

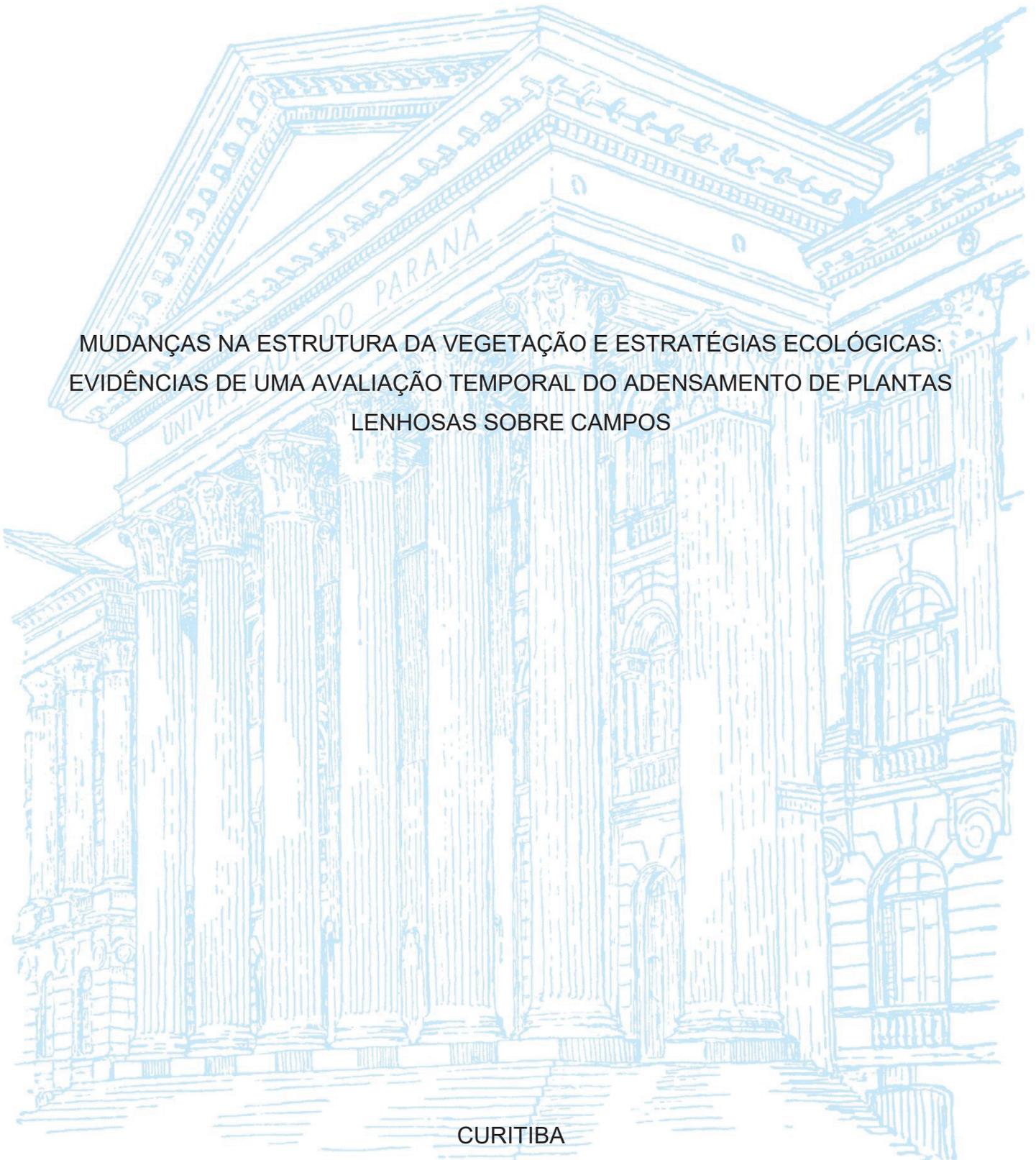
UNIVERSIDADE FEDERAL DO PARANÁ

RAISSA IANA LEITE JARDIM

MUDANÇAS NA ESTRUTURA DA VEGETAÇÃO E ESTRATÉGIAS ECOLÓGICAS:
EVIDÊNCIAS DE UMA AVALIAÇÃO TEMPORAL DO ADENSAMENTO DE PLANTAS
LENHOSAS SOBRE CAMPOS

CURITIBA

2023



RAISSA IANA LEITE JARDIM

MUDANÇAS NA ESTRUTURA DA VEGETAÇÃO E ESTRATÉGIAS ECOLÓGICAS:
EVIDÊNCIAS DE UMA AVALIAÇÃO TEMPORAL DO ADENSAMENTO DE PLANTAS
LENHOSAS SOBRE CAMPOS

Dissertação apresentada ao curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ecologia e Conservação.

Orientador: Prof. Dr. Marcos Bergmann Carlucci

CURITIBA

2023

DEDICATÓRIA

Sou herdeira de uma bióloga. Mas não me refiro a bióloga de formação. Sou herdeira de uma bióloga de coração, amante e observadora da natureza, que cresceu no interior do Norte de Minas Gerais, na transição do Cerrado com a Caatinga, lá no Vale do Jequitinhonha. Desde criança escuto histórias sobre a sua infância e adolescência no mato, sobre os diversos e saborosos frutos nativos, sobre os encantos, perigos e belezas da natureza. Relatos de uma infância preciosa e cheia de aventuras. Cresci na selva de pedra, na periferia de São Paulo, e sempre fui apaixonada por um ambiente que desconhecia, mas que fui ensinada a amar. Histórias sobre uma natureza que, em grande parte, já não existe mais. Dona *Emília Prates Jardim*, minha amada vó, mãe de meu pai, mulher sábia e contadora de tantas vivências sagradas pra mim, a ela dedico esta tese. Com o seu saber tradicional sobre a biodiversidade brasileira, ela me encanta e ensina, muito mais do que posso expressar. Conhecedora de tantos remédios sagrados, praticante de um respeito profundo pelo meio ambiente, sou grata por ser sua neta. És exemplar, por sua força em lutar por uma mudança de vida, por sua garra em conseguir furar tantas barreiras sociais, de gênero, de raça, de existência, pela mulher de luta que és. Hoje, entregar esta dissertação de mestrado, só é possível pela luta dos que vieram antes de mim. Com eles aprendi sobre a importância de ter raízes profundas, e de saber de onde se vem. A ela dedico esta dissertação.

AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001. Agradeço pelo apoio que viabilizou meu ingresso e permanência na pós-graduação. Agradeço à Pró-reitoria de Pesquisa e Pós-graduação da Universidade Federal do Paraná, pelo financiamento das atividades de pesquisa que oportunizou a execução das expedições de campo, apoio que foi fundamental para o projeto, visto que sem este não seríamos capazes de ir a campo coletar dados tão importantes como os que coletamos.

Agradeço imensamente o meu orientador, Dr. Marcos Carlucci, por todos os ensinamentos, pela grande dedicação na missão de me orientar neste projeto desafiador, e pela empatia que teve nos momentos mais adversos desta trajetória. És um cientista, e um ser humano, admirável. Obrigada!

Agradeço ao Instituto Água e Terra pela concessão da autorização para realizar pesquisa em unidade de conservação, no Parque Estadual de Vila Velha e Parque Estadual do Guartelá. Aos gestores sr. Juarez Baskoski e ao sr. Gelson de Oliveira, deixo meu agradecimento especial, por toda colaboração, parceria e generosidade, vocês tornaram as minhas saídas de campo muito mais leves e tranquilas, obrigada por sempre estarem preocupados com nosso bem-estar e por fazerem o possível para nos ajudar nos campos, ao longo de quase 10 meses de saídas de campo quinzenais contínuas. A toda equipe dos parques, agradeço imensamente por todos cafés, conversas, lendas e risadas. O amor de vocês pela natureza me motivou, e me ensinou a pensar nas estratégias ecológicas das plantas pelas lentes de quem observa a natureza, e sabe descrever padrões complexos, de forma tão simples. Aprendi demais, com seres humanos exemplares, sou grata por isso!

A precisa determinação da identidade de algumas espécies só foi possível com o auxílio de especialistas botânicos, aos quais deixo meu agradecimento. Ao Dr. Marcelo Brotto e ao Ms. Felipe Marinero, do Museu Botânico Municipal de Curitiba, responsáveis pela determinação de Lauraceae e Rubiaceae, respectivamente. Ao Dr. Fabrício Schmitz Meyer e Dr. Renato Goldenberg, do Herbário da Universidade Federal do Paraná (UPCB), pela determinação de Melastomataceae e confirmação da identificação de espécies de outras famílias. Ao Ms. Fábio Christiano Speck Vieira, pela determinação de Myrtaceae.

Aos meus amigos do Laboratório de Ecologia Funcional de Comunidades (LABEF), um agradecimento especial. Em todos campos que realizei em meu projeto de mestrado, eu nunca estive sozinha. El@s foram para campo comigo, estiveram ao meu lado nos perrengues, ajudando a medir atributos e a tornar esta pesquisa possível. Aos que tive o

prazer de dividir experiências incríveis, deixo o meu muito obrigada pela parceria e colaboração: Adler Barboza, Josué Araldi, Licet Trochez, Pedro Kotovicz, Ricardo Manosso e Ursula Souza. Agradeço em especial a Bruna da Silva e Ingrid de Nascimento, que me acompanharam ao longo de grande parte dos campos e desenvolveram projetos de iniciação científica conjuntamente, e ao Elielson Ferreira, que foi fundamental na fase final do meu mestrado, agradeço pela amizade e ajuda.

Sou imensamente grata aos meus pais, João Luiz Jardim e Eurides Jardim, por sempre me incentivarem a lutar pelo que acredito e a seguir os meus sonhos. A certeza do amor de vocês, em qualquer circunstância, me sustenta e motiva, pois sei que posso voar para onde for, sempre terei vocês como porto seguro para voltar. Obrigada por abrirem tantas portas para mim, com muita luta, esforço e amor. Aos meus irmãos, Raphaela e João Pedro, agradeço por estarem ao meu lado em todos os momentos, meus primeiros melhores amigos. À minha grande família Leite e Jardim, minhas raízes mais profundas, sou grata por me ensinarem que na vida, o que existe de mais precioso, é muito simples.

Às minhas fieis amigas, que fizeram parte de tantas etapas das da minha vida, sou muito feliz por ter vocês, por terem permanecido ao meu lado durante tantos anos, por compartilharem o encerramento de mais um ciclo, em especial agradeço: Priscila Vitoretti, Maiara Matilde, Jackeline Fernanda, Sarah Spindola, Beatriz Mota e Gabriela Negreiros, ter certeza da amizade e lealdade de vocês torna a minha vida mais feliz e bonita. Em especial, agradeço a Manuella Yamamoto e a Fernanda Vitorio, meu trio da graduação, e agora do mestrado (cada uma em sua área de estudo), vocês foram meu refúgio e porto seguro, sou grata pela amizade de vocês.

Ao meu companheiro, Guilherme Lua, sem palavras. Sabes que foi essencial para a conclusão deste trabalho. Muito obrigada pela ajuda em campo, pela ajuda em lavar raízes, pela criação das figuras e esquemas que desenvolvemos, que tornam a síntese dos resultados da minha pesquisa muito mais clara e acessível. Obrigada pelo companheirismo, em todas as dimensões da vida. Amo você.

Me levanto sobre o sacrifício
de um milhão de mulheres que vieram antes
e penso, o que é que eu faço
para tornar essa montanha mais alta
para que as mulheres que vierem depois de mim
possam ver além – legado

(Rupi Kaur, 2018)

RESUMO

Campos nativos e florestas podem ocorrer lado a lado na paisagem, formando um complexo sistema de mosaicos. Ambos são considerados estados estáveis alternativos da vegetação, mantidos por diferentes feedbacks estabilizadores. Na ausência, ou sob uma baixa frequência de distúrbios, ocorre o processo de adensamento de lenhosas. Esse processo resulta na mudança gradual da fisionomia da vegetação, que passa de uma vegetação campestre com poucos indivíduos lenhosos, para uma floresta, criando ecótonos caracterizados por condições ambientais contrastantes. Espécies lenhosas que ocorrem ao longo do gradiente de adensamento precisam adotar estratégias ecológicas que sejam viáveis, o que pode ser contrastante dadas condições ambientais de cada habitat. Para isso, as plantas tem o desafio de coordenar de forma eficiente o funcionamento de dimensões interdependentes da planta, acima e abaixo do solo. No presente trabalho buscamos avaliar o que acontece com a estrutura da vegetação e com a funcionalidade das espécies ao longo de um gradiente de adensamento de lenhosas sobre campos nativos. A nossa hipótese é de que após 10 anos sem distúrbios, ocorram mudanças estruturais na vegetação, que são associadas com mudanças nos padrões funcionais da comunidade e nas estratégias ecológicas das espécies lenhosas. Para avaliar isso, amostramos 60 parcelas em duas áreas com vegetação formada por mosaicos campo-floresta em dois períodos de tempo diferentes (2012-2022), para coletar dados temporais do adensamento de lenhosas. Utilizamos uma abordagem integradora para acessar as diferentes dimensões de variação dos atributos funcionais das plantas, incluindo atributos acima e abaixo do solo (*whole-plant*, folha, caule e raízes). Correlações bivariadas e análises multivariadas foram utilizadas para acessar a variação dos atributos. Nossos resultados mostraram que a densidade de indivíduos lenhosos aumentou no campo, que atualmente possui uma vegetação com estrutura vegetacional similar a uma floresta secundária. Em geral, espécies lenhosas colonizando o campo tiveram folhas conservativas, como estratégia para proteção contra alta incidência luminosa e seca; raízes aquisitivas para competir de forma eficiente por nutrientes, e casca espessa, como um importante atributo de resistência ao fogo. Em contrapartida, as espécies de floresta foram maiores em altura e tiveram folhas aquisitivas, indicando uma alta habilidade de competir por luz no ambiente florestal, onde a luz é um recurso limitante. Nossos resultados demonstram que campo e floresta possuem comunidades formadas por espécies lenhosas com diferentes estratégias ecológicas, e que na ausência de distúrbios os campos sofrem mudanças estruturais e na fisionomia da vegetação, em consequência do adensamento. Essa mudança favorece a chegada de novas espécies com diferentes estratégias ecológicas, que tornaram-se viáveis considerando as novas condições ambientais resultado pelo processo de adensamento de lenhosas.

Palavras-chave: Mosaicos campo-floresta. *Trade-off* funcional. Atributos acima do solo. Atributos abaixo do solo. Coordenação funcional.

ABSTRACT

Forests and grasslands often occur side by side in the landscape forming a complex mosaic system. These alternative vegetation states are maintained by different disturbances stabilizing feedbacks. Under absence or very low frequency of disturbances, woody plant encroachment (WPE) takes place, and the vegetation changes from grassland with few woody individuals to forest, thereby creating ecotones with contrasting environmental conditions. Woody species that occur along this gradient adopt viable ecological strategies which may be contrasting in each habitat. For this, plants are challenged to efficiently coordinate the functioning of interdependent plant strategy dimensions above and belowground. Here we aim to evaluate what happens to vegetation structure and functionality of woody species along gradient of WPE over grasslands. We hypothesised that after 10 years without disturbances, the vegetation experiences structural changes that are associated to shifts in the community functional pattern and in species ecological strategies. We surveyed 60 plots in forest-grassland mosaics at two different times (2012-2022), capturing a gradient from open grasslands, to encroached grassland and to closed forest, to collect temporal field data about WPE process happening. We used an integrated functional approach to assess the different dimensions of plant trait variation, including above and belowground traits (whole-plant, leaf, stem, and root traits). Bivariate correlations and multivariate analyses were used to assess trait coordination. Our results show that the density of woody individuals increased in the former grassland, which today has a structure similar to a young forest. In general, woody species colonising the grassland had conservative leaves, a strategy for protection against high solar incidence and drought; acquisitive roots for efficient competition for nutrients; and thick barks, that is an important fire-resistance trait. On the other hand, forest species usually were taller and had acquisitive leaves, indicating highly competitive ability for light in the light-limited forest habitat. Interestingly, our results indicate that woody species occurring in forest-grassland mosaics exhibited a clear trade-off between leaf and belowground. Overall, our results demonstrate that forest and grassland harbour woody species with different ecological strategies, and that without disturbance grasslands pass to gradual changes in vegetation physiognomy, which allowed the arrival of species that have different ecological strategies that become viable under the new environmental conditions formed by WPE.

Keywords: Forest-grassland mosaic. Functional trade-off. Aboveground trait. Belowground trait. Functional coordination.

LISTA DE FIGURAS

- Figure 1:** Location of study areas.....25
- Figure 2:** Illustrative scheme of vegetation sampling design carried out in forest-grassland mosaics.27
- Figure 3:** Synthesis of vegetation structural changes after 10 years of woody plant encroachment in forest-grassland mosaics. A) Violin plot (boxplots combined with kernel density plots) comparing the number of individuals in each habitat. Data of Guartelá State Park. B) Violin plot comparing total basal area (m²) per subplot sampled (100 m²), estimated for 1 hectare. Data of Vila Velha State Park. Distinct letters represent significant differences between habitats in the pairwise permutation Tukey's HSD post hoc test, all $p < 0.05$; C) Illustrative drawing of the vertical profile of vegetation height through a temporal gradient of changes in vegetation under WPE; D) Photographic record of grassland in 2012, in 2022 and forest, respectively. All photos are from Vila Velha State Park.....33
- Figure 4:** Functional community pattern of grassland and forest habitat. Analysed at the community level, the dots represent the subplots sampled in each habitat and sampling year. CWM values were calculated considering species abundance for each subplot. Detailed results of the PCA at the community level are available in Appendix H. Grassland is identified with beige colours (light beige for 2012 sample, and brownish beige for 2022 sample). Forest is identified with green colours (light green for 2012 sample and dark green for 2022 sample). Legend: GUA: Guartelá State Park; VV: Vila Velha State Park. Functional traits: LA – leaf area, SLA – specific leaf area, LDMC – leaf dry matter content, SSD – stem-specific density, BT – bark thickness, SRL – specific root length, D – average diameter, RBF – root branching frequency, Hmax - maximum height. Details are available in Appendix F.....37
- Figure 5:** Changes in functional pattern of community during woody plant encroachment. Violin plot (boxplots combined with kernel density plots) comparing functional traits weighted by species abundance (CWM values for each trait), scale-up at the community level, along a spatial and temporal gradient of woody plant encroachment, in forest-grassland mosaic located in (A) Guartelá State Park (GUA) and (B) Vila Velha State Park. Boxplots showing median and 25^o and 75^o percentiles. The vertical curves display the kernel density estimates for

each parameter's probability density function. Distinct letters represent significant differences between habitats in the pairwise permutation Tukey's HSD post hoc test, all $p < 0.05$. Traits legend is the same as above..... 38

Figure 6: Functional distinctions between forest and grassland species ecological strategies associated with woody plant encroachment over time (2012-2022) in southern Brazil. Study areas: Guartelá State Park (GUA) and Vila Velha State Park (VV). Dark colours indicate functional hotspots in the multivariate functional space, i.e. regions with a higher density of species in the functional space. Legend: Functional traits: LA – leaf area, SLA – specific leaf area, LDMC – leaf dry matter content, SSD – stem-specific density, BT – bark thickness, SRL – specific root length, D – average diameter, RBF – root branching frequency, Hmax - maximum height. Details are available in Appendix G..... 39

Figure SM 1: Collection of (A) leaves, (B) stem measurements and (C) roots in the field. After collection the samples were taken to the laboratory, where measurements of leaf (D), stem (E) and root (F) traits were taken..... 62

Figure SM 2: Violin plot (boxplots combined with kernel density plots) comparing structural parameters of vegetation along a spatial and temporal gradient of woody plant encroachment, in forest-grassland mosaic located in Guartelá State Park (GUA). 66

Figure SM 3: Violin plot (boxplots combined with kernel density plots) comparing structural parameters of vegetation along a spatial and temporal gradient of woody plant encroachment, in forest-grassland mosaic located in Vila Velha State Park (VV). 67

Figure SM 4: Spatial correlograms for Guartelá State Park, for structural parameters (mean height, total basal area and total number of individuals per plot), and also considering the residuals generated by the models..... 68

Figure SM 5: Spatial correlograms for Vila Velha State Park, for structural parameters (mean height, total basal area and total number of individuals per plot), and also considering the residuals generated by the models. 69

Figure SM 6: Principal Component Analysis (PCA) for structural parameters of vegetation in 2012 and 2022 in forest-grassland mosaics. A) Guartelá State Park (GUA); B) Vila Velha State Park (VV). 70

Figure SM 7: Correlation between functional traits at community level, for Guartelá (above) and Vila Velha State Park (below).	73
Figure SM 8: Board with a photographic record of species with characteristic functional traits of grassland and forest habitat.	74
Figure SM 9: Board with photographic record of landscape and community structure of forest-grassland mosaics.....	75

LISTA DE TABELAS

Table 1: Functional traits selected to describe above and belowground ecological strategies of species, to evaluate changes along a gradient of woody plant encroachment in forest-grassland mosaics. Abb. – abbreviation, G – grassland, F – forest.....	28
Table 2: Temporal changes in vegetation structure along the woody plant encroachment process. Values are the mean and standard deviation. The mean value per subplot represents data collected per subplot of 100 m ² sampled. Distinct letters represent significant differences between habitats in the pairwise permutation Tukey's HSD post hoc test, all $p < 0.05$	34
Table 3: Functional index of β -diversity among habitats (grassland and forest) and sampling year (2012 and 2022). Total functional β -diversity was decomposed in turnover (turn.) and nestedness (nest.) components. The values in percentages are presented.....	37
Table SM 1: Details of functional PCA at the community level. Only present loadings >0.20	71
Table SM 2: Details of PCA at species level. Only present loadings >0.20	72

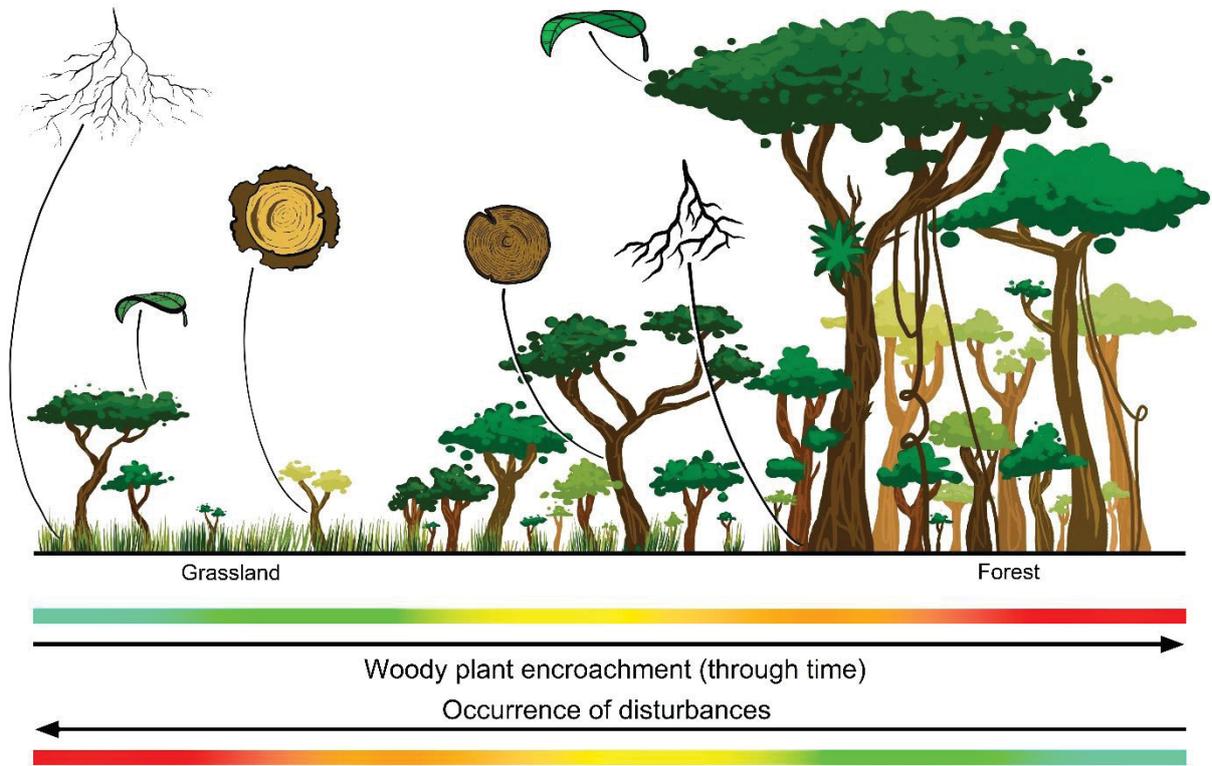
LISTA DE MATERIAL SUPLEMENTAR – APÊNDICES

Appendix A: Detailed description of field and laboratory methods for functional trait data collection.....	58
Appendix B: Description of spatial autocorrelation analysis.	65
Appendix C: Changes in vegetation structural parameters during woody plant encroachment.....	66
Appendix D: Results of spatial autocorrelation analysis.	68
Appendix E: Structural PCA, showing changes in vegetation physiognomy along woody plant encroachment.	70
Appendix F: Results of the principal component analysis of community functional patterns in forest-grassland mosaics, presented in Figure 4.	71
Appendix G: Results of the principal component analysis of functional traits variation at species level, presented in Figure 6.	72
Appendix H: Correlation between functional traits at interspecific and community level. .	73
Appendix J: Board with photographic record of species with characteristic functional traits of grassland and forest, and of the mosaics.	74

SUMÁRIO

1 GRAPHICAL ABSTRACT	16
2 INTRODUCTION	17
3 MATERIAL AND METHODS	23
3.1 STUDY SITES	23
3.2 VEGETATION SURVEY	26
3.3 COLLECTION OF FUNCTIONAL TRAIT DATA	27
3.4 STATISTICAL ANALYSIS.....	28
3.4.1 Vegetation structural analysis	28
3.4.2 Community functional analysis	30
3.4.3 Species functional analysis	31
4 RESULTS	32
4.1 CHANGES IN VEGETATION STRUCTURE ACROSS SPACE AND TIME	32
4.2 SHIFTS IN COMMUNITY FUNCTIONAL PATTERNS UNDERLYING WOODY PLANT ENCROACHMENT	34
4.3 SHIFTS IN SPECIES FUNCTIONAL TRAITS UNDERLYING WOODY PLANT ENCROACHMENT	39
5 DISCUSSION	40
5.1 FUNCTIONAL TRAJECTORY AND STRUCTURAL CHANGES IN VEGETATION SUBMITTED TO WOOD PLANT ENCROACHMENT.....	40
5.2 FUNCTIONAL COMMUNITY CHANGES THROUGH SPACE AND TIME IN A MOSAIC SYSTEM SUBMITTED TO ENCROACHMENT	42
5.3 MAJOR DIMENSIONS OF TRAIT VARIATION SUGGEST A TRADE-OFF ABOVE AND BELOWGROUND.....	46
6 CONCLUSIONS	48
REFERENCES	49
SUPPLEMENTARY MATERIAL	58

1 GRAPHICAL ABSTRACT



2 INTRODUCTION

Woody encroachment in open ecosystems, such as grasslands and savannas, has increased worldwide over the past 100-200 years (Archer et al. 2001, 2017, Bond 2008, 2016, Stevens et al. 2017). Woody plant encroachment (hereafter WPE) is defined as an increase of woody biomass and forest coverage in an ancient and high biodiverse open ecosystem (Archer et al. 1988, Bowman et al. 2001, Roques et al. 2001, Bond 2008, Stevens et al. 2017, García Criado et al. 2020). Open ecosystems form mosaics of vegetation with different forest types, maintained by complex stabilizing feedbacks related to disturbance regime (that favour grasslands and savannas) and shadiness (that favour forests) threshold (Blanco et al. 2014, Dantas et al. 2016, Pausas and Dantas 2017, Pausas and Bond 2020). The rate of woody individuals increment varies according to regional environmental conditions and disturbance regimes (Stevens et al. 2017). In Brazil, open ecosystems have experienced higher rates of woody encroachment in the last decades (Stevens et al. 2017), which seems to be a consequence of an environmental policy of fire suppression (Overbeck et al. 2007, Fidelis and Pivello 2011, Parr et al. 2014). These policies do not consider the origin and evolutionary history of fire-prone ecosystems (Bond 2016, 2021), such as Cerrado, the Central Brazilian savanna, and the South Brazilian grasslands (Overbeck et al. 2007, 2015, 2022, Fidelis and Pivello 2011, Durigan 2020, Pivello et al. 2021). Grasslands and savannas evolved under a natural regime of disturbances by fire, which is a major factor in shaping plant functional traits and mosaics of vegetation (Bond and Keeley 2005a, Keeley et al. 2011). Species are subject to contrasting environmental conditions across forest-grassland and savannas mosaics (hereafter forest-grassland mosaics, for simplification, but referring to mosaics with savanna as well), where both habitats occur side by side in the landscape (Whittaker and Levin 1977, Dantas et al. 2016, Bond 2021). Considering the current threats to open ecosystems and the complex dynamics and feedbacks that maintain forest-grassland mosaics, we must advance in our understanding of general changes of species and community functional composition related to encroachment process, to better manage and conserve these two contrasting ecosystems.

The Alternative State Theory postulates that contrasting vegetation types, such as forests and grasslands and/or savannas, can occur as mosaics with sharp boundaries, side by side in the landscape, under the same environmental conditions, as long as they are submitted to different disturbance regimes (Whittaker and Levin 1977, Dantas et al. 2016, Pausas and Bond 2020, Bond 2021). In Brazil, specifically in the Cerrado and the South Brazilian grasslands, open vegetation forms mosaics with different forest types, which

seems to be associated with the current climate conditions and absence of disturbances, favouring encroachment to occur (Behling 1997, Pillar 2003, Behling et al. 2004, Blanco et al. 2014, Souza 2021). Fire and herbivory act as major consumers of vegetation, helping to shape the spatial distribution, structure, composition and functioning of communities (Bond and Keeley 2005). Savanna and grassland species possess several adaptations to fire (Bond and Keeley 2005b, Strömberg 2011, Keeley et al. 2011, Simon and Pennington 2012, Dantas et al. 2016). The evolution and diversification of C₄ grasses are associated with an increase in the occurrence of fires during the Miocene in South America (Strömberg 2011). There is evidence from dated phylogenies that fire played a central role in shaping ecosystems of the Cerrado since at least 4 million years ago, with a peak of diversification of species with fire-resistant traits (Simon and Pennington 2012). Megafaunal extinction during the Holocene in the Neotropics released woody species from the pressure exerted by large herbivores (Dantas and Pausas 2020, 2022) and was associated with the arrival of the first Amerindians, which probably used fire, according to registers showing that palaeofires continued to be frequent at the beginning of the Holocene (Behling 1997). Grasslands and savannas were once thought of as secondary vegetation produced by deforestation but are now seen as having an evolutionary history of coevolution with disturbances long before human arrival, which shows that they are ancient vegetations, with rich endemic biota adapted to a natural disturbances regime (Simon and Pennington 2012, Overbeck et al. 2015, Durigan 2020, Sühs et al. 2020).

Forest-grassland mosaics are considered alternative states maintained by fire stabilizing feedback (Dantas et al. 2013, 2016, Blanco et al. 2014, Bond 2016, Pausas and Bond 2020, Bernardino et al. 2022). The feedback that stabilizes a particular habitat creates the necessary conditions for it while simultaneously impeding conditions for another habitat, as each habitat is resistant to changes in state (Pausas and Bond 2020, Bond 2021). This process occurs at a local scale, in a landscape of local plant communities (forest-grassland mosaics) connected by the dispersal of their species (Pausas and Dantas 2017). On one hand, in grasslands, a natural-based fire regime contributes to maintaining the conditions for open vegetation, i.e. with high light availability propitiating the dominance of shade-intolerant flammable grasses that further increase the probability of fires (Bond and Keeley 2005a, Dantas et al. 2013, 2016, Blanco et al. 2014, Bond 2021, Pilon et al. 2021, Bernardino et al. 2022). Once the natural disturbance regime is maintained, it contributes to generating stable open ecosystems. In contrast, if a grassland goes without burning for a long time, fire suppression interval will allow tree colonization (Pillar 2003, Müller et al. 2007, 2013, Blanco et al. 2014, Dantas et al. 2016, Pausas and Dantas 2017, Bernardino et al.

2022). As trees grow taller and block more light, the ecosystem may undergo significant changes in the composition of species and the structure of vegetation (Bond 2021, Pilon et al. 2021). When shade-intolerant, flammable grasses are completely excluded by the shading of the forest, the fire-suppression threshold has been reached (Hoffmann et al. 2012). Shade generated by the tree canopy hinders the establishment of shade-intolerant grasses and generates conditions for stable closed ecosystems, with shade-tolerant species (Pausas and Bond 2020, Bond 2021). The switch from a highly flammable open ecosystem to a non-flammable closed forest, therefore, results in a fundamental transition in the behaviour of the system (Hoffmann et al. 2012, Dantas et al. 2013), from a state dependent on and maintained by a fire regime with fire-resistant plants to a state that is considered sensitive to it, as tropical and subtropical forests (Oliveras and Malhi 2016, Pivello et al. 2021).

Forest-grassland mosaics experiencing encroachment exhibit significant changes in vegetation physiognomy and microclimate conditions along a local spatial gradient, ranging from open to closed vegetation (Müller et al. 2007, Carlucci et al. 2012, Dantas et al. 2013). In addition to the spatial gradient, it is also possible to assess changes that occur as a result of the WPE process over the years, i.e. temporal changes in vegetation associated with a long disturbances-free interval (Maracahipes et al. 2018b, Goncalves et al. 2021, Bernardino et al. 2022, Raymundo et al. 2023). Due to both spatial and temporal variations can be assessed to better understand the WPE process, these complex mosaics of vegetation present an ideal opportunity to study general community patterns and functional adjustments of species to changing environmental conditions (Carlucci et al. 2012, Dantas et al. 2013, Maracahipes et al. 2018a, Flake et al. 2021, Carrijo et al. 2021, Bernardino et al. 2022, Raymundo et al. 2023). The distinct environmental conditions of each habitat may select and favour the establishment of different sets of species, thus impacting the community's functional composition and ultimately, the whole ecosystem (Wieczorkowski and Lehmann 2022). In the absence of disturbance, if the fire-suppression threshold is surpassed (Hoffmann et al. 2012), the theory predicts grasslands tend to follow a successional trajectory resulting from encroachment until it becomes a forest (Müller et al. 2013). However, information is still scarce on how this trajectory is, considering that there are few temporal studies. To better understand vegetation dynamics and promote conservation, it is crucial to assess functional changes in community and species composition along spatial and temporal gradients of vegetation mosaics experiencing WPE.

Beta diversity (hereafter β -diversity) is a measure of variation in species composition between sites (Whittaker, 1960), and compares inventory diversity at two different scales

(alpha and gamma diversity) (Baselga 2010). Functional β -diversity is a key facet of biodiversity as it allows us to understand community assembly processes across environmental gradients or spatial scales (Villéger et al. 2013, Socolar et al. 2016). Functional β -diversity considers functional traits of species in the analysis of community composition dissimilarities and can be decomposed in turnover and nestedness-resultant components (Baselga 2010, Baselga and Orme 2012, Villéger et al. 2013). Turnover is described as the species replacement between sites, and nestedness is described as the richness difference between sites, both components of β -diversity help to understand community structure and changes through time and space (Villéger et al. 2013). Assessing functional β -diversity changes over time offer a unique opportunity to understand community changes caused by species replacement and the arrival of species with different ecological strategies. Despite this, few studies assess this facet of biodiversity (Socolar et al. 2016).

Resource-use ecological strategy is related to how plants allocate limited resources to achieve better performance, so it can indicate what limits or favours plants in each habitat given local environmental conditions and disturbance history (Dantas et al. 2013, Bernardino et al. 2022). Resource-use ecological strategy permits the assessment of why a plant invests in one dimension to the detriment of investment in others (trade-off); alternatively, how plants may invest in a coordinated way between plant dimensions (positive covariance) (Reich 2014). Species subject to different environmental conditions can adopt distinct ecological strategies to use available resources, depending on costs and benefits, that range from an acquisitive (fast-growing) to a conservative (slow-growing) spectrum (Wright et al. 2004, Reich 2014, Rosado and de Mattos 2017). Resource-conservative species have slow growth but high survival rates, due to high investment in dry matter content of dense and hydraulically safe tissues (Perez-Harguindeguy et al. 2016), which increases the capacity to tolerate stress and toughest conditions (Grime 1977) to the detriment of low photosynthesis and respiration rates (Wright et al. 2004, Reich 2014). Conversely, resource-acquisitive strategies are characterised by quick returns on investments, with high rates of photosynthesis and respiration and low dry matter content (Wright et al. 2004, Reich 2014, Rosado and de Mattos 2017). Regarding ecological strategies of species in forest-grassland mosaics, grassland species tend to have conservative strategy for leaves and traits that contribute to tolerance of frequent fire, such as thick barks and resprout ability (Carlucci et al. 2012, 2015, Dantas et al. 2013, Silva 2015, Pausas 2015, Maracahipes et al. 2018a, Flake et al. 2021, Carrijo et al. 2021, Scalon et al. 2021, Bernardino et al. 2022, Raymundo et al. 2023). Forest species, on the other hand, tend to be taller, grow faster and higher specific leaf area, indicating a strategy for efficient competition for light (Maracahipes et al.

2018b, Rodriguez-Cubillo et al. 2021, Flake et al. 2021, Carrijo et al. 2021, Bernardino et al. 2022). Both habitats are occupied by functionally different species, with disturbance playing a key role in shaping and maintaining this complex and dynamic mosaic system (Whittaker and Levin 1977, Dantas et al. 2016, Pausas and Bond 2020). However, if there occurs a change in the disturbance regime, this can modify the environmental conditions and the vegetation state. Once the fire suppression or the fire-resistance threshold is reached (Hoffmann et al. 2012, Raymundo et al. 2023), there is a tendency for a change in community composition and function as well (Dantas et al. 2013, Raymundo et al. 2023), which tends to become forest or grassland habitats, respectively. To better understand changes in species composition that occur over time associated with encroachment, long-term temporal studies are needed.

Despite major advances in understanding the dynamics of forest-grassland mosaics in the last two decades, some knowledge gaps still exist on how community composition and ecological strategies of woody species may change over time along a spatial gradient formed by forest-grassland mosaics, due to the scarcity of temporal studies. Also, we still need to advance in functional ecology by including more plant dimensions, in specific exploring the roots dimension. To our knowledge, existing information about functional patterns of woody species along forest-grassland mosaics gradient is based on static (non-temporal) studies, and mainly limited to traits related to the leaf economics spectrum (Müller et al. 2007, Carlucci et al. 2012, 2015, Silva 2015). There is a knowledge gap regarding other dimensions of form and function of plants (Díaz et al. 2016), especially in belowground (Carmona et al. 2021, Freschet et al. 2021), that was considered the 'black hole' in plant ecology (Weemstra et al. 2022). Integrating above and belowground traits render a more complete understanding of plant performance and ultimately, community and ecosystem processes, than separate assessments of plant dimensions (leaf, stem, root and whole-plant) (Carmona et al. 2021, Freschet et al. 2021, Weemstra et al. 2022).

We conducted a study in Southern Brazilian forest-grassland mosaics to examine how vegetation has changed structurally and functionally over time due to the absence of disturbance, observing the encroachment process occur. For this, we adopted an integrated functional approach to evaluate leaf, stem, root and whole-plant traits, relevant to the performance of woody plants. We aimed to answer the following questions:

(1) How does the vegetation structure of the grassland subject to encroachment change in space and time? With the interruption of the disturbance, we expected to observe the process of WPE happening, with the gradual colonisation of woody species that may

transform the grassland into a forest physiognomy, marked by an increase in the mean height of vegetation, mean density and total basal area of woody individuals over the years.

(2) How does the functional composition of the community change along a spatial gradient of forest-grasslands mosaics under WPE? And how does this composition change through time along WPE? We expected to find high values of β -diversity comparing grassland and forest communities mainly due to the turnover component because forest and grassland communities have distinct sets of species, although some species occur in both habitats. Over time, we expect β -diversity values to decrease as a result of the increasing similarity of functional composition of grasslands encroached and forest communities, result of colonization of forest species.

(3) How are community functional patterns and species ecological strategies distributed in a spatial gradient of forest-grasslands mosaics under WPE? And how do they change over time along WPE? Different ecological strategies enable the occurrence of species along a gradient between both habitats. Species that occur in grasslands would present adaptations to survive in open ecosystems, with early investments in bark thickness and higher leaf dry matter content, indicating a strategy to protect stem and leaf tissues against fire. For the forest, we predicted light competition among trees would select for increasing height and specific leaf, traits related to the ability to compete for light. Roots should be coordinated to acquire resources fast and exhibit an acquisitive trait in forests too. We expected a turnover in ecological strategies of woody species in grasslands that were encroached because shifts in environmental conditions associated with WPE would allow the establishment of species with other ecological strategies, mainly forest species. For forests, we do not expect significant changes in functional patterns as they are in a stable state and did not suffer any disturbances during the study period.

Finally, (4) we aimed to assess the whole-plant functional strategy, including belowground, to understand possible coordination between above and belowground dimensions. We are not expecting to find coupled resource use strategy for all plant dimensions (as expected according to Reich 2014 and Weigelt et al. 2021). Instead, we expect that each dimension responds to different selective pressures and environmental conditions, with limited coordination between above and below-ground functional traits. These expectations were built considering recent studies that showed that above and belowground are not necessarily coupled (Carmona et al. 2021) and that a single conservation-acquisition axis of variation cannot capture the variability of functions and environmental pressures to which the root system is subjected (Weemstra et al. 2016, Arrieta-González et al. 2021).

3 MATERIAL AND METHODS

3.1 STUDY SITES

We conducted this study in two sites of forest-grassland mosaics, located in the state of Paraná, southern Brazil, inside protected areas: Guartelá State Park (GUA) and Vila Velha State Park (VV) (Figure 1). The GUA is a protected area (24°33'45.52"S - 50°15'27.70" W), with 790 ha situated along the Iapó River canyon and elevations ranging from 912 to 1,000 m. The VV (25°14'8.94"S - 49°59'50.23" W) has a total area of 3.803 ha with elevations ranging from 800 to 1160 m. VV was created in 1990 and is subject to prescribed fire every 4 to 5 years by the state environmental agency since ca. 2010. The study area in VV was burned last time in 2017, i.e. between the two sampling years (2012 and 2022). However, the fire did not reach either forest or grassland now under woody encroachment (personal observation and satellite images), possibly due to the proximity of a river course that acted as a fire breaker. In opposition to VV, GUA had a zero-fire policy until 2022, with no record of disturbance since the implementation of the protected area (in 1992, > 30 years). We consider that both study areas have not been disturbed and thus we aim to verify the effect of this absence of management on vegetation dynamics. Also, there were no domestic cattle or large native grazers in the study area, which could impact the WPE process as well. The actual vegetation spatial gradient of forest-grassland mosaics is a result of at least 30 years of WPE process due to fire and cattle suppression.

Study areas were located approximately 80 km apart from one another. However, both areas are located under different climate classifications: Cfa for GUA and Cfb for VV (Cruz 2007, Alvares et al. 2013). Cfa is a subtropical climate with an average temperature (Temp.) in the coldest month below 18° C, and an average Temp. in the hottest month above 22° C, with hot summers, infrequent frosts and a tendency to concentrate rainfall in the summer months, with no defined dry season. Cfb is a temperate climate with an average temperature in the coldest month below 18° C, with cool summers and an average temperature in the hottest month below 22° C, without a defined dry season (Cruz 2007, Alvares et al. 2013). The climatic data of study areas were obtained from meteorological stations close to the study areas, in the municipalities of Telêmaco Borba (GUA) and Ponta Grossa (VV), respectively (IDR-Paraná, available at <https://www.idrparana.pr.gov.br/Pagina/Dados-Meteorologicos-Historicos-e-Atuais>). The mean annual rainfall is 1646 (GUA) and 1554 (VV), and are well distributed over the year,

with only two months (July and August) with < 105 mm in both study areas. The mean annual temperature is 17.8°C (VV) and 18.6°C (GUA), whereas the monthly minimum and maximum mean temperatures range between 13.5°C to 26.0°C (GUA), and 13.3°C to 24.1°C (VV).

Both study areas are located in the region of *Campos Gerais* (Maack, 1948), which is formed by sedimentary rocks with a high diversity of soil types (Moro & Carmo, 2007). Soils in both areas include haplic cambisols and humid litholic neosols derived predominantly from sandy sedimentary rocks of the Paraná and Itararé groups (Carmo 2006, Cervi et al. 2007, Moro and Carmo 2008). Previous soil analysis in study areas concluded that the soil chemicals and structural characteristics are similar, however, VV soils are considered a little more fertile than those in GUA, because of their lower sandiness and higher concentrations of K and Mn, while GUA was characterised by sandier and acidic soils (Moraes et al. 2016).

The vegetation in the study areas is composed of patches of grasslands, shrublands and forests, which all together form a complex mosaic system (Figure 1). South Brazilian grassland is the collective term used to refer to grassland vegetation in the Southern part of Brazil (Rio Grande do Sul, Santa Catarina, and Paraná states). South Brazilian grassland included the *Campos Gerais* region, where our sites are located (see further details in Overbeck et al. 2022). The vegetation of the *Campos Gerais* region has been gradually replaced by different forest types as a consequence of changes from a drier to a more humid climate in the last millennia (Maack 1948, Klein 1960, Behling 1997, Pillar 2003, Behling et al. 2004b, Carlucci et al. 2021, Souza 2021). It is distinguished mainly by the presence of a continuous layer formed by grasses, high species richness, and low tree density, without a gradient of tree cover (Müller et al. 2013). The northern portion of the *Campos Gerais*, where our sites are located, is a transitional zone including patches of *Araucaria* Forest, native grasslands, and a strong floristic influence of Cerrado, especially in GUA, that is inside Cerrado ecoregion. The Southern limits of Cerrado is in the *Campo Gerais* region, represented by small disjunction patches with great affinities and floristic similarity with the Northern savannah formations of São Paulo and core area of the ecoregion (Cervi et al. 2007, Moro and Carmo 2008, Maris et al. 2010, Moraes et al. 2016). Cerrado is characterized by different vegetation physiognomies that form a gradient of tree cover, from pure grasslands (called *campo limpo*), to grassland with scattered woody species (called *campo sujo*), to savanna until reaching an open forest physiognomy (called *cerradão*) (Batalha 2011, IBGE 2012). This gradient does not exist in South Brazilian grasslands, where the woody component is almost exclusively of shrubs and trees of pioneer species

from forests that are not fully adapted to fire, and who are able to establish in grasslands with a sufficient interval without fire (Müller et al. 2013). By contrast in Cerrado exist a lot of wood species typical of savannas, that have adaptations to fire (Simon and Pennington 2012, Dantas et al. 2013, Maracahipes et al. 2018b, Goncalves et al. 2021, Bernardino et al. 2022). Floristic studies that have assessed the flora of the *Campos Gerais* region identify the proportion of shared species recorded may reach 40% for the flora as a whole and 60% for grasses (Cervi et al. 2007, Ritter et al. 2010), and identify a phytogeographic barrier between the phytocenoses of GUA and its surroundings and those of VV and its surroundings (Ritter et al. 2010). This floristic influence is stronger in GUA, where a typical savannah flora was found in previous surveys, (Moro and Carmo 2008, Ritter et al. 2010, Moraes et al. 2016). The cooccurrence of these different vegetation types reflects the confluence of subtropical and tropical floras, with the occurrence of typical elements of the savanna together with grasslands (Moro and Carmo 2008). These floristic differences justified our decision to analyse both study areas separately.

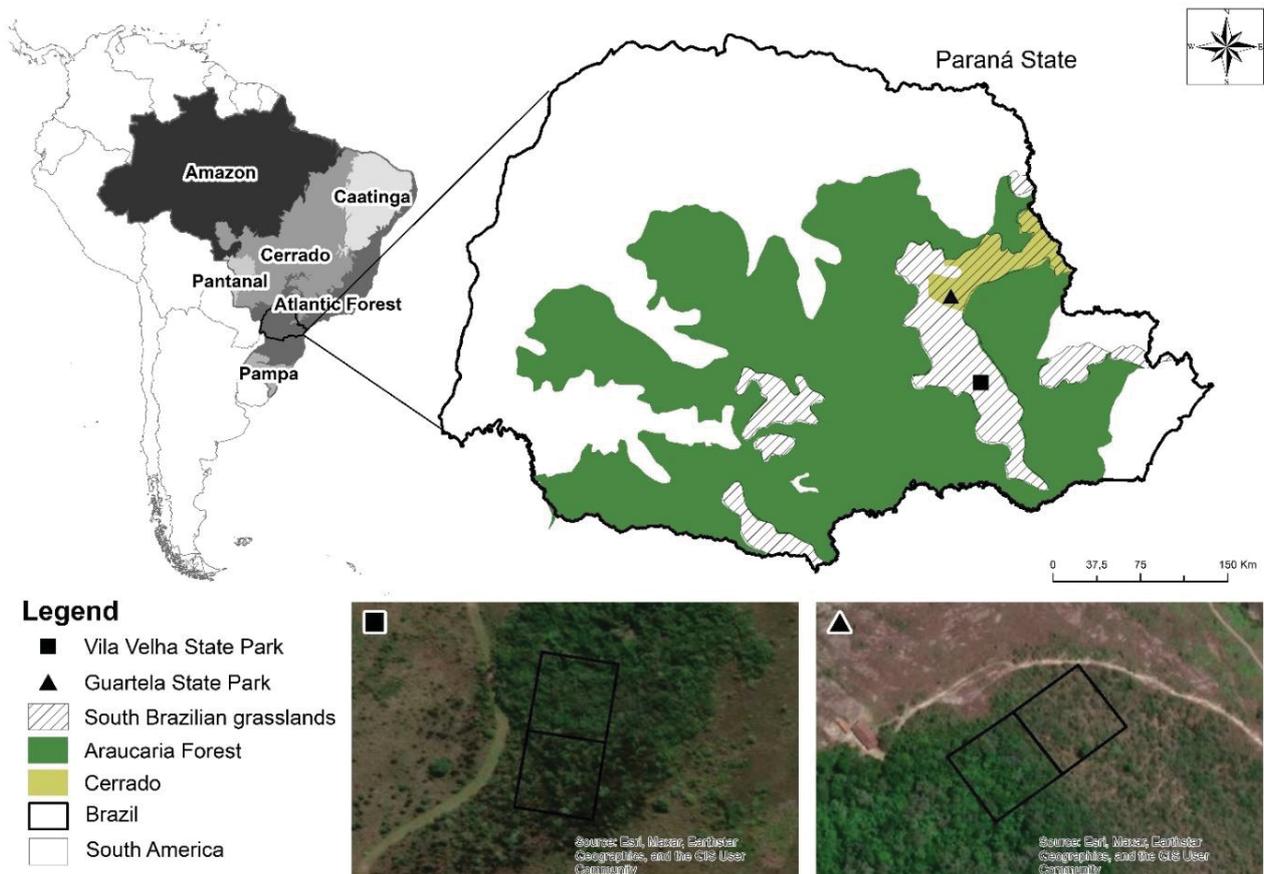


Figure 1: Location of study areas. Map of South America, with the delimitation of Paraná State, Brazil; Location of the sampling sites in Vila Velha State Park (VV - square symbols) and Guartelá State Park (GUA - triangle symbols), in the transition region between Cerrado (light beige area), Araucaria Forest (green) and South Brazilian grasslands (delimited striped area). Satellite images from both areas in 2022 (Google Earth Pro). Biomes delimited according to IBGE (2012) classification.

3.2 VEGETATION SURVEY

The first sampling was carried out in 2012 by the SISBIOTA Project *Campos Sulinos*, a national project focused on surveying forest-grassland mosaics in South Brazilian grassland. The project has established permanent plots located in forest-grassland mosaics so that half of the plot covered forest habitat (hereafter forest) and the other half covered grassland habitat (hereafter grassland). The transition between each habitat was determined considering that grassland started when the continuous canopy from the forest ended and the grass layer could be identified as the predominant cover in the ground (Silva 2015). The vegetation mosaic formed between grassland and forest is therefore the spatial gradient of study. In addition to the spatial gradient, we also have the temporal gradient: the areas were sampled in 2012, and in 2022 we returned and resurveyed the same sites to obtain current data on vegetation function, structure and composition to compare what has changed over these 10 years.

The sampling design consisted of one block of 70 m x 140 m per study area. Each block was divided into two adjacent habitats, one covering forest and the other covering grassland, each with 0,49 ha (70 m x 70 m). Each habitat was divided into 49 subplots with 10m x 10m, out of which 15 subplots were randomly selected for sampling. We sampled 30 subplots, considering both habitats, per study area, totalizing 60 subplots sampled. The subplots were demarcated in 2012 with metal vertex and species identification plaques, which allowed us to return to the same subplots in 2022 to do a second census of all previously marked woody individuals. In each selected subplot, we recorded the total plant height and diameter at the base height (DBH) of each stem for every woody individual whose main stem had a DBH \geq 5 cm. Geographic coordinates were obtained at the centre of each subplot using a GPS device.

To assess our assumption that temporal changes had occurred in vegetation structure, and to better characterise the dynamics of WPE over grasslands, we calculated the following community structural parameters per subplot: total number of adult tree individuals, average plant height (m), mean number of stems and total basal area (m²/ha), indicators of stand density (IBGE 2012). Forests' successional stage was determined for each habitat according to the total basal area per hectare estimated (CONAMA n^o 002/1994).

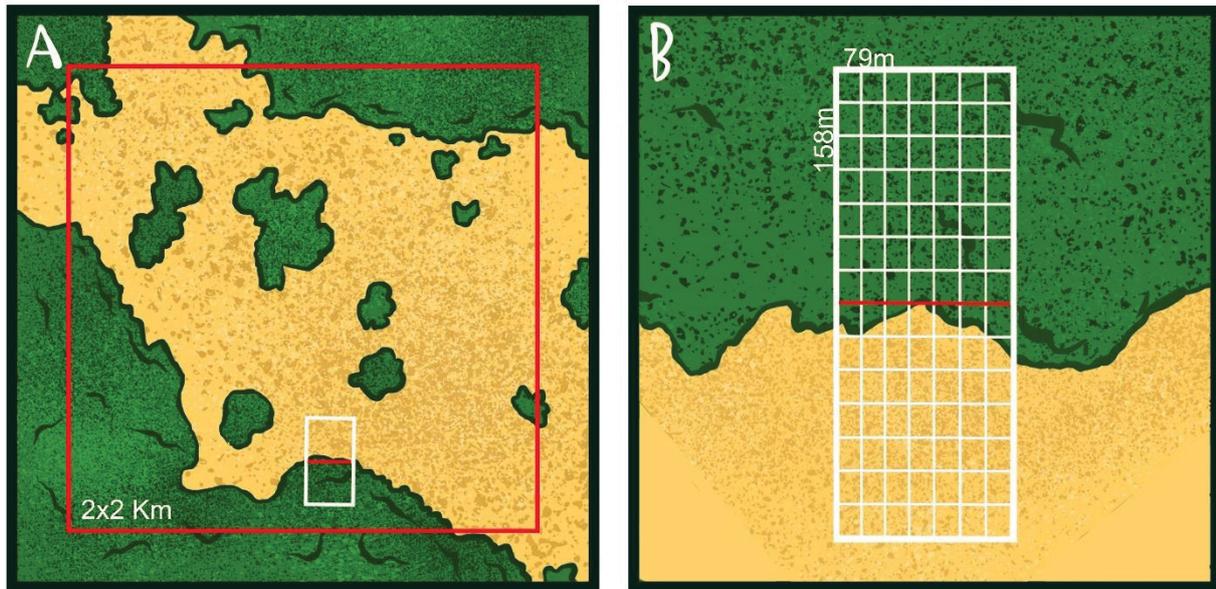


Figure 2: Illustrative scheme of vegetation sampling design carried out in forest-grassland mosaics. A) Sampling plots established in forest-grassland mosaic areas with a grassland and a forest block; B) Example of distribution of plot permanent plots, divided into subplots, of which some were randomly selected.

3.3 COLLECTION OF FUNCTIONAL TRAIT DATA

We chose seven functional traits that are good descriptors of the performance of species along environmental gradients, according to studies that evaluated global form and functional plant patterns (Westoby 1998, Westoby et al. 2002, Wright et al. 2004, McGill et al. 2006, Moles et al. 2006, 2009, Chave et al. 2009, Perez-Harguindeguy et al. 2016, Díaz et al. 2016, Salguero-Gómez 2017, Carmona et al. 2021). We measured the following functional traits: leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), stem-specific density (SSD), bark thickness (BT), maximum height (Hmax), specific root length (SRL) and mean diameter of root (D) (Table 1). Hence, we measured functional traits related to the whole-plant form and function spectrum (leaf – LA, SLA and LDMC; stem – SSD and BT; root – SRL and D; and whole-plant – Hmax). The measurement of traits in 2012 was restricted to leaf traits and maximum height, and in 2022 applied to the whole plant. Trait data collections were carried out between October-2021 and May-2022 and followed the global protocol (Perez-Harguindeguy et al. 2016) and the standardised procedures indicated by the TRY Database (Kattge et al. 2020). We adopted the minimum number of >5 individuals sampled per habitat, as recommended by the global protocol (Perez-Harguindeguy et al. 2016). For species that occurred in both habitats, we collected data in each habitat to account for intraspecific variability (Violle et al. 2012). In the absence of individuals to complete the minimum sampling effort for a given species inside the subplots, we reached for individuals in the same block, then nearby the block but always in

the same habitat, and finally in other blocks of the SISBIOTA Project, surveyed following the same protocol (necessary just for two rare species in GUA). A detailed description of the methodology for trait collection considering intraspecific variation is available in Appendix A.

Table 1: Functional traits selected to describe above and belowground ecological strategies of species, to evaluate changes along a gradient of woody plant encroachment in forest-grassland mosaics. Abb. – abbreviation, G – grassland, F – forest.

Functional trait	Abb.	Unit	Functional significance	Reference	Hypothesis	
					G	F
Maximum height	Hmax	m	Assess to resources and competitive vigour for light.	(Westoby 1998, Moles et al. 2009, Díaz et al. 2016)	Low	High
Leaf area	LA	cm ²	Related to responses to nutrient stress, harsh environmental conditions and disturbances, a trait of the leaf economic spectrum	(Westoby 1998, Wright et al. 2004, Donovan et al. 2011, Reich 2014, Díaz et al. 2016)	Low	High
Specific leaf area	SLA	cm ² . g ⁻¹	Leaf economic spectrum, a trade-off between C gain and leaf longevity, efficiency in the use of resources, competitive vigour for light.	same as LA	Low	High
Leaf dry matter content	LDMC	g . g ⁻¹	Leaf economic spectrum, resistance to leaf damage.	same as LA	High	Low
Stem-specific density	SSD	cm ³ . g ⁻¹	A trade-off between growth vs. biomechanical and hydraulic resistance.	(Chave et al. 2009, Díaz et al. 2016)	Low	High
Bark thickness	BT	mm	Allocation of resources in the protection of vital tissues against damage, fire-resistance threshold	(Chave et al. 2009, Hoffmann et al. 2012, Pausas 2015)	High	Low
Specific root length	SRL	m . g ⁻¹	Fine-root trait related to resource-use strategy and capacity to acquire resources in the soil.	(Bergmann et al. 2020, Carmona et al. 2021, Weigelt et al. 2021, Freschet et al. 2021, Weemstra et al. 2022)	Low	High
Mean root diameter	D	mm	Fine-root trait related to resource-use strategy and fungal collaboration interaction to acquire resources from the soil.	same as SRL	High	Low
Root branching frequency	RBF	mm	Fine-root trait related to resource-use strategy and capacity to acquire resources in the soil.	same as SRL	Low	High

3.4 STATISTICAL ANALYSIS

3.4.1 Vegetation structural analysis

We performed a principal component analysis (PCA) to reduce the multidimensionality of vegetation structural parameters, considering the existence of some

correlation between them i.e., the known correlation between the average height and total basal of the community (Dantas et al. 2013). Data of vegetation inventory was organized per subplot sampled, considering the total number of individuals, average height of the community (m), total basal area ($\text{m}^2/\text{ha}^{-1}$) and the mean number of stems per subplot. The PCA axis was used as an index that synthesizes structural change in vegetation that exists in a gradient formed by mosaics of grassland-to-forest, by summarizing the variety of important densitometric and structural parameters of the community, combining the variation of basal area, plant height and tree density, consider good predictors of canopy closer and vegetation structure (IBGE 2012, Dantas et al. 2013). We expected that the structural PCA axis synthesizes and reflects the general changes in vegetation structure and in the light environment along the spatial gradient formed by grasslands and savannas to closed forests and along the temporal gradient of WPE. Hereafter the first PCA axis is called 'vegetation structure index'. The structural PCA was built using the 'prcomp' function of the 'stats' package, the basic package of R.

We construct a model to evaluate how much variation in structural data of vegetation can be explained by habitat (forest vs. grassland), and the two sampling years (2012 and 2022), that were our predictor variables, to answer question 1. As a response variable, we used the vegetation structure index. We performed a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. As we have the same subplots sampled in two periods of time, we used a two-way PERMANOVA with repeated measures, which is recommended whenever multiple observations on the same replicate were collected at different times (Gotelli & Ellison, 2016). Models were performed per study area (considering the floristic differences discussed), using 'lmp' function of the package 'lmPerm' (Wheeler et al. 2022). We calculated the proportion of variance explained by our predictor variables, considering only significant results. In addition to the temporal autocorrelation controlled with repeated measures, we assessed spatial autocorrelation between plots by using Moran's spatial auto-correlograms (Diniz-Filho et al. 2003) (see supporting information for details, in Appendix B).

To assess differences in averages between habitat and year of sampling, we performed a post hoc analysis using a pairwise two-sample permutation test, with the 'pairwisePermutationTest' function of package 'rcompanion' (Mangiafico 2017), applying the Bonferroni correction to *P*-values for multiple tests, to test pairwise differences.

3.4.2 Community functional analysis

We aim to understand how the general functional pattern of the community and the ecological strategies of species may change along spatial and temporal gradients of vegetation under WPE.

For functional community analysis, it was necessary to scale up traits measured at the species level to the community level. We have functional trait data at species level, incorporating intraspecific variation of species that occur in both habitats and/or in both sampling years (details in Appendix A). We scaled up species trait information to the community level by computing mean trait value per subplot, incorporating species abundance, using community-weighted trait means index (hereafter CWM; Lavorel et al. 2008). With this, we have the mean value of each trait, considering the species composition of each subplot, and the respective abundance of species. We first calculated CWM with functional data in their real scales, and later standardised the CWM matrix to zero mean and unit variance, and carried out a PCA with the CWM standardised matrix (hereafter called the functional community PCA). We used the function 'functcomp' of the 'FD' package.

To test how the functional pattern of community shifts in response to structural changes in vegetation as a consequence of the WPE process, we performed a PERMANOVA. The first main axes of the functional community PCA were used as response variables in our model and as predictor variables was used the vegetation structure index. Thus, we can understand how functional changes in communities are associated with encroachment along space and along time.

Furthermore, we assessed functional beta diversity (hereafter functional β -div). We used the approach by Villéger et al. (2013), which adopted the taxonomic β -div framework by Baselga (2012) to functional β -div using multivariate trait spaces. Functional β -div computes distances matrices accounting for total β -div, considering β -div = (functional space not shared / total functional space filled) and decomposes it into turnover and nestedness components (Villéger et al. 2013). High functional turnover means that the communities host different ecological strategies, while low functional turnover and high nestedness indicate that one community hosts a small subset of the ecological strategies present at the other (Villéger et al. 2013). We measured functional β -div and their respective turnover and nestedness-resultant components among habitats (forest or grassland) and sampling year (2012 or 2022), using the standardised trait matrix. We used the function 'functional.beta.pair' of the 'betapart' package (Baselga and Orme 2012).

3.4.3 Species functional analysis

We followed the analytical methodology proposed by Diaz et al. (2016) in the global spectrum of plant form and function and by Carmona et al. (2021) in the global spectrum including fine-root traits. Functional data were organized in a matrix of the mean trait value per specie, per habitat, and per sampling year, to do the functional analysis. Thus, we were able to maintain variation for species that occurred in both habitats and both sampling periods. We performed a PCA for above and belowground traits to extract the main axes of variation and evaluate functional trade-offs or covariances between traits at the species level, related to our question four.

First, we standardised the functional trait matrix to zero mean and unit variance, using the 'decostand' function of the 'vegan' package. We applied a PCA on the trait standardised matrix to assess functional trait variation as a consequence of WPE, hereafter called the functional species PCA. This analysis was used to reduce trait dimensionality to significant axes of trait variation and to characterise the trait space occupied by species in each habitat and possible changes that occur along WPE. The strength of the correlation between functional traits and principal components was used to infer gradients in life-history trade-offs at the species level. We retained functional traits with correlations ≥ 0.4 with a given axis for further interpretation. To visualize the occurrence probability of given combinations of functional traits in a two-dimensional space defined by PCA axes, we used multivariate kernel density estimation, which revealed areas of high density of occupation. We performed the kernel density estimation using 'kde2d' function ('MASS' package) and produced plots using 'stat_density_2d' function of 'ggplot2' package. To test for pairwise correlations among traits above and below-ground, we performed the analysis of correlation using Pearson correlations with the 'corrplot' function from the corrplot package (Wei et al. 2021).

Analyses were conducted in R software version 2022.02.3+492 (R Core Team, 2022).

4 RESULTS

4.1 CHANGES IN VEGETATION STRUCTURE ACROSS SPACE AND TIME

Our results showed that both study areas had undergone a considerable increase in woody cover over 10 years without disturbance (Table 2). Woody individuals were rare in the grasslands in 2012. In the absence of disturbances, forest species have been able to colonise the grassland (Figure 3). We found clear structural differences between grassland in 2012, grassland in 2022 and forest in GUA (pseudo-F = 70.46, $R^2 = 0.78$, $p = 0.001$) and in VV (pseudo-F = 85.21, $R^2 = 0.74$, $p = 0.001$). Both models had a good fit and no sign of spatial autocorrelation in the residuals (Figure SM 4 and Figure SM 5). In Guartelá State Park (GUA), we recorded 395 individuals in 2012: 72 in the grassland and 323 in the forest. After 10 years, we recorded 711 individuals: 294 in the grassland and 417 in the forest, representing an increase of 302.76% and 29.10% in the density of woody individuals, in the grassland and the forest, respectively. A total of 65 species were registered in GUA, among which 17 occurred exclusively in the grassland, 31 occurred exclusively in the forest, and 17 were found in both habitats. In Vila Velha State Park (VV), we recorded 441 individuals in 2012: 67 in the grassland and 374 in the forest. Similarly, in VV was evident the impact of WPE led to a substantial change in vegetation structure: in 2022 we recorded 647 woody individuals, among which 224 occurred in the grassland and 417 in the forest, representing an increase of 234.32% the density of woody individuals in the grassland and 11.76% in the forest. These individuals belong to 49 species, among which five species occurred exclusively in the grassland, 31 exclusively in the forest, and 13 occurred in both habitats.

We found clear structural differences between forest and grassland over time (the structural PCA results; Figure SM 6). PC1 differentiated forest from grassland for both sampling years. It was positively related to higher values of basal area, height and number of individuals per plot, which characterise forests, and negatively related to higher mean values of tillers number, that separate grassland subplots. Considering the high correlation in structural parameters of vegetation, and clear differences between grassland and forest, a single axis of the structural PCA was able to capture 69.1% and 67.8% of vegetation variation between habitat and through time, in GUA and VV, respectively. This PC1 was used as an vegetation structure index that synthesizes vegetation structural changes in subsequent functional analyses. Details about the structural PCA available in Appendix E.

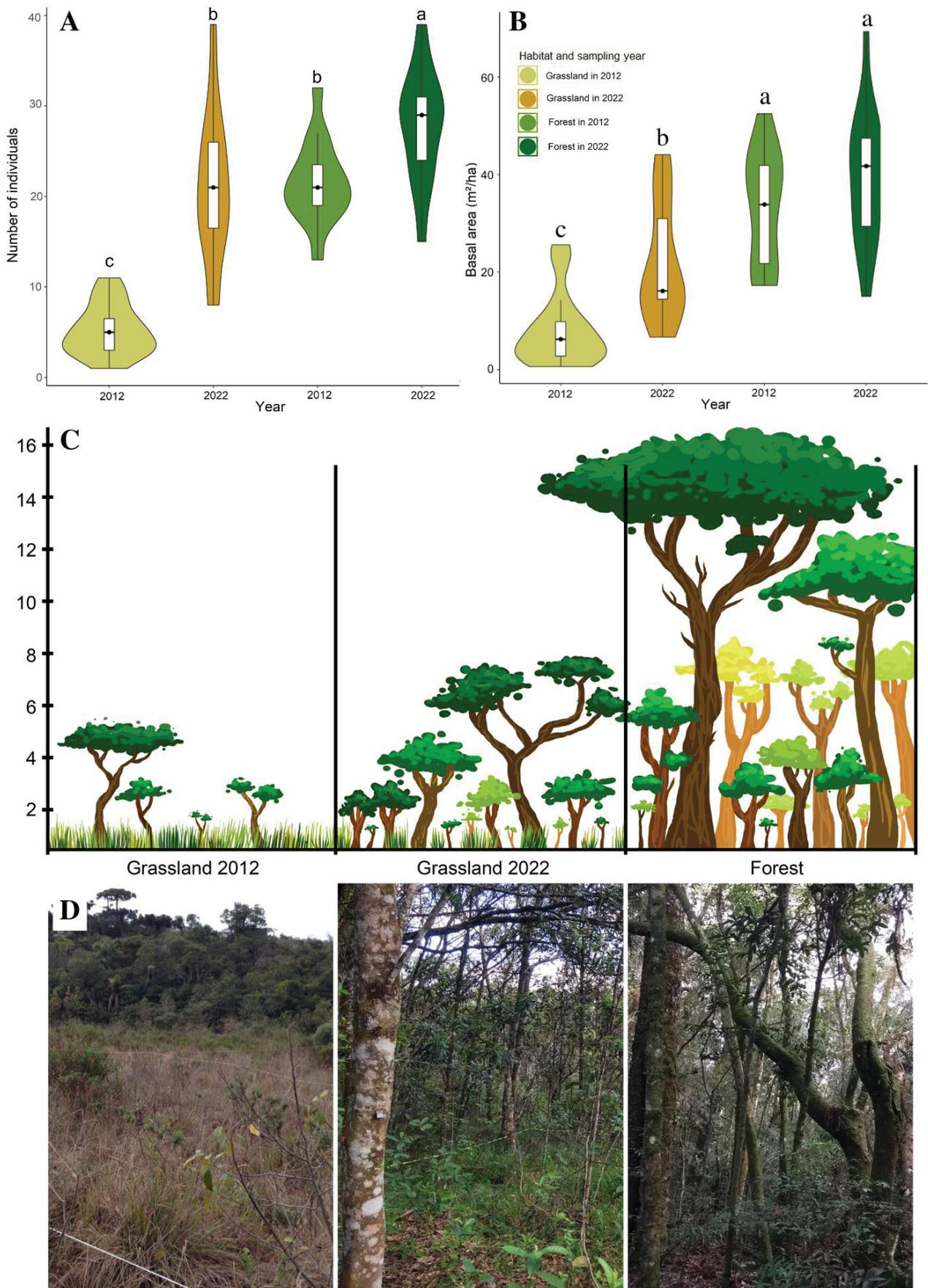


Figure 3: Synthesis of vegetation structural changes after 10 years of woody plant encroachment in forest-grassland mosaics. A) Violin plot (boxplots combined with kernel density plots) comparing the number of individuals in each habitat. Data of Guartelá State Park. B) Violin plot comparing total basal area (m^2) per subplot sampled ($100 m^2$), estimated for 1 hectare. Data of Vila Velha State Park. Distinct letters represent significant differences between habitats in the pairwise permutation Tukey's HSD post hoc test, all $p < 0.05$;

C) Illustrative drawing of the vertical profile of vegetation height through a temporal gradient of changes in vegetation under WPE; D) Photographic record of grassland in 2012, in 2022 and forest, respectively. All photos are from Vila Velha State Park.

Table 2: Temporal changes in vegetation structure along the woody plant encroachment process. Values are the mean and standard deviation. The mean value per subplot represents data collected per subplot of 100 m² sampled. Distinct letters represent significant differences between habitats in the pairwise permutation Tukey's HSD post hoc test, all $p < 0.05$.

Guartelá State Park	Grassland		Forest	
	2012	2022	2012	2022
Total number of woody species	21	32	40	44
Mean number of woody individuals per subplot	5.21 ± 2.91 a	21.00 ± 8.63 b	21.53 ± 4.56 b	27.80 ± 6.14 c
Increment of woody individuals per subplot, in percentage	-	302.73%	-	29.10%
Mean number of stems per individual per subplot	2.57 ± 2.30 c	3.80 ± 4.50 b	1.15 ± 0.52 a	1.27 ± 0.85 a
Mean height (m)	3.95 ± 1.50 a	3.41 ± 1.21 a	6.66 ± 2.40 b	6.93 ± 3.44 c
Total basal area estimated per hectare (m ² /ha ⁻¹)	5.21 ± 3.94 a	16.21 ± 7.45 a	40.53 ± 13.95 b	45.00 ± 17.25 b
Forest successional stage	initial	intermediate	advanced	advanced
Vila Velha State Park	Grassland		Forest	
	2012	2022	2012	2022
Total number of woody species	12	16	40	41
Mean number of woody individuals per subplot	4.46 ± 2.58 c	14.93 ± 6.76 b	24.93 ± 5.99 a	27.86 ± 5.69 a
Increment of woody individuals per subplot, in percentage	-	234.32%	-	11.76%
Mean number of stems per individual per subplot	3.37 ± 2.80 b	2.33 ± 1.92 b	1.43 ± 0.98 a	1.54 ± 1.15 a
Mean height (m)	6.20 ± 1.27 b	6.68 ± 1.32 b	7.87 ± 0.91 a	8.74 ± 1.15 a
Total basal area estimated per hectare (m ² /ha ⁻¹)	8.14 ± 7.40 c	21.99 ± 12.06 b	33.29 ± 11.66 a	39.59 ± 13.65 a
Forest successional stage	initial	intermediate	advanced	advanced

4.2 SHIFTS IN COMMUNITY FUNCTIONAL PATTERNS UNDERLYING WOODY PLANT ENCROACHMENT

The structural and physiognomic changes that occurred associated with the WPE process were accompanied by shifts in the functional pattern of the community, resulting in trait-based distinctions between forest and grassland habitats.

The PCA revealed two main axes of variation, which primarily separated grassland communities from forest communities (PC1). The first axes of the functional community PCA were capable to synthesize 74.2% and 65.4% of the variation in functional traits scale-up at the community level, in GUA and VV, respectively. In GUA the first axis was positively correlated to higher CWM values of LDMC, SRL and RBF, which distinguished grassland communities, and negatively related to higher CWM values of LA, SLA, D and Hmax, related to forest communities. In VV the first axis was positively correlated to SRL, RBF and BT, and negatively correlated to SLA and Hmax, and also clearly separate forest and

grasslands, that barely overlap in the functional community PCA. Also, in VV the second axis is important (according to broken-stick criteria), and synthesizes a negative relationship between D, LA and Hmax. The first axis, in both study areas, separated grassland and forest communities, with the first being characterized by acquisitive roots traits, conservative leaves and high resistance against fire events (high BT), from forest communities, which possess traits values associated with competition for light, with acquisitive leaves, higher Hmax and conservative roots traits.

There were considerable changes in CWM trait values along the spatial gradient of forest-grassland mosaics and over time, showing a functional trajectory of changes associated with encroachment. We found a considerable decrease in LDMC, BT, RBF and SRL values, which were high in grassland communities in 2012 and tend to decrease in grassland in 2022, with lower values found in forest communities (Figure 5). The opposite pattern is observed for SLA and Hmax which tend to increase associated with the advancement in encroachment, in both study areas. In GUA, LA and SSD had a significant increase comparing grassland in 2012 with encroached grassland and forest, however in VV this trait did not have significant changes, as shown in the pairwise analyses. The functional trajectory of change in the community was similar for most of the functional traits assess above and below ground. However, for D we obtain a contradictory pattern: D was higher in forest communities in GUA and higher in grassland communities in VV. Detailed results of the PCA at the community level are available in Appendix F.

We test if the clear functional differences between grassland and forest communities identify in functional community PCA were correlated to the WPE process, performing a model where the response variable was the axes that synthesize the functional variation and the predictor variable was the vegetation structural index. We found that a significant portion of shifts in community functional patterns was explained by the structural vegetation index in GUA (pseudo-F = 166.5, $R^2 = 0.74$, $p = 0.001$) and in VV (pseudo-F = 120.4, $R^2 = 0.67$, $p = 0.001$). In other words, there is a general change in the community functional pattern and a large part of this change is directly associated with the process of WPE that resulted in shifts in vegetation structure and physiognomy. Both models had a good fit and no sign of spatial autocorrelation in the residuals. The vegetation structural index was strongly correlated with the first axis of functional community PCA (PC1) in GUA ($r = -0.86$, $P < 0.001$) and VV ($r = -0.76$, $P < 0.001$). Considering the second axis, this correlation is not significant in GUA ($r = -0.02$, $P = 0.863$) or diminish in VV ($r = -0.37$, $P = 0.003$). This negative correlation between functional patterns and structural shifts means that with an increase in vegetation encroachment (high vegetation structural index values) occur a decrease in the

typical functional characteristics of grassland communities (high values of PC1), which tend to become similar to forests communities. Community-weighted trait means changed linearly with forest development. Also, the strong correlation between the vegetation structural index and PC1 in both study areas indicates that this may be the main functional trade-off in the community – a trade-off between being acquisitive in leaves or roots traits since both had negative covariance.

The grassland presented a considerable reduction in functional space between 2012 and 2022, in both study areas. Grassland in 2022 seems to follow a functional trajectory that is leading it to become more similar to the forest. The forest presented a similar functional pattern in both sampling years. Comparing both study areas, GUA is characterized by higher functional dispersion in grassland, while VV is characterized by higher functional dispersion in forest.

High functional β -div between habitats and sampling years was found in both study areas, mainly due to a high level of functional turnover (Table 3). We observed the highest values of turnover when comparing the grassland with the forest in both periods. It means that species found in grassland and forest are functionally very different. In contrast, we found that nestedness accounted for most of the total functional β -div when comparing grassland in 2012 and 2022, which seems that the grassland community in 2022 fills only a small portion of the functional space filled by grassland in 2012 (Table 3).

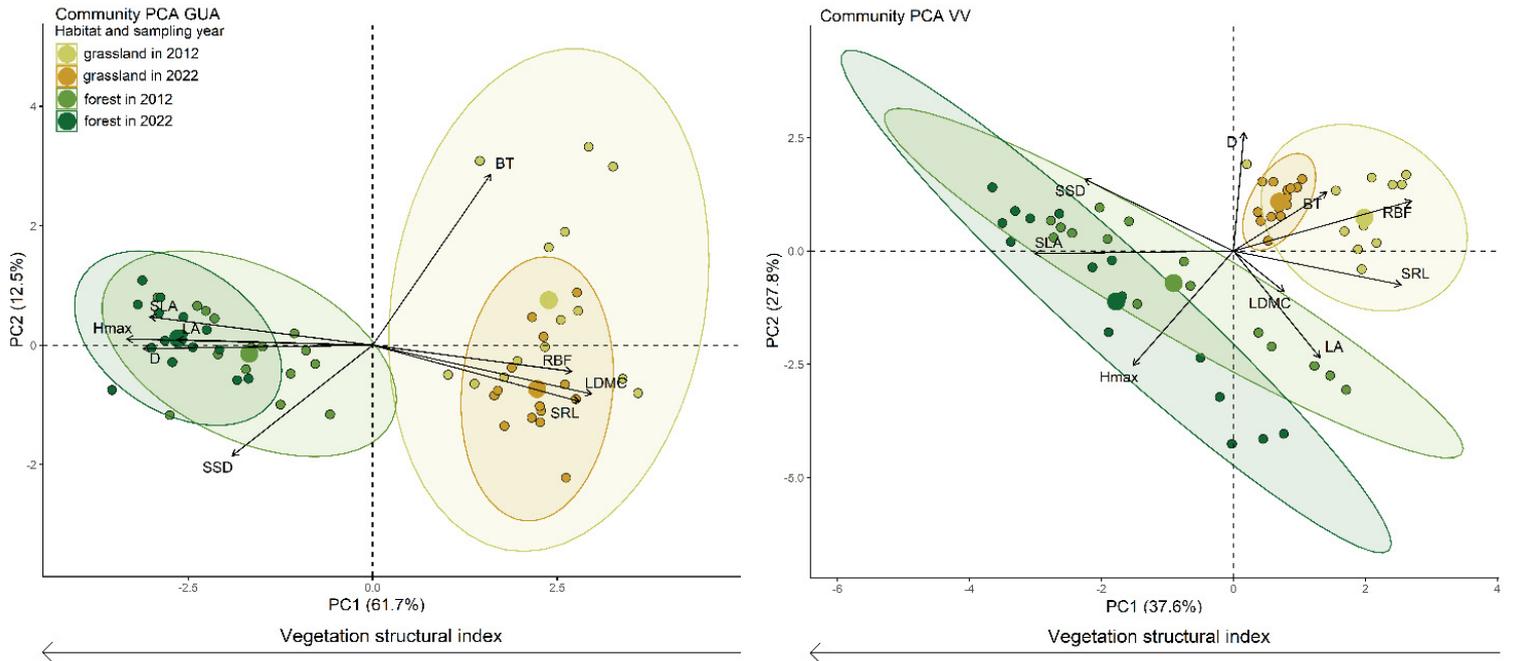


Figure 4: Functional community pattern of grassland and forest habitat. Analysed at the community level, the dots represent the subplots sampled in each habitat and sampling year. CWM values were calculated considering species abundance for each subplot. Detailed results of the PCA at the community level are available in Appendix H. Grassland is identified with beige colours (light beige for 2012 sample, and brownish beige for 2022 sample). Forest is identified with green colours (light green for 2012 sample and dark green for 2022 sample). Legend: GUA: Guartelá State Park; VV: Vila Velha State Park. Functional traits: LA – leaf area, SLA – specific leaf area, LDMC – leaf dry matter content, SSD – stem-specific density, BT – bark thickness, SRL – specific root length, D – average diameter, RBF – root branching frequency, Hmax - maximum height. Details are available in Appendix F.

Table 3: Functional index of β -diversity among habitats (grassland and forest) and sampling year (2012 and 2022). Total functional β -diversity was decomposed in turnover (turn.) and nestedness (nest.) components. The values in percentages are presented.

Pairwise comparison between habitat and year	GUA					VV				
	β -div	Turn.	Nest	Turn %	Nest %	β -div	Turn	Nest	Turn %	Nest %
Grassland 2012 and Grassland 2022	52.06	1.72	50.34	3.30	96.70	76.85	44.72	32.13	58.19	41.81
Grassland 2012 and Forest 2012	73.12	44.23	28.89	60.49	39.51	96.33	82.10	14.22	85.23	14.76
Grassland 2012 and Forest 2022	99.55	99.38	0.16	99.83	0.16	98.63	94.19	4.43	95.50	4.49
Grassland 2022 and Forest 2012	38.51	38.03	0.48	98.75	1.25	70.66	51.32	19.34	72.63	27.37
Grassland 2022 and Forest 2022	87.06	81.97	5.09	94.15	5.85	82.71	74.18	8.52	89.69	10.30
Forest 2012 and Forest 2022	52.81	33.57	19.24	63.57	36.43	33.09	27.49	5.60	83.08	16.92

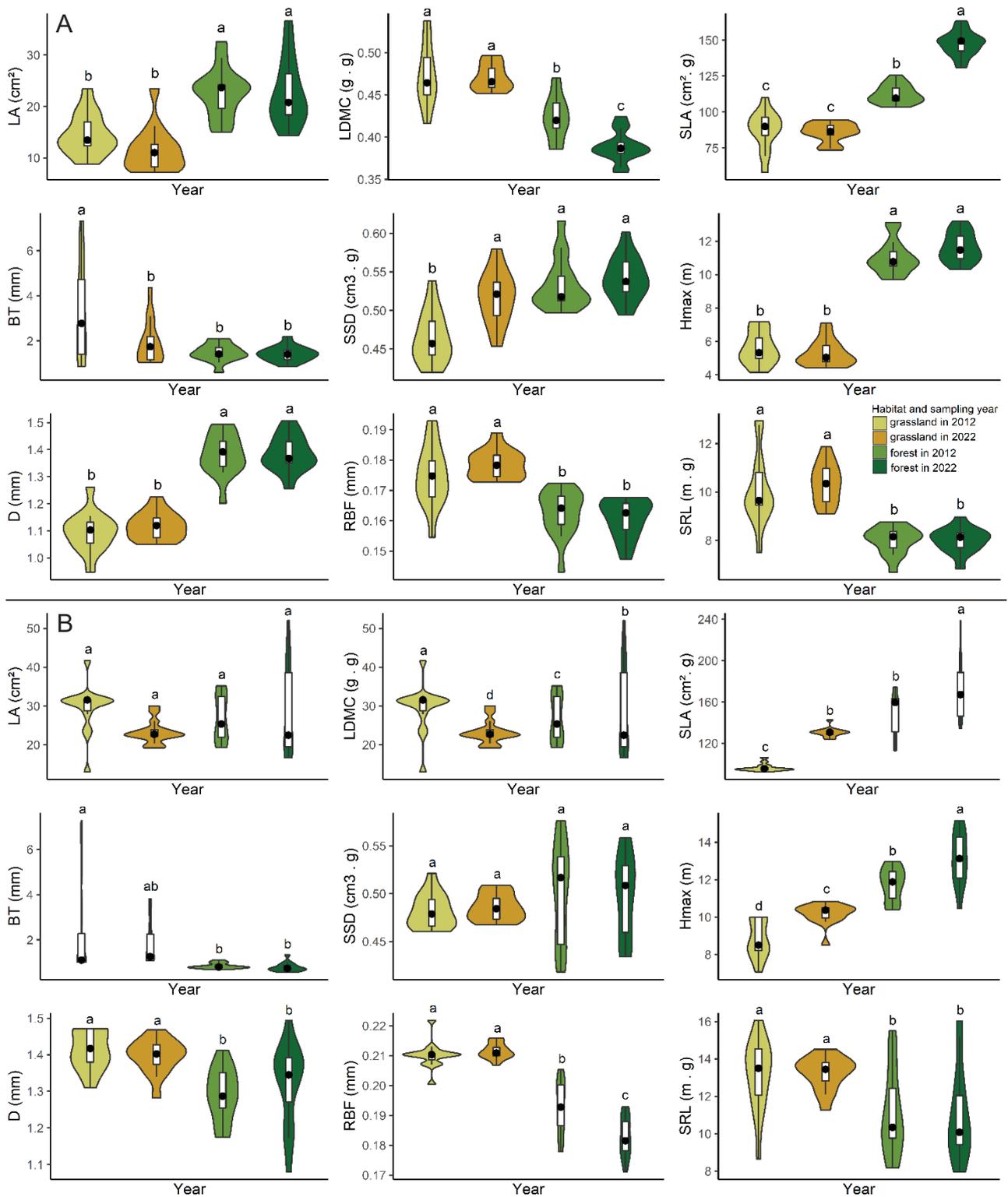


Figure 5: Changes in functional pattern of community during woody plant encroachment. Violin plot (boxplots combined with kernel density plots) comparing functional traits weighted by species abundance (CWM values for each trait), scale-up at the community level, along a spatial and temporal gradient of woody plant encroachment, in forest-grassland mosaic located in (A) Guartelá State Park (GUA) and (B) Vila Velha State Park. Boxplots showing median and 25^o and 75^o percentiles. The vertical curves display the kernel density estimates for each parameter's probability density function. Distinct letters represent significant differences between habitats in the pairwise permutation Tukey's HSD post hoc test, all $p < 0.05$. Traits legend is the same as above.

4.3 SHIFTS IN SPECIES FUNCTIONAL TRAITS UNDERLYING WOODY PLANT ENCROACHMENT

Variation of leaf, stem, root and whole-plant traits was summarised in ordination axes that represent important functional trade-offs at the species level.

Functional analysis at the species level showed that grassland and forest formed distinct functional hotspots in the functional trait space (Figure 6). Exist some degree of overlap between habitats and sampling years, which can suggest that despite the existence of an overall functional pattern in the community, with no overlap between forest and grassland in the community functional analysis (Figure 4), many species that were present in the community may not fit into it.

The functional pattern of acquisitive roots (high SRL and RBF values) in grasslands and acquisitive leaves in forest (high SLA values), identify at the community level, was found also considering species level. Curiously, D was the only trait that has a contradictory pattern in community analysis comparing both study areas. However, in species-level analysis, this trait had the same functional pattern: higher D values in forest and lower in grassland, with a negative covariance with the other two root traits (SRL and RBF), which had higher values in grassland and lower values in forest (Figure 6).

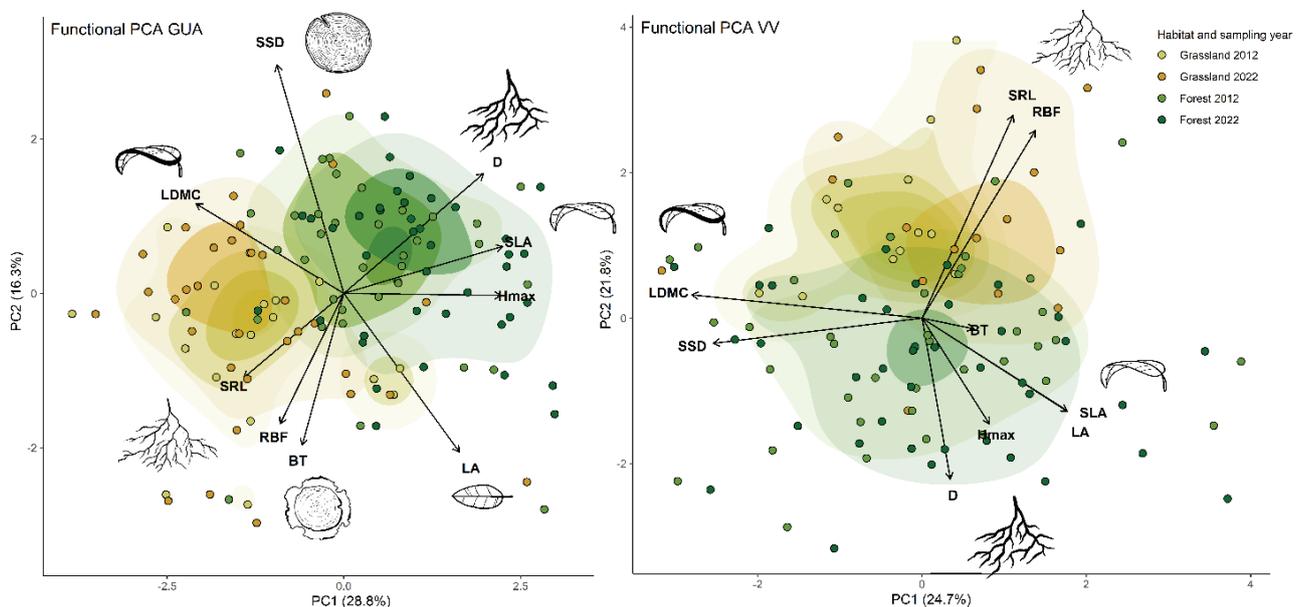


Figure 6: Functional distinctions between forest and grassland species ecological strategies associated with woody plant encroachment over time (2012-2022) in southern Brazil. Study areas: Guartelá State Park (GUA) and Vila Velha State Park (VV). Dark colours indicate functional hotspots in the multivariate functional space, i.e. regions with a higher density of species in the functional space. Legend: Functional traits: LA – leaf area, SLA – specific leaf area, LDMC – leaf dry matter content, SSD – stem-specific density, BT – bark thickness, SRL – specific root length, D – average diameter, RBF – root branching frequency, Hmax - maximum height. Details are available in Appendix G.

5 DISCUSSION

5.1 FUNCTIONAL TRAJECTORY AND STRUCTURAL CHANGES IN VEGETATION SUBMITTED TO WOOD PLANT ENCROACHMENT

In this paper, our goal was to evaluate how the vegetation structure changes in response to the WPE process and its relation of this with shifts in community functional patterns and species ecological strategies. By comparing vegetation plots sampled with 10-year intervals in areas under WPE, we were able to record significant changes in vegetation structure that occur due to the absence of disturbance. The grassland had been colonised by woody individuals and now presents a vegetation structure similar to a young forest, regarding woody density, community mean height and basal area. Surprisingly, the density of woody individuals in the grassland increased by 302.73% in GUA and 234.32% in VV. This increase in density, as well as the mean community height and basal area, is typically associated with increased shade conditions (Carlucci et al. 2012, Dantas et al. 2013, Flake et al. 2021). The encroachment process leads to the exclusion of grasses and shade-intolerant species, resulting in changes in vegetation-fire feedback (Pilon et al. 2021). The threshold of tree basal area ($\text{m}^2 \text{ha}^{-1}$) for Neotropical ecosystems know to be 6.85 ± 3.74 for savannas and 26.96 ± 10.22 for forests (Dantas et al. 2016). Forest species are generally absent from communities with a basal area $< 8 \text{ m}^2 \text{ha}^{-1}$, a medium density for *cerrado sensu stricto* savanna (Flake et al. 2021). Once this threshold is surpassed, the expectation is that vegetation physiognomy shifts will lead to a change in the vegetation state, from open to closed vegetation. Although the basal area was lower in the grassland in 2012, we observed a 67.85% increase in GUA and a 62.98% increase in VV, surpassing the recognized basal area threshold for open ecosystems (Dantas et al. 2016, Passos et al. 2018). The temporal evaluation of the WPE process allowed us to confirm, for the first time for mosaics in South Brazilian grassland, that a shift in vegetation alternative state occur over ten years, in a landscape where fire suppression had been implemented for 30 years (since the creation of the protected area). These findings support the predictions of the Alternative States Theory (Bond and Keeley 2005a, Dantas et al. 2013, 2016, Pausas and Bond 2020, Bond 2021), indicating that a vegetation state change occurs through time. Over time, grasslands subject to WPE became similar to a forest, regarding both vegetation structure, community functional pattern and ecological strategies of species. The grassland habitat nowadays is following the successional processes of a secondary forest, resembling the structure of Cerradão in GUA and young Araucaria Forest in VV.

Our results demonstrate that the WPE process not only changes vegetation structure, state and physiognomy, but also drive substantial changes in community functional patterns and species ecological strategies, leading to directional shifts in the dominant life-history strategies in the community. As environmental conditions gradually shift from open to closed environments, forest species with different ecological strategies are able to establish themselves. Without fire suppression, these forest species would hardly survive due to insufficient time to surpass the fire-resistance threshold (Hoffman et al. 2012). However, the suppression of fire allows forest species to successfully establish and significantly reshape the functional pattern of the community. In grasslands, the prevailing functional pattern is characterized by conservative leaves (high LDMC and low SLA values) for protection against high solar incidence and drought, acquisitive roots (high RBF and SRL values) for efficient nutrient competition, and thick barks (high BT values) for protection against fire. The encroachment is correlated with a decrease in these sets of traits, while causing an increase in others (Dantas et al. 2013, Flake et al. 2021, Raymundo et al. 2023). Over time, we observed an increase in Hmax, with increase investment in acquisitive leaves (high SLA values) to efficiently acquire resources in a light-limited environment, in detriment of decrease in BT values and in root trait values (with high D and lower SRL and RBF), indicating a conservative strategy for roots. Notably, even forest communities undergo changes, as indicated by increased SLA and Hmax and decreased LDMC values when comparing forests from 2012 to the present. This functional pattern is very clear at community level, however exist a wide range of variation at the species level, which can indicate the arrival of species with other ecological strategies, due to the encroachment that changes environmental conditions, corroborating functional β -div results that show a high functional turnover. Our results indicate the existence of life-history strategies that are vulnerable to encroachment, represented by woody species typical of grasslands and savannas, and life-history strategies that are favoured by encroachment, represented by woody species typical of forest habitat. Species that evolve under different selective pressures may develop different ecological strategies that allow them to have a better performance under certain conditions (Grime 1977, Grime and Pierce 2012, Díaz et al. 2016). Grasslands and savannas evolved under regimes of disturbances by fire, which is a major factor shaping plant functional traits (Bond and Keeley 2005a, Keeley et al. 2011), and possess functional strategies to survive and have a good performance in open ecosystems. However, once the environment changes, species adapted to open environments tend to be gradually excluded and replaced by forest species in the encroached habitat.

5.2 FUNCTIONAL COMMUNITY CHANGES THROUGH SPACE AND TIME IN A MOSAIC SYSTEM SUBMITTED TO ENCROACHMENT

Species with lower SLA and higher LDMC values dominate in grassland habitat, indicating a conservative strategy for leaves, i.e. high investment in dry matter in relation to leaf area, which enables woody species to establish under high solar incidence in grasslands. Leaf protection is fundamental for plants that grow in open habitat, to avoid damage of high exposition to direct sunlight on leaf tissue or water loss via evapotranspiration (Wright et al. 2004, Donovan et al. 2011). We corroborated our hypothesis that the average tree leaf in the sunny grassland had a conservative strategy. Conservative leaves have long leaf lifetimes in detriment of slow growth, low photosynthesis and respiration rates (Wright et al. 2004, Donovan et al. 2011, Reich 2014, Díaz et al. 2016). Higher LDMC may be due to thicker epidermis, cuticle and cell walls, which increase resistance against desiccation and herbivory with better protection from damage (Reich 2014, Perez-Harguindeguy et al. 2016, Weigelt et al. 2021). Hotter and drier microhabitats under open conditions (low basal area, low tree cover) may limit survival of plants with acquisitive leaves, due to a high risk of dehydration under such conditions (Carlucci et al. 2012). This pattern have been recorded in many studies of forest-grassland and forest-savanna mosaics (Carlucci et al. 2012, 2015, Dantas et al. 2013, Silva 2015, Maracahipes et al. 2018b, 2018a, Carrijo et al. 2021), since the leaf economics spectrum is far more exploited than other plant dimensions (Weemstra et al. 2016, Freschet et al. 2021). Over time we register the increased in dominance of species with higher SLA, with gradual decrease in LDMC values. Species that could suffer dehydration in the open habitat due to more vulnerable acquisitive leaves were able to establish in the encroached habitat over time. Also, this decrease in LDMC values can indicate the mortality of typical grassland species with conservative leaves (Flake et al. 2021, Raymundo et al. 2023).

As a consequence of encroachment, the shade condition created by the continuous canopy in the forest may give an adaptative gain for species with acquisitive leaves and higher H_{max} , that were able to increase light interception and photosynthetic rates (Westoby 1998, Donovan et al. 2011, Díaz et al. 2016). Light varies significantly along a vertical gradient in a forest, as light is intercepted by taller individuals and gradually decreases in each successive layer of leaves (Craine and Dybzinski 2013). Because light is supplied from above the plants, taller woody individuals better intercept and compete for the light available along forest strata (Moles et al. 2009), with an increase in photosynthetic rates and indirectly reduce the growth of neighbours via shade (Craine and Dybzinski 2013). Forest species

had, in general, acquisitive leaves and higher Hmax to increase light capture under shade conditions (Westoby 1998, Wright et al. 2004, Donovan et al. 2011). Similar results were obtained in grassland-forest mosaics in the Pampa, the southernmost region of Brazil, where higher LA and SLA values were found in forest communities under denser canopies, whereas leaf thickness and LDMC values were higher in grasslands, among saplings (Carlucci et al. 2012, 2015) and adult tree communities (Silva 2015). Across a fire gradient in savanna-forest mosaics in the Cerrado, woody species had mainly conservative leaves in grasslands and acquisitive leaves in forests (Bernardino et al. 2022), the same functional result obtained in our study. As a consequence of the change in vegetation due to the WPE process, we were able to document the gradual change in the functional pattern described above: the dominant pattern of conservative leaves had been gradually replaced by acquisitive leaves, at the community scale, a directional shift from grassland to forest.

Grasslands are adapted to fire and harbour woody species with fire-resistance traits (Pausas 2015), while forests are considered sensitive to fire (Pivello et al. 2021). Fire is predicted to have a key role in shaping BT due to its efficiency in causing tree mortality, while thicker barks are effective heat insulators that ensure the protection of stem tissues in fire-prone ecosystems (Pausas 2015). At the global scale, a significant proportion of the variability in BT is explained by the variability in fire regimes (Lawes et al. 2013, Pausas 2015). Studies in fire-prone habitats consistently show a dominant strategy of greater investment in thicker bark in areas more susceptible and with a history of fire, such as grasslands and savannas (Hoffmann et al. 2012, Dantas et al. 2013, Lawes et al. 2013, Maracahipes et al. 2018a, Carrijo et al. 2021, Scalon et al. 2021, Bernardino et al. 2022, Raymundo et al. 2022). In our study areas, we record high BT values in grassland in 2012, meanwhile the encroached habitat experiences a significant decrease in bark thickness (that reach 65.52% decrease comparing grassland in 2012 with forest in 2022), mainly due to the establishment of thin-barked forest species. The decrease in bark thickness indicates an increase in fire-sensitive species dominance, rendering the community more vulnerable to fire disturbances (Flake et al. 2021). Concurrently, as BT decreases, Hmax tends to increase in encroached communities, indicating a change in life-story strategy dominance associated with the WPE process, allowing woody species to survive under dense shade. Negative relationships between BT and Hmax were already reported in many studies in Cerrado (Maracahipes et al. 2018b, 2018a, Rodriguez-Cubillo et al. 2021, Flake et al. 2021, Carrijo et al. 2021, Scalon et al. 2021, Bernardino et al. 2022, Raymundo et al. 2023, Chiminazzo et al. 2023).

While tissue protection is fundamental for leaves and stems above the ground, individuals growing in grassland must compete directly with grasses for soil nutrients and water belowground. The possible explanation for the success of grasses in limiting trees and dominating grasslands is related to their advantage in exploring soil nutrients more intensively than trees, better protection against fire and herbivory, and faster regrowth rates (Bond 2008). Grasses have dense fibrous root systems, with higher SRL and lower root diameter than woody species (Freschet et al., 2017), being formidable competitors to woody species (Bond 2008). We noticed in the field while digging the soil to collect the samples, a dense cluster of grass fine roots in the grassland, which indicates woody individuals that establish in grasslands face high competition for resources with grasses. We found that woody species that occur in grassland generally invest in acquisitive roots which allows to explore larger portions of the soil, with high RBF and SRL values. Acquisitive species have cheaper root tissues, with lower investment in dry matter per length of root build and root lifespan (Weemstra et al. 2016, Freschet et al. 2021). Plants with high RBF and SRL may have an advantage in the competition for soil nutrients, because their roots occupy a larger volume of soil with less energy spent in biomass construction (Perez-Harguindeguy et al. 2016, Freschet et al. 2021), which seems to have been an adaptation for direct competition with grasses (Bergmann et al. 2020, Freschet et al. 2021). Unlike the traits of other plant organs, root traits did not change the functional pattern comparing grassland in 2012 and the encroached habitat in 2022, where there is still a predominance of an acquisitive strategy for roots.

On the opposite, in forest habitat predominate a conservative strategy for roots, with lower values of RBF and SRL, and higher values of D (just for GUA and at the species level for VV). A conservative strategy for roots is characterised by a high investment in thick roots with more dry matter invested per length, with lower RBF and SRL values. Belowground is more complex than assumed, as the roots may perform a variety of functions and respond to wide variations in environmental conditions and resource availability below ground (Weemstra et al. 2016, 2022, Bergmann et al. 2020, Carmona et al. 2021). The root economics spectrum has two main axes of variation: a first related to fast-slow trade-off, which may be similar to aboveground dimensions (Reich 2014, Kong et al. 2015), while a second related to the collaboration gradient with mycorrhizal fungi, with no equivalent above ground (Bergmann et al. 2020, Carmona et al. 2021). The fungal collaboration gradient dominates the root economic spectrum in a large data set of species (Bergmann et al. 2020). Plant collaboration strategies in this gradient range from 1) 'do it yourself', with thin, high RBF and SRL roots for efficient resource uptake; 2) 'outsourcing', with thicker roots with high

D and lower RBF and SRL values, which favour the colonization by mycorrhizal fungi, as a strategy to acquire resources via collaboration interaction (Bergmann et al. 2020, Weigelt et al. 2021). Our results indicate that in grassland communities predominate the strategy to 'do it yourself', while in forest communities predominates the strategy of 'outsourcing' and collaboration with mycorrhizal fungi, although we did not assess the incidence of mycorrhizae in the species, which deserves further investigation.

Studies that have evaluated root traits for woody species have almost been limited to temperate grasslands and forests (Iversen and McCormack 2021). For tropical and subtropical ecosystems, there has been found that the decrease in fire frequency was associated with an increase in mycorrhizal colonization and a decrease in root branching and fine root development in grasses species, suggesting an important ecological role of mycorrhizal symbiosis during WPE in African savannas (Hartnett et al. 2004). Along a fire-history gradient in Cerrado, there has been found that fire stimulates investment in fine root biomass in grasses in response to the higher demand for belowground resources (le Stradic et al. 2021). The increase in shrub biomass cover was related to time without fire and higher SRL values (le Stradic et al. 2021). We assessed belowground strategies and obtained a clear pattern of acquisitive roots for grassland communities and conservative roots for forest communities, based on three fine-roots traits. To advance the understanding of this functional tendency and its relative importance to understand the WPE process, future studies with forest-grassland mosaics should include more root traits (Freschet et al. 2021, Weemstra et al. 2022) and consider fungal collaboration gradient (Bergmann et al. 2020), to better understand what happens in woody species root systems along WPE.

The functional trajectories of changes describe above synthesize the general pattern of the community. At community level we observe no overlap between grassland and forest communities, indicating that besides encroachment, grassland in 2022 still functional different of forest. However, at species level, we obtain highly variation between species, which considerable overlapped between grassland and forest species in the multivariate functional space. This result indicates that a wide variety of viable ecological strategies rather than a unique strategy, reflecting environmental filtering for competitive traits at the expense of stress-tolerant ones along gradients of canopy closure.

5.3 MAJOR DIMENSIONS OF TRAIT VARIATION SUGGEST A TRADE-OFF ABOVE AND BELOWGROUND

One of the most important findings was that species that occur in forest-grassland mosaics exhibited a trade-off resource-use strategy among whole-plant trait (Hmax), leaf and belowground, with a negative correlation between, Hmax, leaf and root traits, contrary to what was predicted by the worldwide plant economics spectrum (Chave et al. 2009, Reich 2014, Weigelt et al. 2021). The whole-tree performance relies on the coordinated functioning of interdependent plant dimensions (whole-plant, leaves, stems and roots), who performs functions and is subject to unique pressures (Reich 2014, Kong et al. 2015, Díaz et al. 2016, Weemstra et al. 2022). Changes in environmental conditions over time, associate and subjacent to WPE process, may favour woody species with different ecological strategies. Interestingly, we found a clear trade-off between above and belowground in both habitats: acquisitive root and conservative leaves in grassland 2012 and in the encroached grassland in 2022, and conservative root and acquisitive leaves in the forest, with species exhibiting a wide trait variation across this broad spectrum.

The worldwide plant economics spectrum has been extended to leaves (Grime 1977, Westoby 1998, Wright et al. 2004, Reich 2014, Díaz et al. 2016), to stems (Chave et al. 2009, Díaz et al. 2016) and, more recently, to roots (Laughlin et al. 2021, Carmona et al. 2021, Freschet et al. 2021, Weemstra et al. 2022). It assumes that different plant dimensions covary in a one-dimensional whole-plant economics spectrum, respectively, ranging from species with acquisitive to conservative traits (Wright et al. 2004, Chave et al. 2009, Reich 2014). A species that has a fast resource investment in one respect generally is fast in others, as 'being fast or slow is a general feature of species' (Reich 2014). The wood economics spectrum also agrees about the coordination between plant dimensions and expect that if a species has conservative leaf traits, it would present conservative wood traits too (Chave et al. 2009). More recently, studies have taken into account root traits to assess above and belowground coordination, and found that both dimensions are not necessarily coupled (Carmona et al. 2021, Freschet et al. 2021, Weemstra et al. 2022). A global evaluation of coordination above and belowground found that species were more functional redundant below than aboveground, with many species with similar fine-root traits exhibiting contrasting aboveground traits (Carmona et al. 2021). Moreover, there was one more axis of variation for belowground than for aboveground traits, related to the fungal collaboration gradient (Bergmann et al. 2020).

A negative covariance between roots and leaves was found at species and at community level, which shows that the hypothesis of the worldwide plant economics spectrum that a fast vs. slow (acquisitive vs. conservative) strategy would be coordinated among all plant organs (Reich 2014) is not corroborated in our study system, contrary to our hypothesis. Studies that evaluated above and belowground trait variation found contradictory results regarding whole-plant coordination. No consistent evidence of coordination was found in many studies (Weemstra et al. 2016, Carmona et al. 2021, Ávila-Lovera et al. 2022). In contrast, consistent evidence of a whole-plant integrative resource economy across plant dimensions was found in subarctic flora species (Freschet et al. 2010) and for Mediterranean woody communities (de la Riva et al. 2018). We found that leaf traits had a clear trade-off with root traits, with a negative correlation between SRL-SLA. A global meta-analysis found that ~40% of studies reported positive correlations between SRL-SLA, ~51% reported nonsignificant results and only 9% reported negative correlation between SRL-SLA (Weigelt et al. 2021). Thus, conclusions regarding trait coordination above and belowground remain ambiguous despite the large number of the studies reviewed (Kong et al. 2015, Weemstra et al. 2016, 2022, Carmona et al. 2021, Freschet et al. 2021), deserving further investigation.

6 CONCLUSIONS

Grasslands and forest had different vegetation structures, and the community is composed of different sets of species' ecological strategies. After 10 years, we recorded gradual changes in the main ecological strategies of species in each habitat. In the absence of disturbances, over time, the encroached grassland will increasingly resemble a forest. We documented the establishment of typical forest species in the former grassland in 2022, with ecological strategies like thin bark and acquisitive leaves, that without encroachment would not be viable in grasslands. Associated with encroachment, there is also a change in the life strategies of woody species, with an impact on the entire ecosystem. Our integrative functional approach, which considers above and belowground dimensions of trait variation, was very useful to identify functional patterns in forest-grassland mosaics and shifts that occur associated with the WPE process. In summary, we provided field-based evidence of negative covariation between leaves and roots. The inclusion of root traits sheds light on aspects of the ecological strategies of species that were not assessed before when only aboveground dimensions were considered. Further studies should consider the inclusion of more root traits (Freschet et al. 2021) and also consider traits to directly evaluate the collaboration between plants and mycorrhizal fungi (Bergmann et al. 2020), to confirm if this functional pattern of acquisitive roots in woody species occurring in grassland and conservative roots in forest is found in other mosaics areas. Future studies that assess the belowground dimension and the collaboration gradient would advance the understanding of how belowground interactions may favour or limit the WPE process to occur.

REFERENCES

- Alvares, C. A., J. L. Stape, P. C. Sentelhas, J. L. De Moraes Gonçalves, and G. Sparovek. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22:711–728.
- Archer, S., T. W. Boutton, and K. A. Hibbard. 2001. Trees in Grasslands: Biogeochemical Consequences of Woody Plant Expansion.
- Archer, S. R., E. M. Andersen, K. I. Predick, S. Schwinning, R. J. Steidl, and S. R. Woods. 2017. Woody Plant Encroachment: Causes and Consequences. In *Rangeland systems* (Book).
- Archer, S., C. Scifres, and C. R. Bassham. 1988. Autogenic succession in a subtropical savanna: conversion of grassland to thorn woodland. *Page Ecological Monographs*.
- Ávila-Lovera, E., G. R. Goldsmith, K. M. Kay, and J. L. Funk. 2022. Above- and below-ground functional trait coordination in the Neotropical understory genus *Costus*. *AoB PLANTS* 14.
- Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography* 19:134–143.
- Baselga, A. 2012. The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. *Global Ecology and Biogeography* 21:1223–1232.
- Baselga, A., and C. D. L. Orme. 2012. Betapart: An R package for the study of beta diversity. *Methods in Ecology and Evolution* 3:808–812.
- Batalha, M. A. 2011. O cerrado não é um bioma. *Page Biota Neotrop.*
- Behling, H. 1997. Late Quaternary vegetation, climate and fire history of the Araucaria forest and campos region from Serra Campos Gerais, Paraná State (South Brazil). *Page Hermann Behling/Review of Palaeobotany and Palynology*.
- Behling, H., V. D. P. Pillar, L. Orlóci, and S. G. Bauermann. 2004. Late Quaternary Araucaria forest, grassland (Campos), fire and climate dynamics, studied by high-resolution pollen, charcoal and multivariate analysis of the Cambará do Sul core in southern Brazil. *Palaeogeography, Palaeoclimatology, Palaeoecology* 203:277–297.
- Bergmann, J., A. Weigelt, F. Van Der Plas, D. C. Laughlin, T. W. Kuyper, N. Guerrero-Ramirez, O. J. Valverde-Barrantes, H. Bruehlheide, G. T. Freschet, C. M. Iversen, J. Kattge, M. L. McCormack, I. C. Meier, M. C. Rillig, C. Roumet, M. Semchenko, C. J. Sweeney, J. Van Ruijven, L. M. York, and L. Mommer. 2020. The fungal collaboration gradient dominates the root economics space in plants. *Page Sci. Adv.*
- Bernardino, P. N., V. L. Dantas, M. Hirota, J. G. Pausas, and R. S. Oliveira. 2022. Savanna-Forest Coexistence Across a Fire Gradient. *Ecosystems* 25:279–290.
- Bjornstad, N., and J. Cai. 2022. *Title Spatial Covariance Functions*.

- Blanco, C. C., S. Scheiter, E. Sosinski, A. Fidelis, M. Anand, and V. D. Pillar. 2014. Feedbacks between vegetation and disturbance processes promote long-term persistence of forest-grassland mosaics in south Brazil. *Ecological Modelling* 291:224–232.
- Bond, W. J. 2008. What limits trees in C4 grasslands and savannas?
- Bond, W. J. 2016, January 8. Ancient grasslands at risk. American Association for the Advancement of Science.
- Bond, W. J. 2021. *Out of the shadows: ecology of open ecosystems*. Taylor and Francis Ltd.
- Bond, W. J., and J. E. Keeley. 2005a, July. Fire as a global “herbivore”: The ecology and evolution of flammable ecosystems.
- Bond, W. J., and J. E. Keeley. 2005b, July. Fire as a global “herbivore”: The ecology and evolution of flammable ecosystems.
- Bowman, D. M. J. S., A. Walsh, and D. J. Milne. 2001. Forest expansion and grassland contraction within a Eucalyptus savanna matrix between 1941 and 1994 at Litchfield National Park in the Australian monsoon tropics. *Global Ecology and Biogeography* 10:535–548.
- Carlucci, M. B., V. J. Debastiani, V. D. Pillar, and L. D. S. Duarte. 2015. Between- and within-species trait variability and the assembly of sapling communities in forest patches. *Journal of Vegetation Science* 26:21–31.
- Carlucci, M. B., V. Marcilio-Silva, and J. M. Torezan. 2021. The Southern Atlantic Forest: Use, Degradation, and Perspectives for Conservation. Pages 91–111 *The Atlantic Forest*. Springer International Publishing.
- Carlucci, M. B., H. Streit, L. D. S. Duarte, and V. D. Pillar. 2012. Individual-based trait analyses reveal assembly patterns in tree sapling communities. *Journal of Vegetation Science* 23:176–186.
- Carmo, M. R. B. 2006. *Caracterização fitofisionômica do Parque Estadual do Guartelá, município de Tibagi, estado do Paraná*.
- Carmona, C. P., C. G. Bueno, A. Toussaint, S. Träger, S. Díaz, M. Moora, A. D. Munson, M. Pärtel, M. Zobel, and R. Tamme. 2021. Fine-root traits in the global spectrum of plant form and function. *Nature* 597:683–687.
- Carrizo, J. N., L. Maracahipes, M. C. Scalon, D. V. Silvério, A. C. Abadia, M. V. Fagundes, A. A. Veríssimo, L. A. Gonçalves, D. Carrizo, J. Martins, and E. Lenza. 2021. Functional traits as indicators of ecological strategies of savanna woody species under contrasting substrate conditions. *Flora: Morphology, Distribution, Functional Ecology of Plants* 284.
- Cervi, A. C., L. Von Linsingen, G. Hatschbach, and O. S. Ribas. 2007. *A Vegetação do Parque Estadual de Vila Velha, Município de Ponta Grossa, Paraná, Brasil*. Curitiba.

- Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12:351–366.
- Chiminazzo, M. A., A. B. Bombo, T. Charles-Dominique, and A. Fidelis. 2023. Bark production of generalist and specialist species across savannas and forests in the Cerrado. *Annals of Botany*.
- CONAMA. 1994. Define as formações vegetais primárias, bem como os estágios sucessionais de vegetação secundária nativa no Estado do Paraná.
- Craine, J. M., and R. Dybzinski. 2013. Mechanisms of plant competition for nutrients, water and light. *Functional Ecology* 27:833–840.
- Cruz, G. C. F. 2007. Alguns Aspectos do Clima na região dos Campos Gerais - Patrimônio Natural dos Campos Gerais do Paraná. Editora UEPG:59–72.
- Dantas, V. L., M. A. Batalha, and J. G. Pausas. 2013. Fire drives functional thresholds on the savanna-forest transition. *Ecology* 94:2454–2463.
- Dantas, V. de L., M. Hirota, R. S. Oliveira, and J. G. Pausas. 2016. Disturbance maintains alternative biome states. *Ecology Letters* 19:12–19.
- Dantas, V. L., and J. G. Pausas. 2020. Megafauna biogeography explains plant functional trait variability in the tropics. *Global Ecology and Biogeography* 29:1288–1298.
- Dantas, V. L., and J. G. Pausas. 2022. The legacy of the extinct Neotropical megafauna on plants and biomes. *Nature Communications* 13.
- Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönsch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet Ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. *Nature* 529:167–171.
- Diniz-Filho, J. A. F., L. M. Bini, and B. A. Hawkins. 2003. Spatial autocorrelation and red herrings in geographical ecology. *Global Ecology and Biogeography* 12:53–64.
- Donovan, L. A., H. Maherali, C. M. Caruso, H. Huber, and H. de Kroon. 2011, February. The evolution of the worldwide leaf economics spectrum.
- Durigan, G. 2020. Zero-fire: Not possible nor desirable in the Cerrado of Brazil. *Flora: Morphology, Distribution, Functional Ecology of Plants* 268.
- Fidelis, A., and V. Pivello. 2011. Deve-se usar o fogo como instrumento de manejo no Cerrado e Campos Sulinos?
- Flake, S. W., R. C. R. Abreu, G. Durigan, and W. A. Hoffmann. 2021. Savannas are not old fields: Functional trajectories of forest expansion in a fire-suppressed Brazilian savanna are driven by habitat generalists. *Functional Ecology* 35:1797–1809.

- Freschet, G. T., J. H. C. Cornelissen, R. S. P. van Logtestijn, and R. Aerts. 2010. Evidence of the “plant economics spectrum” in a subarctic flora. *Journal of Ecology* 98:362–373.
- Freschet, G. T., et al. 2021, November 1. A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. John Wiley and Sons Inc.
- García Criado, M., I. H. Myers-Smith, A. D. Bjorkman, C. E. R. Lehmann, and N. Stevens. 2020. Woody plant encroachment intensifies under climate change across tundra and savanna biomes. *Global Ecology and Biogeography* 29:925–943.
- Goncalves, R. V. S., J. C. F. Cardoso, P. Eugênio Oliveira, and D. Coelho Oliveira. 2021. Changes in the Cerrado vegetation structure: Insights from more than three decades of ecological succession. *Web Ecology* 21:55–64.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Page Amer. Natur.*
- Grime, J. P., and S. Pierce. 2012. Primary Adaptive Strategies in Plants.
- Hartnett, D. C., A. F. Potgieter, and G. W. T. Wilson. 2004. Fire effects on mycorrhizal symbiosis and root system architecture in southern African savanna grasses.
- Hoffmann, W. A., E. L. Geiger, S. G. Gotsch, D. R. Rossatto, L. C. R. Silva, O. L. Lau, M. Haridasan, and A. C. Franco. 2012. Ecological thresholds at the savanna-forest boundary: How plant traits, resources and fire govern the distribution of tropical biomes. *Ecology Letters* 15:759–768.
- IBGE, I. B. de B. F. 2012. Manual técnico da vegetação brasileira. Rio de Janeiro.
- Iversen, C. M., and M. L. McCormack. 2021. Filling gaps in our understanding of belowground plant traits across the world.
- Kattge, Jen., and et al. 2020. TRY plant trait database – enhanced coverage and open access. *Global Change Biology* 26:119–188.
- Keeley, J. E., J. G. Pausas, P. W. Rundel, W. J. Bond, and R. A. Bradstock. 2011, August. Fire as an evolutionary pressure shaping plant traits.
- Klein, R. M. 1960. Aspecto dinâmico do pinheiro brasileiro. *Sellowia* 12.
- Kong, D., J. Wang, P. Kardol, H. Wu, H. Zeng, X. Deng, and Y. Deng. 2015. The root economics spectrum: divergence of absorptive root strategies with root diameter. *Biogeosciences Discuss* 12:13041–13067.
- de la Riva, E. G., T. Marañón, I. M. Pérez-Ramos, C. M. Navarro-Fernández, M. Olmo, and R. Villar. 2018. Root traits across environmental gradients in Mediterranean woody communities: are they aligned along the root economics spectrum? *Plant and Soil* 424:35–48.
- Laughlin, D. C., L. Mommer, F. M. Sabatini, H. Bruelheide, T. W. Kuyper, M. L. McCormack, J. Bergmann, G. T. Freschet, N. R. Guerrero-Ramírez, C. M. Iversen, J. Kattge, I. C.

- Meier, H. Poorter, C. Roumet, M. Semchenko, C. J. Sweeney, O. J. Valverde-Barrantes, F. van der Plas, J. van Ruijven, L. M. York, I. Aubin, O. R. Burge, C. Byun, R. Čušterevska, J. Dengler, E. Forey, G. R. Guerin, B. Hérault, R. B. Jackson, D. N. Karger, J. Lenoir, T. Lysenko, P. Meir, Ü. Niinemets, W. A. Ozinga, J. Peñuelas, P. B. Reich, M. Schmidt, F. Schrod, E. Velázquez, and A. Weigelt. 2021. Root traits explain plant species distributions along climatic gradients yet challenge the nature of ecological trade-offs. *Nature Ecology and Evolution* 5:1123–1134.
- Lavorel, S., K. Grigulis, S. McIntyre, N. S. G. Williams, D. Garden, J. Dorrough, S. Berman, F. Quétier, A. Thébault, and A. Bonis. 2008. Assessing functional diversity in the field - Methodology matters! Blackwell Publishing Ltd.
- Lawes, M. J., J. J. Midgley, and P. J. Clarke. 2013. Costs and benefits of relative bark thickness in relation to fire damage: A savanna/forest contrast. *Journal of Ecology* 101:517–524.
- Legendre, P. 1993. Spatial autocorrelation: Trouble or new paradigm? Page Source: *Ecology*.
- Maack, R. 1948. *Geografia Física do Paraná*. BADEP-Universidade Federal do Paraná, Curitiba.
- Mangiafico, S. 2017. *An R Companion for the handbook of Biological Statistics*.
- Maracahipes, L., M. B. Carlucci, E. Lenza, B. S. Marimon, B. H. Marimon, F. A. G. Guimarães, and M. V. Cianciaruso. 2018a. How to live in contrasting habitats? Acquisitive and conservative strategies emerge at inter- and intraspecific levels in savanna and forest woody plants. *Perspectives in Plant Ecology, Evolution and Systematics* 34:17–25.
- Maracahipes, L., J. O. Dos Santos, S. M. Reis, and E. Lenza. 2018b. Temporal changes in species composition, diversity, and woody vegetation structure of savannas in the cerrado–amazon transition zone. *Acta Botanica Brasilica* 32:254–263.
- McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21:178–185.
- Moles, A. T., D. D. Ackerly, J. C. Tweddle, J. B. Dickie, R. Smith, M. R. Leishman, M. M. Mayfield, A. Pitman, J. T. Wood, and M. Westoby. 2006. Global patterns in seed size. *Global Ecology and Biogeography*.
- Moles, A. T., D. I. Warton, L. Warman, N. G. Swenson, S. W. Laffan, A. E. Zanne, A. Pitman, F. A. Hemmings, and M. R. Leishman. 2009. Global patterns in plant height. *Journal of Ecology* 97:923–932.
- Moraes, D. A., P. O. Cavalin, R. S. Moro, R. A. C. Oliveira, M. R. B. Carmo, and M. C. M. Marques. 2016. Edaphic filters and the functional structure of plant assemblages in grasslands in southern Brazil. *Journal of Vegetation Science* 27:100–110.
- Moro, R. S., and M. R. B. do Carmo. 2008. *A vegetação campestre nos Campos Gerais*. Editora UEPG.

- Müller, S. C., G. E. Overbeck, J. Pfadenhauer, and V. D. Pillar. 2007. Plant functional types of woody species related to fire disturbance in forest-grassland ecotones. *Plant Ecology* 189:1–14.
- Müller, S. C., G. E. Overbeck, C. C. Blanco, J. M. De Oliveira, and V. D. Pillar. 2013. South Brazilian forest-grassland ecotones: Dynamics affected by climate, disturbance, and woody species traits. Pages 167–187 *Ecotones Between Forest and Grassland*. Springer New York.
- Oliveras, I., and Y. Malhi. 2016, September 19. Many shades of green: The dynamic tropical forest–savannah transition zones. Royal Society of London.
- Overbeck, G. E., S. C. Müller, A. Fidelis, J. Pfadenhauer, V. D. Pillar, C. C. Blanco, I. I. Boldrini, R. Both, and E. D. Forneck. 2007. Brazil's neglected biome: The South Brazilian Campos. *Perspectives in Plant Ecology, Evolution and Systematics* 9:101–116.
- Overbeck, G. E., E. Vélez-Martin, L. da S. Menezes, M. Anand, S. Baeza, M. B. Carlucci, M. S. Dechoum, G. Durigan, A. Fidelis, A. Guido, M. F. Moro, C. B. R. Munhoz, M. Reginato, R. S. Rodrigues, M. F. Rosenfield, A. B. Sampaio, F. H. Barbosa da Silva, F. A. O. Silveira, Ê. E. Sosinski, I. R. Staude, V. M. Temperton, C. Turchetto, J. W. Veldman, P. L. Viana, D. C. Zappi, and S. C. Müller. 2022, September 1. Placing Brazil's grasslands and savannas on the map of science and conservation. Elsevier GmbH.
- Overbeck, G. E., E. Vélez-Martin, F. R. Scarano, T. M. Lewinsohn, C. R. Fonseca, S. T. Meyer, S. C. Müller, P. Ceotto, L. Dadalt, G. Durigan, G. Ganade, M. M. Gossner, D. L. Guadagnin, K. Lorenzen, C. M. Jacobi, W. W. Weisser, and V. D. Pillar. 2015, December 1. Conservation in Brazil needs to include non-forest ecosystems.
- Parr, C. L., C. E. R. Lehmann, W. J. Bond, W. A. Hoffmann, and A. N. Andersen. 2014. Tropical grassy biomes: Misunderstood, neglected, and under threat. Elsevier Ltd.
- Passos, F. B., B. S. Marimon, O. L. Phillips, P. S. Morandi, E. C. das Neves, F. Elias, S. M. Reis, B. de Oliveira, T. R. Feldpausch, and B. H. Marimon Júnior. 2018. Savanna turning into forest: concerted vegetation change at the ecotone between the Amazon and “Cerrado” biomes. *Revista Brasileira de Botânica* 41:611–619.
- Pausas, J. G. 2015, March 1. Bark thickness and fire regime.
- Pausas, J. G., and V. de L. Dantas. 2017. Scale matters: fire–vegetation feedbacks are needed to explain tropical tree cover at the local scale. *Global Ecology and Biogeography* 26:395–399.
- Pausas, J. G., and W. J. Bond. 2020, March 1. Alternative Biome States in Terrestrial Ecosystems. Elsevier Ltd.
- Perez-Harguindeguy, N., S. Diaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, and J. H. C. Cornelissen. 2016. Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. CSIRO.

- Pillar, V. P. 2003. Dinâmica da expansão florestal em mosaicos de floresta e campos no sul do Brasil.
- Pilon, N. A. L., G. Durigan, J. Rickenback, R. T. Pennington, K. G. Dexter, W. A. Hoffmann, R. C. R. Abreu, and C. E. R. Lehmann. 2021. Shade alters savanna grass layer structure and function along a gradient of canopy cover. *Journal of Vegetation Science* 32.
- Pivello, V. R., I. Vieira, A. v. Christianini, D. B. Ribeiro, L. da Silva Menezes, C. N. Berlinck, F. P. L. Melo, J. A. Marengo, C. G. Tornquist, W. M. Tomas, and G. E. Overbeck. 2021. Understanding Brazil's catastrophic fires: Causes, consequences and policy needed to prevent future tragedies. *Associação Brasileira de Ciência Ecológica e Conservação*.
- Raymundo, D., C. E. R. Lehmann, N. E. de Oliveira-Neto, V. C. Martini, M. Altomare, J. Prado-Junior, and P. E. Oliveira. 2023. Temporal changes in the dominance of tree functional traits, but no changes in species diversity during woody plant encroachment in a Brazilian savanna. *Journal of Vegetation Science*.
- Raymundo, D., N. E. Oliveira-Neto, V. Martini, T. N. Araújo, D. Calaça, and D. C. de Oliveira. 2022. Assessing woody plant encroachment by comparing adult and juvenile tree components in a Brazilian savanna. *Flora: Morphology, Distribution, Functional Ecology of Plants* 291.
- Reich, P. B. 2014. The world-wide “fast-slow” plant economics spectrum: A traits manifesto. *Journal of Ecology* 102:275–301.
- Ritter, L. M. O., M. C. Ribeiro, and R. S. Moro. 2010. Composição florística e fitofisionomia de remanescentes disjuntos de Cerrado nos Campos Gerais, PR, Brasil - limite austral do bioma. *Page Biota Neotrop.*
- Rodriguez-Cubillo, D., N. A. L. Pilon, and G. Durigan. 2021. Tree height is more important than bark thickness, leaf habit or habitat preference to survive fire in the cerrado of south-east Brazil. *International Journal of Wildland Fire* 30:899–910.
- Roques, K. G., T. G. O'Connor, and A. R. Watkinson. 2001. Dynamics of shrub encroachment in an African savanna: Relative influences of fire, herbivory, rainfall and density dependence. *Journal of Applied Ecology* 38:268–280.
- Rosado, B. H. P., and E. A. de Mattos. 2017. On the relative importance of CSR ecological strategies and integrative traits to explain species dominance at local scales. *Functional Ecology* 31:1969–1974.
- Salguero-Gómez, R. 2017, March 1. Applications of the fast–slow continuum and reproductive strategy framework of plant life histories. Blackwell Publishing Ltd.
- Scalon, M. C., D. R. Rossatto, I. Oliveras, R. C. Miatto, E. F. Gray, F. M. C. B. Domingos, F. T. Brum, M. B. Carlucci, W. A. Hoffmann, B. H. Marimon-Júnior, B. S. Marimon, and A. C. Franco. 2021. Fire and drought: Shifts in bark investment across a broad geographical scale for Neotropical savanna trees. *Basic and Applied Ecology* 56:110–121.

- Silva, M. G. 2015. Padrões funcionais de comunidades de plantas lenhosas em transições floresta-campo em resposta a gradientes ambientais no sul do Brasil. Porto Alegre.
- Simon, M. F., and T. Pennington. 2012. Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado.
- Socolar, J. B., J. J. Gilroy, W. E. Kunin, and D. P. Edwards. 2016, January 1. How Should Beta-Diversity Inform Biodiversity Conservation? Elsevier Ltd.
- Souza, A. F. 2021. A review of the structure and dynamics of araucaria mixed forests in southern Brazil and northern Argentina. Taylor and Francis Ltd.
- Stevens, N., C. E. R. Lehmann, B. P. Murphy, and G. Durigan. 2017. Savanna woody encroachment is widespread across three continents. *Global Change Biology* 23:235–244.
- le Stradic, S., C. Roumet, G. Durigan, L. Cancian, and A. Fidelis. 2021. Variation in biomass allocation and root functional parameters in response to fire history in Brazilian savannas. *Journal of Ecology* 109:4143–4157.
- Strömberg, C. A. E. 2011. Evolution of grasses and grassland ecosystems. *Annual Review of Earth and Planetary Sciences* 39:517–544.
- Sühs, R. B., E. L. H. Giehl, and N. Peroni. 2020. Preventing traditional management can cause grassland loss within 30 years in southern Brazil. *Scientific Reports* 10.
- Villéger, S., G. Grenouillet, and S. Brosse. 2013. Decomposing functional β -diversity reveals that low functional β -diversity is driven by low functional turnover in European fish assemblages. *Global Ecology and Biogeography* 22:671–681.
- Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012, April. The return of the variance: Intraspecific variability in community ecology.
- Weemstra, M., T. W. Kuyper, F. J. Sterck, and M. N. Umaña. 2022. Incorporating belowground traits: avenues towards a whole-tree perspective on performance. *Oikos*.
- Weemstra, M., L. Mommer, E. J. W. Visser, J. van Ruijven, T. W. Kuyper, G. M. J. Mohren, and F. J. Sterck. 2016, September 1. Towards a multidimensional root trait framework: a tree root review. Blackwell Publishing Ltd.
- Weigelt, A., L. Mommer, K. Andraczek, C. M. Iversen, J. Bergmann, H. Bruelheide, Y. Fan, G. T. Freschet, N. R. Guerrero-Ramírez, J. Kattge, T. W. Kuyper, D. C. Laughlin, I. C. Meier, F. van der Plas, H. Poorter, C. Roumet, J. van Ruijven, F. M. Sabatini, M. Semchenko, C. J. Sweeney, O. J. Valverde-Barrantes, L. M. York, and M. L. McCormack. 2021, October 1. An integrated framework of plant form and function: the belowground perspective. John Wiley and Sons Inc.
- Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Page Plant and Soil*.

- Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: Some leading dimensions of variation between species.
- Wheeler, B., M. Torchiano, and M. M. Torchiano. 2022. Package 'ImPerm.'
- Whittaker, R. H., and S. A. Levin. 1977. The Role of Mosaic Phenomena in Natural Communities. Page POPULATION BIOLOGY.
- Wieczorkowski, J. D., and C. E. R. Lehmann. 2022. Encroachment diminishes herbaceous plant diversity in grassy ecosystems worldwide. *Global Change Biology* 28:5532–5546.
- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum.

SUPPLEMENTARY MATERIAL

Appendix A: Detailed description of field and laboratory methods for functional trait data collection.

Plant functional traits measurements followed the global protocol (Perez-Harguindeguy et al. 2016) and the standardised procedures indicated by the TRY Database (Kattge et al. 2020). We adopted the minimum number of >5 individuals sampled per habitat, as recommended by the global protocol, and collected data considering the intraspecific variability of species in each habitat, for species that occur in both (Violle et al. 2012).

We evaluate three functional traits of the leaf economic spectrum: leaf area (LA), specific leaf area (SLA) and leaf dry mass content (LDMC). These traits are central to the leaf economic spectrum, which summarizes the variation and coordination of several leaf traits concerning carbon fixation and nutrient use and is related to fundamental trade-offs between leaf longevity vs. production costs (Westoby 1998, Wright et al. 2004, Donovan et al. 2011). To evaluate SLA and LA, we collected ten fully expanded sun leaves for individuals. To collect leaf samples from the canopy of very tall trees we used a telescopic pruning saw that reach branches of trees up to 12 meters, and also a bottle filled with rocks with rope, harnessed to the highest branches of the canopy. The branch was then pulled up with the ropes. We selected completely expanded and hardened mature leaves in the branch, haphazardly selected from the outer canopy. Leaves were placed in moist paper and stored in sealed plastic bags until processing. To obtain fresh mass we weighed the fresh leaves, individually, using a digital scale balance with a precision of 0.001 g. To calculate the leaf area, we scanned fresh leaves using a digital scanner. Then, to calculate the total LA, we measured the scanned images using the software ImageJ (by W. Rasband, vl.43u, available at <https://imagej.nih.gov/ij/index.html>). Subsequently, leaves were dried at 70°C for at least 72 h, until obtain constant weight. With fresh and dry mass and leaf area data, we were able to obtain three functional leaf traits: LA, SLA and LDMC. LA is defined as the projected area of an individual leaf, expressed in cm². SLA (cm²/g) represents the light-capturing area deployed per dry mass allocated and is obtained with $SLA = LA \div leaf\ dry\ mass$, as the one-sided area of a fresh leaf (cm²), divided by its oven-dry mass (g). LDMC (g/g) is related to the average density (fresh mass per fresh volume)

of the leaf tissues and is obtained with $LDMC = \text{leaf dry mass} \div \text{leaf fresh mass}$ (Perez-Harguindeguy et al. 2016).

We evaluate two functional traits of the stem economic spectrum: stem-specific density (g/cm^3) (SSD) and bark thickness (BT). SSD (g/cm^3) is the oven-dry mass (g) of a section of the lignified plant stem divided by the volume of the same section, when still fresh (Perez-Harguindeguy et al. 2016). It is an important trait to understand the stability, defence, architecture, hydraulics, C gain and growth potential of plants (Perez-Harguindeguy et al. 2016), and describes the carbon investment or carbon storage per unit volume of stem and underlies the growth-survival trade-off (Chave et al. 2009). To obtain SSD, we collected a sample from a terminal branch with evident secondary growth and >2cm regular diameter, in > 5 individuals per species, per habitat. Based on Archimedes' principle, we used the water-displacement method, since this procedure allows us to measure the volume of irregularly shaped samples (Perez-Harguindeguy et al. 2016). Each fresh sample was submerged under distilled water and the weight of displaced water was registered by the precision balance. The samples were dried in an oven at 70° C for 72 h until the material reached a constant weight, and the weight of dry mass was registered using a precision balance. SSD is the ratio between dry mass / fresh volume. The other stem trait measured was bark thickness (BT), which refers to all tissues in the main stem, branches and twigs, that were outside the vascular cambium. This trait is fundamental to protecting internal tissues, acting as an insulator of living tissues against desiccation and damages caused by fire, pathogens, herbivores, frost or drought (Pausas 2015, Perez-Harguindeguy et al. 2016, de Antonio et al. 2020, Scalon et al. 2021). BT is the thickness of the total bark, measured with a digital calliper in mm. Investing in a thicker bark can be a potential strategy to survive in fire-prone environments, such as grasslands and savannas. In the field, we measured total bark thickness using a digital calliper (± 0.0001 mm), always at 40 cm above the soil, in > 5 individuals per species, per habitat (Perez-Harguindeguy et al. 2016).

Adult plant height is the shortest distance between the upper boundary of the main photosynthetic tissues at maturity (excluding inflorescences) on a plant at the ground level, expressed in metres (Perez-Harguindeguy et al. 2016). Height conditions how plants make a living, is the most common measure of whole-plant morphological trait and indicates ability to pre-empt resources, position of the species in the vertical light gradient of the vegetation, reproductive size and dispersal ability (Westoby 1998, Moles et al. 2009, Díaz et al. 2016). Although taller plants intercept more light, exist a trade-off given the increase of construction and maintenance costs and high risk of breakage (Moles et al.

2009). For this trait, all individuals inside the 15 subplots sampled per habitat were measured, to obtain measures of at least ~25 individuals per species, that cover the entire range of height and diameter (Perez-Harguindeguy et al. 2016). We used a telescopic pruning of 10 meters as a reference unit of known height to estimate the total height of individuals. For less abundant species, we obtain the total height of individuals that occur near the subplots, in the same habitat (forest or grasslands), and when necessary, complement with the SISBIOTA Project Database, which contains data of other study areas sampled under the same methodology and in the same system of study. Maximum height (Hmax) was used as a proxy for species size and was calculated according to Poorter et al. (2003) method, as the 95th percentile of the height values in each species' population.

We assess the belowground, including only fine absorptive roots, classified as roots of the first-three orders, generally with diameter-based cut-off ≤ 2 mm (Freschet and Roumet 2017). We collect ≥ 5 individual samples per specie, per habitat. We dug approximately 20 cm and collected an amount of fine roots that fit in the palm of one hand for sampling (Perez-Harguindeguy et al. 2016). The roots were washed in running water, carefully, to remove aggregated soil particles. We scanned fresh roots using a digital scanner. The images were analysed using RhizoVision Explorer v2.0.3 (available at <https://www.rhizovision.com/home>), using the algorithms described by Seethepalli et al. (2021). With the digitalized root images in high resolution (600 pixels), RhizoVision software evaluates each pixel of the skeletonized image and provides a range of root functional traits. We used data of the total root length, that is obtained by computation of the total number of pixels in the digitalized image, the branching frequency that is measured considering the number of branch points divided by the total root length, and the average diameter of root, that measures the distance to the nearest non-root pixel using this distance as the radius of a circle is fitted. For more details about root traits consult RhizoVision Explorer Guide (Seethepalli et al. 2021). After that, root samples were dried at 60° C for 48h, to obtain dry mass that was determined using a digital scale balance. SRL is the ratio of root length to dry mass of fine roots (m/g), related to the amount of resource investment (mass) (g), per area of roots (root length) (m), and is considered the belowground equivalent of SLA for leaves, because their main role in acquiring resources (Perez-Harguindeguy et al. 2016, Carmona et al. 2021, Freschet et al. 2021). To assess the ecological strategies of woody species as a whole, we evaluated three belowground traits: specific root length (SRL), mean root diameter (D) and root branching frequency (RBF). SRL is a proxy for the volume of soil under the influence of

the root system and is related to soil resource uptake efficiency, foraging capacity, plant resistance to uprooting, and plant resistance to avoidance of drought (details in Weigelt et al. 2021, Freschet et al. 2021) and describes part of the root economics spectrum (Carmona et al. 2021). ROT is related to branching density and soil exploitation, once that higher branching frequency can increase local soil exploitation (Freschet et al. 2021). Mean root diameter (D , in mm) is a simple fine-root trait obtained with digitalized roots and is related to the thickness and tissue density of the roots, and can be negatively related to SRL – thicker roots with low values of SRL. Thicker roots favour fungal colonization, a collaborative interaction which can increase the absorption of nutrients by the plant, with the help of mycorrhizae. On the other hand, thin roots are cheaper and faster to construct, with minimal biomass investment but high metabolic rates, to the detriment of low lifespan (Bergmann et al. 2020, Weigelt et al. 2021, Freschet et al. 2021).

For functional analysis, we organised a matrix with an individual code per specie sampled, as “area_habitat_speciesname”, to maintain the intraspecific variation of species between both habitats (grasslands and forest), and between both years (2012 and 2022) in statistical analysis (Violle et al., 2012), since individuals from the same species but from different habitats and sampling years may exhibit differences in trait values. By doing this, we include intraspecific variability of species in the analysis.



Figure SM 1: Collection of (A) leaves, (B) stem measurements and (C) roots in the field. After collection the samples were taken to the laboratory, where measurements of leaf (D), stem (E) and root (F) traits were taken.

References of Appendix A

de Antonio, A. C., M. C. Scalon, and D. R. Rossatto. 2020. The role of bud protection and bark density in frost resistance of savanna trees. *Plant Biology* 22:55–61.

Bergmann, J., A. Weigelt, F. van der Plas, D. C. Laughlin, T. W. Kuyper, N. Guerrero-Ramirez, O. J. Valverde-Barrantes, H. Bruelheide, G. T. Freschet, C. M. Iversen, J. Kattge, M. L. McCormack, I. C. Meier, M. C. Rillig, C. Roumet, M. Semchenko, C. J. Sweeney, J. van Ruijven, L. M. York, and L. Mommer. 2020. The fungal collaboration gradient dominates the root economics space in plants. *Page Sci. Adv.*

Carmona, C. P., C. G. Bueno, A. Toussaint, S. Träger, S. Díaz, M. Moora, A. D. Munson, M. Pärtel, M. Zobel, and R. Tamme. 2021. Fine-root traits in the global spectrum of plant form and function. *Nature* 597:683–687.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12:351–366.

- Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet Ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. *Nature* 529:167–171.
- Donovan, L. A., H. Maherali, C. M. Caruso, H. Huber, and H. de Kroon. 2011, February. The evolution of the worldwide leaf economics spectrum.
- Freschet, G. T., L. Pagès, C. M. Iversen, L. H. Comas, B. Rewald, C. Roumet, J. Klimešová, M. Zadworny, H. Poorter, J. A. Postma, T. S. Adams, A. Bagniewska-Zadworna, A. G. Bengough, E. B. Blancaflor, I. Brunner, J. H. C. Cornelissen, E. Garnier, A. Gessler, S. E. Hobbie, I. C. Meier, L. Mommer, C. Picon-Cochard, L. Rose, P. Ryser, M. Scherer-Lorenzen, N. A. Soudzilovskaia, A. Stokes, T. Sun, O. J. Valverde-Barrantes, M. Weemstra, A. Weigelt, N. Wurzbürger, L. M. York, S. A. Batterman, M. Gomes de Moraes, Š. Janeček, H. Lambers, V. Salmon, N. Tharayil, and M. L. McCormack. 2021, November 1. A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. John Wiley and Sons Inc.
- Freschet, G. T., and C. Roumet. 2017, August 1. Sampling roots to capture plant and soil functions. Blackwell Publishing Ltd.
- Kattge, Jen., and et al. 2020. TRY plant trait database – enhanced coverage and open access. *Global Change Biology* 26:119–188.
- Moles, A. T., D. I. Warton, L. Warman, N. G. Swenson, S. W. Laffan, A. E. Zanne, A. Pitman, F. A. Hemmings, and M. R. Leishman. 2009. Global patterns in plant height. *Journal of Ecology* 97:923–932.
- Pausas, J. G. 2015, March 1. Bark thickness and fire regime.
- Perez-Harguindeguy, N., S. Diaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, and J. H. C. Cornelissen. 2016. New handbook for standardised measurement of plant functional traits worldwide. CSIRO.
- Poorter, L., F. Bongers, F. J. Sterck, and H. Wöll. 2003. Architecture of 53 rain forest tree species differing in adult stature and shade tolerance. *Ecology* 84:602–608.
- Scalon, M. C., D. R. Rossatto, I. Oliveras, R. C. Miatto, E. F. Gray, F. M. C. B. Domingos, F. T. Brum, M. B. Carlucci, W. A. Hoffmann, B. H. Marimon-Júnior, B. S. Marimon, and A. C. Franco. 2021. Fire and drought: Shifts in bark investment across a broad geographical scale for Neotropical savanna trees. *Basic and Applied Ecology* 56:110–121.
- Seethepalli, A., K. Dhakal, M. Griffiths, H. Guo, G. T. Freschet, and L. M. York. 2021. RhizoVision Explorer: Open-source software for root image analysis and measurement standardization. *AoB PLANTS* 13.
- Weigelt, A., L. Mommer, K. Andrzejek, C. M. Iversen, J. Bergmann, H. Bruehlheide, Y. Fan, G. T. Freschet, N. R. Guerrero-Ramírez, J. Kattge, T. W. Kuyper, D. C. Laughlin, I. C.

Meier, F. van der Plas, H. Poorter, C. Roumet, J. van Ruijven, F. M. Sabatini, M. Semchenko, C. J. Sweeney, O. J. Valverde-Barrantes, L. M. York, and M. L. McCormack. 2021, October 1. An integrated framework of plant form and function: the belowground perspective. John Wiley and Sons Inc.

Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum.

Appendix B: Description of spatial autocorrelation analysis.

In addition to the temporal autocorrelation, we dealt with the spatial autocorrelation between plots. The sampling design was planned to enable identifying changes resulting from woody plant encroachment at the local scale, so some level of spatial autocorrelation was expected. Autocorrelation is the lack of independence between pairs of observations at given distances in time or space and is commonly found in ecological data (Legendre 1993). If data is spatially autocorrelated and predictors do not account for spatial dependency entirely, then an assumption of the statistical model is not met (Diniz-Filho et al. 2003). For this, we first generated spatial auto-correlograms, using Moran's I coefficients, for the response variables of our ANOVA models. Moran's I coefficients usually vary between -1.0 and 1.0 for maximum negative and positive autocorrelation, respectively. Non-zero values of Moran's I indicate that the values of variables in plots at a given geographical distance are more similar (positive autocorrelation) or less similar (negative autocorrelation) than randomly expected (Diniz-Filho et al. 2003). Strong spatial structure in the residuals would suggest that other non-measured factors can cause variation of the response variable, while low autocorrelation of the residuals indicates that the model is fitted (Diniz-Filho et al. 2003). We analysed spatial autocorrelation for all response variables (mean height, total basal area, total number of individuals per plot and mean number of stems per plot), and also considered the residuals generated by the models, using the 'spline.correlog' function in the 'ncf' package (Spatial Nonparametric Covariance Functions) (Bjornstad and Cai 2022).

References of Appendix B

- Bjornstad, N., and J. Cai. 2022. Title Spatial Covariance Functions.
- Diniz-Filho, J. A. F., L. M. Bini, and B. A. Hawkins. 2003. Spatial autocorrelation and red herrings in geographical ecology. *Global Ecology and Biogeography* 12:53–64.
- Legendre, P. 1993. Spatial autocorrelation: Trouble or new paradigm? Page Source: *Ecology*.

Appendix C: Changes in vegetation structural parameters during woody plant encroachment.

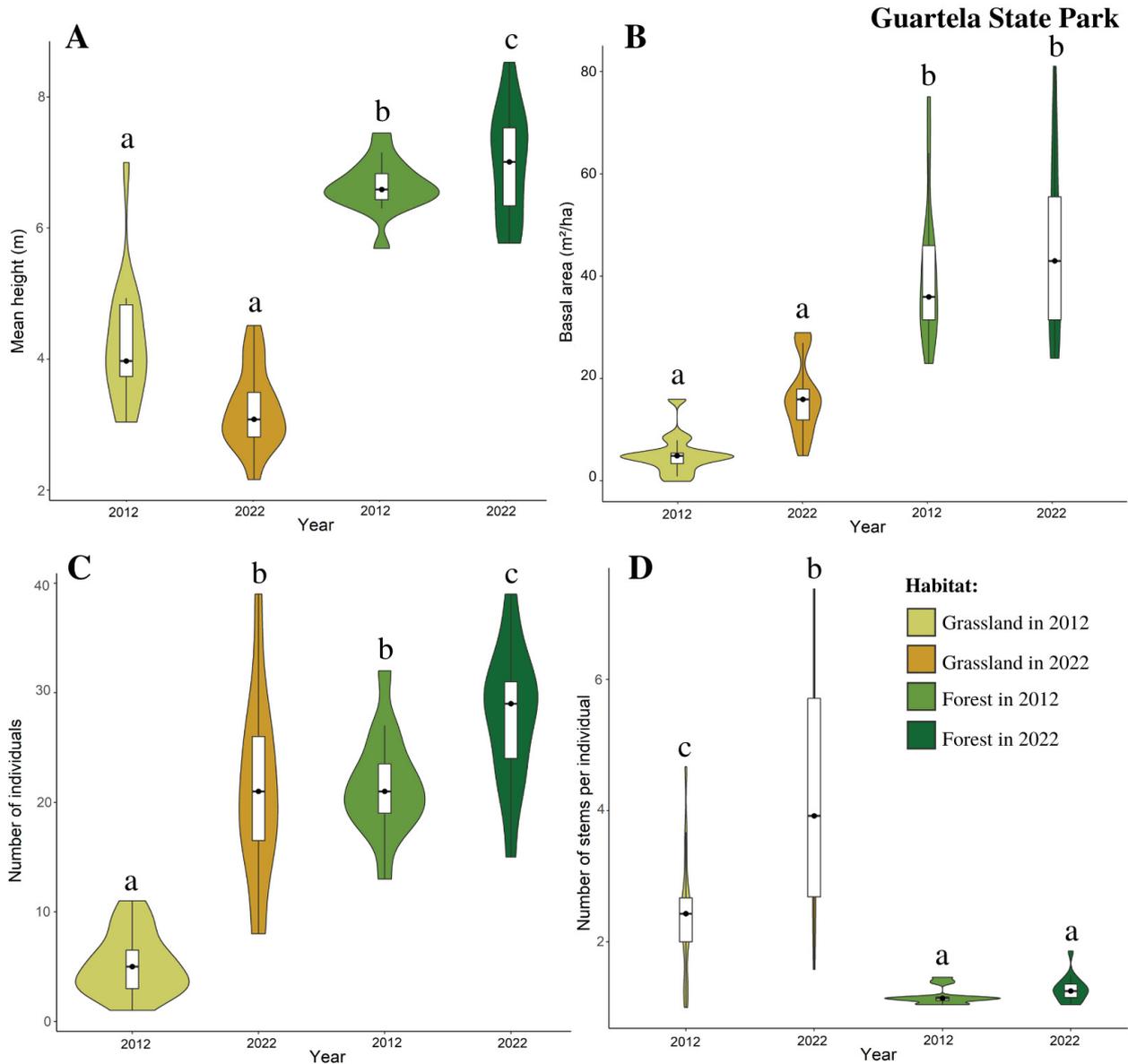


Figure SM 2: Violin plot (boxplots combined with kernel density plots) comparing structural parameters of vegetation along a spatial and temporal gradient of woody plant encroachment, in forest-grassland mosaic located in Guartelá State Park (GUA).

Boxplots showing median and 25^o and 75^o percentiles. The vertical curves display the kernel density estimates for each parameter's probability density function. Grassland is identified with beige colours (light beige for 2012 sample, and brownish beige for 2022 sample). Forest is identified with green colours (light green for 2012 sample and dark green for 2022 sample). Distinct letters represent significant differences between habitats in the pairwise permutation post hoc test ($p < 0.05$).

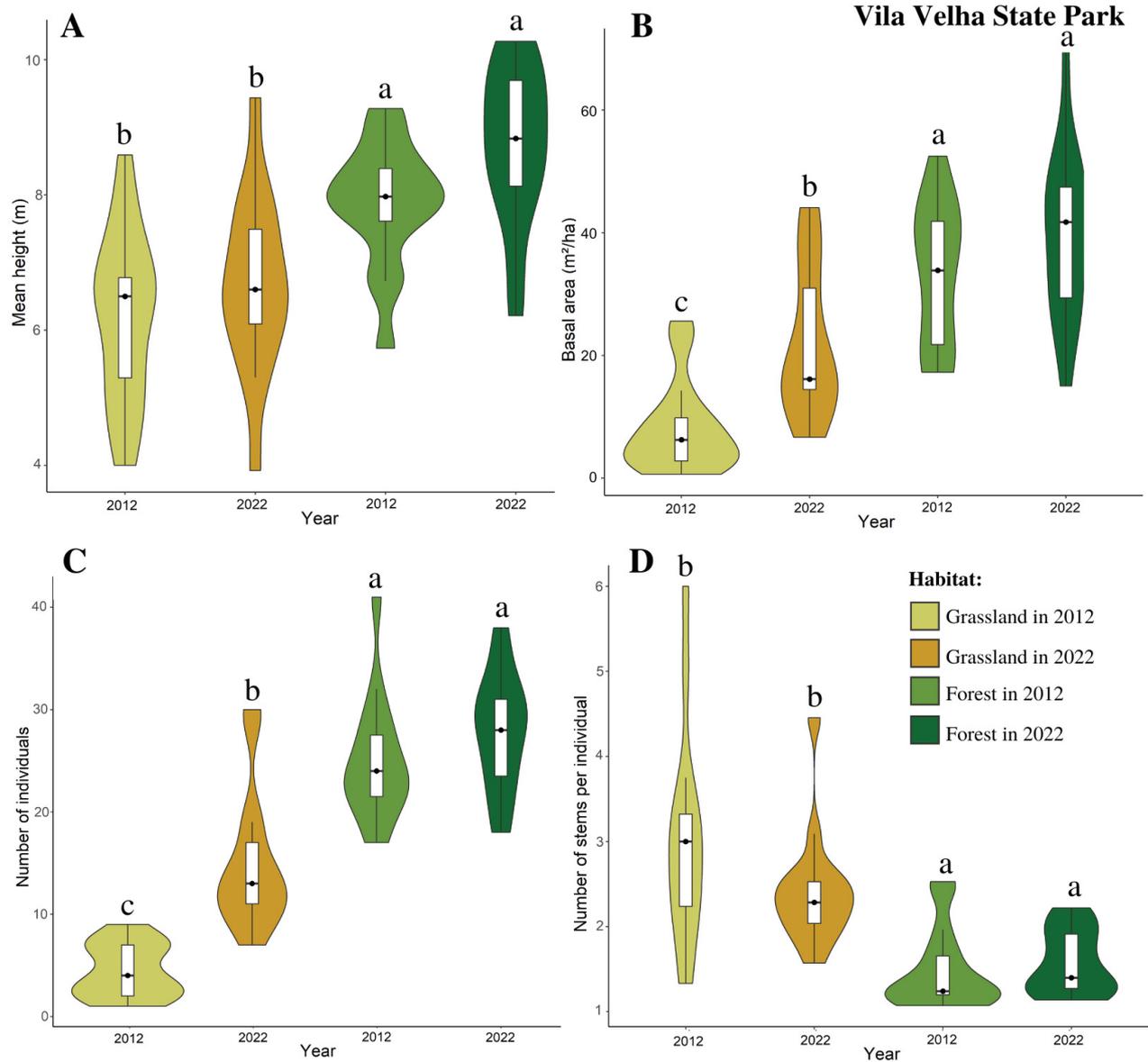


Figure SM 3: Violin plot (boxplots combined with kernel density plots) comparing structural parameters of vegetation along a spatial and temporal gradient of woody plant encroachment, in forest-grassland mosaic located in Vila Velha State Park (VV).

Boxplots showing median and 25^o and 75^o percentiles. The vertical curves display the kernel density estimates for each parameter's probability density function. Grassland is identified with beige colours (light beige for 2012 sample, and brownish beige for 2022 sample). Forest is identified with green colours (light green for 2012 sample and dark green for 2022 sample). Distinct letters represent significant differences between habitats in the pairwise permutation post hoc test ($p < 0.05$)

Appendix D: Results of spatial autocorrelation analysis.

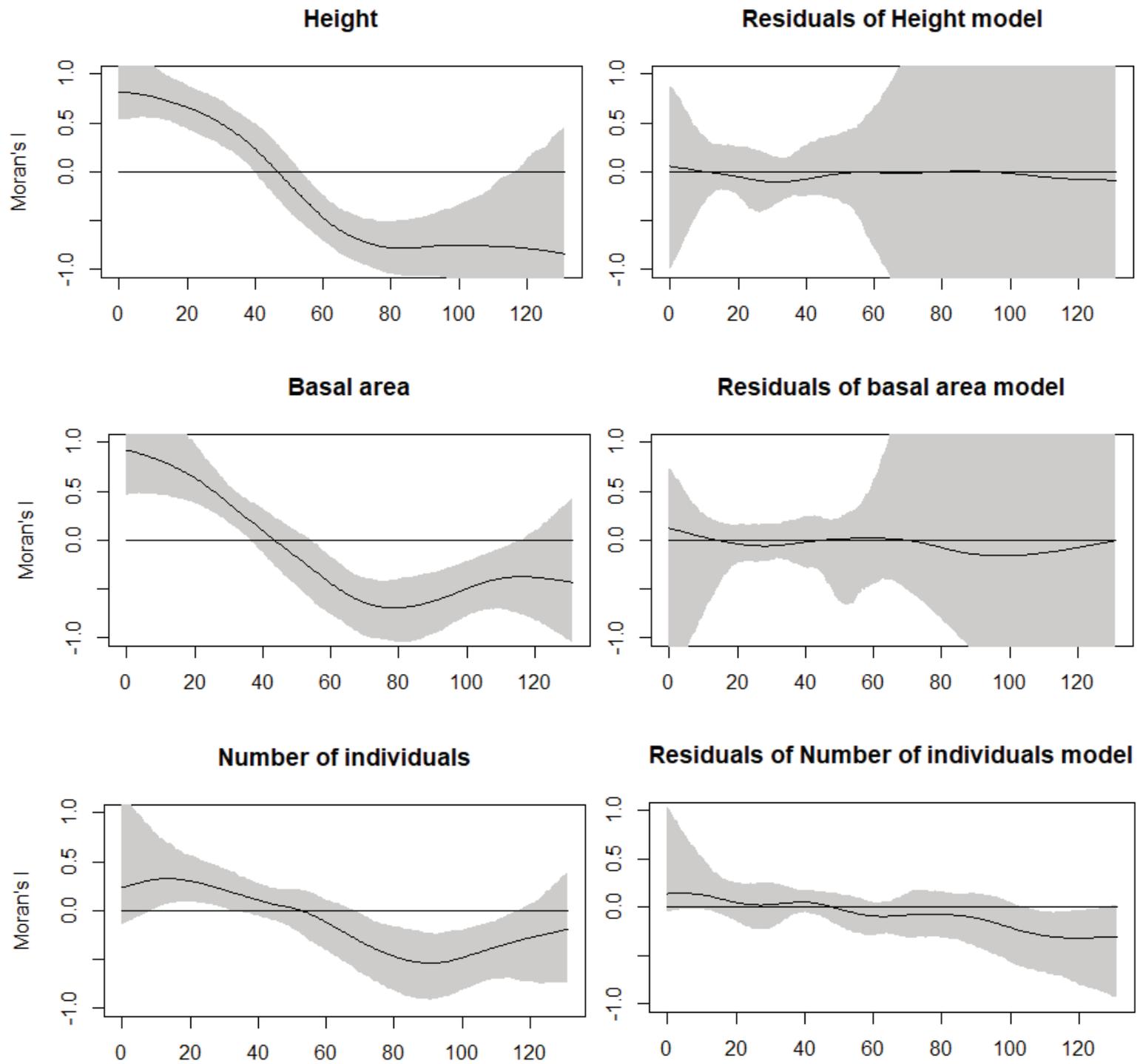


Figure SM 4: Spatial correlograms for Guartelá State Park, for structural parameters (mean height, total basal area and total number of individuals per plot), and also considering the residuals generated by the models.

The x-axis represents the distance between plots, in metres, and the y-axis represents Moran's I coefficients, that vary between -1.0 and 1.0 for maximum negative and positive spatial autocorrelation, respectively.

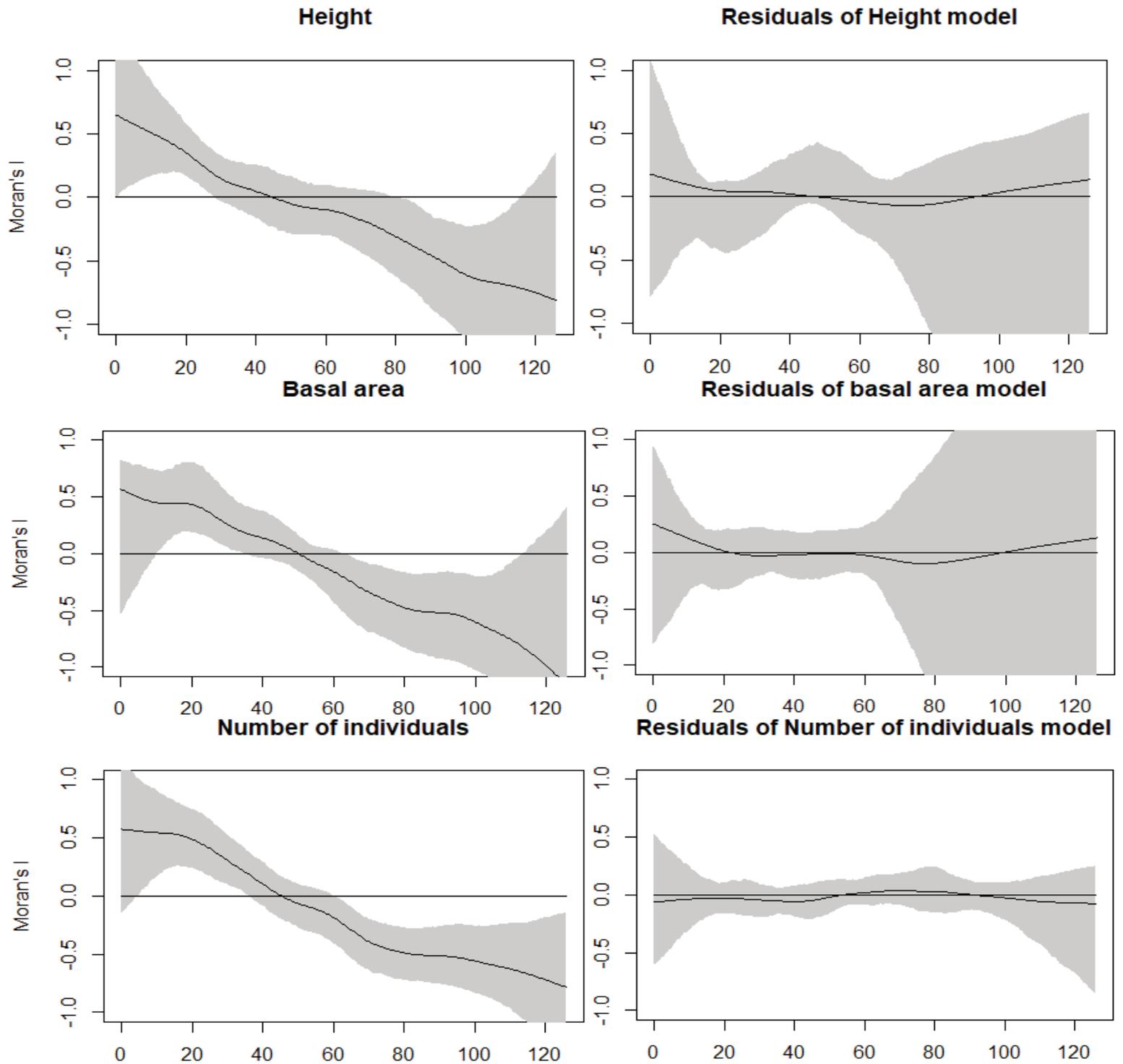


Figure SM 5: Spatial correlograms for Vila Velha State Park, for structural parameters (mean height, total basal area and total number of individuals per plot), and also considering the residuals generated by the models.

The x-axis represents the distance between plots, in metres, and the y-axis represents Moran's I coefficients, that vary between -1.0 and 1.0 for maximum negative and positive spatial autocorrelation, respectively.

Appendix E: Structural PCA, showing changes in vegetation physiognomy along woody plant encroachment.

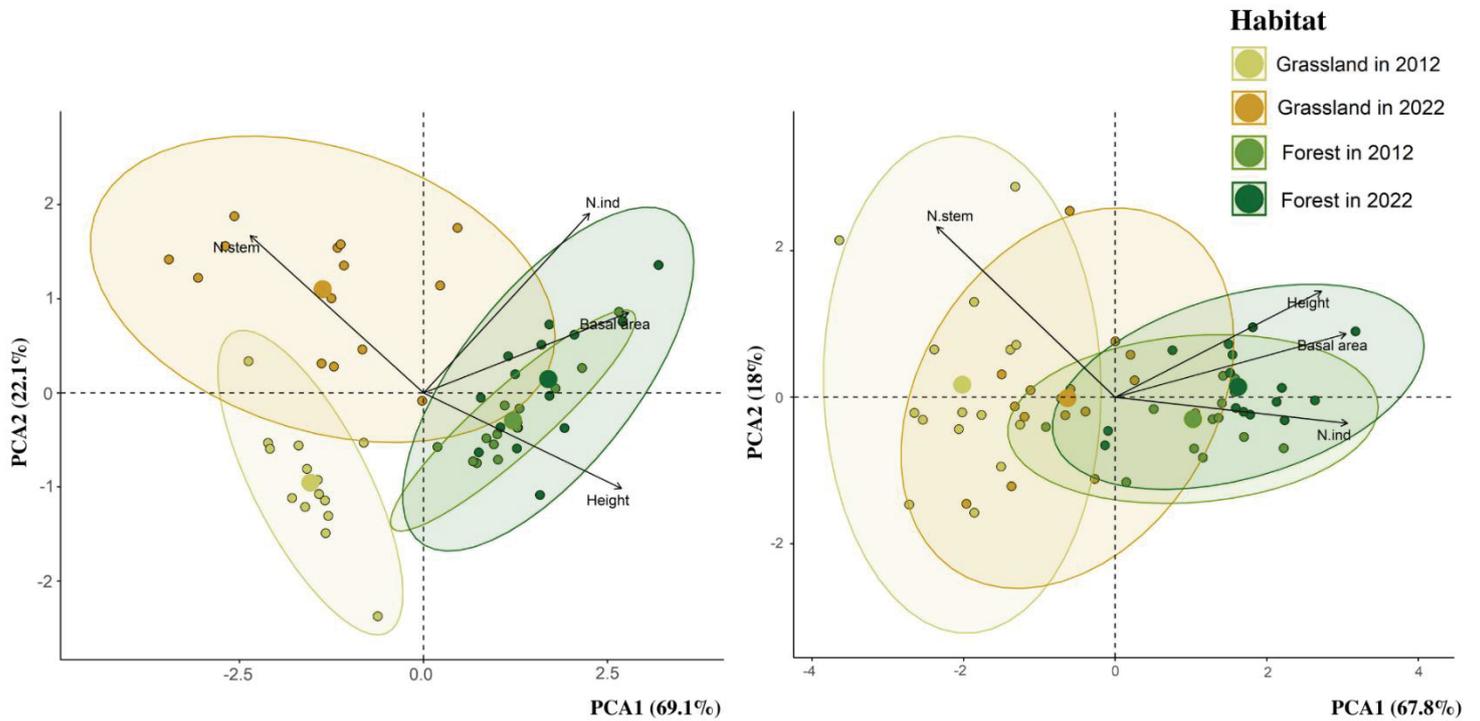


Figure SM 6: Principal Component Analysis (PCA) for structural parameters of vegetation in 2012 and 2022 in forest-grassland mosaics. A) Guartelá State Park (GUA); B) Vila Velha State Park (VV).

Appendix F: Results of the principal component analysis of community functional patterns in forest-grassland mosaics, presented in Figure 4.

This PCA was built considering community-weighted trait mean values, as each subplot was considered a community. CWM values were calculated considering species abundance. Only present loadings >0.20. Legend: GUA: Guartelá State Park; VV: Vila Velha State Park. Functional traits: LA – leaf area, SLA – specific leaf area, LDMC – leaf dry matter content, SSD – stem-specific density, BT – bark thickness, SRL – specific root length, D – average diameter, RBF – root branching frequency, Hmax - maximum height.

Table SM 1: Details of functional PCA at the community level. Only present loadings >0.20.

	PC1	PC2
GUA:		
Proportion explained	0.61	0.12
Cumulative proportion explained	0.61	0.74
Positively correlated trait	LDMC, SRL and RBF	BT
Negatively correlated trait	LA, SLA, D and Hmax	SSD
Eigenvalues	5.54	1.12
Broken-stick	2.82	1.82
Trait loadings		
LA	-0.32	
SLA	-0.36	
LDMC	0.36	-0.22
SSD	-0.23	-0.50
BT		0.77
SRL	0.34	-0.25
D	-0.37	
RBF	0.32	
Hmax	-0.40	
VV:		
Proportion explained	0.37	0.22
Cumulative proportion explained	0.37	0.69
Positively correlated trait	SRL and RBF	D
Negatively correlated trait	SLA and SSD	LA and Hmax
Eigenvalues	3.38	2.50
Broken-stick	2.82	1.82
Trait loadings		
LA	0.22	-0.46
SLA	-0.51	
LDMC		
SSD	-0.38	0.31
BT	0.24	0.25
SRL	0.43	
D		0.51
RBF	0.45	0.21
Hmax	-0.25	-0.49

Appendix G: Results of the principal component analysis of functional traits variation at species level, presented in Figure 6.

Table SM 2: Details of PCA at species level. Only present loadings >0.20.

	PC1	PC2
GUA:		
Proportion explained	0.28	0.16
Cumulative proportion explained	0.28	0.45
Positive correlated trait	LA, SLA, D and Hmax	SSD and D
Negative correlated trait	LDMC and SRL	LA, BT, RBF
Eigenvalues	2.59	1.46
Broken-stick	2.82	1.82
Trait loadings		
LA	0.32	-0.41
SLA	0.44	
LDMC	-0.41	
SSD		0.59
BT		-0.39
SRL	-0.28	-0.21
D	0.39	0.30
RBF		-0.33
Hmax	0.44	
VV:		
Proportion explained	0.24	0.21
Cumulative proportion explained	0.24	0.46
Positive correlated trait	LA and SLA	SRL and RBF
Negative correlated trait	LDMC and SSD	D
Eigenvalues	2.22	1.96
Broken-stick	2.82	1.82
Trait loadings		
LA	0.35	-0.25
SLA	0.36	-0.25
LDMC	-0.56	
SSD	-0.50	
BT		
SRL	0.22	0.55
D		-0.44
RBF	0.27	0.51
Hmax		-0.29

Appendix H: Correlation between functional traits at interspecific and community level.

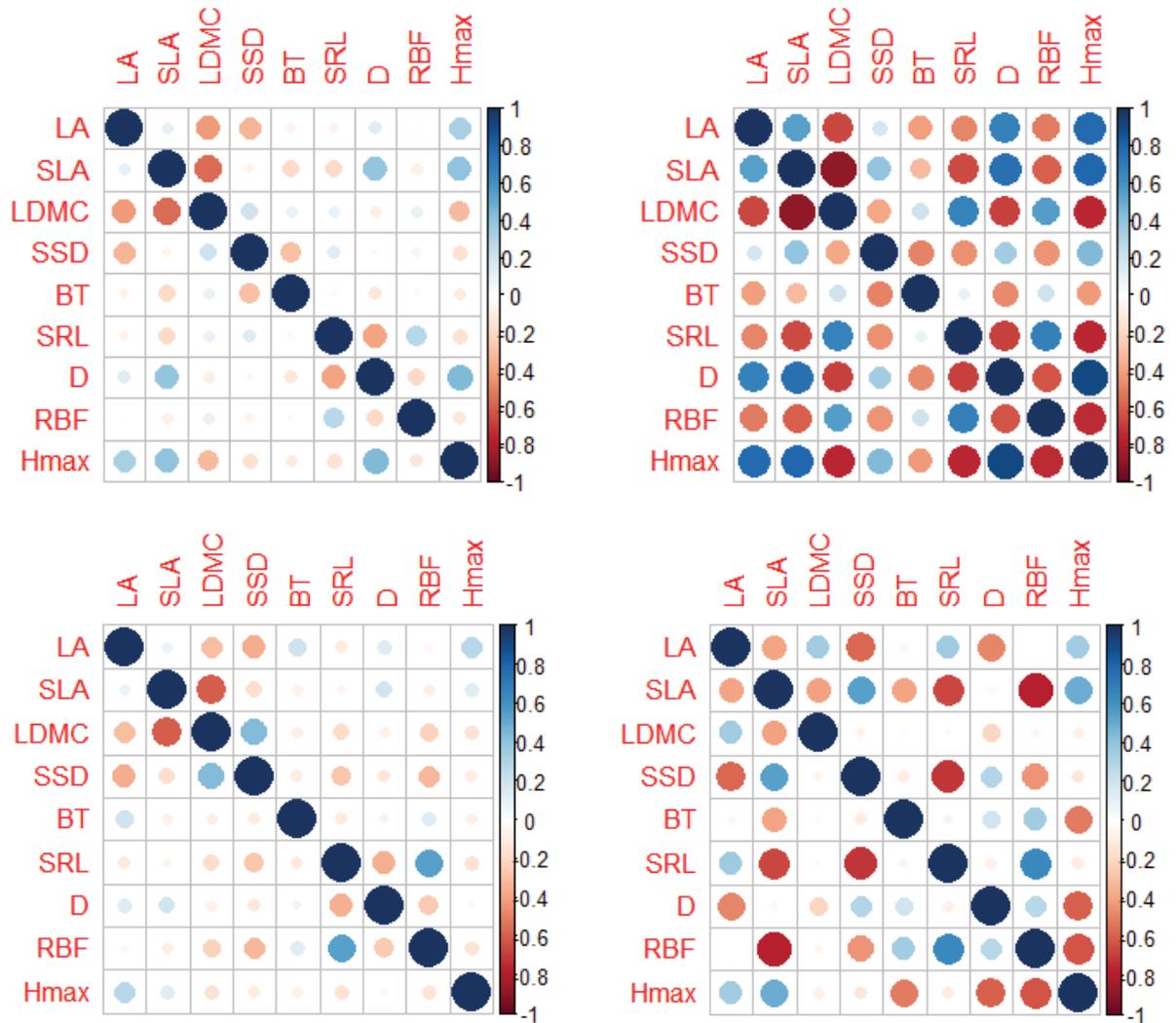


Figure SM 7: Correlation between functional traits at community level, for Guartelá (above) and Vila Velha State Park (below).

Appendix I: Board with photographic record of species with characteristic functional traits of grassland and forest, and of the mosaics.

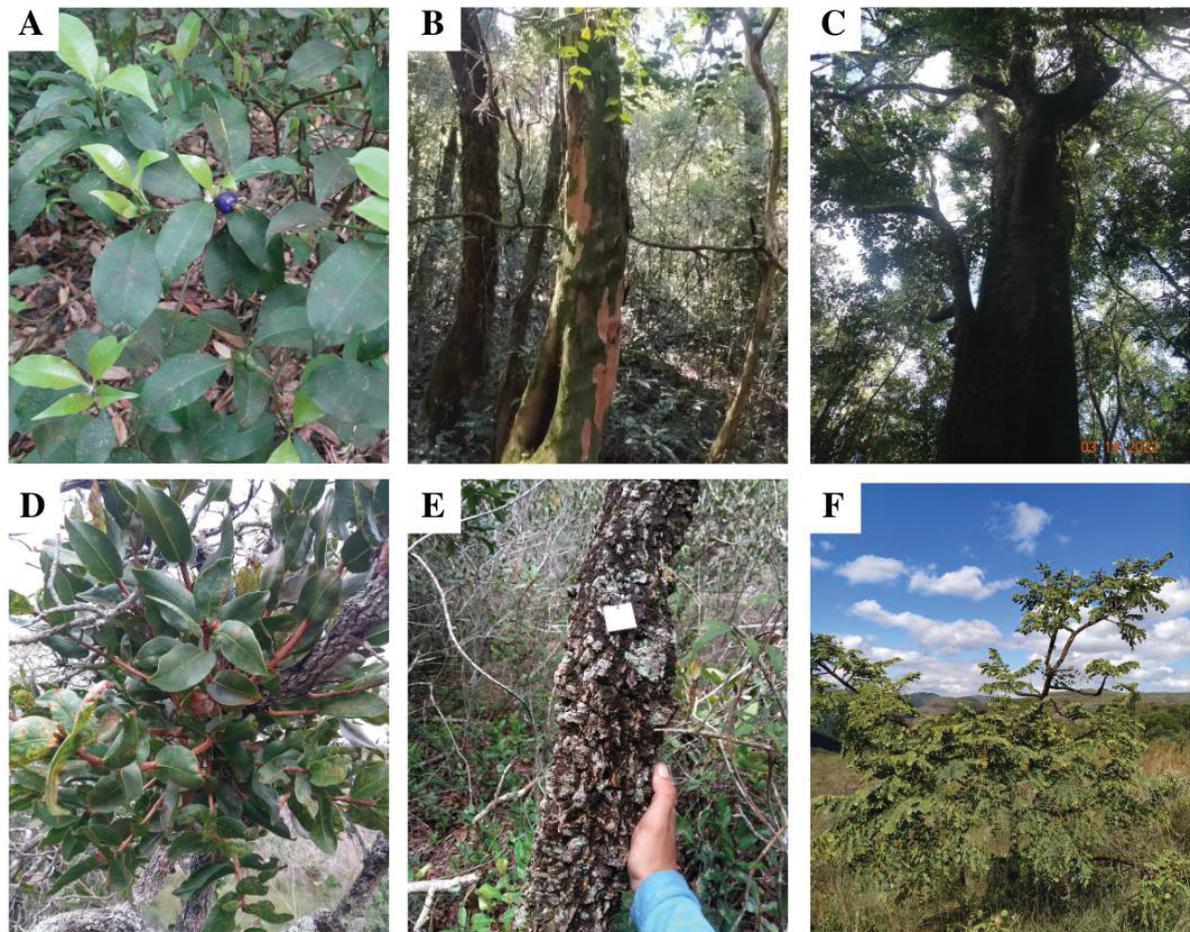


Figure SM 8: Board with a photographic record of species with characteristic functional traits of grassland and forest habitat.

Forest species have acquisitive leaves, with high specific leaf area, conservative woody with high woody density and thin bark, and are taller when compared to grassland species. In opposition, grassland species have conservative leaves, with high leaf dry matter content values, lower woody density and in compensation very thick bark to protect vital tissues against fire, and are smaller than forest species. Forest species traits: A = *Psychotria suterella* Müll.Arg. (Rubiaceae) with acquisitive leaves, B = *Myrciaria floribunda* (H.West ex Willd.) O.Berg (Myrtaceae) with thin bark, and C = *Pera glabrata* (Schott) Baill. (Peraceae) with high height. Grassland species: D = *Erythroxylum suberosum* A.St.-Hil. (Erythroxylaceae) with conservative leaves; E = *Qualea cordata* Spreng. (Vochysiaceae) with thicker bark that protects internal tissues from fire; and F = *Stryphnodendron adstringens* (Mart.) Coville (Fabaceae) with small height and twisted branches, characteristics of grassland species.

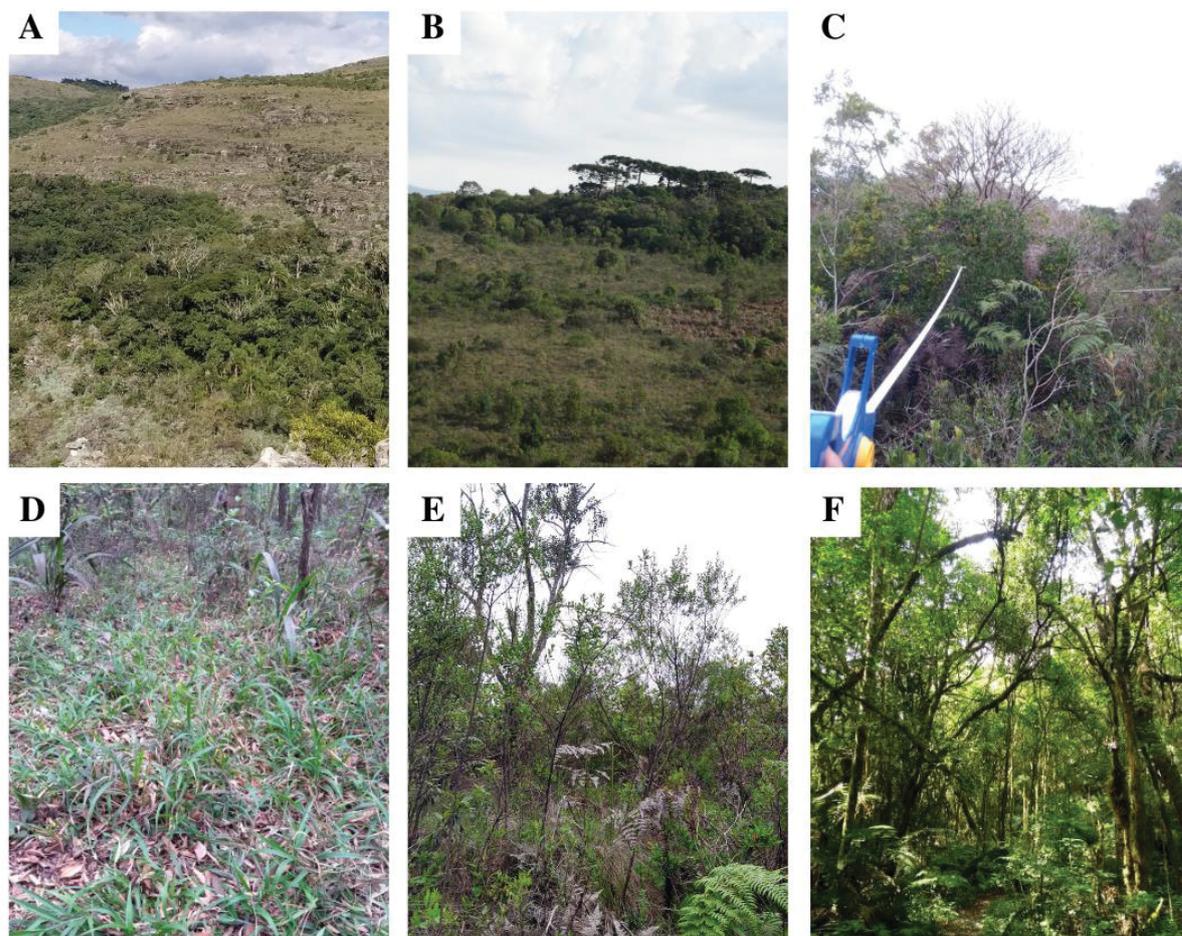


Figure SM 9: Board with photographic record of landscape and community structure of forest-grassland mosaics.

A) A patch of Araucaria Forest and South Brazilian grasslands, occurring along the Iapó river canyon in Guartelá State Park. B) Forest expansion through nucleation in Vila Velha State Park. C and E) Structure of grassland in 2022, with many wood individuals colonizing, however the canopy remains discontinuous. D) Herbaceous strata with grasses in grassland in 2012. F) Forest habitat, with closed canopy, structure understory and shade condition, characteristics of this habitat.