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Tese de Doutorado

Suscetibilidade de comunidades campestres à invasão por plantas exóticas invasoras

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“ Me gusta sentarme en la tierra porque sé que estoy firme y sentir la naturaleza en mí. Palparla con mis manos y sentirme cerca de ella para poder olerla ”

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Resumo

O objetivo geral desta tese foi examinar diferentes questões associadas ao processo de invasão de plantas para investigar os mecanismos, impactos e medidas de recuperação da comunidade vegetal, abordando diferentes estratégias metodológicas que incluem estudos observacionais, experimentos de remoção e uma revisão metodológica. Os resultados do **Capítulo 1** mostraram como as relações entre condições climáticas e estrutura da paisagem podem determinar o grau de invasão de plantas na escala regional. Observou-se que os padrões de invasão dos campos sulinos estão principalmente relacionados com maior densidade de estradas, menor cobertura de campo nativo e com o aumento do déficit hídrico. Além disso, constatou-se que a gramínea *Eragrostis plana* é a planta invasora mais abundante dos campos sulinos. No entanto, os resultados do experimento do **Capítulo 2** mostraram que sua invasão não pode ser explicada pela riqueza de espécies ou composição dos grupos funcionais de plantas da comunidade residente. A invasão de *E. plana* foi principalmente associada ao distúrbio causado pela remoção de biomassa na comunidade. Neste sentido, as comunidades campestres poderiam ser resistentes à invasão de *E. plana* até que algum distúrbio aumente sua vulnerabilidade. Por outro lado, através da comparação entre comunidades invadidas, removidas e não-invadidas, os resultados do experimento do **Capítulo 3** contribuíram no entendimento do impacto real da invasão de *E. plana,* em termos de redução da riqueza e cobertura de plantas nativas na comunidade. No entanto, embora os métodos de remoção utilizados reduziram a cobertura da invasora, não foram suficientes para conseguir sua erradicação local. Além disso, após três anos de remoção de *E. plana,* as

comunidades se tornaram distintas às invadidas mas não foram semelhantes às comunidades não invadias, o qual poderia indicar que outras medidas de restauração são ainda necessárias. Embora os experimentos de remoção de espécies sejam úteis para investigar questões associadas ao processo de invasão, existem limitações importantes a considerar, como foi evidenciado nos **Capítulos 2** e **3**. Neste sentido, oferecemos a revisão bibliográfica sistemática do **Capítulo 4,** onde se discute o potencial dos métodos de remoção utilizados para estudar a resistência e a recuperação da comunidade à invasão, apontando algumas limitações. Como resultado, o **Capítulo 4** mostrou que a maioria dos trabalhos não propõem controles adequados nos experimentos, o que pode dar lugar a confundimento de efeitos. Desta forma, foram desenvolvidas algumas sugestões para serem consideradas nos experimentos de remoção de espécies, com o objetivo de continuar avançando nesta temática. As informações geradas nesta tese podem contribuir para o entendimento do processo de invasão de plantas nos ecossistemas campestres, com vistas ao manejo, à conservação e à restauração das comunidades invadidas, adquirindo um senso crítico no planejamento de desenhos experimentas.

Palavras-chaves: ecossistemas campestres; *Eragrostis plana*; experimentos de remoção; invasibilidade; impacto; plantas invasoras.

Abstract

The general aim of this thesis was to investigate different issues associated with plant invasion process to understand the mechanisms, impacts and community recovery, by employing different methodological strategies such as observational studies, removal experiments and a literature review. The results from **Chapter 1** showed how the interactions between climate and landscape structure can determine the level of invasion of South Brazilian grasslands, highlighting that invasion is mainly related to high road density, less native grassland cover and increased aridity. Furthermore, it was confirmed that *Eragrostis plana* is the most important invasive species in the South Brazilian grasslands. However, the results from the experiment of **Chapter 2** showed that its invasion could not be explained by the species richness or functional group composition in the community. *Eragrostis plana* invasion was associated with the disturbance effect caused by the amount of removed biomass. Thus, grassland communities may be resistant to *E. plana* invasion until some disturbance increases their vulnerability. Moreover, by comparing invaded, removed and non-invaded communities, the experimental results of **Chapter 3** highlighted the ecological impact of *E. plana* invasion, in terms of richness reduction and native species cover. However, although removals methods reduced the cover of the invasive species, they were not enough to locally extinct it. Moreover, after three years of the invasive removal, communities became different from invaded ones but not resembling non-invaded references, which suggest that community recovery may require restoration strategies. Although removal experiments have been useful to investigate certain issues associated with invasion process, there

are important limitations to consider, as was shown in **Chapter 2** and **3**. For this purpose, our systematic review presented in **Chapter 4** discussed the potential of removal methods for assessing community resistance and recovery from invasion. There, we showed that most of the studies did not use adequate controls in removal experiments, which can lead to confounding effects. Thus, we developed suggestions to be considered in experimental designs to advance the methodological technique of removals. The information generated in this thesis can contribute to the understanding of plant invasion process in South Brazilian grasslands and, consequently, aid to management, conservation and restoration of invaded communities by acquiring a critical sense in experimental designs.

Key words: grassland ecosystems; *Eragrostis plana*; removal experiments; invasibility; impact; invasive plants.

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Introdução geral

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Processo de invasão em plantas

O processo de invasão começa com a introdução de uma espécie exótica pela ação humana, seja esta intencional ou acidental, num ambiente natural (ver Figura 1; Richardson et al. 2000; Richardson & Pysek 2006). Uma vez introduzida, a espécie pode se estabelecer e se propagar, gerando assim populações de plantas adultas capazes de se reproduzir e se dispersar em áreas distantes ao local de introdução (Richardson et al. 2000; Richardson & Pysek 2006; Figura 1a). Desta forma, as plantas introduzidas devem transpor barreiras geográficas, barreiras bióticas (e.g. de dispersão e reprodução) e abióticas (e.g. do ambiente), para invadir ambientes naturais (Richardson et al. 2000; Richardson & Pysek 2006), o que, frequentemente está associado a determinados impactos ecológicos. Assim, a compreensão das diferentes etapas do processo de invasão requer tanto conhecimento sobre os atributos da planta invasora, quanto características do ambiente e da comunidade residente (Richardson & Pysek 2006).

Conceito de invasibilidade

O conceito de invasibilidade descreve a suscetibilidade de um ambiente à colonização e ao estabelecimento de plantas exóticas invasoras em uma comunidade natural (Richardson 2000; Davis 2005). Configura uma propriedade emergente de um determinado ambiente, e, portanto, a invasibilidade constitui uma condição variável no tempo e no espaço (Davis et al. 2005). Muitos esforços têm sido realizados para determinar o papel da resistência de comunidades à invasão, desde o ponto de vista biótico e abiótico, para assim identificar que tipo de ambientes e quais comunidades seriam mais suscetíveis a serem invadidos (Lonsdale 1999; Davis et al. 2000). Porém, alguns trabalhos têm mostrado resultados contrastantes (Fridley et al. 2007), indicando que ainda não existem mecanismos universais que expliquem diferenças em invasibilidade.

Mecanismos de invasão

Os principais mecanismos e processos associados à introdução e ao estabelecimento das espécies exóticas invasoras são diversos. A riqueza e a identidade das espécies da comunidade têm sido propostas como os principais fatores de resistência biótica (Elton 1958; Dukes 2002). Neste sentido, alguns trabalhos têm manipulado os componentes da comunidade nativa para avaliar o efeito das mudanças na composição e/ou riqueza de espécies no sucesso de invasão (Figura 1b; Guido & Pillar 2015). No entanto, diversos trabalhos têm demostrado que a invasibilidade é determinada por vários fatores, envolvendo agentes locais e regionais. Neste contexto, além da composição e riqueza da comunidade, as condições climáticas (e.g. Hellmann et al. 2007), estrutura da paisagem (e.g. With 2002), regime de perturbação (e.g. Burke & Grime 1996) e disponibilidade de recursos (Davis et al 2000) têm sido importantes para determinar a invasibilidade. Neste sentido, a compreensão do processo de invasão requer preferencialmente diferentes perspectivas e abordagens complementares (Lonsdale 1999; Foxcroft et al. 2011). Desta forma, esta teste propõe tanto uma abordagem regional e local, quanto estudos observacionais e experimentais para manipular alguns fatores, com vistas a potencializar o entendimento do processo.

Impacto da invasão

A invasão de habitats naturais por espécies exóticas é uma das principais ameaças à biodiversidade no mundo (Vitousek et al. 1996, Mack et al. 2000), por frequentemente causar mudanças na composição, estrutura e funcionamento dos ecossistemas (Hejda et al. 2009; Pysek et al. 2012). Porém, muitas vezes o impacto de uma determinada espécie é desconhecido, o que faz com que o problema e medidas preventivas sejam negligenciados. Neste sentido, avaliar os impactos é um passo chave para a conscientização do problema. Na maioria dos estudos que avaliam o impacto de uma planta invasora na comunidade, o desenho amostral se baseia geralmente em estudos observacionais que comparam áreas invadidas e não invadidas (e.g. Davis & Svejcar 2008; Hejda et al. 2009). Embora alguns têm trazido resultados interessantes, as comparações não permitem estabelecer relações de causais, devido a que possíveis efeitos de diferenças entre as comunidades pré-invasão não podem ser avaliados. Desta forma, uma compreensão abrangente do impacto de espécies invasoras deveria, preferencialmente, contemplar estudos que integrem abordagens observacionais e experimentais, assim, comparando comunidades invadidas, comunidades onde a espécie invasora foi experimentalmente removida e comunidades não invadidas (Andreu & Vilà 2011; Guido & Pillar 2015). Deste modo, é possível saber qual é a resposta da comunidade à invasão e à remoção da espécie exótica invasora, inferindo seu impacto real (Flory & Clay 2009; Guido & Pillar 2015; ver Figura 1c).

Plantas invasoras nos campos do sul do Brasil

Os campos do *Rio da Prata*, os mais importantes de América do Sul localizados no sul do Brasil, leste da Argentina e Uruguai (Soriano 1992; Overbeck et al. 2007), têm sofrido perturbações associadas a mudanças do uso da terra, manejo pecuário inadequado, e adição de nutrientes, o que possivelmente aumenta a vulnerabilidade à invasão. Porém, existem grandes variações no grau de invasão de plantas entre estas regiões fitogeográficas (Fonseca et al. 2013). Na região da *Pampa Inundable* na Argentina, as plantas exóticas constituem praticamente 20% da flora (Chaneton et al. 2002; Perelman et al. 2007), enquanto no Uruguai foi encontrado apenas 7% (Bresciano et al. 2014). Estas diferenças no grau de invasão indicam que o processo é causado por múltiplos fatores, sendo então um grande desafio propor abordagens ecológicas que integrem o entendimento dos principais mecanismos e impactos associados. Em particular, nos campos do sul do Brasil, situados na parte subtropical, existem poucos estudos que quantifiquem o grau de invasão destes ecossistemas campestres (Rolim et al. 2014), o que faz com que o problema e as medidas preventivas sejam negligenciadas. Neste contexto foi que surgiu a motivação e o desafio de realizar esta tese de doutorado.

Objetivo geral

A partir de uma abordagem experimental e observacional, considerando escalas regional e local, a tese propõe abordar diferentes etapas do processo de invasão (Figura 1) para entender que tipo de ambientes (**Capítulo 1**) e quais comunidades (**Capítulo 2**) são mais propensas à invasão, e avaliar o impacto de uma das plantas invasoras mais importantes dos campos sulinos (**Capítulo 3**). Além disso, a tese aborda questões metodológicas relacionadas a experimentos de manipulação, onde através de uma revisão sistemática se propõe avaliar o potencial dos experimentos de remoção como ferramentas metodológicas chaves no estudo do processo de invasão (**Capítulo 4**).

Objetivos específicos

- **Capítulo 1**: Analisar a influência das condições climáticas e da estrutura da paisagem como fatores determinantes do grau de invasão dos campos do Rio Grande do Sul.
- **Capítulo 2:** Avaliar o efeito da supressão de grupos funcionais de plantas no processo de invasão (ver Figura 1b).
- **Capítulo 3:** Avaliar o efeito da invasão de uma planta exótica invasora na comunidade vegetal campestre (ver Figura 1c).
- **Capítulo 4**: Discutir o potencial dos experimentos de remoção de espécies como abordagens para estudar o processo de invasão em plantas (ver Figura 1b e c).

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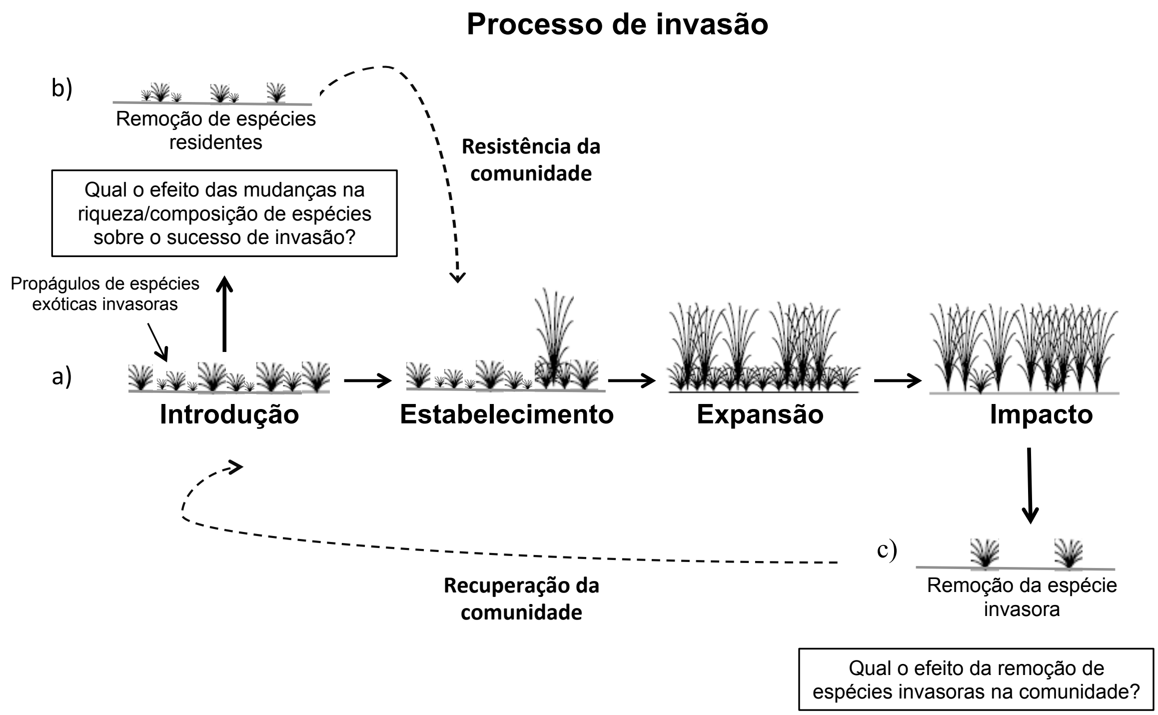

Figura 1. Experimentos de remoção para avaliar diferentes etapas do processo de invasão. (a) Etapas do processo de invasão. (b) Remoção de espécies residentes da comunidade nativa para avaliar resistência biótica à invasão. (c) Remoção da espécie invasora para avaliar o impacto e a recuperação da comunidade.

Capítulo 1

“Plant invasion in South Brazilian grasslands: a landscape approach”

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Chapter 1: Plant invasion in South Brazilian grasslands: a landscape approach

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Abstract

How interactions among climate and habitat structure can determine invasion patters is a key step to understand and predict the level of invasion across landscapes. We evaluated the invasion of the four most important invasive plants of South Brazilian natural grasslands in relation to climate and landscape variables. We selected 20 plots (2 x 2 km each), situated in ten different grassland types in southern Brazil. In each grassland type, one plot was located in a region with remnant grassland cover < 30% (not conserved grasslands) and another in a region with remnant grassland cover >60% (conserved grasslands), in a paired design. In each plot, we surveyed the presence and cover of the four most important invasive species of Rio Grande do Sul grasslands. We used 12 variables related to climate and landscape structure for testing causal models of plant invasion using Akaike's information criterion for path analyses. *Eragrostis plana* was the most important invasive species in the grasslands. The paired sampling design revealed that natural grassland cover in the surrounding landscape affected invasion. We proposed six causal models, which were tested as valid, indicating that road density, natural grassland cover and global aridity index directly influenced invasive species cover. Our results identified the general conditions that might promote successful population growth of the most invasive species in south Brazilian grasslands. We highlighted how interactions among climate, landscape structure and human activity can determine differences in the level of invasion across grassland types.

Key-words: exotic species; invasive species; invasion pattern; invasiveness; fragmentation; land use

Introduction

The level of invasion across landscapes is determined by several drivers, involving local and regional factors, which mainly include propagule pressure, resident community composition, climate conditions, habitat configuration, disturbance regime and resource availability (Lonsdale 1999; Alpert et al. 2000; Davis et al. 2000; Theoharides and Dukes 2007). A wide range of approaches contributes to the understanding and prediction of the occurrence and spread of invasive species in natural communities, including observational studies (Lonsdale 1999; Stohlgren et al. 1999; Stohlgren et al. 2002), experimental approaches (Naeem et al. 2000) and modeling (Peterson and Vieglais 2001; Barbosa et al. 2013). Although many hypotheses have been proposed to explain why some communities are more invaded than others (see Jeschke 2014 and Hierro et al. 2005 for reviews), results from field studies have been controversial and no general theory of community invasibility has yet emerged (Lonsdale 1999; Vila and Ibáñez 2011).

At regional and continental scales, climate has been considered the major driver of invasive species occurrence (Hellmann et al. 2007; Ibáñez et al. 2009), as temperature and precipitation are key factors of resource availability, which limits survival, growth and reproduction of plants (Woodward 1987). Populations of invasive species are considered more likely to survive whenever introduced in areas with climatic conditions similar to their native range. Nonetheless, climate effects on plant invasions are also mediated by the ecological characteristics of different recipient habitats, such as biotic resistance or other abiotic constraints. In regions with low annual precipitation, increased presence of exotic species has been observed in grasslands, rocky ecosystems and habitats influenced by agricultural use; and a lower presence in coastal, scrubland, woodlands and ruderal habitats (Ibáñez et al. 2009). Levels of invasion thus are not constant under a given climate region, but depend also on other factors that are simultaneously operating at the landscape (Ibáñez et al. 2009).

The composition and configuration of habitats at landscape scale play an important role on invasive species establishment and spread (With 2002; Bradley and Mustard 2006; Theoharides and Dukes 2007; Chytry et al. 2008; Vila and Ibáñez 2011). Landscape structure affects population connectivity, and thus dispersal and availability of propagules and species interactions, favouring invasion or reducing the resistance of communities to invasion (With 2002; Vilà and Ibáñez 2011). Land-use changes are usually linked to reduction of native vegetation in terms of total extent and patch size, and also to increased edges between original and modified areas. The surrounding native vegetation may act as a physical barrier for invasive species establishment and spread (Ohlemüller et al. 2006; Vila and Ibáñez 2011). Ohlemüller et al. (2006) found that patch size was the best predictor of invasive species occurrence, suggesting that smaller fragments are more likely to be invaded. Edge effects are more pronounced in smaller and narrower patches, resulting in more open and disturbed vegetation, which facilitates the influx of invasive species (Hobbs 2000; Vila and Ibáñez 2011). Habitat fragmentation may directly affect the population viability of native species and increase the risk of local extinctions, turning these communities more susceptible to invasions (Elton 1958; With 2002; Theoharides and Dukes 2007).

Moreover, there is abundant evidence showing that the level of invasion is also associated to landscapes that are highly altered by humans (Mack and D'Antonio 1998; Levine and D'Antonio 1999; Theoharides and Dukes 2007; Vilà et al. 2007). Human activities constitute accidental or intentional vectors of exotic plant dispersion, and may also directly influence their establishment due to the perturbation of native communities, that might otherwise be resistant to invasion (MacDougall and Turkington 2005). Therefore, increasing levels of human transformation of ecosystems, such as density of urban areas (Cilliers et al. 2008; Bartuszevige et al. 2006), roads and roadsides (Gelbard and Belnap 2003; Vilà and Ibañéz 2011), as well as human population density (Decker et al. 2012), provide opportunities for invasion.

To predict which environments are more susceptible to be invaded, climate and habitat-dependent effects in the landscape should be taken into account simultaneously. This is specially important in heterogeneous regions such as Rio de la Plata grasslands, located in southern Brazil, east of Argentina and Uruguay, in which variations of plant invasion level were reported (Perelman et al. 2007; Fonseca et al. 2013; Bresciano et al. 2014; Rolim et al. 2014). Although South Brazilian grasslands support very high plant biodiversity (more than 2500 vascular plant species; Ilsi Boldrini unpublished data) and are also the main resource for livestock production, an important economic activity of Rio Grande do Sul state, they are not adequately protected under current conservation policies (Overbeck et al. 2007, 2015). Consequently, these grasslands are currently threatened, mainly by changes and intensification of land use, which may also promote invasive species establishment. However, the patterns of plant invasion and the mechanism behind the establishment success are poorly known. Lack of field data is a serious impediment to an evaluation of the risk posed by invasive species for the South Brazilian grasslands region (Rolim et al. 2014).

In this study we evaluated the role of climatic conditions and landscape structure as drivers of plant invasion in grassland communities, based on a regional survey in southern Brazil. Specifically, we asked the following questions:

i) How severely are South Brazilian grasslands invaded by those plants considered to be the most invasive in the region?

ii) Does native grassland extent in the surrounding landscape matrix affect the level of plant invasion?

iii) Which drivers related with climate and landscape structure are better predictors of plant invasion?

Methods

Study area

The study area comprised the native grassland remnants in the state of Rio Grande do Sul (RS), Brazil, which belong to one of largest areas of natural temperate sub-humid grasslands in the world, extending from southern Brazil to Uruguay and east of Argentina (Soriano et al. 1992; Overbeck et al. 2007). These grasslands constitute an ancient vegetation type, but forests have expanded over the past few thousand years due to a more humid and warmer climate since the late Holocene (Behling and Pillar 2007). As a consequence, in addition to grasslands, forest-grassland mosaics, shrublands and forests characterize the vegetation in RS (Teixeira et al. 1986; Leite and Klein 1990). The climate in southern Brazil is characterized as subtropical (Cfa in Köppen classification). The southern half of RS state has both lower annual precipitation (ca. 1200–1600 mm) and mean annual temperature (13–17 ^{0}C), while in the northern half has higher precipitation (ca. 1500–2000 mm) and the mean annual temperature ranges from 16 to 22 ^{0}C (Overbeck et al. 2007). Cattle grazing and fire are the main factors shaping the vegetation, historically and currently, with fire now restricted to the northern half of RS (Overbeck et al. 2007). The whole region has suffered disturbances associated with land use changes, overgrazing, nutrient addition and non-native forage species introduction, increasing the risk of invasive species colonization (see Fonseca et al. 2013 for a review).

Sampling design

To represent the heterogeneity of grassland vegetation types across an area of about 60.000 km2 occupied by grassland remnants in Rio Grande do Sul state, we stratified the whole area into 10 recognized grasslands regional types (Hasenack et al. unpublished; see Fig. 1 and Fig. S1 from Supplemental material 1). Each one of these grassland types was divided into grid cells (8.5 x 8.5 km) and the extent of grassland remnants was calculated using a classification based on Landsat 5 satellite data from 2002 (Cordeiro and Hasenack 2009). The grids were classified into: 1) conserved landscapes, those with more than 60% of grassland remnants and 2) not conserved landscapes, those with less than 30% of remnants. For each grassland type, we randomly selected one grid cell of the conserved and one grid cell of the not conserved class, resulting in 20 grids in a paired design. Within each grid, we set a plot of 2 x 2 km, in which we performed the invasive species survey (Fig. 1).

Invasive species survey

We selected 12 variables including climate and landscape configuration variables (Table 1). The selection of these variables was based on the main factors that might influence the level of invasion across landscapes (With 2002; Ibáñez et al. 2009; Vilà and Ibáñez 2011). Climate variables were obtained from WorldClim database (Hijmans et al. 2005). For each plot we considered annual precipitation, mean annual temperature and the global aridity index. Global aridity index is the ratio between the mean annual precipitation and mean annual evapotranspiration from CGIAR-CSI Global-Aridity database (Zomer et al. 2008), values <0.03 indicate hyper arid environments while values >0.65 indicate humid conditions.

Landscape variables included: mean elevation, percentage of grassland cover, mean grassland patch size, total grassland edge, road density, density of urban areas, distance of the closest city and population density in the municipality (See Table S1 from Supplemental material 5 for variables variation within plots). Mean elevation was the average value of elevation above sea level in the 2 x 2 km plot. Percentage of grassland cover corresponds to the relative area of grasslands within the regional unit (8.5 x 8.5 km) including the 2 x 2 km plot. Mean grassland patch size and the total grassland edge (i.e. summed perimeter of all grassland patches) were measured within the same regional units. Percentage of agriculture cover corresponds to the relative area of agriculture within a regional unit, considering all type of crops and silviculture. Density of urban areas was evaluated considering a distance of 42.5 km around each plot (this radius was the largest minimum distance from a 2 x 2 km plot and any urban area) and the size of urban areas ranged from 0.11 to 915 ha. All these landscape variables were based on land-cover data from Cordeiro and Hasenack (2009). The distance to the closest city was the Euclidean distance from de center of each plot to the edge of the closest city. Road density included paved roads, unpaved roads, railways, urban roads and paths or trails and was estimated from the vector base map of Rio Grande do Sul, 1:50.000 (Hasenack and Weber 2010). Population municipality density data were obtained from IBGE (available at http://www.cidades.ibge.gov.br).

Data analyses

We compared not conserved to conserved landscapes (< 30% and > 60% of native grassland remnants) regarding mean invasive species per transect in each area (n=20) by using analysis of variance with permutation tests (Manly 2007), restricting permutations to paired plots within each grassland type (blocks).

We proposed causal models linking climate, landscape and a combination of both variables to invasive species mean cover and number. We used path analysis for testing the validity of each proposed causal model. Path analysis represents a useful tool to test for an agreement between specific causal hypotheses and empirical data. For that, we adopted the d-separation approach (Shipley 2000) in which for each proposed causal model a set of independent relationships between the variables was defined. Each of these independent relationships involved correlations and partial correlations that were tested by permutation tests (Manly 2007). Each proposed causal model generated a value for a composite probability statistic (Fisher's C statistic; Shipley 2000), which follows the χ2 probability distribution. A valid causal model must present a P-value larger than an acceptable probability threshold; we adopted P>0.1 as was suggested by Pillar et al. (2013). Linear regression models were used to determine the path coefficient and the corresponding probability found by permutation (Manly 2007) for each causal link, and a non-determination coefficient for each response variable ($U = 1 – R^2$). Predictor variables were centered and standardized to unit variance; therefore, the path coefficients were comparable across predictors and models. Among valid models, we selected those that showed significant, or marginally significant, path coefficients ($P<0.05$). Then, we used the AIC model selection method, applied to path analytic models, to select which ones had more support (Shipley 2013). For each proposed model, we calculated the $AICc = C + 2K(n/(n-K-1))$; where C is the Fisher's C statistic, K is the number of maximum-likelihood parameters that are estimated using the empirical data and n the sample size. Given competing models, the one with the smallest AIC value is preferred and the relative support of the different models is based on the differences in the AIC values relative to the preferred model (see Shipley 2013). All data analyses were done with MULTIV software (available at http://ecoqua.ecologia.ufrgs.br), using 10,000 permutations, which were restricted to paired plots within each grassland type.

Results

In most of the survey plots (18 from 20 plots) and in 61% of total transects was record at least one of the four invasive species. *Eragrostis plana, Cynodon dactylon* and *Senecio madagascariensis* were the most important invasive species in terms of frequency and mean cover per transect (Table 2). *Eragrostis plana* was dominant, with records in 43% of the transects and with mean cover of 14% per transect. *Ulex europaeus* was observed in only two transects.

Among transects in which we recorded at least one invasive species, 61% were from not conserved landscapes. Not conserved landscapes (< 30% of grassland cover) had higher invasive species cover than conserved ones (> 60% of grassland cover) (Fig. 2). Besides, plots within not conserved landscapes also showed higher data dispersion (Fig. 2).

We proposed 17 causal models linking climate (model 11; see Fig. S3 from Supplemental material 3), landscape (models 2, 3, 5, 6, 7, 8, 9, 10, 13 and 15; see Fig. 3 and Fig. S3 from Supplemental material 3) and a combination of these variables (models 1, 4, 12, 14, 16 and 17; see Fig. 3 and Fig. S3 from Supplemental material 3) to the level of plant invasion. A total of 16 models were considered valid ($P>0.1$ for C; see Table 3) but only six of them showed all path coefficients significant, or marginally significant ($P<0.05$ for β; see Fig. 3). The cover of natural grassland in the surrounding landscape was the most important landscape variable, appearing in all of the selected causal models. In addition, global aridity index was the only climate variable, among the ones we considered, with a significant effect on the level of invasion. According to ΔAICc values and W, models 1 and 2 were the most plausible hypothesis (Fig. 3 and Table 3).

Model 1 was the most plausible causal model with AICc = 14.61 and W = 0.61 (see Fig. 3 and Table 3). It included both climate and landscape variables (i.e. global aridity index and percentage of natural grassland cover) and total invasive species cover, as a measure of the level of invasion. The model suggests that global aridity index and natural grassland cover in the surrounding landscape affect invasive species cover, both variables with negative significant path coefficients (global aridity index: $\beta=-0.47$, $P=0.04$; grassland cover: $\beta=-0.46$, $P=0.04$). Yet 55% of invasive species cover could not be explained by these two variables ($U=0.55$).

Model 2 was the second most plausible causal model with AICc = 15.58 and W=0.38 (Fig. 3 and Table 3). It included only landscape variables (i.e. road density and natural grassland cover). The model suggests that both density of roads and natural grassland cover affect invasive species cover, with positive significant (β=0.50, P=0.0027) and negative marginally significant (β=-0.41, P=0.069) path coefficients, respectively. Yet 52% of invasive species cover could not be explained by these two landscape variables (U=0.52).

Although models 3, 4, 5 and 6 did not present any weight (W=0; see Table 3), they were also valid (P>0.1 for C) and showed significant path coefficients linking variables. These models add information about the possible causes of grassland cover reduction, being density of urban areas and agriculture cover the main factors (Fig. 3).

Other postulated causal models were also valid (P>0.1 for C) but they did not show significant path coefficients between some or all the proposed variables (P>0.05 for β; see Fig. S3 and Table S3 form Supplemental material 3).

Discussion

Our study is the first contribution that quantified the level of plant invasions in south Brazilian grasslands by using a systematic sampling design, which represented all native grassland remnants in the state of Rio Grande do Sul. Our results identified the general conditions that promote successful population growth of the most invasive species, and confirmed that *E. plana* is the most important invasive species in these grasslands, as was suggested by other authors (Medeiros and Focht 2007; Guido and Guadagnin 2015). Most of the surveyed plots (18 from 20 plots) and transects (61%) showed presence of invasive species, which underlines the level of invasion of south Brazilian grasslands. Moreover, invasion was negative affected by grassland cover in the surrounding landscape. On the basis of climate and landscape structure, we postulated causal models indicating that road density, native grassland cover and aridity, are the main factors that increase invasive species cover across grassland types.

Eragrostis plana is invasive in other regions of the world, with high potential of expansion, mainly in southern of South America (Barbosa et al. 2013). It was accidently introduced in 1957 from South Africa to southern Brazil, and during the 1970s farmers used *E. plana* seeds for cultivated pastures due to its frost resistance. However, it spread and became a problem for grazing livestock production in view of the low forage quality (Reis and Coelho 2000; Medeiros and Focht 2007; Medeiros et al. 2009). In 1979, the sale of seeds and seedlings of the species was banned in Rio Grande do Sul by the Brazilian Ministry of Agriculture (Reis and Coelho 2000). Therefore, in some of the surveyed areas, current records of *E. plana* may be the result of the intentional introduction in past decades, as propagule pressure could not be controlled in our sampling design. More effort is need to separate the actual level of invasion from habitat susceptibility (Chytry et al. 2008). Further, it has been shown before, that invaded areas by *E. plana* are often associated with previous high disturbance, such as along roadsides (Medeiros and Ferreira 2011), past agricultural use and even overgrazing (Focht and Medeiros 2012). *Eragrostis plana* has been rarely observed in well-conserved natural grasslands, what suggests that native communities may be resistant to invasion until some human perturbation takes place.

It is well known that land transformation enhances invasion success (Hobbs 2000; With 2002; Vilà and Ibáñez 2011). The sampling paired design and also the proposed casual models revealed that reduced grassland remnant cover in the surrounding landscape might increase the level of invasion of grassland communities. In the past three decades, approximately 25% of the grassland cover in our study region was lost due to the expansion of agricultural activities (Overbeck et al. 2007), and probably this value is currently much higher. The surrounding native grassland vegetation may act as a barrier for invasive species establishment and spread (Ohlemüller et al. 2006; Vilà and Ibáñez 2011). Land transformation promotes biotic change through system disruption that provides the opportunity for biological invasions, and also by bringing new species from different biogeographic regions into contact with altered ecosystems (Hobbs 2000). Understanding the effect of native grassland loss on invasion levels may thus be important for predicting and halting the additional spread of invasive species in southern Brazil grasslands.

Many studies have found a positive association between anthropogenic disturbance features (e.g. city distance, urban area, density of road) in the surrounding landscape and the level of invasion at a site (Gelbard and Belna 2003; Bartuszevige et al. 2006; Cilliers et al. 2008; Decker et al. 2012). Some of the valid causal models that we proposed suggest that density of urban areas and agriculture cover caused a reduction in natural grassland cover, facilitating then the increase of invasive species cover. Moreover, the invasion was also related with the density of roads in the landscape. The anthropogenic activities and development could act as a source of invasive species propagules and a facilitator of its spread (Vilà & Ibañez 2011). Roadsides have long been identified as key pathways of invasion species occurrence in nearby ecosystems, due to alteration of adjacent ecosystem and facilitation of seed dispersal (Tyser and Worley 1992; Parendes and Jones 2000; Gelbard and Belna 2003; Bradley and Mustard 2006; Vilà and Ibáñez 2011; von der Lippe et al. 2013).

Numerous studies had documented that fluctuation in resource availability (e.g. water, light and nutrients) is a key factor for controlling plant community invasion (Davis et al. 2000; Blumenthal 2006). Davis et al. (2000) hypothesized that communities become more susceptible to invasion whenever there is an increase in the amount of unused resources. Therefore, most studies have focused on analysing the role of resource enrichment on plant invasion (e.g. Adair et al. 2008), but only few have aimed to respond how stressful conditions, such as drought periods (e.g. Jimenez et al. 2011), affect community invasibility. The most plausible causal model proposed that lower levels of water availability have a positive effect on invasive species cover, which was the only climate variable with a significant direct effect on the level of invasion. Contrastingly, Alpert et al. (2000) proposed in their review on the role of environmental stress in the spread of non-native plants, suggesting that low water availability results in low invasion degree. This result was also reported in other studies (Milchunas and Lauenroth 1995; Sheley et al. 1997), suggesting that drought tolerance is lower for invasive than native species. However, many of the studies that suggested this negative relationship between invasion and water availability were performed in areas with lower resource levels (e.g. mesic to xeric habitats) than southern Brazil grasslands. Although climate in southern Brazil is characterized mainly as subtropical, with a generally well distributed rainfall regime (Alvares et al. 2013), in the last few decades some prolonged periods of water deficit occurred, mainly in the southwest of Rio Grande do Sul (Leivas et al. 2006; Albuquerque and Mendes 2009). As the vegetation of southern Brazil grasslands may not be naturally resistant to such extreme events, many native species might be adversely affected, which could have promoted invasion success by opening gaps for invasive species. We can thus hypothesize that these water deficit events provide a temporal heterogeneity in resource availability, opening a window for invasive species colonization.

We conclude that the level of plant invasion in South Brazilian grasslands are affected by climate conditions and landscape structure, and especially those variables related with human activity, which can determine differences in plant invasion levels across grassland types. Thus, our results provide general and basic information to identify which factors may promote invasion spread in South Brazilian grasslands. This information could be useful to determinate which areas are already invaded for the most important invasive plants, and thus, should take priority for control and eradication efforts. Finally, more surveyed areas within each grassland type are fundamental to have more information about its invasion patters at local scale.

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Figures and Tables

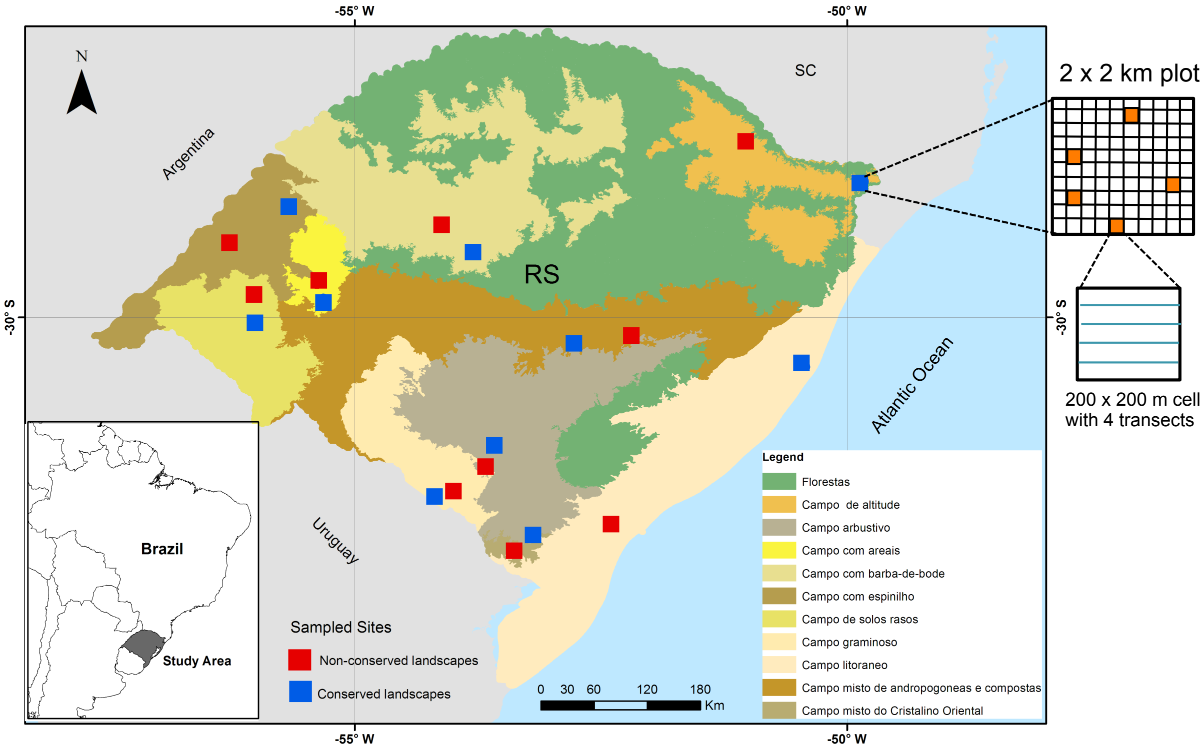

Fig. 1. Sampled plots (n=20) in Rio Grande do Sul state, Brazil. For each grassland type we set two plots (2 x 2 km) in a paired design, one in a conserved landscape (>60% grassland remnant cover) and another in a not conserved landscape (<30% grassland remnant cover). Each plot was divided into 200 x 200 m cells from which five were randomly selected. Inside each cell we sampled four parallel transects (200 m long, 10 m width and 40 m apart). See Fig. 1S in Supplemental material 1. Hasenack H; Weber E; Boldrini I; Trevisan R (unpublished) Mapa de sistemas ecológicos do estado do Rio Grande do Sul.

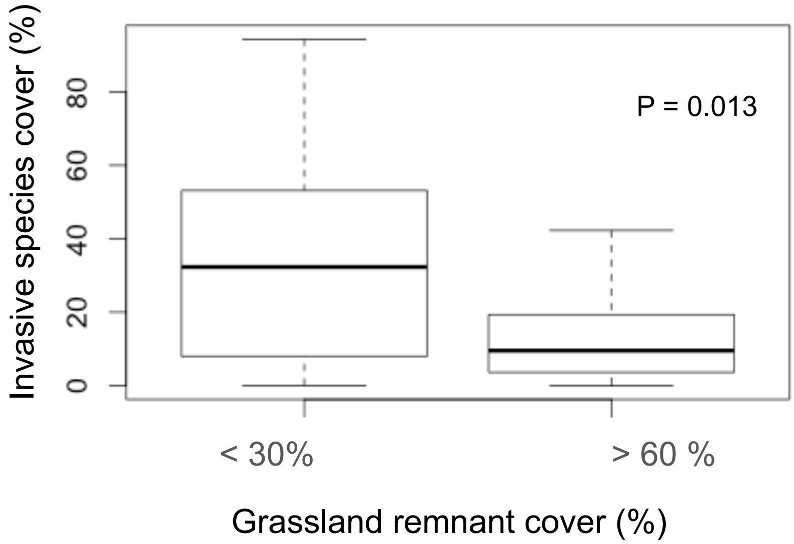

Fig. 2 Mean of invasive species cover (%) for not conserved (<30% of remnants) and conserved (>60% of remnants) native grassland landscapes, based on the four most invasive species in Rio Grande do Sul grasslands (*Eragrostis plana; Cynodon dactylon, Senecio madagascariensis* and *Ulex europaeus*). Results obtained from the 200 m transect (4 transects in each cell x 5 cells in each plot x 20 plots = 400 transects).

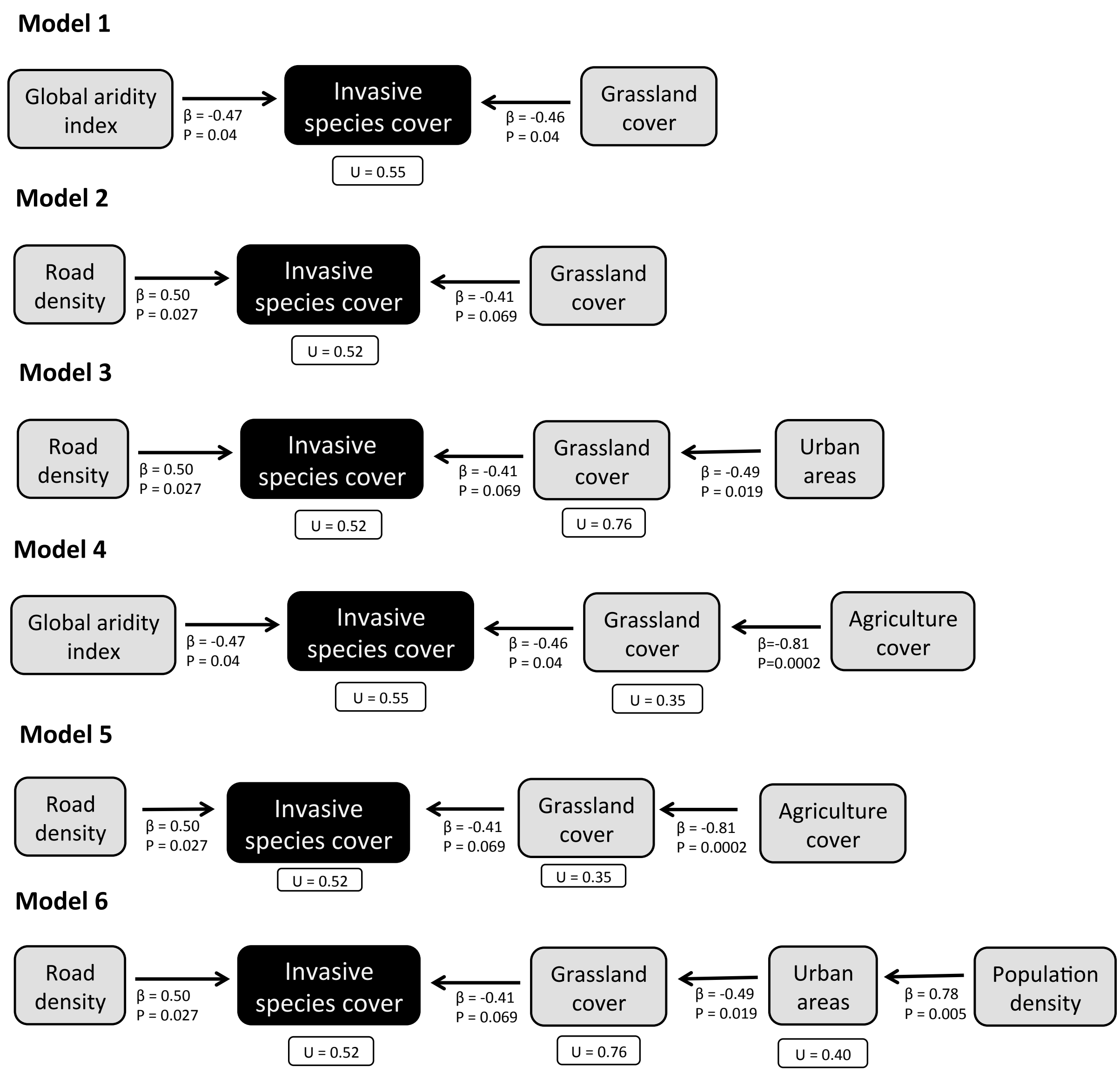

Figure 3. Causal relationships linking climate and landscape to invasibility by different path models (n=20). The postulated independent relationships were tested by correlations and partial correlations (Shipley 2000). U is the non-determination coefficient of the response variable. Path coefficients (β) are indicated, which are regression coefficients using predictor and response variables that were previously centered and standardized to unit variance.

Table 1. Climate and landscape variables included in data analyses.

Variables	Scale	Unit
Climate		
Annual precipitation	2 x 2 km	mm
Mean annual temperature	2 x 2 km	^oC
Global aridity index	2 x 2 km	
Landscape		
Grassland cover	8.5 x 8.5 km	%
Mean grassland patch size	8.5 x 8.5 km	ha
Total grassland edge	8.5 x 8.5 km	m
Agriculture cover	8.5 x 8.5 km	%
Urban areas density	42.5 km radius	number of areas
Mean elevation	2 x 2 km	m
Road density	2 x 2 km	km/km^2
City distance	_	km
Population density	municipality	$inhabitants/km^2$

Table 2. Frequency (%) and mean cover per transect (%) with its standard deviation (±) for each invasive species. Results obtained from the 200 m transect (4 transects in each cell x 5 cells in each plot x 20 plots = 400 transects).

Invasive species	**Frequency**	**Mean cover**
Eragrostis plana	43	14.20 ± 24.76
Cynodon dactylon	24	7.81 ± 17.26
Senecio madagascariensis	16	3.43 ± 10.32
Ulex europaeus	1	0.06 ± 0.86

Table 3. Model fit of six competing path models that are represented in Fig. 3 The C (df, P) gives the Fisher's C statistic and, in parentheses, its degrees of freedom (df) and the null probability (P). K is the number of parameters needed to fit the model. AICc and Δ AICc are the Akaike values and the difference in AICc relative to model 1, respectively. W gives the model weights.

Model	**C**	**df**	**P**	**K**	**AICc**	**ΔAICc**	**W**
1	0.33	2	0.85	5	14.62	0.00	0.61
2	1.30	2	0.52	5	15.58	0.97	0.38
3	2.76	6	0.84	7	26.09	11.47	0.00
4	4.35	6	0.63	7	27.68	13.07	0.00
5	4.72	6	0.58	7	28.05	13.43	0.00
6	5.31	12	0.95	9	41.31	26.70	0.00

Supplemental material 1

Plant invasion in South Brazilian grasslands: a landscape approach, authored by Anaclara Guido*, Eduardo Vélez-Martin, Gerhard E. Overbeck & Valério D. Pillar.

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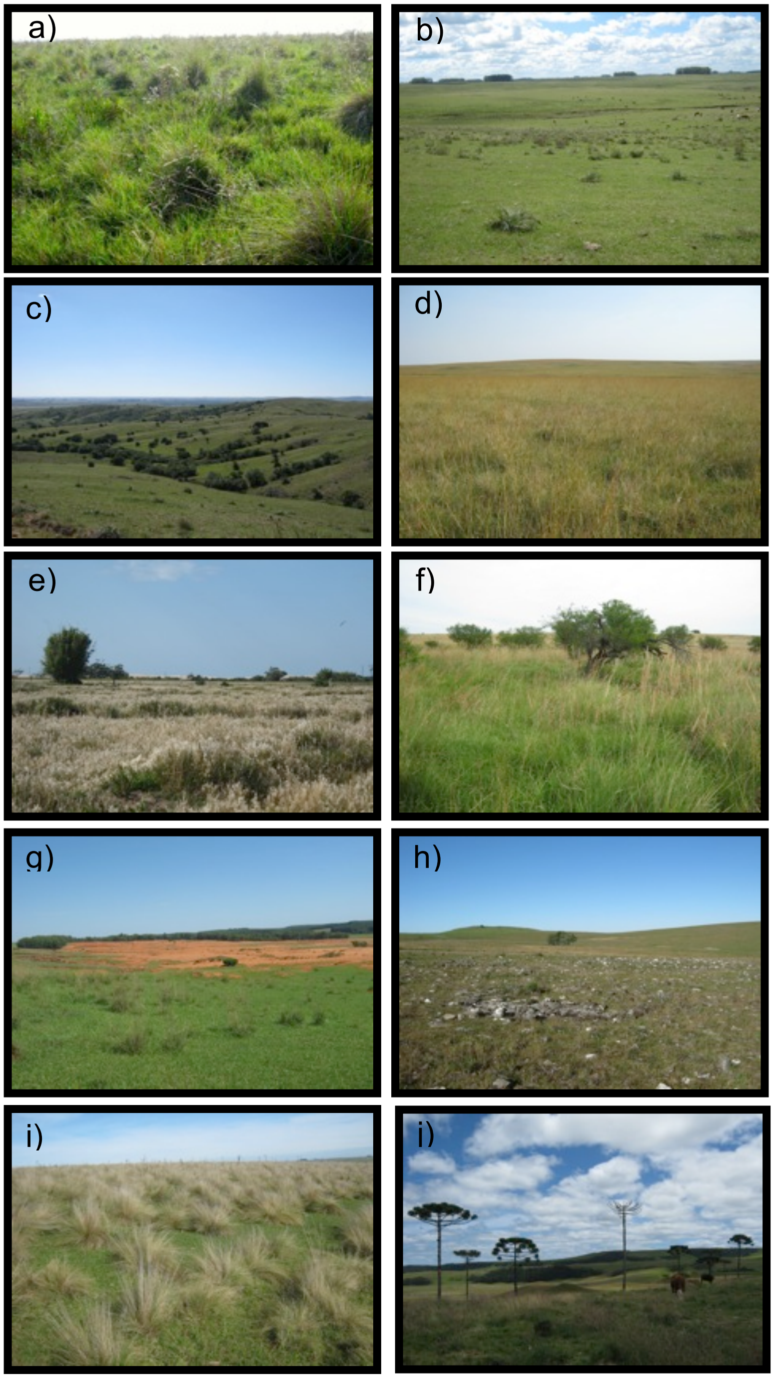

Figure 1S. Physiognomy of each grassland type (n=10); a) campos mistos de andropogôneas e compostas; b) campos graminosos; c) campos arbustivos; d) campos de solos rasos; e) campos litorâneos; f) campos com espinilho; g) campos com areais; h) campos mistos do cristalino oriental; i) campos de barba de bode; j) campos de altitude. From a) to i) are in Pampa biome and j) in Atlantic forest biome. Photos: Anaclara Guido.

Supplemental material 2

Plant invasion in South Brazilian grasslands: a landscape approach, authored by Anaclara Guido*, Eduardo Vélez-Martin, Gerhard E. Overbeck & Valério D. Pillar.

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Eragrostis plana *("capim annoni"; "South African lovegrass")*

Perennial grass from South Africa, accidentally introduced in the Rio Grande do Sul state in the 1950s with a mixture of commercial seeds. It forms dense fiber tussocks with flat base. It flowers in summer, producing high quantity of longevous seeds. The environments most susceptible to invasion are open areas with high disturbance, especially with over-grazing and high compaction soils.

Cynodon dactylon *("bermuda grass")*

Perennial grass from Africa and Europe, intentionally introduced in South America by the British in the early twentieth century to prevent erosion in railways. It creeps along the ground forming a dense mat with a high rate of vegetative reproduction. Its inflorescence is formed by 2-7 spikes, usually violet color. The environments most susceptible to invasion are areas with high human disturbance, especially urban areas.

Senecio madagascariensis *("senecio")*

Annual or biannual subshrub from South Africa and Madagascar, accidentally introduced in the 1950s mixed with others natural materials. It has alternate leaves usually toothed on the edge and a showy yellow inflorescence (daisy flowers). It is considered toxic to livestock because of the high alkaloids concentration in its flowers. The environments most susceptible to invasion are areas with high human disturbance, such as roadsides.

Ulex europaeus *("tojo"; "gorse")*

Perennial shrub from Europe, intentionally introduced in the 1990s as an ornamental plant, mainly for use as a hedge. It has 1-3 m height, its leaves are reduced to thorns and its showy yellow flowers result in 1.5-2 cm pilose legumes. The environments most susceptible to invasion are areas with high human disturbance, such as roadsides.

Figure 2S. Invasive species considered in the study: *Eragrostis plana* (Poaceae), *Cynodon dactylon* (Poaceae), *Senecio madagascariensis* (Asteraceae) and *Ulex europaeus* (Fabaceae). Photos: Anaclara Guido.

Supplemental material 3

Plant invasion in South Brazilian grasslands: a landscape approach, authored by Anaclara Guido*, Eduardo Vélez-Martin, Gerhard E. Overbeck & Valério D. Pillar.

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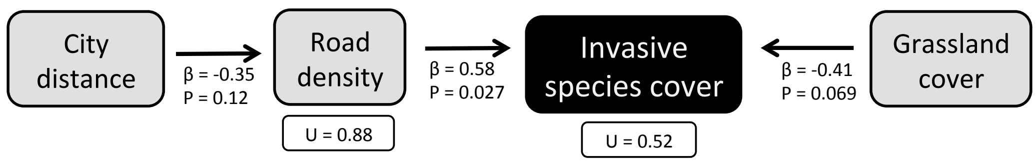
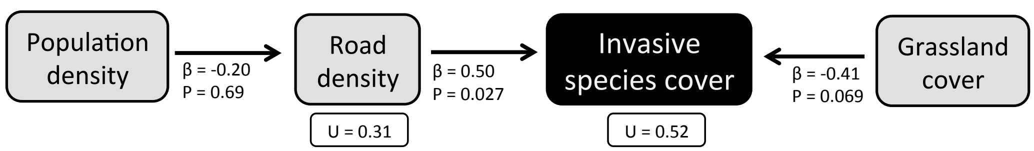
Model 7



Model 8



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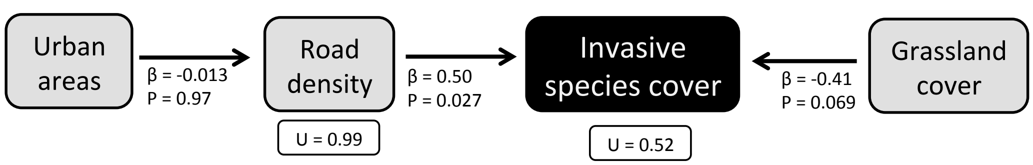

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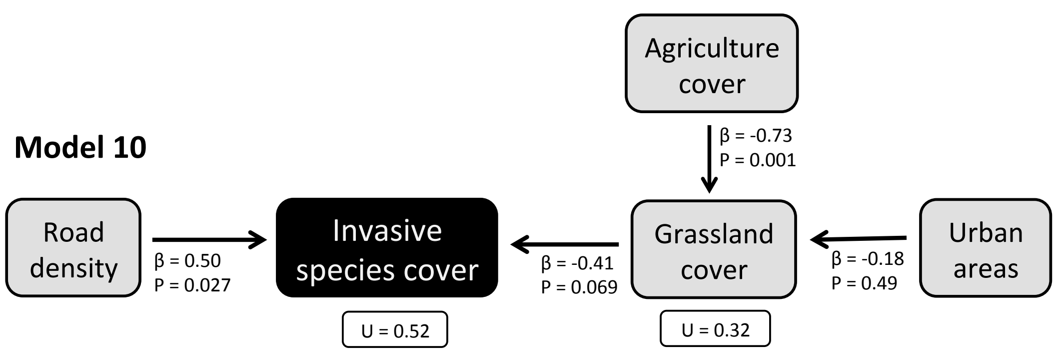
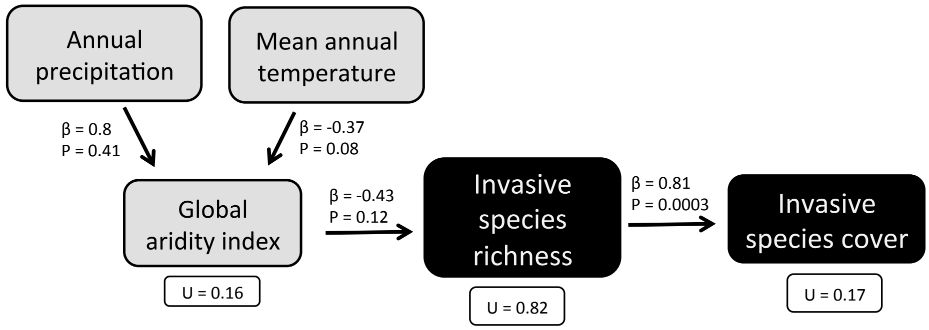


Model 11



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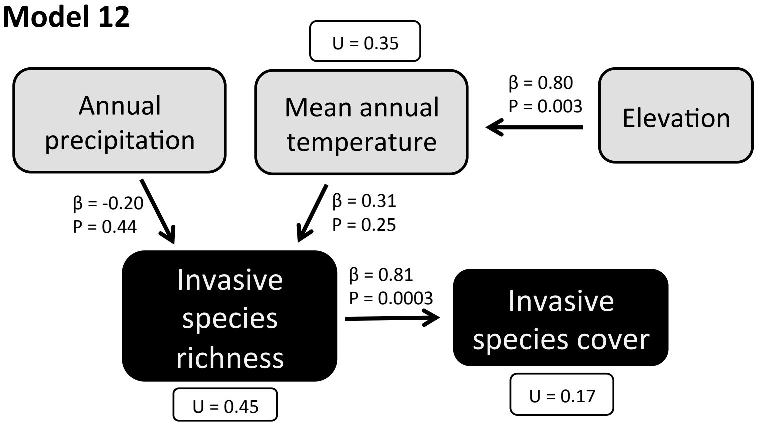
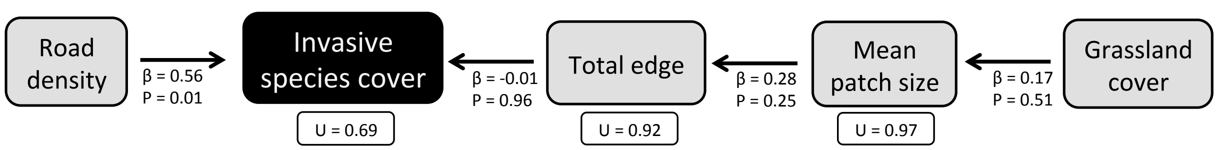
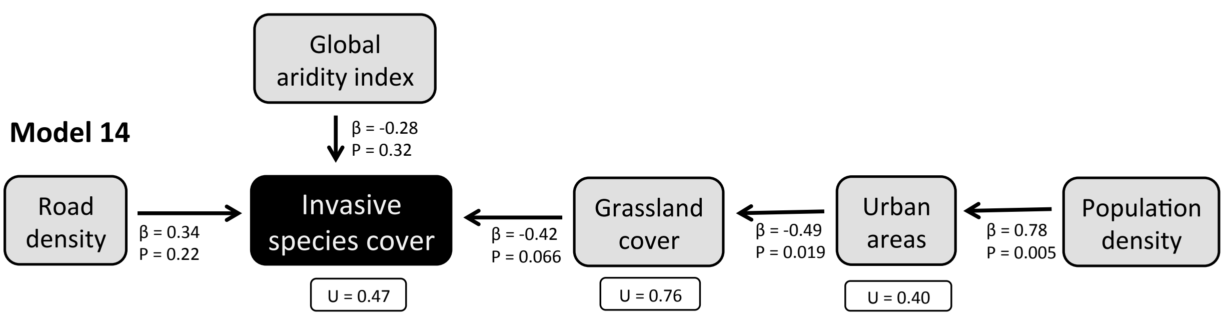


Model 13



Model 14



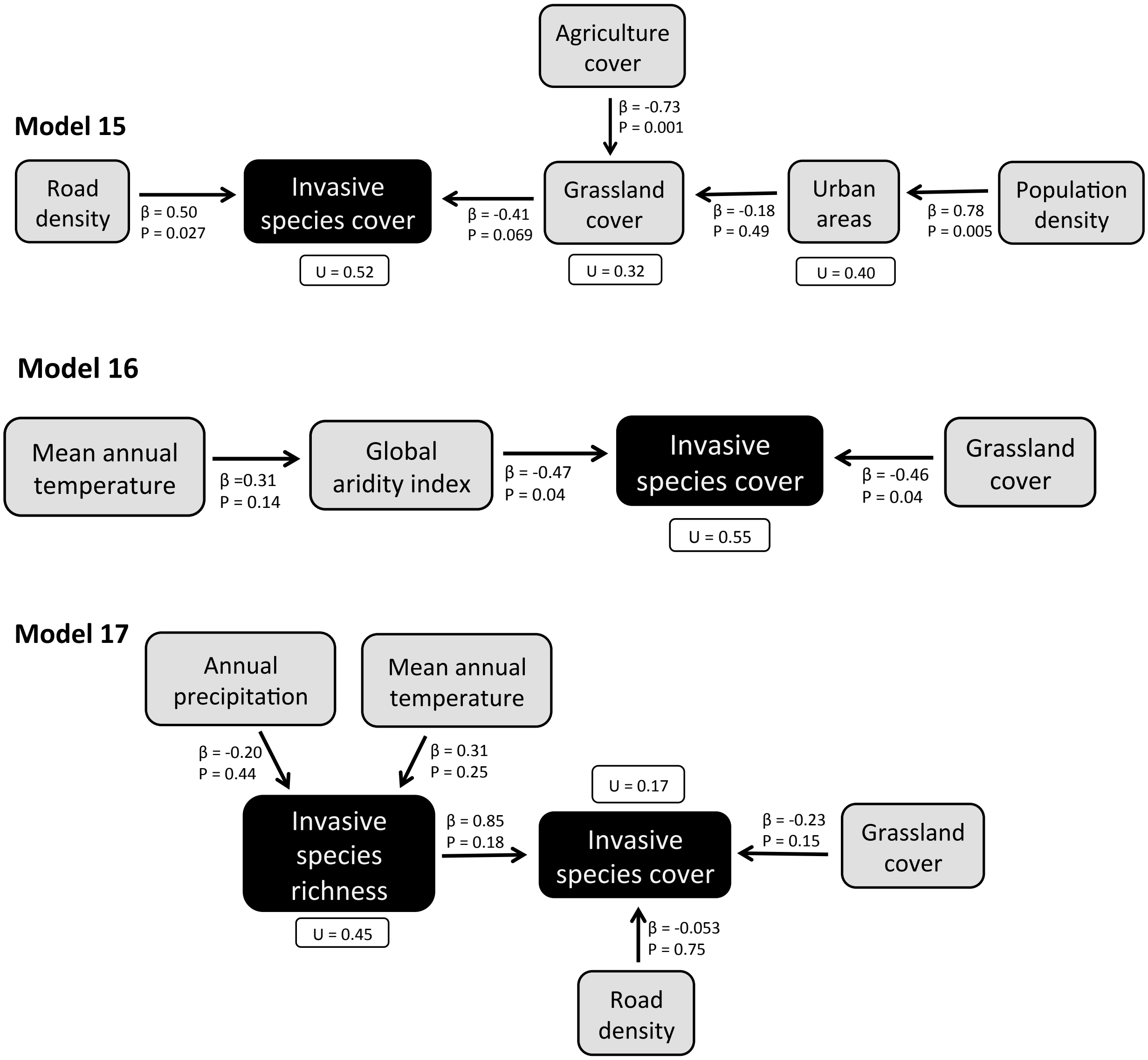


Figure 3S. Causal relationships linking climate and landscape to invasibility through different path models that were not considered to have enough support (n=20). The postulated independent relationships were tested by correlations and partial correlations (Shipley 2000). U is the non-determination coefficient of the response variable. Path coefficients (β) are indicated, which are regression coefficients using predictor and response variables that were previously centered and standardized to unit variance.

Table S3. Model fit of 11 path models that are represented in Fig. S3 and were not considered to have enough support. The C (df, P) gives the Fisher's C statistic and, in parentheses, its degrees of freedom (df) and the null probability (P). K is the number of parameters needed to fit the model. AICc and ΔAICc are the Akaike values and the difference in AICc relative to model 1. W gives the model weights.

Model	**C**	**df**	**P**	**K**	**AICc**	**ΔAICc**	**W**
7	2.09	6	0.91	7	25.42	10.80	0.00
8	5.63	6	0.46	7	28.97	14.35	0.00
9	6.05	6	0.41	7	29.38	14.76	0.00
10	9.85	6	0.13	7	33.18	18.57	0.00
11	0.39	12	0.39	10	44.83	30.22	0.00
12	13.17	12	0.36	9	49.17	34.56	0.00
13	13.79	12	0.31	9	49.79	35.17	0.00
14	17.12	12	0.14	9	53.12	38.50	0.00
15	12.39	20	0.9	11	67.39	52.78	0.00
16	14.69	20	0.79	11	69.69	55.07	0.00
17	35.31	20	0.018	11	90.31	75.70	0.00

Supplemental material 4

Plant invasion in South Brazilian grasslands: a landscape approach, authored by

Anaclara Guido*, Eduardo Vélez-Martin, Gerhard E. Overbeck & Valério D. Pillar.

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Table S4. Cover (%) and mean number of each invasive species per transect in each grassland plot. Data about municipality location, grassland regional type and landscape grassland cover class is provided (CMM: campos mistos de andropogôneas e compostas; CGR: campos graminosos; CAR: campos arbustivos; CSR: campos de solos rasos; CLI: campos litorâneos; CCE: campos com espinilho; CCA: campos com areais; CMC: campos mistos do cristalino oriental; CBB: campos de barba de bode; CAL: campos de altitude). Results obtained from the 200 m transects (4 transects in each cell x 5 cells in each plot x 20 plots = 400 transects).

Municipality	**Type**	**Class**	**Invasive species cover**				**Total cover**	**Mean species number**
			E. plana	*C. dactylon*	*S. madagascariensis*	*U. europaeus*		
Rio Pardo	CMM	<30%	87	3.60	5.40	0	96	1.75
Aceguá	CGR	<30%	6.15	48.25	12.25	0	66.65	1.70
Candiota	CAR	<30%	30.80	4.95	27.05	0	62.80	1.90
Alegrete	CSR	<30%	48.25	7.30	0	0	55.55	1.40
Cachoeira do Sul	CMM	>60%	25.75	7.30	9.75	0	42.80	1.90
Rio Grande	CLI	<30%	2.40	38.95	0.60	0	41.95	1.1
Aceguá	CGR	>60%	0	21	6.05	0	27.05	0.90
Itaqui	CCE	<30%	22.05	0.60	0	0	22.65	0.95
Alegrete	CSR	>60%	17.88	1.41	0	0	19.29	1.10
Alegrete	CCA	<30%	17.42	1.71	0	0	19.13	0.90
Alegrete	CCA	>60%	10.85	0	0	0	10.85	0.80
Palmares do Sul	CLI	>60%	0.60	9.80	0	0	10.40	0.45
Arroio Grande	CMC	>60%	3.70	3.70	0	1.20	8.60	0.30
Tupanceritã	CBB	<30%	6.15	1.80	0	0	7.95	0.35
Pinheiro Machado	CAR	>60%	0	0	6.05	0	6.05	0.40
Júlio de Castilhos	CBB	>60%	3.60	0	0	0	3.60	0.30
Jaguarão	CMC	<30%	0	3.05	0	0	3.05	0.15
São Borja	CCE	>60%	3	0	0	0	3	0.25
S. J. dos Ausentes	CAL	>60%	0	0	0	0	0	0
Vacaria	CAL	<30%	0	0	0	0	0	0

Capítulo 2

"Does functional group suppression affect community invasibility?"

Anaclara Guido
Cristiane Jurinitz
Valério D. Pillar

Chapter 2: Does functional group suppression affect community invasibility?

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This manuscript will be submitted to *Natureza e Conservação.*

Abstract

The relationship between invasibility and the different components of community diversity is central for understanding how invasion processes start and for identifying the principal mechanisms of biotic resistance. The aim of this study is to evaluate the role of plant functional group suppression on community invasibility. We report the results of a removal experiment in natural grassland of southern Brazil to evaluate the effect of repeated clipping of aboveground biomass of target functional group(s) on *Eragrostis plana* invasion. The experiment consisted in a randomized block design (n=18) with two-factors and eight treatments. One factor consisted in four removal treatments (forb removal, graminoid removal, total removal and no removal as a control), and the other was *E. plana* seed addition (with, without). We controlled for non-target effects that varied between treatments (i.e. manipulation disturbance and differences in community richness) using statistics tools. For that, we used generalized linear mixed-effects models (response variable: number of *E. plana* tillers) to assess the relative effects of factors (removal treatments, community richness and removal biomass). We used Akaike's Information Criterion to select a model that could better predict community invasibility. Our results indicated that community composition, in terms of functional groups, and species richness are insufficient to help understanding community resistance to *E. plana* invasion. Community invasibility was mostly explained by the disturbance effect caused by the amount of removed biomass. We suggested that grassland communities may be resistant to *E. plana* invasion until some disturbance occurs.

Key words: Biotic resistance; *Eragrostis plana;* Functional diversity; Grassland; Invasion; Removal experiments

Introduction

Biotic resistance has been postulated to be a mechanism through which resident community can reduce invasion success (see Levine *et. al.* 2004 for a review). Species richness and functional group diversity and composition can limit the susceptibility of a plant community to invasion success, acting as barriers by altering levels of limiting resources (Elton 1958). In particular, species richness has long been hypothesized to be negatively related to invasion, mostly because resources are used more efficiently in richer communities, not being available for the colonization of potentially invasive species (Elton 1958; Davis *et. al.* 2000). Hence, invader success has been shown to decrease across richness gradients, both in natural communities surveys (Perelman *et. al.* 2007) and in manipulated experiments (Naeem *et. al.* 2000). Nonetheless, some studies have shown contrasting results between community richness and invasion, suggesting also positive (Stohlgren *et. al.* 2003) and even no relationship (Lavorel *et. al.* 1999).

As species could be redundant in their functions, to consider only species richness may be insufficient to explain invasion resistance (Diaz & Cabido 2001). Increased plant functional group diversity has been presumed to result in greater niche occupation and consequently, in more efficient total resource utilization, mechanisms which may reduce invasion success (Symstad & Tilman 2001). Moreover, some evidences suggest that functional similarity between invaders and native communities reduces invasion success (Byun *et al.* 2013). This is mainly based on the principle of limiting similarity, which proposes that species should be functionally different to coexist (MacArthur & Levins 1967). Hence, functional group similarities between invasive species and resident species in plant communities are good indicators of biotic resistance to invasion, since they may indicate overlap in resource utilization (Byun *et al.* 2013).

The results of many studies raise questions about the role of biotic resistance and/or reinforce the need for further experimentation with natural communities to enhance the control of extrinsic factors. In this context, removal experiments offer an effective experimental alternative to the well-known synthetic-assemblage communities (Diaz *et. al.* 2003; Guido *et. al.* 2015). Removal experiments consist in removing some components of the community to assess the effects of non-random local extinctions on community proprieties and ecosystem processes. Considering that scarce studies have evaluated resident species removal to quantify community resistance to invasion, a key factor in early stage of the invasion process (Guido & Pillar 2015), we highlight the importance of experimental approaches for studying invasion ecology in natural communities. For having an adequate interpretation of data in removal experiments, it is important to also evaluate for non-target effects (e.g. manipulation disturbance) using appropriate experimental controls (Guido & Pillar 2015) or statistics tools.

Here we examine the invasion of *Eragrostis plana* ("capim-annoni"), currently the most problematic invasive species in southern Brazilian grasslands (Guido & Guadagnin 2015), considered also invasive in other regions of the world with high potential of expansion (Barbosa *et. al.* 2013). *E. plana* is a perennial grass, which was accidently introduced from South Africa to southern Brazil in 1957 (Appendix 1). As it has low forage quality compared with native species, invaded areas became a problem for cattle production (Medeiros & Focht 2007). Invasion is often associated with high disturbance, such as roadsides (Medeiros & Ferreira 2011) and overgrazing (Focht & Medeiros 2012), and is rarely observed in well-conserved native grasslands. However, the main drivers of *E. plana* invasion success are still unknown. Most experimental studies have focused on an agronomic approach, often involving differences in grazing intensity and/or other factors associated with production activities and invasion success (Bremm *et. al.* 2012; Focht & Medeiros 2012). Information is lacking on the role of resident community richness and functional group composition in preventing *E. plana* invasion, which is linked to the testing of biotic resistance hypotheses.

In this study we evaluate the role of functional group suppression (i.e. forbs or graminoids) on community invasibility. We report the results of a removal experiment in natural grassland vegetation in southern Brazil to evaluate the effect of repeated clipping of aboveground biomass of target functional group(s) on *E. plana* invasion. We control for non-target effects that may vary between treatments (i.e. manipulation disturbance and differences in community richness) using statistics tools. We hypothesized that removing one or both functional groups would increase *E. plana* invasion because more resources would be available for its colonization. We also hypothesized that, according to the limiting similarity hypothesis, removing graminoids would increase community invasibility more than removing forbs.

Methods

The experiment was located in southern Brazil, in a 5-ha natural grassland area at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul

(30°07'10"S, 51°41'06"W, 63 m a.s.l). Vegetation is typical of the biome characterizing the Rio de la Plata grasslands, which extends from southern Brazil to Uruguay and east of Argentina (Soriano *et. al.* 1992). Physiognomically the vegetation of the experimental site was homogeneous, composed essentially of perennial graminoids, forbs and shrubs sparsely arranged. The site has been grazed by cattle, which was maintained at the same grazing intensity during the experiment. Considering ethical concerns of introducing invasive species into non-invaded communities, we chose a grassland area that was already partially invaded by *E. plana*. However, we selected non-invaded patches to install the experimental plots.

Experimental design

Herbaceous community composition was classified in two general functional groups: graminoids and forbs. Graminoids included Poaceae and Cyperaceae families, and forbs the species of other plant families. We employed a randomized block design with 18 blocks of 2 × 2 m, each with eight 0.2 × 0.2 m plots. Complete factorial arrangements of treatments (4 removal treatments × 2 *E. plana* seed addition treatments) were randomly located among the plots, totaling eight treatments. Blocks were at least 3 m apart and were located in grazed patches, i.e. avoiding shrubs and large tussocks. Plots were at least 0.2 m apart and were positioned in such a way to maximize within-block homogeneity and to present about 50% cover of graminoids and 50% of forbs. Removal treatments were: forb removal (FR), graminoid removal (GR), total removal (TR), and no removal as a control (NR) (Appendix 2). *E. plana* seed addition treatments consisted in seeding (700 seeds per plot) and non-seeding the plots, the latter being a control with seeds from the environment.

Vegetation sampling and data collection

Removal was done by monthly clipping aboveground biomass at soil level with plant pruning shears, from November 2012 to April 2013 (six successive monthly clippings). The period of clipping was related to the stabilization of the percentage of the removed biomass for each treatment. After six months of successive clipping, the treatments did not differ in the proportion of removed biomass (see Appendix 3). We chose clipping as removal method to prevent soil disturbance and to avoid using unknown toxic residues by herbicide application. Thus, after the periodically suppression of the target(s) functional group(s), we expect a dominance of the remaining group in that plot (e.g. FR treatment means graminoid dominance). The removed biomass was oven-dried at 70° C during 48-72 hours and weighed.

In May 2012 we collected *E. plana* seeds in an invaded area at the same study site. The seeds were transported to the laboratory, dried naturally and counted. We verified seed viability by planting a known number o seeds in a greenhouse and counting the number of germinated seeds (more than 90% of germination). In April 2013, after the last removal, we seeded half of the plots (four per block). In September 2013 we seeded *E. plana* again in order to guarantee the availability of invasive species seeds. The total number of seeds added was approximately 700 per plot.

In November 2013, vegetation composition was evaluated in order to assess species composition in each plot. We visually estimated species cover adopting the following classes: <1%, 2%, 4%, 10%, 20%, and subsequently in intervals of 10%. We also evaluated the invasion success by counting the number of tillers of *E. plana* (Appendix 4).

Data analyses

Non-seeded plots barely presented *E. plana* seedlings, showing that autochthonous seeds were experimentally controlled. Thus, for data analysis we employed only seeded plots (n=72). To test for statistical effects, we used generalized linear mixed-effects models approach (GLMM) with Poisson distribution, since the response variable was number of *E. plana* tillers (counting data). GLMM are a useful statistical tool, as they combine the proprieties of generalized linear models and linear mixed models, providing a flexible approach for analyzing non-normal data when with random effects (Bolker et al. 2008). We proposed four different models that have the same random factor (blocks) but vary in the fixed predictor variable: 1) Invasion ~ treatment; 2) Invasion ~ total resident species richness; 3) Invasion ~ removed biomass; 4) Invasion ~ block (null model). Thus, besides removal treatments, we evaluated the effect of species richness and manipulation disturbance (i.e. removed biomass) on *E. plana* invasion, as both variables differed between treatments (P=0.0001; P=0.0001, respectively). We conducted a model selection using Akaike's Information Criterion (AIC) to decide which of the proposed models can better predict community invasibility. All analyses were done in R (R Development Core Team 2012), employing the package *'lme4'* for GLMM.

Results

During the experiment, a total of 105 species was recorded, being 82 forbs and 23 graminoids (Appendix 5). Removal treatments did suppress the cover of each target functional group (Figure 1). According to the 2013 survey, the forb removal treatment was dominated by graminoid species (83%) and graminoid removal was dominated by forb species (70%).

The selected model for predicting *E. plana* invasion in community grasslands included the disturbance effect as the fixed explanatory variable (lowest AIC) (Table 1; Figure 2). The model indicated a positive and exponential relationship between the number of *E. plana* tillers and the amount of removed biomass, suggesting that communities in which total removal was done are more susceptible to be invaded (Figure 2).

Discussion

Our results indicate that functional group suppression increased the invasibility of the studied grassland communities mostly due to the disturbance effect produced by the removal of biomass, at least in terms of *E. plana* invasion and in a small spatial scale. Therefore, the invasion of *E. plana* in manipulated communities with different group dominance composition was not explained by the biotic resistance hypothesis. Although information is lacking on which factors influence *E. plana* invasion, the few available studies suggested soil disturbance and management conditions as the main drivers, which indicate that grassland communities may be resistant to *E. plana* invasion until some disturbance takes place.

Our hypotheses on the effect of functional group diversity and composition were not supported by the results. The recruitment of *E. plana* increased with the level of manipulation disturbance measured as the total amount of removal biomass. It is well known that disturbance promotes invasion success by increasing the level of available resources, mainly space and light (Burke & Grime 1996). Moreover, manipulation artifacts in removal experiments were also evidenced by Symstad (2000), in which the effect of functional group richness and composition on invasion resistance was apparently due to an interaction between functional group composition and disturbance effect. Thus, we suggest that differences in functional group richness and composition are insufficient to help understanding why some communities are more resistant to *E. plana* invasion.

Disturbance affecting invasion success seems to be more important in grassland ecosystems, as disturbance regime and intensity (mostly associated with grazing management conditions) vary in short space and time scales, promoting important differences in vegetation structure (Hobbs & Huenneke 1992). In particular, community resistance to *E. plana* invasion in southern Brazil grasslands is more associated with differences in disturbance and not community composition. Studies in Rio Grande do Sul suggested that the grazing regime and soil disturbance are crucial to prevent *E. plana* introduction (Focht & Medeiros 2012). Once established, the invasive dominance increases principally as a result of the selective grazing behavior of cattle, which in the best period for growing (spring), prefer to ingest native species with higher nutritional value rather than *E. plana*. Consequently, *E. plana* grows more increasing its propagule pressure and the chances of new recruitments in the following growing seasons. Therefore, we suggest that grassland communities may be resistant to *E. plana* invasion until some disturbance takes place, especially those including biomass removal.

As invasion success involves many drivers, it is difficult to control and manipulate all the factors in a randomized experiment. However, although we cannot control all manipulation artifacts, it is important to test for some important non-target effects, as we did, to avoid interpret data in a wrong way. Testing for the effect of the removed biomass on community invasibility was crucial to understand our results. However, additional controls should be included in removal experiments to avoid non-target effects (Guido & Pillar 2015), such as the removal of the same amount biomass/richness of resident species without altering functional group richness/composition, in order to separate the effect of manipulation from the effect of treatments.

Acknowledgments

We thank people who helped in fieldwork, Laboratory for Grassland Vegetation of UFRGS for species identification, and Agronomic Experimental Station for logistic support. V. Pillar received funding from CNPq (grant 478742/2012-6), and A. Guido and C.F. Jurinitz a fellowship from CAPES, Brazil.

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Figures and Tables

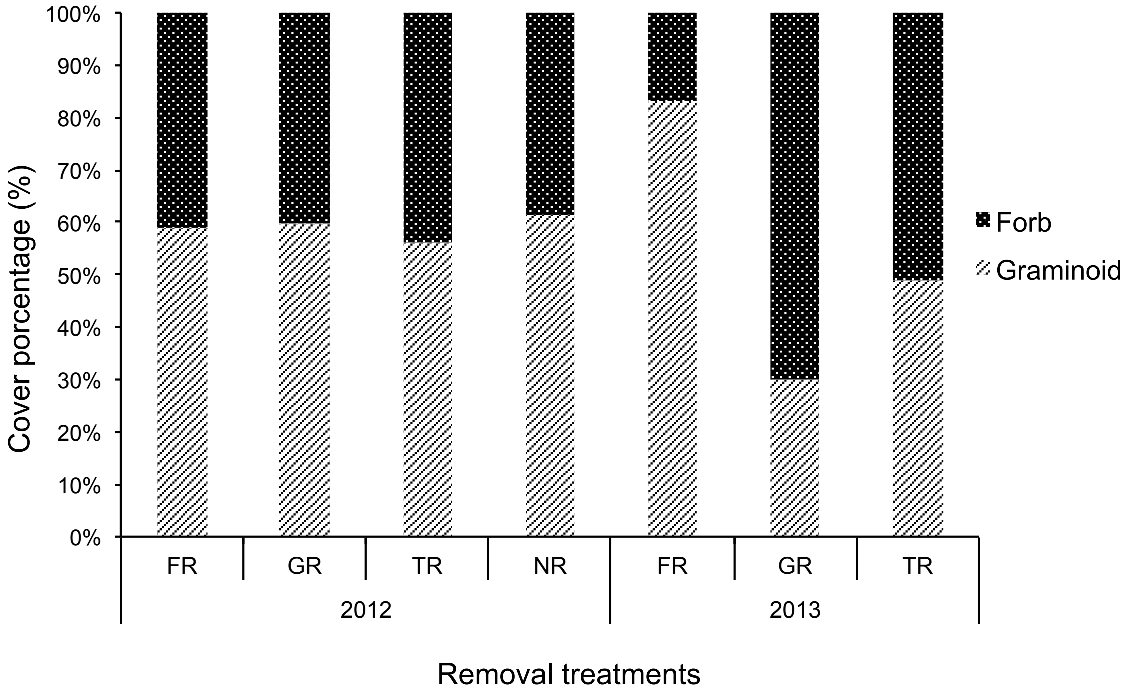


Figure 1. Mean of the proportion of cover (%) for graminoids and forbs in each removal treatment in 2012 (before removals) and 2013 (after removals) per plot (0.2 x 0.2 m; n=18) (FR: forb removal; GR: graminoid removal; TR: total removal; NR no removal).

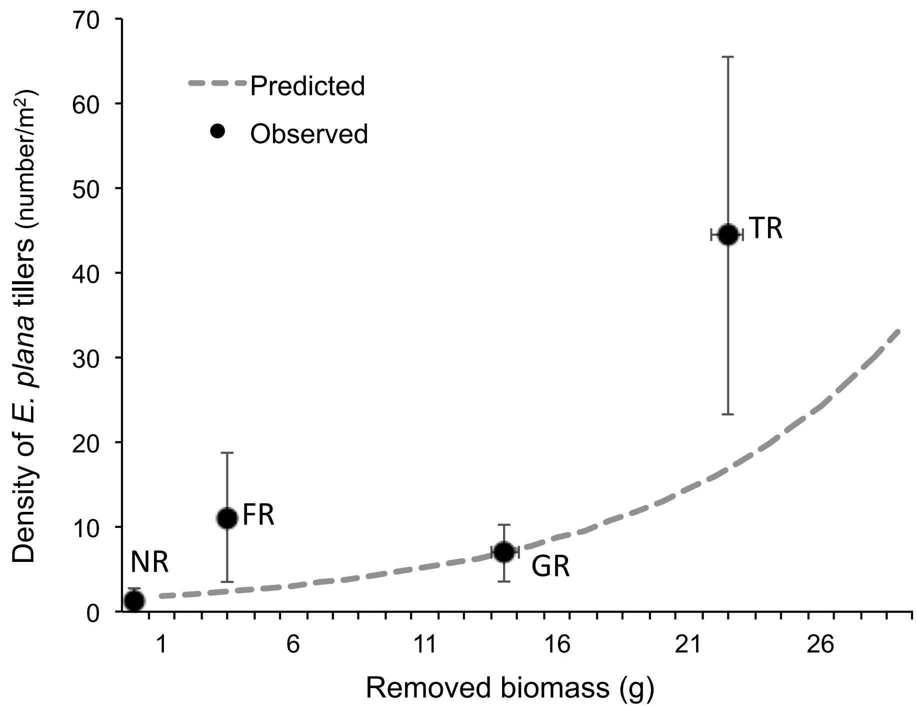


Figure 2. Observed and predicted values ($\hat{y} = e^{(-2.704 + 0.103x)}$) for the number of *E. plana* tillers (m^2) *vs.* the removed biomass (g). The observed data represent the number of *E. plana* tillers for each removal treatment (n=18; FR: forb removal; GR: graminoid removal; TR: total removal; NR: no removal), which have different removed biomass. Bars represent the standard errors.

Table 1. Model selection for the predicted number of *E. plana* tillers as a function of treatment, richness, biomass or block (null model). AIC = Akaike's information criterion; ΔAIC = AIC of each model - AIC best model (lowest AIC value). The best model is shaded.

Model	**Fixed effect**	**AIC**	**ΔAIC**
1	Treatment	156.3	30.9
2	Richness	144.3	18.9
3	Biomass	125.4	0.0
4 (Null)	Block	154.0	28.6

Supplemental material to the manuscript: Guido A; Jurinitz C & Pillar V. Does functional group suppression affect community invasibility?

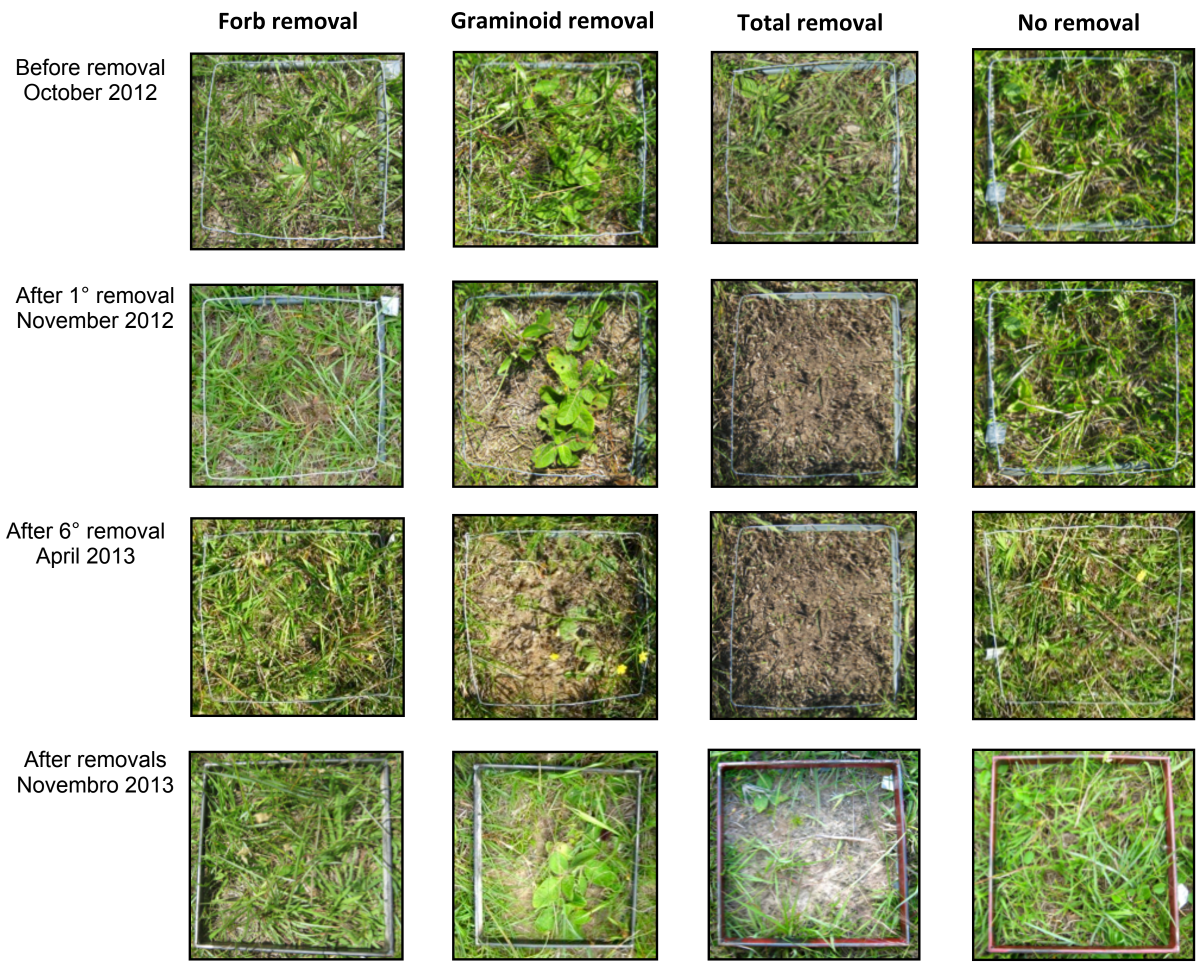
Appendix 1: Illustrations and information about the invasive plant *Eragrostis plana* in South Brazilian grasslands.

Eragrostis plana

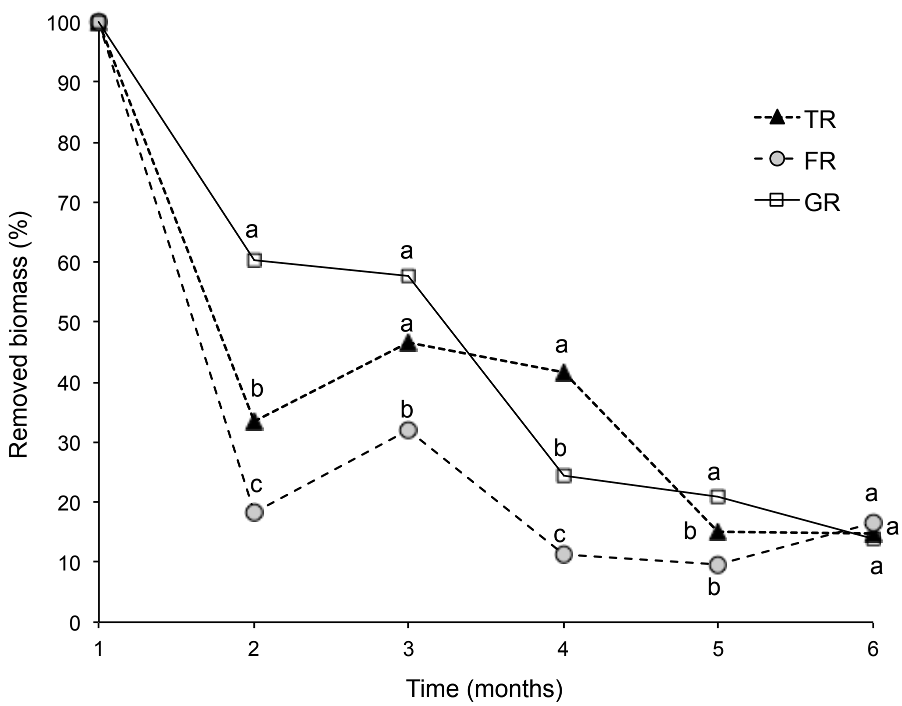
(“capim-annoni”)

Perennial grass from South Africa, accidentally introduced in the Rio Grande do Sul state in the 1950s with a mixture of commercial seeds. It forms dense fiber tussocks with flat base. It flowers in summer, producing high quantity of longevous seeds. The environments most susceptible to invasion are open areas with high disturbance, especially with over-grazing and high compaction soils.

Appendix 2: Illustrations about before and after removal treatments application in 0.2 x 0.2 m plots by periodic clipping at soil level (removal treatments: forb removal, graminoid removal, total removal and no removal).



Appendix 3: Mean percentage of removed biomass over time (months) for each removal treatment (FR: forbs removal, GR: graminoids removal and TR: total removal). Each month represents one removal. Different letters indicate significance difference in percentage of removed biomass between removal treatments (P<0.05).



Appendix 4: Illustration of *Eragrostis plana* germination.

Appenidx 5: List of plant species found during the experiment classified into functional groups (NI: non-identified species).

Species	Functional group
Acmella bellidioides (Sm.) R.K. Jansen	Forb
Andropogon lateralis Nees	Graminoid
Andropogon ternatus (Spreng.) Nees	Graminoid
Aristida venustula Arechav.	Graminoid
Aspilia montevidensis (Spreng.) Kuntze	Forb
Axonopus fissifolius (Raddi) Kuhlm	Graminoid
Baccharis trimera (Less.) DC.	Forb
Briza subaristata Lam.	Graminoid
Bulbostylis capillaris (L.) Kunth ex C.B.Clarke	Graminoid
Bulbostylis sphaerocephala (Boeckeler) Lindm.	Graminoid
Carex phalaroides Kunth	Graminoid
Centella asiatica (L.) Urb.	Forb
Chamaecrista repens (Vogel) H.S.Irwin & Barneby	Forb
Chaptalia exscapa (Pers.) Baker	Forb
Chaptalia piloselloides (Vahl) Baker	Forb
Chaptalia runcinata Kunth	Forb
Chevreulia acuminata Less.	Forb
Chevreulia sarmentosa (Pers.) S.F.Blake	Forb
Chromolaena ascendens (Sch.Bip. ex Baker) R.M.King & H.Rob.	Forb
Cliococca selaginoides (Lam.) C.M.Rogers & Mildner	Forb
Coelorachis selloana (Hack.) A.Camus	Graminoid
Cuphea glutinosa Cham. & Schltdl.	Forb
Danthonia cirrata Hack. & Arechav.	Graminoid
Desmanthus virgatus (L.) Willd.	Forb
Desmodium incanum DC.	Forb
Dorstenia brasiliensis Lam.	Forb
Eragrostis neesii Trin.	Graminoid
Erigeron primulifolium (Lam.) Greuter	Forb
Eryngium ciliatum Cham. & Schltdl.	Forb
Eryngium elegans Cham. & Schltdl.	Forb
Eryngium horridum Malme	Forb
Euphorbia selloi (Klotzsch & Garcke) Boiss.	Forb
Evolvulus sericeus Sw.	Forb

Facelis retusa (Lam.) Sch.Bip.	Forb
Fimbristylis dichotoma (L.) Vahl	Graminoid
Galactia marginalis Benth.	Forb
Galianthe fastigiata Griseb.	Forb
Galium richardianum (Gillies ex Hook. & Arn.) Endl. ex Walp.	Forb
Gamochaeta sp. Wedd.	Forb
Glandularia marrubioides (Cham.) Tronc.	Forb
Gnaphalium americanum Mill.	Forb
Habenaria parviflora Lindl.	Forb
Helianthemum brasiliense (Lam.) Pers.	Forb
Herbertia lahue (Molina) Goldblatt	Forb
Hydrocotyle exigua Malme	Forb
Hypochaeris sp. L.	Forb
Hypoxis decumbens L.	Forb
Justicia axillaris (Nees) Lindau	Forb
Kyllinga sp. Rottb.	Graminoid
Lippia coarctata Tronc.	Forb
Mecardonia tenella (Cham. & Schltdl.) Pennell	Forb
Orthopappus angustifolius	Forb
Oxalis brasiliensis G. Lodd.	Forb
Oxalis conorrhiza Jacq.	Forb
Oxalis eriocarpa DC.	Forb
Oxalis perdicaria (Molina) Bertero	Forb
Oxypetalum solanoides Hook. & Arn.	Forb
Panicum sabulorum Lam.	Graminoid
Paspalum notatum Flüggé	Graminoid
Paspalum paucifolium Swallen	Graminoid
Paspalum plicatulum Michx.	Graminoid
Peltodon longipes A.St.-Hil. ex Benth.	Forb
Pfaffia tuberosa (Spreng.) Hicken	Forb
Piptochaetium montevidense (Spreng.) Parodi	Graminoid
Piriqueta suborbicularis (A. St.-Hil. & Naudin) Arbo	Forb
Plantago myosuros Lam.	Forb
Plantago tomentosa Lam.	Forb
Polygala australis A.W. Benn.	Forb
Pomaria sp. Cav.	Forb
Psidium salutare var. mucronatum (Cambess.) Landrum	Forb
Pterocaulom sp. Ell.	Forb

Rhynchospora rugosa (Vahl) Gale	Graminoid
Richardia grandiflora (Cham. & Schltdl.) Steud.	Forb
Richardia humistrata (Cham. & Schltdl.) Steud	Forb
Richardia stellaris (Cham. & Schltdl.) Steud.	Forb
Ruellia hypericoides (Nees) Lindau	Forb
Ruellia morongii Britton	Forb
Senecio madagascariensis Poir.	Forb
Senecio selloi (Spreng.) DC.	Forb
Setaria parviflora (Poir.) M.Kerguelen	Graminoid
Sisyrinchium micranthum Cav.	Forb
Sisyrinchium sp. L.	Forb
Soliva sessilis Ruiz & Pav.	Forb
Spermacoce verticillata L.	Forb
Sporobolus indicus (L.) R.Br.	Graminoid
Steinchisma hians (Elliott) Nash	Forb
Stenandrium diphyllum Nees	Forb
Stylosanthes leiocarpa Vogel	Forb
Stylosanthes montevidensis Vogel	Forb
Tibouchina gracilis (Bonpl.) Cogn.	Forb
Verbena montevidensis Spreng.	Forb
Vernonanthura discolor (Spreng.) H.Rob.	Forb
Vernonanthura nudiflora (Less.) H.Rob.	Forb
Vernonanthura tweedieana (Baker) H.Rob.	Forb
Zornia sp. J.F. Gmel.	Forb
NI 1	Forb
NI 2	Forb
NI 3	Forb
NI 4	Forb
NI 5	Forb
NI 6	Forb
NI 7	Forb
NI 8	Forb
NI 9	Graminoid
NI 10	Graminoid

Capítulo 3

Invasive species removal for assessing community impact and recovery from invasion

Anaclara Guido

Valério D. Pillar

Chapter 3: Invasive species removal for assessing community impact and recovery from invasion

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Summary

1. Invasive species spread on natural ecosystems is one of the most important causes of biodiversity loss. To disentangle the real invasive plant impact on natural communities it is essential to implement appropriate sampling and experimental designs, which enable correct result interpretations and lead to right decisions for management.

2. We examined South Brazilian grasslands invasion by *Eragrostis plana*, the currently most problematic invasive species in the region. We assessed *E. plana* impact on vegetation, evaluated community response to its removal and discussed removal methods effectiveness, through an experiment on invaded communities complemented by observation of non-invaded communities. Fifty permanent 1 x 1 m plots were located at natural grassland that was partially invaded by *E. plana*. Removal was done annually from 2012 to 2015 and consisted in five treatments (n=10): (i) clipping aboveground biomass at once; (ii) clipping aboveground biomass periodically; (iii) herbicide and (iv) hand-pulling, plus (v) control treatment with no-removal. Additionally, 10 plots located in an adjacent non-invaded area were monitored. Vegetation surveys were done before treatment application (2012) and one (2013), two (2014) and three years (2015) after removals.

3. All removal treatments reduced *E. plana* cover across years, but were not enough to eradicate it. Our results revealed not only differences in observational comparisons between invaded and non-invaded communities, but also an effect of *E. plana* removal on native species richness and cover.

4. We demonstrated the impact of *E. plana* invasion on grassland vegetation, suggesting a reduction of native species richness and cover. These results reinforce the hypothesis that invasive plants replace native species in the communities they invade. Invasive species removal turned communities different from invaded ones, but not resembling non-invaded references, suggesting that community recovery may needs more time for reestablishment or that some restoration strategies are required.

5. *Synthesis*: This study demonstrated the impact on vegetation of the most important invasive species in South Brazilian natural grasslands, highlighting the importance of including observational and experimental comparative studies between invaded, invasive removal and non-invaded communities in ecological invasion research. We expect our study contribute for inferring causal effects in invasion species research.

Key words: *Eragrostis plana;* exotic species; grassland; invasive effect; non-native species; removal experiment; richness; southern Brazil

Introduction

The spread of invasive species on natural ecosystems is one of the most important causes of biodiversity loss (Vitousek *et al*. 1997; Mack *et al.* 2000). Given the current spread of invasive plants worldwide, it is important to evaluate and disentangle the magnitude of the potential impacts of invasion on natural community and ecosystem processes. For this, it is essential to adopt appropriate sampling and experimental designs that enable correct interpretations and lead to right decisions.

Frequently, studies have utilized an observational approach to assess invasive species impact, which consist in comparing certain characteristics (e.g. species composition, richness, cover, diversity) between invaded and non-invaded reference communities (see reviews Levine *et al.* 2003 and Vilà *et al.* 2011). Although some studies have found consistent results, comparisons between invaded and non-invaded communities do not allow for inferring causal relations. Hence, other studies have used randomized experiments in which the invasive species is removed, and comparisons between removal and no removal communities are conducted (Ostertag *et al.* 2009; Flory & Clay 2009; Pavlovic *et al.* 2009; see also Guido & Pillar 2015 for a review). Nevertheless, these experiments involve other confounding factors, such as soil disturbance after removals (Díaz *et al.* 2003; Andreu & Vilà 2011). Further, non-invaded reference communities cannot be properly included as a control treatment in a randomized experiment. Hence, it has been proposed that the comprehensive understanding of an invasive species impact would be improved by combining observational and experimental comparative studies between invaded, invasive removal and non-invaded communities (Andreu & Vilà 2011). Although few studies have successfully used this approach (Hejda & Pysek 2006; Andreu *et al.* 2010; Hejda 2012), the logic of the interpretation of results based on observational and experimental methods is not sufficiently developed in the literature. In Fig. 1 we conceptually explain the possible causal interpretations based on the outcomes of combined observational and experimental comparisons between non-invaded communities and removal and no removal treatments on invaded communities. We expect that such a complementary approach may offer significant opportunities for inferring causal effects in invasion species research.

The removal of an invasive species has become an important challenge, not only for evaluating the potential impacts on the communities, but also for being a frequent component of restoration ecology efforts (Zavaleta *et al.* 2001). Some studies have suggested that the suppression of an invasive species had notable positive effects on the resident community, in terms of increasing species diversity, richness, cover and seedling recruitment (e.g. D'Antonio *et al.* 1998; Flory 2010). Based on a meta-analysis, Andreu & Vilà (2011) showed that invaded plots contained on average 30% fewer species than plots where the invasive species was removed. This may suggest that invasive removal increases species richness and makes the native plant community more similar to non-invaded sites (Andreu & Vilà 2011). Complementary, the removal of invasive species has helped to quantify the response of invasive species and native communities to different removal methods, mainly as a way to test restoration strategies (see Kettenring & Adams 2011 for a meta-analysis). Thus, to consider a removal effort as successful, both the effective elimination of the invasive species and the recovery of the native plant community to its reference composition and function may be required (Zavaleta *et al.* 2001).

The invasion of South Brazilian grasslands by *Eragrostis plana* Nees (Poaceae; commonly named as “capim annoni” or “love grass”) is currently the most problematic invasive species in the region (Medeiros & Focht 2007; Guido & Guadagnin 2015). Further, *E. plana* is considered an invasive species with high potential of expansion in other regions of South America, including Uruguay and the Mesopotamia region in northeastern Argentina (Barbosa et al. 2013). *Eragrostis plana* is a perennial grass, which was accidently introduced in 1957 from South Africa to the state of Rio Grande do Sul, in southern Brazil. During the 1970s, farmers used *E. plana* seeds for cultivated pastures, mainly due to its fast growth and frost resistance. As a result, the species was propagated and marketed until 1979. Since then, the sale of seeds and seedlings was banned in Rio Grande do Sul by the Brazilian Ministry of Agriculture, on account of low forage quality compared to native species, which became a problem for grazing livestock (Reis & Coelho 2000; Medeiros & Focht 2007; Medeiros *et al.* 2009). Although *E. plana* invasion is currently one of the main ecological and economical threats for southern Brazil natural grasslands, there is an apparent lack of information assessing its impact on vegetation community and also regarding methods that can potentially reduce its spread.

In this study we assessed the impact of *E. plana* invasion and the recovery of natural grassland communities after its removal, considering observation and experimental approaches that included invaded, removal and non-invaded communities. We also evaluated the differences of removal methods effectiveness, in terms of *E.*

plana suppression and community recovery. More specifically, we asked the following questions: (i) How different are invaded and non-invaded communities? (ii) Does *E. plana* removal increase species richness and change community composition? (iii) Do removal communities resemble non-invaded ones? and (iv) Which removal method is more efficient to reduce *E. plana* and enhance community recovery? Our hypothesis is that *E. plana* has an impact on natural grassland vegetation, which is revealed not only by observational differences between invaded and non-invaded communities, but also by community changes after its removal (see Fig. 1). After *E. plana* removal, we expect the communities will recover from invasion, which should be demonstrated by similarities between removal and non-invaded communities (see Fig. 1).

Methods

Study site

The experiment was located in a natural grassland site at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (30°07'10"S, 51°41'06"W, 63 m a.s.l) in southern Brazil, which was partially invaded by *E. plana* (see Fig. S1 from Appendix S1 in Supporting Information). The climate is subtropical and humid with the normal mean annual precipitation being 1455 mm year^{-1} and the monthly mean temperature ranging from 13.6 °C in winter to 23.7 °C in summer. The vegetation is part of the Rio de la Plata grasslands biome, which extends from southern Brazil to Uruguay and east of Argentina (Soriano *et al.* 1992; Overbeck *et al.* 2007). Vegetation physiognomy in the study site was homogeneous and composed mostly of perennial C_4 grasses, forbs and scarce shrubs sparsely arranged (see Fig. S1 from Appendix S1). The area has been historically grazed by livestock (mostly bovines), which was maintained at the same intensity during the experiment, as the suppression of grazing would have produced remarkable effects on vegetation structure (e.g. Blanco *et al.* 2007).

Experimental design

Fifty permanent 1 x 1 m plots were sparsely located in the study site (see Fig. S2 from Appendix 1). Plots were at least 4 m apart and were positioned in such a way to maximize homogeneity between them, i.e. avoiding shrubs and large tussocks, and to have about 30-40% of *E. plana* cover in each plot. Five *E. plana* removal treatments were randomly allocated between plots (n = 10 per treatment): (i) clipping aboveground biomass at once; (ii) clipping aboveground biomass periodically (i.e., we divided each plot in five fractions and every 15 days we clipped 1/5); (iii) herbicide application (i.e. sprayed glyphosate precisely on the leaves and stems); (iv) hand-pulling (i.e. removing above and below ground biomass); and (v) no-removal control (see Appendix S2). Removals took place annually at one event (between October and November) from 2012 to 2015, except the treatment of clipping aboveground biomass periodically, which in each year was applied five times every 15 days. In addition, we located 10 permanent 1 x 1 m plots in a non-invaded patch (i.e. non-invaded communities) in the same study site, as a possible reference for communities before invasion (see Fig. S2 from Appendix S1).

Data collection

In the spring of 2012, before the application of the treatments, we assessed the plant composition of each plot by recording species presence and visually estimating their cover, which may overlap, using the following classes: < 1%, 2%, 4%, 10%, and subsequently in intervals of 10% (modified from Londo 1976). Additionally, five height measurements (i.e. one in each quadrant and the other in the middle of the plot) were done in each plot. Plant composition recording and height measurements were repeated annually during the spring of 2013, 2014 and 2015, always before the annual removals. As the monitoring was delayed by almost one year after the invasive species removals, we expect that transient responses to disturbances caused by the removals should have passed.

Data analysis

We compared the state of the plots before (2012) and after three years of annual removals (2015) between the three types of communities: no removal (n=10), removal (n=40) and non-invaded (n=10), in terms of: (i) community composition; (ii) Shannon diversity index; (iii) Pielou's evenness index; (iv) species richness and (v) total species cover. In these analyses we excluded *E. plana* from the species lists irrespective of treatment. For comparing species composition between communities we used MANOVA (Pillar & Orlóci 1996) based on chord distances between communities, and for the other variables we used ANOVA, both with permutation testing (Manly 2007).

We analyzed *E. plana* cover in each treatment across years considering the 50 invaded plots: 2012 (before removals), 2013 (one year of removals), 2014 (two years of removals) and 2015 (three years of removals). In addition, for comparing the effectiveness of *E. plana* control between removal methods, we calculated for each plot the cover differences between after (2015) and before (2012) removals, resulting negative values for cover decreases, positive for increases and zeroes for no change in *E. plana* cover. We used ANOVA with permutation test (Manly 2007) in which permutations were restricted within plots (i.e. restricted comparison to the same plot over time) for comparing years (from 2012 to 2015), and were unrestricted for comparing treatments.

Furthermore, we analyzed the effect of the different removal methods on: (i) community richness across years (2012-2015), and (ii) species extinctions and (iii) species colonization after three years of annual removals. Species extinctions and colonization were obtained comparing community composition between before (2012) and after removals (2015). Extinction was defined as the absence of a species after removals, while colonization was the record of a new species present after removals. For this, we considered the 40 plots with removals, plus 10 from removal control, and used ANOVA with permutation test (Manly 2007). Again, permutations were unrestricted.

Finally, for each year (2012-2015), based on species composition (previously removing *E. plana* from the species list) we calculated the chord distances between each plot under a removal treatment (clipping, clipping periodically, herbicide and hand-pulling) and all the plots of the no removal control. We took the average of these distances as a descriptor of each removal plot in a given year. We repeated the same procedure for comparing removal and non-invaded plots in each year. We used ANOVAs with permutation test (Manly 2007) to contrast the differences in species composition across years.

All analyses were implemented by MULTIV software (available at http://ecoqua.ecologia.ufrgs.br). For all permutation tests we used 10 000 permutations.

Results

During the experiment, the total number of species was 160, belonging to 38 botanical families (see Appendix S3). As only 10 species (6.2 %) of the list were non-native (see Appendix S3), which never represented more than 20% of the total cover, we adopted the terms native species richness and native species cover through the text. Before removals (2012), not considering *E. plana* in the species lists, invaded communities (i.e. removal and no removal) were remarkable different from non-invaded communities, in terms of community composition, diversity, richness and native species cover (Table 1a). Compared to invaded plots, non-invaded reference communities were more diverse (2.94 ± 0.30), richer (43.10 ± 3.96) and with more native species cover (100.93 % ± 0.73). Besides, the vegetation of invaded communities (9.79 cm ± 0.49) was taller than non-invaded ones (5.02 cm ± 1.53). Before removals (2012), as expected, invaded communities (i.e. removal and no removal) were not different from each other (Table 1b). Yet, after three years of annual removals (2015), there was a significant effect of removal on native species richness and cover, when contrasted to no removal invaded communities (Table 1b). However, these effects were not enough for making removal plots similar to non-invaded reference plots (Table 1c).

In all removal treatments, *E. plana* cover decreased significantly across years, mostly after two and three years of treatment application, while for the no removal control the increasing trend was not significant (Fig. 2). Further, contrasted to the no removal control, all removal treatments significantly reduced *E. plana* cover between 2012 and 2015, but the removal methods did not differ each other in their reduction effect (Fig. 3).

Across years, all removal treatments significantly increased species richness (Fig. 4). However, richness also increased in non-invaded plots, while remained unchanged in no-removal control treatment (Fig. 4). In addition, we did not detect significant effects on species extinction (P=0.128) and colonization (P=0.136) between removal methods (see Appendix S4).

Differences in species composition between each removal treatment and no removal communities increased throughout years, revealing that *E. plana* suppression had a significant effect on plant community composition (Fig. 5). However, after three years of cutting, cutting periodically or hand-pulling *E. plana,* the community composition did not become more similar to non-invaded plots (Figs. 5a, b & d). Herbicide application was the only treatment that increased the differences in species composition from non-invaded communities (Fig. 5c). Additionally, the differences in species composition between invaded with no removal and non-invaded communities remained constant across years (P=0.189; data not shown)

Discussion

This study is the first to asses the impact of *E. plana* invasion on natural grassland vegetation in Southern Brazil, highlighting the importance of including observational and experimental comparative studies between invaded, removal and non-invaded communities in ecological invasion research (Andreu & Vilà 2011; Guido & Pillar 2015; Fig. 1). This approach is particularly important for evaluating short-term invasive species impact, as its introduction in non-invaded areas is not a desirable strategy with regards to conservation, and the effects of invasion may take longer than short-term experiments. Our results indicate that despite the significant reduction of *E.*

plana cover across years, removal treatments were not effective enough to eradicate the invasive species at the local scale. Additionally, removal methods did not differ in their effectiveness to control *E. plana* cover, but we observed differences in community response between methods. More importantly, our results revealed significant differences in community characteristics between invaded, removal and non-invaded plots, suggesting that *E. plana* invasion has an impact on grassland vegetation, since its removal turned communities different from invaded ones.

Comparisons between invaded (no removal) and non-invaded communities revealed differences in terms of species composition, diversity, richness and native cover (Table 1). However, we cannot attribute these differences as an effect of *E. plana* invasion, as we do not know how were the pre-invasion conditions. Therefore, community differences could be a cause (e.g. lower richness causes invasion) or a consequence (e.g. lower richness is an effect) of *E. plana* invasion. Further, despite three years of annual removals were not enough to locally eradicate *E. plana*, our results (Table 1) showed a significant positive effect of its suppression on native species richness and cover. Thus, if we assume that the effect of *E. plana* removal causing the increase in species richness and cover is reversible, i.e. *E. plana* invasion caused their reduction, then we may infer that the reduction of species richness and cover observed in invaded communities is a consequence of *E. plana* invasion, revealing the invasive species impact on natural vegetation (Fig. 1). Yet, despite the suppression of the invasive species, invaded communities remained significantly different from non-invaded reference communities in terms of community composition, diversity, and native species richness and cover, indicating that either the effects of the invasive species are not reversible, at least at the temporal scale of the experiment, or that the reference communities do not represent pre-invasion states.

Although our study did not evaluate impact related mechanisms, we suggest that *E. plana* invasion may affect light availability, as it mostly grows taller than other native grasses due to livestock rejection, which could decrease germination and the survival of certain species beneath its canopy. After invasive species removal, native plants were able to use the bare space and the released resources, increasing resident community richness and cover. Thus, in agreement with previous works, our results corroborate the hypothesis that invasive plants replace native species in the communities they invade (Flory & Clay 2009; Andreu *et al.* 2010; Vilà *et al.* 2011). In this context, a study has suggested an allelopathic effect of *E. plana* on native vegetation (Favaretto *et al.* 2011), although this observation was not verified in the field. Thus, further research is needed to understand the mechanisms that are responsible for changes in community characteristics in invaded communities.

After *E. plana* removal, the differences in species composition between removal and no removal invaded communities increased across years, indicating that annual invasive species suppression had a significant effect on plant community composition. This corroborates the well-known impact that invasive plants spread has on native communities (Vilà *et al.* 2011; Pysek *et al.* 2012). Nevertheless, three years of different removals were not enough to decrease the differences between each removal treatment and non-invaded communities, which may suggest that longer-term experiments and/or further restoration strategies are needed. Additionally, herbicide removal was the only treatment that increased the differences in species composition to non-invaded communities, suggesting that besides removing *E. plana,* glyphosate application may have other non-target effects on natural vegetation (Rodriguez & Jacobo 2013). This finding has practical relevance, as glyphosate application is the most common method used for *E. plana* control in South Brazilian grasslands, which may have further important implications for biodiversity conservation.

Finally, our work demonstrated the impact of *E. plana* invasion on grassland communities, mostly in terms of species richness and cover, in a small spatial (1 m^2) and temporal scale (three years). Additionally, we showed multiple techniques that can be used to reduce *E. plana* cover, but are not enough for its locally eradication, while simultaneously pointing out the responses of plant communities to different removal methods. An open question is whether the same effects of invasive removal would be observed if a native species with similar dominance level were removed. Such a control would allow disentangle the effects of invasive and non-invasive species removal on the remaining community (see Guido & Pillar 2015 for a review). The combined interpretation of both the effect of removal method on *E. plana* reduction and on community response, we recommend not using glyphosate application, as it has shown non-target effects on community recovery. However, since the eradication was not achieved, it is possible that some isolated removal events cannot result in native community recovery from invasion. Hence, more research effort towards effective methods of *E. plana*'s control in invaded areas is urgently needed. Longer-term experiments are crucial, not only to eradicate the invasive species but also to turn invaded communities more similar to non-invaded references plots. Complementary, the evaluations of different restoration strategies (e.g. invasive species removal and reintroduction of native species) are important to further progress on controlling *E. plana* in natural grasslands.

Acknowledgments

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Figures and Tables

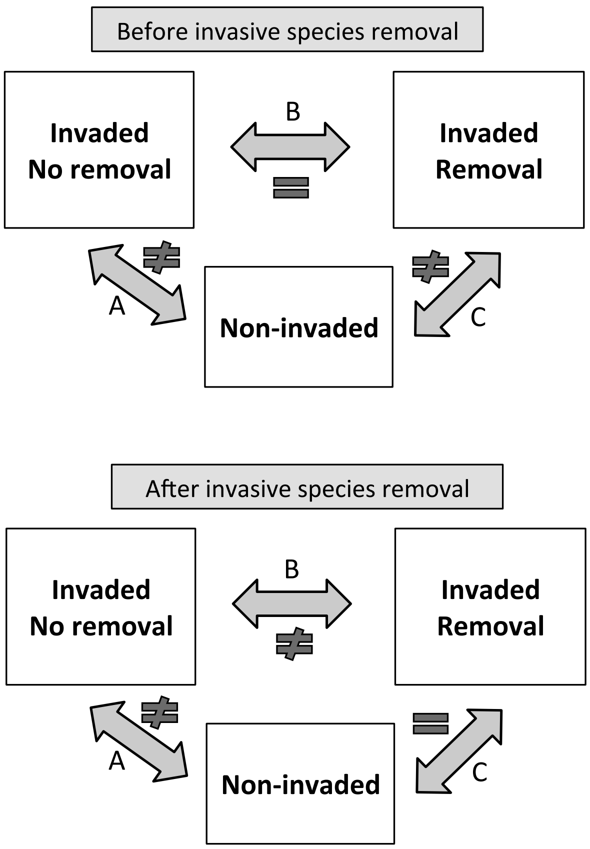

Figure 1. Conceptual diagram showing possible causal interpretations by comparisons between non-invaded communities and invasive species removal and no removal treatments on invaded communities. Comparisons may be based on variables such as native species composition, richness, evenness, or abundance. (A) Observational approach: no removal and non-invaded communities are different, before and after removal, but whether the differences were caused by the invasion or by pre-existing conditions that facilitated the invasion cannot be disentangled; (B): Experimental approach: before removal, the removal and the no removal communities should be similar, as expected in a randomized experiment; after removal, if they become different we can conclude, with a nominal error specified by the *P*-value of the statistical test, that the removal caused the effect (e.g. increased native species richness); whether the invasion would cause a reversed effect (e.g. decreased native species richness) can be inferred by examining the comparison (C): Observational approach: when before removal, non-invaded and removal communities are different and after removal they become similar. Expanded from Andreu & Vilà 2011.

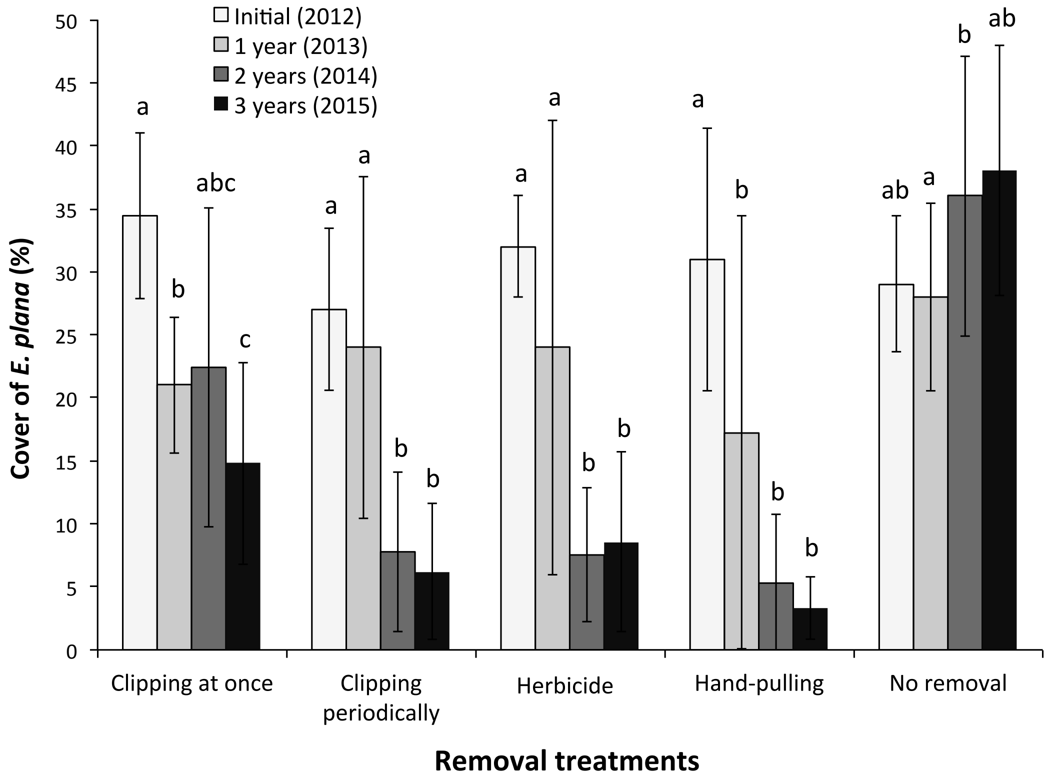

Figure 2. Percentage of cover of *Eragrostis plana* in the four years of the experiment for each removal treatment (2012: before removals; 2013: 1 year after removals; 2014: 2 years after removals and 2015: 3 years after removals). Years identified with same letters did not differ significantly each other within treatments (P>0.05).

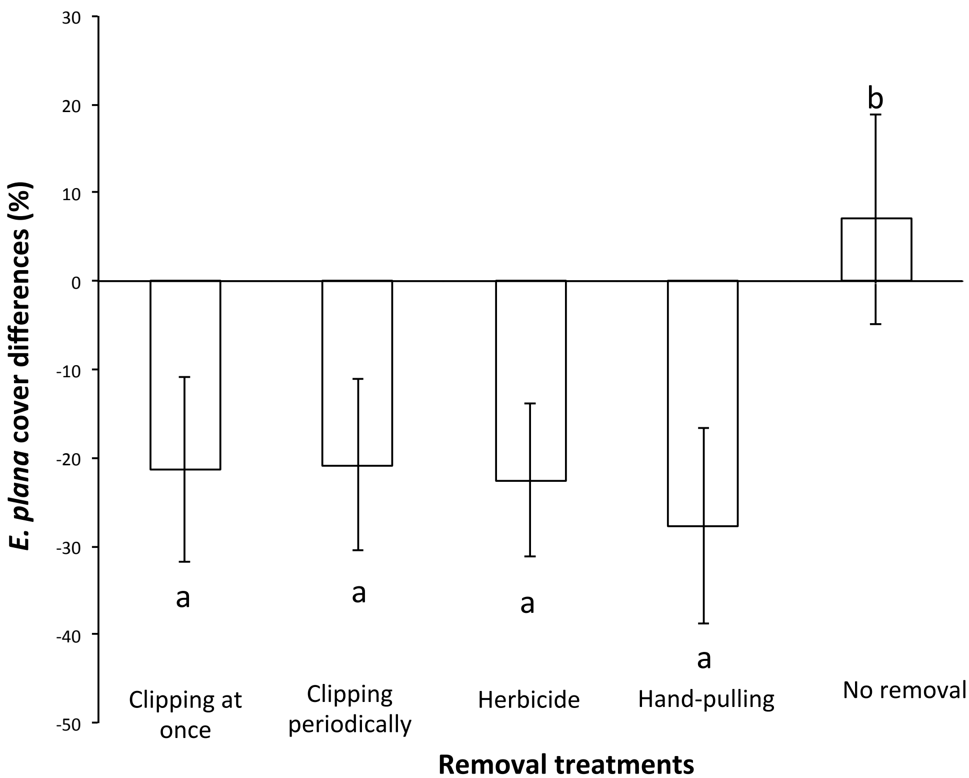

Figure 3. *Eragrostis plana* cover differences between 2015 and 2012 for each removal treatment. Treatments identified with same letters did not differ significantly each other (P>0.05).

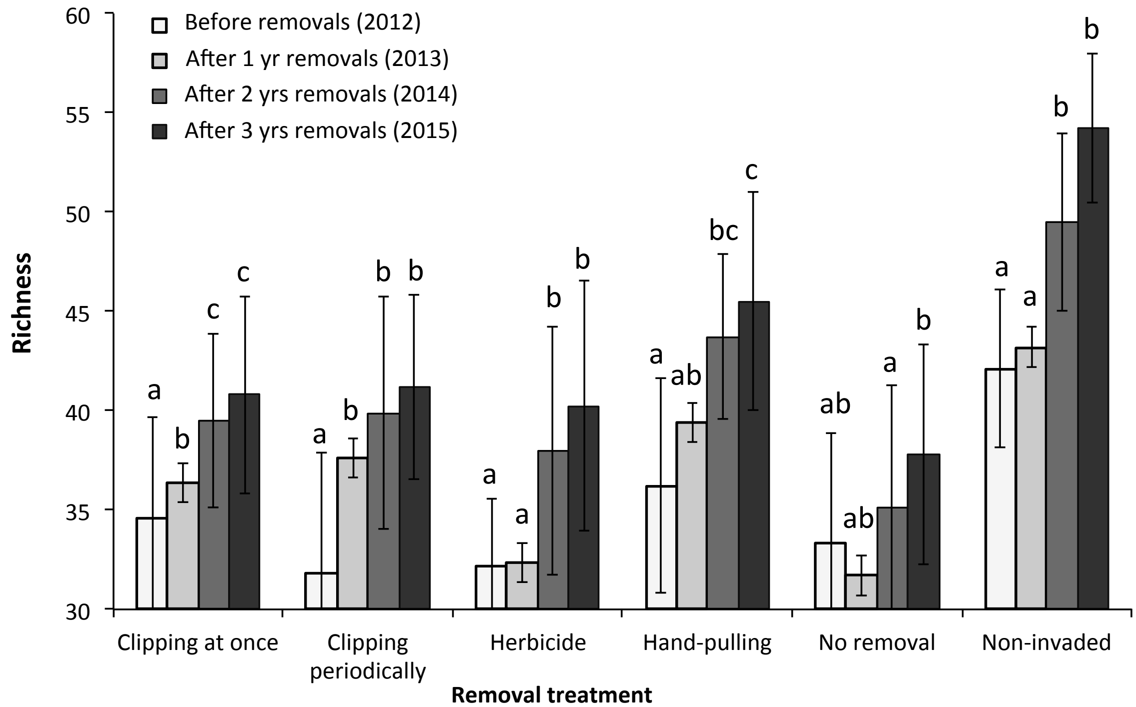

Figure 4. Community richness (per 1 m^2 plot) during the four years of the experiment for each removal treatment. Years identified with same letters did not differ significantly within treatments (P>0.05).

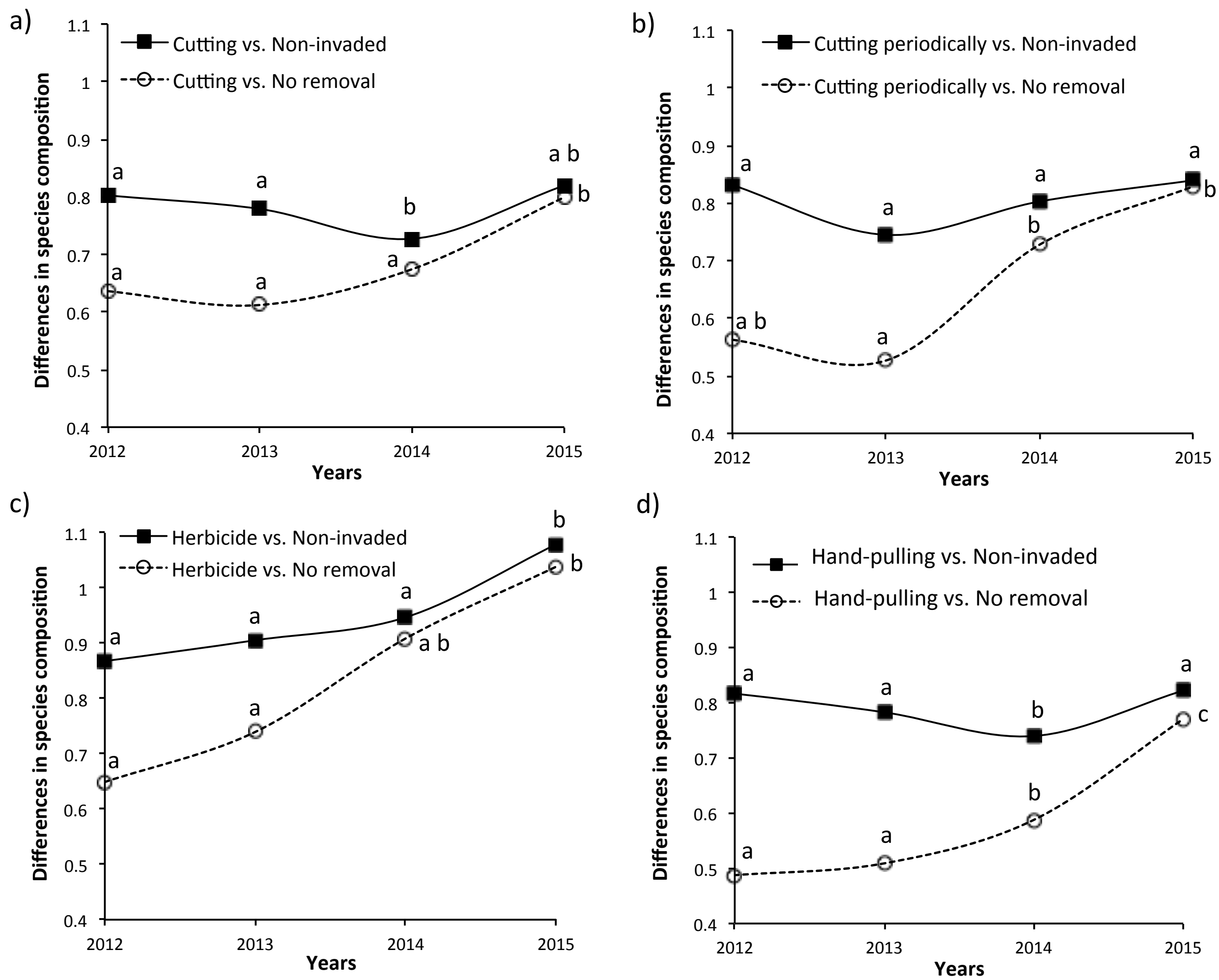

Figure 5. Differences in species composition between each removal treatment and both controls across years (2012: before removals; 2013: one year of removals; 2014: two years of removals; 2015: three years of removals). Chord distance was used as resemblance measure, excluding *Eragrostis plana* in the species list. Years identified by same letters did not differ significantly (P>0.05).

Table 1. Comparisons between non-invaded (n=10) and invaded communities with removal (n=40) and no removal (n=10) treatments, before (2012) and after three years of *Eragorstis plana* annual removal (2015). P-values in bold indicate significant differences (P<0.05) between the contrasted communities for each response variable (or variables in multivariate composition) for the same year. For all variables, *E. plana* was excluded from the species lists irrespective of treatment.

Type of comparison	Response variable	Before removals (2012) P value	After 3 yrs removals (2015) P value
(a) Invaded with no removal *vs* non-invaded	Community composition	**0.001**	**0.047**
	Diversity	**0.012**	**0.005**
	Evenness	0.160	0.763
	Species richness	**0.023**	**0.003**
	Native species cover	**0.002**	**0.001**
(b) Invaded with removal *vs* no removal	Community composition	0.732	0.869
	Diversity	0.874	0.653
	Evenness	0.931	0.149
	Species richness	0.843	**0.049**
	Native species cover	0.450	**0.017**
(c) Invaded with removal *vs* non-invaded	Community composition	**0.001**	**0.003**
	Diversity	**0.006**	**0.001**
	Evenness	0.105	0.078
	Species richness	**0.001**	**0.044**
	Native species cover	**0.004**	**0.018**

Supporting Information may be found in the online version of this article: Invasive species removal: assessing community impact and recovery from invasion. Anaclara Guido & Valério D. Pillar.

Appendix S1: Study site information at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul (30°07'10"S, 51°41'06"W, 63 m a.s.l) in southern Brazil.

Figure S1. Photos from the study site. Agronomic Experimental Station of the Federal University of Rio Grande do Sul (30°07'10"S, 51°41'06"W, 63 m a.s.l) in southern Brazil.

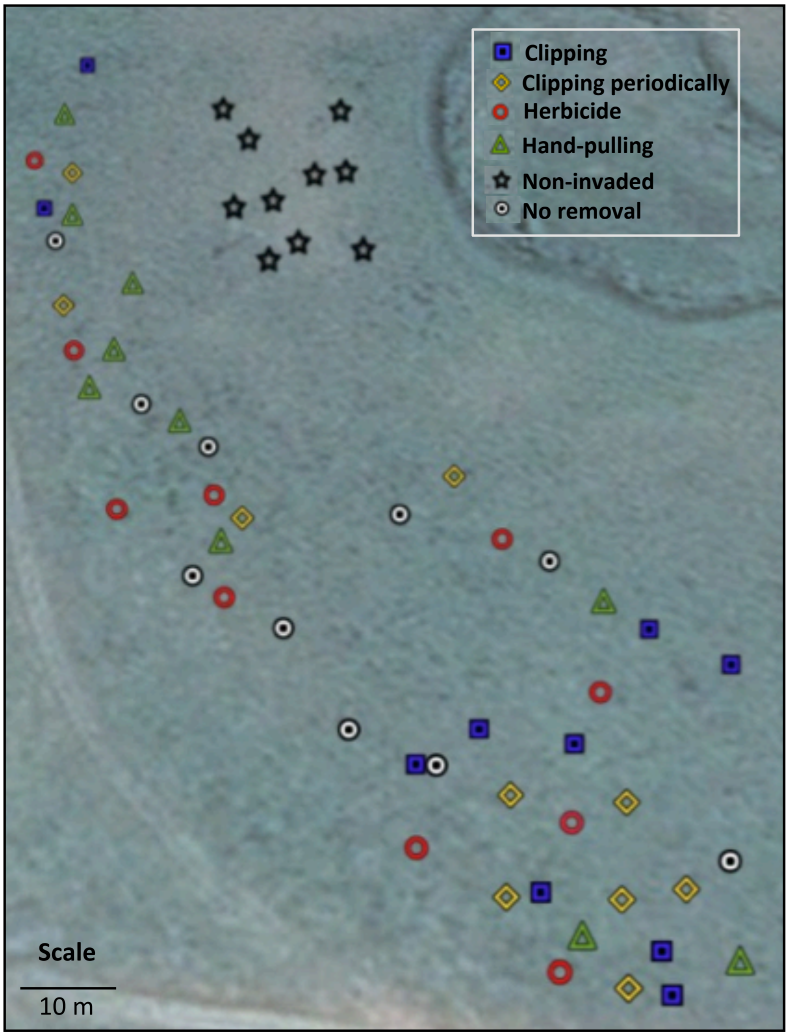

Figure S2. Plots distribution on the study site (n=60). Agronomic Experimental Station of the Federal University of Rio Grande do Sul (30°07'10"S, 51°41'06"W, 63 m a.s.l) in southern Brazil.

Appendix S2: Removal methods photos

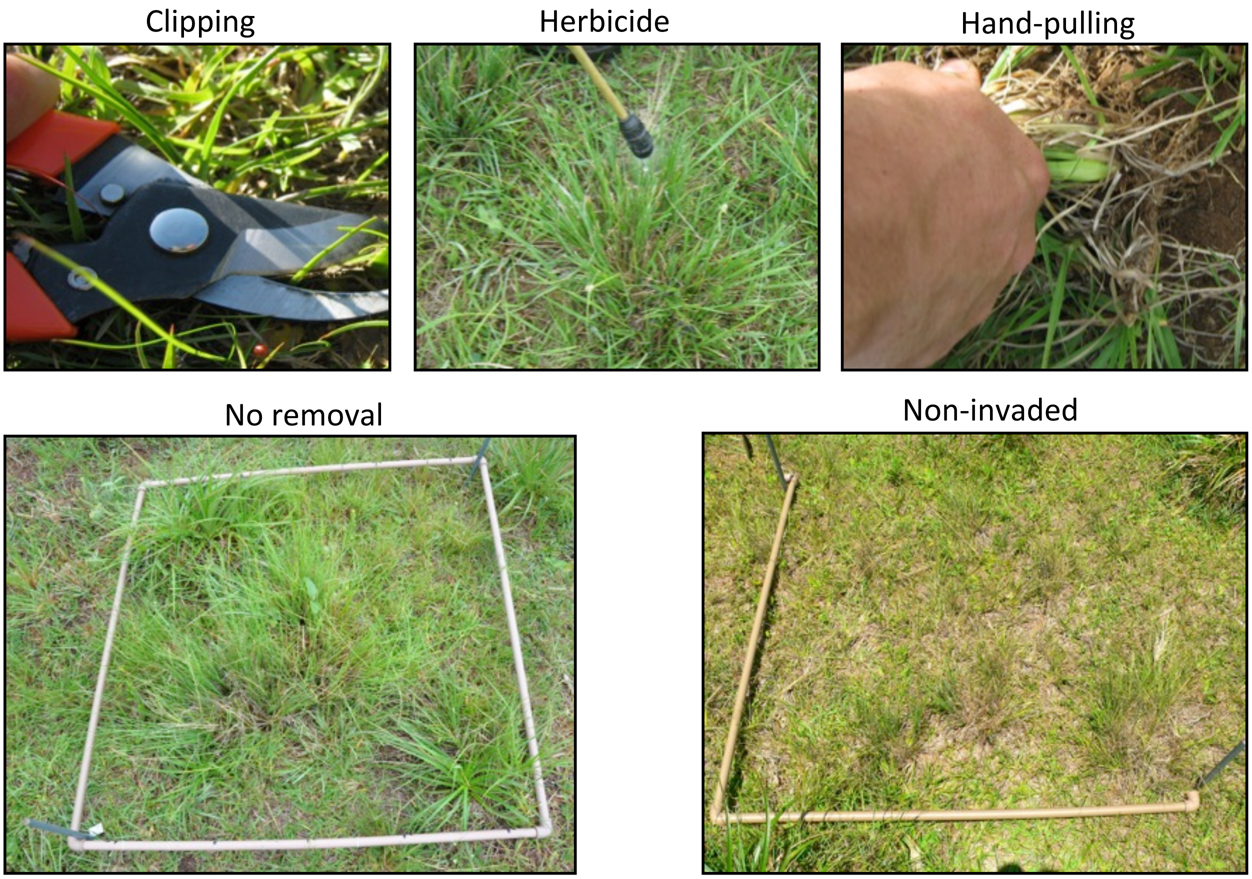

Figure S1. Photos illustrating *Eragrostis plana* removal methods (clipping, herbicide and hand-pulling), no removal treatment, and non-invaded reference communities.

Appendix S3: Species list

Table S1. List of plant species registered during the experiment (19 species could not be identified). * non-native species; ** target species in the removal experiment.

Species	Family
Acmella bellidioides (Sm.) R.K. Jansen	Asteraceae
Anagallis arvensis L.	Primulaceae
Andropogon lateralis Nees	Poaceae
Andropogon ternatus (Spreng.) Nees	Poaceae
Aristida flaccida Trin. & Rupr.	Poaceae
Aristida laevis (Nees) Kunth	Poaceae
Aristida venustula Arechav.	Poaceae
Aristolochia sessilifolia (Klotzsch) Duch.	Aristolochiaceae
Aspilia montevidensis (Spreng.) Kuntze	Asteraceae
Avena sativa L. *	Poaceae
Axonopus fissifolius (Raddi) Kuhlm	Poaceae
Baccharis trimera (Less.) DC.	Asteraceae
Briza subaristata Lam.	Poaceae
Briza uniolae (Nees) Steud.	Poaceae
Bromus sp. L.	Poaceae
Bulbostylis capillaris (L.) Kunth ex C.B.Clarke	Cyperaceae
Bulbostylis sphaerocephala (Boeckeler) Lindm.	Cyperaceae
Calamagrostis viridiflavescens (Poir.) Steud.	Poaceae
Cardamine chenopodifolia Pers. *	Brassicaceae
Carex phalaroides Kunth	Cyperaceae
Carex sororia Kunth	Cyperaceae
Centella asiatica (L.) Urb. *	Apiaceae
Cerastium glomeratum Thuill. *	Caryophyllaceae

Chamaecrista repens (Vogel) H.S.Irwin & Barneby	Fabaceae
Chaptalia exscapa (Pers.) Baker	Asteraceae
Chaptalia piloselloides (Vahl) Baker	Asteraceae
Chaptalia runcinata Kunth	Asteraceae
Chevreulia acuminata Less.	Asteraceae
Chevreulia sarmentosa (Pers.) S.F.Blake	Asteraceae
Chromolaena ascendens (Sch.Bip. ex Baker) R.M.King & H.Rob.	Asteraceae
Cliococca selaginoides (Lam.) C.M.Rogers & Mildner	Linaceae
Clitoria nana Benth.	Fabaceae
Coelorachis selloana (Hack.) A.Camus	Poaceae
Cuphea campylocentra Griseb.	Lithraceae
Cuphea glutinosa Cham. & Schltdl.	Lithraceae
Cyclospermum leptophyllum (Pers.) Sprague	Apiaceae
Cynodon dactylon (L.) Pers. *	Poaceae
Cypella herbertii (Lindl.) Herb.	Iridaceae
Danthonia cirrata Hack. & Arechav.	Poaceae
Desmanthus virgatus (L.) Willd.	Fabaceae
Desmodium incanum DC.	Fabaceae
Dichondra microcalyx (Hallier f.) Fabris	Convolvulaceae
Dichondra sericea Sw.	Convolvulaceae
Dorstenia brasiliensis Lam.	Moraceae
Elephantopus mollis Kunth	Asteraceae
Eleusine tristachya (Lam.) Lam. *	Poaceae
Elionurus muticus (Spreng.) Kuntze	Poaceae
Eragrostis neesii Trin.	Poaceae
Eragrostis plana Nees **	Poaceae
Erigeron primulifolium (Lam.) Greuter	Asteraceae
Eryngium ciliatum Cham. & Schltdl.	Apiaceae

Eryngium horridum Malme	Apiaceae
Euphorbia selloi (Klotzsch & Garcke) Boiss.	Euphorbiaceae
Evolvulus sericeus Sw.	Convolvulaceae
Facelis retusa (Lam.) Sch.Bip.	Asteraceae
Fimbristylis dichotoma (L.) Vahl	Cyperaceae
Fimbristylis ovata (Burm.f.) J.Kern	Cyperaceae
Galactia gracillima Benth.	Fabaceae
Galactia marginalis Benth.	Fabaceae
Galianthe fastigiata Griseb.	Rubiaceae
Galium richardianum (Gillies ex Hook. & Arn.) Endl. ex Walp.	Rubiaceae
Gamochaeta sp. Wedd.	Asteraceae
Glandularia marrubioides (Cham.) Tronc.	Verbenaceae
Habenaria parviflora Lindl.	Orquideaceae
Helianthemum brasiliense (Lam.) Pers.	Cistaceae
Herbertia lahue (Molina) Goldblatt	Iridaceae
Hydrocotyle bonariensis Comm. ex Lam.	Arialiaceae
Hydrocotyle exigua Malme	Arialiaceae
Hypochaeris sp. L. *	Asteraceae
Hypoxis decumbens L.	Hypoxidaceae
Juncus capillaceus Lam.	Juncaceae
Justicia axillaris (Nees) Lindau	Acanthaceae
Kyllinga sp. Rottb.	Cyperaceae
Lippia coarctata Tronc.	Verbenaceae
Lolium multiflorum Lam. *	Poaceae
Mecardonia tenella (Cham. & Schltdl.) Pennell	Plantaginaceae
Micropsis spathulata (Pers.) Cabrera	Asteraceae
Nothoscordum montevidense Beauverd	Amaryllidaceae
Ophioglossum nudicaule L. f.	Ophioglossaceae

Oxalis brasiliensis G. Lodd.	Oxalidaceae
Oxalis conorrhiza Jacq.	Oxalidaceae
Oxalis eriocarpa DC.	Oxalidaceae
Oxalis lasiopetala Zucc.	Oxalidaceae
Oxalis perdicaria (Molina) Bertero	Oxalidaceae
Oxypetalum solanoides Hook. & Arn.	Apocynaceae
Panicum sabulorum Lam.	Poaceae
Paspalum dilatatum Poir.	Poaceae
Paspalum notatum Flüggé	Poaceae
Paspalum paucifolium Swallen	Poaceae
Paspalum plicatulum Michx.	Poaceae
Paspalum umbrosum Trin.	Poaceae
Paspalum urvillei Steud.	Poaceae
Peltodon longipes A.St.-Hil. ex Benth.	Lamiaceae
Pfaffia tuberosa (Spreng.) Hicken	Amaranthaceae
Piptochaetium montevidense (Spreng.) Parodi	Poaceae
Piptochaetium stipoides (Trin. & Rupr.) Hack. & Arechav.	Poaceae
Piriqueta suborbicularis (A. St.-Hil. & Naudin) Arbo	Passifloraceae
Plantago myosuros Lam.	Plantaginaceae
Plantago tomentosa Lam.	Plantaginaceae
Polygala australis A.W. Benn.	Polygalaceae
Pomaria sp. Cav.	Fabaceae
Psidium salutare var. mucronatum (Cambess.) Landrum	Myrtaceae
Pterocaulom sp. Ell.	Asteraceae
Rhynchospora rugosa (Vahl) Gale	Cyperaceae
Rhynchospora sp. Vahl	Cyperaceae
Richardia grandiflora (Cham. & Schltdl.) Steud.	Rubiaceae
Richardia humistrata (Cham. & Schltdl.) Steud	Rubiaceae

Richardia stellaris (Cham. & Schltdl.) Steud.	Rubiaceae
Ruellia hypericoides (Nees) Lindau	Acanthaceae
Ruellia morongii Britton	Acanthaceae
Rumex sp. L. *	Poligonaceae
Schizachyrium tenerum Nees	Poaceae
Scutellaria racemosa Pers.	Lamiaceae
Senecio brasiliensis (Spreng.) Less.	Asteraceae
Senecio madagascariensis Poir. *	Asteraceae
Senecio selloi (Spreng.) DC.	Asteraceae
Setaria parviflora (Poir.) M.Kerguelen	Poaceae
Setaria vaginata Spreng.	Poaceae
Sida rhombifolia L.	Malvaceae
Sisyrinchium micranthum Cav.	Iridaceae
Sisyrinchium sp. L.	Iridaceae
Solanum sp. L.	Solanaceae
Soliva sessilis Ruiz & Pav.	Asteraceae
Spermacoce eryngioides (Cham. & Schltdl.) Kuntze	Rubiaceae
Spermacoce verticillata L.	Rubiaceae
Sporobolus indicus (L.) R.Br.	Poaceae
Steinchisma hians (Elliott) Nash	Poaceae
Stenandrium diphyllum Nees	Acanthaceae
Stenocephalum megapotamicum (Spreng.) Sch.Bip.	Asteraceae
Stipa setigera J.Presl	Poaceae
Stylosanthes leiocarpa Vogel	Fabaceae
Stylosanthes montevidensis Vogel	Fabaceae
Syagrus romanzoffiana (Cham.) Glassman	Arecaceae
Tibouchina gracilis (Bonpl.) Cogn.	Melastomataceae
Trachypogon montufarii (Kunth) Nees	Poaceae

Tragia bahiensis Müll.Arg.	Euphorbiaceae
Trifolium polymorphum Poir.	Fabaceae
Turnera sidoides L.	Passifloraceae
Verbena montevidensis Spreng.	Verbenaceae
Vernonanthura discolor (Spreng.) H.Rob.	Asteraceae
Vernonanthura nudiflora (Less.) H.Rob.	Asteraceae
Vernonanthura tweedieana (Baker) H.Rob.	Asteraceae
Zornia sp. J.F. Gmel.	Fabaceae

Appendix S4: Species extinction and colonization

Table S1. Mean number of species extinction and colonization per plot ($1 m^2$) for each removal treatment and for non-invaded communities (n=10), which did not differ significantly (P>0.05).

Treatments	**Extinction**	**Colonization**
Clipping at once	7.50	13.50
Clipping periodically	7.30	15.80
Herbicide	8.00	16.67
Hand-pulling	7.20	17.00
No removal	8.40	13.00
Non-invaded	4.70	16.70

Capítulo 4

"Are removal experiments effective tools for assessing plant community resistance and recovery from invasion?"

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Chapter 4: Are removal experiments effective tools for assessing plant community resistance and recovery from invasion?

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Abstract

Removal experiments are useful tools for assessing two important aspects of plant community invasion: (1) resistance to invasion and (2) recovery after invasive species removal. We discuss the potential of such experiments based on a brief systematic review of the literature on community resistance, as measured by invasibility after removal of resident species (reduction of taxonomic/functional richness), and on community recovery, as measured by resident community response after invasive species removal. We found 62 research articles, most of them related to invasive species removal. Few studies used removals to test biotic resistance, despite the importance of resident removals for identifying community components that play key roles in the often controversial invasion resistance hypotheses. Furthermore, appropriate experimental controls were rarely used, which would allow separation of the effect of local species extinction from that of disturbance. We hope this review stimulates plant ecologists to adopt removal experiments for studying invasion processes.

Keywords: Biodiversity experiments; Biotic resistance; Community recovery; Invasion process; Local extinction; Species removal

Introduction

Experimental manipulation involving plant removal has been used to gain insight into fundamental community and ecosystem processes (e.g. Armesto & Pickett 1986). The method consists of the removal of certain components of the plant community for evaluating the effects of differences in abundance and interactions among species and the effects of nonrandom local extinctions on ecosystem and community processes, as well as the factors constraining the response of natural systems to species removal (Díaz et al. 2003). In contrast to the synthetic communities frequently used in biodiversity experiments (e.g. Weigelt et al. 2010), removal experiments involve naturally assembled communities, in which the effects of environmental filters, species recruitment, dispersal and other assembly processes are likely more realistic (see Diaz et al. 2003 for a review).

Within the study of plant invasions, certain community components can be manipulated in order to test hypotheses of the complex invasion process. Two main approaches exist: (i) removal of native species from the resident community to evaluate biotic resistance, usually involving experimental introduction of an invasive species, and (ii) removal of invasive species to evaluate the effect on the native community and/or its potential for recovery (Fig. 1). Some of the key questions often proposed in these two contexts are: How does removal of native species affect the colonization and establishment of non-native invasive species? Are some species or functional groups more important for determining biotic resistance? How does the community recover after non-native invasive species removal?

Here we discuss the potential of removal experiments after a brief review and literature survey of the context in which such experiments have been applied. We consider both removals of resident and invasive species, as they deal with similar methodological difficulties for studying the effects of local extinction, allowing for a better understanding of the underlying mechanisms involved in plant invasion. We assess how many studies in plant invasion ecology have adopted a removal experiment approach, the kinds of removal methods that have been used, and if there is any common method adopted across vegetation types and species groups. We also discuss whether the results of removal experiments contribute to ecological theories of invasion ecology. Finally, we discuss the critical need for adopting proper control treatments in removal experiments and offer recommendations for further studies.

Community resistance: resident removal approach

Biotic resistance has been hypothesized to be a mechanism by which invasion success can be reduced by resident plants in the community (see Levine et al. 2004 for a review). Invasion resistance has been linked to the ability of the native community to maintain low levels of limiting resources (Davis et al. 2000). One mechanism limiting community invasibility, i.e. susceptibility of a community to invasion, is species and functional richness that act as barriers to invasion and should thus be appropriate predictors of biotic resistance. Therefore, studying the relationship between resource availability and different components of biodiversity (i.e. taxonomic and functional diversity) is relevant for the understanding of invasion processes.

Species richness has long been hypothesized to reduce invasion, mainly because resources are used more efficiently in richer communities and thus are not available for potentially invasive organisms (Elton 1958). Hence, invader success has been shown to decrease across diversity gradients both in natural communities (e.g. Perelman et al. 2007) and where diversity has been experimentally manipulated (e.g. Symstad 2000). Nonetheless, studies show contrasting results, involving different mechanisms (i.e. biotic resistance, limiting similarity and competitive exclusion) and a range of factors (i.e. spatial heterogeneity, neutral processes and productivity) for explaining invasibility. Some studies have found a positive correlation between the number of native and non-native species (e.g. Stohlgren et al. 2002), or the absence of any relationship (e.g. Lavorel et al. 1999). This relationship may depend upon the scale of analysis (Byers & Noonburg 2003) and can vary with resource availability (Davis et al. 2000; Grime & Price 2012) and disturbance (Burke & Grime 1996), which makes any generalization difficult. As a result, there is no apparent overall pattern related to the effect of taxonomic richness on invasibility (Fridley et al. 2007) and the results are often influenced by sampling effects (Wardle 2001).

For evaluating the effect of functional diversity, species are allocated to certain functional groups, either *a priori* based on observed morphological and phenological traits (e.g. Symstad 2000) or through multivariate trait analysis (e.g. Byun et al. 2013). Species-rich communities often display high functional diversity when species show different functional traits and, thereby, diverse strategies to acquire resources. Increased functional group diversity has been postulated to result in greater niche occupation and thus more efficient total resource utilization (Symstad 2000). It has been suggested that functional similarity between invaders and resident communities reduces invasion (see Price & Pärtel 2013 for a meta-analyses), based on the principle of limiting similarity, which suggests that species should be functionally different in order to coexist (MacArthur & Levins 1967). In this sense, species similar to the invader should provide greater invasion resistance due to overlap in resource utilization. However, Price & Pärtel (2013) showed that the experimental design can dramatically influence the results, as evidence for limiting similarity was found only in artificially assembled communities. These results raise questions about the role of biotic resistance through limiting similarity in natural plant communities and/or reinforce the need for further experimentation with natural communities.

Removing certain components of the resident community seems to be a promising approach to gain insight about which species or functional types play a key role in biotic resistance. This experimental approach permits control of extrinsic factors that affect plant diversity (e.g. disturbance and resource levels), which may otherwise confound results of non-experimental studies. Therefore, removal experiments are useful for manipulating richness via simulation of local extinctions in order to test biotic resistance hypothesis. Biotic resistance may be tested based on the recruitment of an invasive species that has been experimentally introduced into the resident community, in which the taxonomic or functional group richness is manipulated by removal.

Community recovery: invasive species removal approach

The response of native communities after invasive species removal offers useful insights for processes of community recovery (Zavaleta et al. 2001) and ecological restoration. Many studies suggest that the suppression of invasive species could have remarkable positive effects on the resident community (e.g. richness, abundance, cover, and seedling recruitment) (e.g. Flory 2010). Based on a meta-analysis, Andreu & Vilà

(2011) showed that invaded plots contained in average 30% fewer species than plots where invasive species were removed. This suggests that removal could increase species richness and make the native plant community more similar to non-invaded sites. Nonetheless, the experimental design for testing the effect of invasive species removal should also include controls in which native species of similar dominance level are also removed, which is rarely found in the literature.

Frequently, studies compare invaded, non-invaded and removal sites to assess the impacts of a non-native invasive plant, and the resulting native species assemblage after its removal (Andreu & Vilà 2011). Similarly, observational studies have compared invaded communities with non-invaded reference plots to infer invasive species impacts (e.g. Davis & Svejcar 2008). Although some studies found interesting and consistent results, comparisons between invaded and non-invaded communities do not allow for disentangling the effects of invasive species from unevaluated site differences prior to invasion. By comparing removal and non-removal treatments in a controlled experiment we can make unambiguous conclusions about these effects. This is not equivalent to comparing naturally invaded to un-invaded plots. Complementarily, the removal of an invasive species has helped to quantify the response of invasive species and native communities to different removal methods, mainly as a way for testing restoration strategies (see Kettenring & Adams 2011 for a meta-analysis). Throughout the text, we will use “invasive removal” as shorthand for “removal of invasive species”.

Literature survey

We compiled data on the prevalence of the two removal approaches by searching for papers in ISI Web of Knowledge database in August 2013, with no restriction on publication year. We used the following search terms: remov* AND (invas* OR exotic OR alien OR non-native OR nonnative OR nonindigenous OR non-indigenous) AND (recovery OR resilience OR impact* OR effect* response* OR resistance OR success OR restor*) AND (plant community OR vegetation community). Among the retrieved papers we retained for close examination those meeting the following criteria: (i) invasive species removal in a community recovery context without any other manipulation (e.g. native species introduction), (ii) native species removal to test biotic resistance to invasion in whereby the invasive species was experimentally introduced and (iii) natural field experiments (not a laboratory, greenhouse or agricultural setting). We used chi-square tests to compare removal methods used. For this, we used permutation tests in MULTIV software (available at http://ecoqua.ecologia.ufrgs.br).

Results

We retrieved a total of 510 articles, among which 52 studies met our criteria, seven articles were related to resident removal, and 45 to invasive species removal (Appendices S1 and S2). Additional articles were found associated with invasive removal by screening the reference lists from the retrieved papers. Therefore, we considered 62 articles in total, seven studies related to resident removal and 55 to invasive species removal. We found an increasing number of studies from 1998 to the present, with 2010 having the highest number of published articles. Surprisingly, we did not find a dominant method for resident or invasive species removal; the most common were hand-weeding (24%), herbicide application (23%) and clipping (16%). None of the studies used more than one method to remove resident species, and only 29% of the papers compared method effectiveness for invasive species removal (Appendices S1 and S2), but responses were not consistent among studies nor systems.

Among the seven articles with resident removal, two involved manipulation of taxonomic richness and five measured functional group richness (Appendix S1). Interestingly, all these studies were conducted in grassland ecosystems. The two studies that manipulated taxonomic richness eliminated rare species to simulate local extinction but no consistent results were found. One study showed that rare species removal increased community invasibility, and the other did not find a clear response. In the other studies, removed functional groups were defined by easily observed morphological traits (mainly life forms), mostly to test for an effect of limiting similarity. All the studies that manipulated functional richness found an effect of resident removal on invasibility, but results did not always corroborate the limiting similarity hypothesis. Three of the studies reported that communities without a removal treatment were less invaded than manipulated communities (Pokorny et al. 2005; Rinella et al. 2007, Sheley & James 2010).

Among the 55 invasive removal studies, 58% were centered on invasive species removal in forest ecosystems (Appendix S2). The removed species were mostly shrubs (37%), forbs (34%) and grasses (18%). Clipping, hand-weeding and herbicide application, or frequently a combination of these, were the most commonly used removal methods. Considering these three most common methods, we did not find a significant difference in removal method frequency between forests and grasslands (χ^2 = 1.28; P-value = 0.60) nor between life forms (χ^2 = 7.66; P-value = 0.10). Almost all studies found notable positive effects of invasive removal on the resident community, increasing the number of native plant seedlings and increasing community species richness (see Appendix S2). This pattern was highly consistent among studies.

Discussion

The two approaches adopted in removal experiments (native community resistance to invasion and community recovery after invasive removal) provide complementary insights for understanding the different stages of the invasion process (see Fig. 1). Resident removal provides information on biotic causes of invasive species establishment, while the invasive removal allows for inference on the impacts of removal on the resident community and associated ecosystem processes.

The sole occurrence of resident removal experiments in grasslands and not in forests, may be related to the feasibility of removal of species or specific life forms (i.e. herbaceous plants) in grasslands versus ecosystems dominated by shrubs and trees. Nonetheless, we did not expect to find so few studies with resident species removal. One reason could be related to the ethical concerns of introducing invasive species into un-invaded communities. However, experimental sites that are already partially invaded may be available, which may allow selecting, at a finer scale, experimental plots on sites that have not been invaded. We noted that most of the experiments manipulating community diversity were conducted in artificially assembled communities. Although these provide a good test of the strength of competitive interactions in a controlled environment, the action of long-term climate conditions, disturbance regimes and biotic interactions may rarely be considered and thus, limiting inference for early successional stages (Díaz et al. 2003). These limitations have not changed in recent years, thus it is often difficult to extrapolate empirical results observed in artificial communities to real communities.

Very few studies evaluated resident species removal with an interest in quantifying community resistance, which is critical at the early stage of the invasion process. Most of the studies involved invasive species removal, where the interest was detecting the effect of invasive species on community resilience. This dominant interest might be linked to its immediate applicability in management and restoration ecology. Experiments removing invasive plants were largely conducted in forest ecosystems, while all experiments removing native plants were in grasslands. Forest ecosystems are more often the focus of restoration ecology, where the control of invasive species is one of the most important issues (e.g. Hartman and McCarthy 2004). In some countries grassland systems are often still neglected in conservation biology and ecological restoration (Overbeck et al. 2007, 2013).

Our review supports, in part, the conclusion that removing invasive species has positive effects on native species richness and abundance in the remaining community. However, invasive removal can promote the establishment of other non-native species in the community (e.g. Ogden & Rejmánek 2005) and can also have other significant effects in the context of the whole ecosystem (Zavaleta et al. 2001). Nevertheless, removal experiments are useful as a methodological tool for studying the recovery of resident communities.

Although removal experiments are often the approach available to gain insight into certain aspects of the invasion process, there are methodological limitations that should be considered for proper interpretation of results. Diaz et al. (2003) suggested that the removal effect might be the result of at least three components, although unfortunately not many studies have considered them. Firstly, the effect of the loss of a certain functional type, which is the primary interest of most of the studies; how does the community respond to that absence? Secondly, the effect mediated by the response of other remaining plants, which depends on which plants occupy the space and the resources released by the removed plants. Finally, the disturbance effect itself, involving changes in resource supply or physical interference with habitat structure for the remaining organisms. This final issue is related to the removal method used. Hand-weeding often disturbs the soil environment, while chemical treatments might leave unknown toxic residues that could inhibit native plant recruitment (Rodriguez & Jacobo 2012). Alternatively, if only aboveground clipping is employed, belowground parts may continue to be active and eventually resprout aboveground biomass, which can require repeated clipping (Joner et al. 2011). Using removal methods without thoroughly testing their effectiveness and non-target effects might lead to ambiguous responses.

As a way to deal with disturbance caused by the removal, adequate experimental controls should be implemented, which has been scarcely considered according to our literature search. Including a treatment without removal does not mean that non-target removal effects are controlled. Regarding resident removal, compositional effects of species or functional group removal with dissimilar biomass amount might confound dominance and species identity effects. We found only one study that included an appropriate treatment to control for these effects, in which diversity was altered by removal of less abundant species and the resulting disturbance was controlled by removal of an equivalent amount of biomass of the most common species (Lyons & Schwartz 2001). Regarding invasive removal, how can we distinguish community response to invasive species local extinction from disturbance effect caused by biomass removal? We found two studies that evaluated the response of non-target species followed by herbicide treatment to control an invasive species. Herbicide treatments either reduced species richness, evenness and diversity after herbicide application (Almquist & Lym 2010) or increased forb abundance and grass cover (Ruffner & Barnes 2010). Similarly, Wilke & Irwin (2010) removed proportional amounts of resident biomass in un-invaded plots to control for invasive removal disturbance, and based on lack of a difference between removal treatments (invasive vs. resident removal) they suggested there was a negligible disturbance effect on the evaluated variables. Skurski et al. (2013) established ground disturbance similar to manual removal effects, suggesting that disturbance tended to have a slight negative effect on native grass cover. Considering these controls, it is possible to filter confounding factors and obtain more solid conclusions, distinguishing the effects of local extinction from disturbance. Unfortunately none of the studies described here have invested in a detailed discussion about such experimental controls.

We support the broad conclusion that removal experiments are effective tools for assessing plant community resistance and recovery from invasion, as long as the experimental design is adequate. For future studies, we strongly recommend inclusion of a removal method control to distinguish the effects of biomass removal disturbance from the local extinction treatment effect. For the resident removal approach, the control could include removal of the same resident biomass without altering taxonomic or functional richness, and for invasive removal the removal of equivalent resident biomass.

We hope this review stimulates discussion on the approaches used in removal experiments for studying invasion processes. We highlight the implication of including both types of removals in field experiments, emphasizing the importance of increasing the number of resident removal experiments, and the significance of using appropriate experimental controls. More studies in which the effectiveness of the removal method is tested are needed to gain insight regarding their non-target effects and hence, provide further information about removal method selection**.**

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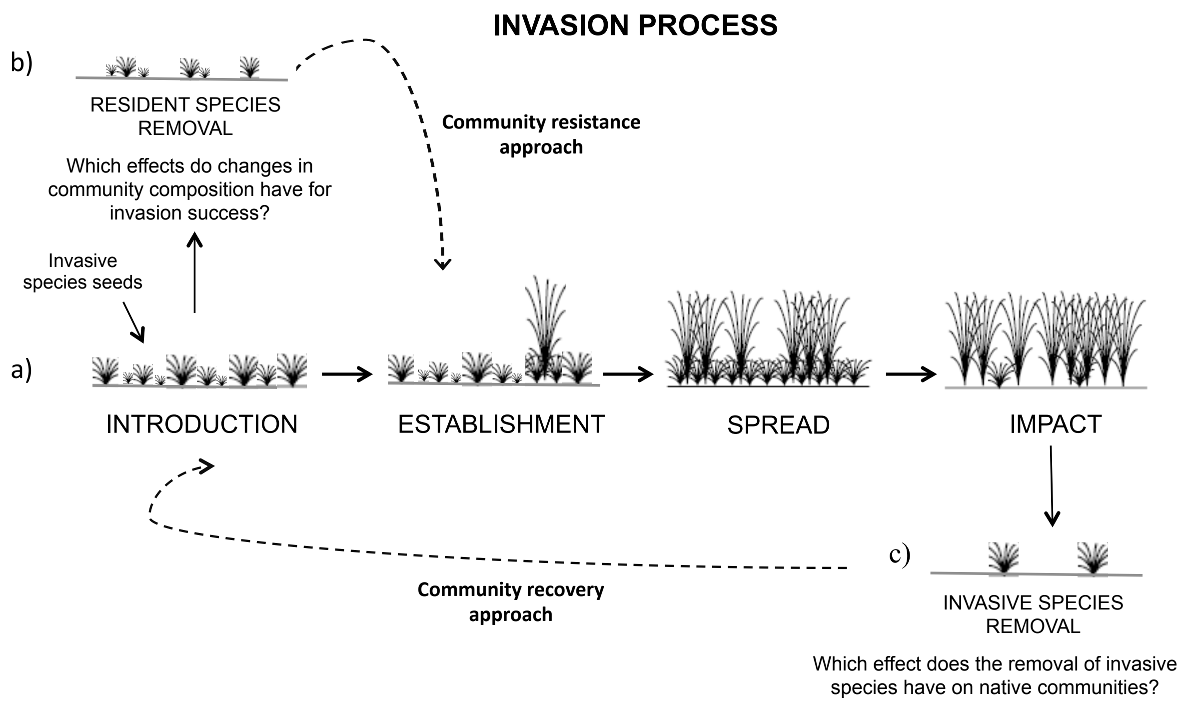

Figure 1. Removal experiments approach to study invasion process. (a) Invasion process stages (modified from Levine et al. 2004). (b) Removal of resident species to evaluate biotic resistance. (c) Removal of invasive species to evaluate community recovery.

Electronic appendices

Appendix S1. Studies included in our review about the invasive species response to resident removal treatment (taxonomic richness or functional group manipulation).

Appendix S2. Studies included in our review about resident community recovery to invasive species removal.

Appendix S1

Supporting Information to the paper Guido, A. & Pillar, V.D. Are removal experiments effective tools for assessing plant community resistance and recovery from invasion? *Journal of Vegetation Science*. Studies included in our review about the invasive species response to resident removal treatment (taxonomic richness or functional group manipulation).

System	**Invasive species**	**Manipulated factor**	**Removal treatments**	**Removal method**	**Invasive species response**	**References**
Grassland	*Lolium temulentum*	Taxonomic richness	Rare species	Hand-weeding	Highest density	Lyons & Schwartz 2001
Grassland	*Melilotus officinalis*	Taxonomic richness	Rare species	Clipping + Herbicide	None	Smith et al. 2004
Grassland	*Cytisus scoparius*	Functional group	Shrubs	Clipping		Bellingham & Coomes 2003
			Tussocks			
			Shrubs + Tussocks		Lowest seedling density	
			None			
Grassland	*Centaurea maculosa*	Functional group	All plants	Herbicide	Highest density and biomass	Pokorny et al. 2005
			All forbs			
			Grasses			
			Deep forbs			
			Shallow forbs			
			Spikemoss			
			None		Lowest density and biomass	

Grassland	*Centaurea maculosa*	Functional group	All plants	Herbicide	Highest density	Rinella et al. 2007
			All forbs			
			Grasses			
			Cryptogam layers			
			Shallow-rooted forbs			
			Spikemoss			
			None		Lowest density and biomass	
Grassland	*Taeniatherum caput-medusae*	Functional group	Annual forbs	Herbicide		James et al. 2008
			Perennials forbs			
			Bunchgrasses		Highest density	
			None			
Grassland	*Taeniatherum caput-medusae*	Functional group	All plants	Herbicide		Sheley & James 2010
			Shrubs			
			Perennial grasses		Highest density	
			Taprooted forbs			
			Rhizomatous forbs			
			Annual forbs			
			Mosses			
			None			

Appendix S2

Supporting Information to the paper Guido, A. & Pillar, V.D. Are removal experiments effective tools for assessing plant community resistance and recovery from invasion? *Journal of Vegetation Science*. Studies included in our review about the resident community recovery to invasive species removal (*additional studies not found in the systematic search but considered in the review).

System	Invasive species removed	Life form	Removal method	Community main response	Reference
Forest	*Schizachyrium condensatum*	Grass	Hand-weeding	Increase shrubs size, leaf tissue nitrogen and seedling density	D'Antonio et al. 1998
Forest	*Lonicera maackii*	Shrub	Clipping	Increase tree seedling survival	Gorchov & Trisel 2003
Forest	*Alliaria petiolata*	Forb	Herbicide	Increase cover of some native species	Carlson & Grochov 2004*
Forest	*Rhamnus frangula*	Shrub	Clipping	Increased tree seedling	Frappier et al. 2004*
Forest	*Lonicera maackii*	Shrub	Clipping Herbicide	Increase seedling survival	Hartman & McCarthy 2004*
Forest	*Hedera helix*	Shrub	Hand-weeding	Increase species cover	Dlugosch 2005
Forest	*Tamarix* spp.	Shrub	Clipping Burning	Negligible	Harms & Hiebert 2006
Forest	*Impatiens glandulifera*	Forb	Hand-weeding	Negligible	Hejda & Pysek 2006
Forest	*Impatiens glandulifera*	Forb	Clipping	Increase seedling density, diversity and non-native species proportion	Hulme & Bremner 2006 *
Forest	*Hedera helix*	Shrub	Herbicide Hand-weeding	Increase seedling density and diversity	Biggerstaff & Beck 2007*
Forest	*Alliaria petiolata*	Forb	Hand-weeding	Increase diversity	Stinson et al. 2007

Forest	*Elaeagnus umbellata*	Shrub	Clipping + Hand-weeding	Changes in species composition	Vidra et al. 2007
	Lonicera japonica	Shrub	Clipping + Hand-weeding		
	Ligustrum sinense	Shrub	Clipping + Hand-weeding		
	Microstegium vimineum	Grass	Clipping + Hand-weeding		
Forest	*Microstegium vimineum*	Grass	Hand-weeding	Increase species richness	Judge et al. 2008
			Herbicide		
Forest	*Lonicera maackii*	Shrub	Clipping + Herbicide	Not significant	Swab et al. 2008*
Forest	*Mimulus guttatus*	Forb	Hand-weeding	Increase species richness	Truscott et al. 2008
Forest	*Lantana camara*	Shrub	Unexplained	Increase species richness, abundance and recruitment	Gooden et al. 2009
Forest	*Microstegium vimineum*	Grass	Hand-weeding	Increase diversity	Flory & Clay 2009
			Herbicide		
Forest	*Microstegium vimineum*	Grass	Clipping	Not significant	Flory & Lewis 2009
			Hand-weeding		
			Burning		
Forest	*Ligustrum sinense*	Shrub	Mulching + Herbicide	Increase species cover	Hanula et al. 2009
			Hand-weeding + Herbicide		
Forest	*Psidium cattleianum*	Tree	Clipping + Herbicide	Lower leaf area index, less litterfall mass	Ostertag et al. 2009*
	Macaranga mappa	Shrub	Clipping + Herbicide		
	Melastoma septemnervium	Shrub	Hand-weeding + Herbicide		
	Falcataria moluccana	Tree	Clipping + Herbicide		
	Clidemia hirta	Shrub	Hand-weeding + Herbicide		

Forest	*Hesperis matronalis*	Forb	Hand-weeding	Increase woody exotic cover	Pavlovic et al. 2009
Forest	*Microstegium vimineum*	Grass	Hand-weeding	Increase species richness	DeMeester & Richter 2010
Forest	*Microstegium vimineum*	Grass	Hand-weeding Herbicide	Increased productivity	Flory 2010
Forest	*Pittosporum undulatum* *Hedychium gardneranum* *Clethra arborea* *Acacia melanoxylon*	Tree Forb Tree Shrub	Hand-weeding + Herbicide Hand-weeding + Herbicide Hand-weeding + Herbicide Hand-weeding + Herbicide	Increase seeds abundance	Heleno et al. 2010
Forest	*Hedychium gardnerianum*	Forb	Clipping + Herbicide	Increase tree seedling density	Minden et al. 2010*
Forest	*Lantana camara*	Shrub	Clipping + Hand-weeding	Changes in species composition	Prasad 2010
Forest	Unspecified		Unexplained	Increase seedling richness and abundance	Baider & Florens 2011
Forest	*Rubus ulmifolius*	Shrub	Clipping + Herbicide	Increase proportion of natives species	Mazzolari et al. 2011
Forest	*Melilotus alba*	Forb	Clipping	Increase seedling survival	Spellman & Wurtz 2011
Forest	*Impatiens parviflora*	Forb	Unexplained	Changes in species composition	Hejda 2012
Forest	*Falcataria moluccana*	Tree	Girdling	Increase in native species biomass	Hughes et al. 2012
Forest	*Megathyrsus maximus*	Grass	Hand-weeding	Increase species richness	Rojas-Sandoval et al. 2012
Forest	*Polygonum cuspidatum*	Forb	Herbicide	Increase exotic species richness and total vegetation cover	Claeson & Bisson 2013
Forest	*Aristotelia chilensis* *Rubus ulmifolius*	Shrub Shrub	Clipping + Herbicide Clipping + Herbicide	Increase native and exotic species richness	Vargas et al. 2013

Grassland	*Cytisus scoparius*	Forb	Hand-weeding	Increase seedling density	Ussery & Krannitz 1998
			Clipping		
Grassland	*Foeniculum vulgare*	Forb	Burning + Herbicide	Changes in species composition	Ogden & Rejmánek 2005
Grassland	*Erodium cicutarium*	Forb	Hand-weeding	Increase abundance and richness of native annual plants	Schutzenhofer & Valone 2006
Grassland	*Potentilla recta*	Forb	Herbicide	Increase native perennial grass cover and biomass	Sheley & Denny 2006
			Hand-weeding	Increased species richness	
Grassland	*Artemisia tridentata* subsp. *wyomingensis*	Shrub	Burning	Increase herbaceous aboveground annual production and cover	Davies et al. 2007
Grassland	*Juniperus occidentalis*	Tree	Burning	Increased total grass cover and productivity	Coultrap et al. 200
			Clipping		
			Herbicide		
Grassland	*Eragrostis lehmanniana*	Grass	Herbicide	Increase species richness and cover	Crimmins & McPherson 2008
Grassland	*Lonicera morrowii*	Shrub	Hand-weeding	Increased metrics of herbaceous community quality	Love & Anderson 2009
			Clipping	Increased metrics of herbaceous community quality	
			Herbicide	Reduced metrics of herbaceous community quality	
Grassland	*Cirsium arvense*	Forb	Herbicide	Decrease species richness, evenness and diversity	Almquist & Lym 2010
Grassland	*Schedonorus phoenix*	Grass	Herbicide	Increase native grass cover and forbs abundance	Ruffner & Barnes 2010
Grassland	*Linaria vulgaris*	Forb	Hand-weeding	Alters flowering patters	Wike & Irwin 2010

Grassland	*Frangula alnus*	Shrub	Herbicide	Increase forbs cover and changes in species composition and abundance	Friedler et al. 2012
	Phalaris arundinacea	Grass	Herbicide		
	Rosa multiflora	Shrub	Herbicide		
	Cirsium arvense	Forb	Herbicide		
	Typha sp.	Forb	Clipping + Herbicide		
	Populus tremuloides	Tree	Clipping + Herbicide		
Grassland	*Solidago gigantea*	Forb	Clipping	Increase cover, abundance and seedling density	Saito & Tsuyuzaki 2012
Grassland	*Centaurea stoebe*	Forb	Hand-weeding	Increase native forb cover	Skurski et al. 2013
			Herbicide	Decrease native forb cover and increase exotic grass cover	
Coastal dune	*Carpobrotus* sp.	Forb	Hand-weeding	Increase species richness, especially annual plants	Andreu et al. 2010*
Coastal dune	*Acacia longifolia*	Shrub	Clipping	Changes in seed banks	Marchante et al. 2011a*
Coastal dune	*Acacia longifolia*	Shrub	Clipping	Increase native and exotic species richness, cover and diversity	Marchante et al. 2011b
Savanna	*Poa pratensis*	Grass	Clipping	Increase species richness	MacDougall & Turkington 2005
			Hand-weeding		
	Dactylis glomerata	Grass	Clipping		
			Hand-weeding		
Desert	*Brassica tournefortii*	Forb	Hand-weeding	Increase in native plant reproduction and annual plants richness	Barrows et al. 2009
Island	*Cinchona pubescens*	Forb	Hand-weeding	Increase species richness and diversity	Jäger & Kowarik 2010
Shrubland	Multiple		Herbicide	Increase native annual dominance and perennial abundance	Steers & Allen 2010
			Hand-weeding + Herbicide		
			Raking		

Considerações finais

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De forma geral, nesta tese investigamos diferentes questões associadas ao processo de invasão de plantas para entender os mecanismos, impactos e medidas de recuperação da comunidade, abordando diferentes estratégias metodológicas que incluem estudos observacionais, experimentos de remoção e uma revisão bibliográfica. Esta tese é um dos poucos trabalhos que se propõe a analisar a invasão de *Eragrostis plana,* planta invasora mais frequente dos campos sulinos, utilizando uma abordagem ecológica, integrando diferentes questões associadas ao processo de invasão biológica e desenhos experimentais. Os resultados obtidos contribuem para o entendimento do processo de invasão de plantas nos campos sulinos, com vistas ao manejo, à conservação e à restauração das comunidades invadidas, e recomendado um melhor planejamento de desenhos experimentais para obter resultados mais consistentes.

O **Capítulo 1** é a primeira contribuição que, mediante uma metodologia adequada na escala regional, quantifica o grau de invasão real dos campos sulinos por plantas exóticas invasoras. Os resultados identificaram como as relações entre o clima e a estrutura da paisagem podem determinar o grau de invasão dos ecossistemas campestres. Observou-se que os padrões de invasão estão principalmente relacionados com maior densidade de estradas, menor cobertura de campo nativo na paisagem e com o aumento do déficit hídrico do ambiente. Os resultados encontrados são consistentes com a literatura, salientando que tanto o clima quanto as modificações antrópicas na paisagem podem determinar o grau de invasão dos ecossistemas naturais (e.g. Hobbs 2000; With 2002). Entretanto, a inclusão de mais espécies invasoras e maior número de áreas amostradas seria fundamental para continuar avançando no entendimento da invasibilidade dos ecossistemas campestres do sul do

Brasil. Por outro lado, constatou-se que *Eragrostis plana* é a planta invasora mais frequente e abundante dos campos sulinos. No entanto, os resultados do experimento do **Capítulo 2** mostraram que sua invasão não pode ser explicada pela riqueza de espécies ou composição dos grupos funcionais de plantas da comunidade. A invasão de *E. plana* foi principalmente associada ao distúrbio causado pela remoção da biomassa, o qual já tinha sido evidenciado em outros trabalhos de abordagem agronômica (e.g. Medeiros & Focht 2007; Focht & Medeiros 2012). Neste sentido, as comunidades campestres poderiam ser resistentes à invasão de *E. plana* até que algum distúrbio aumente sua vulnerabilidade. Com o aumento do nível de distúrbio, as comunidades tornam-se mais suscetíveis à invasão por *E. plana*, permitindo seu estabelecimento e expansão, causando assim um impacto na vegetação nativa. Neste contexto, mediante um experimento de três anos de duração, o **Capítulo 3** contribui no entendimento do impacto real de *E. plana* na vegetação campestre. Os resultados mostraram uma redução na riqueza e cobertura de plantas nativas nas comunidades invadidas. Estes resultados são consistentes com outros estudos, onde indicam que as plantas invasoras substituem as espécies nativas no local onde invadem (e.g. Flory & Clay 2009; Andreu et al. 2010; Vilà et al. 2011).

Embora os métodos de remoção de espécies sejam úteis para investigar questões associadas ao processo de invasão, existem limitações importantes a considerar, como foi evidenciado nos **Capítulos 2** e **3**. Neste sentido, na revisão bibliográfica sistemática do **Capítulo 4** foi discutido o potencial dos métodos de remoção utilizados para estudar a resistência e a recuperação da comunidade à invasão. Como resultado, o **Capítulo 4** mostra que a maioria dos trabalhos não propõem controles adequados nos experimentos, o que pode confundir seus efeitos.

Desta forma, foram desenvolvidas algumas sugestões a serem consideradas nos experimentos de remoção de espécies, com o objetivo de continuar avançando nesta temática.

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