

Composition and phylogenetic structure of Pampean Grasslands under distinct land use and presence of alien species

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Abstract

Alien species can modify ecosystem functions and ecological processes in natural communities, and potentially become invasive. In the Brazilian Pampean grasslands, reports of changes in land use and invasions of alien plant species are becoming more frequent. This study aimed to investigate species composition and phylogenetic relationships between native and alien plants across four sites of Brazilian Pampean grasslands under distinct land uses (NOM: no agricultural management; GRZ: grazed pastures; AGR: agrarian crops; ROAD: roadside). The phylogenetic relationship between native and alien species was analyzed at two scales: inter-site (large scale) and intra-site (small scale). We found inter-site differences in phylogenetic diversity. Overall, across all sites we found random phylogenetic relationships among alien and native species. In the most disturbed site (ROAD) we found significant phylogenetic clustering among all species (alien and natives), while at the small scale, clustering was only found among natives. We conclude that clustering of phylogenetic relationships among alien and native species is only evident at small sampling scales in environments subject to high levels of disturbance (i.e., road sides) in the studied Pampean Grasslands, suggesting that environmental filtering plays an important role in local community assembly.

Keywords: disturbance, land use, phylogenetic pattern, plant invasion, native communities

Introduction

Plant communities are driven by factors such as environmental abiotic filters, plant-interactions, coevolution of functional traits, and by chance (Hubbell 2001; Kraft et al. 2007; Cavender-Bares et al. 2009; Occhipinti 2013). Alien species can modify ecological processes in natural communities (Vilà et al. 2011) by altering resource availability (e.g. soil nutrients), thus changing the environmental prerequisites which determine plant establishment and naturalization, and consequently, modifying the vegetation structure (Gordon 1998;

Alpert et al. 2000; Richardson et al. 2000; Liao et al. 2008; Fonseca et al. 2013; Lapiedra et al. 2015). In view of this, changes in local conditions might potentially facilitate a successful invasion by an alien species (Lapiedra et al. 2015). Many studies have reported alien dominance over native species (Ghazoul 2004; Stinson et al. 2006), which may be because a particular alien species might be a more efficient competitor than those in the established native cohort (Callaway and Aschehoug 2000; Pyšek et al. 2017; Bartz and Kowarik 2019). This alien dominance might cause the displacement of native plants due to the negative effects of habitat alterations imposed by factors such as increasing alien propagule pressure and alterations to the nutrient cycle, which subsequently stifles the growth of native seedlings and adult plants (Vilà et al. 2011; Rai and Singh 2020). On the other hand, alien species can also benefit the native communities by extending flowering season, enhancing resources for pollinators when native species are not flowering (Salisbury et al. 2015) and increasing pollinator visitation (Lopezaraiza-Mikel et al. 2007). Furthermore, it is not uncommon to observe native tolerance of the establishment of exotic species in grasslands (Martín-Forés et al. 2016).

Two contrasting hypotheses can be posed with respect to plant community assembly in the presence of alien plant species. First, environmental filtering associated with mutualistic interactions established by native species could be exploited by alien species (Traveset and Richardson 2014). This increases the occurrence of alien species which are functionally (e.g., life form, morphology, physiology) and phylogenetically more closely related to the native species set, than would be expected by chance (phylogenetic clustering) (Kraft et al. 2007; Leibold et al. 2010), which may reduce the potential invasive success of the alien species (Strauss et al. 2006). Second, negative plant interactions (e.g., competition) result in a community composed of species more phylogenetically distant than would be expected by chance, i.e., phylogenetic overdispersion (Novotny et al. 2002; Webb et al. 2002; Peters 2003; Valiente-Banuet and Verdú 2007; Mayfield and Levine 2010; Godoy et al. 2014). However, if these factors are not significant drivers, stochastic factors (Hubbell 2001) such as dispersal limitation and disturbance will result in a community with a phylogenetic structure not significantly different than would be expected by chance (Kembel and Hubbell 2006; Mi et al. 2016). Further, sampling scale can also influence the detection of these specific functional and phylogenetic trends (e.g., clustering or overdispersion), since the strength of factors driving community assembly such as competition, is expected to be more dominant at small i.e., neighborhood scale, and environmental filtering is assumed to exert stronger influence at larger (i.e., habitat/community) scales (Cavender-Bares et al. 2009; Vilà et al. 2011; Diniz et al. 2021). Therefore, opposing results at two scales might be indicative of the differing influence of abiotic and biotic effects on the relationships between native and alien species, and the community assembly, at different spatial scales (Thuiller et al. 2010; Loiola et al. 2018).

Disturbances caused by distinct land use types can facilitate the probability of the successful establishment of alien species (Fonseca et al. 2013). If we take into account the Naturalization Hypothesis (Darwin 1859), confirmed in several important studies (Strauss et al. 2006; Park and Potter 2013; Bezeng et al. 2015), one can assume that disturbances occurring in phylogenetically poorer communities might favor alien species with trait distinctiveness that allows them to more efficiently exploit the newly created empty niches (Elton 1958). Thus, in a phylogenetic community randomly assembled by disturbance events, alien species might have a higher probability of establishment success, because environmental constrictions and competitive interactions will exert a weak force (Tilman 2004). However, after disturbance, the assembly of the resultant community also depends upon the trait plasticity of the alien species and the remaining pool of native species and their functional and phylogenetic similarity (Prinzing et al. 2002; Strauss et al. 2006; Lapiedra et al. 2015). When alien species are closely related and present similar traits to the native species, naturalization of the former might be expected, thus leading to community functional and phylogenetic clustering (Lososová et al. 2005; Liendo et al. 2021). Conversely, under a scenario of functional and phylogenetic distinctiveness between alien and native species, invasion success (i.e., invasiveness) tends to be favored over naturalization and results in community functional and phylogenetic overdispersion (Gerhold et al. 2011; Park and, Potter et al. 2013. Bezeng et al. 2015).

Pampean grasslands occupy a vast area across Southern Brazil (60 % of Rio Grande do Sul state approximately), Argentina and Uruguay. Historically, intense anthropogenic land use has occurred in such grasslands, a circumstance which facilitates biological invasion (Fonseca et al. 2013), offering an interesting scenario to

investigate issues concerning relationships among alien and native species. Southern Brazil is renowned for intensive agriculture and livestock production, which leads to significant disturbances that favor the establishment of ruderal and weedy plants (Hobbs and Huenneke 1992; Overbeck et al. 2007). Past and current land uses of Pampean grasslands have remarkably altered disturbance regimes, thus affecting present environmental conditions and influencing ecological processes (e.g., environmental filtering and interactions) that might lead to alien plant invasion (Pauchard and Alabak 2004). The presence of roads (Trombulak and Frissell 2000), intensive grazing and agrarian activities associated with fire management (Hobbs and Huenneke 1992; Baldissera et al. 2010) are strong determinants of the enhancement of alien species dispersal on Pampean grasslands. However, for the large part these alien species are naturalized and co-occur in balance with native species in Rio Grande do Sul state, though some species have become aggressive invaders (Rolim et al. 2015).

A total of 356 invasive alien plant species have been recorded in the Pampean grasslands, of which 153 species have been recorded in the Brazilian Pampas (Fonseca et al. 2013). Nevertheless, there is a lack of studies exploring phylogenetic relatedness between native and alien species, and in view of relatedness, the effect of distinct land uses on the interactions between native and alien species and the subsequent implications for the susceptibility of the Pampa grasslands to invasion or naturalization of alien species. Most studies seeking to explore the human influence on the susceptibility of ecosystems to the alien species' invasiveness and potential to naturalize, have been conducted on other types of vegetation e.g. forests (Pauchard and Alabak 2004), roadsides (Christen and Matlack 2009; Meunier and Lavoie 2012) or pasture (Lunt 1990; DiTomaso 2000; Lisboa et al. 2009).

This study sought to provide new insights into the consequences of the occurrence of alien plant species (naturalized and invasive) in Southern Brazilian grasslands and the implications of phylogenetic relatedness between native and alien species. We investigated species composition and phylogenetic diversity and structure at four sites with distinct land uses in the Brazilian Pampean grasslands. The central questions we raised were: a) are the pools of alien and native species evenly distributed among sites? b) is overall (alien and native species) phylogenetic diversity similar across sites? c) are there significant phylogenetic relationships (i.e. clustering or overdispersion) among alien and native species at different sites? c) are there phylogenetic patterns (diversity and structure) dependent upon the sampling scale (small or large) under consideration?

Material and Methods

Study sites

This study was conducted at four different sites within the Pampa biome, each with different land uses, and consequently, distinct levels and intensity of disturbance conditions specific to each one (Fig. 1). Thus, these sites were classified as: NOM (no agricultural management; 30°20'4.19"S and 54°21'33.76"W), which is a site that has not been used for pasture or agricultural purposes, and had no current management regime, therefore being the least disturbed site; GRZ (grazed pastures; 30°04'80.88"S 55°55'42.94"O) represents a site under low density or moderate density cattle and sheep grazing; AGR (agrarian crops; 30°22'15.74"S and 54°19'47.32"W), which is subject to more intense anthropogenic activity in the landscape, mainly soybean cultivation. Finally, ROAD represented a site that might be subjected to several sources of disturbance. Samples for this site were collected along the roadside of Br 290 (30°19'48.94"S and 54°21'36.52"W). AGR, NOM and ROAD sampled sites were located in the municipality of São Gabriel, while GRZ was located in the municipality of Alegrete, both municipalities in Rio Grande do Sul state, Brazil.

The Pampa biome occupies 2.07 % of the Brazilian territory and 63% of the state of Rio Grande do Sul (IBGE 2004), the only state dominated by this biome in Brazil. Southern Brazil ranges between temperate and subtropical climates (Overbeck et al. 2007) and is characterized by warm summers and cool winters with no dry season (Mack 1989; Overbeck et al. 2007; Pillar et al. 2009). The predominant vegetation of the

Pampa biome is grasslands, comprising a high number of herbaceous species, some shrubs and small trees (Pillar *et al.* 2009).

Data collection

Experimental design

We created transects within each of the four study sites. At the ROAD site, 2 x 200 m transects on either side of, and parallel to the road, were established. At both the GRZ and NOM site, we established 400 m long transects, traversing the study site. At the AGR site, we created a 400m transect along the edge of the soybean cultivation. Within each site, we positioned 20 x 1 m² subplots at 20 m intervals along the transect, and sampled and quantified the plant cover-abundance within each subplot using the Braun-Blanquet method (Braun-Blanquet). Each of the 1 m² subplots was subdivided into a 10 x 10 cm grid to quantify the percentage of cover for each plant species (hereafter, plant cover). Plant cover was estimated and allocated to one of thirteen grades of cover where 0.1 = <1%, 0.2 = 1-3%, 0.4 = 3-5%, 1 = 5-15%, 2 = 15-25%, 3 = 25-35%, 4 = 35-45%, 5 = 45-55%, 6 = 55-65%, 7 = 65-75%, 8 = 75-85%, 9 = 85-95% and 10 = 95-100%. All the plants were identified either in the field or with the assistance of a plant specialist, and placed in the HBEI herbarium at the Universidade Federal do Pampa.

Phylogeny reconstruction

We pruned the megatree “GBOTB.extended” to all tree taxa from our metacommunity (pooled species of the four sites totaling 104 species) in order to generate our phylogenetic tree, using the phylo.maker function from the V.PhyloMaker package (Jin and Qian 2019) in R version 3.6.1 (R Development Core Team 2019). We used the data frame of this megatree (generated with the function build.nodes.1) and applied the phylogenetic hypothesis under scenario 3 (using the function phylo.maker) to extract the genus- or family-level largest cluster’s root and basal node information (Jin and Qian 2019). Scenario 3 binds the tip for a new genus between the family root node and basal node (Qian and Jin 2016). Since the mega-tree embedded in the algorithm of V.phyloMaker is built upon fossil records contained in GenBank and the Open Tree of Life (Smith and Brown 2018) including all plant families and majority of genera, the resultant phylogenetic tree, by the insertion of species lists, has all families and most of genera fully resolved (Jin & Quian 2019). Our resultant phylogeny had 104 tips and 95 internal nodes, i.e. 8 remaining unresolved taxa or 7.69% of unresolved. Thus, our phylogeny holds a high percentage (92.31%) of phylogenetic resolution.

Data analyses

Phylogenetic Diversity

We analyzed phylogenetic diversity patterns across the four sites using the phylogenetic dissimilarity metric provided by evolutionary PCA (Principal component analysis) with the function evoPCAHellinger of the package adiv (Pavoine 2020) based on Hellinger distance (Rao 1995; Pavoine 2016). The Hellinger distance centres the data of the community matrix based on the presence or absence of the species in the subplots of the sites, thus normalizing data by communities’ size (Rao 1995). In practice, the Hellinger distance is recommended when analyzing ordination of species abundance data (e.g., evoPCA) in heterogeneous sampled data, since it gives more even weights for the species by dividing the abundance of each species by the site total abundance, resulting in a square root transformation (Legendre and Gallagher 2001; Borcard *et al.* 2011). Therefore, the computed phylogenetic dissimilarity with evoPCAHellinger offers a robust index that enables the investigation of phylogenetic patterns in gradients (e.g., disturbance) along the geographic space

(Pavoine 2016). We then evaluated the individual contribution of the study sites and of the species to the variance in the evoPCA by decomposing the inertia for its axes using the function `inertia.dudi` of the package `ade4` (Dray and Dufour 2007).

Phylogenetic community structure

We analyzed the phylogenetic diversity and structure for each of the four sites. The analyses were performed for each of the four sites by first splitting the set of species into two pools: all plant species (alien and native plant species) and just natives. The combined whole sample (all species and just natives) was used to test for overall trends in phylogenetic relationships. In the pool of all species, we included the native and naturalized alien species, and additionally the two invasive species we recorded (i.e., *Echiumplantagineum* and *Eragrostis plana*; see Results section for details), which we excluded from the natives only pool. We created these pools to enable us to disentangle the influence of native and non-native invasive species on phylogenetic dispersion. This takes into account the fact that even a small number of, or single highly competitive and productive alien species, can exert influence in the phylogenetic structure of local communities, especially if they are phylogenetically distinct (i.e., from distant clades) and do not coexist with close relatives in these communities (Vilà et al. 2011; Loiola et al. 2018; Baroughy et al. 2021).

To test for the influence of sampling scale on the phylogenetic relationships between species (all species and just natives), we computed phylogenetic metrics per subplots within each site (small scale) and for site level (large scale). This allowed us to test whether increasing the sampling scale (from small to large) affected the observed phylogenetic patterns and inferred some dependent-scale trend (i.e., clustering or overdispersion), as might be expected in accordance with the known influence of distinct spatial scale and sampling subsets in the phylogenetic relationships (Cavender-Bares et al. 2009; Diniz et al. 2021). Throughout the text we refer to large scale as the single values of the phylogenetic metrics representing the average per site derived from all its subplots, whereas the small scale refers to the values calculated and evaluated for each of all subplots of the sites. We calculated the phylogenetic mean pairwise distance (MPD) between different taxa (Webb et al. 2002), using the function `mpd` in the package `picante` (Kembel et al. 2010). The MPD represents the deepest evolutionary splits encompassing the oldest clades and basal nodes (Webb et al. 2002). The larger the MPD value, the larger the phylogenetic overdispersion, i.e., community mostly assembled by distant phylogenetic relatives. Based on the mean and standard deviation of MPD, we computed its standardized size effect with the function `ses.mpd` from `picante`. The `sesMPD` was computed using the unconstrained “phylogeny.pool” null model, performed with 10,000 randomizations, in which species identities were shuffled within conducted surveys (Kembel and Hubbell 2006). Thus, using an unconstrained null model, all species have equal probability of being included in the randomized communities (Webb et al. 2011). In order to keep and correctly use the widely employed NRI (Net relatedness index) nomenclature from `Phylocom` (Webb et al. 2002, 2008), we multiplied the resultant scores of `sesMPD` by -1. Negative values of NRI imply phylogenetic overdispersion (i.e. distantly related), while positive values imply phylogenetic clustering (i.e. closely related).

We performed `Simper` analysis on plant cover using the function `simper` of the package `vegan` (Oksanen et al. 2018) in order to determine the degree to which each plant species contributed to the richness found in the sites, and in which site they were most influential (Clarke 1993). The significance of the mean NRI, calculated from subplots within each site (small scale) for both pools of species (all and only natives), was tested by applying a One Sample T-test computed in R version 3.6.1. A mean (positive or negative) significantly different from zero indicates a significant phylogenetic pattern (clustering or overdispersion). We evaluated the significance of single values of NRI averaged per site (large scale), by applying the 95% confidence interval, which ranged between 1.96 and -1.96. Values outside this range are considered to deviate from the mean and are therefore significant (Forthofer et al. 2007; Zar 2010). Thus, significant overdispersion was represented by a value lower than -1.96 and significant clustering by a value higher than 1.96 (Gotelli and Entsminger 2003). Significance of single values of MPD per site were interpreted by evaluating the percent of

the MPD ranked values (observed values vs. randomized values from the null communities), computed by the “phylogeny.pool” null model, falling into the range (2.5% = significance level of 0.05) of a 97.5 % confidence interval (Zar 2010). Thus, we considered single MPD values to indicate overdispersion if less than 2.5% of the ranked values were lower than the total of randomizations (i.e., 10,000) computed by the null model. Clustering is indicated when less than 2.5% of the ranked values are higher than total of randomizations (Gotelli and Entsminger 2003). We conducted comparisons of the means of the phylogenetic metrics (MPD and NRI) between subplots (small scale) within each site for both pools (all species and only natives) by applying the Welch Two Sample t-test in R. Throughout the following text we refer to comparisons at the large scale as inter-site, and comparisons at the small scale as intra-site.

Results

Floristic composition

We found a total of 104 herbaceous plant species (Table S1 in Supplementary material) across the four sites, of which, 12 are alien species. We recorded eight different families of alien species (Table 1) of which ten species are naturalized and two are considered invasive species. The ROAD site demonstrated the greater richness of naturalized alien species (6 species) followed by AGR (5 species) (Table 2). Further, we found both of the alien invasive species recorded in our sampling, *Eragrostis plana* Nees (Poaceae) and *Echium plantagineum* L. (Boraginaceae) (Table 1), in the AGR and ROAD sites (Table 2). The highest number and proportion of native species was recorded in the least disturbed sites, GRZ and NOM respectively (Table 2). Asteraceae and Poaceae were the most commonly recorded alien plant families (Table 1).

The results of the SIMPER analysis revealed that the invasive species *Eragrostis plana* Nees was the greatest contributor to the inter-site overall composition differences, followed by native *Paspalum notatum* Flugge (Poaceae) and the invasive *Echium plantagineum* (Table 3). *E. plana* and *E. plantagineum* were mostly associated with the ROAD site, while *P. notatum* was more associated with the NOM and GRZ sites (Table 3). The native species *Dichondra sericea* Sw. (Convolvulaceae) represented the lowest contribution to the overall inter-site dissimilarity (Table 3).

Phylogenetic diversity and structure

We found a clear inter-site separation in the evolutionary principal component analyses (Fig. 2), which indicates phylogenetic diversity dissimilarity across these sites. Axis 1 (Dim1) explained the largest portion (13.1%) of the observed phylogenetic variation in diversity, followed by axis 2 (Dim2) that contributed 9% (Fig. 2). The third axis (Dim3) explained 7.8% of the variance, with subsequent axes each explaining <5.8% (Fig. S1 in Supplementary material).

The site without agricultural management (NOM) contributed to the largest portion of the variation in axis 1, while the site with grazed pasture (GRZ) showed the lowest contribution to this axis (Table 4). Conversely, the site at roadside (ROAD) contributed to the largest portion of variation in axis 2, whereas NOM had the lowest contribution to this axis (Table 4). Table S2 in supplementary material details the contributions of the sites for the subsequent axes. The alien species *Echium plantagineum*, which is considered invasive, contributed to 4.28% of the variation in axis 1 and 6.33% in axis 2. However, the largest variation (11.82%) in axis 2 was explained by the native species *Pfaffia tuberosa* (See Table S3 in supplementary material to assess the contributions of all species for the variation in the axes).

We found non-significant phylogenetic patterns (clustering or overdispersion) across all sites (large scale), in both the combined pool (all alien and native species) and the native pool (only natives) (Table 5). We also found non-significant differences with regards to MPD and NRI among subplots at the intra-site level (small scale). (Table 6). However, at the small scale (1m² subplots) at the ROAD site, the combined pool (alien and native species) and the pool containing just native species did show significant phylogenetic clustering using NRI (All species: $t = 3.28$, $p=0.03$; Natives: $t = 2.16$, $p=0.04$, Table 6).

Discussion

Floristic composition

Our results demonstrate that the sites under the lowest disturbance (NOM and GRZ) shelter the highest number of native species, while the recorded alien invasive species occur in the more disturbed sites (AGR and ROAD) (Tables 2 and 3). For AGR and ROAD this higher richness of alien species would be expected due to the intensity of their disturbance regimes i.e., ploughing and weeding reducing the native weeds cover in the agrarian sites and regular clearing of roadside vegetation as a part of highway maintenance. Such disturbance regimes promote a fast turnover of environmental conditions, which presents an opportunity for a wide range of alien and generalist species to establish (Leger and Espeland 2010; Deak et al. 2020), e.g. the alien species *E. plantagineum* and *E. plana*, both considered invasive.

Baldișsera et al. (2010) conducted a study in grassland communities in the Plateau region of Rio Grande do Sul State and found that the more disturbed communities (e.g., subjected to pressure of grazing and firing) demonstrated higher species evenness (i.e., homogeneity of the abundance distribution among species). Among the main explanatory factors for such evenness, these authors highlight the exclusion of strong competitive species (e.g., the native *Paspalum plicatulum*). Thus, our observations from the Simper analysis indicate that the competitive native species (*Paspalum plicatulum*, *Paspalum notatum* and *Piptochaetium montevidense*) display an important role in reducing the species evenness in the less disturbed sites, GRZ and NOM, and consequently, enhancing the inter-site composition differences. Under the low disturbance regime of the NOM sites, the tussock forming nature of these native species allows them to dominate, reducing species richness and inhibiting the establishment of a variety of other, potentially alien species (Baldișsera et al. 2010).

Conversely, the higher intensity of disturbance endured at the AGR and ROAD sites coincides with our finding for the invasive alien species *Eragrostis plana* and *Echium plantagineum*, which were accountable for the highest proportion of the overall difference in composition between sites. *Eragrostis plana* (Poaceae) is one of the most remarkable invasive species of South Brazilian grasslands (Rolim et al. 2015). It typically occurs in highly disturbed sites with intense agrarian crop activity and it was the most abundant alien species in our study. However, we also found individuals of this species on the roadsides. The high abundance of *E. plana* can be explained by the theory that roads act as corridors for dispersal of invasive plants (Meunier and Lavoie 2012). Another invasive species is *E. plantagineum* (Boraginaceae), which was the most abundant species recorded at the ROAD site and which is known to produce larger amounts of seed in ungrazed areas (Piggin 1978).

The most highly represented families of alien plants that we recorded in our survey were Poaceae (23%) and Asteraceae (23%), which concurs with the findings of other studies in both Rio Grande do Sul State (Schneider and Irgang 2005; Schneider 2007; Rolim et al. 2015) and worldwide (Pyšek 1998). There are various traits which confer invasiveness, being therefore typical of species which are considered to be highly invasive, e.g. short juvenile period and small seed mass (Rejmánek and Richardson 2014). Many species of Poaceae are considered highly invasive and their successful establishment as invasive species has been attributed to such characteristics as the highly evolved shape of their inflorescence and pollination system (Pyšek 1998). *Briza minor* was most commonly recorded in NOM sites (no agriculture or grazing). This plant is an example of the adaptability of Poaceae plants, since it is not constrained by specific habitat and nutrients requirements, and it is able to germinate throughout the least disturbed grasslands (Morgan 1998).

Among the species of Asteraceae, *Coleostephus myconis* was the third most abundant alien species across sites and was highly influential in the Simper analysis. It was most abundant in AGR sites and is considered common on agrarian and pastoral land throughout southern Brazil (USDA 2017). Conversely, *Hypochaeris glabra*, also in the Asteraceae family, occurred in GRZ, but at low levels of abundance, possibly due to the grazed pasture in this site. Despite the observed low abundance of *H. glabra* in the GRZ site, monitoring here would be useful to gain insight into its invasive potential, particularly since this species is known to

possess phenotypic plasticity allowing it to adapt and spread in sites with varied and distinct conditions (Martin-Fores et al. 2018 a,b). However, only the continuous monitoring of a site may be effective for the prediction of the invasive potential of species and control of the spread of undesirable weeds (DiTomaso 2000). Further, the high number of native species we found in the GRZ sites supports the assumption that grazing is important for the maintenance of the phytophysionomy of southern Brazilian grasslands (Hobbs and Huenneke 1992), since it promotes evenness of species richness and hinders invasion (Baldissera et al. 2010).

We found that the most abundant native species, *P. plicatulum*, *P. notatum* and *P. montevidense*, were mainly found in sites with moderate to low (GRZ and NOM) human interference (Pillar et al. 2009; Boldrini et al. 2010). *Paspalum plicatulum* is also commonly found along road margins (Pillar et al. 2009), while *P. notatum* is typical of the well managed and less disturbed native fields of the center of Rio Grande do Sul State (Boldrini et al. 2010). *Paspalum montevidense* also presented high abundance in the agrarian crop site (AGR) suggesting a resistance to such disturbance.

The inter-site differences and similarities in composition and its relation to the presence of alien species discussed here, indicates that alterations to the local environment (e.g. high turnover of vegetation removal and replacement) that occur with different land-uses (and levels of disturbance), shape the studied grassland communities (Catford et al. 2012). Since recruitment and dispersal affect species composition, abundance and diversity in grasslands, the action of processes that disrupt natural systems of dispersal are expected to cause changes in native species abundance and composition (Tilman 1997).

Phylogenetic diversity and structure

Our findings indicated dissimilar phylogenetic diversity across the four Pampean sites under distinct land use (Fig. 2). The distinct intensities of disturbance can create a wide range of local environmental constraints and, consequently determine community phylogenetic composition (Carreño-Rocabado et al. 2012). This phylogenetic diversity might be dominated by phylogenetically and functionally closely related species under strong actions of local environmental filters (e.g., soil nutrient supply after disturbance), or by phylogenetically distant but functionally complementary species under widespread and rapidly changing environmental conditions (e.g., increased solar radiation in the soil after crop rotation) (Baraloto et al. 2012; Parmentier et al. 2014). Thus, depending on the intensity and rapidness of the action of the local filters promoted by disturbance in the study sites on alien and native species, the resultant communities might be phylogenetically similar within (i.e., intra) sites while differing across sites (Leger and Espeland 2010).

Our least disturbed site, NOM (no agricultural management), explained the largest proportion of the variation in the main axis (axis 1) of the evoPCA, indicating higher phylogenetic diversity at this site. This can be partially attributed to the habitability of NOM for native species, which presented the highest percentage of contribution towards composition differences across sites, as determined by the Simper analysis. It is generally expected that sites protected from intense disturbance, such as this one, may conserve higher native phylogenetic diversity (Rolland et al. 2012; Lishawa et al. 2019), which has been shown to reduce the likelihood of successful establishment of alien species (Levine et al. 2004; Bennett et al. 2014; Venail et al. 2015). Nevertheless, as mentioned previously, alien and native plants can coexist over time in post-agricultural grasslands, particularly through differential resources use (Martín-Forés et al. 2016). However, though there may have been previous disturbances, the current vegetative structure may be representative of a late successional stage where native species have regained dominance over aliens, as suggested by Martín-Forés et al. (2016). *Pfaffia tuberosa*, which contributed to the largest variation found for the second axis of the evoPCA, is an example of a native species with considerable importance along areas with a historic of disturbance in the Pampean Grasslands (Altesora et al. 2005; Setubal and Boldrini 2010; Andrade et al. 2016). This species is relatively widespread in South-America, especially in grasslands of southern Brazil, Uruguay and Argentina (Marchioretto et al. 2009).

We found a trend for phylogenetic randomness (NRI) for all sites in both pools, i.e., all species and only native ones, at site level (large scale) (Table 3). This suggests that at the larger sampling scales, the importance of relatedness between the species does not differ from what would be expected by chance. Such phylogenetic randomness might reflect the degree to which the species assemblages of our Pampean communities are influenced by dynamic environmental conditions caused by recent and frequent disturbances under distinct land use. When local conditions of plant communities change abruptly as a result of a disturbance event, their ecological drivers (i.e., environmental filtering and plant-plant interactions) might reach a balance in the degree to which they influence the community assembly (Zhang *et al.* 2014; Soliveres *et al.* 2012; Liendo *et al.* 2020). Therefore, a random phylogenetic structure can be expected, since the force of influence of each of these drivers may counteract one another, balancing their influence on the assembling communities (Soliveres *et al.* 2012).

Following disturbance, initial environmental conditions commonly favor the competitive alien species (Hobbs and Huenneke 1992), whereas competition creates conditions less suitable for the alien species (Leger and Espeland 2010; Soliveres *et al.* 2012). When environmental filtering and competitive interaction influencing the community assembly are in balance, clustering and overdispersion are inhibited and the phylogenetic structure of the community does not differ from what would be expected by chance (Soliveres *et al.* 2012). The phylogenetic randomness observed in our study might be caused by local environmental conditions promoted by disturbance effect, strong enough in most sites to counteract the effects of natural ecological processes (environmental filtering and competition), thus preventing functional and phylogenetic clustering or overdispersion.

The ROAD site, which was particularly subject to disturbance through cutting and removal of vegetation, recorded the only significant phylogenetic pattern (clustering) at the small scale (1m² subplots) for both pools (all species and only natives). A possible explanation for this finding relies on the assumption that a local community frequently subjected to strong disturbances, such as those sustained by the ROAD site, is expected to be mostly composed by phylogenetically closely related species, which have similar functional attributes that allow them to better establish in those conditions (Verdú and Pausas 2007; Zhang *et al.* 2014). Further, the high degree of relatedness between the alien and native species within the community may confer some resistance to invasion if intense disturbance is less frequent (Brunbjerg *et al.* 2012; Gerhold *et al.* 2011; Liendo *et al.* 2021). Closely related alien and native species with high trait overlap tend to compete for similar resources in moderate environmental conditions, and this can result in competitive exclusion, causing a shift in species composition in the community (Violle *et al.* 2011; Guerin *et al.* 2019). However, whether or not the relatedness between alien and native species facilitates or impedes invasion, also depends on the degree of trait similarity in evolutionary history (Strauss *et al.* 2006), which was not evaluated in this study.

Other explanations for the above discussed findings on phylogenetic diversity and structure across the four sites, include factors not here investigated, such as coevolution of functional traits, generalist habits and the functional complementarity of co-occurring species. We recorded the invasive species *E. plana* e *E. plantagineum* in our sample, which are considered to be habitat generalists and have high plasticity of functional characteristics (Piggin 1978; Sharma and Esler 2008; Rolim *et al.* 2015; Zhu *et al.* 2017). In agroecosystems many native and alien species are known to co-evolve functional traits in adaptation to disturbances and the resultant changes in local environmental conditions (Ghersa *et al.* 1994). Thus, species frequently subjected to the same environmental pressures might co-evolve specific and complementary traits (e.g. dense seed bank, high dispersal ability and high regeneration potential) that confer them the capacity to easily establish in the new local conditions (Cousins 2006; Leger and Espeland 2010; Occhipinti 2013; Deák *et al.* 2020). These co-evolved traits might lead to phylogenetically clustered or overdispersed communities, depending on whether they are conserved in the lineages of the species (Cavender-Bares *et al.* 2009; Lankau 2012; Occhipinti 2013).

Overall, the findings of this study suggest that the phylogenetic relationship, and consequently reduced phylogenetic diversity, among alien and native species in the Pampean Grasslands is significant only at the small scale and in local conditions under the strongest disturbance regimes (i.e. ROAD). This pattern is

possibly a result of environmental filtering of phylogenetically and functionally closely related species which are adapted to these conditions. However, to confirm these assumptions, it is necessary to conduct long-term studies and monitoring of the local native communities and their relationships with alien species, as well as to determine the possibility of the expansion (i.e., broad scaling) of the alien species from one poorer phylogenetically community (e.g., ROAD) to surrounding communities.

Caveats, limits and implications

Since the trends and patterns regarding composition and phylogenetic relationships in this study have not previously been described for southern Brazil grasslands, we hope that this study encourages further research in Pampa grasslands, where ecological interactions are not yet fully understood. Further studies, with scope for more extensive data collection are necessary to determine whether the trends we found are consistent when tested at a larger scale, since our results may be influenced by the limitations of our study, i.e., sampling based on pseudoreplication, considerable geographic distance of GRZ to other sites and an unbalanced number of alien and native species in the pools analyzed. While evoPCA does allow one to investigate consistent phylogenetic patterns along the geographic space in several and distinct scales (Pavoine 2016), the use of a fully replicated experimental design in conjunction with a more balanced number of alien and native species, might assist in disentangling further phylogenetic trends not captured here. Our pool of alien species is considerably smaller than the native pool, which can interfere with the outcomes of the analysis of phylogenetic relationships between them. Nevertheless, it is acknowledged that a few highly competitive alien species can determine the phylogenetic community structure, particularly if they are distantly related to species in the local assemblage (Vilà et al. 2011 ; Loiola et al. 2018; Baroughy et al. 2021). Therefore, further studies can also assist in clarifying whether a few, but competitive and phylogenetically distantly related, alien species, in fact determine the phylogenetic structure of Pampean Grasslands.

Further analysis of other factors that may influence community assembly, not recorded in this study, can also provide further insights into the effects of the local environmental conditions of the sites under distinct land use and levels of disturbance on the phylogenetic relationships and potential susceptibility of Brazilian Pampas to alien species. Among these factors, differences in habitat conditions (e.g. soil, microclimate, water content) and levels of similarity between functional traits to alien species can also have some impact on community assembly and thus the conclusions of this investigation.

It is also feasible to reach further clarity regarding the influence of phylogenetic relatedness on invasiveness of the species, by analyzing the degree to which alien and native plant trait values are shared in their evolutionary history (i.e. conservatism or convergence) (Blomberg et al. 2003; Losos 2008; Münkemüller et al. 2012). Therefore, the effort to gather trait data, e.g: regenerative traits (Grime 2006; Rolim et al. 2015); leaf nitrogen production in native species (James 2008; Leffler et al. 2013); traits linked to competitive ability such as seeders versus resprouters (Verdú et al. 2009) and traits conferring invasiveness, e.g. life form and height of the invader (Pyšek et al. 2012), besides data of local habitat conditions, can contribute to an improved understanding of the evolutionary history and present assembly of Pampa communities. This may provide important ecological information that could be used to predict which of the sites under different land uses are more susceptible to plant invasion, and potentially prevent or mitigate for the most negative impacts.

Conclusions

Our findings lead us to conclude that phylogenetic pattern (i.e. clustering) in the relationships among alien and native species is only significant at the small sampling scale (1m² subplots) under the most disturbed site (i.e. ROAD) in the studied Pampean Grasslands. This suggests that strong local environmental constraints at this scale sort only phylogenetically closely related species with similar functional capabilities,

allowing them to establish in those conditions. This demonstrates the need to monitor the composition of native communities at the local level. Additionally, the random phylogenetic relationships observed across the other sites with distinct land use might be a result of factors not evaluated in this study, such as specific local conditions and species trait similarities. Roads cause considerable pressure on the plant community by sorting phylogenetically closely related species adapted to these conditions, thus reducing functional and phylogenetic diversity. This environment therefore holds great potential to study the implications of disturbance on community assembly from a phylogenetic and evolutionary perspective. We expect our findings to encourage further research into the influence of phylogenetic relatedness on invasiveness of species in disturbed habitats.

Conflicts of Interest

The authors declare no conflicts of interest.

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Data Availability Statement

The data that support this study will be shared upon reasonable request to the corresponding author.

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List of Figures and Tables

Figure 1 : Location of the four study sites of Brazilian Pampean Grasslands. The land use at each site is described as follows: AGR: agrarian crop; GRZ: grazed pasture; NOM: no agricultural management; ROAD: roadside. Figure created using ArcMap from Esri ArcGIS 10.6.

Figure 2 : Phylogenetic diversity across the four studied Pampa communities demonstrated by evolutionary principal component analysis. AGR = agrarian crop; GRZ = low intensity grazing; NOM = site without agricultural management; ROAD = roadside.

Table 1 : Alien plant species recorded in this study and the site in which they occurred, October and November, 2015. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Table 2: Number of species (native, naturalized alien and invasive alien) recorded in the study sites.

N = Number of species; % = proportion based on the number of species per site in relation to the total species sampled in the study. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Table 3: Most important species derived from Simper Analysis and their contributions (%) to the composition dissimilarity among sites (inter-site).

% contrib. = contribution to the overall inter-site dissimilarity; x = site to which the species was mostly related. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Table 4: Percentage of contributions of the four studied Pampa communities for the variations in the two first axes of the evolutionary principal component analysis (evoPCA). AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Table 5 : Phylogenetic structure of the four studied Pampa communities. Phylogenetic structure calculated for both the pool with all species (aliens and natives) and the pool of just native species. MPD: Mean pairwise distance; NRI: Net relatedness index. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Table 6 : Table 4 : Phylogenetic structure for pooled species (all species and just natives) per subplot in the Pampa communities. MPD: Mean pairwise distance; NRI: Net relatedness index. Values within parentheses represent the standard deviation. Values in bold indicate significant difference from zero under One Sample T-test.

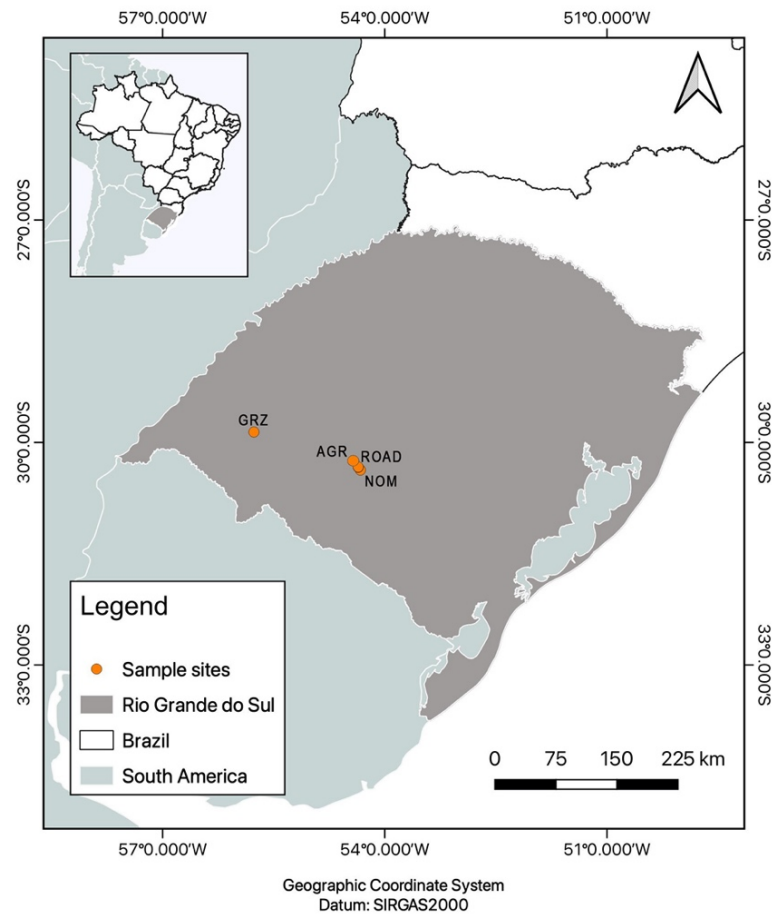


Figure 1 : Location of the four study sites of Brazilian Pampean Grasslands. The land use at each site is described as follows; AGR: agrarian crop; GRZ: grazed pasture; NOM: no agricultural management; ROAD: roadside. Figure created using QGIS v.3.16.

Table 1: Alien plant species recorded in this study and the site in which they occurred.

AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Plant species	Family	Occurrence	Status	Life cycle
<i>Ammi majus</i> L.	Apiaceae	ROAD	Naturalized	Annual
<i>Anagallis arvensis</i> L.	Primulaceae	AGR/ROAD	Naturalized	Annual
<i>Briza minor</i> L.	Poaceae	NOM	Naturalized	Annual
<i>Centaurium pulchellum</i> (Sw.) Druce	Gentianaceae	ROAD	Naturalized	Annual
<i>Cirsium vulgare</i> (Savi) Ten.	Asteraceae	ROAD	Naturalized	Annual/ Biannual
<i>Coleostephus myconis</i> (L.) Rchb.f.	Asteraceae	AGR	Naturalized	Annual
<i>Echium plantagineum</i> L.	Boraginaceae	AGR/ROAD	Invasive	Biannual
<i>Eleusine tristachya</i> (Lam.) Lam.	Poaceae	AGR	Naturalized	Perennial
<i>Eragrostis plana</i> Nees	Poaceae	AGR/ROAD	Invasive	Perennial
<i>Hypochaeris glabra</i> L.	Asteraceae	GRZ	Naturalized	Annual

Plant species	Family	Occurrence	Status	Life cycle
<i>Polycarpon tetraphyllum</i> (L.) L.	Caryophyllaceae	NOM	Naturalized	Annual/Perennial
<i>Rumex obtusifolius</i> L.	Polygonaceae	AGR	Naturalized	Perennial

Table 2: Number of species (native, naturalized alien and invasive alien) recorded in the study sites.

N = Number of species; % = proportion based on the number of species per site in relation to the total species sampled in the study. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

	Native	Native	Naturalized	Naturalized	Invasive	Invasive
	N	%	N	%	N	%
NOM	32	30.77	2	1.92	0	0
GRZ	46	44.23	4	3.85	0	0
AGR	26	25.00	5	4.81	2	1.92
ROAD	34	32.69	6	5.77	2	1.92

Table 3: Most important species derived from Simper Analysis and their contributions (%) to the composition dissimilarity among sites (inter-site) .

% contrib. = contribution to the overall inter-site dissimilarity; x = site to which the species was mostly related. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Species	Family	status	% contrib.	NOM	GRZ	AGR	ROAD
<i>Ammi majus</i> L.	Apiaceae	Naturalized	8.76				x
<i>Anagallis arvensis</i> L.	Primulaceae	Naturalized	9.35				x
<i>Briza minor</i> L.	Poaceae	Naturalized	9.22	x			
<i>Coleostephus myconis</i> L.	Asteraceae	Naturalized	11.31			x	
<i>Dichondra sericea</i> Sw.	Convolvulaceae	Native	4		x		
<i>Echium plantagineum</i> L.	Boraginaceae	Invasive	15.83				x
<i>Eragrostis plana</i> Nees	Poaceae	Invasive	24.45			x	x
<i>Hypochaeris glabra</i> L.	Asteraceae	Naturalized	8.03		x		
<i>Paspalum notatum</i> Flügge	Poaceae	Native	18	x	x		
<i>Paspalum plicatulum</i> Michx.	Poaceae	Native	8	x			
<i>Piptochaetium montevidense</i> (Spreng.) Parodi.	Poaceae	Native	7.9	x	x	x	

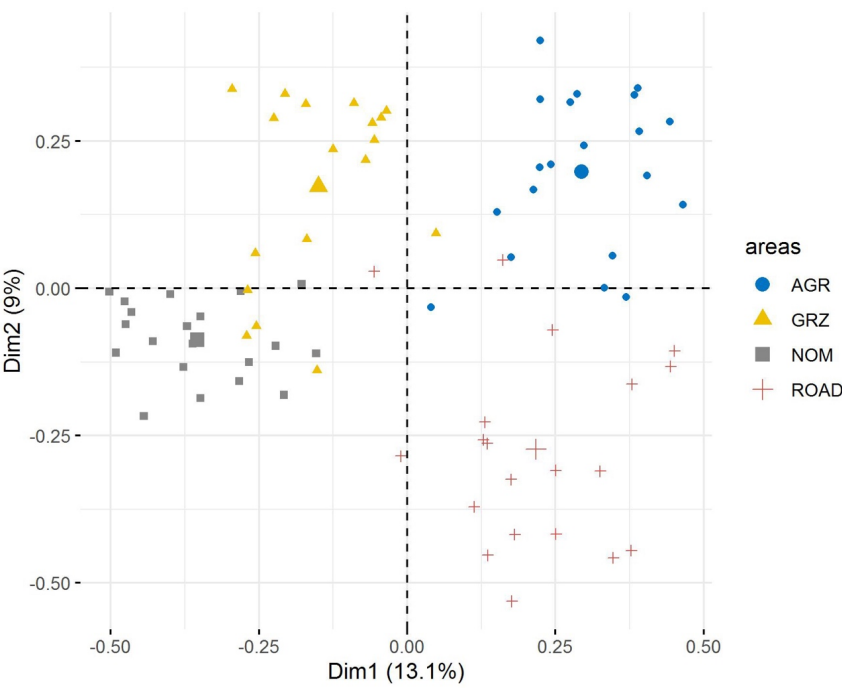


Figure 2 : Phylogenetic diversity across the four studied Pampa communities demonstrated by evolutionary principal component analysis. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Table 4: Percentage of contributions of the four studied Pampa communities for the variations in the two first axes of the evolutionary principal component analysis (evoPCA).

AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

Site	Axis1	Axis2
ROAD	22.76	45.97
NOM	41.70	5.10
AGR	25.75	23.82
GRZ	9.78	25.09

Table 5 : Phylogenetic structure of the four studied Pampa communities.

Phylogenetic structure calculated for both the pool with all species (aliens and natives) and the pool of just native species. MPD: Mean pairwise distance; NRI: Net relatedness index. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

All species plot	All species N taxa	All species MPD	All species NRI
ROAD	41	234.49	0.38
NOM	35	236.46	-0.18
GRZ	49	239.09	-0.91

AGR	31	231.68	1.33
Natives	Natives	Natives	Natives
ROAD	34	234.97	0.31
NOM	33	236.89	-0.18
GRZ	45	240.01	-0.84
AGR	26	230.55	1.42

Table 6 : Phylogenetic structure for pooled species (all species and just natives) per subplot in the Pampa communities.

MPD: Mean pairwise distance; NRI: Net relatedness index. Values within parentheses represent the standard deviation. Values in bold indicate significant difference from zero under One Sample T-test. AGR = agrarian crop; GRZ = grazed pasture; NOM = site without agricultural management; ROAD = roadside.

	MPD	NRI
All Species		
ROAD	224.07 (-27.47)	0.77 (-1.06)
NOM	237.22 (-11.34)	-0.05 (-0.80)
GRZ	239.32 (-6.17)	-0.27 (-0.57)
AGR	236.73 (-7.59)	0.02 (-0.59)
Natives		
ROAD	228.13 (-31.89)	0.55 (-1.11)
NOM	236.73 (-11.35)	-0.02 (-0.80)
GRZ	239.36 (-7.07)	-0.28 (-0.64)
AGR	232.28 (-31.53)	0.04 (-0.87)