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Dissertação de Mestrado

Predizendo a performance de espécies nativas dos Campos Sulinos: uma
abordagem funcional com aplicação à ecologia da restauração

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Porto Alegre, Março de 2019.

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Mestre em Ecologia.

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Predicting the performance of *Campos Sulinos* native species: a functional approach applied to restoration ecology

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Resumo

A importância dos ecossistemas campestres supera seu valor intrínscio dado por sua tamanha diversidade, devido ao seu papel fundamental na manutenção de recursos alimentícios, hídricos e econômicos. No Brasil, tal importância tem sido pouco valorizada em termos de inclusão de sua proteção e restauração em políticas públicas Brasileiras, o que reflete na pesquisa. Atualmente, 60% das áreas campestres no sul do Brasil foram convertidas para outros fins, e os remanescentes se encontram ameaçados. Há demanda por restauração, porém esta ainda é uma ciência jovem para áreas campestres subtropicais Brasileiras, necessitando de fundamentação para práticas de maior sucesso. Para este estudo nosso objetivo geral foi dar suporte à prática da restauração através da avaliação da performance de espécies nativas, por meio de uma abordagem funcional, que permite uma ampla aplicação do conhecimento adquirido. No primeiro capítulo nós avaliamos a performance das espécies durante as primeiras 16 semanas do estabelecimento em três experimentos distintos: câmara de germinação, casa de vegetação e em campo. Os nossos objetivos foram identificar o potencial de atributos regenerativos (características das sementes) de prever a performance destas espécies em seus estágios iniciais de estabelecimento. O segundo capítulo visou expandir o acompanhamento para estágios mais tardios do estabelecimento inicial, sob condições de campo. Foram monitorados o crescimento vegetativo dos indivíduos e avaliada a influência de atributos foliares e estruturais na predição da performance. Nossos objetivos foram avaliar o potencial de predição de atributos funcionais ao longo de dois estágios de estabelecimento (inicial e tardio) e correlacionar a performance das espécies com estratégias ecológicas mais abrangentes, utilizando o esquema CSR (Competitividade- Tolerância ao estress- Ruderalidade). Os resultados dos capítulos indicam que: (i) atributos regenerativos ligados à forma da semente são melhores preditores de performance no estabelecimento inicial do que a massa de semente; (ii) experimentos de campo devem ser considerados quando o objetivo for a predição de variáveis de performance associadas ao estabelecimento das espécies; (iii) atributos foliares associados à estratégia competitiva (componente C do esquema CSR) predizem a biomassa acima do solo, preditor associado ao estágio tardio do estabelecimento; (iv) atributos de fácil mensuração predizem a performance das espécies em ambos os estágios de estabelecimento (inicial e tardio), porém alguns aspectos da performance permaneceram inexplicados; (v) as estratégias CSR são úteis para explicar a performance em ambos os estágios de vida das espécies campestres. Nosso estudo representa um avanço em termos de predição baseada em atributos funcionais para aplicação em restauração de ecossistemas campestres, assim como amplia a possibilidade de comparação dos fatores envolvidos no sucesso da restauração para uma escala global. A ecologia baseada em atributos demonstra ser uma ferramenta de ampla aplicação e os estudos que utilizam essa abordagem para entender a performance das espécies devem avançar de forma a incluir mais preditores, diferentes estágios de vida, tempo de monitoramento, condições variadas e uma gama maior de espécies.

Palavras-Chave: CSR, atributos funcionais, atributos regenerativos, atributos foliares, performance, campos subtropicais.

Abstract

The importance of grassland ecosystems surpasses its intrinsic environmental value due its large diversity, as they have a fundamental role in the maintenance of hydric, food and economical resources. In Brazil its relevance has been underestimated; grasslands have received few attention by public policies in terms of its conservation and restoration, which in turn reflect on research. Currently, 60% of grassland areas have already been converted into alternative land uses, and remnants are threatened. Despite of the demands for restoration, this is still a young science on subtropical grasslands and requires theoretical support for successful practices. In this study, we aimed to provide theoretical support to restoration practices by evaluating native species performance using a functional approach, which allows a wide application of the results. In the first chapter, we evaluated species performance during the first 16 weeks of establishment after seeding in three distinct experiments: germination cabinet, greenhouse and field. Our main objectives were to identify the potential of regenerative traits (seed characteristics) on predicting performance during an early establishment stage. In the second chapter, we aimed to expand the monitoring to a later establishment stage under field conditions, including the evaluation of the vegetative growth of individuals and the influence of leaf and whole-plant traits as predictors of performance. More specifically, we aimed to evaluate the predictive potential of functional traits across two establishment stages (early and later) and the correlation of performance with wide ecological strategies by using the Grime's CSR triangle. The chapters results point out that: (i) regenerative traits associated to the seed shape are better predictors of early establishment performance than seed mass; (ii) field experiments should be considered when the objective is prediction of parameters associated to the species establishment performance; (iii) leaf traits associated to a competitive strategy (C-component of the CSR scheme) are predictors of aboveground biomass, measured in the later establishment of species; (iv) easy to measure traits can predict performance in both establishment stages (early and later), but some aspects of performance remain unexplained; (v) CSR-strategies are useful on explaining performance across life stages of grassland species. Our study represents advances in terms of trait-based prediction applied to restoration of grassy ecosystems, so as expands to a global scale the comparison of determinants of restoration success. The trait-based ecology seems to be a tool of wide application, and we suggest that such studies applied to understand the performance of species should advance by including further predictors, distinct life stages, monitoring time, various conditions, and a larger set of species.

Key-words: CSR, functional traits, regenerative traits, leaf traits, whole-plant traits, subtropical grasslands, performance.

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Introdução Geral

A restauração ecológica é um processo conduzido/assistido, com objetivos variados, baseado no conhecimento ecológico (McDonald et al. 2016). A ciência que guia essa prática é chamada Ecologia da Restauração, a qual engloba estudos teóricos e empíricos de ecologia gerando conceitos, métodos, modelos, entre outros embasamentos (SER 2004). O estudo da Ecologia da Restauração permite tanto o avanço das técnicas de restauração como o da teoria ecológica. Uma analogia utilizada por Bradshaw (1987) e (Palmer et al. 2006) é que a restauração ecológica é como um parquinho de diversões para ecologistas, pois é uma oportunidade única de testar teoria ecológica em condições naturais. No contexto da prática da restauração a previsibilidade dos resultados é de suma importância para melhor alocação de recursos.

A Ecologia Funcional é uma área do conhecimento na ecologia que tem como seu principal objetivo a predição generalizada com base nos atributos funcionais das espécies (Daniel C. Laughlin 2014; Shipley et al. 2016). Esses atributos são definidos como, qualquer característica morfo-fisiológica, fenológica e até comportamental que reflita o *fitness* da espécie, sua capacidade de deixar descendentes (Violle et al. 2007). A predição em nível de espécie, ainda é impraticável, mesmo onde o conhecimento biológico local é avançado, milhares de possíveis comportamentos ecológicos e suas interações com os mais diversos ambientes deveriam ser unificados para aplicação em restauração (Daniel C. Laughlin 2014). Uma abordagem funcional aplicada à Ecologia da Restauração nos permite superar o nível taxonômico e local, o conhecimento ecológico sobre cada espécie não é imprescindível é possível fazer predições baseadas apenas em características mais gerais, portanto comparáveis, passível de ser testadas, em qualquer lugar no globo com características ambientais similares (Shipley et al. 2016). Isso também faz com que possamos estreitar o nosso conhecimento de padrões, mecanismos e processos ecológicos mais rapidamente (Shipley et al. 2016). Pesquisas anteriores têm demonstrado o potencial de aplicação dos atributos funcionais na predição da performance de

espécies em restauração (Pywell et al. 2003; Roberts et al. 2010), predição da composição das comunidades frente a mudanças climáticas (Daniel C. Laughlin 2014) e no monitoramento e entendimento de trajetórias em restauração (Rosenfield & Müller 2018; Kahmen & Poschlod 2004), entre outras possibilidades.

Entretanto, embora a Ecologia Funcional tenha como pressuposto a força de correlação entre atributos funcionais e o *fitness* das espécies, isso permanece pouco testado empiricamente (Shipley et al. 2016; Zirbel et al. 2017). Além disso, a maioria dos estudos tem focado seus esforços em uma pequena gama de atributos funcionais (Larson & Funk 2016; Shipley et al. 2016; Saatkamp et al. 2018). Os atributos funcionais amplamente utilizados podem primeiramente ser separados em atributos regenerativos, estruturais (ex. altura e densidade da madeira) e de órgãos (ex. folhas e raízes) (Daniel C Laughlin 2014). Os atributos regenerativos são aqueles que têm sua função mais associada ao chamado 'nicho regenerativo' (Pierce et al. 2014), definido por Grubb (1977) como as condições bióticas e abióticas necessárias para um indivíduo germinar e se estabelecer como um jovem na comunidade. Como exemplo, massa da semente, forma da semente, apêndices das sementes, requerimentos como luz e temperatura para germinar (Saatkamp et al. 2018). Esses atributos têm sido os menos estudados, se comparados a atributos foliares e estruturais, apesar de vários trabalhos terem chamado a atenção da importância deles para a montagem de comunidades (Pérez-Harguindeguy et al. 2013; Poschlod et al. 2013; Larson et al. 2015; Saatkamp et al. 2018).

Os atributos estruturais e de órgãos são aqueles que só a partir da emergência dos indivíduos passam a exercer sua função. Eles, portanto, aumentam em importância nas fases de sobrevivência e posterior crescimento/reprodução dos indivíduos em estágios tardios do desenvolvimento (Kitajima & Fenner 2000). Os atributos estruturais e de órgãos estão entre os mais estudados, sendo representado por área foliar, conteúdo de massa seca, área foliar específica e ângulo das folhas, altura potencial da planta, atributos de sustentação, como densidade da madeira e condutividade do xilema, e atributos de raízes, como densidade e comprimento específico das raízes (Pérez-Harguindeguy et al.

2013). De forma geral, os atributos refletem a eficiência no uso de recursos assim como hierarquias competitivas entre as espécies e ainda a forma como afetam seu ambiente (função), como a facilidade para se decompor, por exemplo (Pérez-Harguindeguy et al. 2013). Para possibilitar a aplicação dos atributos funcionais à ecologia da restauração é relevante saber como os atributos afetam a performance das espécies em diferentes condições ambientais, também associando-os a diferentes fases de vida e estratégias ecológicas amplamente reconhecidas, facilitando assim a predição das trajetórias de restauração.

Em termos de necessidade de restauração, as formações vegetais campestres estão entre as mais extensas e degradadas do mundo (FAO 2005; Gibson 2009; Gang et al. 2014). As formações campestres são aquelas em que há predominância de espécies de gramíneas na cobertura vegetal (Gibson 2009). No sul do Brasil ocorrem campos subtropicais conhecidos como *Campos Sulinos*, termo este que engloba os campos presentes nos estados de Santa Catarina (SC), Paraná (PR) e Rio Grande do Sul (RS) (Overbeck et al. 2007; Pillar et al. 2009). Ainda os campos da parcela sul do estado do Rio Grande do Sul se estendem por todo o Uruguai e leste da Argentina, formando a unidade ecológica do Río de la Plata (Soriano 1991; Andrade et al. 2018). Se comparado a outras formações não-florestais no Brasil, como o Cerrado, os campos subtropicais tem sido pouco estudados (Overbeck et al. 2007). Através do anos tem sido extensamente degradado, principalmente, fruto da desvalorização deste tipo de formação vegetal e da aptidão agrícola das terras (Andrade et al. 2015). No Rio Grande do Sul, 60% das áreas de campos já foram convertidas para usos alternativos do solo (Andrade et al. 2015), principalmente pelo avanço da cultura da soja (Silveira et al. 2017; Kuplich et al. 2018), mas também pelo cultivo de arroz e a silvicultura com *Pinus* e *Eucalyptus* (Andrade et al. 2015). Outras ameaças incluem a fragmentação (Staude et al. 2017), espécies invasoras (de Medeiros & Ferreira 2011) e o avanço de espécies arbóreas sobre o campo (Guido, Salengue, et al. 2017).

A conversão do uso do solo, em áreas de vegetação campestre, causam mudanças na composição das espécies (bióticas) e em propriedades do solo e microclimáticas (abióticas) que

podem resultar na diminuição da resiliência, sua capacidade de voltar a organização anterior a partir de uma mudança, sendo então necessárias intervenções ativas para restaurar a funcionalidade do ambiente (Walker et al. 2004; Andrade et al. 2015). A primeira mudança, nos casos de conversão para lavoura, é a completa remoção da cobertura vegetal (Andrade et al. 2015). Estudos realizados na região demonstram que o banco de sementes em áreas utilizadas para cultivo é principalmente composto por espécies exóticas ou nativas ruderais (Favreto & de Medeiros 2006; Vieira et al. 2015). Isto limita a contribuição do banco de sementes como fonte de propágulos para restauração destas áreas. Além disso, as principais formas de regeneração dessas comunidades tem sido atribuída a estruturas subterrâneas (ex. bulbos, xilopódios e rizomas) e estolões, estruturas que se perdem com o intensivo uso do solo para agricultura (Maia et al. 2003; Favreto & de Medeiros 2006; Fidelis et al. 2009; Vieira et al. 2015). Esta transformação da comunidade vegetal *per se* (promovida por lavoura, silvicultura, ou devido a outros usos) também causa alterações físicas e químicas nas propriedades do solo. Adicionalmente, o uso de agrotóxicos e fertilizantes altera rapidamente a constituição química do solo, agindo como um filtro abiótico importante à seleção das espécies na reorganização das comunidades (Kahmen & Poschlod 2004; Walker et al. 2004). O abandono de áreas cultivadas leva à colonização de espécies exóticas e nativas ruderais, generalistas, que são favorecidas pelo excessivo aumento de nutrientes (Walker et al. 2004). Além disso, devido também a fragmentação do habitat, a dispersão e colonização natural de espécies representativas (dominantes estruturais) da comunidade original é limitada, necessitando da adição ativa de espécies de interesse, associada a técnicas de manejo em curto e médio prazo (Kiehl et al. 2010; Andrade et al. 2015; Staude et al. 2017).

A restauração e conservação de áreas campestres é de suma importância devido a sua diversidade e papel na manutenção de processos ecossistêmicos. Essas áreas constituem grandes extensões de área permeável, sendo relevantes para manutenção do ciclo da água, assim como também são importantes áreas de armazenamento de carbono (White et al. 2000; Overbeck et al. 2015). Parte de sua diversidade é atribuída a flora, a qual se conhece apenas uma parcela, sendo estimadas entre 3000 e 4000 espécies para os três estados que constituem os Campos Sulinos

(Overbeck et al. 2007). Um extenso levantamento quantitativo de espécies dos Campos Sulinos foi realizado por Andrade, Bonilha, et al. (2018), encontrou 905 espécies, em ambientes de campo no sentido mais restrito (excluindo habitats mais específicos, por exemplo, afloramentos rochosos).

São parte da manutenção de sua diversidade, distúrbios como o fogo, a ocorrência e intensidade de geadas e o pastejo. Esses fatores são decorrentes de um passado onde, do Pleistoceno ao Holoceno, o campo foi predominante devido ao clima frio e seco e, depois, quente e seco (Behling & Pillar 2007). A exemplo disso, temos a coexistência de espécies de gramíneas C3 e C4, com dominância de espécies C4, dadas as suas adaptações ao regime de fogo observado nos campos subtropicais (Overbeck et al. 2005, 2018). Entre o Pleistoceno e o Holoceno, também havia a presença de grandes mamíferos pastadores (Behling & Pillar 2007), hoje os ruminantes domésticos ocupam seu lugar e função (Pillar et al. 2009). Sendo a produção pecuária sustentável, além de economicamente viável, parte da manutenção e conservação da diversidade desta formação vegetal (O'Mara 2012; Pillar et al. 2009). As espécies vegetais co-evoluíram com esses distúrbios, tanto o pastejo quanto o fogo resultam em uma diminuição na dominância das espécies competitivas, o que leva a um aumento da riqueza de espécies devido ao aumento da heterogeneidade espacial do ambiente (Behling & Pillar 2007; Overbeck et al. 2007). Os distúrbios criam oportunidades de colonização para espécies menos competitivas, determinando, portanto, a dinâmica desse ecossistema (Pillar et al. 2010).

Contudo, no Brasil, apenas um ínfima parcela dos campos subtropicais se encontra sob proteção legal (Overbeck et al. 2007; Ministério do Meio Ambiente 2000; Pillar et al. 2010). O descaso quanto a essas áreas em políticas públicas também reflete nos esforços de pesquisa, portanto a ecologia da restauração para campos subtropicais no Brasil, é ainda uma ciência jovem. Já existe uma gama de esforços teóricos e empíricos em pesquisas voltados a métodos de restauração, controle de espécies invasoras, o que restaurar e onde restaurar para os campos subtropicais (Maia et al. 2003; Overbeck et al. 2005, 2007; Pillar et al. 2009; Falleiros et al. 2011; de Medeiros & Ferreira 2011; Andrade et al. 2015; Vieira et al. 2015; Fidelis et al. 2016; Bonilha et al. 2017; Guido, Hoss, et al. 2017;

Staude et al. 2017; Thomas et al. 2018). Porém, ainda insuficiente para fundamentar a prática da restauração ecológica em áreas campestres subtropicais.

Referente ao estabelecimento de espécies campestres nativas, estudos de banco de sementes em áreas campestres naturais revelaram a dissimilaridade do banco de sementes com a vegetação estabelecida (Maia et al. 2003; Vieira et al. 2015). Espécies campestres dominantes, representantes da fisionomia, estão frequentemente ausentes ou pouco representadas no banco de sementes, o que limita o uso do banco de sementes para promover a recuperação da comunidade (Maia et al. 2003; Vieira et al. 2015). Em um estudo onde se avaliou métodos para o controle de uma espécie invasora (*Urochloa decubens*) em área degradada, também foram avaliados semeadura e transferência de feno para adição de propágulos (Thomas et al. 2018), dois dos métodos mais usados na restauração de áreas campestres temperadas (Török et al. 2011). Nenhuma das técnicas, semeadura ou feno, resultou em um aumento da riqueza de espécies nativas, como era o esperado. No entanto, ambos resultaram no estabelecimento das espécies adicionadas (Thomas et al. 2018). Ainda, experimentos anteriores que mediram a taxa de germinação de espécies nativas encontraram taxas que variam de baixas (abaixo de 20%) a altas (acima de 90%) (Overbeck et al. 2006; Fidelis et al. 2016; Fior & Schafer 2016; Guido, Hoss, et al. 2017). Estes estudos indicam tanto a viabilidade do uso de espécies nativas, como também a necessidade de ampliação do conhecimento acerca dos determinantes do estabelecimento dessas espécies para o uso em restauração.

Devido à demanda de desenvolvimento de uma ciência da restauração que se adeque aos campos subtropicais, e também à necessidade de aprimorar o conhecimento acerca das relações entre atributos funcionais e o *fitness* das espécies, esta dissertação tem como objetivo geral testar a capacidade de predição de atributos funcionais na performance de espécies nativas do Campos Sulinos. Esta dissertação está dividida em dois capítulos. O primeiro, “Predicting plant performance in ecological restoration of grasslands: the role of regenerative traits”, em português, “Predizendo a performance de plantas em restauração ecológica: o papel dos atributos regenerativos”. O nosso

objetivo foi identificar o potencial de atributos regenerativos (características das sementes) de prever a performance destas espécies em seus estágios iniciais de estabelecimento. O segundo, “Functional traits, CSR strategies and performance across different establishment stages of grassland species”, em português, “Atributos funcionais, estratégias CSR e performance através de diferentes estágios do estabelecimento de espécies campestres”. Esse capítulo teve como objetivo a inclusão de estágios mais tardios do estabelecimento inicial das espécies, em condições experimentais de campo. Os objetivos específicos foram: 1. Testar se a variação do espaço multidimensional em função dos atributos funcionais poderiam explicar performance em diferentes estágios do estabelecimento inicial das espécies campestres em condições ambientais não ideais. 2. Testar se as estratégias CSR estão associadas a performance das espécies nos diferentes estágios do estabelecimento.

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Capítulo 1. Predizendo a performance de plantas em restauração ecológica: o papel dos atributos regenerativos

Title: Predicting plant performance for ecological restoration of grasslands: the role of regenerative traits

Running Head: Regenerative traits predict initial plant performance

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IS, AG, SM conceived and designed the research; IS performed the experiments, analysed the data, and led the manuscript writing; AG, SM critically reviewed and edited the manuscript.

Abstract

When addition of species is required, the conditions shaping performance in the first life stage of species is central in the prediction of restoration success. Regenerative traits such as seed traits, though understudied compared to mature plant traits, have the potential to reflect important performance parameters related to the initial establishment of species in a given environment. The overall objective was to test the predictive potential of regenerative traits on the performance of grassland species for restoration. We carried out a multi-experimental approach (cabinet, greenhouse and field), where we evaluated plant performance using three variables: Final Germination Percentage (FGP), Germination Rate Index (GRI) and Survival. The potential of seed traits as predictors were evaluated by linear regression models. Seed mass and seed shape influenced performance. Seed circularity had a positive effect on GRI. We also found a strong interaction between seed circularity

and mass leading to a higher GRI. Neither of the traits predicted FGP and survival. Our multi-experimental approach showed that we may under or overestimate germination parameters under controlled conditions, highlighting the importance of experimentation under field conditions when the objective is predictability. Our results reinforce the need of including regenerative traits in research approaches in order to improve trait-based ecological restoration, as so field conditions and further life-stages.

Key words: establishment, functional ecology, germination, subtropical, seed mass, seed shape, survival

Implications

- Low germination was consistent among species and may impose challenges for grassland restoration, requiring further research and a broader number of species.
- Include field conditions on testing predictions is essential to accurately draw conclusions about traits-fitness relationships.
- Seed shape and seed mass best correlate with germination performance and should be widely considered in future studies, especially for species' choices for ecological restoration projects.
- By empirically testing multiple traits and its association with plant performance we were able to evaluate the strength of regenerative traits in determine individual fitness in the first development phase.
- This research fills a gap in restoration of Brazilian subtropical grasslands concerning the potential of trait-based approaches for ecological restoration practices.

1. Introduction

Ecological restoration is an assisted process supported by ecological knowledge, where predictive outcomes are desirable for optimizing the success of ecosystem proprieties recovery (Mcdonald et al. 2016; Török et al. 2018). By the use of functional traits, defined as any morpho-physio-phenological characteristic which reflects individual fitness (Violle et al. 2007), it is possible to

reach generalized prediction beyond taxonomy and geographical location that may be suitable for elucidate ecological drivers and processes associated to restoration success (Laughlin 2014; Shipley et al. 2016). It is known that the niche of a species, defined as all biotic and abiotic conditions required for the continuing of its kind in the form of reproductive and establishment success (individual fitness), is central for the understanding of community assemblage (Silvertown 2004; Larson & Funk 2016; Valdez et al. 2019). Although, in plant ecology the fact that different life stages may occupy different niches is quite unexplored (Poschlod et al. 2013; Jiménez-Alfaro, Fernando A.O. Silveira, et al. 2016; Larson & Funk 2016). When the introduction of species in active restoration is required, the conditions shaping success in the first life stages might be deterministic to the establishment of the new community since, the first life stage is associated with highest probability of mortality across life stages (Grime et al. 1981; Fenner 2000; Jiménez-Alfaro, Fernando A.O. Silveira, et al. 2016; Török et al. 2018). Those conditions shaping the first life stages are reidealized by Grubb (1977) as the regeneration niche of a species, defined by as the required biotic and abiotic conditions for a species to germinate and to establish as a juvenile. For that, it may be important to identify which regenerative traits enhance germination and survival success, which are crucial performance parameters for the regenerative stage (Jiménez-Alfaro, Fernando A. O. Silveira, et al. 2016; Saatkamp et al. 2018).

Researches have shown the importance of regenerative traits such as seed mass and seed shape for determining species performance in restoration (Pywell et al. 2003; Barak et al. 2018). There is a general trend that heavier seeds outperform under environmental hazards as drought, fire and herbivory (Armstrong & Westoby 1993; Leishman & Westoby 1994), and that they improve germination and survival, though with lower growth rates (Fenner 2000; Moles & Westoby 2004; Kempel et al. 2013). Seed shape is closely related to the velocity of germination given by a relationship between shape and mass which facilitates or inhibit burial in the soil. Round light seeds are easily buried thus escaping predation, which led to persistence in seed banks allowing to slow germination, whether elongated heavy seeds tend to germinate faster given their short persistence and predation vulnerability (Thompson & Hodgson 1993; Barak et al. 2018). As these traits influence

populations demography and dynamics, which in turn affect community assembly processes, they may hold an important predictive power on restoration outcomes (Pywell et al. 2003; Laughlin 2014; Larson et al. 2015; Wainwright et al. 2018).

The predictive power of traits, however, is derived from one of the functional ecology fundamental assumptions: the degree that traits determine individual fitness (Shipley et al. 2016), whose strength is still weak or unknown in many cases as they are based on few experimental researches, especially for regenerative traits (Jiménez-Alfaro, Fernando A.O. Silveira, et al. 2016; Shipley et al. 2016; Saatkamp et al. 2018). Particularly, southern Brazil subtropical grasslands have not received enough attention over public politics and ecological research that may help its recovery after degradation (Overbeck et al. 2013, 2015; Andrade et al. 2015). These grasslands have already decreased in more than 60% of its original area, mainly by its conversion in agricultural land (Andrade et al. 2015). For the Brazilian subtropical grasslands restoration ecology is a young science, in its initial steps means of selection of species and methods still have to be defined. Therefore regenerative traits might play an important role for the advancement of ecological restoration of these grasslands and similar grasslands.

Thus, our main aim here is to advance in trait-based ecological restoration and test the predictive potential of seed traits on the performance of Brazilian subtropical grassland species through a multi-experimental approach (cabinet, greenhouse and field). We tested the hypothesis that seed traits are able to predict performance of species in the first life stage. We expect heavier seeds having lower Germination Rate Index (GRI), higher survival and Final Germination Percentage (FGP). When lighter, seeds will have higher GRI, but lower survival and FGP. Elongated seeds, will lead to lower GRI, whether round seeds will have higher GRI. The combined effect of mass and shape would lead those same expectations as for only mass, where elongated heavy seeds will have lower GRI, but higher survival and FGP, when light round seed will have higher GRI, but lower survival and FGP.

2. Methods

2.2 Study area

The study area comprised the subtropical grasslands in south Brazil, which comprehend three states: Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS) (Overbeck et al. 2007). The south portion of Rio Grande do Sul is considered as part of the Río de la Plata grasslands ecoregion, which extends from southern Brazil to Uruguay and eastern Argentina (Soriano 1991; Andrade et al. 2018). Climate is subtropical characterized by hot summers and rain equally distributed throughout the year (Alvares et al. 2013). We conducted a field experiment which was located at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (EEA-UFRGS), situated between 30°04' S – 30°07' S and 51°39'18" W – 51°42' 18" W (Bergamaschi et al. 2013). The annual mean rainfall is 1445 mm and during the warmer months the maximum mean temperature can vary from 25 to 30 °C (Bergamaschi et al. 2013). The soil is classified as *Argissolo vermelho distrófico*, which is a red soil characterized by high percentage of clay on the surface, conferring a poor infiltration capacity (Santos et al. 2013). The greenhouse experiment was located at the campus of the Federal University of Rio Grande do Sul in Porto Alegre, Rio Grande do Sul (30°4'1.1" S and 51°7'13.4" W). During the warmer months the maximum mean temperature varied from 28 to 30 °C and annual mean rainfall is 1450 mm (National Institute of Meteorology). Porto Alegre is 62 km away from EEA-UFRGS. The cabinet germination experiment was realized at Laboratory of Plant Ecology, at the same university campus.

2.3 Experimental design

We performed three separated experiments under distinct conditions (cabinet, greenhouse and field). For the three experiments, 15 perennial species of the Brazilian subtropical grasslands were selected based on their frequency and broad distribution patterns, and availability of seeds (Table 1). The origin of the seeds was distinct among species (Table 1). Seeds were collected in the field soon after they became available and were stored for less than one year. Only full seeds were sown (visually

selected under a stereomicroscope trial), and all seed coats and attachments were removed with exception of *Desmodium*. As a standard procedure, all seeds were washed with chlorine 1%, emerged in distilled water for at least 12 hours and sown within a 24h period. All Fabaceae species have their seeds scarified using a sandpaper since physical dormancy is common in this family (Baskin et al. 2006).

Table 1 List of the species used in the experiments with the information of seed collection origin.

Family	Species	Seed origin
FABACEAE	<i>Adesmia latifolia</i> (Spreng.) Vogel	EMBRAPA-Bagé
	<i>Crotalaria hilariana</i> Benth	¹ National Forest of São Francisco de Paula
	<i>Crotalaria tweediana</i> Benth	Morro Santana ² and Sain't Hilaire Park ³
	<i>Desmanthus tatuhyensis</i> Hoehne	Morro do Osso ³ and Sain't Hilaire Park ³
	<i>Desmodium incanum</i> (Sw.) DC.	Campus UFRGS
PLANTAGINACEAE	<i>Plantago myosuroides</i> Lam.	EEA-UFRGS
	<i>Angelonia integerrima</i> Spreng.	Morro Santana ²
POACEAE	<i>Axonopus affinis</i> Chase	EMBRAPA-Bagé
	<i>Anthraenantia lanata</i> (Kunth) Nees	Sain't Hilaire Park ³
	<i>Aristida jubata</i> (Arechav.) Herter	Sain't Hilaire Park ³
	<i>Aristida laevis</i> (Nees) Kunth	Morro Santana ² and EEA-UFRGS
	<i>Axonopus argentinus</i> Parodi	Sain't Hilaire Park ³
	<i>Paspalum notatum</i>	Commercial seller ⁴
	<i>Paspalum regnellii</i> (Mez)	EMBRAPA-Bagé
	<i>Schizachyrium microstachyum</i> (Desv. ex Ham) Rosenberg B.R. Arril. & Izag.	Morro Santana ¹

¹Mata Atlântica biome; ²Is part of Agronomia campus – UFRGS, Porto Alegre; ³Conservation Units in the Porto Alegre region; ⁴The same used in (Guido, Hoss, et al. 2017)

In the cabinet experiment, six replicates of 25 seeds per species were placed within Petri dishes on absorbent paper moistened with distilled water and recovered with a transparent plastic film to keep moisture. Cabinet conditions were 25°C with 12h light and 12h dark. Petri dishes were placed into the cabinet and randomly rearranged during the experiment. Individuals were recorded as germinated by the visual identification of the emergence of a radicle. The recordings were performed once a week for two weeks. Due to seeds of *C. tweediana*, it was not used in the cabinet experiment. Just FGP (Final Germination Percentage) was recorded.

In the greenhouse experiment, species were sown in plug trays with one seed three centimeters deep per plug, totalizing 20 seeds per species with five replicates. Trays were randomly distributed in the greenhouse. A standard soil for seedlings cultivation was used consisting of 2:1 sand and organic soil. Temperatures inside the greenhouse may have reached more than 40°C in some days in the warmer months. Irrigation was provided by automatized systems with a fine spray of water, which frequency was increased or decreased as necessary. The species were sown in February 2018 and the performance parameters, FGP (final germination percentage), GRI (growth rate index) and survival, were recorded during 16 weeks for all 15 species.

In the field, our experimental area was inserted in a matrix of native grasslands, was converted for experiments with forage plants, but was in disuse for more than five years. The secondary vegetation that was covering the area was mechanically removed twice before our experiment. In February 2018, the 15 species were sown, being each species randomly assigned to a plot within each of the five blocks (our replications). In order to increase germination chances, five seeds were sown per hole (of 3 cm deep, with a total of 20 holes per plot), 30 cm away from each other. Thus, a total of 100 seeds were used per species in each block. Weeding was done manually during all experiment, avoiding the establishment of non-target species. Irrigation was provided by automatized systems

when rainfall was insufficient. Similar to the greenhouse experiment, FGP, GRI and survival were recorded during 16 weeks for all 15 species.

2.4 Regenerative traits and performance variables

The regenerative traits considered here were seed traits, being predictors of the performance parameters (response variables), which here were Final Germination Percentage (FGP), survival, and Germination Rate Index (GRI), below described in details. The seed traits were mean values of seed mass and shape per species. Seed mass was measured as the dry weight of a thousand seeds (g) (Pérez-Harguindeguy et al. 2013). Shape measurements were done using the software ImageJ: area (mm²), height (mm), width (mm), and circularity (an index from 0 to 1, where 1 indicates a perfect spherical seed (Rasband 2000)). These seed traits are considered ‘soft traits’ in contrast to ‘hard traits’, which are performance parameters that are better correlated to the fitness itself (Shipley et al. 2016; Saatkamp et al. 2018). Here, to avoid term confusion, we are referring to traits only for the predictor variables. Our performance parameters were measured separately in all experiments for each of the species and used as response variables. FGP was measured as the proportion of germinated individuals (0 to 1). The FGP might represent a numerical effect – species which recruit more are likely to affect coexistence outcomes through dominance (Turnbull et al. 1999; Donath & Eckstein 2010). Survival also ranges from 0 to 1 and consist the proportion of individuals surviving until the last day of the experiment, weighted by the total number of sown seeds. This parameter is a first stage proxy of establishment success (Fenner 2000; Larson et al. 2015). The last parameter is GRI, calculated as:

$$GRI (\%/day) = \frac{G_1}{1} + \frac{G_2}{2} \dots G_x/x$$

Where G1 is the percentage of individuals on the first day after sowing, divided by the number of days, G2 is the percentage in the second day, and so on (Kader 2005). The GRI is how fast and clustered germination is, it has a strong impact on coexistence outcomes in the first life stages, since it is related to the potential priority effects the winners may have on resources use (Kader 2005; Kos & Poschlod 2010; Barak et al. 2018).

2.5 Data analyses

Person's correlation coefficient was first estimated between the measured regenerative traits to retain only independent predictors (Fig. S1). For any pair of traits with a correlation higher than 0.7, we dropped the more redundant (Barak et al. 2018). We kept seed area, circularity, and seed mass. Then a Principal Component Analysis (PCA) was performed using the matrix of mean trait values of species, previously centred and standardized within variables, to describe the general relationship between the 15 studied species and its seed traits. Also, we derivate germination curves for each of the 15 species with the raw data to visualize individual patterns (Figs. S2 to S16).

Linear models were used to evaluate the effect of regenerative traits (predictors) on performance parameters (response variables). Each model was specific to the distribution of its response variable and performed separately for each experiment. Thus, three models were done for each of the field and greenhouse experiment (three response variables: FGP, survival, GRI), and one for the cabinet (only FGP). For all FGP (proportions) models were analysed using a Generalized Additive Model (GAM), as it allows the use of mixed effects when considering a quasi-binomial family, indicated to deal with the distribution of proportion data regarded to an additional parameter for overdispersal (Gail et al. 2009). GRI and Survival data followed the assumption of normal distribution, so the simplest allowed model was used.

In the cabinet greenhouse experiment, linear models were used assuming homogeneity, given they were undertaken under controlled or similar conditions; average parameter values of each species were used. GRI data of the greenhouse experiment was transformed using Square Root in order to increase model adjustment. In the field experiment, the block design was considered, adding a mixed term in the model (the block). GRI data was $\text{Log}_{10}(x+1)$ transformed, increasing model adjustment.

For each model, we started with the most complex, considering all interactions among predictive variables. Then we used model averaging as a model inference approach, keeping candidate models within the top 2 AICc units (Burnham & Anderson 2002). The relative variable importance (Table S1) is given by the model averaging approach, and can be interpreted as the probability that the variable will be within the global model, or into the best model (Burnham & Anderson 2002). Then, we evaluated the significance of the variables using the global model generated by the model averaging criteria. In sequence the confidence intervals of each selected variable were plotted in effect size plots. All analyses were performed using R version 3.5.0 (R Core Team 2018).

3. Results

The PCA (Figure 1) shows the relationship among the 15 studied species and its traits. At the top right there are species with larger seeds, at the bottom right species with higher seed mass values and at the bottom left species with more rounded seeds. At the opposite direction of the arrows are species with the lower values for the respective traits. As example, *A. latifolia* seeds are the heaviest and roundest of the dataset, whereas *C. tweediana* and *D. incanum* seeds presented the greatest values of seed mass of the dataset. The seeds of *A. argentinus* presented the most circular seeds. Both the *Aristida* species were characterized by most elongated seeds and larger values of area, but not seed mass.

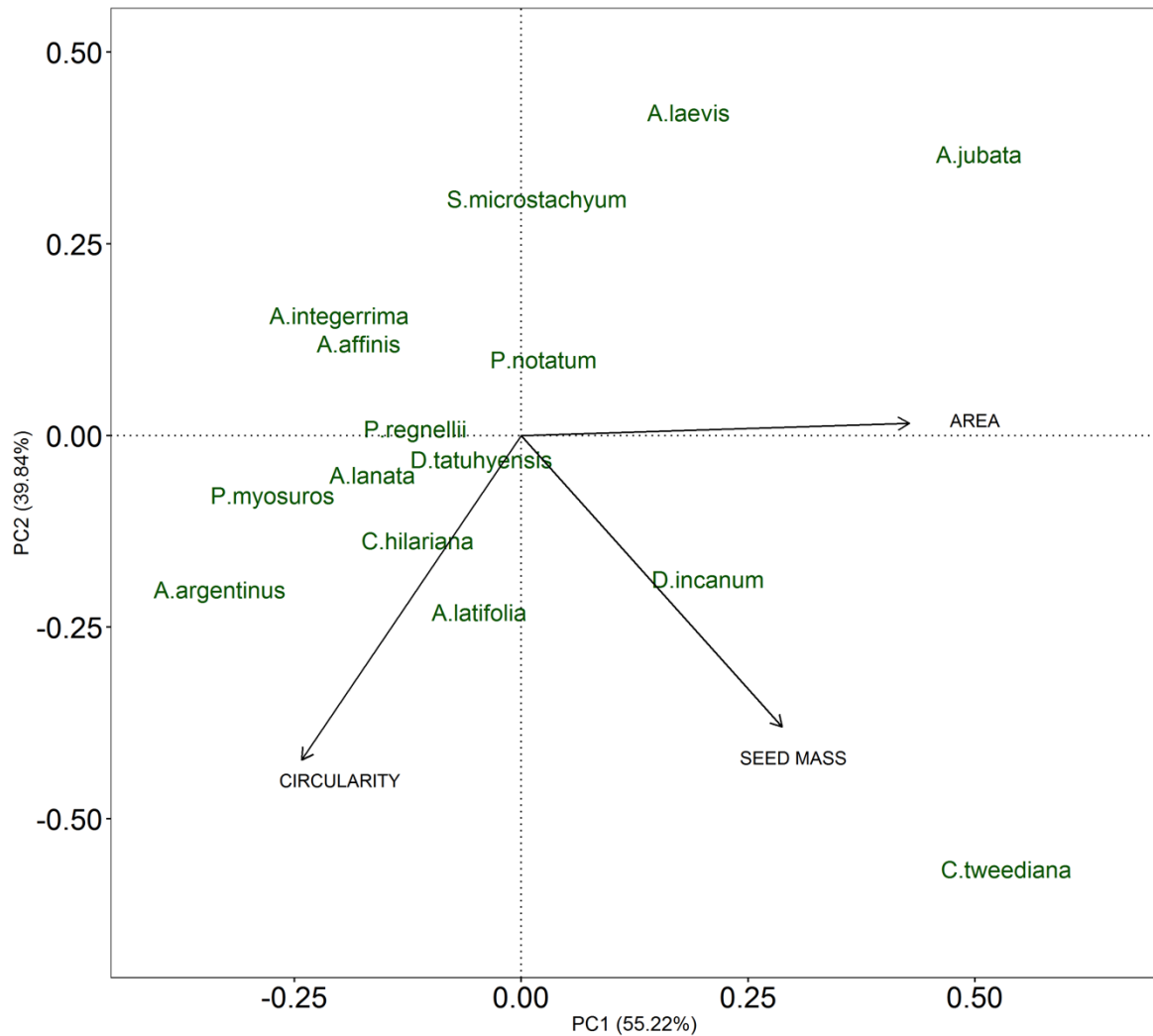


Figure 1 Ordination diagram of the Principal component analysis (PCA) of 15 grassland species of the Southern Brazilian Campos described by three seed traits: seed mass (g), area (mm²) and circularity (an index from 0 to 1, where 1 represents a perfect spherical seed). Complete species names can be found in Table 1.

Germination mean was consistently low in all experiments and for most species. In the cabinet, germination percentage varied from 0 to 90.6%, in the greenhouse from 2 to 28% and in the field from 0.8 to 16% (Table 2). General FGP mean was higher in the cabinet compared to the greenhouse and the field experiment (the lowest mean). Survival mean was higher in the greenhouse compared to the field experiment. GRI mean was slightly higher in the greenhouse compared to the field.

Table 2 Averaged values for the performance parameters of each species in each experiment. Performance parameters are: Final Germination Percentage (FGP), Germination Rate Index (GRI), and Survival.

Performance parameters	FGP (%)			GRI		Survival (%)	
	Cabinet	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
<i>Adesmia latifolia</i>	35	9.8	1.6	2.33	0.45	40.8	50
<i>Angelonia integerrima</i>	50.3	0.8	2	0.03	0.13	100	100
<i>Anthraenantia lanata</i>	90.6	16	8	2.4	1.3	36.8	60
<i>Aristida jubata</i>	3.9	5	4	0.41	0.53	19.3	75
<i>Aristida laevis</i>	27	12.8	11	2.03	2.15	45.9	74.2
<i>Axonopus affinis</i>	13.1	5.4	3	0.53	0.31	4	100
<i>Axonopus argentinus</i>	13.6	6.2	3	0.71	0.72	30	25
<i>Crotalaria hilariana</i>	8.8	4.2	3	0.68	0.28	36.3	100
<i>Crotalaria tweediana</i>	NA	9.4	28	1.3	2.62	62.4	87.9
<i>Desmanthus tatuhyensis</i>	51.8	2.4	5	0.34	1.55	74.2	25
<i>Desmodium incanum</i>	12.8	8.6	23	1.44	3.57	60.7	80.7
<i>Paspalum notatum</i>	0.6	3.6	11	0.26	1.68	31.7	30
<i>Paspalum regnellii</i>	36.5	3.8	25	0.49	5.02	4	67.1
<i>Plantago myosuroides</i>	0	3.6	5	0.11	0.05	100	100
<i>Schizachyrium microstachyum</i>	49.3	3.6	6	0.36	1.46	44	75
General Result	28.09	6.35	9.24	0.89	1.45	46.01	69.99

FGP was not explained for any seed trait on neither of the experiments (Table S4). In the cabinet, seed area appears as a potential predictor, as it was considered informative, though not significant ($\pm 95\%$ CI=-0.1034; 0.03290). In the greenhouse, the GRI was not explained by any trait (Figure 2), however seed mass and area were considered informative on predicting the GRI. In the field experiment, all seed traits and its interactions were present in the final model of GRI, holding

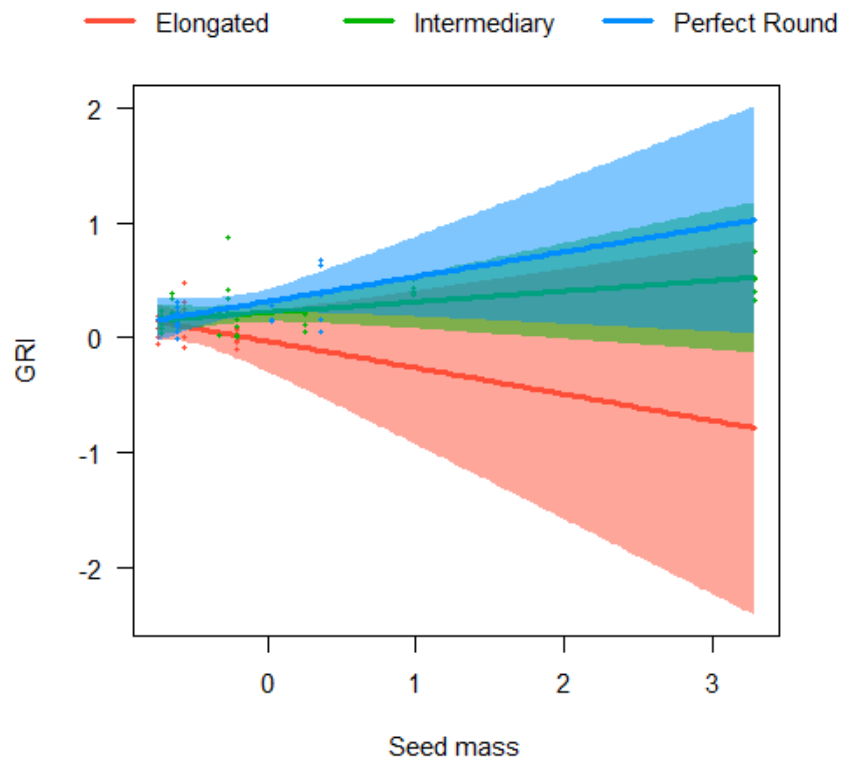


Figure 3 Effect of the interaction between seed mass (g) and seed circularity (an index from 0 to 1, where 1 represents a perfect spherical seed) on GRI in the Field experiment. The red coloured area represents seeds with lower values of circularity, while the green and blue one represents respectively intermediary and higher values of circularity. Shaded areas are fitted regression lines \pm 95% CI.

None of the regenerative traits significantly predicted the survival in both the greenhouse and the field experiments (Table S1). In the field experiment, the final model included individual effects and seed traits interactions, although they were not significant (Figure 4).

exception, *A. laevis* had an unexpected low germination compared to a previous studies: (98%) (Overbeck et al. 2006) and (83.3%) (Guido et al. 2017). Whether, *A. lanata* (90.6%) presented an elevated germination percentage compared with a previous study (77%) (Overbeck et al. 2006). Perenniality has been reported as one reason for low germination as it allows species to spread the risk of good and bad years throughout its lifespan (Fenner 2000). Furthermore, the main regeneration strategy among subtropical Brazilian grassland species is by vegetative regrowth from rhizomes or underground storage organs after disturbances, not by seeds (Overbeck et al. 2007; Maia et al. 2003; Vieira et al. 2015). Nonetheless, among the available methods for grassland restoration, sowing is the cheapest successful one (Török et al. 2011). Thus further research should include more years of seed sampling, and track of good and bad years to confirm rates of germination since in this study all seeds were collected in only one year.

4.3 The experimental conditions affect species responses and must be considered when the objective is predictability

One critic to germination experiments is the common attempt to extrapolate results from controlled conditions to natural ones (Baskin et al. 2006), which can be particularly important in restoration applied studies. Past research shows that patterns found under controlled natural conditions may differ from nature. Hence in Clarke & Davison (2004) study, laboratory germination percentages were similar to field ones, but time to germinate differed significantly. Analogously, Baskin et. al. (2006) suggested that experiments under controlled conditions may under or overestimate germination parameters. In our study we observed four reasons why this might happen. First, it is the existence of seasonal regeneration niche, since *Plantago* was reported to be a temperate genera, thus may respond to temperature variation (Safford 2007; Poschlod et al. 2013). We observed in the field and in the greenhouse experiment a clear delay in the germination timing of *P. myosuroides*, coinciding with the start of the fall season (Fig. S5). Resulting in the observed performance of *Plantago myosuroides* (0% in cabinet experiment, but better in the other two). A study in California found 100% of germination for *Plantago erecta* (Gulmon & Url 1992) and according to our observations, it may

represent the potential for our species too if enough time and conditions are given. Thus, our cabinet experiment might have underestimated the potential for this species given its specific requirements to germinate.

To exemplify a second reason, we have the *Angelonia integerrima* case. It had a higher germination in the cabinet experiment (50.3%) compared to greenhouse (2%) and field (0.8%). In the cabinet, the seeds were directly exposed to light, in opposition to the other two, where seeds were buried. This could mean that early access to light may be a major factor of its regeneration niche, determining competitive outcomes (Fenner 2000; Eckstein 2005). Third, an alternative explanation is the different criteria used to detect germination – in cabinet, radicle emission, in field or greenhouse seedlings may emerge, by overcoming the soil barrier to be detected. Plant emergence is an important cause of death for many species (Larson et al. 2015). In addition the soil in field experiment is a red soil with a significant amount of clay, which imposes a stronger physical barrier than the standard soil used in the greenhouse. Donath & Eckstein (2010), in a burial experiment, found heavier seeds having an advantage in final germination and timing to germinate, emerging earlier. *Angelonia* seeds are among the smaller and lighter in the dataset, thus the physical barrier seems to have a significant effect on its requirements to germinate.

The fourth reason is that only under field conditions effects of traits could be really observed. The study of Rodríguez-Pérez & Traveset (2007) show that the effect of seed traits on fitness was more relevant under the most stressful condition. Thus, natural conditions must be considered in order to best understand the correlations between traits and performance.

4.4 Seed traits matter as predictors of initial performance, but some implications for restoration might be quite unpredictable

Seed shape was relevant on predicting performance in all considered models. Regarded to circularity, past researches have shown that elongated seeds would led to faster germination to avoid

seed predation, as they are selected against burial and its anatomy tend to be easier handled by predators (e.g. ants) (Liu et al. 2007; Barak et al. 2018; Penn & Crist 2018). However, in our study elongated seeds germinated slower (lower GRI) than rounded seeds. As it is not expected, it may lead to reduced fitness, which in a restoration context may need to be reseeded in later years (Barak et al. 2018).

Seed mass has been found to have a positive effect on survival and germination (Westoby et al. 1992; Moles & Westoby 2004) and a lower GRI (Goldberg & Landa 1991; Leishman et al. 2000; Barak et al. 2018). Surprisingly, our results showed that seed mass alone was not a good predictor of initial performance, although it was relevant in both field final models (GRI and survival). Seed mass effect was stronger when interacting with circularity, where heavier rounder seeds presented higher GRI compared to lighter ones, and elongated seeds presented a general lower germination than rounded ones, but faster compared to heavy elongated seeds. Contrary to our results, past researches stated that lighter elongated seeds germinate faster/earlier compared to round heavy ones (Thompson & Hodgson 1993; Liu et al. 2007). A higher GRI can generate priority effects, as early the species germinate, earlier they have access to available resources above and below ground, which might allow they growth earlier (Grubb 1977; Grime et al. 1981; Kimball et al. 2011; Barak et al. 2018). As seed mass is also correlated to biomass, there is a tendency that the earlier arrival of these rounded heavy seeded species lead to overcome competition by shadowing elongated seeded neighbours (Westoby et al. 1992). However, the prediction power related to the interaction between elongated seeds and seed mass was lower than the observed for rounded seeds (Figure 4). As the interactions between these (and other) traits and performance have been rarely studied together, and the observed effects can change in the transition to adulthood phase in the community (Moles & Westoby 2006; Larson et al. 2015), to better understand long-term interplays under real conditions in terms of abiotic factors and species interactions, further experiments must be realized.

To conclude, we found that regenerative traits influence initial performance of grassland species and might thus drive community assembly processes in ecological restoration projects. Our results showed that seed mass alone is not a good predictor of performance, but it is a determinant of performance when interacting with seed shape. This interaction results in a discrimination of species performance by classes of shape and weight, where rounded heavy seeds showed a competitive advantage in relation to elongated ones by earlier 'arrival' in the initial regeneration phase of seeded communities. Here, seed shape was a better predictor than seed mass, which is currently the most studied regenerative trait (Wang et al. 2016; Barak et al. 2018), and should thus be further considered in traits-fitness correlation studies. In ecological restoration, an earlier arrival is a desirable characteristic, as such species can pre-empty niches preventing the establishment of undesirable species during the restoration process (Mcglone et al. 2011; Seahra et al. 2018), but it can also have a negative effect on community assembly if priority effects of early species prevent the arrival and development of complementary desirable species (Grman & Suding 2010).

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Anexo I

Tabelas e gráficos referentes ao material suplementar do capítulo 1.

Figura S1- Gráfico de correlação de Pearson para os atributos de sementes.

Figuras S2:S16- Curvas de germinação para as 15 espécies estudadas.

Tabela S1- Coeficientes padronizados dos modelos finais de regressão. Variáveis resposta estão indicadas acima (FGP: porcentagem final de germinação, varia de 0 a 1; GRI: índice de velocidade de germinação; proporção de sobreviventes, varia de 0 a 1). Os respectivos modelos para cada um dos experimentos estão representados nas colunas com as correspondentes médias estimadas e intervalos de confiança para cada preditor (SM: massa de sementes, g; Área, mm²; Circularidade, um índice de 0 a 1, where 1 represents a perfect spherical seed).

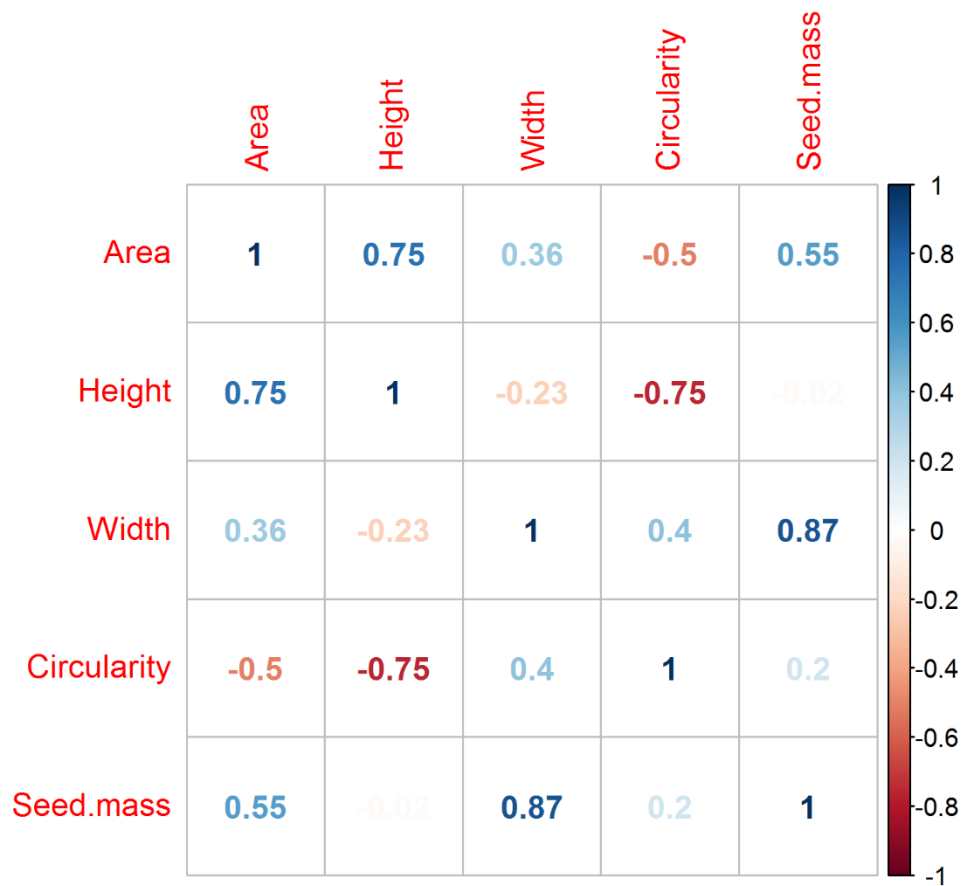


Figure S1- Seed traits Pearson's correlation plot. Seed mass (g); Width (mm); Height (mm); Area (mm²); Circularity (an index from 0 to 1, where 1 represents a perfect spherical seed).

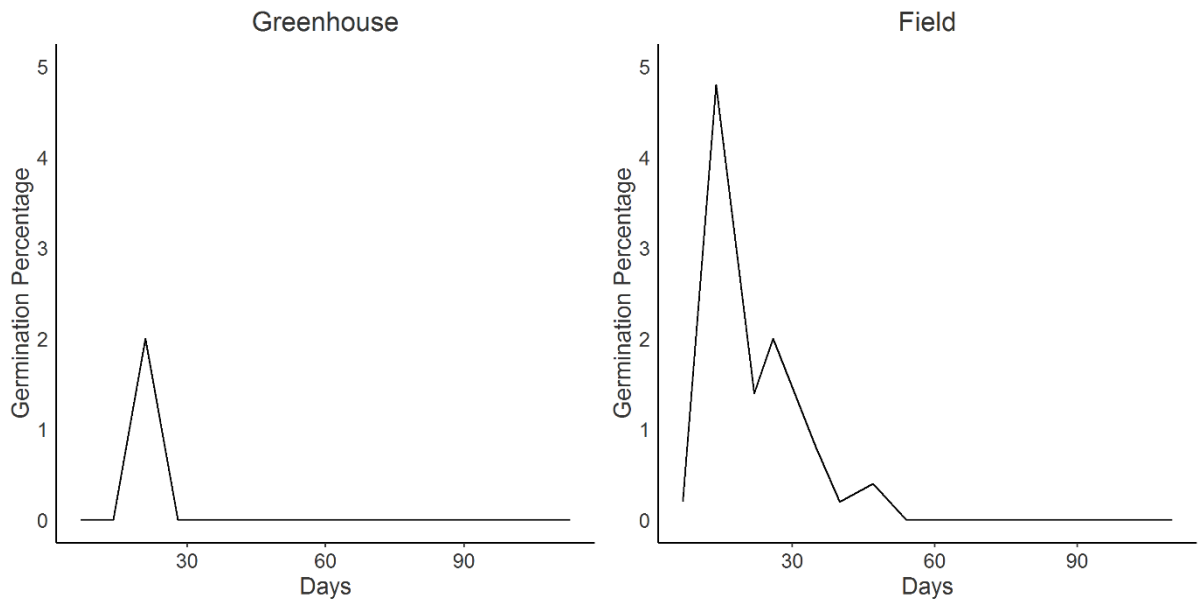


Figure S2 *Adesmia latifolia* germination curves from both experiments.

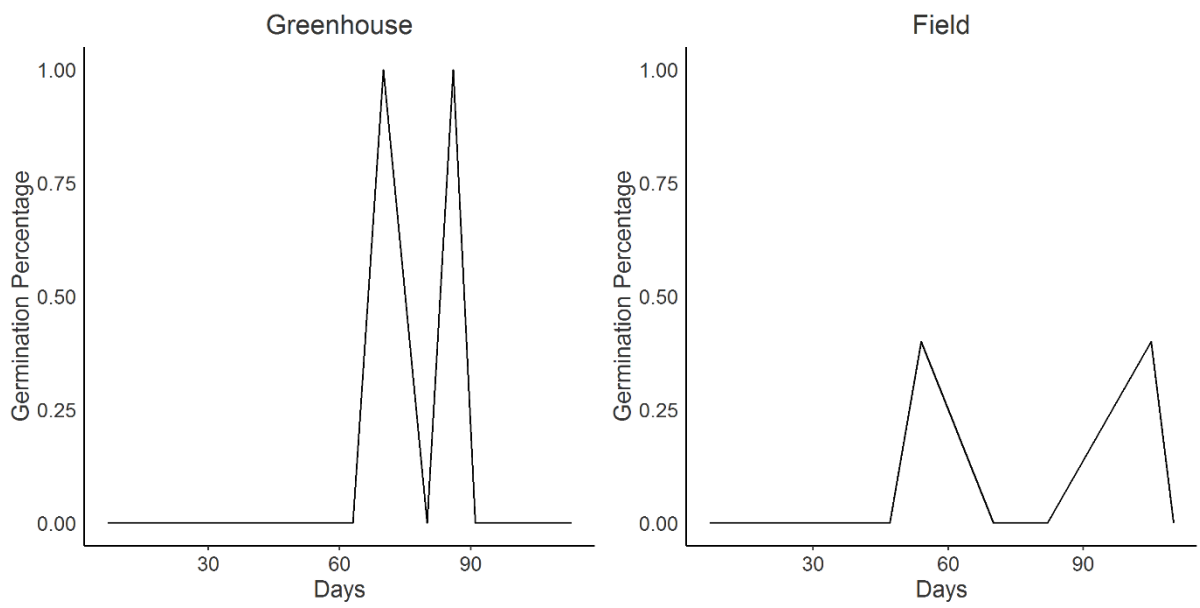


Figure S3 *Angelonia integerrima* germination curves from both experiments.

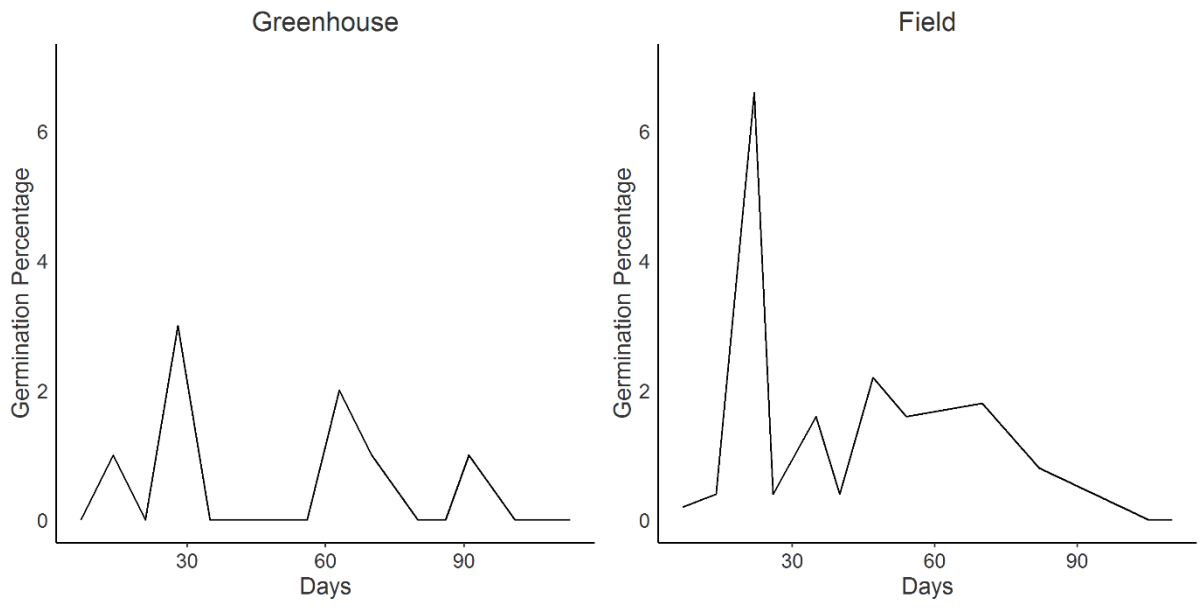


Figure S4 *Anthaenantia lanata* germination curves from both experiments.

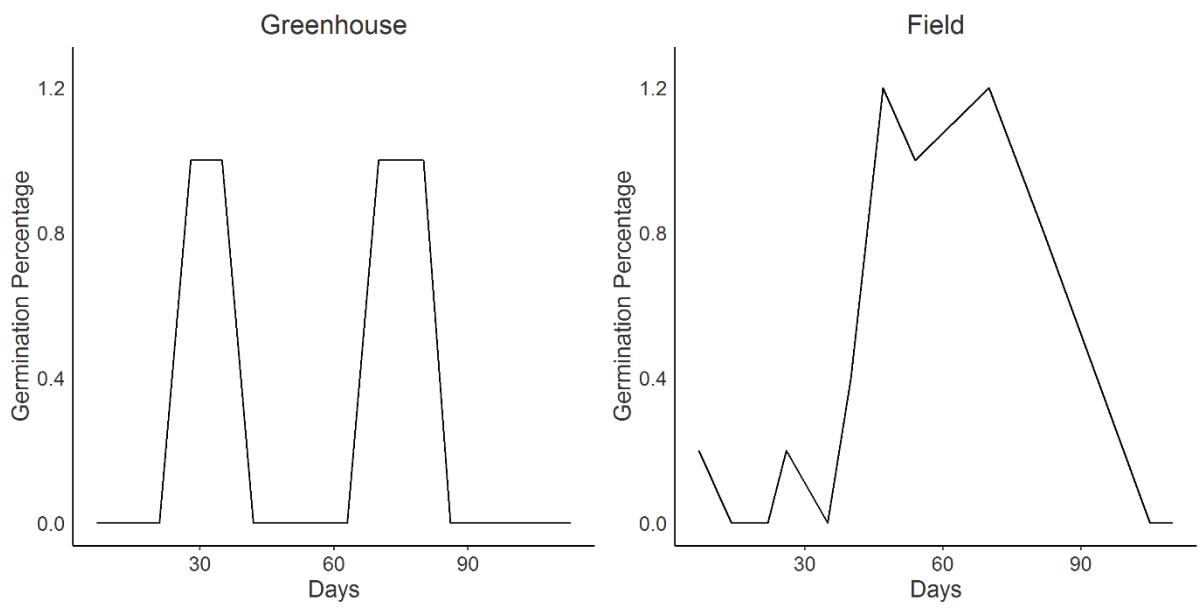


Figure S5 *Aristida jubata* germination curves from both experiments.

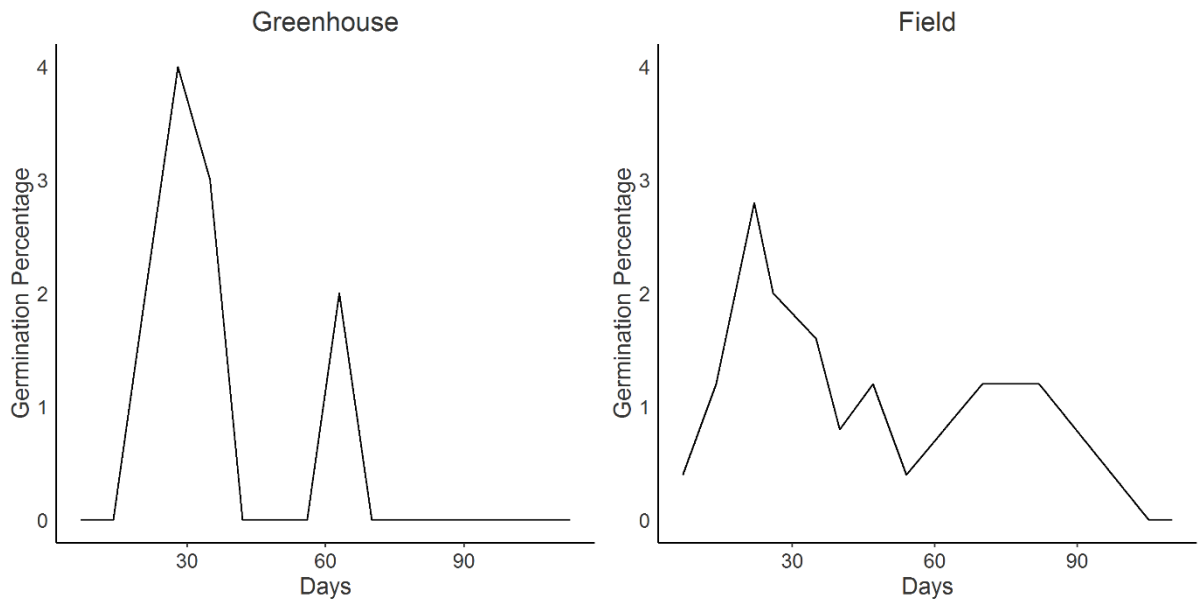


Figure S6 *Aristida laevis* germination curves from both experiments.

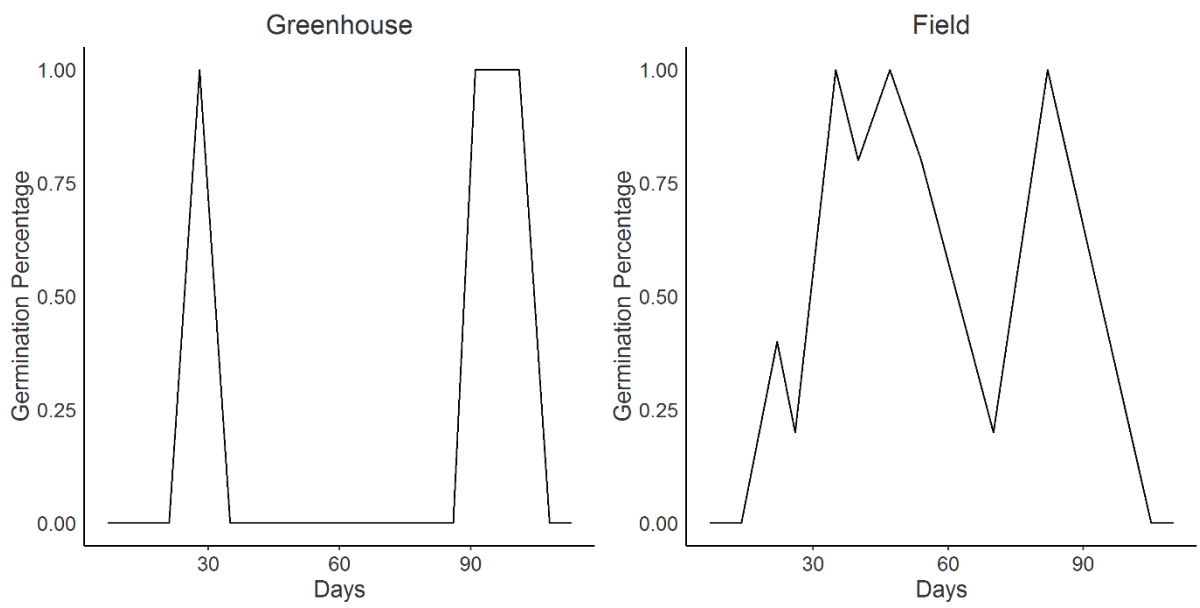


Figure S7 *Axonopus affinis* germination curves from both experiments.

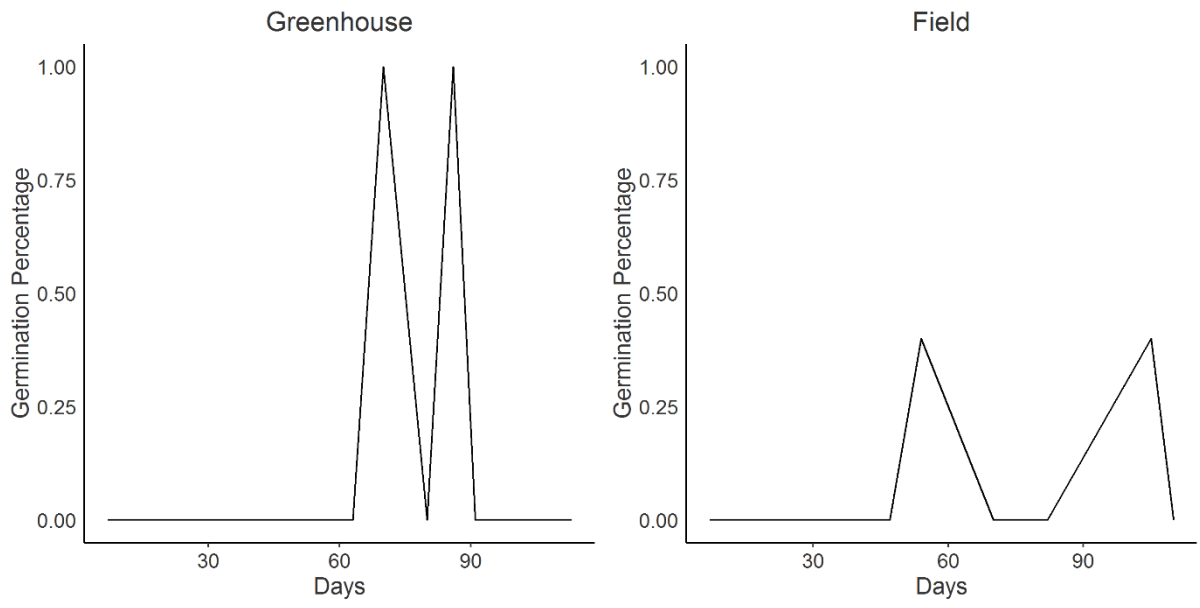


Figure S8 *Axonopus argentinus* germination curves from both experiments.

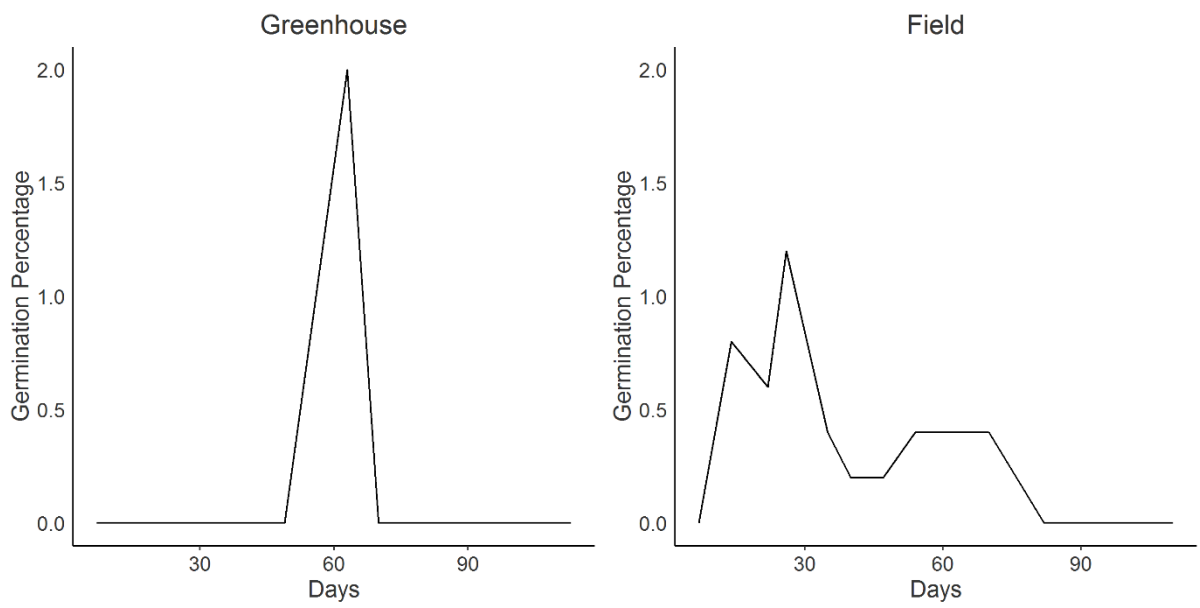


Figure S9 *Crotalaria hilariana* germination curves from both experiments.

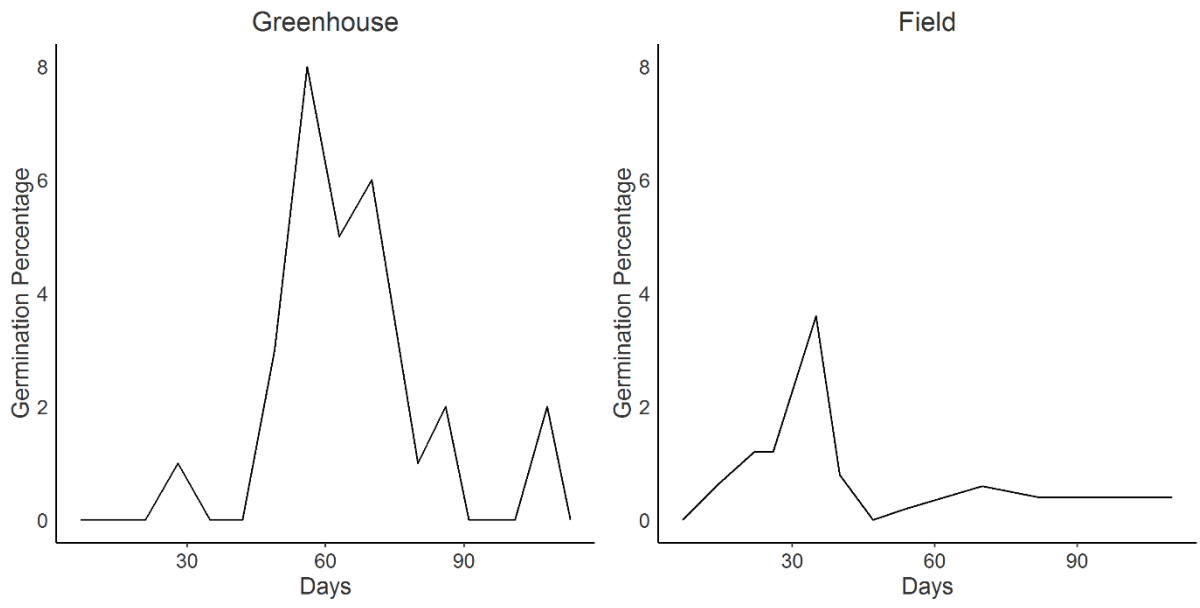


Figure S10 *Crotalaria tweediana* germination curves from both experiments.

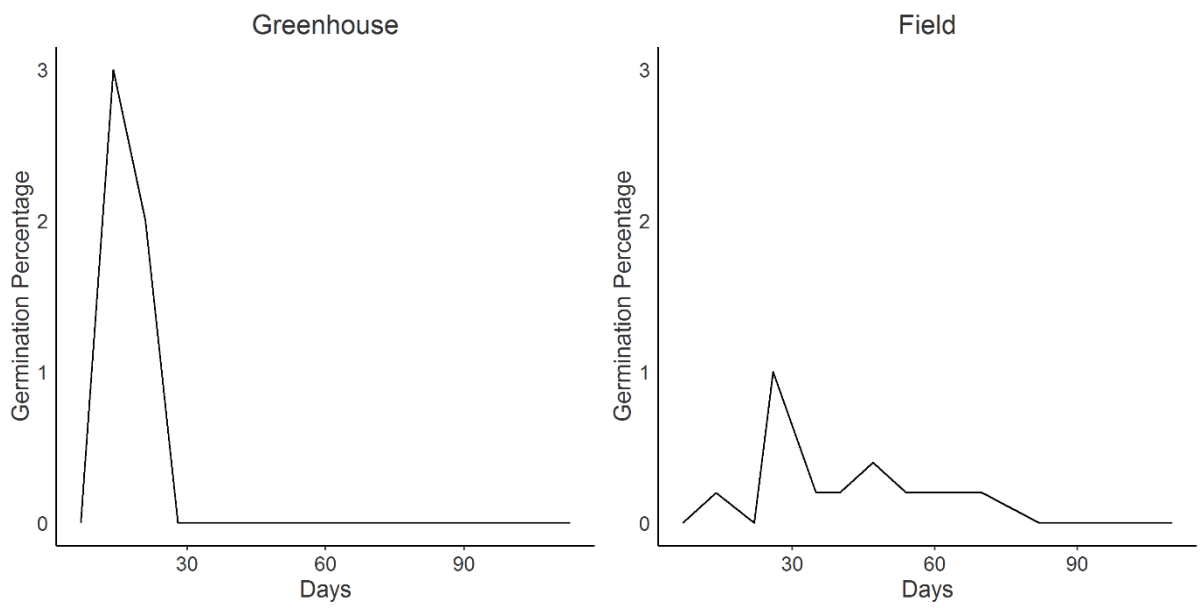


Figure S11 *Desmanthus tathuyensis* germination curves from both experiments.

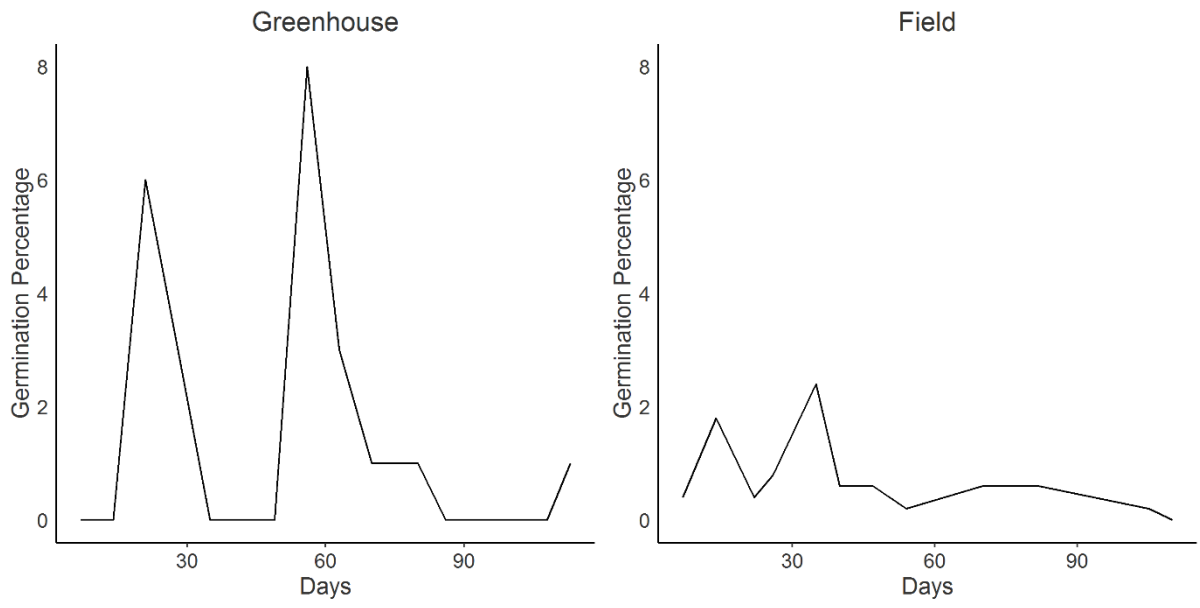


Figure S12 *Desmodium incanum* germination curves from both experiments.

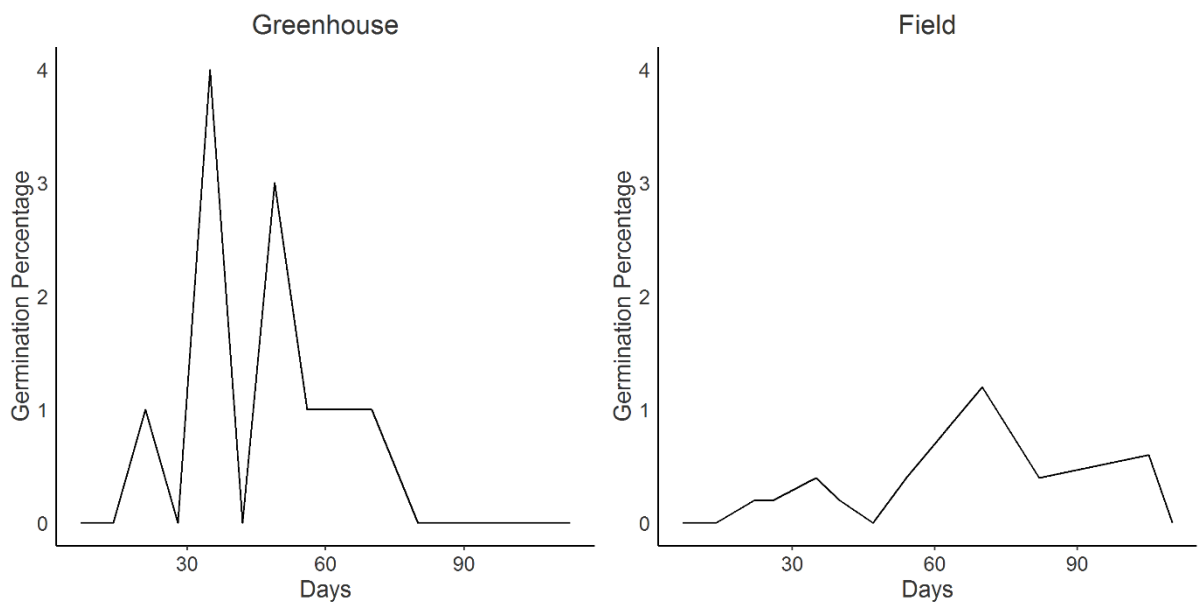


Figure S13 *Paspalum notatum* germination curves from both experiments.

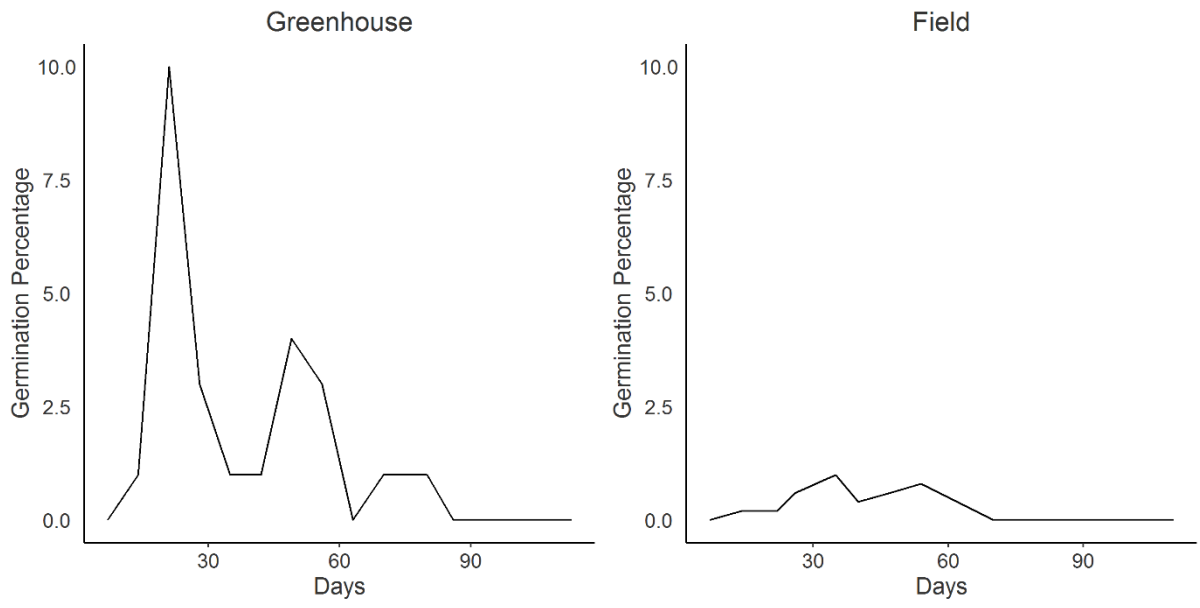


Figure S14 *Paspalum regnellii* germination curves from both experiments.

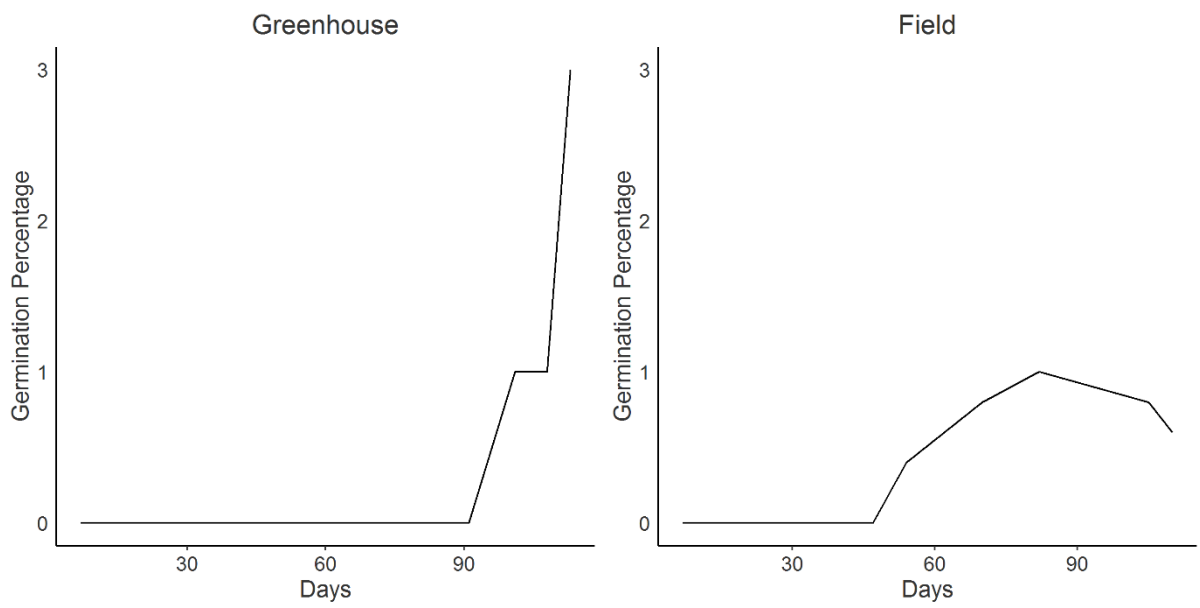


Figure S15 *Plantago myosuroides* germination curves from both experiments.

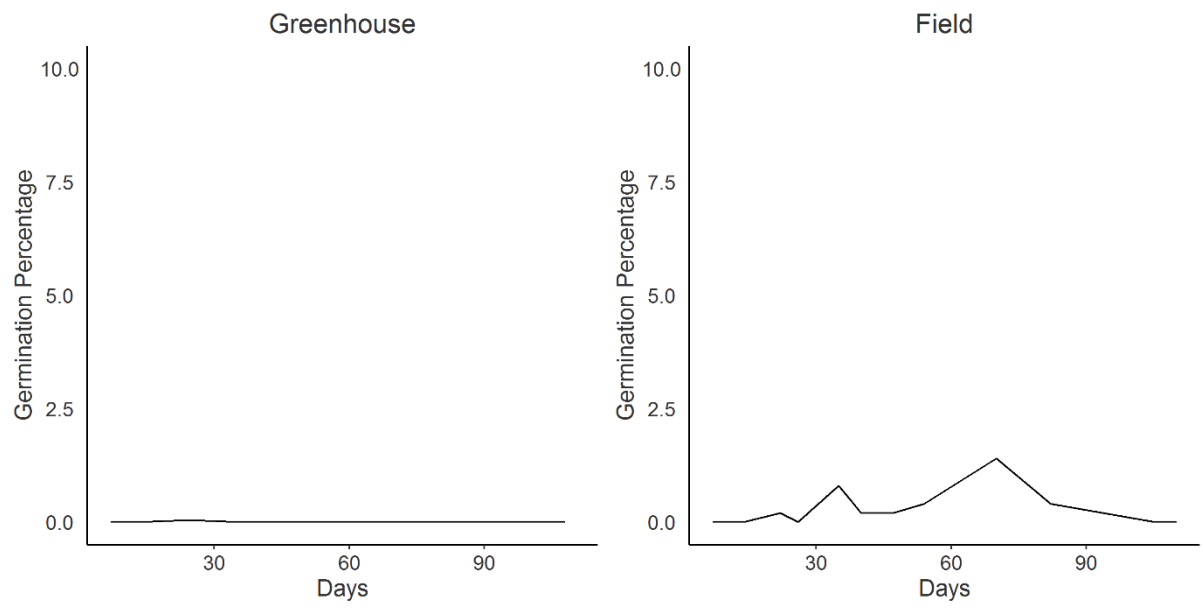


Figure S16 *Schizachyrium microstachyum* germination curves from both experiments.

Table S1 Standard coefficients from the final regression models. Response variables are indicated above (FGP: Final Germination Percentage, range from 0 to 1; GRI: Germination Rate Index; Survival, range from 0 to 1). The respective models for each of the experiments are represented in the columns with the correspondent averaged estimates and confidence intervals for each predictor (SM: seed mass, g; Area, mm²; Circularity, an index from 0 to 1, where 1 represents a perfect spherical seed).

a) Response Variable FGP				
Models	Predictors	Averaged estimates	95% CI	Relative variable importance
Cabinet	Intercept	0.3121	(0.0999, 0.5242)	NA
	Area	-0.0352	(-0.1034, 0.03290)	0.28
Greenhouse	Null model			
Field	Null model			
b) Response Variable GRI				
Greenhouse	Intercept	1.0673	(0.7504, 1.3842)	NA
	SM	0.2286	(-0.0914, 0.5487)	0.33
	Area	0.1895	(-0.1396, 0.5186)	0.22
Field	Intercept	0.2021	(0.1070, 0.2971)	NA
	SM	-0.0146	(-0.1775, 0.1482)	1
	Area	0.0568	(-0.0665, 0.1802)	1
	Circularity	0.1479	(0.0366, 0.2593)	1
	Area:SM	-0.0930	(-0.2018, 0.0157)	0.81
	Circularity:SM	0.2285	(0.0564, 0.4005)	1
	Area:Circularity	0.0504	(-0.0704, 0.1712)	0.30
	Area:Circularity:SM	0.1933	(-0.0114, 0.3981)	0.30
c) Response Variable Survival				
Greenhouse	Null model			
Field	Intercept	0.4516	(0.3576, 0.5454)	NA
	SM	0.1379	(-0.0849, 0.3607)	0.72

Area	-0.1448	(-0.3788, 0.0890)	0.83
Circularity	-0.1047	(-0.2571, 0.0478)	0.33
Area:Circularity	-0.0876	(-0.2332, 0.0579)	0.13
Area:SM	0.0474	(-0.0755, 0.1705)	0.12

Capítulo 2. Atributos funcionais, estratégias CSR e performance através de diferentes estágios do estabelecimento de espécies campestres

Title: Functional traits, CSR strategies and performance across different establishment stages of grassland species

Short running title: Determinants of grassland species performance

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Abstract

Questions: The initial establishment of species is considered a limiting factor for the success of restoration projects. However, few is known about how different traits are linked with the ontogenetic stages and plant performance during the initial establishment stage. Our specific questions were: (i) Can species' multidimensional variation in terms of functional traits explain early and later establishment performance of herbaceous grassland species? (ii) Are the CSR components associated to plant performance in the initial development phase of grassland species?

Local: Southern Brazilian Subtropical Grasslands.

Methods: The experiment consisted in seeding 12 species with different functional traits and life strategies in field permanent plots. Each plant was monitored to evaluate early and late performance of species. Functional traits along three multidimensional axes were used as predictors, and CSR strategies were correlated to them and to the performance variables.

Results: Principal component 2 (PC2), mainly represented by Leaf area (LA) was positively correlated to above ground biomass (AGB), a later performance variable, which was also positive correlated to

the C-strategy within the Grime's CSR-triangle. PC1 was related to regenerative traits, which, neither correlated to CSR-triangle or performance. S-strategy was correlated to growth rate index and final germination percentage (early establishment performance) and PC3 (specific leaf area).

Conclusion: By using easily measurable traits, we were able to confirm leaf traits as predictors of later performance and integrate it to the CSR scheme, in order to understand success in initial establishment phases of grassland species. The CSR scheme was well associated to performance in both establishment stages. We could not link a multidimensional axis expressing regenerative traits as predictors to neither of the performance stages. We encourage further investigation of species performance and ecological strategies based on functional traits to support ecological restoration practices.

Key-words: Height, SLA, LA, LDMC, Seed Mass, Seed Shape, Restoration, Biomass, Growth Rate, Germination, Survival

6. Introduction

Ecological restoration broadly aim at the successful reassemble of plant communities, which should be based on ecological knowledge (Laughlin, 2014; Mcdonald, Gann, Jonson, & Dixon, 2016; Rosenfield & Müller, 2018). Functional ecology is currently playing an important role on the understanding of ecosystem processes, and the associated mechanisms and patterns (Huang, Liu, Bradford, Huxman, & Venable, 2016; Shipley et al., 2016; Zirbel, Bassett, Grman, & Brudvig, 2017). This approach makes use of functional traits, which are defined as morpho-physiological and phenological characteristics that influence individual fitness (Violle et al., 2007), in order to achieve general predictability about rules of community assembly and ecosystem functioning (S Díaz & Cabido, 2001; Shipley et al., 2016). One of the main assumptions of the trait-based ecology is the strength of the relationship between functional traits and fitness, which may be proxies of plant performance (Shipley et al., 2016). However, it has still been poorly empirically tested (Shipley et al., 2016). Plant traits can represent characteristics of the whole-plant, such as height, or organs as leaves (e.g. specific

leaf area - SLA, leaf dry matter content - LDMC) and seeds (e.g. seed mass – SM, dispersal mode, seed appendices, seed shape, germination clues), also called regenerative traits (Jiménez-Alfaro, Fernando A.O. Silveira et al. 2016; Larson & Funk 2016; Shipley et al. 2016).

However, regenerative traits have been the most neglected traits in functional ecology studies (Barak, Lichtenberger, Wellman-Houde, Kramer, & Larkin, 2018; Jiménez-Alfaro, Silveira, Fidelis, Poschlod, & Commander, 2016b; Saatkamp, Cochrane, Commander, Guja, & Jimenez-, 2018). The term “regenerative traits” include traits which are associated with the regeneration niche, defined by Grubb, (1977) as the biotic and abiotic requirements for an individual to germinate and establish as a seedling in a given community. On the other hand, whole-plant and leaf traits have been widely studied and linked to performance in later stages of life. For instance, the relationship between productivity and leaf and root traits (Sandra Díaz et al., 2016; Mark Westoby, Falster, Moles, Vesk, & Wright, 2002; Wright et al., 2004), as well as growth rates (Campbell & Rochefort, 2003; M Westoby, 1998). Whether, height is related to competitive hierarchies, by facilitated access to light resources (M Westoby, 1998; Mark Westoby et al., 2002).

In restoration ecology, the community reassembly usually starts with seed addition, thus regenerative traits are thought to play an important role in this phase, where its effects can long last and influence ecological patterns in a broad scale (Huang et al., 2016; Kiehl, Kirmer, Donath, Rasran, & Hölzel, 2010; Wainwright et al., 2018). Seed germination, emergency and survival during the first weeks are thought to be an important bottleneck to plant establishment, mainly determined by regenerative traits (Kempel, Chrobock, Fischer, Rohr, & van Kleunen, 2013; Larson, Sheley, Hardegree, Doescher, & James, 2015; Moles & Westoby, 2004). On the other hand, as soon as first weeks survival was succeed, whole-plant traits and leaf traits start to influence plant growth and fitness in further stages of development (Craine et al., 2002; Kitajima & Fenner, 2000). This is known as developmental niche, the order and magnitude in which traits are expressed influence performance along life stages (Donohue, 2013; Larson et al., 2015; Valdez, Hartig, Fennel, & Poschlod, 2019). As a result, we could

expect that the effect of regenerative traits on performance may indirectly be extended to further stages of development (i.e. not restricted to early phases), whereas whole-plant and leaf traits may only influence performance after its emergence, the first-weeks survival and further (i.e. restricted to late performance) (Donohue, 2013; Larson et al., 2015; Pierce, Bottinelli, & Cerabolini, 2014; Zirbel et al., 2017). Those differences across life stage have been rarely considered (Larson et al., 2015; Valdez et al., 2019).

In order to understand the relationship among traits and its effects on species performance, we can consider two broadly studied and applied approaches. First, by meaningful correlations among traits expressed on multidimensional spaces describing plant strategies (Sandra Díaz et al., 2016; Pierce et al., 2014; Shipley et al., 2016; M Westoby, 1998; Mark Westoby et al., 2002), that mostly reflect variation on an economic spectrum of plant acquisition *versus* conservation of resources (Sandra Díaz et al., 2016; Pierce et al., 2017). Second, by plant strategies schemes, which consist of a set of traits that respond or affect their environment in a similar way (Reich et al., 2003; Mark Westoby et al., 2002). One ecological strategy scheme empirically developed and wide-world known is the competitive-stress tolerant-ruderal (CSR) classification proposed by Grime (1974, 1977). In this classification, a *competitive-strategy* is mostly characterized by plants with higher canopy height (CH) and higher biomass, whereas a *stress tolerant-strategy* is characterized by small stature plants with low relative growth rates (RGR) and small tick leaves, and a *ruderal-strategy* is mainly composed by annuals or perennials plants with small, but acquisitive leaves, high RGR and small seeds (Grime, 1977; Pierce et al., 2014). The CSR scheme was developed for the understanding of species strategies given its distribution in an environmental gradient (Grime, 1977; Pierce et al., 2014, 2017). Recently, a tool for the derivation of species strategies based on easy to measure leaf traits was developed by Pierce et al. (2017), allowing wider applications of the CSR scheme, as well as for the understanding of species performance.

Particularly, in the subtropical Brazilian grasslands, restoration has been recognized as an urgent need (Andrade et al., 2015; Overbeck et al., 2013), as currently about 60% of native grasslands have been converted to alternative soil uses (Andrade et al., 2015). Although grasslands are highly diverse, with about 4000 plant species estimated for this region, there are few researches available to support ecological restoration projects (Andrade et al., 2015, 2018; Overbeck et al., 2013, 2015). In this study we sought to address two main questions, which in turn can be broadly applicable to other species or ecosystems: (i) Can species multidimensional variation in terms of functional traits (whole-plant, leaf, and seed traits) explain early and later establishment performance of herbaceous grassland species? (ii) Are the CSR components associated to plant performance across life stages of grassland species?

7. Methods

7.2 Study area

The experimental plots were located at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (EEA-UFRGS), situated between 30°04' S – 30°07' S and 51°39'18" W – 51°42' 18". The soil of the experimental area is classified as Argissolo vermelho distrófico, a red soil with high percentage of clay on the surface (Santos, Zaroni, & Almeida, 2013). The area is within the southern Brazilian subtropical grasslands, which comprises the states of Paraná (PR) and Santa Catarina (SC) and Rio Grande do Sul (Overbeck et al., 2007). The southern tip of Rio Grande do Sul encompass the Uruguay and eastern Argentina grasslands either, forming the Rio de la Plata grasslands ecoregion (Andrade et al., 2018; Soriano, 1991). Climate is subtropical humid characterized by hot summers and rainfall equally distributed throughout the year (Alvares et al. 2013). The annual mean precipitation is 1445 mm and the annual mean temperature is 18.8 °C, but during the warmer months the maximum mean temperature can vary from 25 to 30°C (Bergamaschi et al. 2013).

7.3 Experimental Design

For this study, 12 perennial herbaceous species of southern Brazilian subtropical grasslands were selected based on their frequency and distribution patterns, and availability of seeds at collection time (Table 1). The performance variables included in this study regarded to two distinct stages of species establishment, early establishment and later establishment, were monitored under distinct experimental designs.

The performance variables related to the early establishment stage were monitored during the first four months of the experiment, where species were sown in a block design experiment. Seeds were collected in natural grassland areas soon after they became available (i.e. at maturity). They were stored less than one year. The origin of the seeds was distinct (Table 1). Seeds were then individually selected, in order to use only full and healthy seeds. Seed coats and attachments were removed with exception of *Desmodium*. Previous to sowing, all seeds were washed with chlorine 1%, following emersion in distilled water for at least 12 hours. In addition, all Fabaceae species had their seeds scarified using a sandpaper (Baskin, Thompson, & M. Baskin, 2006). In February 2018, species were sown, with each species randomly assigned to a plot within each of the five blocks (our replications). In order to increase germination chances, five seeds were sown per hole (of 3 cm deep, with a total of 20 holes per plot), 30 cm away from each other. Thus, a total of 100 seeds were used per species in each block. Weeding non target species was done manually during all experiment. Irrigation was provided by automatized systems when rainfall was insufficient. The monitored parameters here were: Final Germination Percentage (FGP), Growth Rate Index (GRI) and survival.

In July 2018 the early establishment stage was ceased, since for most species any new germination was recorded for at least two consecutive weeks. Following, the experiment was adapted for the later establishment monitoring. Individuals of species remaining in the plots were randomly planted in the experimental area, increasing space among individuals in order to avoid overlap of above and below ground organs. From July/2018 to January/2019, individuals had their individual

growth monitored (Table 1). Induced irrigation was not provided during this period. Total monitoring period was 6 months (156 days), enough time to most species reach their reproductive stage. For the later establishment stage, growth rate (GR), above ground biomass (AGB) and below ground biomass (BGB) were recorded as performance parameters.

Table 1 List of the species and families used in the experiment, local of seed's origin and the number of individuals monitored during later establishment stage.

Family	Species	Seed Origin	Number of individuals
FABACEAE	<i>Adesmia latifolia</i> (Spreng.) Vogel	EMBRAPA-Bagé	6
	<i>Crotalaria hilariana</i> Benth.	National Forest of São Chico	6
	<i>Crotalaria tweediana</i> Benth.	Morro Santana ¹ and Sain't Hilaire Park ²	10
	<i>Desmanthus tathuyensis</i> Hoehne	Morro do Osso ² and Sain't Hilaire Park ²	6
	<i>Desmodium incanum</i> (Sw.) DC.	Campus UFRGS	9
PLANTAGINACEAE	<i>Angelonia integerrima</i> Spreng.	Morro Santana ¹	3
	<i>Plantago myosuroides</i> Lam.	EEA-UFRGS	6
POACEAE	<i>Anthaenantia lanata</i> (Kunth) Benth.	Sain't Hilaire Park ²	4
	<i>Aristida laevis</i> (Nees) Kunth	Morro Santana ¹ and EEA-UFRGS	4
	<i>Axonopus affinis</i> Chase	EMBRAPA-Bagé	3
	<i>Paspalum regnellii</i> Mez	EMBRAPA-Bagé	7
	<i>Schizachyrium microstachyum</i> (Desv. ex Ham) Rosenberg B.R. Arril. & Izag.	Morro Santana ¹	5

¹Located within the Agronomia campus- UFRGS; Porto Alegre; ²Conservation Units in the Porto Alegre region

7.2 Functional traits

Our predictor variables were a set of regenerative traits, whole-plant and leaf traits. The regenerative traits were seed traits, which consisted in mean values of seed mass and shape per species (see Chapter 1). Both seed traits are thought to influence germination, germination rate, survival, biomass, abundance and population dynamics (Barak et al., 2018; Guo, Brown, Valone, & Kachman, 2000; Huang et al., 2016; Wang et al., 2016). Seed mass (SM, mg) was measured as the dry weight of a thousand seeds, for that all attachments were removed (Pérez-Harguindeguy et al., 2013).

Shape measurements were done using the software ImageJ: seed area (SA, mm²), height (mm), width (mm), and circularity (an index from 0 to 1, where 1 indicates a perfect spherical seed (Rasband, 2000)).

To measure whole-plant and leaf traits we sampled three to six individuals per species, from those of our own experiment. Leaf traits included specific leaf area (SLA, mm² mg⁻¹), leaf area (LA mm²), leaf dry matter content (LDMC, mg mg⁻¹), leaf thickness (TC, mm), and leaf tissue thickness (LTD, mg mm³). All selected leaf traits are related to the leaf economic spectrum and also to the ecological strategies of species (Sandra Díaz et al., 2016; Grime, 1977). They are related to nutrient cycle rates and investment in biomass mediated by light interception and use (Craine et al., 2002; Pérez-Harguindeguy et al., 2013). For measuring leaf traits, three to five fully expanded leaves in the fully lit proportion of the canopy were collected per individual and kept moist until measurements in the lab. The smallest photosynthetic area was used, thus for compound leaves the values correspond to their leaflets. Leaf thickness was determined using a digital calliper on fresh leaves, positioning the calliper between the border and the midrib, and between the tip and base of the leaf (Pérez-Harguindeguy et al., 2013). Following, leaves were fresh weighted and scanned with a portable scanner with 600 dpi. Leaf area was estimated using ImageJ software. In sequence, leaves were dried at 80°C for 48h (or until the stabilization of the weight) and weighted to determine leaf mass (LM, mg). Compound leaves were weighted without rachis. Specific leaf area was calculated as the ratio of leaf area to leaf mass (Pérez-Harguindeguy et al., 2013). Leaf dry matter content was calculated as the ratio of leaf fresh weight to leaf mass. Leaf tissue thickness was calculated as the ratio of leaf mass to leaf volume (mm³), where leaf volume is the product of leaf thickness and leaf area (Craine, Froehle, Tilman, Wedin, & Chapin, 2001). The whole-plant trait included canopy height (CH, cm), which was measured in the field, at the last day of the experiment (in January), as the mean canopy height from three to five individuals of each species. Height is expect to reflect a species competitive ability were taller species earn a competition by accessing light resources, also representing a great proportion of the variation found among species (Sandra Díaz et al., 2016; L. Poorter, 2007).

7.3 Performance variables

Performance variables mainly associated to the early establishment stage were measured during the first 4 months of the experiment under the block design, which were: Growth Rate Index (GRI), Final Germination Percentage (FGP), and survival. Although, survival can be associated with both stages. GRI was calculated as:

$$GRI (\%/day) = \frac{G_1}{1} + \frac{G_2}{2} \dots Gx/x$$

where G1 is the percentage of individuals on the first day after sowing, divided by the number of days, G2 is the percentage in the second day and et cetera (Kader, 2005). The GRI indicates how fast and clustered germination is. FGP was measured as the proportion of germinated individuals (0 to 1). And survival is the proportion of individuals surviving on the last day of the experiment (week 16th), weighted by the total number of sown seeds (range from 0 to 1). Both survival and FGP, result in a numerical effect – a hierarchy based on the number of individuals that reach the vegetative growth stage, likely leading to dominance (Donath & Eckstein, 2010; Turnbull, Rees, & Crawley, 1999).

Performance variables mainly associated to the later establishment stage were measured during the last 6 months of the experiment under a random design, which were: Growth Rate (GR), Above Ground Biomass (AGB) and Bellow Ground Biomass. GR is a proxy for the relative growth rate (RGR), which is related to an acquisitive strategy – efficient conversion of light into increase in biomass–, which is thought to respond to whole-plant and leaf traits (H. Poorter & Remkes, 1990). As we had a small number of individuals per species, we avoided the destructive measurement of RGR (Pérez-Harguindeguy et al., 2013). We used digital photographs (Klassen, G., Frantz, Pinnock, & Bugbee, 2003) in a way it allowed us to couple with both horizontal and vertical spread, given the different life-form of our species. The camera position was fixed throughout the time in relation to each individual. All photos were taken monthly by the same equipment; each photo was 2992 x 2000 pixels and 300 dpi. Photos were then analysed by a fixed digital grid of 1 x 1 cm. The increase in cover

was measured in relation to each individual first measurement. This method allowed estimating the GR by using a linear regression of each individual increase in cover by day (growth/day), which is the regression β . Three to ten individuals were monitored per species, according to the number of available individuals (Tab. 1).

By the end of the experiment, AGB (kg) was measured by clipping all aboveground plant material, including the reproductive parts when present, dried at 80 °C for 48h and weighted. Species which did not include reproductive parts were: *A. integerrima*; *P. regnellii*; *A. lanata*; *A. latifolia*. Following, the BGB (kg) was estimated by collecting the belowground structures by using metallic structures 10 cm deep and 12 cm of diameter positioned on the main shoot, then pressed until 10 cm deep and removed with a shovel. These soil cores passed through a 2 mm sieve to collect all roots attached to the main shoot. Roots were washed and dried at 80 °C for 48h before weight. For AGB and BGB, three individuals per species were sampled. Biomass directly reflect productivity, more productive species are expect to drive important ecosystem processes (S Díaz & Cabido, 2001; McGill, Enquist, Weiher, & Westoby, 2006), and this is also desirable for ecological restoration purposes (Kardol & Wardle, 2010).

7.4 Data Analyses

First, we performed a Pearson's correlation and consider only traits which had correlations below 0.7 to other traits (Fig. S1) (Barak et al., 2018). With regard to regenerative traits, SM and circularity were considered. For whole-plant and leaf traits we maintained LA, LDMC, SLA and CH. With all these functional traits, we performed a principal component analysis (PCA) using the matrix of mean trait values of species, previously centred and standardized. The axes of a PCA can be interpreted as orthogonal variables of the multidimensional space occupied by the studied species, here representing ecological strategies (variations along trait spectrums), where the relative loading of the trait in the eigenvector gives the importance of each trait along the ordination axis. The first three axes were used as predictive variables in the further analysis (see below), as this approach allows

us to use the explanatory power of a set of traits (i.e. each axis represent overall plant strategies) in models with a small sample size.

Then we performed linear regression models for each of our response variables: FGP, GRI, survival (early stage performance), AGB, BGB, and GR (later stage performance), considering mean values of each of the 12 species. In sequence the confidence intervals were plotted in effect size plots. All analyses were performed using R version 3.5.0 (R Core Team 2018).

We further estimated the CSR classification for the studied species, using the LA, SLA and LDMC traits within the StrategyFy tool (Pierce et al. 2017). This tool is a spreadsheet, calibrated with a global data set, where we included our leaf trait values for each species and it estimates the CSR strategies (Table S1 and Figure S2) (Pierce et al., 2017). Following that, we performed Person's correlations between the CSR estimated percentages of each species and the performance variables, as well as the three PCA axes used in the linear regression models. By doing that, we have a worldwide common ground of function comparison, which here might reflect (if associated) the actual performance of species in a field experiment that aim to subsidize ecological restoration of subtropical grasslands.

8. Results

8.2 Species multidimensional variation in terms of functional traits

The first three axes of the PCA are representing the main species variation within their multidimensional trait space, accounting for 83% of total variation (Figure 1). Axis 1 comprises the main spectrum of distinct strategies, varying from plants with lighter elongated seeds that invest in height (CH) and in conservative leaves (higher LDMC), in the left side, to plants that are smaller and have lower LDMC, but with heavier and more rounded seeds (high SM and circularity) (Fig. 1). Axis 2 expresses a variation in LA and SLA, from more acquisitive plants (larger leaf area and higher SLA, on the top of the ordination plot in the Fig. 1a) to more conservative species (small leaves, low SLA and

high LDMC) that also present heavier seeds. Axis 3 is better representing the SLA variation, from plants with higher SLA, but small leaves (on the bottom of the ordination plot in the Fig. 1b) to plants with larger leaves and lower SLA, but higher CH and heavier seeds. Species scores along the first three axes can be seen in Table S2:3.

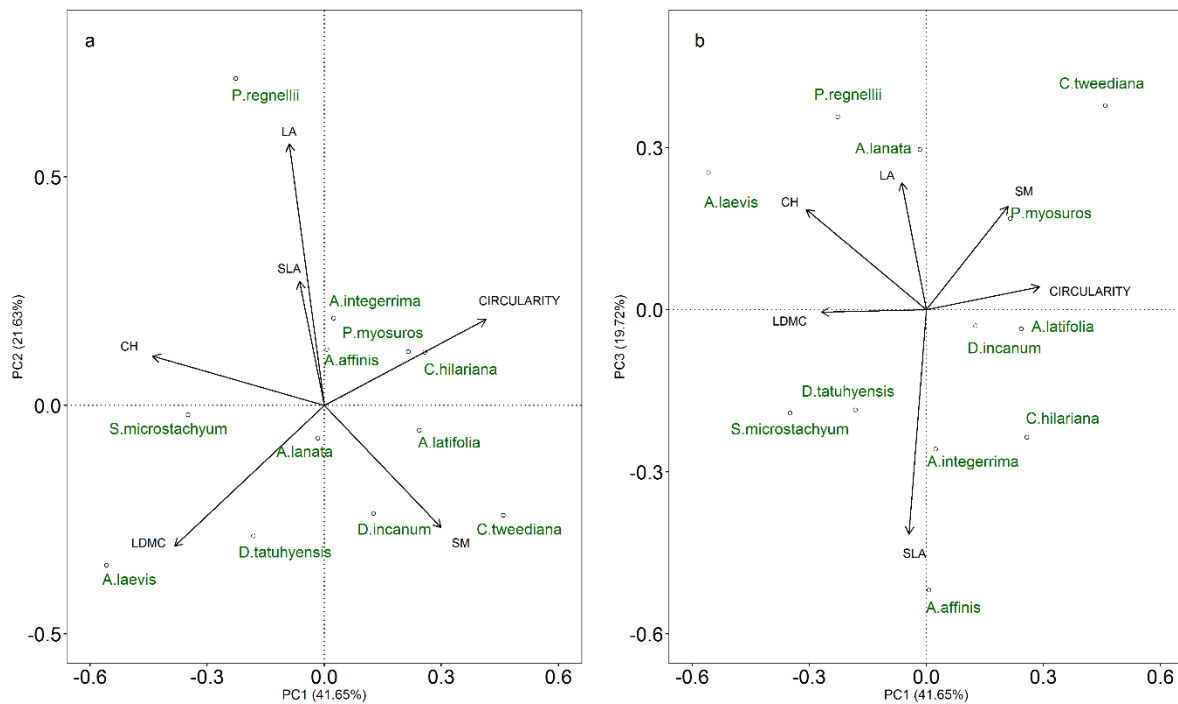


Figure 1 Ordination diagrams of the Principal Component Analysis (PCA) of functional traits variation for 12 herbaceous perennial species from subtropical Brazilian grasslands. In (a) we show PC1 versus PC2, whereas in (b) is PC1 versus PC3. Traits are: LA- leaf area (mm²); LDMC- leaf dry matter content (mg mg⁻¹); CH- canopy height (cm); SM- seed mass (mg); Circularity (0-1 index, 1 is a perfect round seed). Complete species names can be seen in Table 1.

8.3 Determinants of establishment performance

Overall performance parameters can be seen in Table 2. For the early establishment phase, *A. lanata* had the highest FGP and GRI, i.e. germinated more and faster, whereas *A. integerrima* had the lowest FGP and GRI. On the other hand, *A. integerrima* and *P. myosuroides* presented a higher survival performance, as emerged individuals had 100% of establishment success. This contrasted to *P. regnellii* that presented the lowest survival performance (Tab. 2). For the later establishment phase, we highlighted the performance of *P. regnellii* with the highest AGB and had the second fastest GR. The

highest GR was for *A. latifolia*. Regarding to BGB, *D. tatuhyensis* followed by *D. incanum* (both are Fabaceae species) presented the higher values (Tab. 2).

Table 2 Averaged values for the performance parameters of each species. Performance parameters are: Final Germination Percentage (FGP), Germination Rate Index (GRI), Survival, Above Ground Biomass (AGB), Below Ground Biomass (BGB) and Growth Rate (GR).

Species	Early establishment			Later establishment		
	FGP (%)	GRI	Survival (%)	AGB (kg)	BGB (kg)	GR (rate/day)
<i>Adesmia latifolia</i>	9.8	2.329	40.8	0.0318	0.0016	0.5952
<i>Angelonia integerrima</i>	0.8	0.033	100	0.0028	0.0005	0.0172
<i>Anthaenantia lanata</i>	16	2.403	36.8	0.0051	0.0013	0.0617
<i>Aristida laevis</i>	12.8	2.031	45.9	0.0205	0.0006	0.0955
<i>Axonopus affinis</i>	5.4	0.526	4	0.0079	0.001	0.0277
<i>Crotalaria hilariana</i>	8.8	0.68	36.3	0.0555	0.003	0.1164
<i>Crotalaria tweediana</i>	9.4	1.303	62.4	0.0118	0.0026	0.0413
<i>Desmanthus tatuhyensis</i>	2.4	0.341	74.2	0.0033	0.0035	0.0675
<i>Desmodium incanum</i>	8.6	1.436	60.7	0.003	0.0034	0.0534
<i>Paspalum regnellii</i>	3.8	0.494	4	0.0676	0.0032	0.1215
<i>Plantago myosuroides</i>	3.6	0.113	100	0.0184	0.0026	0.023
<i>Schizachyriu microstachyum</i>	3.6	0.359	44	0.0231	0.0009	0.0809

The selected models explaining each performance variable can be seeing in Table S4 and Figures 2 and 3. The only model containing a significative predictor variable was that for the AGB, a later performance stage variable, where axis PC2 had a positive effect on AGB (Fig. 3, Table S4). As the highest loading on the discriminant axis PC2 is LA, we can infer that the highest the LA, higher the above ground biomass. Further correlations with this axis also reveal that species with higher

performance in terms of AGB tend to have lower LDMC and SM, and also higher SLA (Table S3). Other models were not clearly explained by the PCA axes.

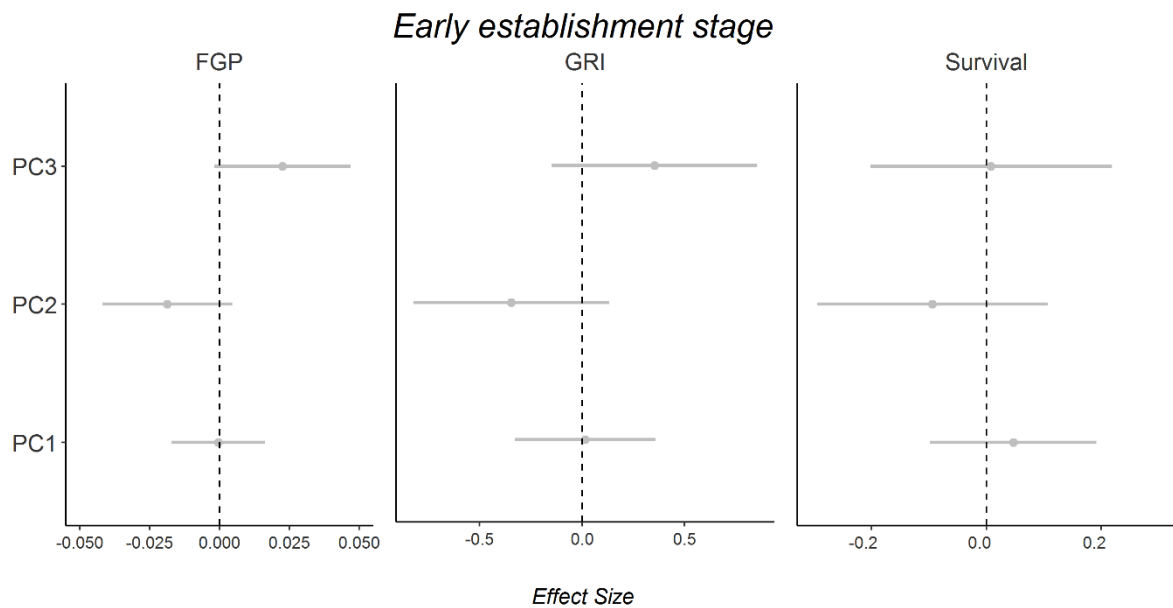


Figure 2 Standard coefficient plots from the final regression models of performance in early establishment stage: FGP (final germination percentage, range from 0 to 1); GRI (germination rate index); Survival (range from 0 to 1). Predictive variables are: Axis PC1, PC2 and PC3 (see Figure 1). Points are averaged coefficient estimates and bars correspond to 95% CIs. Grey circles and bars correspond to effect sizes that overlap with zero (i.e. not significant).

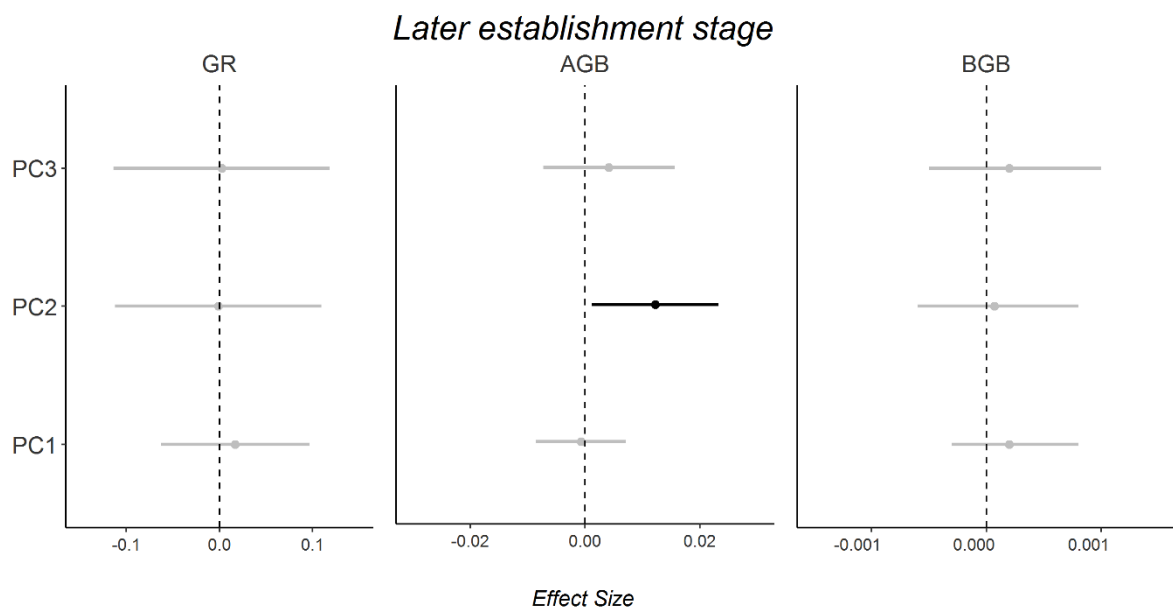


Figure 3 Standard coefficient plots from the final regression models of performance in later establishment stage: GR (growth rate); AGB (above ground biomass, kg); BGB (below ground biomass, kg). Predictive variables are:

Axis PC1, PC2 and PC3 (see Figure 1). Points are averaged coefficient estimates and bars are corresponding to 95% CIs. Black coloured circles and bars correspond to effect sizes that do not overlap with zero, whereas grey circles and bars correspond to effect sizes that overlap with zero. When confidence intervals do not overlap with zero they represent strong and large effects.

The association between the CSR components to each of the performance variables or even to the three PCA axes can be seen in Table 3. Both the components C and S were associated to axis PC2, being positive with C (0.69) and negative with S (-0.68). Component R was strongly associated to axis PC3 (-0.80). Axis PC1 presented low values of correlation for either of the CRS strategies (Tab. 3), as it mostly represents the variation in regenerative traits (Fig. 1). Concerning the performance variables, we highlight the positive correlation between the S-component and the FGP and GRI (that were highly correlated, $r = 0.94$), which are from the early establishment phase. Among the later establishment variables, the highest correlation value was for AGB, and also with the S-component (-0.37).

Table 3-Pearson's correlation for the PCA axes, Competitive-Stress-Tolerance-Ruderal (CSR%) Grime's strategy, later performance variables (GR: growth rate, rate/day; AGB: above ground biomass, kg; BGB: below ground biomass, kg) and early performance variables (FGP: final germination percentage, range from 0 to 1; GRI: growth rate index; Survival, range from 0 to 1).

	C	S	R	GR	AGB	BGB	FGP	GRI	Survival
PC1	0.14	-0.20	0.17	0.17	-0.06	0.37	-0.02	0.03	0.24
PC2	0.69	-0.68	0.33	-0.01	0.66	0.08	-0.46	-0.46	-0.35
PC3	0.00	0.49	-0.80	0.02	0.21	0.22	0.53	0.44	0.03
C	1.00	-0.80	0.17	-0.27	0.28	-0.11	-0.44	-0.49	0.29
S	-0.80	1.00	-0.73	0.00	-0.37	0.13	0.61	0.52	-0.03
R	0.17	-0.73	1.00	0.30	0.29	-0.09	-0.49	-0.30	-0.28

9. Discussion

Here we evaluated how multidimensional variation of species concerning their functional traits explains the performance of 12 perennial species of the subtropical Brazilian grasslands in the first phases of plant development under field conditions. Our results confirmed the expectation that mainly leaf traits variation (PC2) influence plant performance in later stages (i.e. between 4 to 12

months after germination), whereas regenerative traits (seed traits) and whole-plant traits (height) did not. However, we could not confirm mainly regenerative traits dimension as predictors of early establishment performance (i.e. first 4 months after seeding), as initially expected. The first PCA axis (PC1) mostly captured the regenerative traits variation, but also a variation in canopy height (CH) and LDMC, however this multidimensional vector was not able to predict the performance variables in the present study. Additionally, we confirmed that performance parameters fit into a wider ecological strategy within the CSR scheme, facilitating world-wide comparisons. We observed a positive association between S-strategy and early performance parameters, and a positive association between C-strategy and PC2, which explained aboveground biomass (later performance).

9.2 Multidimensional variation of functional traits is a weak predictor of early establishment stages

The only significant model was observed for a parameter of the later establishment stage, the aboveground biomass (AGB), predicted by the PC2. This dimension explained 22% of total trait variation observed among the studied species, and is mainly associated to the size of the leaves (LA), but also to SLA and LDMC, reflecting the leaf economic spectrum (positive scores represent more acquisitive species: high LA and SLA, low LDMC). Axis PC2 presented a positive effect on AGB, a measure that represents the total aboveground growth of the species, related to the individual capacity in transform available resources into biomass, due to its functional structures that appear later in their development (Donohue, 2013; Fenner, 2000). The highest loading in this axis was the positive correlation with LA (0.73), whereas the SLA and LDMC had smaller values (0.35 and -0.39, respectively) and the SM had a similar value to LDMC (-0.34). This reflects that fast-growth species are producing more biomass, and might be well adapted for the establishment even under harsh field conditions often observed in the first phases of ecological restoration. Once the individuals have survived, leaf traits dimension is thus a good predictor of the later stage of grassland species establishment. As in our results, high biomass production has often been related to species with high LA and SLA (Sandra Díaz et al., 2016; Mark Westoby et al., 2002; Wright et al., 2004).

The SLA had a secondary role on this positive association to AGB, in comparison to LA, as PC3 was not significantly associated with the performance variables (the greatest loading of PC3 is SLA; -0.75). SLA is thought to be strongly correlated to growth rates in a later establishment stage, which also include studies comprising of seedlings (Campbell & Rochefort, 2003; M Westoby, 1998), being better than LA or LDMC in predicting performance (Pérez-Harguindeguy et al., 2013; H. Poorter & Jong, 1999). However, other studies found that the effect of SLA is dependent on the light incidence and other components of the growth rate; LAR (leaf area ratio) and NAR (net assimilation rate) might better express variation in the growth rate or biomass production of species under field conditions, where light incidence is high (H. Poorter & Remkes, 1990; Ryser & Wahl, 2001).

As already said, the first axis did not correlate with neither of the performance variables related to the early establishment stage. An independent axis having larger loadings of regenerative traits (Pierce et al. 2014) and influencing the early establishment stage was expected as seed traits mostly influence germination velocity and rate (Barak et al., 2018; Cornelissen et al., 2003; Moles & Westoby, 2004), as also observed in our own previous study (Chapter 1). However, here, this first dimension also includes whole-plant and leaf traits (canopy height and LDMC) into the variation between the species. This multiple strategy dimension is not directly influencing plant performance in the very early phase (just after seeding), as interactions effects between regenerative traits may be most relevant in explaining the success of related regeneration processes. In the Chapter 1, we could see that the interaction between seed circularity and SM was relevant for GRI (rounded heavier seeds). Therefore, the use of combined variables of multiple traits (as the use of PCA axis) may not always be an adequate approach to relate biodiversity-ecosystem functions (Rosado, Mattos, & Xavier, 2017). Moreover, given the variation in life forms herbaceous species have, competition for resources may not be well determined by vertical height (as we have assumed by considering the canopy height trait), as for example lateral spread can be linked to success in restoration (fast soil coverage), which turn the canopy height a poor predictor of performance (Clark et al., 2012; Pianka, 1970). Thus, the

influence of canopy height on the PC1 variation may be a barrier for the first axis to exert an effect on early performance.

Belowground biomass (BGB) is one of the components of primary production and for grasslands can be proportionally higher than above ground biomass (Castro & Kauffman, 1998). However, it has been rarely studied in Brazil (Fidelis & Fernanda, 2013; Titlyanova, Romanova, Kosykh, & Mironycheva-Tokareva, 1999). BGB patterns seemed to respond to ecosystem dynamics associated to disturbances, such as fire regimes (Fidelis, Müller, Pillar, & Pfadenhauer, 2006), and grazing (López-Mársico, Altesor, Oyarzabal, Baldassini, & Paruelo, 2015), and can be also linked to leaf traits (Craine et al., 2001; Husáková, Weiner, & Münzbergová, 2018). However, variation on BGB was not explained by neither of the axis. Possibly because belowground structures are complex, compounded by different organs, which can differ in function across ontogenetic stages (Klimešová & Martínková, 2018). Biomass allocation to roots tend to increase with age and, for young plants, above ground biomass may be a better performance parameter at early stages of establishment (Husáková et al., 2018; Niinemets, 2004).

9.3 Species performance correlates with the CSR triangle trade-off

The CSR classification can currently be applied to all species that have measures of three common leaf traits: LA, SLA, and LDMC, allowing broad comparisons on ecological strategies of distinct species and ecosystems (Pierce et al. 2017). To our knowledge, it is the first time this classification is performed to species of the subtropical Brazilian grasslands. Our studied species are classified in a gradient of stress-tolerant to competitive status (Tab. S1 and Fig. S2). However, many also had a considerable perceptual of ruderal strategy (Tab. S1 and Fig. S2). This is consistent with other subtropical and tropical grasslands, including a study performed in a tropical Brazilian grassland (Negreiros, Stradic, Fernandes, & Rennó, 2014; Pierce et al., 2017), which characterized ecosystems where light competition is considered a structural factor of communities, and so the presence of

disturbances that control individuals height, as cattle grazing and fire events, is very determinant of community assembly (Gibson, 2009; Overbeck et al., 2007).

Here we found axis PC2 (which influenced AGB) strongly correlated to the C-S strategy (0.69 to C, and -0.68 to S-component), which confirm that species positively correlated to this axis, representing acquisitive individuals that may be linked to the occurrence (and with a better development) in relative fertile habitats (Grime, 1974, 1977; Pierce et al., 2014). Furthermore, as this axis showed to be a predictor of AGB performance, thus a C-strategy is linked to performance in the later stage establishment. It is consistent with other studies that correlated a C-strategy to the success of restoration in sites after agriculture (Boatman, Jones, Conyers, & Pietravalle, 2011; Pierce et al., 2017; Pywell et al., 2003). We also had positive correlations between S-strategy and GRI and FGP, which are linked to early establishment performance. This strategy has been previously correlated with colonization ability, thus with early establishment stages in restoration (Boatman et al., 2011; Grime, 1977; Pywell et al., 2003). The biotic and abiotic context are relevant on favouring each one of the strategies, and the S-strategy is mainly correlated to stressful and resource-limited environments (Caccianiga, Luzzaro, Pierce, Ceriani, & Cerabolini, 2006). Although our experiment was in a region under mild environmental conditions, the field experiment characteristics were harsh considering the initial establishment phases, as individuals were scattered over a bare soil susceptible to torrential rainfalls and full sunlight. In addition, as expected, our mostly regenerative axis (PC1) did not present high correlations with any of the CSR-strategies. (Pierce et al., 2014) have already concluded that regenerative strategies operate independently of the CSR-scheme.

To conclude, in a restoration context it is important to understand success and failure across life stages by using something as simple as easy measured functional traits to predict performance of native species that should first establish into the new community. Joint leaf traits correspond to wider ecological strategies, linked to the evolutionary process of the species that can be resumed in the CSR scheme. The integration of performance and the CSR-strategies would facilitate comparisons within

worldwide restoration projects, allowing further steps on research and restoration instead of starting from scratch regard to restoration success. Our study was successful on showing that a leaf traits dimension, especially influenced by LA, is able to predict performance in a later establishment stage and it was also correlated to the C-strategy within the Grime's CSR-triangle. Further studies concerning the relationships between functional traits and performance under field conditions, with a great set of species and traits should contribute more to the understanding of the species success in grassland restoration projects.

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Anexo II

Tabelas e gráficos referentes ao material suplementar do capítulo 2.

Figura S1- Gráfico de correlação de Pearson para os potenciais preditores da performance contendo atributos regenerativos (SM: massa de sementes, mg; SA: área da semente, mm²; Height: altura da semente, mm; Width: largura da semente, mm; Circularidade: índice de 0-1, onde 1 são sementes perfeitamente redondas), atributos estruturais e de folha (TC: espessura da folha, mm; LA: área da folha, mm²; SLA: área específica da folha, mm² mg⁻¹; LTD: densidade do tecido foliar, mg mm³; LDMC: conteúdo de matéria seca foliar, mg mg⁻¹; CH: altura da copa, cm).

Figura S2- Triângulo de Grime para as 12 espécies campestres subtropicais estudadas. Representação gráfica das estratégias Competitivo-Estresse-Tolerante-Ruderal (CSR) proposta por Grime (1977).

Tabela S1- Resultado da classificação nas estratégias ecológicas proposta por Grime: Competitivo-Estresse-Tolerante-Ruderal (CSR). Estimativas feitas na ferramenta de análise Stratify para as 12 espécies pertencentes aos campos subtropicais brasileiros- Campos Sulinos.

Tabela S2- Valores estimados pela análise de componentes principais (PCA) para cada das 12 espécies estudadas pertencentes aos campos subtropicais brasileiros- Campos Sulinos. PCA1 explicou 41.65% da variação, PCA2 explicou 21.63% e PCA3 explicou 19.73%.

Tabela S3- Valores estimados pela análise de componentes principais (PCA) para cada dos atributos funcionais. Atributos regenerativos (SM: massa de sementes; Circularidade: índice de 0-1, onde 1 são sementes perfeitamente redondas), atributos estruturais e de folha (LA: área da folha; SLA: área específica da folha; LDMC: conteúdo de matéria seca foliar; CH: altura da copa). PCA1 explicou 41.65% da variação, PCA2 explicou 21.63% e PCA3 explicou 19.73%.

Tabela S4- Coeficientes padronizados dos modelos finais de regressão. Variáveis resposta estão indicadas acima: Performance em estágio inicial: (FGP: porcentagem final de germinação, range from 0 to 1; GRI: índice de velocidade de germinação; proporção de sobreviventes, range from 0 to 1),

performance tardia (GR: taxa de crescimento, taxa/dia; AGB: biomassa aérea,kg; BGB: biomassa subterrânea, kg). Os respectivos modelos para cada um dos experimentos estão representados nas colunas com as correspondentes médias estimadas e intervalos de confiança para cada preditor: Atributos regenerativos (SM: massa de sementes, mg; Circularidade: índice de 0-1, onde 1 são sementes perfeitamente redondas), atributos estruturais e de folha (LA: área da folha, mm²; SLA: área específica da folha, mm² mg⁻¹; LDMC: conteúdo de matéria seca foliar, mg mg⁻¹; CH: altura da copa, cm).

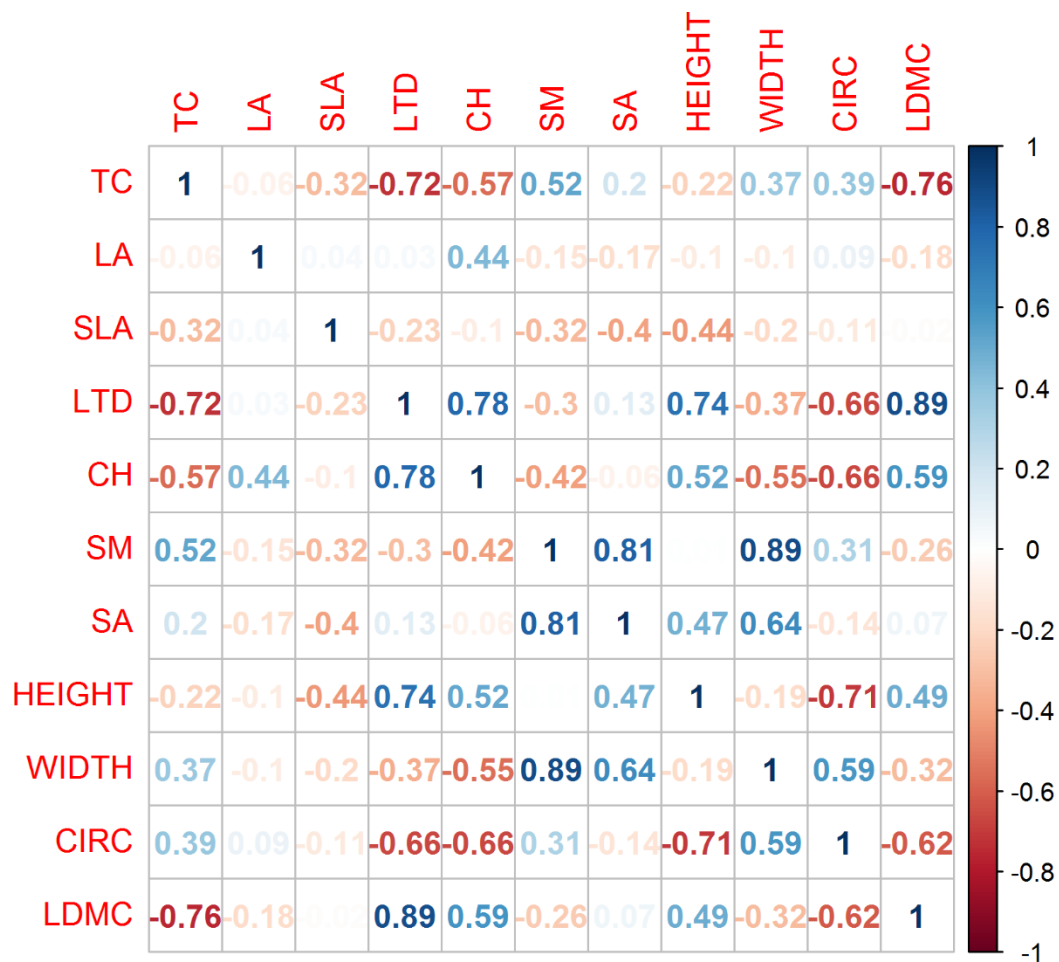


Figure S1- Pearson's correlation chart for potential predictors of performance containing the following regenerative traits (SM: seed mass, mg; SA: seed area, mm²; Height, mm; Width, mm; Circularity: 0-1 index, 1 is a perfect round seed) and whole plant and leaf traits (TC: thickness, mm; LA: leaf area mm²; SLA: specific leaf area, mm² mg⁻¹; LTD: leaf tissue density, mg mm³; LDMC: leaf dry matter content, mg mg⁻¹; CH: canopy height, cm).

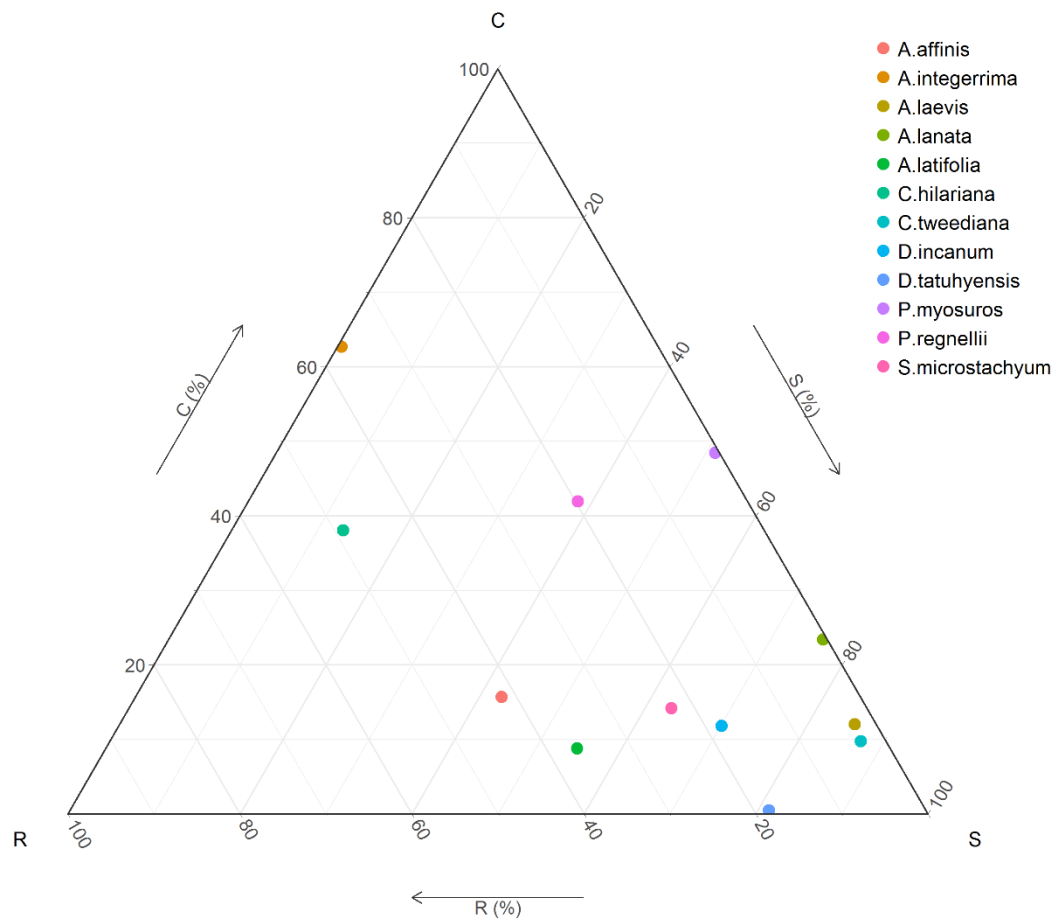


Figure S2- Grime's triangle for the 12 native subtropical grassland species studied. Graphic representation of the Competitive-Stress-Tolerant- Ruderal (CSR) strategies proposed by Grime (1977).

Table S1 Competitive-Stress-Tolerant-Ruderal (CSR) strategy scheme analysis output, estimated by StrateFy for 12 herbaceous perennial native species from subtropical Brazilian grasslands- Campos Sulinos.

Species	C (%)	S (%)	R (%)	C : S : R =	Strategy class
<i>Adesmia latifolia</i>	8.8	54.8	36.4	9 : 55 : 36 %	SR
<i>Angelonia integerrima</i>	63	0	37	63 : 0 : 37 %	C/CR
<i>Anthaenantia lanata</i>	23.5	76.5	0	24 : 76 : 0 %	S/CS
<i>Aristida laevis</i>	12	85.4	2.5	12 : 85 : 3 %	S
<i>Axonopus affinis</i>	15.7	42.6	41.7	16 : 43 : 42 %	SR/CSR
<i>Crotalaria hilariana</i>	38.1	13	49	38 : 13 : 49 %	CR/CSR
<i>Crotalaria tweediana</i>	9.8	87.4	2.9	10 : 87 : 3 %	S

<i>Desmanthus tatuhyensis</i>	0	81.7	18.3	0 : 82 : 18 %	S/SR
<i>Desmodium incanum</i>	11.8	70.1	18.1	12 : 70 : 18 %	S/SR
<i>Paspalum regnellii</i>	41.9	38.3	19.7	42 : 38 : 20 %	CS/CSR
<i>Plantago myosuroides</i>	48.7	51.3	0	49 : 51 : 0 %	CS
<i>Schizachyrium microstachyum</i>	14.2	63.1	22.7	14 : 63 : 23 %	S/CSR

Table S2 Species scores for each axis of the principal component analysis (PCA) of 12 herbaceous perennial native species from subtropical Brazilian grasslands- Campos Sulinos. PC1 explained 41.65% of the variation, PC2 explained 21.63% and PC3 explained 19.73%.

Species	PC1	PC2	PC3
<i>Adesmia latifolia</i>	1.33394	-0.21655	-0.13309
<i>Angelonia integerrima</i>	0.13042	0.74846	-0.9725
<i>Anthaenantia lanata</i>	-0.09112	-0.28007	1.11743
<i>Aristida laevis</i>	-3.05552	-1.37921	0.95947
<i>Axonopus affinis</i>	0.0361	0.47819	-1.95669
<i>Crotalaria hilariana</i>	1.4113	0.45672	-0.89053
<i>Crotalaria tweediana</i>	2.51197	-0.9486	1.42712
<i>Desmanthus tatuhyensis</i>	-0.99399	-1.12983	-0.69717
<i>Desmodium incanum</i>	0.68967	-0.93352	-0.11068
<i>Paspalum regnellii</i>	-1.24336	2.82478	1.34001
<i>Plantago myosuroides</i>	1.18045	0.46293	0.63769
<i>Schizachyrium microstachyum</i>	-1.90987	-0.08329	-0.72105

Table S3 Trait scores for each axis of the principal component analysis (PCA). PCA1 explained 41.65% of the variation, PC2 explained 21.63% and PCA3 explained 19.73%. Traits are: LA- leaf area, mm²; LDMC- leaf dry matter content, mg mg⁻¹; CH- canopy height, cm; SM- seed mass, mg; Circularity (0-1 index, 1 is a perfect round seed).

Functional Traits	PC1	PC2	PC3
SLA	-0.0813	0.3463	-0.7574
LA	-0.1149	0.7299	0.4286
CH	-0.5613	0.1377	0.3389
SM	0.3817	-0.3408	0.3489
Circularity	0.5287	0.2397	0.0771
LDMC	-0.4899	-0.3933	-0.0081

Table S4 Standard coefficients from the final regression models. Response variables are indicated above: Early establishment performance (FGP: Final Germination Percentage, range from 0 to 1; GRI: Germination Rate Index; Survival, range from 0 to 1), later establishment performance (GR: Growth Rate, rate/day; AGB: Above Ground Biomass, kg; BGB: Below Ground Biomass, kg). Significant results, estimated intervals which do not overlap zero, are in bold face.

Predictors	Averaged estimates	95% CI
Response Variable: FGP		
Intercept	0.0670	(0.0416, 0.0924)
PC1	-0.0005	(-0.0173, 0.0163)
PC2	-0.0187	(-0.0420, 0.0046)
PC3	0.0226	(-0.0019, 0.0470)
Response Variable: GRI		
Intercept	1.0040	(0.4833, 1.5248)
PC1	0.0154	(-0.3286, 0.3594)
PC2	-0.3460	(-0.8234, 0.1315)
PC3	0.3532	(-0.1469, 0.8533)
Response Variable: Survival		
Intercept	0.5076	(0.2882, 0.7270)
PC1	0.0470	(-0.0980, 0.1919)
PC2	-0.0945	(-0.2956, 0.1066)
PC3	0.0079	(-0.2027, 0.2186)
Response Variable: GR		
Intercept	-0.0124	(-0.0124, 0.2293)
PC1	0.01670	(-0.0631, 0.0966)
PC2	-0.0012	(-0.1120, 0.1097)
PC3	0.0024	(-0.1137, 0.1185)
Response Variable: AGB		
Intercept	0.0208	(0.0089, 0.0328)
PC1	-0.0007	(-0.0086, 0.0071)
PC2	0.0122	(0.0012, 0.0232)
PC3	0.0041	(-0.0073, 0.0156)
Response Variable: BGB		
Intercept	0.0020	(0.0012, 0.0028)
PC1	0.0002	(-0.0003, 0.0008)
PC2	0.00007	(-0.0006, 0.0008)
PC3	0.0002	(-0.0005, 0.0010)

Considerações finais

A ecologia funcional abrange aspectos associados a processos evolutivos adaptativos das espécies a certas condições bióticas e abióticas. Inferências nos permitem prever como melhor manejar espécies tanto no presente quanto no futuro, frente a mudanças no ambiente local e global. Existem infinitas combinações pelas quais podemos acertar ou errar quanto à possibilidade de estabelecimento de um conjunto de espécies. No entanto, a ecologia funcional nos permite sintetizar boa parte dessa informação e reduzir nosso espaço para erros. Esta é uma disciplina da ecologia que vem derrubando barreiras ligadas aos nossos limites geográficos e sistemáticos. Para os ecossistemas campestres do Brasil, frente aos desafios presentes e futuros, a ecologia funcional é um trampolim para o avanço da ciência da restauração. Este estudo demonstrou empiricamente as implicações do uso de diferentes desenhos experimentais e a possibilidade de uso de atributos funcionais de plantas na predição da performance de espécies nativas dos Campos Sulinos, assim como avançou quanto a associação da performance às estratégias ecológicas amplamente utilizadas em práticas de restauração.

O capítulo 1 explorou as fases iniciais do estabelecimento das espécies, sendo considerada uma importante barreira para restabelecer comunidades a partir da restauração ativa por semeadura. Esse estágio inicial tem recebido pouca atenção e, portanto, nosso objetivo também foi ampliar o estudo de possíveis atributos preditores de performance, afim de compreender quais características permitiriam o sucesso dessa fase. Para tal, três experimentos realizados em condições distintas (Germinador, Casa de vegetação e Campo) foram conduzidos para responder se atributos de sementes seriam preditores do estabelecimento inicial das espécies. Os preditores foram um conjunto de atributos regenerativos relacionados à semente: massa de sementes e forma da semente (circularidade e área). As variáveis resposta foram parâmetros de performance: porcentagem final de germinação, índice de velocidade de germinação e proporção de sobreviventes. Concluímos que as condições experimentais afetam a performance e supõem distintas barreiras, portanto a experimentação em condições de campo é recomendada para um melhor entendimento de como

ocorre a influência dos atributos na performance das espécies mediante condições sob as quais elas evoluíram. Encontramos que atributos regenerativos são preditores da performance nesse estágio inicial. Circularidade e uma interação entre circularidade e massa de sementes afetam a velocidade de germinação em campo. Como outros atributos regenerativos, além da massa de sementes, são raramente incluídos na pesquisa em ecologia funcional, nossos resultados indicam que a inclusão de mais atributos é desejável para entendermos o estabelecimento das espécies e a subsequente montagem de comunidades no processo de restauração ecológica.

No capítulo 2 expandimos a análise para incluir estágios tardios do estabelecimento inicial das espécies, assim como atributos foliares e estruturais pertinentes a esta fase. Neste capítulo, apenas a performance em campo foi considerada, onde as espécies foram monitoradas por mais seis meses, considerando o desenvolvimento de indivíduos. Os parâmetros de performance incluídos para caracterizar essa fase (*later performance*) foram: Taxa de crescimento; Biomassa aérea; Biomassa subterrânea. Além dos parâmetros de performance inicial (*early performance*): porcentagem final de germinação, índice de velocidade de germinação, e proporção de sobreviventes. Os preditores foram atributos regenerativos, foliares e estruturais: massa de semente; circularidade da semente; área da folha (LA), área específica da folha (SLA), conteúdo de matéria seca (LDMC), altura da copa (CH). O objetivo foi avaliar o poder de predição dos atributos funcionais, conjugados em eixos de ordenação (dimensões multifuncionais), em diferentes fases do desenvolvimento, a fim de se aprimorar o direcionamento de ações durante o manejo e monitoramento da restauração. Além disso, objetivou-se relacionar a performance com as estratégias que compõem o triângulo de Grime (CSR), a fim de prover um cenário comum para comparação dos determinantes de performance em restauração em distintas espécies e ecossistemas. A LA demonstrou ser um preditor da biomassa aérea (efeito positivo), sendo também positivamente correlacionada com o componente C das estratégias CSR (competitividade). Os atributos regenerativos estiveram associados a uma dimensão de atributos (a de maior explicação da variação total), que também incluiu CH e LDMC, porém esta dimensão não foi significativa para a predição da performance nos estágios iniciais de estabelecimento, conforme

esperado. Da mesma maneira, os atributos regenerativos não se mostraram correlacionados com os componentes do triângulo de Grime (CSR). As variáveis de performance GRI e FGP correlacionaram com o componente S das estratégias CSR, mas não estiveram associadas a preditores da performance. Concluimos que a dimensão de atributos foliares, especialmente representada pela variação em LA, prediz adequadamente a performance de espécies campestres durante o estabelecimento tardio, sendo também correlacionada com as estratégias ecológicas propostas por Grime (CSR). Estes resultados expandem o potencial de predição e comparação da performance de espécies em ecologia da restauração.

Considera-se que este foi um estudo de curto prazo em monitoramento e também com um número limitado de espécies, dada sua inovação e, conseqüentemente, desafios que transcorreram. Outros aspectos a serem observados foi a baixa germinação das espécies utilizadas e o fato que o estudo teve como critério apenas o uso de atributos funcionais de fácil mensuração como preditores da performance. Nesse contexto, a maior parte da performance medida permaneceu inexplicada, o que poderia nos levar a novas perguntas e outras abordagens complementares. Com isso, esse estudo proporciona uma base para continuar com o desenvolvimento de pesquisas voltadas a restauração de áreas campestres, incentivando o uso de mais espécies, de distintos atributos funcionais relacionados às diferentes fases de estabelecimento e investindo em monitoramentos de longa duração. Ainda assim, e considerando os aspectos acima apresentados, concluimos que a ecologia funcional é uma ferramenta capaz de estreitar nossa capacidade de prever a performance de espécies para uso em restauração, considerando as distintas barreiras que cada etapa de estabelecimento pode apresentar. Esta pesquisa representa um avanço para restauração ecológica regional e internacional. Do ponto de vista regional, esse é o primeiro experimento multi-espécies, com espécies campestres nativas dos Campos Sulinos, em que são medidos parâmetros de performance e a relação com seus atributos funcionais que podem ser usados em restauração. Assim como é primeira vez que há o enquadramento dessas espécies no triângulo de Grime (CSR), expandindo o entendimento das correlações evolutivas ecológicas das espécies em uma linguagem de

amplo entendimento na ecologia. Além disso, esse trabalho contribui para o estreitamento da correlação entre atributos funcionais e o *fitness* das espécies, ainda pouco explorado empiricamente, sendo esta relação uma das bases da ecologia funcional. Igualmente, contribui no entendimento dos atributos regenerativos e seu papel para predizer a performance das espécies durante os estágios iniciais de estabelecimento e, portanto da contribuição dos mesmos para a montagem de comunidades.