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INSTITUTO DE BIOCÊNCIAS  
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Tese de Doutorado

**PADRÕES DE DIVERSIDADE DE COMUNIDADES CAMPESTRES DO SUDESTE DA AMÉRICA DO SUL -  
UMA ABORDAGEM FILOGENÉTICA**

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Porto Alegre, maio de 2022

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**Padrões de diversidade de comunidades campestres do sudeste da América do Sul: uma abordagem filogenética**

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*“Jamais poderão aprisionar os nossos sonhos.”*

(Luiz Inácio Lula da Silva, 2018)

## RESUMO

Compreender como processos ecológicos e evolutivos atuam é fundamental para entender os padrões globais de diversidade e para prever suas variações ante mudanças climáticas e processos antrópicos como invasões biológicas. Processos ecológicos envolvem a dispersão e a relação dos organismos com fatores abióticos e bióticos. Processos evolutivos influenciam a história dos organismos através das taxas de especiação/extinção e eventos geológicos. A filogenia pode ser incorporada a estudos ecológicos como uma abordagem evolutiva, fazendo uma ligação entre passado e presente que indique possíveis explicações históricas e evolutivas nos padrões observados. Nesta tese, apresento resultados de uma pesquisa em ampla escala sobre a biodiversidade dos campos do sudeste da América do Sul a partir da base de dados Transcampos, construída por um esforço conjunto de pesquisadores brasileiros, argentinos e uruguaios. No **primeiro capítulo**, busquei entender as relações entre diversidade filogenética alfa e as condições ecológicas do presente (fatores climáticos e edáficos), estabilidade climática histórica e impacto humano. Demonstrei que fatores ambientais do presente e do passado influenciam os padrões de diversidade filogenética de comunidades campestres da América do Sul. Áreas de maior estabilidade histórica da temperatura e áreas com menor temperatura mínima apresentaram comunidades com padrão de agrupamento filogenético. No **segundo capítulo**, investiguei os fatores que determinam os padrões de diversidade filogenética beta através da distribuição de clados e grupos funcionais de gramíneas. A contribuição de espécies  $C_3$  aumenta em direção sul e em áreas mais frias com maior estabilidade histórica de temperatura, refletindo linhagens que se especializaram e irradiaram em ambientes frios. As informações apresentadas nessa tese nos ajudam a entender um pouco mais da história evolutiva da vegetação campestre, mas também adicionam percepções sobre o uso de informações filogenéticas para investigar os fatores que originaram e mantiveram os padrões de diversidade observados.

**Palavras-chave:** agrupamento filogenético; diversidade filogenética; ecologia filogenética de comunidades; ecossistemas campestres; gradientes ambientais; história evolutiva; Poaceae.



## **ABSTRACT**

Understanding how ecological and evolutionary processes act is essential to understand global patterns of diversity and to predict their variations in face of climate change and anthropic processes such as biological invasions. Ecological processes involve the dispersion and relationship of organisms with abiotic and biotic factors. Evolutionary processes influence the history of organisms through speciation/extinction rates and geological events. Phylogeny can be incorporated into ecological studies as an evolutionary approach, making a connection between past and present, indicating possible historical and evolutionary explanations in the observed patterns. In this thesis I present the results of a large-scale survey on the grassland biodiversity of southeastern South America using the Transcampos database, built by a joint effort of researchers from Brazil, Argentina and Uruguay. In the **first chapter**, I aimed to understand the relationships between alpha phylogenetic diversity and present ecological conditions (climatic and edaphic factors), historical climate stability and human impact. I showed that present and past environmental factors influence the patterns of phylogenetic diversity of grassland communities in South America. Areas with higher historical temperature stability and areas with lower minimum temperature hosted communities with pattern of phylogenetic clustering. In the **second chapter**, I investigated environmental factors driving the beta phylogenetic diversity through distribution patterns of grass clades and functional groups. The contribution of C<sub>3</sub> species increased towards south and in colder areas with higher historical temperature stability, reflecting lineages have specialized and radiated in cold environments. The information presented in this thesis helps us understand a little more about the evolutionary history of grassland vegetation, but also adds insights into the use of phylogenetic information to investigate the factors that originated and maintained the observed diversity patterns.

**Keywords:** community phylogenetics; environmental gradients; evolutionary history; grassland ecosystems; phylogenetic diversity; phylogenetic clustering; Poaceae.

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## **INTRODUÇÃO GERAL**

### **Ecologia filogenética: uma breve história**

Os fatores que determinam a distribuição da diversidade biológica é uma das principais questões investigadas por ecólogos e biogeógrafos, remontando ao início do século XX. Entretanto, evolucionistas focaram-se em estudos genéticos, buscando compreender as mudanças evolutivas nos genótipos dos organismos, sem investigar como essas mudanças poderiam influenciar comunidades ecológicas inteiras. Enquanto isso, ecólogos estavam interessados na descrição de padrões em escala local, na qual os processos evolutivos não representam grande influência. Essa dissociação entre estudos ecológicos e evolutivos manteve-se até a metade do século XX, quando se percebeu que os processos evolutivos poderiam determinar as interações entre organismos em uma comunidade (McGill et al. 2019). Foi a partir dos trabalhos de MacArthur sobre a evolução de atributos ecologicamente relevantes que a ecologia evolutiva surgiu como um campo da ciência. Hoje, há um maior entendimento que padrões globais de diversidade e composição de espécies são formados pela interação entre processos ecológicos e evolutivos (Mittelbach & Schemske 2015).

Dentre os processos ecológicos, há duas visões sobre a organização de comunidades. A primeira perspectiva estabelece que as comunidades são resultado das interações determinadas pelo nicho das espécies que as compõem. Essa perspectiva está ligada à teorias em que o nicho tem um papel central, na qual os diferentes processos ambientais, por exemplo, variáveis climáticas ou edáficas, bem como interações entre espécies, agem como filtros, selecionando as espécies adaptadas (MacArthur & Levins 1964; Diamond 1975; Keddy 1992). Por outro lado, a teoria Neutra (Hubbell 2001), considera as espécies como ecologicamente ou funcionalmente equivalentes. Segundo esta teoria, são principalmente processos estocásticos e demográficos derivados da dispersão dos indivíduos (por exemplo, nascimento e morte, imigração e extinção local) que definem a composição de uma comunidade em dado local. Já os processos evolutivos estão relacionados à história evolutiva das espécies,

principalmente àqueles que definem os processos históricos de especiação e dispersão de espécies (Ricklefs 1987; Hubbell 2001; Tilman 2004; Gerhold et al. 2015). Considerados em conjunto, esses diferentes processos influenciam as relações dos organismos com fatores abióticos, bióticos e históricos, no espaço e no tempo (Vellend 2016).

O surgimento da ecologia filogenética de comunidades a partir do trabalho de Webb et al. (2002) e da disponibilidade de filogenias moleculares para diversos grupos biológicos representou um avanço dentro dos estudos em ecologia evolutiva. Uma árvore filogenética reflete a quantidade de história evolutiva compartilhada entre as espécies em relação ao ancestral comum, ou seja, representa as relações de parentesco envolvidas. Dessa forma, duas espécies são mais relacionadas se têm um ancestral comum mais recente e menos relacionadas se têm um ancestral comum menos recente. Essa distância de parentesco entre as espécies reflete a quantidade de passos evolutivos envolvidos na diversificação de uma linhagem. Ao construirmos a informação filogenética para uma comunidade biológica, assumimos que a evolução desempenha um papel importante na organização da comunidade, de forma que a quantidade de informações evolutivas compartilhadas entre todas as espécies presentes possa ser expressa por medidas de estrutura filogenética (Webb et al. 2002). Dessa forma, duas comunidades que possuem o mesmo número de espécies podem diferir consideravelmente em sua estrutura filogenética, devido às diferenças evolutivas entre os conjuntos de espécies. Dizemos que uma comunidade é filogeneticamente agrupada (*clustering*) quando as espécies coexistentes possuem relações evolutivas muito próximas (Webb et al. 2002; Cavender-Bares et al. 2009). Por outro lado, as comunidades podem apresentar um padrão de dispersão filogenética (*overdispersion*) quando as espécies coexistentes são distantemente relacionadas (Webb et al. 2002; Cavender-Bares et al. 2009).

Por mais que a ecologia filogenética tenha proposto desde o início a busca pelo entendimento de como os processos evolutivos interagem com os processos ecológicos locais na organização de comunidades (Webb et al. 2002; Cavender-Bares et al. 2009), por muito tempo a interpretação dos padrões de estrutura filogenética de comunidades focou apenas nos processos ecológicos envolvidos – como interações abióticas e bióticas

do presente, relacionadas à teoria do nicho (Webb et al. 2002). Além disso, baseado no princípio de que espécies mais aparentadas entre si apresentam atributos mais similares, ou seja, os atributos funcionais são conservados ao longo da filogenia, muitos estudos começaram a utilizar a informação filogenética para inferir processos ecológicos quando não dispunham de informação funcional das espécies disponíveis (Gerhold et al. 2015). Dessa forma, entende-se que a ação de um filtro ambiental resultaria em uma comunidade com padrão de agrupamento filogenético, devido à conservação de atributos em determinada linhagem, o que possibilitaria a sobrevivência naquela condição ambiental (Ackerly 2003; Donoghue 2008; Duarte 2011), caracterizando um filtro filogenético de habitats (Duarte 2011). Por outro lado, em comunidades onde a competição biótica seja o fator mais importante na organização, um padrão de dispersão filogenética seria esperado, uma vez que espécies mais similares competem pelos mesmos recursos, levando à exclusão de linhagens menos competitivas (Webb et al. 2002). Entretanto, os atributos nem sempre são conservados na filogenia, nem competição leva necessariamente à dispersão (Mayfield & Levine 2010; Gerhold et al. 2015). Portanto, inferir processos de organização de comunidades a partir de padrões filogenéticos de agrupamento ou dispersão limita as possibilidades de interpretação dos processos de biodiversidade (Gerhold et al. 2015).

Por outro lado, a filogenia pode ser incorporada a estudos ecológicos como uma abordagem evolutiva que agrega um *proxy* temporal, fazendo uma ligação entre passado e presente que indique possíveis explicações históricas e evolutivas nos padrões observados (Cavender-Bares et al. 2009; Gerhold et al. 2015). Sabe-se que processos históricos, como taxas de diversificação de linhagens pode afetar a diversidade filogenética de comunidades (Gerhold et al. 2018). Por exemplo, a ocorrência de uma rápida e recente irradiação leva à uma baixa diversidade filogenética (Yan et al. 2013). Alternativamente, uma baixa taxa de extinção combinada com uma alta especiação, resulta em um acúmulo de linhagens distantemente relacionadas e em comunidades com alta diversidade filogenética (Qian 2014). Outro fator ligado aos processos históricos é a idade e estabilidade do habitat (Gerhold et al. 2015; Lososová et al. 2015). Habitats relativamente jovens na escala de tempo geológico devem apresentar comunidades

agrupadas filogeneticamente, porque apenas algumas linhagens seriam pré-adaptadas a esses habitats, e o tempo muito curto para permitir uma adaptação bem-sucedida de um espectro mais amplo de linhagens distantes relacionadas. Além disso, sabe-se que a estabilidade climática histórica influencia a diversidade de plantas, especificamente a distribuição de espécies ou a diversidade funcional (Costa et al. 2018). Por exemplo, áreas de relativa estabilidade são caracterizadas por endemismo filogeográfico e possuem maior riqueza funcional (Carnaval et al. 2014; Ordonez & Svenning 2015), como por exemplo, as regiões de refúgio no último máximo glacial (Ordonez & Svenning 2015).

Para aprofundar as questões que determinam a organização de comunidades, a associação entre abordagens filogenéticas e funcionais propiciam um melhor entendimento de como atributos funcionais e sua evolução influenciam padrões ecológicos observados atualmente na natureza (Pillar, Duarte, et al. 2009; Pillar & Duarte 2010). Teoricamente, a combinação de filogenia com informações de atributos poderia fornecer ideias sobre a interação entre coexistência local e macroevolução, por exemplo, através da competição entre espécies próximas provocando deslocamento e diversificação de caracteres (de Bello et al. 2015; Gerhold et al. 2015). Portanto, associar a estrutura filogenética com a estrutura funcional pode fornecer uma imagem mais completa dos processos de organização do passado e do presente, e suas consequências evolutivas e ecológicas.

### **Relações evolutivas na macroescala: análise de estrutura e composição filogenética**

A inclusão de métodos filogenéticos comparativos na avaliação da biodiversidade permite explorar a dispersão de linhagens ao longo da distribuição dos organismos. Uma forma de avaliar isso é através das diferentes dimensões da diversidade filogenética, avaliando a relação de parentesco entre as espécies dentro e entre regiões, também denominadas estrutura (diversidade filogenética alfa) e composição filogenética (diversidade filogenética beta; Graham & Fine 2008).

Os padrões de estrutura filogenética das comunidades são comumente avaliados através de dois índices: a distância média entre pares (do inglês *mean pairwise distance*,

MPD) e a distância média do táxon mais próximo (do inglês *mean nearest taxon distance*, MNTD, Webb et al. 2002). O MPD calcula as distâncias filogenéticas em toda a árvore filogenética, computando a média de todas as distâncias de pares de espécies coocorrentes, enquanto o MNTD calcula distâncias em níveis filogenéticos mais rasos (ou seja, nos ramos terminais da árvore filogenética), entre os parentes mais próximos de cada espécie ocorrente em uma comunidade (Webb et al. 2002). Assim, MPD e MNTD são medidas de diversidade filogenética que capturam as relações evolutivas em diferentes intervalos de tempo, com MPD refletindo as associações de longo prazo e potencialmente um período de tempo mais longo para adaptação (Moro et al. 2015). Considerando que essas medidas de diversidade filogenética bruta são diretamente correlacionadas com a diversidade taxonômica (Webb et al. 2002; Kembel et al. 2010), o cálculo de seus tamanhos de efeito padronizados (do inglês *standardized effect size*, SES), informam se os índices de diversidade são maiores ou menores do que o esperado dado a riqueza de espécies da comunidade (Pavoine & Bonsall 2011).

Os padrões de agrupamento e dispersão filogenética podem apresentar uma variação ao longo do espaço e do tempo, caracterizando a diversidade filogenética beta (Kerkhoff et al. 2014; Kubota et al. 2018). A diversidade filogenética beta reflete a informação evolutiva compartilhada entre comunidades no espaço (Graham & Fine 2008), e, combinada com os resultados de diversidade filogenética alfa pode demonstrar a relação entre os clados ao longo do tempo e espaço (Carlucci et al. 2017). Quando a diversidade beta é analisada, podemos verificar se duas comunidades que apresentam estrutura filogenética (diversidade filogenética alfa) semelhantes também se assemelham em relação à sua composição filogenética, ou seja, se são compostas por espécies das mesmas linhagens ou linhagens com histórias evolutivas distintas, caracterizando uma substituição de linhagens ao longo dos gradientes ambientais. Esses padrões podem ser resultantes de condições ambientais favoráveis ou limitantes (atuação de um filtro ambiental), ou da limitação geográfica de dispersão das espécies (limitação de dispersão; Wiens & Donoghue 2004). Os padrões de diversidade filogenética beta podem ser avaliados por meio de coordenadas principais de estrutura filogenética (do inglês *Principal Coordinates of Phylogenetic Structure*, PCPS; Duarte 2011; Duarte et al. 2016).



Esse método consiste em uma análise de coordenadas principais (PCoA) da matriz de composição de espécies ponderada pela filogenia para cada comunidade. A PCoA gera as coordenadas principais da estrutura filogenética (PCPS), autovetores que descrevem um gradiente filogenético no conjunto de dados (Duarte et al. 2016). Principalmente, o método informa se a distribuição de espécies de plantas ao longo do gradiente ambiental é mediada pelas relações filogenéticas envolvidas (Duarte et al. 2016). A representação gráfica dos escores gerados pela PCPS permite a observação da representatividade dos clados ao longo do gradiente. Assim, a aplicação de métodos filogenéticos ajuda a entender como a história macroevolutiva do conjunto regional de espécies interage com os processos ecológicos e biogeográficos para produzir as comunidades observadas (Webb et al. 2002; Cavender-Bares et al. 2009).

### **Região de estudo: Ecossistemas campestres no sudeste da América do Sul**

A região do cone sul da América do Sul é caracterizada pela vegetação campestre predominante, se estendendo amplamente pelo sul do Brasil, nordeste da Argentina e Uruguai (28°S–38°S, 50°W–61°W), sendo uma das maiores regiões de campos temperados do mundo e a maior na América do Sul (Soriano 1992; Azpiroz et al. 2012; Andrade et al. 2018). O clima na região é subtropical, classificado como Cfa e Cfb de acordo com a classificação de Köppen (Alvares et al. 2013). A precipitação média anual varia entre 700 mm no sul da região até 2.200 mm na região nordeste, enquanto a temperatura média anual varia entre 13°C e 20°C, dependendo da altitude (Alvares et al. 2013). A região compreende campos que em geral são estudadas separadamente, apesar de apresentarem as mesmas características ecológicas. Unificadas, as sub-regiões “campos do Rio da Prata”, “Campos de Cima da Serra” e “campos do Chaco” são chamadas de campos do sudeste da América do Sul (*SESA grasslands*; *sensu* Azpiroz et al. 2012). Nesta tese, estudamos a vegetação campestre das sub-regiões dos campos do Rio da Prata e dos Campos de Cima da Serra, devido à disponibilidade de dados (Figura 1).

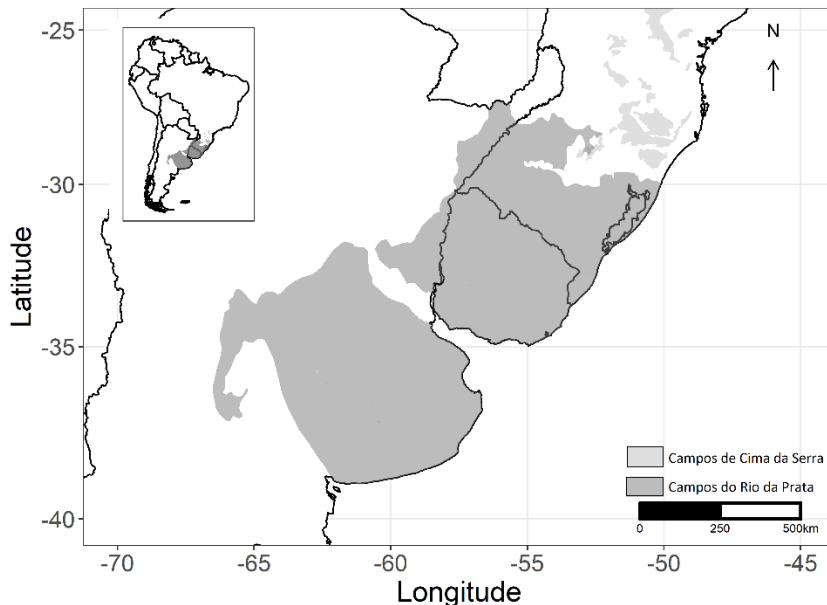


Figura 1. Extensão dos campos do sudeste da América do Sul, evidenciando as sub-regiões dos Campos de Cima da Serra e dos Campos do Rio da Prata.

A porção brasileira dessa região inclui o bioma Pampa na porção sul e oeste do Rio Grande do Sul, e a parte mais sul do bioma Mata Atlântica, incluindo as partes mais altas do planalto Sul-brasileiro, estendendo-se por Santa Catarina e Paraná (28°S–33°S, 48°W–58°W). Na região do bioma Pampa os campos predominam e constituem a matriz da paisagem, com as florestas formando manchas isoladas, ou dispostas ao longo de cursos d'água (Overbeck et al. 2007). Na região da Mata Atlântica há uma maior predominância de florestas, com campos insulares de diferentes tamanhos (Oliveira-Filho et al. 2015). Os campos do Uruguai são muito similares aos campos do Pampa brasileiro, enquanto os campos da Argentina distinguem-se de acordo com a fisionomia, geomorfologia e características edáficas (Soriano 1992). A riqueza de espécies na região segue uma tendência latitudinal, com duas vezes mais espécies de plantas vasculares por unidade de área na porção norte (Brasil e Uruguai) do que na porção sul da Argentina (Oyarzabal et al. 2020). O conjunto dos campos da região do Pampa brasileiro, Argentina e Uruguai denomina-se Campos do Rio da Prata (*Río de la Plata Grasslands*), enquanto

os campos inseridos na Mata Atlântica são denominados Campos de Cima da Serra. Os estudos disponíveis sobre os fatores determinantes da diversidade dos campos da América do Sul geralmente são restritos a locais únicos ou regiões menores (Perelman et al. 2001; Burkart et al. 2011; Lezama et al. 2014; Menezes et al. 2016). Apenas recentemente Andrade et al. (2018) exploraram a composição florística em toda a região dos Campos do Rio da Prata. Entretanto, trabalhos analisando a região dos campos da América do Sul como um todo não foram encontrados, sendo também a análise de outras faces da biodiversidade, como a diversidade filogenética, uma lacuna a ser explorada.

Evidências paleoecológicas desta região indicam que os ecossistemas campestres não eram muito extensos durante a maior parte do período Cenozóico (Barreda & Palazzesi 2007), mas se tornaram o ecossistema dominante desde o Pleistoceno até meados do Holoceno (cerca de 18 Ma) devido a um clima mais seco e frio do que hoje (Behling 2002; Behling et al. 2007; Strömberg et al. 2013). A prevalência histórica de ecossistemas abertos é reconhecida no norte da Argentina e no Brasil, onde as amostragens de pólen apontam para a presença de campos com predomínio de gramíneas no Mioceno tardio (Strömberg 2011). Nos últimos três milênios, com a tendência de mudança para um clima mais quente e úmido, tem se observado uma progressiva expansão das florestas sobre os mesmos, pelo menos nos Campos de Cima da Serra, e nas regiões oeste e sudeste do RS (Behling & Pillar 2007). Processos de expansão florestal sobre os campos nativos são especialmente evidentes em áreas com baixa intensidade ou ausência de queimadas ou pastejo pelo gado (Overbeck et al. 2007).

Em ecossistemas campestres em condições de clima e solo que permitem o desenvolvimento de ecossistemas florestais, distúrbios como fogo e pastejo são necessários para manter sua biodiversidade e características ecológicas. As espécies desses ecossistemas são, de forma geral, adaptadas à ocorrência destes distúrbios, já que evoluíram com o fogo e os grandes herbívoros (Baggio et al. 2021; Pivello et al. 2021). Os campos do cone sul da América do Sul se desenvolveram sob a influência de grandes herbívoros até o Holoceno Inicial, quando a megafauna foi extinta (Sandom et al. 2014). A introdução de gado doméstico pelos colonizadores europeus no século XVII induziu a manutenção da biodiversidade e dos processos ecológicos desses

ecossistemas, e hoje a pecuária é uma das atividades econômicas mais importantes da região (Pillar, Müller, et al. 2009; Oyarzabal et al. 2020). Adicionalmente, ecossistemas campestres dominados por gramíneas em regiões subtropicais e tropicais são dependentes do fogo, e a vegetação apresenta diversas adaptações e sinergias com o fogo (Pivello et al. 2021). Apesar de não ser mais utilizado com uma ferramenta de manejo do campo, o fogo é reconhecido com um papel importante na manutenção dos campos abertos após a extinção dos grandes herbívoros no final do Pleistoceno e início do Holoceno até a introdução do gado doméstico (ca. 10 mil anos depois; Prieto 2000; Bond & Keeley 2005; Paruelo et al. 2022).

Os campos são fisionomicamente caracterizados pelas gramíneas, que constituem o grupo dominante. Poaceae é a quinta família mais diversa entre as angiospermas, com aproximadamente 780 gêneros e 12.000 espécies e distribuição cosmopolita (Christenhusz & Byng 2016). As gramíneas começaram a se propagar a partir do início do Mioceno, entre 10 a 20 milhões de anos atrás (Strömberg 2011), e hoje os ecossistemas dominados por gramíneas cobrem cerca de 31% a 43% da superfície terrestre global (Gibson 2009). A história evolutiva das gramíneas está fortemente ligada ao surgimento da via fotossintética C<sub>4</sub>. A redução do CO<sub>2</sub> atmosférico há cerca de 30 Ma diminuiu a eficiência de muitas plantas terrestres (Sage 2004; Edwards et al. 2010), exigindo modificações anatômicas para melhorar o desempenho fotossintético nessas novas condições (Sage 2004; GPWGII 2012). Assim, a rota fotossintética C<sub>4</sub> evoluiu independentemente em mais de 45 linhagens de plantas com flores, 24 delas em gramíneas (Sage 2004; GPWGII 2012), possibilitando a expansão global dos campos com predomínio de plantas C<sub>4</sub> em substituição da vegetação C<sub>3</sub> durante o Mioceno Superior e Plioceno (3 a 8 Ma; Edwards et al. 2010; Strömberg 2011). A distribuição atual de gramíneas C<sub>3</sub> e C<sub>4</sub> é frequentemente explicada por condições ambientais contrastantes, com gramíneas C<sub>4</sub> dominando ecossistemas campestres tropicais e subtropicais, e gramíneas C<sub>3</sub> dominando as regiões de campos temperados (Gibson 2009; Edwards et al. 2010).

## Objetivos da tese

O principal objetivo desta tese foi avaliar possíveis mecanismos ecológicos e evolutivos envolvidos na estruturação filogenética de comunidades de plantas campestres ao longo de gradientes ambientais. Mais especificamente, a tese visou: (1) entender as relações entre diversidade filogenética e as condições ecológicas do presente (fatores climáticos e edáficos), estabilidade climática histórica e impacto humano; e (2) investigar os fatores que determinam os padrões de distribuição de clados e grupos funcionais de gramíneas.

A tese está estruturada em dois capítulos: (1) "*Past and present drivers of phylogenetic diversity of grassland communities in southeastern South America*" e (2) "*Determinants of biogeographical distribution of grasses in grasslands of South America*". Ambos os capítulos foram elaborados a partir da base de dados Transcampos, construída por um esforço conjunto de pesquisadores brasileiros, argentinos e uruguaios, cobrindo um amplo espectro ambiental nos ecossistemas campestres da América do Sul.

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**CAPÍTULO 1. PAST AND PRESENT DRIVERS OF PHYLOGENETIC DIVERSITY OF GRASSLAND COMMUNITIES IN SOUTHEASTERN SOUTH AMERICA**

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## Abstract

**Aim:** Little is known on patterns of phylogenetic diversity along climatic gradients in grassland communities, especially in the context of evolutionary and macroecological processes. Here, we aimed to understand the relationships of phylogenetic diversity and current climate, soil conditions, human impact, and historical climate stability.

**Location:** South American Temperate and Subtropical grasslands.

**Methods:** We analyzed the phylogenetic structure of 666 grassland vegetation plots across southeastern South America. The metrics we used for phylogenetic diversity were the mean pairwise distance (SESmpd) and mean nearest taxon distance (SESmntd). We then investigated patterns of phylogenetic diversity along gradients of historical and current climatic variables, and edaphic conditions through generalized least-square regressions.

**Results:** The majority of sites showed phylogenetic clustering at deep phylogenetic levels (SESmpd; 76.7%). SESmpd increased with precipitation seasonality, historical temperature instability and historical rainfall instability. Human footprint, soil clay content and sampling effort led to reduced SESmpd values. At shallow phylogenetic levels (SESmntd), relationships were weak with no clear patterns.

**Main Conclusions:** Communities under higher stress conditions showed higher phylodiversity. This suggests that different lineages evolved different strategies to cope with environmental fluctuations. The low phylogenetic diversity found could reflect higher recent speciation, generating many young species within lineages, especially in grasses. Our results offer insights into how ecological and evolutionary processes act to shape current patterns of phylogenetic diversity in grassland ecosystems in South America.

**Keywords:** Brazilian highland grasslands, climatic stability, community phylogenetics, human pressure, phylogenetic clustering, phylogenetic relatedness, Rio de la Plata grasslands.

## **Introduction**

An important issue addressed in ecology is how plant species are organized into communities across space. Environmental and biotic processes related to niche and species coexistence act as filters by selecting adapted species (Diamond, 1975; Keddy, 1992; MacArthur & Levins, 1964). On the other hand, neutral processes associated with stochasticity and demographic rates (e.g. birth and death, migration and local extinction; Hubbell, 2001) act coupled with niche processes assembling plant communities, varying in space and time (Vellend et al., 2014). Moreover, historical and evolutionary factors also influence community assembly (Gerhold et al., 2015, 2018). Although these knowledge sheds light on the initial conditions and historical processes of species speciation and dispersal (Hubbell, 2001; Ricklefs, 1987; Tilman, 2004), placing history and evolution into the explanation of current patterns of community assembly and biodiversity are not an easy task and has still been neglected in community ecology studies (Gerhold et al., 2015). Therefore, the field of community phylogenetics seeks to understand current and evolutionary factors processes generating and maintaining ecological communities (Cavender-Bares et al., 2009; Gerhold et al., 2015).

Plant species that co-occur in a community may be evolutionarily close or distant, resulting in phylogenetically poor or rich communities (Webb, 2000). Co-occurrence of species can be the result from recent assembly processes which are often interpreted as plant responses to environmental conditions and biotic interactions (Keddy, 1992). However, extant plant communities also reflect historical processes, such as effects of diversification or historical barriers (Leibold et al., 2010). A first step towards the disentangling of the factors that are behind these contrasting – or complementary – perspectives is the analysis of phylogenetic patterns across a metacommunity. The metacommunity is a set of local communities that are linked over a larger spatial extent through dispersal processes (Leibold et al., 2010) and that usually includes environmental gradients (Gerhold et al., 2015; Webb et al., 2002). By assessing the diversity of evolutionary lineages along these gradients (Leibold et al., 2010; Massante et al., 2019), a more complete picture of the past and present assembly processes, their evolutionary and ecological consequences could be provided. Additionally, phylogenetic information can be used to understand how macroevolutionary processes impact community

assembly, as well as how community assembly and species interactions influence evolution (Gerhold et al., 2015).

It is known that deep-past processes such as diversification rates can affect the phylogenetic diversity of communities (Gerhold et al., 2018). Recent and rapid radiation leads to low phylodiversity (Yan et al., 2013). In contrast, low extinction rates coupled with high speciation over time results in long-term accumulation of distantly related lineages and communities with high phylogenetic diversity (Qian, 2014). In line with this, recent studies suggested that habitat age is an important factor affecting phylodiversity (Gerhold et al., 2015; Lososová et al., 2015). Habitats that are relatively young on the geological time scale should host communities with clustered phylodiversity because only a few lineages from the existing species pool are preadapted to these habitats, and time was too short to allow for successful adaptation of a broader spectrum of distantly related lineages. Furthermore, impacts of historical climatic stability are known to influence plant diversity, specifically species distribution or functional diversity (Costa et al., 2018). For example, areas of relative stability are characterized by phylogeographic endemism and hold higher functional richness (Carnaval et al., 2014; Ordonez & Svenning, 2015); examples are refuge regions in the last glacial maximum (Ordonez & Svenning, 2015).

Current environmental conditions also shape plant community composition (Keddy, 1992) and phylogenetic patterns (Padullés Cubino et al., 2022). For instance, high soil fertility is pointed out as essential for supporting the colonization of species coming from distant lineages, possibly increasing phylogenetic overdispersion in local communities (Oliveira et al., 2014). Phylogenetic diversity may decline in water-limited regions, as higher water stress eliminates drought-intolerant species (Li et al., 2019). Anthropogenic changes further impact phylogenetic patterns: even moderate habitat loss impacts local plant communities in terms of species richness and beta-diversity, but also phylogenetic diversity (e.g., Staude et al., 2018). However, current knowledge of the processes that affect phylogenetic diversity of grassland communities are limited mainly to Europe (Lososová et al., 2015, 2021; Massante et al., 2019), in ecosystems under contrasting geological history, biogeographical and land use processes than found in tropical and subtropical regions.

Grasslands in South America, which include southern Brazil, northeastern Argentina and Uruguay (28°S–38°S, 50°W–61°W), provide an excellent ecosystem for testing how environmental factors and anthropic disturbances affect plant community phylogenetic diversity. The region is one of the largest temperate/subtropical grassland regions in the world and the largest in South America (Andrade et al., 2018; Azpiroz et al., 2012; Soriano, 1992), harboring high biotic diversity. Palaeoecological studies have demonstrated that grasslands were the dominant ecosystem in this region since the Pleistocene to mid-Holocene due to a drier and colder climate than today (Behling, 2002; Behling et al., 2007; Prates & Perez, 2021). Behling et al. (2004) show that on the northern portion of our study system, climate shifted from cold and dry to warmer and more seasonal from 10,000 to around 4,000 BP, and then became warmer, more humid and less seasonal, resulting in the replacement of dry steppe by humid grassland (Prieto, 2000). The region is characterized by the coexistence of C<sub>3</sub> and C<sub>4</sub> grasses, with changes in the proportion of C<sub>4</sub>:C<sub>3</sub> grasses along the latitudinal gradient (Epstein et al., 2002; Still et al., 2003).

Species richness in the region follows a latitudinal trend, with twice as many vascular plant species per unit area in the northern portion (Brazil and Uruguay) than in the southern portion in Argentina (Oyarzabal et al., 2020). Available studies on the drivers of taxonomic diversity in grasslands of South America usually are restricted to single sites or smaller regions (Burkart et al., 2011; Lezama et al., 2014; Menezes et al., 2016; Perelman et al., 2001). Only recently the floristic composition and drivers of taxonomic diversity over the *Río de la Plata* region are being explored (Andrade et al., 2018; Bergamin et al., 2022), but other facets of biodiversity, such as phylogenetic patterns, have not been explored so far. As nearly 50% of the original vegetation have been lost, and land use change mainly to crop production and forestry is still at high rates (20% has been lost in the last three decades) (Baeza et al. 2022), a better understanding of how human activities affect local biodiversity patterns is urgent.

Here, we investigate patterns of phylogenetic diversity in grassland communities along environmental and land-use gradients in the context of evolutionary and macroecological processes. Importantly, grasslands in the latitudinal range we work on

have not been considered, so far, in broad-scale studies on the relation of phylogenetic diversity and geographic variation (Massante et al., 2019). These questions have previously only been explored locally (Moraes et al., 2016), and the lack of data and understanding on plant evolution and biome assembly in large tracts of South America, including the *Pampa* grasslands have been pointed out (Hughes et al., 2013). Specifically, we aimed to understand the relationships of phylogenetic diversity and present climate, soil conditions, historical climate stability, and human impact. Considering present drivers mentioned above, we expect to find low phylodiversity in assemblages under conditions of environmental stress, such as soils with low fertility, under conditions with high rainfall seasonality with presence of drought periods, and in regions with higher human pressure. As historical climate stability enhances persistence and diversification over geological timescales, we expected that less stable areas should host lower phylodiversity (Jablonski et al., 2006; Svenning et al., 2015).

## Methods

### *Study area*

The study comprises the grassland region in southeastern South America (SESA grasslands *sensu* (Azpiroz et al., 2012), including grasslands located in northeastern Argentina, Uruguay, and southern Brazil. The SESA grasslands comprise two phytogeographically distinct regions: the *Río de la Plata* grasslands that extend over some 750,000 sq. km (28°S–38°S, 50°W–61°W; (Andrade et al., 2018; Soriano, 1992), and the south Brazilian highland grasslands in mosaic with *Araucaria* forest and altitudes from 400m a.s.l. up to above 1000m a.s.l., spreading northwards until 25°S (Andrade et al., 2019; Overbeck et al., 2007). Climate in this region shifts from humid subtropical without a dry season with warm (Cfa) or temperate summers (Cfb) according to the Köppen's classification (Alvares et al., 2013), with a wide range of mean annual temperature (13°C to 20°C) and annual precipitation (777mm to 2027mm; (Fick & Hijmans, 2017)).

Our dataset comprised 666 sites located in latitudinal range from 26°S to a maximum of 38°S of the equator, extending southern Brazil, part of Argentina and Uruguay (see Appendix S1: Figure S1). Brazilian sites are part of a sampling network of *Campos* from Brazil's National System of Research on Biodiversity (Sistema Nacional de



Pesquisa em Biodiversidade, SISBIOTA). Brazilian sites were sampled from 2011 to 2013 during spring and early summer in 167 sampling units, each one described by nine separate 1 m<sup>2</sup> subplots situated within a 5,000 m<sup>2</sup> area and gathered to obtain one sampling unit of 9 m<sup>2</sup>. For details on sampling design and vegetation survey, as well as results on taxonomic diversity and classification, see Andrade et al. (2019). Uruguayan sites are originally from Lezama et al. (2019) and Lezama et al. (2006), comprising 382 sampling units with a single plot of 25 m<sup>2</sup>, sampled between 2001 and 2009 during late spring and early summer. Sites from Argentina are described in Perelman et al. (2017) and Burkart et al. (2011) and comprised 117 sampling units with a single plot of 25 m<sup>2</sup> from inventories carried out during spring and early summer in a 15-yr period (1988–2003). Altogether, sites covered all different subregions of Southeastern South American grasslands, as described in Soriano (1992) and Azpiroz et al. (2012).

### ***Phylogenetic data***

We constructed a phylogeny for our 1,123 species based on the most up-to-date angiosperm mega-phylogeny (Smith & Brown, 2018), which comprises 79,881 taxa. Ferns and lycophytes are also commonly found in South American grassland ecosystems, however, due to their low frequency in the communities, we chose to remove them from the analysis to avoid inflated results due to a few taxa connected to deep phylogenetic nodes (Massante & Gerhold, 2020). Species names in our data set were standardized according to The Plant List using the R package “Taxonstand” (Cayuela et al., 2012).

We used the R function `phylo.maker` (package `V.PhyloMaker`; (Jin & Qian, 2019) to link the species names in our dataset with those in the mega-phylogeny, and the scenario 3 approach to add species to the phylogeny. Scenario 3 adds missing taxa (e.g., genera or species) to the phylogeny within the taxa with known branch lengths, in a similar way to the approach implemented in `Phylomatic` and `BLADJ`. The genus and family nodes information of the backbone phylogeny are included in the function `phylo.maker`. Our tree presented 1,123 tips and 747 internal nodes. The phylogenetic relationships of 632 species had polytomies, all at the genus level. To account for phylogenetic uncertainty stemming from the polytomies in the tree, we used the analytical strategy proposed by Rangel et al., (2015), which consists of simulating a given number of trees and account

for the effect of varying trees in phylogenetic metrics of interest. In each random tree, every phylogenetically uncertain taxon (PUT: in this study, the 632 species located on nodes with three or more branches) is inserted in a random position within its most derived consensus clade (MDCC: the clade in the phylogeny where there is no doubt that the PUT pertains). Thereby, for each polytomy, two branches are chosen at random and reassigned to the same node (within its MDCC), and then each remaining branch from the original polytomy is inserted sequentially, in random order, within the same node (Rangel et al., 2015). This process assures that all new trees are fully resolved. We generated 1,000 trees using this procedure, and subsequent statistical analyses were performed on the 1,000 trees, generating a mean value for each metric used, and an associated confidence interval. Random trees were generated using the 'Sunplin' software (Simulation with Uncertainty for Phylogenetic Investigations, available at <http://wsmartins.net/sunplin/>) and its integrated function in the R environment (R Core Team 2020).

To examine patterns of phylogenetic structure of grassland communities we computed mean pairwise distance (MPD), mean nearest taxon distance (MNTD; Webb et al., 2002) and phylogenetic diversity (PD; Faith, 1992) for each plot using 'picante' package in R version 3.6.3 (R Core Team 2020). MPD provides a method for measuring the mean branch length distance between all pairs of species occurring in a vegetation plot, whereas MNTD calculates the phylogenetic distance between each species occurring in a community and its nearest species in the phylogeny and quantifies the extent of terminal clustering, independent of deep-level clustering (Webb et al., 2002). MPD and MNTD are measures of phylogenetic diversity that capture different timeframes of ecological association and evolutionary adaptation, with MPD representing longer term ecological association and potentially a longer timeframe for adaptation (Moro et al., 2015). PD itself is defined as the sum of branch lengths of all species present in a community (Faith 1992) and provides an evolutionary overview of the relationships among species of an assemblage.

Considering these raw phylogenetic diversity measures are often correlated with taxonomic diversity (PD positively correlates with species richness, MNTD negatively

correlates with species richness, and MPD variance decreases with increasing species richness; Miller et al., 2017; Kembel et al., 2010; Webb, 2000), we furthermore computed their standard effect sizes (SES), which tells us whether our diversity indices are higher or lower than expected given the community species richness (Pavoine & Bonsall, 2011). Indices calculations were performed with 1,000 randomizations using the null model *phylogeny.pool*, in which the community data matrix is randomized by drawing species from the pool of species occurring in the phylogenetic distance matrix with equal probability. For each phylogenetic metric, that is, Faith's PD, MPD and MNTD, we calculated SES as follows (Gotelli & Rohde, 2002):

$$SES = \frac{\text{Observed value} - \text{Mean value (null model)}}{\text{Standard deviation (null model)}}$$

where the observed value corresponds to the mean metric calculated for the 1,000 resolved trees; the mean value (null model) represent the mean values of the expected metrics of the randomized assemblages (n = 1,000); and the standard deviation (null model) represent the standard deviations of the mean values for the randomized assemblages.

Although these indices reflect “community phylogenetic structure” or “phylogenetic relatedness” of coexisting species, we use hereafter the term “phylogenetic diversity” for simplicity. Standard effect sizes estimations were made using the functions *ses.pd*, *ses.mpd* and *ses.mntd* of the R package ‘picante’ (Kembel et al., 2010). We only interpreted SES values that are significantly greater than  $\pm 1.96$ . Since SESpd was significantly correlated with SESmntd (Pearson's  $r > 0.90$ ; Appendix S2: Figure S2), it was subsequently discarded from analysis.

### **Predictor variables**

We selected different predictor variables related to environmental conditions (climate and soils), historical climate stability and human impact that we hypothesized to have direct influence on phylogenetic diversity. We obtained five climatic variables from the Worldclim 2 database (Fick & Hijmans, 2017) that represent both annual patterns and extremes: annual mean temperature, temperature seasonality, minimum temperature of

coldest month, annual mean precipitation and precipitation seasonality. We selected three soil variables related to physical and chemical properties: clay content, cation exchange capacity and pH. These soil variables are proxies of nutrient and water-holding capacity, nutrient availability, and soil toxicity, respectively. Edaphic variables were extracted from SoilGrids (Hengl et al., 2017) with a 1 km<sup>2</sup> resolution and based on a soil depth of 15 cm. Spatial distribution of bioclimatic and soil variables are available as supplementary material (see Appendix S3: Figures S3.1 and S3.2). Climate stability was obtained over the last 21 kyr (Carnaval et al., 2014). We calculated the mean temperature and rainfall over this period at time slices of 1 kyr and then the standard deviation. Lower values of standard deviation indicate greater climatic stability. Hereafter we call this variable “historical temperature/rainfall instability”, which is more intuitive. Paleoclimate data used by Carnaval et al. (2014) is available at Dryad (<http://datadryad.org/resource/doi:10.5061/dryad.8kc1v>). Finally, in order to access the human impact, we obtained data on the distribution of terrestrial human pressure for 2009 from the global Human Footprint maps (Venter et al., 2016). These maps are comprised of a cumulative spatial index of eight key human pressures including built environments, population density, electric infrastructure, crop lands, pasture lands, roads, railways, and navigable waterways. These pressures are scaled based on their estimated environmental impact and summed in 1 km<sup>2</sup> grid cells, resulting in a combined global scale between zero and fifty where zero is little to no human pressure and fifty is extreme urban conglomerates. Furthermore, we considered the sampling design (nine separate plots adding up to 9 m<sup>2</sup> and one single plot of 25 m<sup>2</sup>) as a predictor variable in order to include the potential effect of the sampling unit area on phylogenetic diversity. All variables were at a spatial resolution of 30 seconds (~1 km<sup>2</sup>), except for climate stability that was at 2.5 minutes (~5 km<sup>2</sup>).

### ***Statistical Analysis***

Prior to analysis, we assessed collinearity between predictors using Variance Inflation Factors (VIF; (Graham, 2003), and those highly autocorrelated (correlation threshold of 7) were removed using the ‘vifstep’ R function of the ‘usdm’ package (Naimi et al., 2014). Minimum temperature of the coldest month, annual precipitation and soil pH

had collinearity problem with other variables and were removed from analysis. Retained predictors were standardized to mean zero and unit variance to allow direct comparisons of model coefficients (function 'decostand' from package 'vegan'; Oksanen et al., 2022). Full model with the remaining variables was submitted to a backward stepwise model selection by Akaike Information Criterion (AIC) to fit the best minimal model (function 'stepAIC' from package 'MASS'; Venables & Ripley, 2002).

To assess the role of environment and anthropogenic drivers shaping phylogenetic diversity in SESA grasslands, we performed generalized least-squares (GLS) regressions relating the phylodiversity indices with the set of environmental and anthropogenic variables. GLS allows the non-independence of geographically close observations (Dormann et al., 2007). We tested GLS models with and without distinct spatial autocorrelation structures (spherical, Gaussian and exponential spatial autocorrelation), of which we selected the one having the lowest AIC value (Yuan et al., 2016; Ali & Yan, 2017). The goodness of fit of the GLS model was assessed by pseudo- $R^2$  (Nakagawa et al., 2017). The GLS models were implemented using the *nlme* package (Pinheiro et al., 2022), while pseudo- $R^2$  was calculated with the package *piecewiseSEM* (Lefcheck, 2016) in R v.4.2.1 (R Core Team, 2022).

## Results

The data set comprised 1,123 angiosperm species, belonging to 462 genera and 82 families. Most frequent families are shown in Appendix S4: Figure S4. Overall, 76.7% of sites showed phylogenetic clustering (i.e., negative values below -1.96 are significantly different from the null model) at deep phylogenetic levels (SESmpd), and 49.2% at shallow phylogenetic levels (SESmntd). Neither for SESmpd nor for SESmntd, none of the grassland communities showed phylogenetic overdispersion. Values for SESmpd ranged from -7.88 to 0.61 (Figure 1a), while for SESmntd ranged from -4.6 to 0.52 (Figure 1b).

We found significant relations between the standardized phylogenetic diversity indices with both present and historic climate, as well as soil variables and human footprint (Figure 2; Appendix S5). The pseudo- $R^2$  for the full statistical model was 0.15 and 0.06 in SESmpd and SESmntd, respectively. In the generalized least-square models, SESmpd

increased with increasing precipitation seasonality, historical temperature instability and historical rainfall instability, while soil clay content, human footprint and sampling effort led to reduced SESmpd values (Figure 2a; Appendix S6: Figure S6.1). Sampling effort showed a negative relationship with SESmpd (Figure 2a and Appendix S5: Table S5.3), with lower values of phylogenetic diversity using 25 m<sup>2</sup> plots than the nine pooled 1 m<sup>2</sup> sampling units. Detailed information on estimated effects of the variables in the top-ranked generalized linear model on community phylogenetic diversity are given in Appendix S5: Table S5.3. At shallow phylogenetic levels (SESmntd), relations were weak with no clear patterns (Figure 2b; Appendix S6: Figure S6.2).

## **Discussion**

Recent studies have demonstrated the potential of considering both ecological and evolutionary processes to explain biodiversity patterns in ecological assemblages (Gerhold et al., 2018; Kerkhoff et al., 2014; Kubota et al., 2018). By analyzing the variation in SESmpd along environmental gradients, including historic processes, we were able to show that variations in rainfall regime (seasonality) and soil clay content, as well as historical temperature and rainfall instability are important drivers of the phylogenetic diversity of grassland communities in southeastern South America. To our knowledge, phylogenetic diversity patterns of grassland communities in tropical and subtropical grasslands have not yet been widely explored; our findings represent a first step to understand the contribution of environment and evolutionary history to the phylogenetic diversity of grassland ecosystems in South America. Additionally, we found patterns of phylogenetic diversity for grassland communities similar to those found for tree communities in the same region (Rezende et al., 2017). The greater lineage diversity found for tree communities are related to the Pacific Forest, which are isolated from other forests in South America by the Andes, while Pampean forests showed phylogenetic clustering, pattern attributed to diverse lineages that have a temperate Southern Hemisphere origin and a history of diversification outside of the tropics (Rezende et al., 2017). The stronger clustering found in the core area of our study, and consequently higher or random patterns alongside the limits of the ecoregion may be indicative of the influence of Andean and Austral-Antarctic lineages in the South and tropical clades in the

North (Rezende et al., 2017). Lower phylogenetic diversity in biological communities could occur due to higher recent speciation, generating many young species within lineages (Gerhold et al., 2018; Massante et al., 2019; Rezende et al., 2017). Habitat types such as grasslands are known for producing lineage pools of evolutionarily proximate species, mainly relatively recently radiated grass species (Gerhold et al., 2018; Strömberg, 2011).

### ***Influence of climate and soil features on phylodiversity across SESA grasslands***

Regarding the relationships between phylodiversity and environmental stress, our results were contrary to our predictions. Soil clay content was negatively related with PD, whereas precipitation seasonality was positively related to PD. Both of these variables are related to water availability in the environment. Thus, regions in SESA grasslands with longer periods of drought or soils with lower capacity to retain water and nutrients hosted higher PD. Natural or experimental droughts in grasslands has been shown to lead to losses in plant diversity for taxonomic and phylogenetic levels (Adler & Levine, 2007; Fine & Kembel, 2011; Li et al., 2019), and water availability has been described as the main driving factor of floristic heterogeneity in RPG grasslands (Bergamin et al., 2022; Burkart et al., 2011), with areas under balanced water supply presenting higher species richness (Bergamin et al., 2022). The occurrence of phylogenetic clustering in vegetation types experiencing arid conditions underlines the importance of drought as a driver of phylogenetic structure (Lososová et al., 2021). Here, areas under higher water stress showed higher PD, i.e, areas with more balanced water supply have species more phylogenetically related. Physiological drought tolerance has already been described for grass species of all six continents, suggesting that most native grasslands worldwide are likely to contain a high number of species with drought tolerance (Craine et al., 2013). Physiological drought tolerance in grasses has probably evolved numerous times and is widely distributed phylogenetically and geographically (Craine et al., 2013). Thus, we cannot exclude the possibility of multiple lineages developing strategies to cope with water stress conditions, leading to the higher PD in our study. Considering grasslands as a whole, the diversity of life forms in the most rich-species families includes shrubs, subshrubs, erect and prostrate herbs, in the case of Asteraceae (with different abilities to resprout after stress or disturbance), and rhizomatous, stoloniferous and tussock grasses

(Poaceae). This reflects the many ways that species have evolved in response to their environment, including in response to stress conditions. Altogether, edaphic conditions could be reflecting a selection of species from dry-adapted lineages along water availability gradients and exclusion of those that lack mechanisms to cope not only with drought but also with low soil nutrients availability (Fine & Kembel, 2011).

Both temperature and rainfall gradients are widely known as global drivers of biodiversity (Currie et al., 2004; Francis & Currie, 2003; Hawkins et al., 2003). In our study, temperature seasonality influenced PD. Previous regional studies in the southern portion of the SESA grasslands indicated that other aspects of climatic variation can also influence functional patterns: lower minimum temperature of the coldest month have been found to be related with higher cover of C<sub>3</sub> grasses (Burkart et al., 2011; Perelman et al., 2017). Our dataset included all angiosperm species, not only grasses. Yet, there are evidences of floristic turnover along part of this climatic gradient (Andrade et al., 2019). Cold-adapted lineages, as C<sub>3</sub> grasses, have specialized and radiated into cold environments (Edwards & Smith, 2010). Thus, in areas with higher temperature seasonality, conditions are more propitious to harbor a wide spectrum of lineages, resulting in higher phylodiversity. Therefore, communities with higher PD could be offering more suitable environmental conditions to species with different tolerances, creating a niche overlap of species adapted to warm and cold.

### ***Historical temperature stability***

To our knowledge, studies assessing historical biome or climate stability are focused on forest ecosystems (Colville et al., 2020; Costa et al., 2018; Massante & Gerhold, 2020), and our study is pioneer to consider historical factors as possible drivers assembling present grassland communities. We expected areas with higher climate stability to harbor higher levels of phylodiversity, as they might have acted as historical refugia during restrictive climate period, at least for forest ecosystems (Jablonski et al., 2006; Svenning et al., 2015). Instead, we found PD increasing with both temperature and rainfall instability. In grassland ecosystems, higher historical instability can induce fluctuations between savanna-like physiognomy (i.e., with presence of shrubs and trees in the grassland matrix) and open grasslands. These physiognomies can be considered



as alternative stable states, where diversity variables “fluctuate” around the ecosystem’s equilibrium point (Beisner et al., 2003). The northern portion of our study region consists a region of grassland-forest mosaics (Rambo, 1956), with processes of forest expansion over grassland due to the climatic changes over the last millennia (Behling & Pillar, 2007). While, for our region, the possibility of historical fluctuations to forests thus cannot be excluded, this forest expansion process is considered to be a recent process. These shifts of alternative stable states suggest that thermal instability does not pose a significant adaptive barrier to grassland diversity.

Phylogenetically clustered communities are described in the literature as typical of relatively young habitats, as European grasslands from late Tertiary (Lososová et al., 2015) and other open vegetation types (Lososová et al., 2021). Palaeoecological studies demonstrate that extensive areas of open *Campos* vegetation dominated the landscape in southern Brazil at least from the Late Pleistocene until the Middle–Late Holocene (Behling et al., 2004, 2005; Prieto, 2000), but there is no certainty on the origins of lineages and habitat age. For the Brazilian *Cerrado*, a floristically diverse tropical savanna to which SESA grasslands have some similarities, such as dominance by C<sub>4</sub> grasses with adaptations to fire, but also differences, such as lack of savanna trees in SESA grasslands, the origin of plant lineages has been dated to the late Miocene to Pliocene, i.e., the late Tertiary (Simon et al., 2009). This broadly coincides with the period of expansion of the C<sub>4</sub> grass-dominated savanna and grassland biomes around the world (Beerling & Osborne, 2006; Simon et al., 2009). Our results on the effects of temperature instability are in agreement with our findings related to stressful conditions: areas with higher thermal instability could be offering environmental conditions suitable to species with different tolerances to stress, thus hosting higher PD. Higher environmental stability can lead to narrower niches related to tolerance to abiotic conditions and biotic interactions, which increases speciation rates, resulting in present local communities with a clustering pattern (Fine, 2015; Gerhold et al., 2018).

### ***Human Footprint***

We confirmed that human footprint is an important variable associated with phylodiversity of grassland communities. Areas under high human pressure showed lower

PD. Previous studies concerning the effects of habitat fragmentation on species diversity usually show a homogenization effect on taxonomic diversity, but affecting phylogenetic diversity (Carlson et al., 2014; Dainese et al., 2015). Our findings are consistent with those reported by Staude et al. (2018) for this region: PD was found to decline with landscape fragmentation at both basal and terminal levels. Staude et al. (2018) argued that local extinctions occur nonrandomly in landscapes with less habitat amount, and the erosion of particular evolutionary lineages leads to phylogenetic clustering. However, here we assessed human impact over a major gradient and our human footprint index encompasses different aspects of land use. SESA grasslands are under cattle grazing since the seventeenth century, and today cattle breeding is one of the most important economic activities in the region (Pillar et al., 2009). The effects of grazing over taxonomic and functional patterns of vegetation are well described for our study system (Ferreira et al., 2020; Streit et al., 2022), where traditional management showed lower diversity compared to deferred grazing, i.e., grazing with only periodic access of cattle to simulate rotational grazing. Grazing has been described as a driver of phylodiversity in Eurasian grasslands (Dainese et al., 2015; Wang et al., 2019), with higher pressure leading to a loss in phylodiversity. Therefore, human activities in SESA grasslands could be acting as a filter, selecting disturbed-tolerant species, which could lead also to functional convergence.

### ***Sampling effort***

We recognize that the differences in sampling methods in our dataset may bring limitations to our approach. We observed that phylodiversity was lower at Argentine and Uruguayan sites and higher at Brazilian sites: pooled data from smaller sampling units (nine 1 m<sup>2</sup> plots distributed over 5,000 m<sup>2</sup>) led to higher phylodiversity than single 25 m<sup>2</sup> plot. Similar pattern arose regarding species richness in the region (Bergamin et al., 2022), yet this finding is directly related to the occurrence of more than twice as many species per unit area in the Brazilian part of the RPG than Argentina and Uruguay (Andrade et al., 2018). Regardless considering the different sampling approaches – one 25 m<sup>2</sup> plot vs nine 1 m<sup>2</sup> plots situated within an area of 5,000 m<sup>2</sup> – in our statistical models, it is not possible to assert the influence of these effects.

## **Conclusions**

Our study is one of the first to investigate phylogenetic patterns in subtropical/temperate grasslands. Our results emphasize that both environmental and evolutionary processes are essential to explain patterns of phylogenetic diversity of grassland ecosystems in South America. The positive effect of historical temperature instability showed that communities experiencing unstable conditions host more diverse lineages, likely related to different strategies to cope with temperature fluctuations and ultimately enabling co-occurrence of both cold and warm-adapted species, while areas with higher instability the few lineages adapted were able to speciate. This is in accordance with the results regarding the effect of environmental stress: higher phylodiversity was found for regions with lower clay content in soil and with larger precipitation seasonality, i.e., larger risk of periodic droughts. Our results on the human footprint confirmed that changes in land use lead to a loss of evolutionary history. Further studies should combine trait-based and phylogenetic approach to develop an integrated understanding of plant community dynamics. Finally, our results could be explored in more detail in the context of conservation and restoration strategies of ecological communities.

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**Author Contributions**

H.S., R.S.B., S.C.M. and G.E.O. conceptualized the study; B.O.A. assembled the database; F.L. is curator of data from Uruguay. H.S. analyzed the data; H.S. led the writing of the manuscript. All authors read and approved the final work. This work is part of Helena Streit's PhD thesis in Ecology at Universidade Federal do Rio Grande do Sul.

**Data Availability Statement**

Data used for this study are available from the authors on request.

## Figures

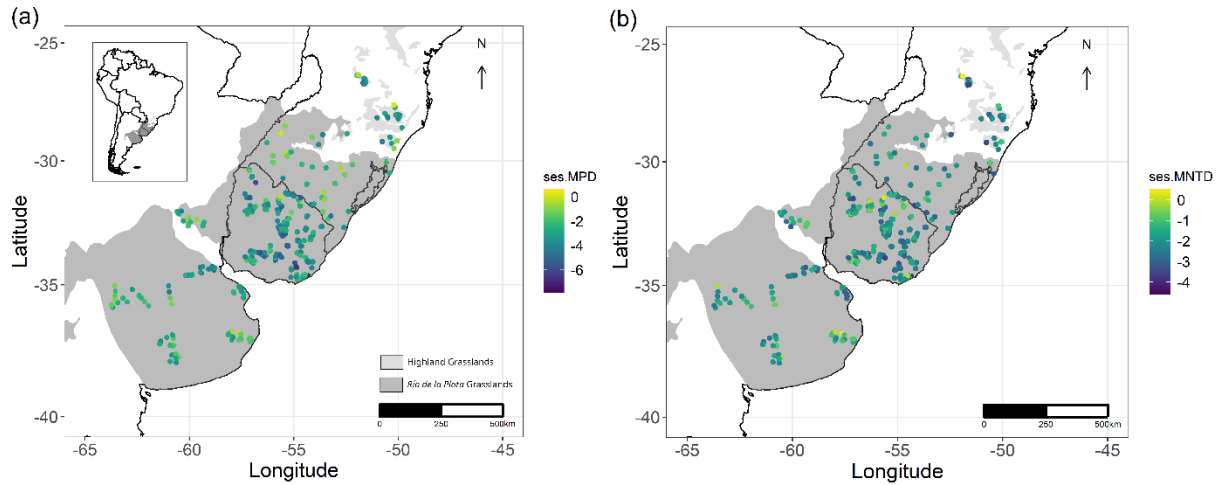


Figure 1: Variation of phylogenetic diversity across sampling sites in Southeastern South America grasslands. (a) Variation of the standardized effect size of the mean phylogenetic distance (SES.mpd) and (b) mean nearest taxon distance (SES.mntd). Communities with mean phylogenetic distance below -1.96 are clustered.

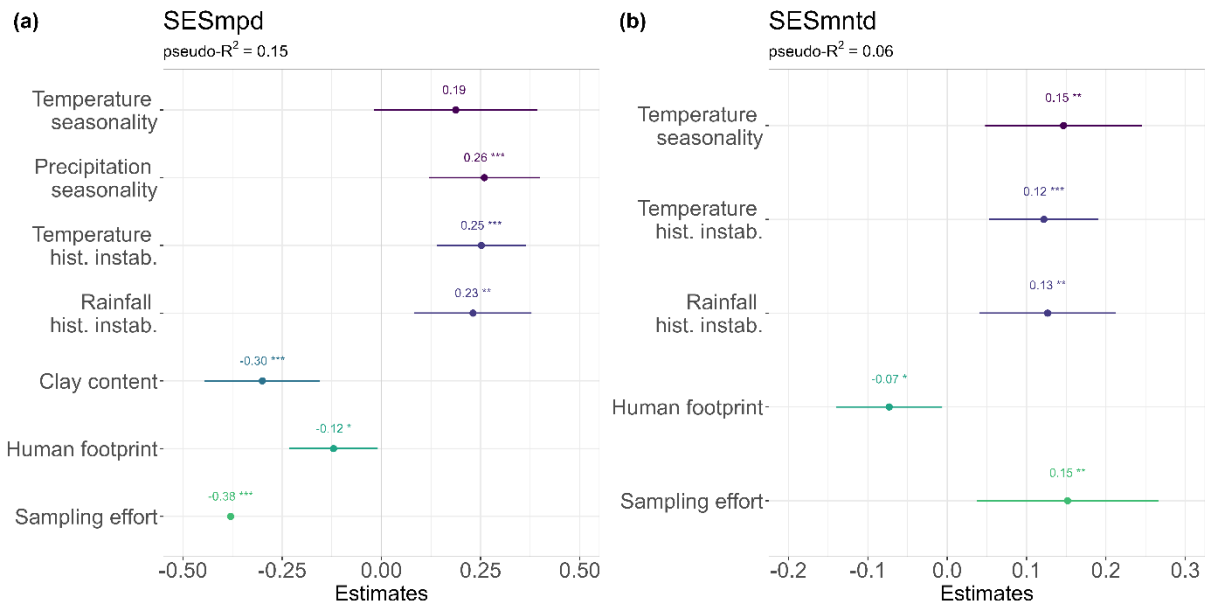


Figure 2: Standardized regression coefficients of generalized least-squares regression model and associated 95% confidence intervals (CI) for phylogenetic diversity of grassland species communities in southeastern South America grasslands. (a) The standardized effect size of the mean pairwise distance (SES.mpd) and (b) the standardized effect size of the mean nearest taxon distance (SES.mntd). Colors represent different sets of predictor variables. CIs that do not cross the zero baseline indicate a statistically significant effects (\*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.005$ ; \*,  $p < 0.05$ ) on changes in phylogenetic diversity.

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<https://doi.org/10.1007/s00442-016-3737-8>

## Supporting Information

Appendix S1. Distribution of study sites in the Southeastern South America Grasslands.

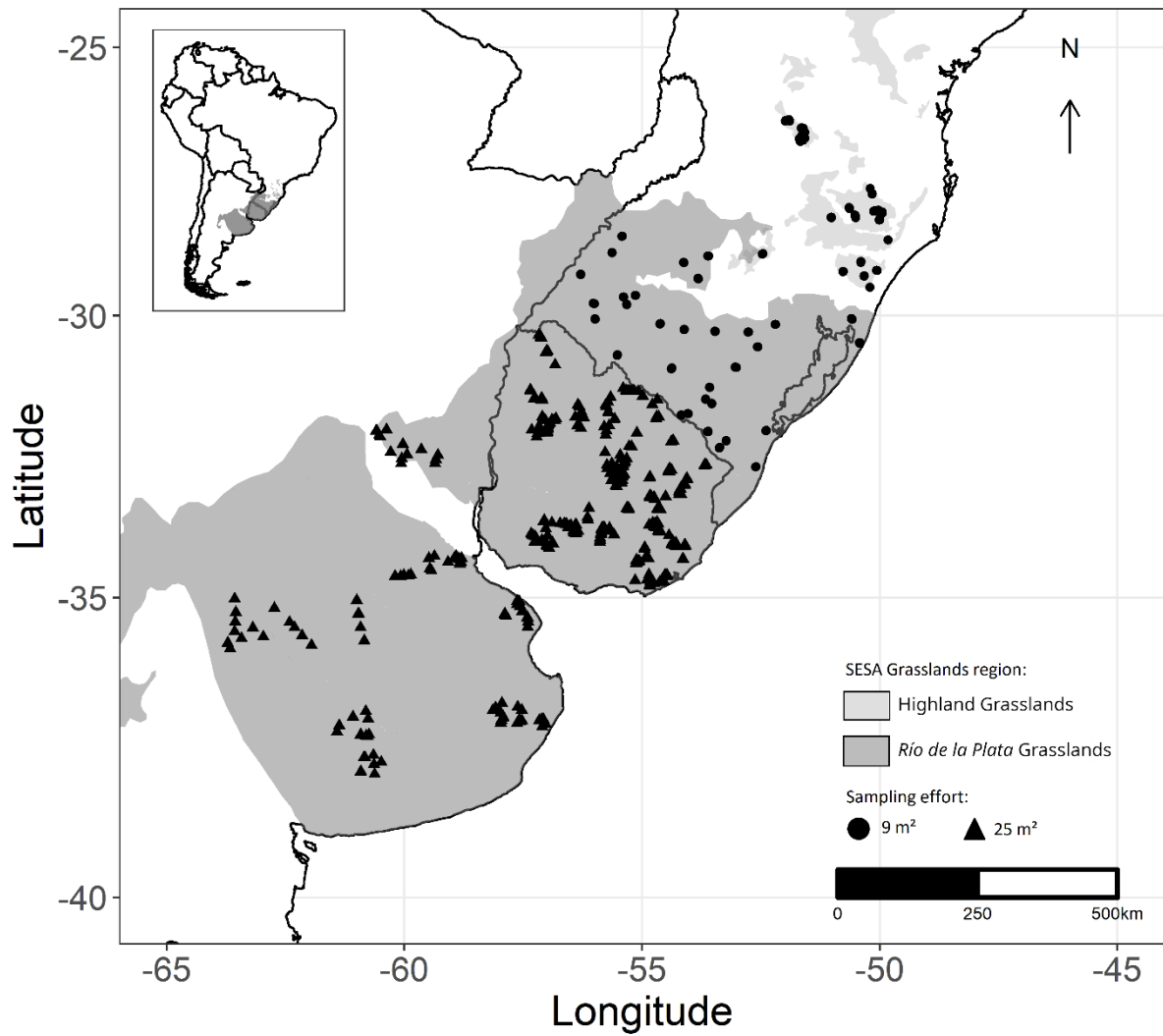


Figure S1. Location of the 666 sampling sites in the Southeastern South America Grasslands, with corresponding sampling unit area.

Appendix S2. Pairwise Pearson's correlation between phylogenetic diversity indexes.

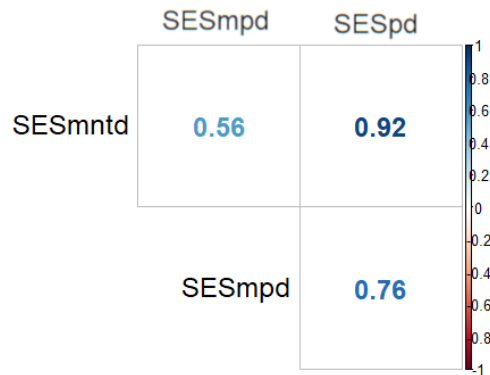


Figure S2: Pairwise Pearson's correlation between standardized effect sizes (SES) of the phylogenetic diversity indexes: mean pairwise distance (MPD); mean nearest taxon distance (MNTD) and Faith's phylogenetic distance (PD).

Appendix S3. Spatial distribution of predictor variables selected in this study.

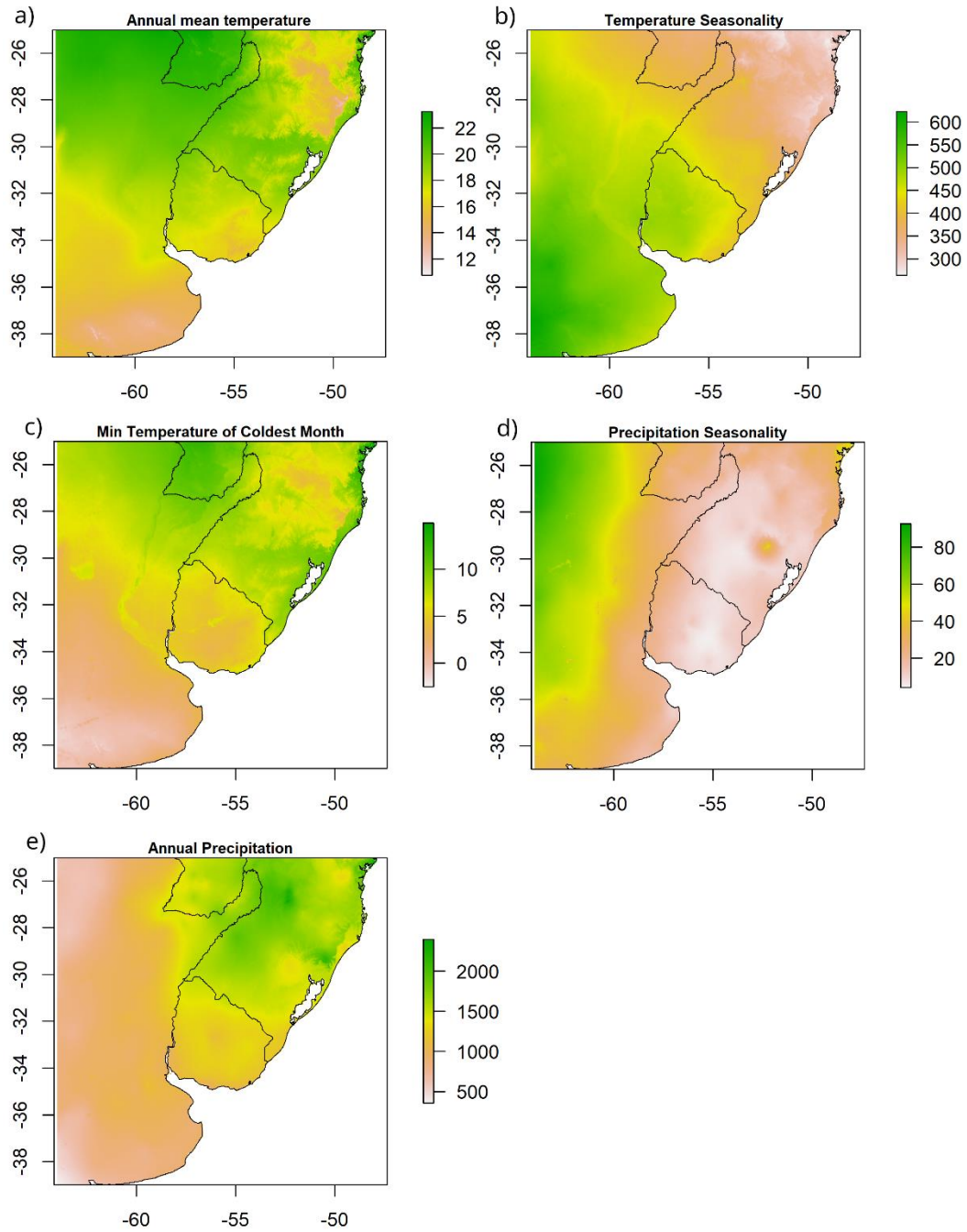


Figure S3.1. Spatial distribution of bioclimatic variables selected as predictor variables in this study. These spatial products were derived from bioclimatic variables available from WorldClim 2 database (Fick & Hijmans, 2017).



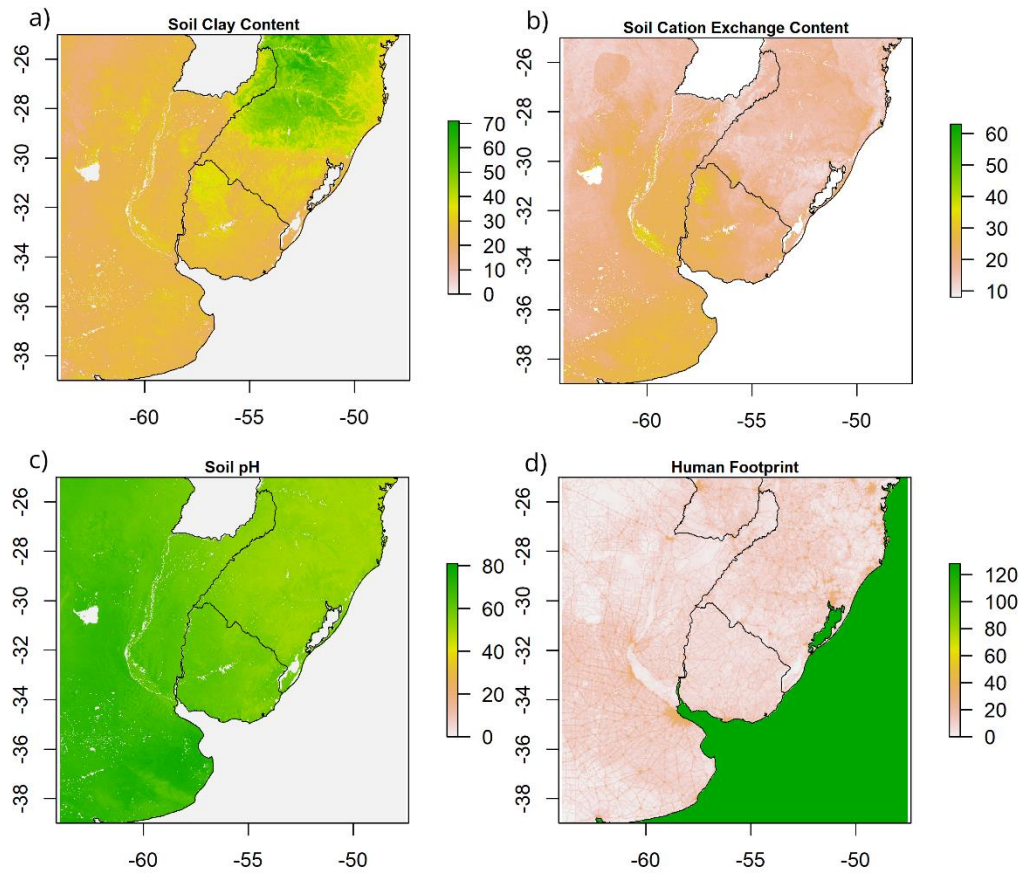


Figure S3.2: Spatial distribution of soil and anthropic variables selected as predictor variables in this study. A, B and C correspond to spatial products derived from soil data available from SoilGrids database (Hengl et al., 2017). D corresponds to data on the distribution of terrestrial human pressure for 2009 from the global Human Footprint maps (Venter et al., 2016).

Appendix S4. Number of species per plant family.

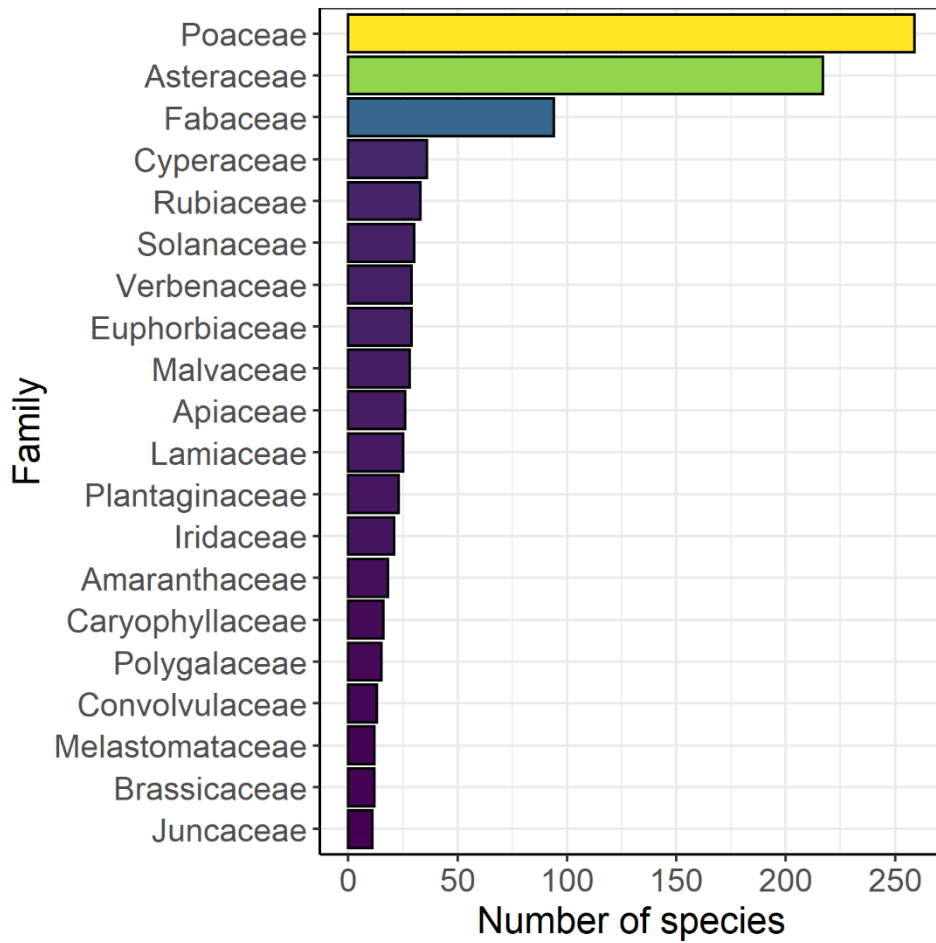


Figure S4. Number of species included in the data set for each plant family. Only the families with more than 10 species are shown.

Appendix S5: Model selection and statistical details for generalized least square evaluating the drivers of phylodiversity.

Table S5.1. Model selection of the GLS models without and with distinct spatial autocorrelation structure for standardized effect size of mean pairwise distance (SES.mpd). The selected model was the Gaussian spatial autocorrelation structure (in bold).

GLS model	df	AIC
Without spatial autocorrelation	9	2317.085
Spherical spatial autocorrelation structure	10	2315.750
<b>Gaussian spatial autocorrelation structure</b>	<b>10</b>	<b>2315.578</b>
Exponential spatial autocorrelation structure	10	2316.233

Table S5.2. Model selection of the GLS models without and with distinct spatial autocorrelation structure for standardized effect size of mean nearest taxon distance (SES.mntd). The selected model was without spatial autocorrelation structure (in bold).

GLS model	df	AIC
<b>Without spatial autocorrelation</b>	<b>7</b>	<b>1681.275</b>
Spherical spatial autocorrelation structure	8	1682.879
Gaussian spatial autocorrelation structure	8	1682.879
Exponential spatial autocorrelation structure	8	1683.275

Table S5.3: Estimated effects of the variables in the top-ranked generalized least-square model on community phylogenetic diversity (standardized effect size of the mean pairwise distance – SES.mpd and standardized effect size of the mean nearest taxon distance – SES.mntd) in Southeastern South American grassland communities. Pseudo-R<sup>2</sup> of the models were 0.15 and 0.06, respectively. Labels: PS: precipitation seasonality; TS: temperature seasonality; CC: soil clay content; HTI: historical temperature instability, HRI: historical rainfall instability; HF: human footprint index; SE: standard error.

Predictor	SES.mpd		SES.mntd	
	Estimate (±SE)	P-value	Estimate (±SE)	P-value
<b>Environmental</b>				
PS	<b>0.259 (0.072)</b>	<b>&lt;0.001</b>	-	-
TS	0.186 (0.105)	0.074	<b>0.146 (0.050)</b>	<b>0.003</b>
CC	<b>-0.299 (0.074)</b>	<b>&lt;0.001</b>	-	-
<b>Historical</b>				
HTI	<b>0.252 (0.057)</b>	<b>&lt;0.001</b>	<b>0.122 (0.035)</b>	<b>&lt;0.001</b>
HRI	<b>0.231 (0.075)</b>	<b>0.002</b>	<b>0.127 (0.044)</b>	<b>0.003</b>
<b>Anthropogenic</b>				
HF	<b>-0.120 (0.057)</b>	<b>0.034</b>	-0.073 (0.034)	0.032
Sampling effort	<b>-0.379 (0.095)</b>	<b>&lt;0.001</b>	<b>0.152 (0.058)</b>	<b>0.009</b>

Appendix S6. Visualization of the fit of generalized least-square regression models.

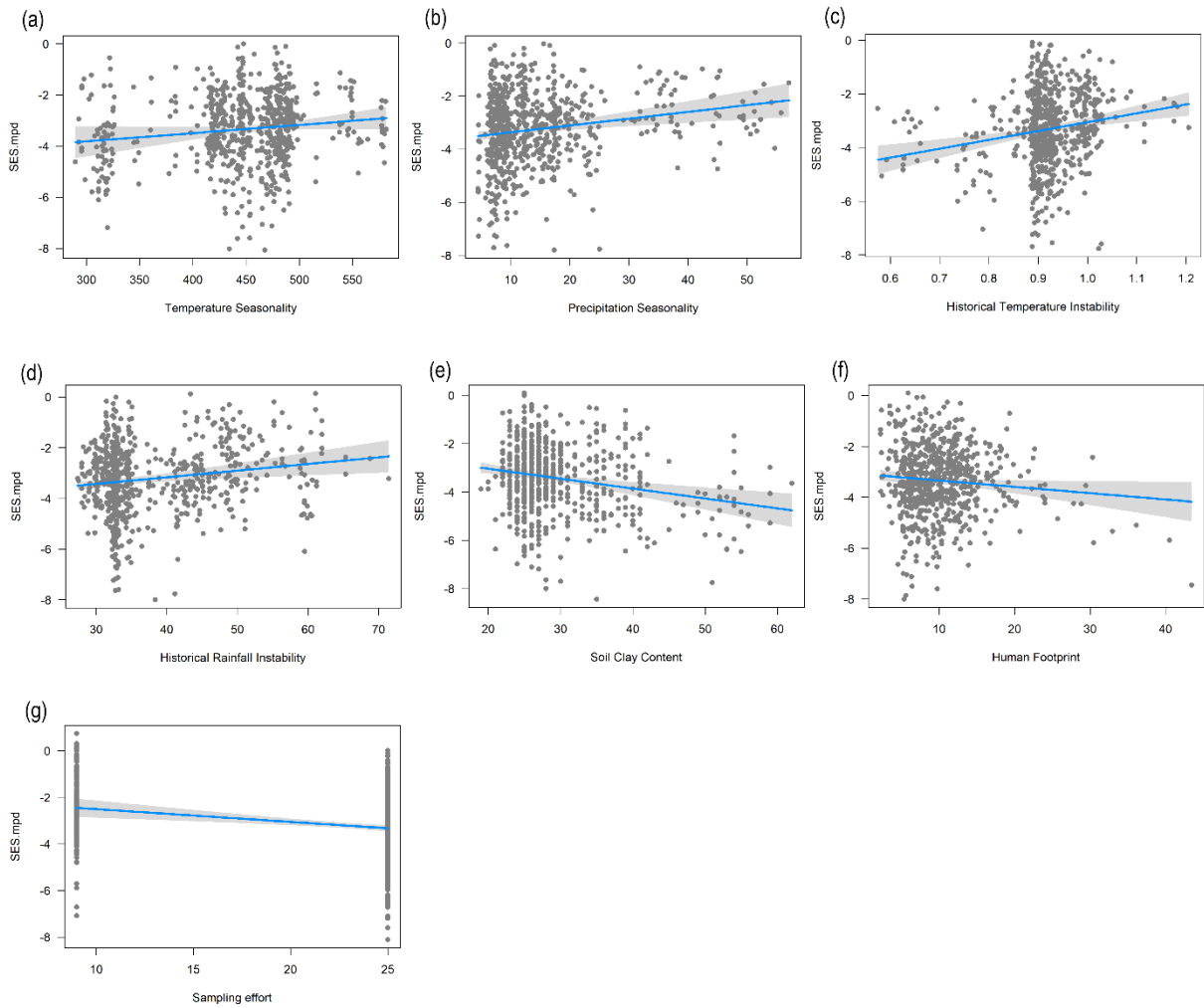


Figure S6.1: Variation of SES.mpd according to (a) temperature seasonality, (b) precipitation seasonality, (c) historical temperature instability, (d) historical rainfall instability, (e) soil clay content, (f) human footprint and (g) sampling effort in South American grasslands. Clustered plots are predominant within communities.

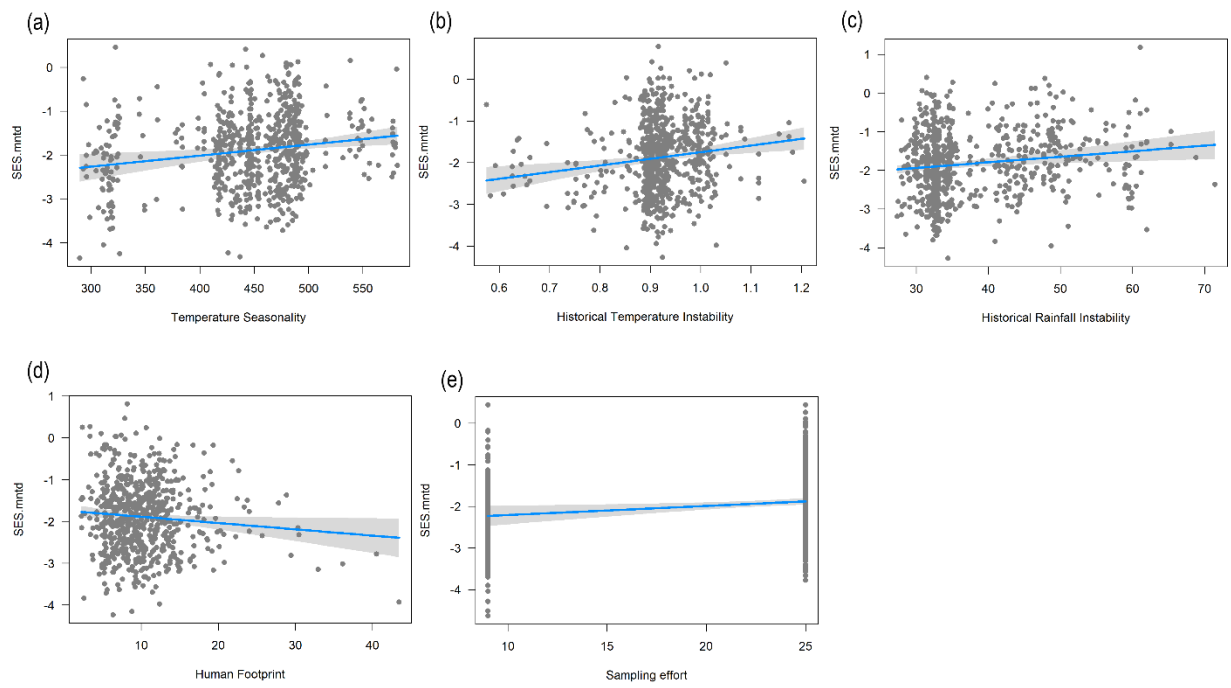


Figure S6.2: Variation of SES.mntd according to (a) temperature seasonality, (b) historical temperature instability, (c) historical rainfall instability, (d) human footprint and (e) sampling effort in South American grasslands. Clustered plots are predominant within communities.

## CAPÍTULO 2. DETERMINANTS OF BIOGEOGRAPHICAL DISTRIBUTION OF GRASSES IN GRASSLANDS OF SOUTH AMERICA

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## Abstract

Current distribution of C<sub>3</sub> and C<sub>4</sub> grasses is often explained by contrasting environmental conditions. Regions where C<sub>3</sub> and C<sub>4</sub> grasses coexist, as the Southeastern South America grasslands (SESA grasslands), provides an excellent opportunity to investigate the evolutionary imprints of grasses through clade distribution patterns. Here, we aimed to understand how ecological and evolutionary processes affect the phylogenetic diversity of grass communities along 666 sites located in a latitudinal gradient ranging from 26°S to 38°S in SESA grasslands (Argentina, Brazil and Uruguay).

We applied generalized linear models (GLM) to understand the role of the different environmental and historical drivers that shape the proportion of C<sub>3</sub> grasses distribution in SESA grasslands. The effect of latitude on phylogenetic beta-diversity patterns among the vegetation surveys was evaluated through principal coordinates of phylogenetic structure.

Contribution of C<sub>3</sub> species increased southwards ( $R^2 = 0.40$ ,  $P < 0.001$ ). C<sub>3</sub> species are more likely to occur in colder areas with higher historical temperature stability, reflecting lineages that have specialized and radiated in cold environments (GLM results:  $R^2 = 0.37$ ,  $P < 0.01$ ). Climatically stable areas are the coldest, while unstable areas include warmer habitats, which enabled colonization by C<sub>4</sub> species. Regarding soil conditions, C<sub>3</sub> grasses are more likely to occur in more fertile soils and with low capacity to retain water (GLM results: pseudo- $R^2 = 0.37$ ,  $P < 0.01$ ). We found that phylogeny has an important role as a structuring agent of grass communities across our study region, indicating turnover of grass lineages along the latitudinal gradient. Grass species found at the northern portion of the gradient belong mostly to the clade which contain both C<sub>4</sub> and C<sub>3</sub> species. At the southern part of the gradient, communities are dominated by grasses belonging to a C<sub>3</sub>-exclusive clade.

The distribution of grass clades across the SESA grasslands is indicative of the environmental gradients found in this region between temperate and tropical zones, describing a climate space where disturbance driven feedbacks play a major role in maintaining open vegetation. Our results contribute to the understanding of ecological and evolutionary drivers of grass distribution in the region that up to now has been poorly described.



**Keywords:** Brazilian highland grasslands, phylogenetic beta-diversity, climatic stability, functional ecology, Poaceae, Río de la Plata grasslands.

## Introduction

Poaceae is the fifth most diverse family among the angiosperms, with approximately 780 genera and 12,000 species and a cosmopolitan distribution (Christenhusz & Byng 2016). Grasses started to spread from the start of the Miocene, 10–20 million years ago (Strömberg 2011), and today, ecosystems dominated by grasses cover about 31%–43% of the global land surface (Gibson 2009). South America comprises one of the largest temperate grassland regions in the world, covering southern Brazil, northeastern Argentina and Uruguay (Soriano 1992; Azpiroz et al. 2012; Andrade et al. 2018). Palaeoecological evidences from this region indicates that grasslands were not widespread during most of the Cenozoic (Barreda & Palazzesi 2007), but became the dominant ecosystem since the Pleistocene to mid-Holocene (about 18 Ma) due to a drier and colder climate than today (Behling 2002; Behling et al. 2007; Strömberg et al. 2013). Historical prevalence of open ecosystems is recognized in northern Argentina and Brazil, where pollen assemblages point to the presence of Late Miocene grassy savannas (Strömberg 2011; Arruda et al. 2018).

The evolutionary history of grasses is strongly connected to the emergence of C<sub>4</sub> photosynthetic pathway. The reduction in atmospheric CO<sub>2</sub> about 30 Ma decreased the efficiency and rate of carbon uptake in many terrestrial plants, especially under high temperatures and water deficits (Sage 2004; Edwards et al. 2010; Edwards & Smith 2010; Palazzesi et al. 2022). This limitation required anatomical modifications to improve photosynthetic performance in those new conditions, promoting the convergent evolution of C<sub>4</sub> photosynthesis in more than 45 independent flowering plant lineages, 24 of them in grasses (Sage 2004; GPWGII 2012). Despite the convergent evolution, origins of C<sub>4</sub> pathway in the phylogeny of Poaceae are not random (Sage 2016). Molecular studies have identified two major clades: one comprising exclusively C<sub>3</sub> grasses (subfamilies Bambusoideae, Oryzoideae and Pooideae; hereafter BOP), and the other containing both C<sub>4</sub> and C<sub>3</sub> grasses (subfamilies Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, and Danthonioideae; hereafter PACMAD; Bouchenak-Khelladi et al. 2010; GPWGII 2012). Therefore, BOP and PACMAD species may exhibit ecological differences relevant to community structure and ecosystem functions (Pau & Still 2014; Cruz et al. 2019), once photosynthetic traits are closely associated with both

growth and survival. This led to worldwide expansion of C<sub>4</sub> grasses in grasslands through the displacement of vegetation dominated by C<sub>3</sub> species during the Late Miocene and Pliocene (3 to 8 Ma) (Edwards et al. 2010; Strömberg 2011).

In grasslands in South America, the early dispersals of Pooideae lineages appear in the late Eocene and early Oligocene (~33.9 Ma, Schubert et al. 2019). Starting at 39.8 Myr ago, members of the open-habitat PACMAD clade Danthonioideae become evident in grass communities (Stromberg et al. 2013), and in early Miocene, morphotypes typical of the Pooideae and Panicoideae clades dominate grass assemblages (Stromberg et al. 2013). Currently, Pooideae is the richest subfamily in southeastern South America, with greater prevalence in the Andean region (Biganzoli & Zuloaga 2015) and Panicoideae the second one, dominating in subtropical and warm temperate areas (Biganzoli & Zuloaga 2015).

Current distribution of C<sub>3</sub> and C<sub>4</sub> grasses is often explained by contrasting environmental conditions, with C<sub>4</sub> grasses dominating tropical and subtropical grasslands and savannas, and C<sub>3</sub> grasses dominating the world's cooler temperate grassland regions (Gibson 2009; Edwards et al. 2010). Regions characterized by the coexistence of C<sub>3</sub> and C<sub>4</sub> grasses, as the Southeastern South America grasslands (hereafter SESA grasslands; Epstein et al. 2002; Still et al. 2003), present unique vegetation dynamics. For example, C<sub>3</sub> grasses may gain a competitive advantage if they can acquire resources early in the growing season before C<sub>4</sub> plants become active, which represents a temporal niche separation (Still et al. 2003; Fargione & Tilman 2005). From the perspective of community assembly ecology, the mechanisms promoting C<sub>3</sub>/C<sub>4</sub> coexistence can be explained by resource partitioning by species with different resources requirements in time or space (Fargione & Tilman 2005). Different species and lineages are likely to have independent responses to temperature, precipitation and other environmental factors that are expected to contribute to the variation in grasses coexistence and distributions (Paruelo & Lauenroth 1996; Edwards & Still 2008; Christin & Osborne 2014).

Although the drivers and patterns of C<sub>3</sub> and C<sub>4</sub> grasses distribution have been broadly explored in distinct geographic distributions, previous studies were either limited in spatial extent and focused largely on climatic and physiological explanations (Paruelo

& Lauenroth 1996; Perelman et al. 2001; Griffith et al. 2015; Perelman et al. 2017) or, in a global perspective, limited to grassy mountain ecosystems (de Deus Vidal et al. 2021). Here, we propose to investigate the patterns of C<sub>3</sub> and C<sub>4</sub> grasses functional groups distribution in grassland communities in the context of evolutionary and macroecological processes. We aimed to understand evolutionary imprints of grasses through clades distribution patterns, as well as distribution of grass functional groups (C<sub>3</sub> and C<sub>4</sub>) along 666 sites located in latitudinal gradient ranging from 26°S to 38°S in SESA grasslands (Argentina, Brazil and Uruguay). We explored latitudinal pattern of grasses functional types and investigated which environmental and historical variables are driving proportions of C<sub>4</sub> and C<sub>3</sub> grasses and the contribution of each subfamily of Poaceae along gradients. We expected to find a shift of co-occurring species, both regarding functional and phylogenetic patterns, mediated by historical drivers, specifically: (1) C<sub>3</sub> grasses should become progressively more abundant in terms of the proportion of species as climatic stability increases along the gradient, while unstable areas include hotter habitats, which enabled colonization by C<sub>4</sub> species; and (2) the distribution of grass subfamilies should be correlated with latitude.

## **Methods**

### ***Community data set***

The study comprises the grassland region in southeastern South America (SESA grasslands *sensu* Azpiroz et al. 2012), including grasslands located in northeastern Argentina, Uruguay, and southern Brazil. The SESA grasslands comprise two phytogeographically distinct regions: the Rio de la Plata grasslands that extend over some 750,000 sq. km (28°S–38°S, 50°W–61°W; Soriano 1992; Andrade et al. 2018), and the south Brazilian highland grasslands in mosaic with Araucaria Forest and altitudes from 400m a.s.l. to above 1,000m a.s.l., spreading northwards until 25°S (Overbeck et al. 2007; Andrade et al. 2019). Climate in this region shifts from humid subtropical without a dry season with hot (Cfa) to temperate summers (Cfb) in the north of the region, according to the Köppen's classification (Alvares et al. 2013), with a wide range of mean annual temperature (13°C to 20°C) and annual precipitation (777mm to 2,027mm; Fick & Hijmans (2017)).

Our dataset comprised 666 sites located in latitudinal range from 26°S to 38°S, extending across southern Brazil, part of Argentina and Uruguay (see Fig. 1). Brazilian sites are part of a sampling network of *Campos* from Brazil's National System of Research on Biodiversity (Sistema Nacional de Pesquisa em Biodiversidade, SISBIOTA). Brazilian sites were sampled from 2011 to 2013 during spring and early summer in 167 plots. In each plot, vegetation was sampled in nine systematically distributed quadrats of 1 m<sup>2</sup>. In each quadrat, all plant species were recorded and identified to the lowest taxonomic hierarchy possible. Species cover was estimated with a modification of Londo's (1976) decimal scale. For details on sampling design and vegetation survey, as well as results on taxonomic diversity, see Andrade et al. (2019). Uruguayan sites are originally from Lezama et al. (2019) and Lezama et al. (2006), comprising 382 plots sampled between 2001 and 2009 during late spring and early summer. In each plot, one 5 m × 5 m quadrat was sampled, and all vascular plant species were recorded and had their coverage visually estimated following the Braun-Blanquet abundance scale (Braun-Blanquet 1950). Sites from Argentina are described in Perelman et al. (2017) and Burkart et al. (2011) and comprised 117 plots from inventories carried out during spring and early summer in a 15-yr period (1988–2003). In each plot, all vascular plants present in the site were recorded and had their cover estimated within an area of 25 m<sup>2</sup> (Mueller-Dombois & Ellenberg 1974). Altogether, sites covered all different subregions of SESA grasslands, as described in Soriano (1992) and Azpiroz et al. (2012).

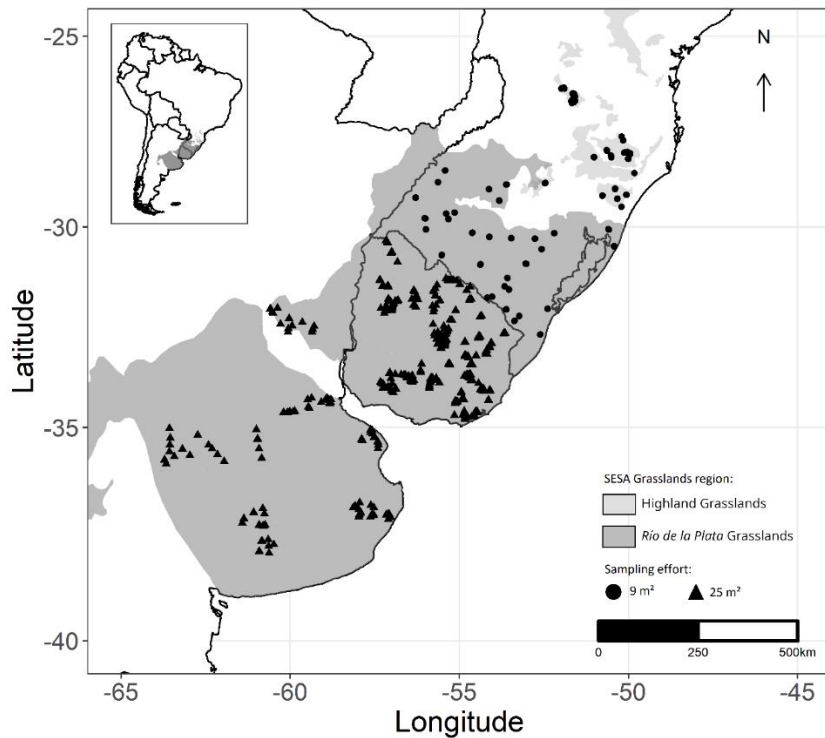


Figure 1: Location of the 666 sampling sites in the Southeastern South America Grasslands, with corresponding sampling unit area.

### ***Taxonomic, functional, and phylogenetic information***

There were 266 species of Poaceae in the 666 assemblages. Occurrences identified only to the genera level and non-native species were excluded. We selected only native species, as their evolutionary history is deeply connected with the region. Species names were standardized according to The Plant List (<http://www.theplantlist.org/>) using the R package “Taxonstand” (Cayuela et al. 2012). The synonyms of eight species were merged with their accepted names before further analysis resulting in a final list with 221 grass species. Photosynthetic functional types C<sub>3</sub>-C<sub>4</sub> were assigned using Osborne et al. (2014) and, whenever necessary, we consult specialists.

A phylogenetic tree for the overall species pool was reconstructed based on the phylogenetic hypothesis proposed by Smith & Brown (2018). We used the R function phylo.maker (package V.PhyloMaker; Jin & Qian 2019) to link the species names in our dataset with those in the megaphylogeny, and the scenario 3 approach to add species to the phylogeny. Scenario 3 adds missing taxa (e.g., genera or species) to the phylogeny

within the taxa with known branch lengths. The phylogeny was then used to evaluate the effect of latitude on phylogenetic beta-diversity patterns among the vegetation surveys.

### ***Environmental and geographical variables***

We selected different explanatory variables related to current environmental conditions (climate and soils), historical climate stability and land use impact that we hypothesized to have direct influence on grasses functional groups distribution. We compiled five climatic variables from Worldclim 2.1 database (Fick & Hijmans 2017) that represent both annual patterns and extremes: annual mean temperature, temperature seasonality (standard deviation of the mean monthly temperature  $\times 100$ ), minimum temperature of coldest month, annual mean precipitation, and precipitation seasonality (coefficient of variation of monthly precipitation over the year). We selected three soil physical and chemical variables: clay content, cation exchange capacity and pH. These soils variables are proxies of nutrient and water retention, nutrient availability, and soil toxicity, respectively. Soil data were downloaded from SoilGrids at a depth of 15cm (Hengl et al. 2017). Climate stability was obtained over the last 21 kyr (Carnaval et al. 2014). We calculated mean temperature and rainfall over this period at time slices of 1,000 years and then the standard deviation. Lower values of standard deviation indicate greater climatic stability. Hereafter we call this variable “historical climate instability”, which is more intuitive: higher values indicate higher climate instability. Paleoclimate data used by Carnaval et al. (2014) is available at Dryad (<http://datadryad.org/resource/doi:10.5061/dryad.8kc1v>). Finally, in order to access the impact of overgrazing, we used cattle density (Robinson et al. 2014). Latitude was considered as a spatial variable to explore the distribution of grass diversity: we assume that the greater the distance between pairs of sampling units, the greater the chance of presenting different grass community composition.

All variables were at a spatial resolution of 30 seconds ( $\sim 1 \text{ km}^2$ ), except for historical climate instability at a resolution of 2.5 minutes ( $\sim 5 \text{ km}^2$ ). The variables were centered and standardized (function ‘decostand’ from package ‘vegan’) to have zero mean and unit variance to make them comparable within models. Variance Inflation Factors (VIF) were determined for the variables in order to evaluate problems related to

the multicollinearity of explanatory variables (Graham 2003), and those highly correlated (VIF > 5) were removed using the 'vifstep' R function of the 'usdm' package (Naimi et al. 2014). We chose a restrictive VIF since bioclimatic variables are known for being multicorrelated. This method removes variables with higher values of VIF one by one until all remaining variables presented VIF values lower than the threshold (Fox and Monette 1992). Latitude, temperature seasonality, minimum temperature of coldest month, annual precipitation and soil pH were highly correlated with other variables and were removed from subsequent analysis. The remaining seven explanatory variables were kept for model selection using the Akaike information criteria (AICc). After excluding the collinear variables, the linear correlation coefficients range between -0.04 and -0.55. Pairwise Pearson's correlation between bioclimatic and soil variables are available at Figure S1.

### ***Data Analysis***

We computed community weighted mean (CWM) of the photosynthetic functional types C<sub>3</sub> and C<sub>4</sub> using the function 'cwm' of 'weimea' package (Zelený, version 0.1.18, <https://github.com/zdealveindy/weimea>). Since CWM was calculated based on a community matrix of presence/absence, values correspond to the proportion of either C<sub>3</sub> or C<sub>4</sub> grasses and the sum is equal to 1. Thus, we applied the analysis to C<sub>3</sub> only. We applied generalized linear models (GLM; function 'glm', from package 'stats') to understand the role of the different drivers that shape C<sub>3</sub> distribution in SESA grasslands. We assured that the response variable had normal distribution. We built one full model predicting C<sub>3</sub> species distribution with all the non-correlated variables, which were: annual mean temperature, precipitation seasonality, soil clay content, soil fertility, cattle density, historical temperature instability and historical precipitation instability. Then, we fitted one minimal adequate model to the full model using the 'step' function. Finally, we ranked the models through Akaike information criteria using 'AICctab' function from 'bbmle' package. R<sup>2</sup> of final model was estimated by 'rsquared' function from 'piecewiseSEM' package. Ranking of full and reduced linear regression models for C<sub>3</sub> species distribution using AICc statistics are available at Table S1.

The effect of latitude on phylogenetic beta-diversity patterns among the vegetation surveys was evaluated through principal coordinates of phylogenetic structure (PCPS;



Duarte 2011; Duarte et al. 2016). We performed this procedure through a set of functions implemented in the R package PCPS (Debastiani & Duarte 2014). This method consists of using pairwise phylogenetic similarities between species to weight their incidence in each survey (Duarte et al. 2016), generating a matrix **P** describing phylogeny-weighted species composition for each survey. Therefore, matrix **P** represents the abundance or frequency of the phylogenetic clades in each community. Matrix **P** is submitted to principal coordinates analysis (PCoA) to generate the principal coordinates of phylogenetic structure (PCPS) the resulting eigenvectors describing an orthogonal phylogenetic gradient in the data set (function 'pcps'; Duarte et al. 2016). Each eigenvector is a phylogenetic gradient for the set of communities, capturing the variations in the entire phylogeny, from basal to terminal nodes (Duarte et al. 2012). Further, through function 'matrix.p.sig', matrix  $D_P$  is generated through pairwise squared-rooted Bray–Curtis dissimilarities between sites. Matrix  $D_P$  is submitted to a modified version of distance-based regression on dissimilarity matrices (Mcardle & Anderson 2001) implemented in the R package PCPS in order to analyse whether plant species distribution across the latitudinal gradient is mediated by phylogenetic relationships between them (Duarte et al. 2016). Hereafter, the method consists of two null model test procedures. Under *site.shuffle* model, the hypothesis that environmental gradient **E** affects the distribution of species across the survey is tested. This procedure is a classical permutation-based process assuming independence between communities and sites. Under *site.shuffle*, if the null hypothesis is rejected ( $P\text{-value} \leq 0.05$ ), we conclude that latitude affects the distribution of species across study plots. In this case, a second null model is performed (*taxa.shuffle*) to test if the association of the environmental gradient and species distribution is phylogenetically structured. This null model allows keeping species composition across sites constant while permuting only the phylogenetic relationships among species (Kembel et al. 2010). Under *taxa.shuffle*, if the null hypothesis is rejected, we conclude that the distribution of species across plots depends on the phylogenetic relatedness among them. Since both null hypotheses were rejected, we also applied the same null model approach used to evaluate the influence of latitude on each of the four first PCPS axes using ordinary least squares (OLS) models (as in Duarte et al. 2016; function

'pcps.sig'). For each null model procedures, 999 permutations were performed for each test.

## Results

The 221 native grass species were distributed in 6 subfamilies and 59 genera. Species richness ranged from 2 to 35 grass species per site. The subfamilies with highest number of taxa across the metacommunity were Panicoideae and Pooideae, with 95 and 73 species, respectively. Within Panicoideae, the most representative tribe was Paniceae, with 11 genera and 37 species; *Panicum* was the richest genus, with 7 species. Within Pooideae, the most representative tribes were Stipeae, with 3 genera and 34 species and Poeae with 10 genera and 24 species; the most representative Stipeae genus was *Nassella* with 23 species. Regarding photosynthetic pathways, 84 species are C<sub>3</sub> and 137 species are C<sub>4</sub>.

### *Drivers of C<sub>3</sub>-C<sub>4</sub> distribution*

Proportion of C<sub>3</sub> species in grassland communities increased with increasing precipitation seasonality and soil fertility, while higher annual mean temperature, soil clay content and historical temperature instability led to communities with reduced presence of C<sub>3</sub> species, and consequently greater contribution of C<sub>4</sub> species (Fig. 2; GLM results: R<sup>2</sup> = 0.37, P<0.01). Cattle density was not selected by the model, while historical rainfall instability was not significant. Additionally, we found a correlation between proportion (CWM) of C<sub>3</sub> and latitude: C<sub>3</sub> species cover increased towards south (Fig. 3; R<sup>2</sup> = 0.40, P<0.001).

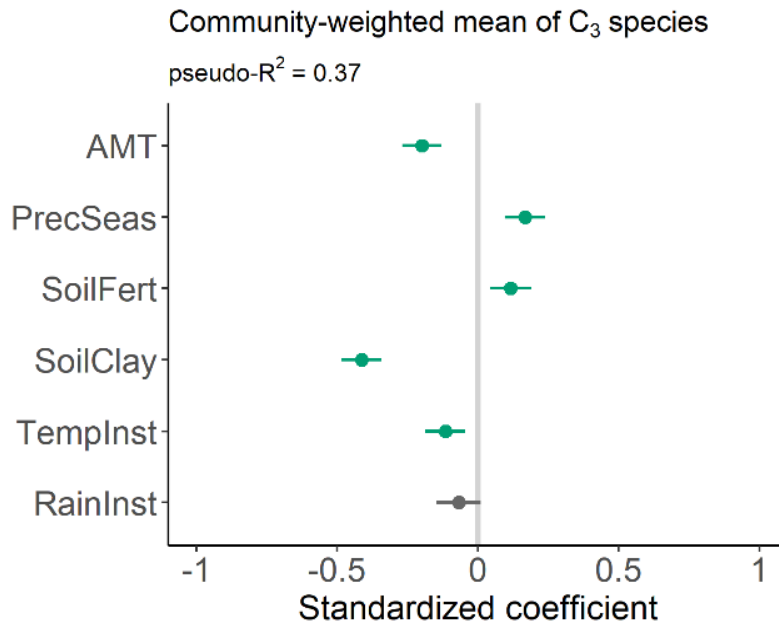


Figure 2. Standardized regression coefficients of model predictors and associated 95% confidence intervals (CI) for C<sub>3</sub> species proportion in southeastern South America grasslands. Labels: AMT: annual mean temperature; PrecSeas: precipitation seasonality, SoilFert: soil fertility, SoilClay: soil clay content, Templnst: historical temperature instability, RainInst: historical rainfall instability. Standardized regression coefficients result from generalized linear model (GLM). Predictors were centered and scaled, so coefficients were directly comparable and can be interpreted as the magnitude of effects of each variable. Cis that do not cross the zero line are colored in green and indicate a statistically significant effect on changes in proportion of C<sub>3</sub> species.

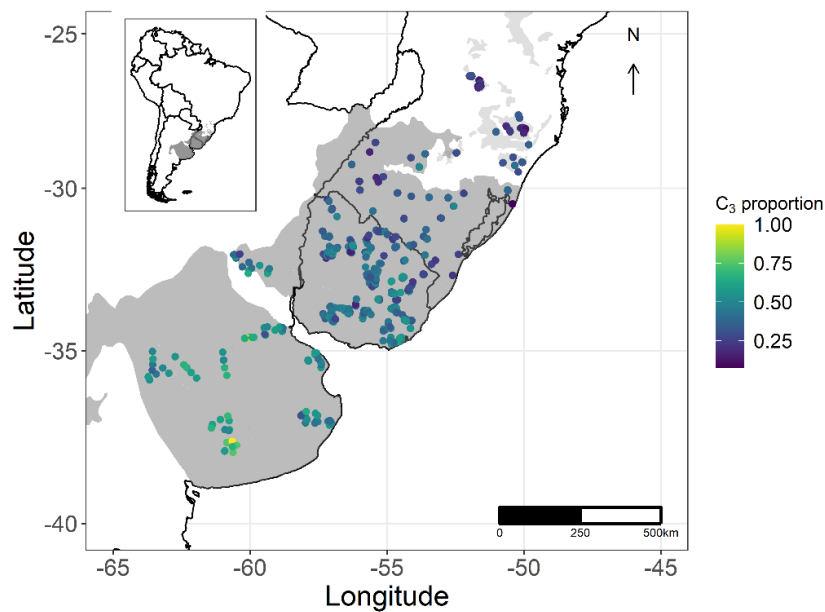


Figure 3. Distribution of C<sub>3</sub> grasses in our study region in Southeastern South America grasslands.

### *Phylogenetic beta diversity*

Distance-based regression performed on matrix DP rejected both null hypotheses (FObs. = 160.79; Psite shuffle = 0.001; Ptaxa shuffle = 0.001), which means that the distribution of species across the latitudinal gradient depends on the phylogenetic relatedness among them. The principal coordinate analysis of the phylogeny-weighted species composition matrix generated 665 PCPS. The first four PCPS synthesized, respectively, 45.2%, 4.1%, 1.6% and 1.1% of the total variation in phylogenetic composition across the metacommunity. Only the first PCPS showed significant positive relationship between latitude and clade distribution across the metacommunity (Table 1; Ptaxa shuffle = 0.002). The PCPS scatter plot based on PCPS axes one and three (Fig. 4) showed that PCPS 1 was positively related to Panicoideae, Aristidoideae, Chloridoideae and Danthonioideae (PACMAD clade, both C3 and C4 species), which were associated with lower latitudes, while Pooideae and Oryzoideae were related to higher latitudes (BOP clade, C3 exclusive).

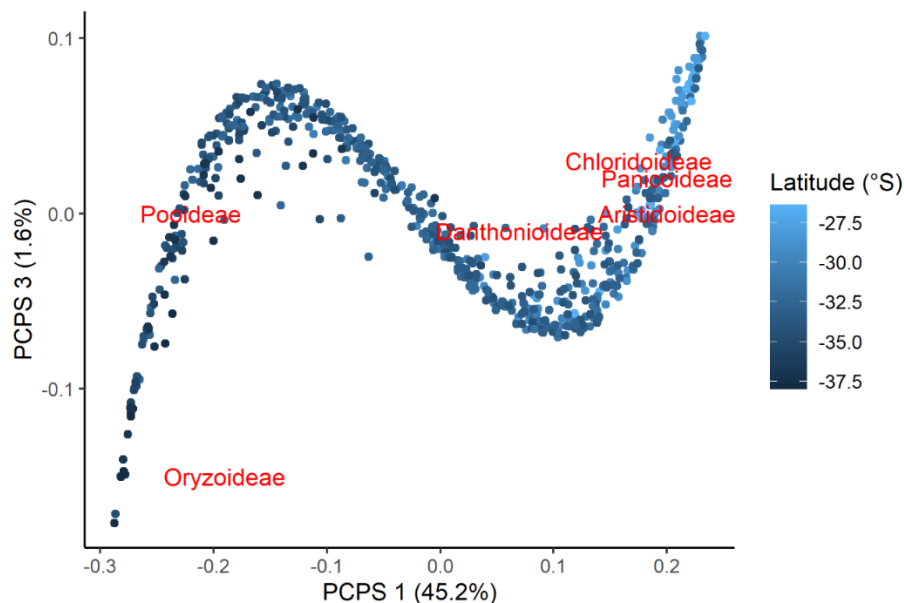


Figure 4. Scatter plot of the PCPS 1 and 3 generated from the ordination of matrix P describing phylogenetic weighted species composition of vegetation surveys distributed across the latitudinal gradient in Southeastern South America grasslands.

Table 1. Null model-based OLS relating the four first PCPS to latitude for 666 vegetation surveys in the Southeastern South America grasslands.

Response variable	Observed F-value	Explained variation	$P_{\text{site shuffle}}$	$P_{\text{taxa shuffle}}$
PCPS 1	488.3	45.2%	0.001	0.002
PCPS 2	1.25	4.1%	0.269	0.8
PCPS 3	25.46	1.6%	0.001	0.297
PCPS 4	0.02	1.1%	0.9	0.982

## Discussion

Over the past decades, several studies explored the current environmental drivers of C<sub>4</sub>/C<sub>3</sub> grass distribution (e.g., Paruelo et al. 1998; Perelman et al. 2001; Cabido et al. 2008; Edwards & Still 2008; Jardine et al. 2021). By including the phylogenetic information, it is possible to integrate historical and evolutionary explanations on the patterns found (Gerhold et al. 2015), as shown by recent studies (e.g., Liu et al. 2019; Donnelly et al. 2023). Here, we observed that both latitude, current and historical environmental conditions affect the distribution of grass species across the SESA grasslands, reflecting the relationships of both phylogenetic and functional strategies among species.

### *Natural drivers of C<sub>3</sub>/C<sub>4</sub> distribution*

The proportion of C<sub>3</sub> grass species are higher in areas with lower annual mean temperature. This pattern is widely described in literature, and has been attributed the evolution of the C<sub>4</sub> pathway which enabled grasses to persist in warmer climates than their C<sub>3</sub> relatives (Edwards & Smith 2010).

Previous studies highlight the associations between the spatial distribution of precipitation and the distributions of the different C<sub>3</sub>/C<sub>4</sub> ratio across communities, as found in this study. The differences in the seasonal occurrence in precipitation are a possible explanation to the proportional distribution of C<sub>3</sub>/C<sub>4</sub> species in areas with relatively small differences in temperature (Cabido et al. 2008; Edwards & Smith 2010; Visser et al. 2012). It has already shown that C<sub>4</sub> grass clades occupy phylogenetically conserved precipitation niches, dominating areas with lower precipitation (Edwards & Smith 2010). In general, C<sub>4</sub> grasses presents a wide tolerance to moisture conditions, occurring in areas with widely differing precipitation regimes (Jardine et al. 2021). However, the tribes Andropogoneae and Paniceae (both tribes included in the subfamily Panicoideae) are described as following the general pattern of higher richness in warm, mesic environments for C<sub>4</sub> species (Jardine et al. 2021). Here we found C<sub>3</sub> species occurring in more seasonal areas. The Pooideae subfamily, which represents the majority of C<sub>3</sub> species in this study, presents cold and drought tolerance (Das et al. 2023), enabling its representatives to cope with precipitation seasonality, especially in temperate regions (Das et al. 2023). This

expected pattern is consistent with our data where precipitation seasonality is combined with lower temperatures, as the southern region of SESA grasslands, in which C<sub>3</sub> grasses are more likely to survive.

In this study we also explored the historical climate fluctuations as possible drivers of present grassland communities, in an attempt to connect functional groups of grasses to the deep-past climatic changes at SESA grasslands. We found C<sub>3</sub> species occurring in more stable areas, considering temperature over the past 21 kyr (data from Carnaval et al. 2014). In our case, climatically stable areas are the colder areas, while unstable areas include hotter habitats, which enabled colonization by C<sub>4</sub> species. The Late glacial – Holocene transition presented the replacement of dry steppe by humid grassland (Prieto 2000). This shift enabled the colonization of C<sub>4</sub> lineages, resulting in the expansion of grassy biomes to habitats with warmer, more humid and less seasonal climatic conditions across a wide span of rainfall (Edwards & Smith 2010). This suggests that climatic conditions have selected particular traits over the evolutionary and biogeographic history affecting historical colonization of these environments (Ricklefs 2006; Hardy et al. 2012). Additionally, higher historical instability can induce fluctuations between savanna-like physiognomies (i.e., with presence of shrubs and trees in the grassland matrix) and open grasslands, coinciding with the transition of Poaceae from closed to open habitats (Elliot et al. 2023).

Regarding soil variables, we found C<sub>3</sub> species occurring in areas with high soil fertility and with lower halomorphic content. In general, C<sub>4</sub> species have a higher water- and nitrogen-use efficiency than C<sub>3</sub> species and are expected to be more abundant in drier environments (Sage 1999), while C<sub>3</sub> species are more abundant on fine-textured soils with relatively high moisture levels. However, C<sub>3</sub> species also developed drought tolerance (Das et al. 2023), which seems to be related to the species that occur in SESA grasslands, as C<sub>3</sub> species are also more abundant in areas with periodic droughts. Soil features (specifically soil pH and salinity) have been found to predict the regional distribution of C<sub>3</sub> and C<sub>4</sub> grasses in the Flooding Pampa, the southern portion of our study area (Perelman et al. 2001; Chaneton et al. 2005). In this case, C<sub>3</sub> grasses were more abundant in acidic soils, while C<sub>4</sub> grasses coverage increased towards more alkaline soils,

and authors suggested the pH gradient was also related to salinity, linked to greater differences in water-use efficiency between C<sub>3</sub> and C<sub>4</sub> species (Perelman et al. 2001).

### ***Anthropogenic drivers of C<sub>3</sub>/C<sub>4</sub> distribution***

We expected cattle density to have an effect on C<sub>3</sub>/C<sub>4</sub> distribution, as in disturbance-dependent ecosystems, integration of climatic variables and land management history is fundamental to understand the drivers of grassland vegetation patterns (Baggio et al. 2021; Pivello et al. 2021). It is known that areas under high grazing intensity and with fire management are dominated by stoloniferous, rhizomatous prostrate and tussock C<sub>4</sub> species (respectively for grazing and fire), typical of rapid resource acquisition and avoidance of grazing (Cruz et al. 2010). Concerning disturbance effect over grass functional groups, higher grazing pressure or fire use has been shown to reduce C<sub>3</sub> productivity, leading to a replacement of C<sub>3</sub> graminoids by less-productive C<sub>4</sub> grasses, indicating that grazing increases competitive ability of C<sub>4</sub> grasses (Irisarri et al. 2015). However, cattle density was not selected by our model, which may indicate that environmental drivers are better determinants of distribution of grass functional groups. On the other hand, the variable could not reflect the real livestock density, which is very variable in space and time.

### ***Phylogenetic beta diversity***

The phylobetadiversity patterns results showed that phylogeny has an important role as a structuring agent of grass communities across our study region, indicating turnover of grass lineages across the assemblages, as PCPS1 was able to capture 45.2% of the phylogenetic variation of grassland communities over the latitudinal gradient (Fig. 4 and Table 1). Grass species found at the northern portion of the gradient belong mostly to the PACMAD clade, which contain both C<sub>4</sub> and C<sub>3</sub> species. At the southern part of the gradient, communities are dominated by grasses belonging to the C<sub>3</sub>-exclusive BOP clade. This pattern suggests that grass clades are responding to different environmental conditions, once the latitudinal gradient is correlated to temperature and precipitation (Fig. S1), and the different clades are described by different photosynthetic pathways. This spatial component could be a result of the post-glacial shift of dry steppe to humid



grassland mainly in the northern portion, enabling lineage colonization in novel climatic conditions and preventing older C<sub>3</sub> lineages to colonize these environments (Prieto 2000; Svenning et al. 2015; Giudicelli et al. 2022). Regarding present conditions, species of the BOP clade are often associated to harsh environments, such as cold and dry habitats, while PACMAD have expanded their niches into warmer climates (Pau & Still 2014; Aagesen et al. 2016). The recurrent evolution of the C<sub>4</sub> pathway in PACMAD lineages represented opportunity for variation, as each origin arose within a unique environment, resulting in dominance of C<sub>4</sub> species, since different climatic niches (Edwards et al. 2010).

Our latitudinal range covers the transitional region between subtropical and temperate zones, which is indicative of climatic effects shaping the phylogenetic structure of the grass assemblages. The evolutionary history of Poaceae lineages is well-known and connected to temperature and precipitation shifts (Edwards & Still 2008; Bouchenak-Khelladi et al. 2010; Edwards & Smith 2010). The Pooideae lineage, the predominant clade at the south of our gradient, has specialized and radiated in cold environments by evolving physiological cold acclimation to protect tissues from freezing damage, and vernalization to synchronize flowering with the growing season (Sandve & Fjellheim 2010; McKeown et al. 2016), and today occupy both the coldest and the driest climate space in Poaceae (Edwards & Smith 2010). Conversely, PACMAD species, predominant at the northern sites, tend to be adapted to warm and mesic environments whether or not they are C<sub>4</sub> (Edwards & Smith 2010; Visser et al. 2012). This suggests that the evolution of cold and drought tolerance in Pooideae may be as important as the metabolic differences in shaping grass species distribution (Edwards & Smith 2010; Das et al. 2023).

Species beta diversity (turnover) has already been registered as a main component of beta-diversity for open vegetation in Southern Brazilian grasslands (Staude et al. 2018), as also for other species groups in high diversity systems from Brazil, as for example trees in Atlantic Forest (Bergamin et al. 2020). The high importance of turnover lies within the past and present favourable environmental conditions that promote high biodiversity (Dobrovolski et al. 2012). A recent example is shown in a study investigating the drivers of genetic structure of *Petunia axillaris* subsp. *parodiia*, an annual herbaceous distributed through the SESA grasslands, showing that the Pleistocene climate changes drove

lineage diversification either through micro-refugia and riverine barriers (Giudicelli et al. 2022).

### ***Implications***

Climate change in grassland ecosystems may lead to divergent response patterns in the abundance and distribution of C<sub>3</sub> and C<sub>4</sub> grasses, due to efficiency differences of carbon sequestration between C<sub>3</sub> and C<sub>4</sub> grasses. The expectation is that C<sub>4</sub> species benefit much less from rising CO<sub>2</sub> than C<sub>3</sub> species (Reich et al. 2018). However, in addition to CO<sub>2</sub>, rising temperature and extreme events of high precipitation and droughts are expected for the region (IPCC 2021), and studies with predictive modelling showed an increase in C<sub>4</sub> abundance in these conditions (Epstein et al. 2002). Additionally, a long-term experimental study documented an unexpected reversal of C<sub>3</sub> versus C<sub>4</sub> grass response to elevated CO<sub>2</sub> in the first 12 years of a 20-year free-air CO<sub>2</sub> enrichment experiment, but with a subsequent increase of biomass production by C<sub>4</sub> species in the last eight years due to soil nitrogen mineralization (Reich et al. 2018). Conversely, future increase in atmospheric CO<sub>2</sub> could favour the expansion of woody C<sub>3</sub> species over the grass canopy, thereby shading the grasses, disrupting fire cycles, and reducing the amount of C<sub>4</sub> habitat (Sage 2016). Direct and indirect climate-induced changes in the relation of C<sub>3</sub> and C<sub>4</sub> grasses may thus lead to shifts in grass communities and in productivity (Pau & Still 2014). For instance, BOP grasses can be less productive aboveground due to the resources allocation to roots when environments become more water-limited environments. Moreover, as observed in our results about AMT-C<sub>3</sub> grasses relationship, the increasing annual mean temperature might lead to an expansion of C<sub>4</sub> grasses over the time, also changing local and regional grassland physiognomies. Another related issue is the potential impact of climate change on invasive species, mediated through C<sub>3</sub>/C<sub>4</sub> relations and through dominance of specific grass growth habits. It has been shown that tall-stature C<sub>4</sub> grasses, as tussocks, are stimulated more by rising CO<sub>2</sub> than short-stature grasses (Polley et al. 2012; Hager et al. 2016). This could be significant in areas where the presence of invasive C<sub>4</sub> tall grasses from Africa outcompete native shorter-statured C<sub>4</sub> grasses (Guido et al. 2019). All these consequences of climate change in the region can be intensified if land use conversion and low protection remain.

Currently, the region has a maximum of 6.8% of protected areas - the Brazilian portion has only 3.2% in protected areas (Overbeck et al. 2015), and the current conversion rate is higher than in Amazon Forest (Mapbiomas 2021). Thus, understanding the drivers of C<sub>3</sub>-C<sub>4</sub> distribution and their interactions can inspire novel research priorities for grassland ecosystems, for example studies aiming to predict how diversity patterns will be affected under climate change scenarios.

## **Conclusion**

The distribution of grass clades across the SESA grasslands is indicative of the environmental gradients found in the Southeastern South American grassland region between temperate and tropical zones, describing a climate space where disturbance driven feedbacks play a major role in maintaining current open vegetation. The distribution of C<sub>3</sub> and C<sub>4</sub> species within particular ranges along current and past environmental conditions is indicative of habitat specialization (Hardy et al. 2012). Most of the explanatory power of our models relied in the current climate and soil conditions, but also in historical temperature stability, indicating how patterns originated in the Pleistocene overlap extensively with current environmental gradients (Cantidio & Souza 2019). Our results contribute to the understanding of ecological heterogeneity in the region that up to now has been poorly described and show that temperature and water availability are the main environmental factor driving C<sub>3</sub>:C<sub>4</sub> grass species distribution along the SESA grasslands region.

## **Author contributions**

H.S., R.S.B., S.C.M. and G.E.O. conceptualized the study; B.O.A. assembled the database; F.L. is curator of data from Uruguay. H.S. analysed the data; H.S. led the writing of the manuscript. All authors read and approved the final work. This work is part of H.S. PhD thesis in Ecology at the Universidade Federal do Rio Grande do Sul.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Supplementary Material

### Appendix S1. Additional information on main results.

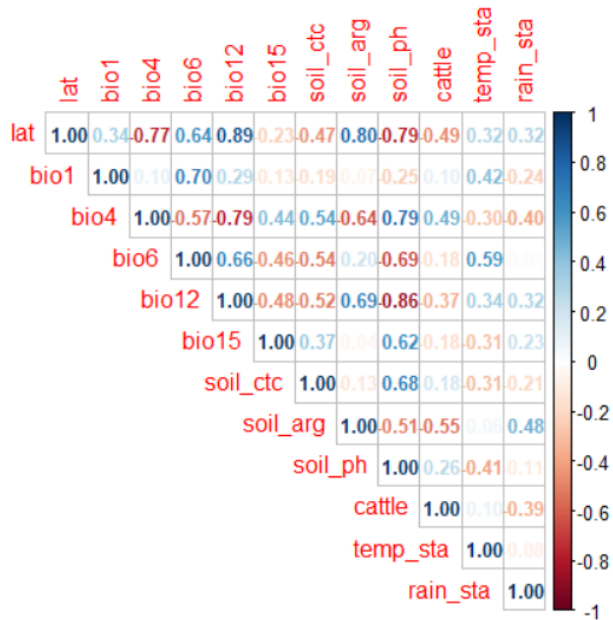


Figure S1: Pairwise Pearson's correlation between bioclimatic and soil variables. Labels: lat, latitude; bio1, annual mean temperature; bio4, temperature seasonality; bio6, minimum temperature of the coldest month; bio12, annual precipitation; bio15, precipitation seasonality; soil\_ctc, soil cation exchange capacity; soil\_arg, soil clay content; soil\_ph, soil pH; cattle, cattle density; temp\_sta, historical temperature instability; rain\_sta, historical rainfall instability. Autocorrelated variables were removed through Variance Inflation Factors (VIF), with threshold of VIF > 5.

Table S1. Variance Inflation Factors (VIF) values for bioclimatic and soil variables. Five variables from the 12 input variables have collinearity problem and were removed from subsequent analysis (latitude, temperature seasonality, minimum temperature of coldest month, annual precipitation and soil pH). After excluding the collinear variables, the linear correlation coefficients range between -0.04 and -0.55. Labels: lat, latitude; bio1, annual mean temperature; bio4, temperature seasonality; bio6, minimum temperature of the coldest month; bio12, annual precipitation; bio15, precipitation seasonality; soil\_ctc, soil cation exchange capacity; soil\_arg, soil clay content; soil\_ph, soil pH; cattle, cattle density; temp\_sta, historical temperature instability; rain\_sta, historical rainfall instability.

Variables	VIF
lat	34.46
bio1	46.10
bio4	60.98
bio6	46.07
bio12	10.00
bio15	4.00
soil_ctc	2.92
soil_arg	7.90
soil_ph	9.49
cattle	2.02
temp_sta	1.69
rain_sta	2.62

Table S2. Ranking of linear regression models for C3 species distribution selected using AICc statistics. Labels: lat, latitude; bio1, annual mean temperature; bio4, temperature seasonality; bio15, precipitation seasonality; soil\_ctc, soil cation exchange capacity; soil\_arg, soil clay content; cattle, cattle density; temp\_sta, historical temperature instability; rain\_sta, historical rainfall instability.

	Model	AICc	df	$\Delta$ AICc	weight	Adj.R2
Mod.min	C3 ~ bio1 + bio15 + soil_ctc + soil_arg + temp_sta + rain_sta	-1055.4	8	0.0	0.58	0.37
Mod.full	C3~bio1+bio15+soil_ctc+soil_arg+cattle+temp_sta+rain_sta	-1054.8	9	0.6	0.42	0.37
Mod.null	C3~1	-756.7	2	298.4	0.0	0.0

## Supplementary results

Appendix S2. Information about additional results. We applied generalized linear models (GLM; function 'glm', from package 'stats') to understand the role of the different drivers that shape C<sub>3</sub> distribution in Southeastern South America grasslands. Dataset considered here included both native and non-native species. Compositional changes of subfamilies along the gradients were evaluated with principal component analysis (PCA). The PCA axes were used as informative of the variances of subfamilies along the gradient to the GLM analysis.

Table S3: Estimated effects of the variables in the top-ranked generalized linear model on community weighted means of C<sub>3</sub> grass species (native and non-native) in Southeastern South American grassland communities. Pseudo-R<sup>2</sup> of the model was 0.45. Labels: AMT: annual mean temperature; PS: precipitation seasonality; SF: soil fertility; CC: soil clay content; HTI: historical temperature instability, SE: standard error.

C3		
Predictor	Estimate (±SE)	P-value
<b>Environmental</b>		
AMT	<b>-0.03 (0.004)</b>	<b>&lt;0.001</b>
PS	0.007 (0.004)	0.09
SF	<b>0.036 (0.004)</b>	<b>&lt;0.001</b>
CC	<b>-0.068 (0.004)</b>	<b>&lt;0.001</b>
<b>Historical</b>		
HTI	<b>-0.017 (0.004)</b>	<b>&lt;0.001</b>

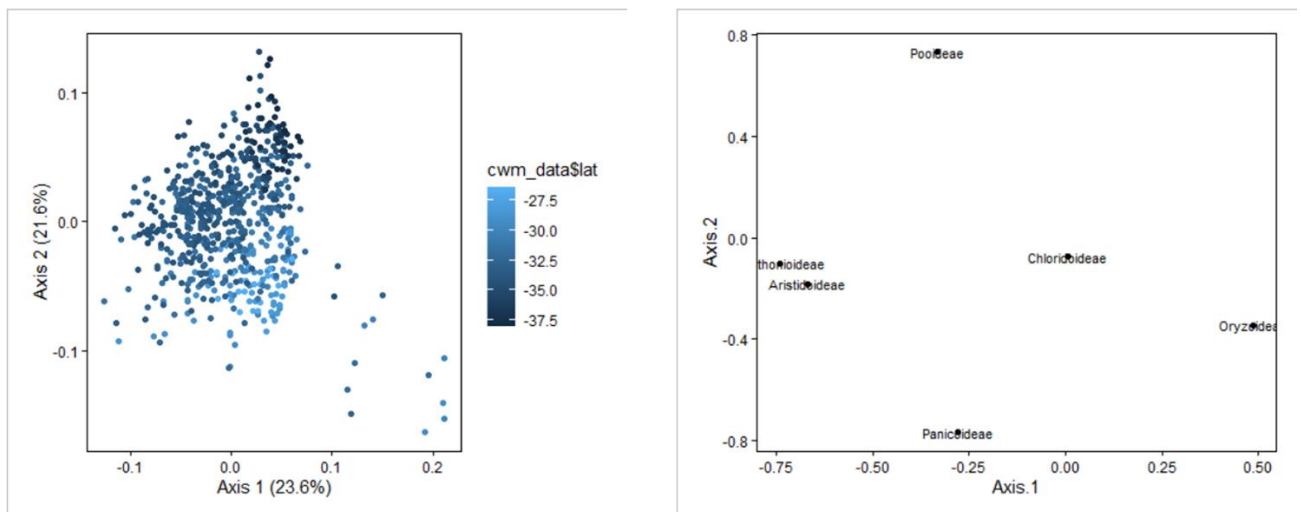


Figure S2. Principal Component Analysis of grassland communities described by Poaceae subfamilies. Axis 1 was able to capture the distribution among Danthonioideae, Aristidoideae, Chloridoideae and Oryzoideae, while Axis 2 captured the distribution gradient of Pooideae and Panicoideae.

Table S4: Estimated effects of the variables in the top-ranked generalized linear model on distribution of subfamilies Danthonioideae, Aristidoideae, Chloridoideae and Oryzoideae in Southeastern South American grassland communities, represented by the axis extracted from Principal Component Analysis. Pseudo-R2 of the model was 0.24. Labels: AMT: annual mean temperature; PS: precipitation seasonality; SF: soil fertility; CC: soil clay content; HRI: historical rainfall instability; Cattle: cattle density; SE: standard error.

Predictor	Axis 1	
	Estimate ( $\pm$ SE)	P-value
<b>Environmental</b>		
AMT	<b>0.009 (0.0016)</b>	<b>&lt;0.001</b>
PS	<b>0.011 (0.002)</b>	<b>&lt;0.001</b>
SF	0.003 (0.001)	0.09
CC	<b>-0.004 (0.002)</b>	<b>&lt;0.05</b>
<b>Historical</b>		
HRI	<b>0.017 (0.002)</b>	<b>&lt;0.001</b>
<b>Anthropogenic</b>		
Cattle	-0.003 (0.002)	0.059

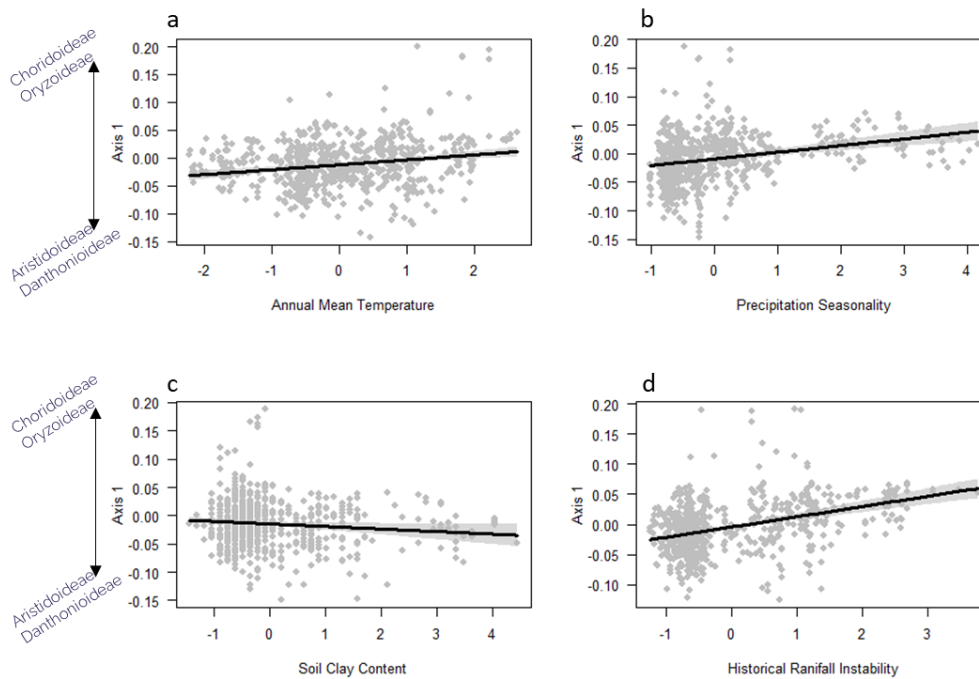


Figure S.3: Variation of PCA Axis 1 according to (a) annual mean temperature, (b) precipitation seasonality, (c) soil clay content and (d) historical rainfall instability.

Table S5: Estimated effects of the variables in the top-ranked generalized linear model on distribution of subfamilies Pooideae and Panicoideae in Southeastern South American grassland communities, represented by the axis extracted from Principal Component Analysis. Pseudo-R2 of the model was 0.51. Labels: AMT: annual mean temperature; PS: precipitation seasonality; SF: soil fertility; CC: soil clay content; HTI: historical temperature instability; HRI: historical rainfall instability; SE: standard error.

Axis 2		
Predictor	Estimate ( $\pm$ SE)	P-value
<b>Environmental</b>		
AMT	<b>-0.008 (0.001)</b>	<b>&lt;0.001</b>
PS	<b>0.010 (0.001)</b>	<b>&lt;0.001</b>
SF	<b>0.014 (0.001)</b>	<b>&lt;0.001</b>
CC	<b>-0.014 (0.001)</b>	<b>&lt;0.001</b>
<b>Historical</b>		
HTI	<b>-0.004 (0.001)</b>	<b>&lt;0.01</b>
HRI	-0.002 (0.001)	0.11

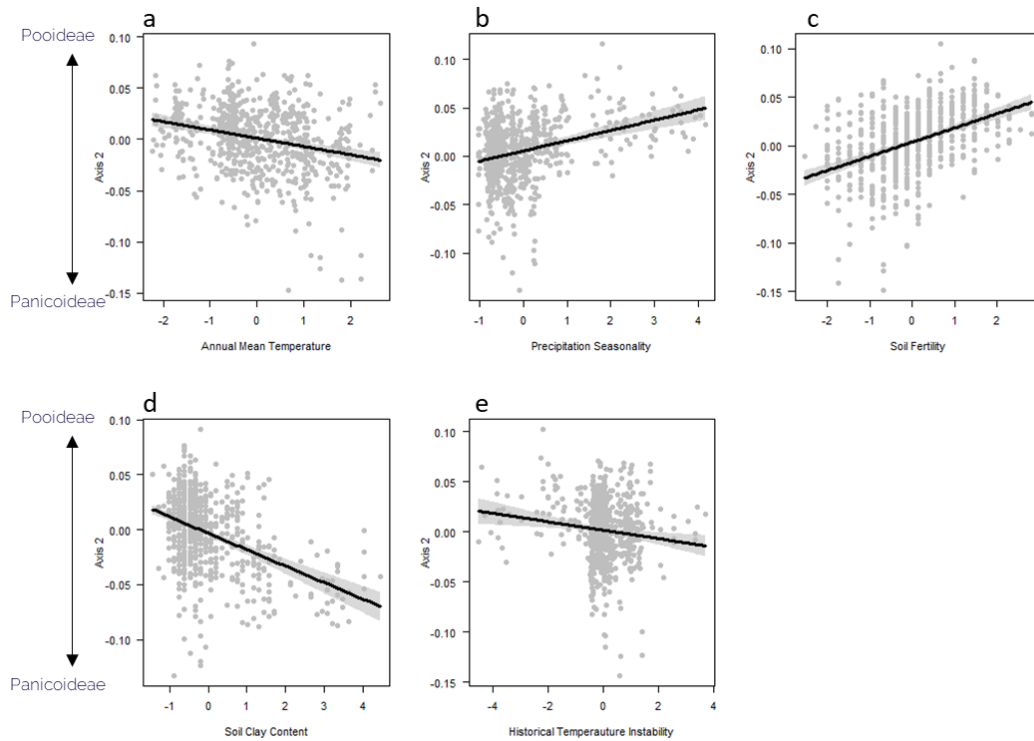


Figure S.4: Variation of PCA Axis 2 according to (a) annual mean temperature, (b) precipitation seasonality, (c) soil clay content, (d) soil clay content and (e) historical temperature instability.

## CONSIDERAÇÕES FINAIS

Nesta tese, avaliei processos ecológicos e evolutivos envolvidos na estruturação filogenética de comunidades de plantas campestres ao longo de gradientes ambientais. Compreender como processos ecológicos e evolutivos atuam é fundamental para entender os padrões globais de diversidade e para prever suas variações ante mudanças climáticas e processos antrópicos, uma vez que esses eventos ameaçam o número de espécies que vivem em diferentes comunidades biológicas, sua função ecossistêmica e a história evolutiva que compartilham. Uma das contribuições mais importantes dessa tese para o conhecimento científico é a análise de padrões de diversidade com dados unificados de três países da América do Sul, seguindo a tendência global de estudos ecológicos de síntese em macroescalas. Além disso, o trabalho desenvolvido nessa tese é inovador ao trazer a perspectiva de biogeografia histórica em estudos ecológicos das comunidades campestres da América do Sul.

No capítulo 1 demonstrei que tanto processos ecológicos, relacionados a filtros ambientais, quanto processos evolutivos explicam os padrões de diversidade filogenética de comunidades campestres da América do Sul. Dentre os processos ecológicos, fatores ambientais do presente e do passado influenciam os padrões. O efeito positivo da instabilidade histórica da temperatura mostrou que as comunidades em áreas que experimentaram condições instáveis comportam uma maior diversidade de linhagens, provavelmente relacionadas a diferentes estratégias para lidar com as flutuações de temperatura e, finalmente, permitindo a co-ocorrência de espécies adaptadas ao frio e ao calor. Em relação às condições atuais, comunidades em áreas com temperatura mínima mais elevada apresentaram maior diversidade filogenética, indicando novamente que as condições são mais propícias para abrigar um amplo espectro de linhagens.

O capítulo 2 trouxe um retrato mais detalhado das relações evolutivas das comunidades campestres com foco em Poaceae, a família botânica mais abundante nesses ecossistemas. A história evolutiva das gramíneas está intrinsicamente ligada ao surgimento da rota fotossintética C<sub>4</sub>, que se deu a partir de mudanças atmosféricas e climáticas a partir de 30 milhões de anos atrás. Estudando os padrões de diversidade

filogenética e de distribuição de espécies C<sub>3</sub> e C<sub>4</sub> no sudeste da América do Sul, pode demonstrar que há uma maior contribuição das espécies C<sub>3</sub> na região sul da área de estudo. Essas comunidades são caracterizadas por serem mais frias e com maior estabilidade histórica de temperatura, refletindo que são linhagens especializadas e irradiadas em ambientes frios. Por outro lado, áreas historicamente instáveis, ou seja, que passaram por flutuações de temperatura, incluem habitats atualmente mais quentes, condições que permitiram a colonização e dominância por espécies C<sub>4</sub>.

De forma conjunta, os resultados dos dois capítulos nos ajudam a entender um pouco mais da história evolutiva da vegetação campestre, mas também adicionam percepções sobre o uso de informações filogenéticas para investigar os fatores que originaram e mantiveram os padrões de diversidade observados. Esses resultados têm implicações relevantes para estudos futuros com o objetivo de prever como os padrões de diversidade de espécies serão afetados em cenários de mudanças climáticas e também podem ser explorados com mais detalhes no contexto de estratégias de conservação e restauração de comunidades ecológicas.