competition for light and taller species perform worse in competition for water (Butterfield & Callaway, 2013). Furthermore, Kunstler et al. (2016) did not find influence of PotH on the competition effect, but they argue that this may be a feature that reflects other time scale, since it would be a long-term effect on the population.

Despite some effects mentioned above, functional traits were weakly related to species performance and did not correspond to our predictions. Even so, many other authors have argued that functional traits could be used to explain and predict outcomes of management and restoration processes of native ecosystems (Pywell et al. 2003; Roberts et al. 2010; Sandel et al. 2011; Burylo et al., 2014), and to base the choice of species for a restoration project (Martinez-Garza et al., 2013). Hence, despite the fact that leaf traits and plant size are considered among the most important functional traits because they are the core of plant life cycle (Pérez-Harguindeguy et al., 2013), it is possible that other traits be more responsive in the initial phases of restoration plantings. For instance, Martínez-Garza et *al*. (2013) pointed small seeds and large and deep crowns as good indicators of growth of species in pasture environments, while leaf traits had only a small influence in species performance.

Another possibility is that other strategies not measured in this work, such as a fast growth belowground, which allow the development of a deep root system (Coll et al., 2008) are being

more important. It is also possible that other traits more complex to measure or multiple-traits (Robert et al., 2010) may relate better to performance,. However, we intended to test the core simple-measurable traits in order to make species selection process more efficient. Since relevant traits may depend largely on environment conditions (Butterfield & Calllaway, 2013), context-dependence possibly plays a major role, which implies a considerable part of the explanations to be species-dependent (Asanok et al., 2013), as we could see in the great variation attributed to the random effects in figs. 1 and 2.

Nevertheless, it is important to mention that most of the works that have found relationship between traits and seedling outcomes have been developed in low diversity communities (Clark et al., 2012; Kraft et al., 2014, Robert et al., 2010). In restoration of far more complex communities, such as tropical forests, there is still uncertainty about the role of functional traits in predicting seedlings outcomes (but see Mártinez-Garza et *al*., 2013). This is especially true when plant interaction is taken into account. In this study, we set up and monitored saplings in an appropriated experimental design to assess this subject. Our results contributed to the debate of this important issue. Considering the applicability of understanding the role of leaf functional traits on the species selection for restoration and on management planning, the subject still demands further research, especially for high diversity tropical forests.
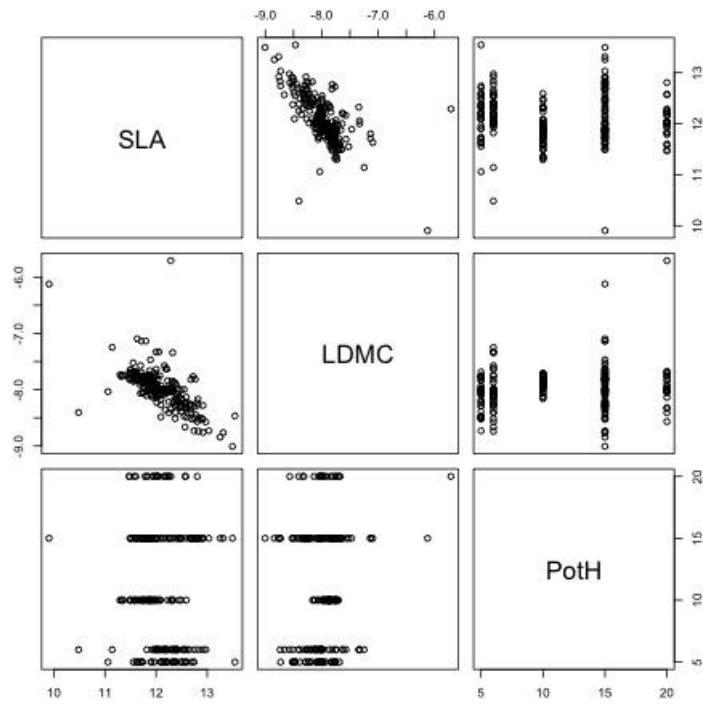
Appendix A. Supplementary data

Figure 1: Pearson correlation between paired samples for pioneer species. SLA=specific leaf area; LDMC=leaf dry matter content; PotH=potential canopy height.
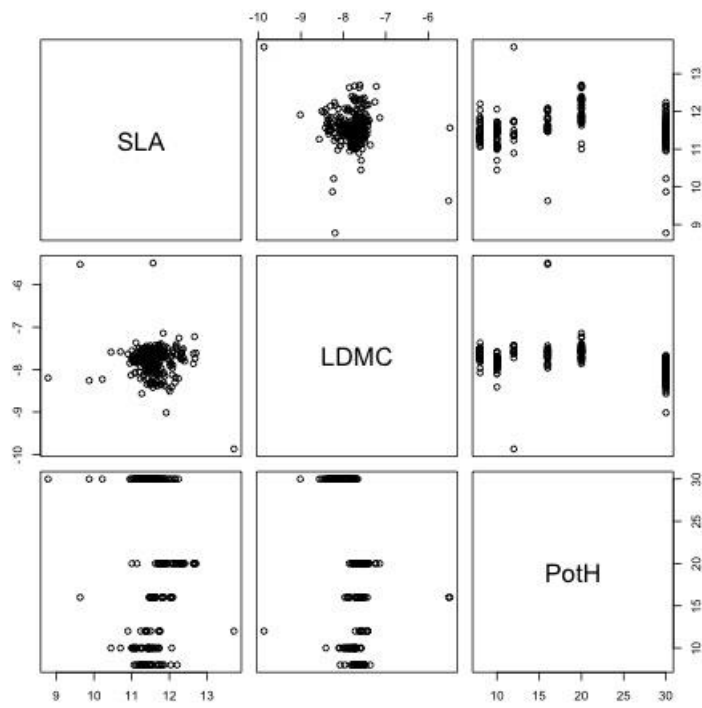
Figure 2: Pearson correlation between paired samples for non-pioneer species. SLA=specific leaf area; LDMC=leaf dry matter content; PotH=potential canopy height.
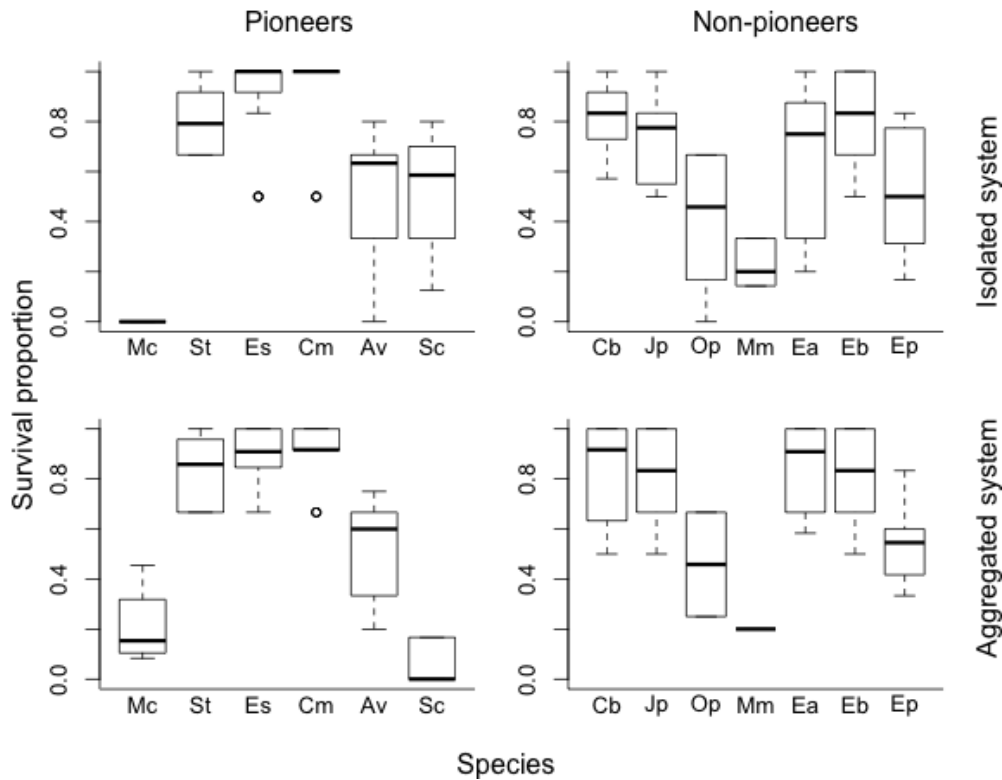
Figure 3: Boxplot of survival proportion data for 6 pioneer and 7 non-pioneer species in aggregated and isolated system, over 18 months (n=8). The box contain the lower and upper quartile, the heavy line inside the box represents the sample median, and open circles are points that fall outside the nominal range of the data inferred from upper and lower quartiles. Pioneer species are: Mc- *Myrsine coriacea*; St-*Schinus terebinthifolius*; Es- *Erythrina speciosa*; Cm-*Citharexylum myrianthum*; Av-*Aegiphila verticillata*; Sc*Solanum cinnamomeum*. Non-pioneer species are: Cb- *Calophyllum brasiliense*; Jp-*Jacaranda puberula*; Op-*Ocotea pulchella*; Mm-*Myrcia multiflora*; Ea-*Eugenia astringens*; Eb-*Eugenia brasiliensis*; Ep-*Eriotheca pentaphylla*.
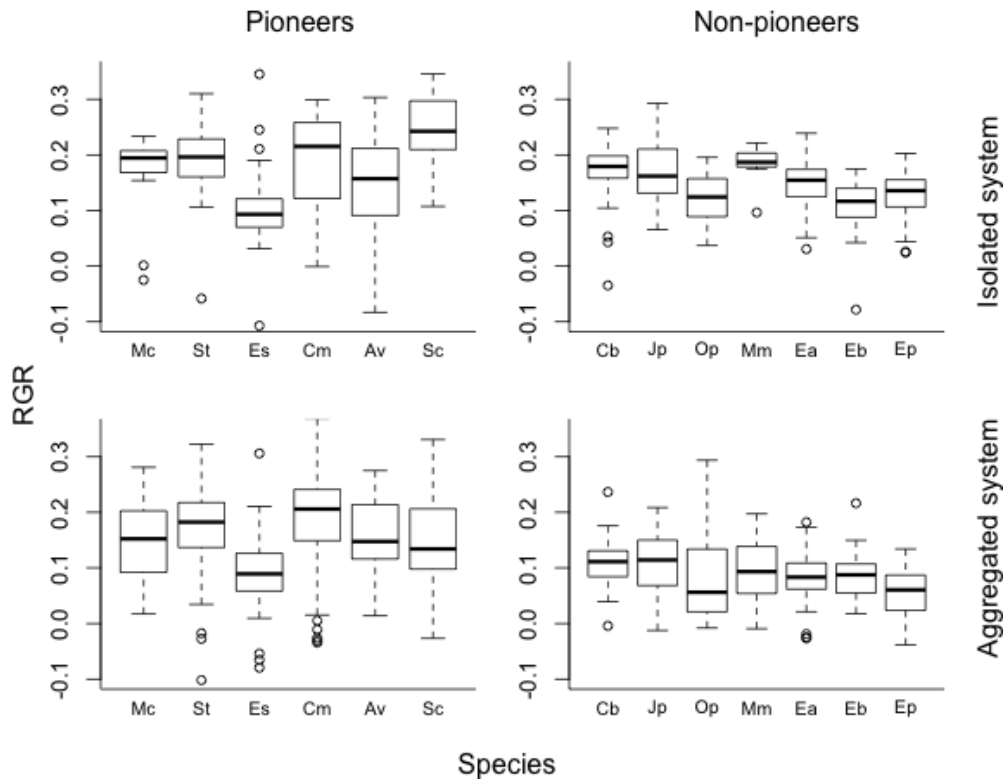
Fig. 4: Boxplot of relative growth rate data (mm x mm - x month-) for 6 pioneer and 7 non pioneer species in aggregated and isolated system, over 18 months. Number of individual was the survivors of a minimum of six individuals per species per plot of isolated and aggregated system in eight blocks (initial n > 1248 individuals). The box contains the lower and upper quartile, the heavy line inside the box represents the sample median, and open circles are points that fall outside the nominal range of the data inferred from upper and lower quartiles. Pioneer species are: Mc- *Myrsine coriacea*; St-*Schinus terebinthifolius*; Es- *Erythrina speciosa*; Cm-*Citharexylum myrianthum*; Av-*Aegiphila verticillata*; Sc-*Solanum cinnamomeum*. Non-pioneer species are: Cb- *Calophyllum brasiliense*; Jp-*Jacaranda puberula*; Op-*Ocotea pulchella*; Mm-*Myrcia multiflora*; Ea-*Eugenia astringens*; Eb-*Eugenia brasiliensis*; Ep-*Eriotheca pentaphylla*.

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4.   Considerações finais

No primeiro capítulo da tese analisamos o resultado do monitoramento de modelos de restauração que avaliaram simultaneamente o papel da distribuição espacial (i.e. interações interespecíficas) e da disponibilidade de recursos sobre espécies de diferentes categorias sucessionais. Constatamos que as espécies não-pioneiras tiveram maior sobrevivência nos sistemas agregados de plantio, indicando que houve a predominância de facilitação nas relações interespecíficas. Entretanto, a interação entre as espécies também resultou em diminuição da taxa de crescimento em diâmetro à altura do solo, indicando a predominância de competição para o crescimento. A adição de nutrientes aparentemente diminuiu essa competição, pois teve um efeito positivo no crescimento de diâmetro e projeção de copa nos sistemas agregados. A maneira como esse padrão observado interage com espécies de diferentes categorias sucessionais pode ser útil para delinear modelos de restauração ecológica. Ao mesmo tempo, esse resultado nos ajuda a interpretar padrões da dinâmica sucessional da estruturação das comunidades no sistema estudado.

No segundo capítulo analisamos os resultados do monitoramento do mesmo modelo de restauração sob a ótica dos atributos funcionais (SLA , LDMC e altura potencial máxima da espécie). Avaliamos o desempenho de espécies de diferentes grupos sucessionais, assim como a influência das interações entre as espécies na relação entre os atributos funcionais e seus desempenhos. A principal relação encontrada foi que espécies pioneiras com maior SLA apresentaram maior sobrevivência quando plantadas isoladamente. Porém, esse padrão foi modificado quando houve interação entre as espécies (plantio agregado), de maneira que espécies com menor SLA apresentaram maior sobrevivência nos núcleos, indicando que estratégias mais conservativas (menor SLA) podem ter relação com a habilidade competitiva

das espécies. Entretanto, os atributos analisados explicaram apenas parcialmente o desempenho das espécies e ainda há diversas lacunas de conhecimento na utilização de atributos funcionais como ferramenta para projetos de restauração em florestas tropicais. Haja vista a importante aplicação prática desse conhecimento, são necessárias mais pesquisas visando elucidar padrões que possam ser generalizados para diferentes áreas tropicais degradadas.

Entender os padrões resultantes dos modelos de restauração, que por sua vez derivam dos padrões de estruturação das comunidades, é um enorme desafio, haja vista a escala temporal necessária para a restauração de uma floresta tropical e a nossa limitação temporal para obter os resultados das pesquisas. Nesse sentido, é muito importante que consigamos cada vez mais incorporar perguntas e adequar os modelos de restauração visando a criação de modelos experimentais replicáveis que possam ser monitorados em médio e longo prazo.