UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

FENOLOGIA REPRODUTIVA E REDES DE INTERAÇÕES PLANTA-POLINIZADOR EM UMA COMUNIDADE CAMPESTRE DO RIO GRANDE DO SUL

Suiane Santos Oleques

Porto Alegre, Março de 2016

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

FENOLOGIA REPRODUTIVA E REDES DE INTERAÇÕES PLANTA-POLINIZADOR EM UMA COMUNIDADE CAMPESTRE DO RIO GRANDE DO SUL

Suiane Santos Oleques

Dissertação apresentada ao Programa de Pós-Graduação em Botânica como um dos requisitos para a obtenção do grau de Mestre em Botânica pela Universidade Federal do Rio Grande do Sul.

Orientador: Prof. Dr. Gerhard Ernest Overbeck

Porto Alegre, Março de 2016

AGRADECIMENTOS

Espero que muito mais que estas palavras eu tenha realmente transmitido minha gratidão a todos que me auxiliaram neste processo. Obedecendo a ordem cronológica dos acontecimentos: agradeço ao Gerhard por ter aceitado me orientar mesmo não sendo sua área de pesquisa e por toda confiança depositada em mim durante o andamento deste trabalho. Sou grata ao Jeferson por todo apoio e disponibilidade. Obrigada pela ajuda em todas as etapas e por estar sempre disponível para sanar minhas dúvidas e me transmitir confiança no eu que estava fazendo. Sou extremamente grata a Graziela, colega de laboratório, que mesmo sem experiência na observação de polinizadores, se dedicou com afinco “na caçada dos bichinhos”. Grazi, muito obrigada mesmo, por tudo! Agradeço também a Daniele pela ajuda no início dos campos e aos meninos da zootecnia/veterinária pelas caronas e pelos mates no final do dia nos primeiros meses do trabalho na EEA. Agradeço também a todo pessoal da Estação Experimental Agronômica: motoristas, cozinheiras, secretários e as meninas da limpeza. Sou muito grata também aos colegas do LevCamp. Cada um com sua particularidade, vocês ganharam espaço no meu coração. Obrigada pelo acolhimento, pelos papos, pela disponibilidade em ouvir e trocar ideias. Um almoço no RU, um mate e uma comilança não são nada banais quando se está longe dos amigos e da família. Essas pequenas coisas tornaram-se rotina valiosa pra mim.

Sabemos que a família não ajuda diretamente em termos práticos da nossa vida acadêmica, mas embora minha mãe nunca tenha ido a campo comigo foi pra ela que eu liguei chorando quando o desespero apertou. Sendo assim, obrigada mãe, vó, vô, pai e mana por serem pessoas importantes na minha vida e sempre apoiarem e incentivarem minhas decisões... O amor de vocês é essencial e indispensável!Agradeço à Gabi e Dani (minhas gatas) pelo carinho, apoio e amizade verdadeira ao longo desses anos que estão na minha vida. Uma ligação, uma mensagem e um tempinho para uma conversa boba fazem a diferença sempre! Por último, mas não menos importante... Agradeço ao meu companheiro, Rubem. Obrigada por ser esta pessoa que és, pois sendo quem és me faz feliz, realizada e confiante de que tudo sempre dará certo. Muito obrigada pela convivência que traz paz e leveza em meio ao caos. Obrigada pelo incentivo, pelo ânimo nas discussões sobre o trabalho e por sempre estar disposto a somar e compartilhar comigo em todos os âmbitos da nossa vida.

SUMÁRIO

Conteúdo

APRESENTAÇÃO

A dissertação encontra-se composta por uma Introdução geral, dois capítulos referentes aos artigos a serem publicados e Conclusões gerais.

Capítulo I:

- Temporal segregation of flowering time and plant-pollinator interactions in a grassland community of Southern Brazil.

Capítulo II:

- Does grazing intensity influence interactions patterns of a plant-pollinator network in a subtropical grassland?

O capítulo I está formatado conforme as normas de submissão exigidas pela revista *Plant Biology*, para qual o artigo foi submetido. O artigo foi aceito para publicação como “minor revision” e encontra-se em vias de ser publicado.

O capítulo II está formatado conforme as normas de submissão exigidas pela revista *Journal of Ecology*.

INTRODUÇÃO GERAL

Foto: *Apis mellifera* em flor de *Richardia grandiflora*

INTRODUÇÃO GERAL

Ecossistemas campestres: panorama geral

Os ecossistemas campestres cobrem em torno de 40% da superfície terrestre e são caracterizados pela alta diversidade de ervas e gramíneas. Estes ecossistemas são mantidos por distúrbios, como pastejo e fogo bem como por climas secos e/ou frios (White *et al*. 2000). Os ecossistemas campestres desempenham importante papel no estoque de carbono atmosférico, na manutenção do ciclo hidrológico, na ciclagem de nutrientes do solo, na oferta de alimentos e na oferta de forragens o que evidencia a importância mundial deste tipo de vegetação na manutenção de funções ecossistêmicas e em termos econômicos (CCSP 2008).

No Brasil, a paisagem campestre distribui-se nas savanas que compõem o bioma Cerrado, nos campos de altitude da Mata Atlântica nas campinaranas e campinas Amazônicas e nas savanas inundáveis do Pantanal (Batalha *et al.* 2001; Alho *et al.* 2008; Campos *et al.* 2012). Porém, é na região sul do país que temos a maior representatividade da vegetação essencialmente campestre. Os denominados campos sulinos estão distribuídos nos estados do Paraná, Santa Catarina e Rio Grande do Sul e são componentes importantes na paisagem e na economia destes estados (Overbeck *et al.* 2007).

No Rio Grande do Sul, os Campos Sulinos dividem-se em duas principais formações: os campos de altitude (bioma Mata Atlântica) e os campos da metade sul (bioma Pampa) (Wagner *et. al.* 2013). Segundo Burkart (1975), os Campos Sulinos constituem uma das regiões mais ricas em gramíneas no mundo e levantamentos contabilizam cerca de 2.200 espécies campestres para o Estado do Rio Grande do Sul (Boldrini *et al.* 2010). Estas paisagens campestres altamente ricas garantem serviços ambientais importantes, como a conservação de recursos hídricos, disponibilidade de polinizadores e o provimento de recursos genéticos. Além disso, estes campos atuam como principal fonte forrageira para a pecuária e possuem alto potencial turístico e cultural (Pillar *et al.*2009).

O bioma Pampa, que se estende desde a metade sul do estado do Rio Grande do Sul até Uruguai e Argentina, é bastante complexo e heterogêneo, contendo diferentes tipos de solo, relevo e fitofisionomias (Santos & Trevisan 2009; Boldrini, *et al.* 2010). Esta heterogeneidade da paisagem resulta em uma grande diversidade de flora e fauna, cuja associações ecológicas com fisionomias específicas ainda carecem de maiores informações (Bencke 2009).A atividade pastoril manejada é considerada a principal atividade para a conservação sustentável da biodiversidade destes campos nativos, tendo em vista a crescente degradação e descaracterização das pastagens naturais através da expansão das atividades agrícolas e silviculturais (Overbeck *et al.* 2007; Santos & Trevisan 2009; Berreta 2009; Baldissera *et al.* 2010; Wagner *et al.* 2013). A dinâmica dos campos está historicamente associada à ocorrência de distúrbios. Deste modo, o pastejo e o fogo, quando adequadamente manejados,têm um papel fundamental, pois atuam no processo de sucessão, impedindo que espécies competitivamente superiores dominem a comunidade. Sendo assim, podemos afirmar que o distúrbio maximiza a diversidade de espécies, promovendo uma maior heterogeneidade na comunidade. Este fato demonstra que as estratégias para conservação da vegetação campestre devem estar associadas ao manejo das atividades pastoris e do fogo e não a sua conservação através da supressão total destes distúrbios (Overbeck *et al.* 2007; Pillar & Vélez 2010).

Os efeitos do manejo das atividades pastoris e do fogo sobre os campos do Rio Grande do Sul estão sendo amplamente estudados (Quadros & Pillar 2002; Overbeck *et al.*2006, Fidelis *et al.* 2010, Fidelis *et al.* 2012, Podgaiski *et al.* 2013), porém ainda não sabemos como estas atividades influenciam nas interações estabelecidas entre plantas e polinizadores. A pressão de pastejo que os animais exercem sobre as plantas reflete em modificações na abundância das espécies e na composição florística do campo. Estudos vêm demonstrando que sob uma oferta de forragem (disponibilidade de forragem seca por kg animal) intermediária (12%) há maiores ganhos tanto para os animais como para as plantas, onde os resultados comprovam uma maior diversidade florística e riqueza específica (Nabinger *et al.* 2009). Porém, necessitamos compreender como essas modificações na composição florística dos campos manejados podem estar relacionadas a mudanças nas interações estabelecidas com polinizadores.

Interações mutualísticas entre plantas e polinizadores

Dentre as diversas interações ecológicas registradas entre animal-planta, a polinização pode ser considerada uma das mais importantes. Isto, porque possui um papel fundamental tanto na reprodução das plantas, sendo essencial para a produção de frutos e sementes, como na oferta de recursos aos polinizadores, sendo muitas vezes o néctar e/ou pólen sua principal fonte energética (Kearns *et al.*1998; Ollerton 2011). Desta forma, a relação entre plantas e seus polinizadores pode ser considerada importante na estruturação de comunidades vegetais e ecossistemas, pois atua na manutenção da biodiversidade e da composição florística (Constanza *et al*. 1997; Kearns 1998 *et al*; Potts 2010), podendo influenciar, por exemplo, a distribuição espacial, a riqueza, a abundância e a fenologia das plantas (Copland & Whelan 1989; Morellato 2004; Moeller *et al*. 2012; Benadi *et al*. 2013).

Estudos ressaltam que mudanças provenientes da ação humana podem influenciar direta ou indiretamente as relações entre plantas e polinizadores. Vázquez e Simberloff (2004) demonstraram que a introdução de herbívoros exóticos em ecossistemas campestres afeta a dinâmica das interações planta-polinizador através de modificações na densidade populacional das plantas. Estas mudanças no número ou abundâncias das espécies vegetais podem influenciar na organização das redes de interações. Diversos estudos tem evidenciado o quanto as redes de interações planta-polinizador podem ser afetadas por modificações na paisagem, como perda de habitat, fragmentação e inserção de espécies exóticas (Memmot & Waser 2002), assim como mudanças climáticas (Thébault & Fontaine 2010, Aizen *et al*. 2012, Spiesman & Inouye 2013), variações altitudinais e de precipitação (Devoto *et al*. 2005).

A descrição e análise das interações através da abordagem de redes complexas e suas estatísticas agregadas permite o entendimento da topologia ou arquitetura das interações no nível das comunidades. Isto possibilita ganhos em relação às percepções sobre sistemas que englobam múltiplas espécies que seriam inviáveis analisando cada espécie isoladamente (Jordano *et al*. 2006; Nielsen & Totland 2013) Além disso, a estrutura das redes de interações pode variar em resposta a alterações na paisagem sem que haja mudança na composição das espécies (Fortuna e Bascompte2006, Aizen *et al*. 2013). Estudos sobre restauração de ecossistemas abordam as redes de interações como importantes ferramentas para avaliação da estrutura das comunidades durante processos de restauração, fornecendo dados sobre um dos mais preocupantes tipos de perda, a perda de interações ecológicas (Hegland *et al.* 2009; Forup *et al.* 2008).

Para compreender a estrutura e o funcionamento das redes de interações utilizamos as chamadas métricas de redes. A conectância das redes, por exemplo, é calculada através do número de interações realizadas (N) em relação ao número de interações possíveis. Neste caso, altos valores de conectância (próximo a 100%) indicam maior generalização e redundância nas interações (Nielsen & Totland 2013). O aninhamento, padrão de organização geralmente encontrado em redes mutualísticas, indica que um grupo de espécies especialistas (que interagem com uma ou duas espécies) associam-se preferencialmente àquelas que interagem com um maior número de espécies. Este padrão pode ser explicado por diferenças na abundância dos indivíduos ou dependências assimétricas (uma alta porcentagem de visitas do polinizador ocorre em uma planta particular (Almeida-Neto *et al*. 2008; Ulrich *et al*. 2009). Outras métricas como a modularidade e especialização, podem indicar o quão especializadas são as interações estabelecidas na rede como um todo (Dormann e Strauss 2013).

O entendimento dos processos ecológicos como a polinização tem importante papel no estabelecimento de estratégias e ações efetivas para conservação e manutenção das comunidades e ecossistemas (Hegland 2010; Menz et al. 2011; Geslin 2013). Sendo assim, compreender como esta relação responde ao manejo em comunidades campestres pode fornecer uma importante contribuição para o entendimento de processos que são importantes na estruturação destas redes. Desta forma, esta abordagem nos permite compreender como os padrões de redes observados podem estar sendo influenciados pelo distúrbio e quais processos estão por trás dos padrões observados (Kaiser-Bundury & Bluthgen 2015). Além disso, ainda é desconhecido como os mecanismos que organizam redes - tais como desacoplamentos nas morfologias e na distribuição espaço-temporal de plantas e polinizadores ou suas abundâncias - contribuem para os padrões de interação entre espécies ao longo de gradientes de perturbação em comunidades campestres do Sul do Brasil.

Padrões fenológicos determinados por interações bióticas

Muito embora esforços venham sendo feitos na tentativa de melhor conhecer e compreender a diversidade vegetal do Pampa pouco sabemos sobre os padrões fenológicos e aspectos reprodutivos das espécies vegetais que compõem os campos, sendo estes importantes fatores na estruturação das interações bióticas e manutenção das comunidades (Elzinga *et al*. 2007). Sabemos que o tempo desempenha um papel fundamental na reprodução das plantas, tanto para dispersão quanto para processo de polinização (Encinas-Viso *et al.* 2012).Sendo assim, a fenologia reprodutiva das espécies é considerada um importante fator na estruturação e evolução das interações bióticas (Olesen *et al*. 2008). Grande parte das espécies de plantas depende completamente ou parcialmente de vetores de polinização para sua reprodução. Deste modo, o período em que a plantas expõem suas estruturas reprodutivas é de fundamental importância na determinação dos potenciais polinizadores o que em muitos casos está relacionado com o sucesso reprodutivo das espécies (Faegri & van der Pijl 1979). Muito embora vários estudos tenham focado no papel de fatores abióticos e filogenéticos (Kochmer & Handell 1986) na determinação dos padrões fenológicos, outros demonstram que em determinadas comunidades as interações bióticas atuam na determinação do padrão fenológico observado (Stiles 1977; Newstron 1994; Elzinga *et al*. 2007). Estudo realizado por Feinsinger *et al*. (1986) demonstrou que 72% das comunidades estudadas apresentam padrão fenológico altamente distribuído ao longo do tempo com sobreposição mínima dos eventos de floração, corroborando a “Hipótese da Competição por Polinizadores”. Deste modo, o padrão fenológico segregado pode ser resultado de forças seletivas que visem diminuir a competição das espécies por polinizadores (Elzinga *et al*. 2007). Por outro lado, as espécies podem concentrar seus eventos reprodutivos de forma agregada no tempo e esta sobreposição pode ser um indício de ocorrência de facilitação na comunidade, otimizando a atração de polinizadores (Feldman 2004).

A alta sazonalidade encontrada nos campos do sul do Brasil e em áreas tropicais sazonais pode reduzir o período de floração (Freitas & Sazima 2006; Pinheiro *et al.* 2008). No entanto, em comunidades sazonais as espécies podem ser mais suscetíveis a forças seletivas que determinam o padrão fenológico, uma vez que o pico dos eventos reprodutivos ocorre em um determinado período do ano e em um curto intervalo de tempo (Freitas & Sazima 2006). Embora Díaz *et al.* (1994), não tenha encontrado evidências de forças bióticas atuantes no padrão fenológico de uma comunidade campestre na Argentina, mais estudos precisam ser conduzidos em comunidades campestres sazonais. É preciso também avaliar como a fenologia pode influenciar em outros aspectos importantes das interações planta-polinizador, como no compartilhamento de polinizadores.

Objetivo geral

O objetivo geral deste trabalho é fornecer dados sobre como o pastejo atua nos padrões e processos estruturadores das redes de interações planta-polinizador em uma comunidade campestre do Sul do Brasil, bem como avaliar o padrão fenológico das espécies vegetais da comunidade campestre como um todo.

Objetivos específicos

O trabalho teve como objetivos responder as seguintes questões:

1) Qual o padrão fenológico apresentado pelas espécies de plantas de uma comunidade campestre do Rio Grande do Sul?

2) Espécies de plantas que apresentam maior sobreposição em suas fases de floração apresentam maior número ou similaridade de visitantes florais?

3) Plantas com flores que apresentam diferentes colorações apresentam um um padrão fenológico em particular?

4) O padrão de organização das redes de interações planta-polinizador é influenciado por diferentes intensidades de pastejo?

5) Como se relacionam aspectos ligados à intensidade de distúrbio com as principais métricas agregadas de redes que descrevem os padrões de interação?

6) Há diferenças na importância relativa dos processos (abundância, fenologia ou morfologia) que estruturam as redes de interações de acordo com a intensidade do distúrbio?

O capítulo I é endereçado a responder as questões referentes ao aspecto fenológico das espécies de plantas da comunidade (1-3), já o capítulo II busca responder as questões referentes a influencia do pastejo sobre as redes de interações planta-polinizador.

REFERÊNCIAS BIBLIOGRÁFICAS

AIZEN, M.A., SABATINO, M. & TYLIANAKIS J. M. 2012. Specialization and rarity predict nonrandom loss of interactions from mutualist networks. **Science** 335: 1486.

ALHO, C. J. R. 2008. Biodiversity of the Pantanal: response to seasonal flooding regime and to environmental degradation. **Brazilian Journal of Biology** 68: 957-966,

ALMEIDA-NETO M. *et al*. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. **Oikos** 117: 1227-1239.

BALDISSERA, R. 2010. Comparison between grassland communities with and without disturbances. **Neotropical Biology and Conservation** 5(1): 3-9.

BATALHA, M. A.; MANTOVANI, W. 2001. Floristic composition of the cerrado in the Pé-de-Gigante Reserve (Santa Rita do Passa Quatro, southeastern Brazil). **Acta Botanica Brasilica** 15: 289-304.

BENADI, G., HOVESTADT, T., POETHKE, H.J. &BLÜTHGEN, N. 2013. Specialization and phenological synchrony of plant–pollinator interactions along an altitudinal gradient.**Journal of Animal Ecology** 83: 639-650.

BENCKE, G.A. (2012) Diversidade e conservação da fauna dos Campos Sulinos. *In:* Pillar, V. P., Muller, S.C., de Souza-Castilhos, Z. M. & Jacques, A. V.A. *Campos Sulinos: conservação e uso sustentável da biodiversidade*. MMA, Brasília, DF, p.101.

BERRETA, E. 2009. Algunos aspectos sobre la biodiversidad de los campos naturales. **Revista INIA Uruguay** 20: 21-25.

BOLDRINI, I.I. *et al*. 2010. Bioma Pampa: diversidade florística e fisionômica. EDU: Porto Alegre pp. 7- 15.

BURKART, A. 1975. Evolution of grasses and grasslands in South America. **Taxon** 24: 53-66.

CAMPOS, M. C. C; RIBEIRO, M. R.; SOUZA-JÚNIOR, V. S; RIBEIRO-FILHO, M. R & ALMEIDA, M. C. 2012. Soil-relief relationships in a grassland/forest transition in the region of Humaitá, Amazonas State, Brazil. **Acta Amazonia**, 42.

CCSP (U.S. Climate Change Science Program). 2008. The effects of climate change on agriculture, land resources, water resources, and biodiversity in the United States.

CONSTANZA, R. *et al.* 1997. The value of the world's ecosystem services and natural capital. **Nature** 387: 253-287.

COPLAND, B.J. & WHELAN, R. J. 1989. Seasonal variation in flowering intensity and pollination limitation of fruit set in four co-occurring *Banksia* species. **Journal of Ecology** 77: 509-523.

DEVOTO, M. & MEDAN, D. 2004. Effects of grazing disturbance on the reproduction of a perennial herb, *Cypella herbertii* (Lindl.) Herb. (Iridaceae). **Plant Systematics and Evolution** 243:165–173.

DÍAZ, S., ACOSTA, A. & CABIDO, M. 1994.Grazing and the phenology of flowering and fruiting in a montane grassland in Argentina: A niche approach. **Oikos**70: 287- 295.

ELZINGA. J.E. *et al.* 2007. Time after time: flowering phenology and biotic interactions. **Trends in Ecology and Evolution** 22: 432- 439.

ENCINAS-VISO, T. A. *et al.*2012.Phenology drives mutualistic network structure and diversity. **Ecology Letters** 15: 198–208.

FAEGRI, K. & VAN DER PIJL L. 1979.*The Principles of Pollination Ecology*. Pergamon Press, Oxford: 245 pp.

FEINSINGER, P., MURRAY, G., KISMAN, S. &BUSBY, W.H. 1986.Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. **Ecology** 67:449-464.

FELDMAN, T.S. *et al.* 2004.When can two plant species facilitate each other's pollination? **Oikos** 105:197- 207.

FIDELIS, A., BLANCO, C.C., MÜLLER, S.C., PILLAR, V.D. & PFADENHAUER, J. 2012. Short-term changes caused by fire and mowing in Brazilian Campos grasslands with different long-term fire histories. **Journal of Vegetation Science** 23: 552–562.

FIDELIS, A.T., MÜLLER, S.C., PILLAR VD & PFADENHAUER, J. 2010. Population biology and regeneration of forbs and shrubs after fire in Brazilian Campos grasslands. **Plant Ecology** 211: 107-117.

FORTUNA, .M.A. & BASCOMPTE, J. 2006 Habitat loss and the structure of plant–animal mutualistic networks. **Ecology Letters** 9: 278–283.

FORUP, M.L., HENSON, K.S.E., CRAZE, P.G. &MEMMOTT, J. 2008. The restoration of ecological interactions: plant-pollinator networks on ancient and restored heathlands. **Journal of Applied Ecololgy** 45:742–752.

FREITAS, L. & SAZIMA, M. 2006. Pollination biology in a tropical high-altitude grassland in Brazil: interactions at the community level. **Annals of the Missouri Botanical Garden** 93:465-516.

HEGLAND, S.J., GRYTNES, J.A. & TOTLAND, Ø. 2009. The relative importance of positive and negative interactions for pollinator attraction in a plant community. **Ecological Research** 24: 929-936.

JORDANO, P. *et al.* 2006. The ecological consequences of complex topology and nested structure in pollination webs. – In: Waser, N. M. & Ollerton, J. (eds), *Plant–pollinator interactions: from specialization to generaliza*tion. University of Chicago Press, pp. 173–199.

KEARNS, C.A. *et al.* 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. **Annual Review of Ecology and Systematics** 29: 83-113.

KOCHMER S. & HANDEL J.P. 1986. Constraints and Competition in the Evolution of Flowering Phenology. **Ecological Monographs** 56: 303- 325.

MOELLER, D.A., GEBER, M.A., ECKHART, V.M. & TIFFIN, P. 2012. Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. **Ecology** 93: 1036-1048.

MORELATTO, L.P.C. 2004. Phenology, sex ratio, and special distribution among dioecious species of *Trichilia* (Meliaceae). **Plant Biology** 6:491-497.

NEWSTRON, L.E. *et al*. 1994.A New Classification for Plant Phenology Based on Flowering Patterns in Lowland Tropical Rain Forest Trees at La Selva, Costa Rica. **Biotropica** 26:141-159.

NIELSEN, A. & TOTLAND, Ø. 2013. Structural properties of mutualistic networks withstand habitat degradation while species functional roles might change. **Oikos** 000: 001- 011.

OLESEN, J.M. & JORDANO, P. 2002. Geographic patterns in plantpollinator mutualistic networks. **Ecology** 83: 2416–2424.

OLLERTON, J. *et al.* 2011. How many flowering plants are pollinated by animals? **Oikos** 120: 321-326.

OVERBECK, G.E. *et al.* 2007. Brazil's neglected biome: the south braziliancampos. perspectives in plant ecology. **Evolution and Systematics** 9:101-116.

OVERBECK, G.E., MULLER, S.C., PILLAR, V.D. & PFADENHAUER, J. 2006. Floristic composition, environmentral variation and species distribution patterns in burned grassland in Southern Brazil. **Brazilian Journal of Biology** 66:1073-1090.

PILLAR, V.P. & VÉLEZ, E. 2010. Extinção dos Campos Sulinos em unidades de conservação: um fenômeno natural ou um problema ético**? Natureza e Conservação** 8: 84-86.

PINHEIRO, M. *et al*. 2008. Floral resources used by insects in a grassland community in Southern Brazil. **Revista Brasileira de Botânica** 31: 469- 489.

PODGAISKI, L.R. *et al*. 2013. Spider trait assembly patterns and resilience under fire-induced vegetation change in south brazilian grasslands. **PLoS ONE** 8: e60207.

POTTS, S.G. *et al*. 2010. Global pollinator declines: trends, impact and drivers. **Trends in Ecology and Evolution** 25: 345-353.

PRANCE, G.T. 1990. Management and conservation of tropical ecosystems requires knowledge of plant-animal interactions: afterword**. Memoirs of the New York Botanical Garden** 55:186-187.

QUADROS, F.L.F. & PILLAR, V.D. 2002.Transições floresta-campo no Rio Grande do Sul: Hipóteses sobre clima e interação fogo-vegetação. **Ciência & Ambiente** 24: 109-118.

SANTOS, T.G .& TREVISAN, R.2009. Eucaliptos versus bioma pampa: compreendendo as diferenças entre lavouras de arbóreas e o campo nativo In: Lavouras de Destruição: a (im)posição do consenso, pp. 299-332.

SPIESMAN. B.J.& ENOUYE, B.D.2013. Habitat loss alters the architecture of plant–pollinator interaction networks. **Ecology** 94: 2688–2696.

STILES, F.G. 1977. Co-adapted pollinators: the flowering seasons of hummingbirds- pollinated plants in tropical forest. **Science** 198:1177-1178

THÉBAULT, E.&FONTAINE, C. 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. **Science** 329: 853.

ULRICH, W., ALMEIDA-NETO, M. & GOTELLI, N.J. 2009. A consumer's guide to nestedness analysis. **Oikos** 118: 3-17.

WAGNER, A.P.L. *et al*. 2013. Tendências temporais de índices de vegetação nos campos do Pampa do Brasil e do Uruguai. **Pesq. agropec. bras.** 48:1192-1200.

WHITE, R. P., MURRAY, S., & ROHWEDER, M. 2000. **Grassland ecosystems** (p. 81). Washington, DC, USA: World Resources Institute.

CAPÍTULO I: Temporal segregation in flowering time and plant-pollinator interactions in a grassland community of Southern Brazil

Foto: *Bombus* sp. em flor de *Senecio heterotrichius*

Temporal segregation in flowering time and plant-pollinator interactions in a grassland community of Southern Brazil

Suiane Santos Oleques[1]; Gerhard Ernst Overbeck[2]; Rubem Samuel de Avila Jr[3]

1. Programa de Pós-graduação em Botânica, Laboratório de Estudos em Vegetação Campestre (LEVCamp), Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

2. Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

3. Laboratório de Estudos em Biodiversidade Pampiana (LEBIP), Universidade Federal do Pampa, Campus São Gabriel, RS Brazil

Correspondence author: suiane.bio@gmail.com, phone number: + 55 (55) 3232- 6075

Short-title: Flowering phenology in subtropical grassland

Abstract Synchrony of phenological events at the community level is a critical aspect for plants. Distinct phenological patterns, such as aggregated and segregated, act in the establishment of plant-pollinator interactions and can play a fundamental role in plant community structure. Working in grazed subtropical grasslands in southern Brazil, our goals were to (1) describe the plant-pollinators interactions in the grassland community, (2) investigate the phenological patterns, (3) evaluate the possible association between flowering synchrony of each plant species with pollinator richness and in the pollinator sharing, and (4) analyze the phenological pattern of species with distinct flower color. All 82 flowering species present in 12 study plots were observed monthly along six months. We used the Co-occurrence index and the Synchrony index to evaluate the phenological community pattern. Both indexes showed that the community presented segregated flowering, which a factor that may reduce pollinator competition. We found no significant relationship between flowering synchrony and similarity of pollinators, that is, plant species that flower for a long time or together with many species did not share more pollinators than plants that flower for short time and alone. Some guilds of flowers visitors preferred flowers with a particular color, which indicated the important role of visual traits in plant-pollinator interactions. This study is the first to report that flowering phenology could be structured by biotic components more than by random processes in these species-rich grasslands. In addition, we show that both the segregation of flowering time and morphological constraints are important to avoid pollinator competition between flowering plants.

Keywords: Plant-pollinator interactions, flowering phenology, grasslands, competition

Introduction

Time plays a fundamental role in plant reproduction, both for the dispersion of propagules and in the pollination process (Encinas-Viso *et al.* 2012). The reproductive phenology of plant species is an important factor for structuring biotic interactions with pollinators and, ultimately, for shaping evolutionary processes (Olesen *et al*. 2008). Even though many studies have focused on the role of abiotic factors, such as temperature, rainfall or light availability, and of phylogenetic constraints on phenological patterns (Kochmer & Handell 1986), biotic interactions have considerable importance for phenological features (Stiles 1977; Newstrom 1994; Elzinga *et al*. 2007).

Most plant species depend completely or partially on pollinators for reproduction (Faegri & van der Pijl 1979). The timing of the exposition of a plant's reproductive structures is of fundamental importance for the respective group of potential pollinators which, in turn, are directly associated with reproductive success of the plant. Plants can present many flowering individuals over a short period of time ("big-bang") or can flower for a long period in low intensities ("steady-state") (*sensu* Gentry 1974). These patterns are extremes of a gradient of flowering strategies defined by flowering intensity and flowering synchrony among individual plants at the population level or among plant species at the community level. This leads to different phenological patterns in the plant community. Concentration of flowering individuals in time results in an aggregated phenological pattern. Long and steady flowering of smaller numbers of individuals results in a scenario where species change their reproductive phases and present low flowering synchrony with others, resulting in segregated phenological pattern. If we consider pollinators to be a resource 'used' by plants and essential elements for their reproductive success, the patterns in the plant community should be expressed and at the same time determined by plant-pollinator interactions. However, interspecific interactions such as competition (e.g. Stiles 1977) or facilitation (e.g. Ratchke & Lacey 1985; Feldman 2004) among plant species may also contribute in shaping phenological patterns. Non-overlapping flowering periods could be advantageous as they decrease the competition effects. However, a high flowering time overlap not always indicates a competitive scenario. Ratchke & Lacey (1985) and Feldman (2004) suggested that facilitation interactions could be observed in some aggregated pattern of flowering time, resulting in an optimization of pollinator attraction by higher display of flowers at a given time. This kind of facilitation could lead to the convergence of phenological time in plant communities (Elzinga *et al*. 2007). Díaz *et al.* (1994), working in mountain grassland of Argentina, found no evidence that suggested the existence of systematic ecological forces leading to divergence in phenologies. However, most studies conducted on the community level (72%) found that flowering periods were distributed over time with a minimum interspecific overlap support the competition-pollinator hypothesis (Feinsinger *et al*. 1986).

Distinct phenological patterns of plants with different flower colors (Warren & Billington 2005) are indicators of the importance of specific interactions between pollinators and plants, and it is well established that pollinator diversity or the number of interactions established by a plant species are defined by different characteristics of the plant that act together, such as resource availability (quantity of nectar, pollen or oil), morphological traits (color, size and shape of flowers) and flowering time (Bosch *et al*. 1997). Robertson (1924) showed that species with greenish flower colors bloom before others along the year. Similarly, Warren & Billington (2005) found a significant association between flower color and flowering phenology. Besides, according to the concept of pollination syndromes, plants have characteristics that reflect their primary pollinators and flower color is a determinant trait in the range of pollinators groups (Faegri & Van der Pijl 1979).

Because of their high species richness (Overbeck *et al.* 2007), subtropical grasslands in southern Brazil are interesting systems for the understanding of phenological patterns and their relation to pollination. The goal of our study was to answer the following questions: i) Does a grassland community under seasonal subtropical climate present a clear phenological pattern in time? Our hypothesis is that the high seasonality found in South Brazilian grassland favours a temporal displacement in flowering events to avoiding pollinator competition in the short period of flowering of individual species. ii) Do plant species with longer flowering times and higher flowering intensities have greater pollinator richness? We expected that species with longer exposure periods could attract more pollinator species along the flowering season. iii) Does overlapping of flowering phases among plant species lead to higher similarity of their flower visitors? We hypothesized that flowering species overlap could lead to sharing flower visitors. iv) Do plants with different flower colors exhibit particular flowering phenological pattern? We expected that the species' phenology did not depend on flower color and that species with distinct flowers colors presented the same pattern at the community as a whole.

Material and Methods

Study area

The study was performed at the UFRGS Experimental Agronomic Station (EEA) in Eldorado do Sul, Rio Grande do Sul state, Brazil (30° 06" 08.68' S, 51° 40" 54.92' W), from September 2014 to February 2015. The area is part of the Brazilian Pampa biome and the specific site presented typical grasslands under cattle grazing (with different grazing intensities). We concentrated our study on the spring and summer months when flowering are much more intense than during the other half of the year (Pinheiro et al. 2008).

Flowering phenology and floral visitors monitoring

In 12 sample plots (100 m² each) all flowering plant species with visually attractive flowers (i.e., species from the Poaceae, Cyperaceae and Juncaceae were not considered) were recorded monthly during the period of six months. For each plant species, we noted abundance (number of individual blooming), flowering period (flower presence or absence) and flower visitors for each plant species. Per plot, each flowering species was observed for 15 minutes during three periods of day (9–12h, 12-14h and 15-17h). In addition to identification of all plants, specimen of all pollinators (when flower visitors contacted the flower reproductive structures) were collected and identified by help of the literature or by specialists on the group in question.

Statistical analysis

We evaluated the existence of community flowering phenology patterns by help of Co-occurrence Analysis (C-Score) using the Software EcoSim 7.0 (Gotelli & Entsminger 2005). The data was organized in a matrix where each plant species represented a row and each month a column and in which occurrence of flowering events was marked. The C-score is the number of checkerboard units for all unique pairs of species. In a community with any phenology pattern, the result should be significantly different than expected by chance. The observed C-score value was compared with the mean of C-score values simulated through 10.000 randomizations. When observed values were lower that the generated values, aggregated phenology pattern occur, and higher observed values indicate segregated phenology pattern (Gotelli & Graves 1996). In order to evaluate the importance of flowering intensity, i.e number of individual plants with open flowers along time, we calculated the Synchrony index (used as “S” at the community level and “*di*” in species level) (Freitas & Bolmgren 2008). The Sørensen index was calculated in order to compare the pollinator compositional similarity with the synchrony of the plant species. We used simple regression analysis to test the association between the variables flowering synchrony and pollinators similarity and flowering synchrony and pollinator richness (Zar 1989). In order to evaluate the flower visitors’ preference for plant species of certain color, we performed a Chi-square analysis.

Results

During the six months of the study, we found 82 flowering plants species from 26 families (Fig. 2). The families with higher number of species were: Asteraceae (22 spp.), Fabaceae (8 spp.), Rubiaceae (7 spp.), Iridaceae, Malvaceae and Verbenaceae (4 spp.). Of all species observed, 64 (78%) presented at least one interaction with flower visitors. We observed 165 morphospecies of flower visitors: Hymenoptera (bees: 22 morphospecies; wasps: 17 spp. and ants: 11 spp.), Diptera (61 morphospecies), Coleoptera (26 morphospecies), Lepidoptera (26 spp.), Blatodea (1 sp.) and Orthoptera (1 sp.). Insects with higher number of plant interaction were: Hymenoptera (35.76%), Diptera (25.8%), Lepidoptera (22.1%) and Coleoptera (15.9%). The plant species with the largest pollinator richness were *Vernonanthura nudiflora* (Less) H. Rob (38), *Aspilia montevidensis* (Spreng.) Kuntze (35), *Senecio leptolobus* DC. (34), *Baccharis crispa* Spreng. (33) and *Baccharis articulata* (Lam.) Pers. (30), all belonging to the Asteraceae (Fig.1).

There was a significant segregation in the peak of flowering phases. October was the month with the highest peak (highest number of species flowering intensively), with 26 species peaking in their phenology events (Fig.2). Both the C-Score and the

Synchrony index indicated a segregated phenology pattern for the plant community (C-Score$_{obs}$= 1.47 < C-Score$_{sim}$= 1.39, p < 0.001 e S = 0.068) (Fig. 3). The synchrony index calculated for each color group showed that plants with the same flower color also showed segregate patterns (Yellow group: 0.068, White group: 0.066, Pink group: S= 0.067 and Purple group= 0.068). We found that guilds of flower visitors had preference for plant species of certain color (Chi-square= 37.50, gl= 15, p= 0.001) (Fig.4).

There was a significant, although very low, association between pollinator richness and synchrony (*di*) of each species in community (R^2 = 0.08, F = 5.49, p = 0.02) (Fig. 5), while the association between similarity (Sørensen) and synchrony (*di*) was not significant (R^2 = 0.02, F = 1.82, p = 0.18) (Fig. 6).

Discussion

The high diversity of flower visitors found in this study corroborates the role of distinct taxonomic groups in pollination of flowering plants in grassland communities (Pinheiro *et al*.2008). Asteraceae was the family with higher flower visitor richness, which underlines the fact that many species of these groups are generalists regarding pollinators (Pinheiro *et al*. 2008; Torres & Galleto 2002).

The staggering of flowering phenology observed in our study increases the existence of a significant temporal segregation of flowering. We know that flowering time and period are fundamental parameters for the reproductive success of plant species (Ratchke & Lacey 1985). The plant species found in our community overlapped minimally concerning their peaks of phenology, and the pattern differed significantly from the null model. This result corroborates our hypothesis that the seasonality of the South Brazilian grassland favours a temporal displacement in flowering events to avoid pollinator competition in the short period of flowering of individual species. This nonrandom pattern may be selected by interspecific interactions, such as competition and sharing pollinators, and favour this temporal displacement in flowering events. Although other factors could be important in the determination of flowering time (Ratchke & Lacey 1985; Kochmer & Handell 1986; Aizen & Vázquez 2006), previous studies have demonstrated that competitive interactions are determinant and fundamental factors in some communities (Stiles 1977; Vázquez *et al*.2006).

Plants can reduce pollinator competition through a lot of strategies, among them, temporal segregation of phenological events, spatial segregation and evolution of distinct floral morphologies. Among various floral traits, flower color is a trait that promotes an easy visual recognition by pollinators and could maximize the chance of plant species receive conspecific pollen (Heinrich 1975; Waser 1978; Arnold *et al*. 2009). Analyses using pseudo-community simulation based on the observed data, as applied here, improve our understanding which biological process are important in structuring phenological patterns (Gotelli & Graves 1996). Here, the approach evidenced that the studied community presented a phenological structure as result of ecological interactions, possibly plant competition for pollinators.

The seasonality found in South Brazil grasslands and even more so in seasonal tropical areas (Croat 1975; Pinheiro *et al*. 2008) could reduce the length of the flowering period. Therefore, grasslands remain susceptible to selective forces related to flower visitors (Freitas & Sazima 2006; Pinheiro *et al.* 2008). In addition, in seasonal communities, species could be more susceptible to variation and to selective forces in the peaks of their reproductive events (Freitas & Sazima 2006). Our results did not support the hypothesis that higher reproductive effort and long flowering time had a positive association with more flower visitors. Although positive and significant, the low value of association between *di* and richness indicates that species with long and intense flowering phenology (higher *di* values) have no advantages in relation to species with short and weak one (low *di* values). This result could be explained by existence of other plant-pollinator restrictions, such as morphology and resource mismatches, only two of the many factors that govern the establishment of plant-pollinator interactions (Vásquez *et al*. 2009). The synchrony values associated to flowering intensity are good indicators of temporal niche overlap in communities (Freitas & Bolmgren 2008). Our prediction was that high synchrony values were associated with greatest pollinator similarity, which, however was not true and leads us to the question why species with high phenology overlap do not share flower visitors. Our results indicate that preference of flower visitors to color could be one important explanation, as found in other studies (Heinrich 1975; Arnold *et al*. 2009). Color is one of the diverse floral traits that could be related to occurrence or not of plant-pollinator interactions (Briscoe &Chittka 2001; Internicola *et al*. 2008). The results indicated a significant preference of some flower visitors in determined color group (Fig.4). Heinrich (1975) and Arnold *et al.* (2009) also found no association between color and phenology. These studies support the hypothesis that selective forces could favour flower with distinct colors in several periods of year (Heinrich 1975, Arnold *et al*. 2009). Furthermore foraging behavior could be associated with the resource offer at any given moment of time rather than an innate preference for certain color. However, flower traits such as color or other visual traits could contribute to plant-pollinator interactions in studied community. This is corroborated by our results that showed non-association between flowering synchrony (*di*) and pollinator similarity (*ps*) in community. Therefore, both temporal segregation of phenological events as well as morphological restrictions seem to be important strategies for plants to avoid pollinator competition in our system (Raguso & Willis 2002). In addition, we showed that color could play an important role for plant-pollinator interaction. Although some groups of pollinators showed a preference for a certain flower color, most plants species, with different colors, were visited by a great range of pollinator guilds that could be associated with a generalization of plant pollinator interactions in this community.

This study is the first to report that flowering phenology could be structured more by biotic components than by random processes in southern Brazilian grasslands. Studies with a network approach seem promising to explore distinctive ecological process, such as morphological matching or mismatching between plant and pollinators, and abundance in the relation of phenological structure of plant communities and of flower visitors in Brazilian Pampa grasslands.

Acknowledgements

We thank to G. Minervini for field work support, M. Pinheiro (bees), B.R Garcete-Barret (wasps), J. Duarte (flies), M. Morales (flies-Syrphidae), M. S. Pires (ants), N. Mega (butterflies) and D. Lucas (plants) for their help in the species identification. SSO received a Master scholarship from the Brazilian CNPq. This study was conducted in the context of the LTER/PELD Campos Sulinos, funded by CNPq (grant 403750/2012-1 to VDP) and received additional funding by CNPq (477618/2013-8 to GEO).

References

Aizen M.A., Vázquez D.P. (2006) Flowering phenologies of hummingbird plants from the temperate forest of southern South America: is there evidence of competitive displacement? *Ecography,* **29**:357-366.

Arnold, S.E.J., Le Comber, S.C., Chittka, L.(2009) Flower color phenology in European grassland and woodland habitats, through the eyes of pollinators.*Israel Journal of Plant Sciences,* **57**:211- 230.

Bosch J., Retana C.X., Cerdá X. (1997) Flowering phenology, floral traits and pollinator composition in a herbaceous Mediterranean plant community. *Oecologia,***109**:583- 591.

Briscoe A.D., Chittka L. (2001) The evolution of color vision in insects. *Annual Review of Entomology,* **46**:471-510.

Croat T.B. (1975) Phenological behavior of habit and habitat classes on Barro Colorado Island (Panama canal zone). *Biotropica*, **7**: 270-277.

Díaz S., Acosta A., Cabido, M. (1994) Grazing and the phenology of flowering and fruiting in a montane grassland in Argentina: A niche approach. Oikos, **70**: 287- 295.

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A.E., Bernasconi, G. (2007) Time after time: flowering phenology and biotic interactions. *Trends in Ecology and Evolution*, **22**: 432- 439.

Encinas-Viso T. A. et al. (2012) Phenology drives mutualistic network structure and diversity. *Ecology Letters,* **15**: 198–208.

Faegri K., Van Der Pijl L. (1979) *The Principles of Pollination Ecology*. Pergamon Press, Oxford: 245 pp.

Feldman, T.S.; Morris, W.F., Wilson, G.W. Feldman T.S. (2004) When can two plant species facilitate each other's pollination? *Oikos*, **105**:197- 207.

Feinsinger P., Murray G., Kisman S., Busby W.H. (1986) Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology*, **67**:449-464.

Freitas L., Bolmgren K. (2008) Synchrony is more than overlap: measuring phenological synchronization considering time length and intensity. *Brazilian Journal of Botany*, **31**: 721-724.

Freitas L., Sazima M. (2006) Pollination biology in a tropical high-altitude grassland in Brazil: interactions at the community level. *Annals of the Missouri Botanical Garden,* **93**:465-516.

Gentry A.H. (1974) Flowering Phenology and Diversity in Tropical Bignoniaceae. *Biotropica*, **6**:64- 68.

Gotelli N.J., Graves G.R. (1996) Null Models in Ecology. Smithsonian Institution Press, Washington D.C.: 360 pp.

Gotelli N.J., Entsminger G.L. (2005) EcoSim: Null models software for ecology.Version 7. Acquired Intelligence Inc. & Kesey-Bear. Jericho.

Heinrich B. (1975) Energetics of Pollination. *Annual Review of Ecology and Systematics*, **6**:139- 170.

Internicola A.I., Bernasconi, G., Gigord, L.D.B. (2008) Should food-deceptive species flower before or after rewarding species? An experimental test of pollinator visitation behavior under contrasting phenologies.*Journal of Evolutionary Biology*, **21**:1358–1365.

Kochmer S., Handel J.P. (1986) Constraints and Competition in the Evolution of Flowering Phenology. *Ecological Monographs,* **56**: 303- 325.

Newstrom L.E., Frankie G.W., Baker H.G. (1994) A New Classification for Plant Phenology Based on Flowering Patterns in Lowland Tropical Rain Forest Trees at La Selva, Costa Rica. *Biotropica,* **26**:141-159.

Olesen J., Bascompte J., Elberling H., Jordano, P.(2008) Temporal dynamics in a pollination network. *Ecology*, **89**:1573- 1582.

Overbeck G.E., Müller S.C., Fidelis A., Pfadenhauer J., Pillar V.D., Blanco C.C., Boldrini I.I., Both R., Forneck E.D. (2007) Brazil’s neglected biome: the south Brazilian campos. *Perspectives in Plant Ecology Evolution and Systematics*, **10**:81-90.

Pinheiro M., Abrão B.E., Harter-Marques B., Miotto S.T.S.(2008) Floral resources used by insects in a grassland community in Southern Brazil. *Revista Brasileira de Botânica,* **31**: 469- 489.

Rathcke B. and Lacey E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology, Evolution and Systematics*, **16**: 179-214.

Raguso R.A., Willis M.A. (2002) Synergy between visual and olfactory cues in nectar feeding by naive hawkmoths, *Manducasexta. Animal Behaviour,* **64**: 685-695.

Robertson C. (1924) Flowers and insects. *Botanical Gazette*, **78**:68–74

Stiles F.G. (1977) Co-adapted pollinators: the flowering seasons of hummingbirds-pollinated plants in tropical forest. *Science,* **198**:1177-1178.

Torres C., Galleto L. (2002) Are Nectar Sugar Composition and Corolla Tube Length Related to the Diversity of Insects that Visit Asteraceae Flowers? *Plant Biology*, **4**: 360-366.

Vázquez D.P., Bluthgen N., Cagnolo L., Chacoff N.P. (2009) Uniting pattern and process in plant-animal mutualistic networks: a review. *Annals of botany*, **103**:1445-1457.

Vázquez D.P., Chacoff N.P., Cagnolo L. (2009) Evaluating multiple determinants of the structure of mutualistic networks. *Ecology*, **90**: 2039–2046.

Warren J., Billington T. (2005) Flower color phenology in British mesotrophic grassland communities. *Grass and Forage Science*, **50**:332-336.

Waser N.M.(1978) Competition for Hummingbird Pollination and Sequential Flowering in Two Colorado Wildflowers. *Ecology*, **59**: 934-944.

Rathke B., Lacey E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, **16**:179-214.

Zar J.H. (I989) Biostatistical analysis Englewood Cliffs, NJ: Prentice-Hall.

Figures legends

Figure 1. Number of flower visitors (pollinator richness) observed for each plant species during months of study. The plants species is abbreviated with first letters of genus and epithet.

Figure 2. Phenogram with number of individuals blooming for each species found during six months of observations (September, October, November, December and February). The thick lines represent the flowering peak and dashed lines months where occur flowering decrease.

Figure 3. C-Score indexes calculated through the randomizations and C-Score observed (arrow). C-Score index observed is different and higher than C-Scores calculated indicating significant result ($p < 0.001$).

Figure 4. Graphic indicate the preference of some flowers visitor for species plants with certain flower colors.

Figure 5.Simple regression between variables pollinator richness (log N +1) and flowering synchrony (*di*). There was a significant, but low relation between analyzed variables.

Figure 6. Simple regression between pollinator similarity (Sorensen index) and flowering synchrony (*di*).The results indicate that no association between these variables.

Figure 1.

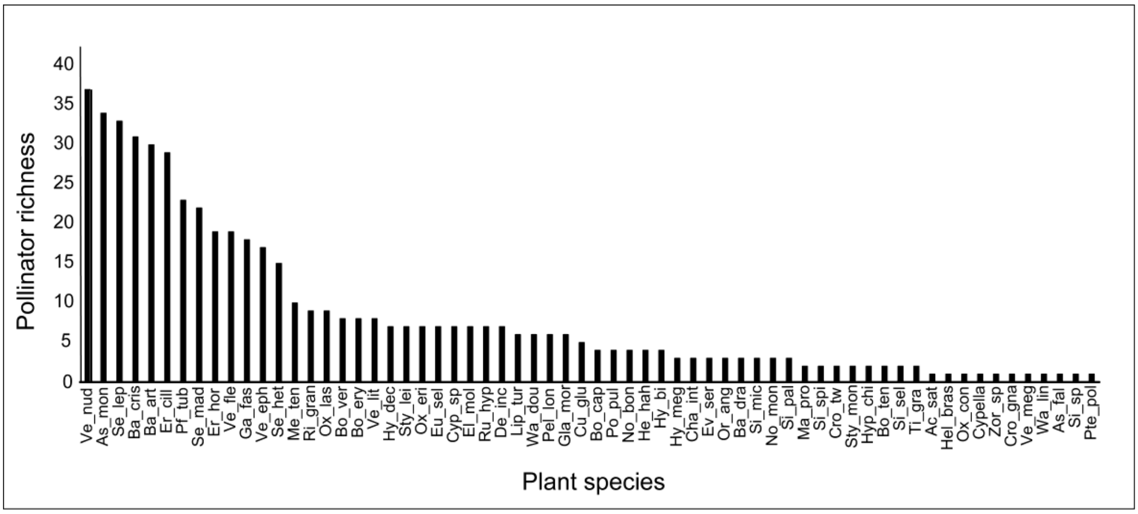

Pollinator richness
40
35
30
25
20
15
10
5
0
Ve_nud
As_mon
Se_lep
Ba_cris
Ba_art
Er_cill
Pf_tub
Se_mad
Er_hor
Ve_fle
Ga_fas
Ve_eph
Se_het
Me_ten
Ri_gran
Ox_las
Bo_ver
Bo_ery
Ve_lit
Hy_dec
Sty_lei
Ox_eri
Eu_sel
Cyp_sp
El_mol
Ru_hyp
De_inc
Lip_tur
Wa_dou
Pel_lon
Gla_mor
Cu_glu
Bo_cap
Po_pul
No_bon
He_hah
Hy_bi
Hy_meg
Cha_int
Ev_ser
Or_ang
Ba_dra
Si_mic
No_mon
Si_pal
Ma_pro
Si_spi
Cro_tw
Sty_mon
Hyp_chi
Bo_ten
Si_sel
Ti_gra
Ac_sat
Hel_bras
Ox_con
Cypella
Zor_sp
Cro_gna
Ve_meg
Wa_lin
As_fal
Si_sp
Pte_pol
Plant species

Figure 2.

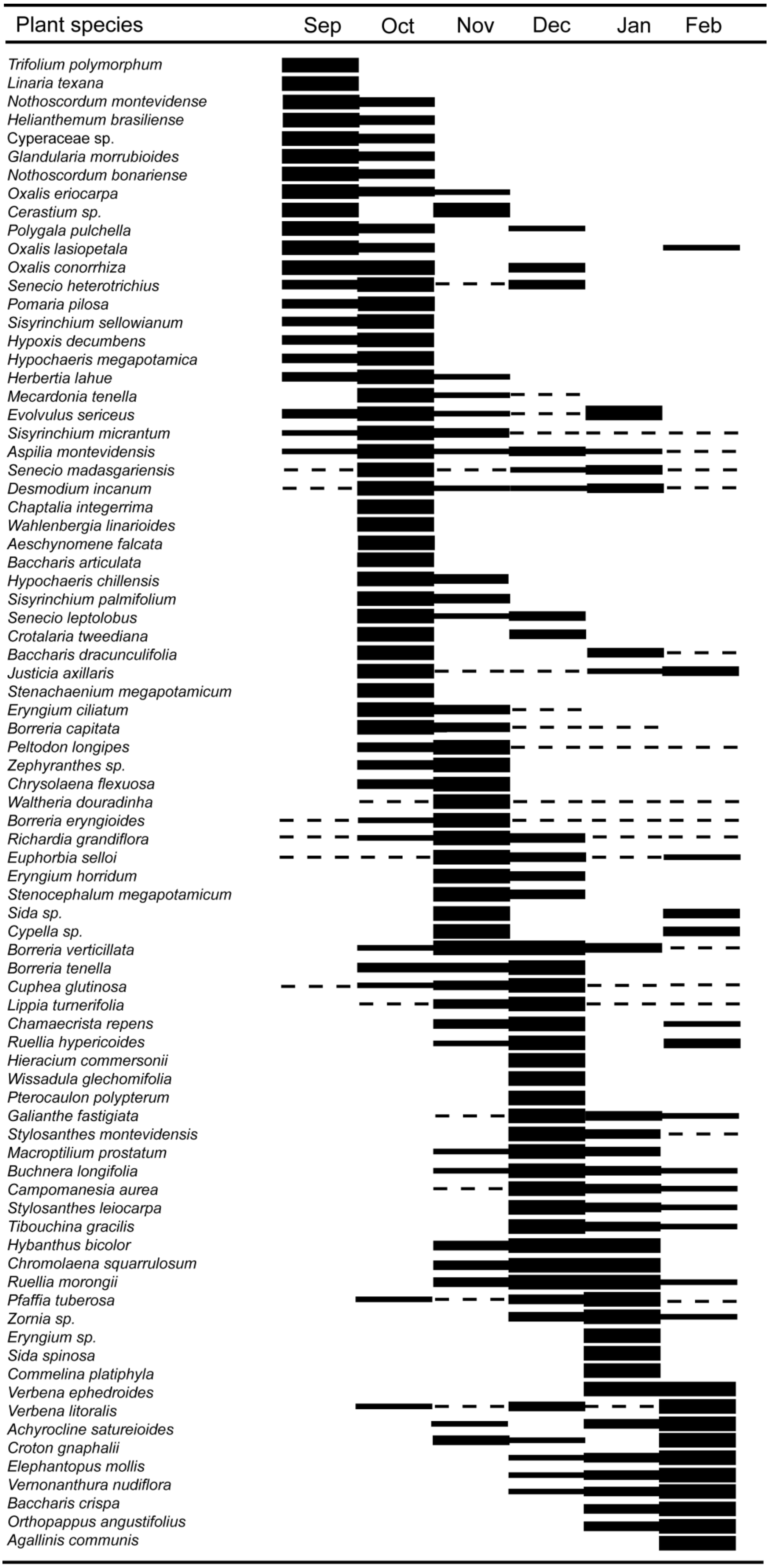

Plant species
Sep
Oct
Nov
Dec
Jan
Feb
Trifolium polymorphum
Linaria texana
Nothoscordum montevidense
Helianthemum brasiliense
Cyperaceae sp.
Glandularia morrubioides
Nothoscordum bonariense
Oxalis eriocarpa
Cerastium sp.
Polygala pulchella
Oxalis lasiopetala
Oxalis conorrhiza
Senecio heterotrichius
Pomaria pilosa
Sisyrinchium sellowianum
Hypoxis decumbens
Hypochaeris megapotamica
Herbertia lahue
Mecardonia tenella
Evolvulus sericeus
Sisyrinchium micrantum
Aspilia montevidensis
Senecio madasgariensis
Desmodium incanum
Chaptalia integerrima
Wahlenbergia linarioides
Aeschynomene falcata
Baccharis articulata
Hypochaeris chillensis
Sisyrinchium palmifolium
Senecio leptolobus
Crotalaria tweediana
Baccharis dracunculifolia
Justicia axillaris
Stenachaenium megapotamicum
Eryngium ciliatum
Borreria capitata
Peltodon longipes
Zephyranthes sp.
Chrysolaena flexuosa
Waltheria douradinha
Borreria eryngioides
Richardia grandiflora
Euphorbia selloi
Eryngium horridum
Stenocephalum megapotamicum
Sida sp.
Cypella sp.
Borreria verticillata
Borreria tenella
Cuphea glutinosa
Lippia turnerifolia
Chamaecrista repens
Ruellia hypericoides
Hieracium commersonii
Wissadula glechomifolia
Pterocaulon polypterum
Galianthe fastigiata
Stylosanthes montevidensis
Macroptilium prostatum
Buchnera longifolia
Campomanesia aurea
Stylosanthes leiocarpa
Tibouchina gracilis
Hybanthus bicolor
Chromolaena squarrulosum
Ruellia morongii
Pfaffia tuberosa
Zornia sp.
Eryngium sp.
Sida spinosa
Commelina platiphyla
Verbena ephedroides
Verbena litoralis
Achyrocline satureioides
Croton gnaphalii
Elephantopus mollis
Vernonanthura nudiflora
Baccharis crispa
Orthopappus angustifolius
Agallinis communis

Figure 3.

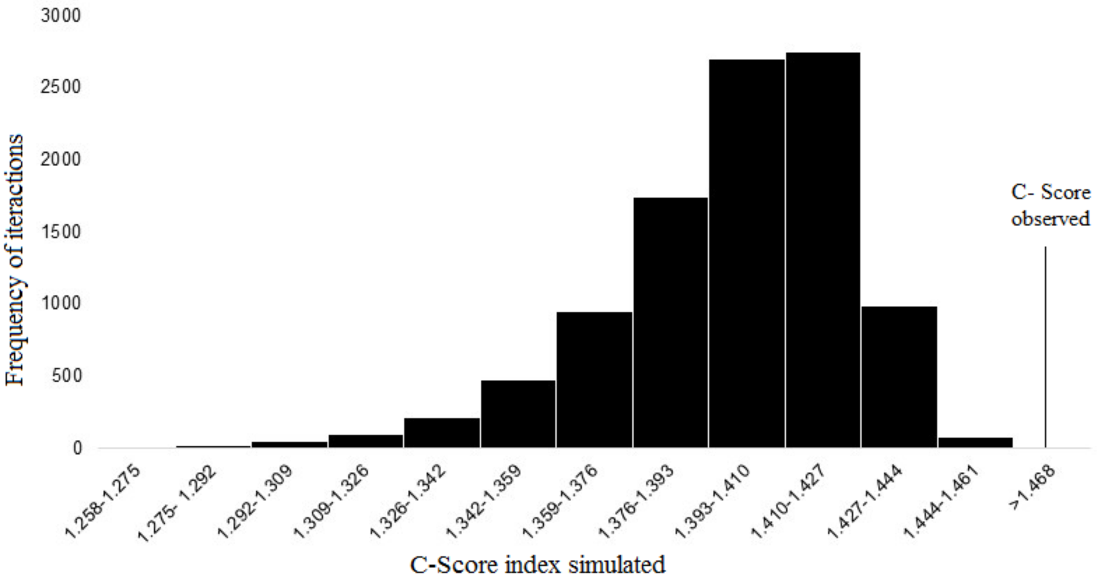

3000
2500
2000
1500
1000
500
0
Frequency of iteractions
C- Score
observed
1.258-1.275
1.275- 1.292
1.292-1.309
1.309-1.326
1.326-1.342
1.342-1.359
1.359-1.376
1.376-1.393
1.393-1.410
1.410-1.427
1.427-1.444
1.444-1.461
>1.468
C-Score index simulated

Figure 4.

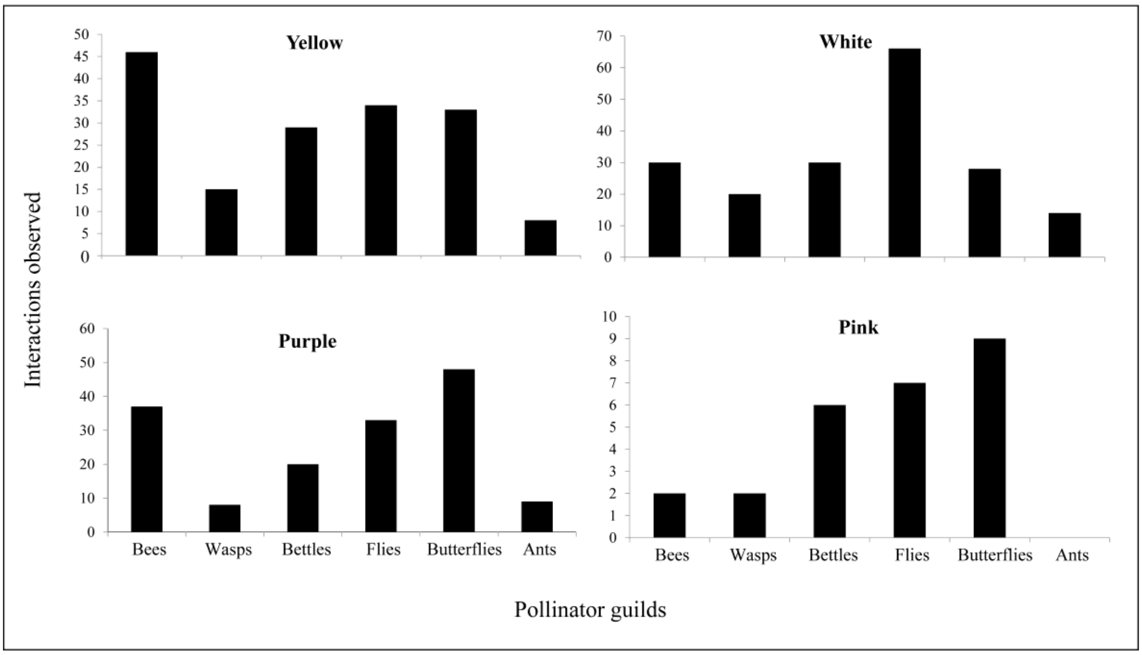

Yellow
50
45
40
35
30
25
20
15
10
5
0
White
70
60
50
40
30
20
10
0
Interactions observed
Purple
60
50
40
30
20
10
0
Pink
10
9
8
7
6
5
4
3
2
1
0
Bees
Wasps
Bettles
Flies
Butterflies
Ants
Bees
Wasps
Bettles
Flies
Butterflies
Ants
Pollinator guilds

Figure 5.

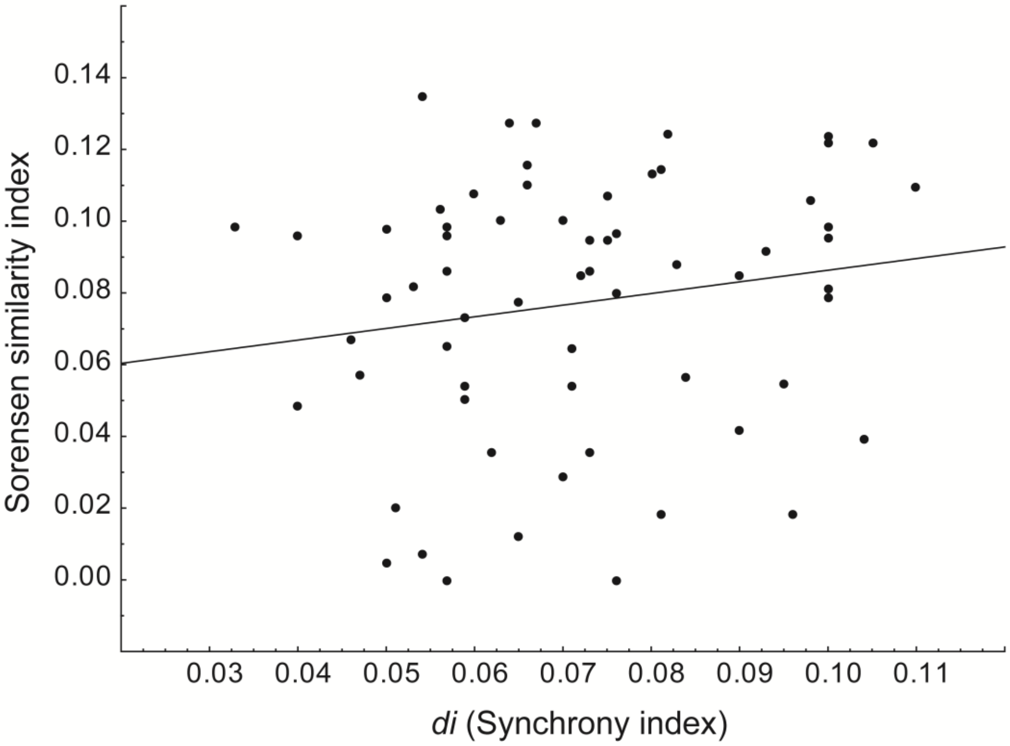
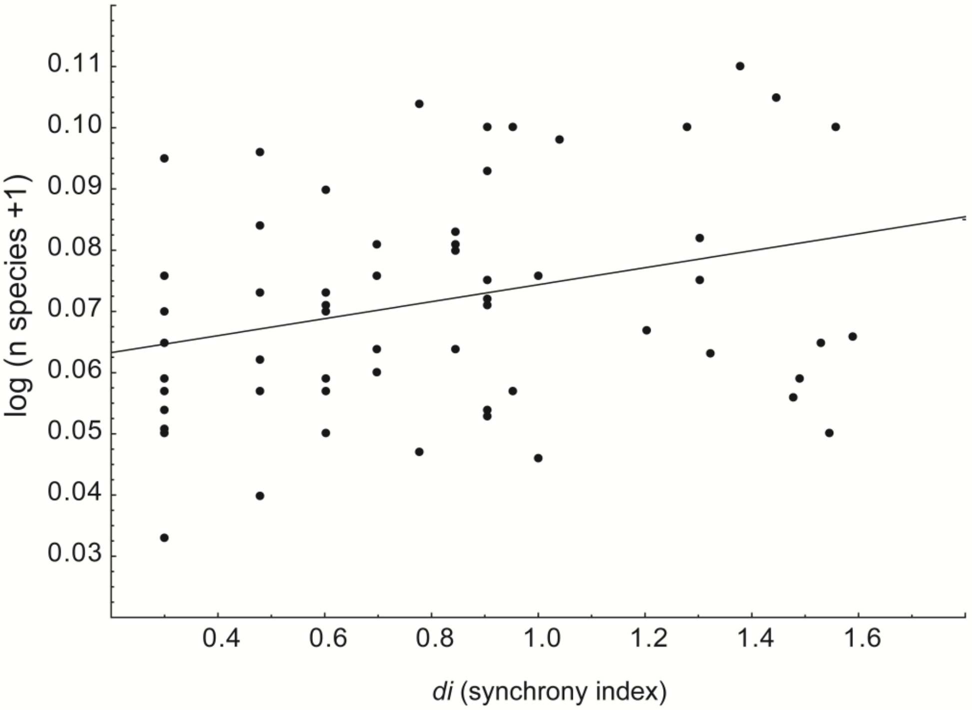

0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Sorensen similarity index
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.11
di (Synchrony index)

Figure 6.



0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
log (n species +1)
0.4
0.6
0.8
1.0
1.2
1.4
1.6
di (synchrony index)

CAPÍTULO II: Does grazing intensity influence interactions patterns of a plant-pollinator network in a subtropical grassland?

Foto: Curculionidae em *Eryngium horridum*

Does grazing intensity influence interactions patterns of a plant-pollinator network in a subtropical grassland?

Suiane Santos Oleques[1]; Jeferson Vizentin-Bugoni[2]; Gerhard Overbeck[13]

1. Programa de Pós-graduação em Botânica, Laboratório de Estudos em Vegetação Campestre (LEVCamp), Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
2. Programa de Pós-graduação em Biologia Vegetal, Universidade Estadual de Campinas, Campinas, SP, Brazil
3. Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Correspondence author: suiane.bio@gmail.com, phone number: + 55 (55) 9676-6748

Summary

a.i.1. Here we studied the structure of plant–pollinator networks along a gradient of cattle grazing intensities in a South Brazil grassland. We hypothesized that networks should be more complex at both ends of the disturbance gradient due to the high dominance of ruderal, unpalatable and generalist species of Asteraceae that attract a wide range of pollinators.

a.i.2. We tested how network metrics changes along the disturbance gradient and the relative importance of processes such as morphological traits, phenology and abundance to predict network structure and interactions.

a.i.3. Of the six metrics evaluated, only connectance and interaction eveness responded significantly. This indicates that most network attributes were preserved along the disturbance gradient in our system and that generalist species were of high importance. Our study provides evidence that abundance plays a fundamental role in network organization in Southern Brazil grassland. Finally, we clarify the role of some dominant Asteraceae plant species that are avoided by cattle, attract a wide range of pollinators and thereby help structure and add resilience to plant-pollinator interactions in these grazing communities.

Synthesis: Plant-pollinator networks changed little along the gradient of grazing intensity, and these interactions thus show high resilience in response to grazing activity. In grassland communities of Southern Brazil, plant-pollinator interactions apparently do not serve as indicator for grassland conservation or degradation. *Key-words*: Grazing management - land use - intermediated disturbance -network interactions – ruderal species

Introduction

Pollination is an important process in the assembly of plant communities. It contributes to maintenance of diversity of both plants and pollinators and influences the species spatial distribution, richness, abundance and phenology (Costanza *et al.* 1997; Potts *et al.* 2010; Moeller *et al.* 2012). At the community level, the variation in diversity or specificity of mutualistic interactions emerges as a complex *network*, specifying who pollinates whom, and who provides resources for whom (Olito & Fox 2015). The analysis of networks between flowering plants and their pollinators has been widely used to understand plant-pollinator relationships at community level and to detect changes in pollination processes along ecological gradients (Basilio *et al.* 2006; Bascompte & Jordano 2007; Nielsen & Bascompte 2007). Pollination interaction networks often exhibit highly conserved structural properties such as nestedness, skewed degree distribution and asymmetry in the strength of interactions (Jordano *et al.* 2003; Vázquez *et al.* 2009; Olito & Fox 2015). These patterns may be driven by neutral (abundance) and niche-based (i.e. morphology and phenology traits) processes. However, in any given network, these processes are not exclusive, and factors such as abundance, species traits, phenology and phylogeny may act mutually on the structure of ecological networks (Verdú & Valiente-Banuet 2011; Vizentin-Bugoni *et al.* 2014).

Several structural properties, i.e.,properties that can be described by network metrics, are known to confer stability to plant-pollinator networks (Tscharntke & Tylianakis 2010; Thébault & Fontaine 2010). For instance, connectance indicates higher generalization levels and redundancy of connections in network (Bascompte *et al.* 2003) while nestedness, i.e. pattern emerging when specialists interact only with generalists, and generalists interact also among themselves, may be linked to higher resilience (Okuyama & Holland 2008). Assessment of these and other metrics, such as interaction evenness, complementary specialization and web asymmetry (Kaiser-Bunbury & Blüthgen 2015), are useful to describe pattern of interactions among assemblages and are especially relevant in a conservation context, because some of these prevalent patterns may be linked to responses to landscape use or habitat fragmentation (Nielsen & Totland 2014; Klein *et al.* 2007; Spieman 2013).

Disturbances are important processes in many natural systems, and particularly in tropical and subtropical grasslands that evolved under fire and grazing pressures (Cingolani *et al.* 2005; Veldman *et al.* 2015). Grazing and fire at intermediate levels often increases plant community diversity and complexity, i.e species richness, composition and vegetation heterogeneity (Overbeck *et al.* 2005), and both processes also affect plant phenology, abundance, composition and diversity of species (Grant *et al.* 1996; Jones, Lawton 1989; Pykala 2004). Indirectly, these disturbances may affect other trophic levels, such as soil organisms (Neilson *et al.* 2002), spiders (Podgaiski *et al.* 2013) and birds (Baker & Guthery 1990; Develey *et al.* 2008), but also pivotal ecological processes such as pollination (Vázquez & Simberloff 2004; Lázaro *et al.* 2015).

The specific effects of grazing on plant–pollinator interactions should depend on grazing intensity, however this hypothesis remains poorly investigated. A recent study in Mediterranean garrigue supported the idea that at intermediate levels of grazing, generalization, diversity, number of links and evenness of interactions are highest, which can be interpreted as indicative of high community stability (Lázaro *et al.* 2015). However, this relationship may not be true in other grazed systems. Contrasting to these findings, for instance, lower diversity and generalization at intermediate grazing levels have been found in a pollination network in Mongolian steppes (Yoshihara *et al.* 2008). A potential explanation may be differences in species richness between both networks as well as distinct effects of different grazing animals, depending also on their role in the evolution of the system (Lázaro *et al.* 2015). Likely, differences in productivity should also be important, as they have been shown to be important drivers of the response of the plant community to disturbances (Lezama *et al.* 2013).

Here, we investigated how and in which extent grazing intensity by cattle influences plant-pollination networks in Pampa grasslands in southern Brazil. In this region, high grazing levels leads to vegetation dominated by low growing grasses, which form a short sward, ruderal species and an increase in abundance of unpalatable Asteraceae species (e.g. from the genera *Vernonanthura* H. Rob. and *Senecio* L.), besides favoring small flowering forbs (e.g. *Mercadonia* Ruiz & Pav., *Oxalis* L. and *Euphorbia* L.) (Boldrini & Eggers 1996). On the other hand, low grazing causes dominance of tussock grasses and shrubs, such as of the genus *Baccharis* L. (Overbeck *et al.* 2007). Some studies have shown that plant species richness is highest at intermediate levels of grazing in this region, where we can observe co-occurrence of species from different functional groups and a decrease in number of dominant and ruderal species (Boldrini & Eggers 1996; Pillar & Focht 2010). This increase in plant diversity at intermediate grazing levels also is beneficial for livestock cattle performance, hence the management strategies focused in maintenance of intermediated grazing levels (Milchunas *et al.* 1988; Soares *et al.* 2005). However, a considerable portion of the increase in plant diversity is related to the grass component (i.e coexistence of tussock grasses and rhizomateous species), non-pollinator dependent species, and the real effect of intermediate grazing on plant pollinator interactions remains unknown.

Based on previous findings of the effect of disturbances on the structural properties of plant-pollinator networks in different ecosystems around the world, we hypothesized that networks should be more complex at both ends of the disturbance gradient (in our case, grazing) because: 1) the high dominance of ruderal, unpalatable species of Asteraceae together with the occurrence of small pollinator dependent herbs enhance the complexity of plant-pollinator network at high grazing levels and 2) the high dominance of abundant and generalist *Baccharis* species promote higher network generalization and pollinator diversity at low grazing levels. In our case, network complexity should be related to larger networks that are more connected, show higher nestedness, higher modularity and are more generalized.

Secondly, we investigate if there were any changes in the dominant processes that structure the networks across the gradient. We hypothesized that species abundances should be the most important factor determining plant-pollinator interactions at low grazing intensity or grazing exclusion. This should be true to the high dominance of some abundant and generalist Asteraceae species, mainly shrubs, that attract a wide range of pollinators at low or no grazing, and due to the increases in the abundance of ruderal Asteraceae species and small insect-pollinated herbs at high grazing intensities. At intermediate grazing levels, on the other hand, processes such as morphology match are expected to play an important role due increase functional diversity (floral traits) promoted by higher plant diversity (Fontaine *et al.* 2006). Finally, we hypothesized that flower phenology of this community could also influence response to grazing, together with other processes. Although the grassland we worked in presents a segregated flowering pattern, plants that flowered for a long time did not have more interactions than shorter flowering ones (Oleques *et al.* accepted). This suggests that other factors, such as abundance or morphology could determine the plant-pollinator interactions in the studied community.

Material and Methods

Study area and grazing intensities

The study was performed at the Agricultural Experimental Station (EEA) of the Universidade Federal do Rio Grande do Sul, Eldorado do Sul, Rio Grande do Sul state, Brazil, from beginning of September to end of February of 2014 (spring and summer season). This region of southern Brazil presents a subtropical climate and is characterized by dominance of grassland vegetation (Overbeck *et al.* 2007). Livestock is one of the main economic activities in the region and has an important role for conservation and maintenance of grasslands and their biodiversity (Nabinger *et al.* 2000). The EEA historically has native grassland managed under different herbage allowance (availability of dry forage per kg animals) and grazing intensities.

We allocated twelve sampling plots with 100 m² (minimum distance between plots: 100m) situated grazing gradient ranging from overgrazed sites to sites in an ungrazed area that has not been grazed by livestock for at last 10 years. Sites with leafcutter ants were avoided. For our gradients, we made use of a long-term experiment of different grazing intensities (Nabinger *et al.* 2009). Location of each plot along the gradient was determined by evaluation of mean value of vegetation height in each sample area. This variable is known to be highly correlated to grazing intensity and plant diversity: increase in mean height is a consequence of decreasing grazing pressure that results in higher plot-scale plant richness and functional diversity (Ferreira-Abreu 2014). After a visual pre-selection of sites, we measured vegetation height at 25 points within each, ordered them according to mean height and then selected twelve plots better scattered along the grazing intensity gradient.

Sampling of Plant-pollinator interactions

In the 12 sampling plots, all flowering plant species with visually attractive flowers (i.e., species from the Poaceae, Cyperaceae and Juncaceae were not considered) were recorded monthly during a period of six months. For each plant species, we measured flower abundances (total number of flowers), flowering phenology (flower presence or absence) and flower visitors for each plant species. Per plot, each flowering species was observed for 15 minutes during three periods of day (9–12h, 12-14h e 15-17h). In addition to identification of all plants, specimen of all potential pollinators (i.e. flower visitors who contacted the reproductive structures of the flowers) were collected and identified with specific literature or by specialists of each group.

Phenology, abundance and morphology of plants and pollinators

Sampling was conducted monthly for six months, summing up to six sampling days per plot. In each plot, we assigned presence/absence of flowers to each plant species as phenology and estimated the number of individuals per flowering species as the number of open inflorescences (single flower or capitulum). The total number of inflorescences was considered as the abundance of each plant species, as the total number of flowers produced and the number of individuals are correlated strongly and linearly and because flower abundance (not abundance of plants per se) matter from a pollinator perspective (Vázquez *et al.* 2009; Vizentin-Bugoni *et al.* 2014; Olito & Fox 2015). Pollinator phenology was assessed by presence/absence of each morphotype during each month and abundance as the frequency of species occurrence in each plot along the study.

In order to construct a morphology coupling matrix, we measured corolla tube length for plants and potential pollinator's tongue length for insects, using two to 10 individuals per species. We classified species from both groups into three length classes, respectively (Olito & Fox 2015): Plants - 1) open corolla (up to 1 mm), 2) small (1 to 5 mm) and 3) large corolla tube length (more than 5 mm). For pollinators - 1) without long mouth apparatus (up to 1 mm), 2) small mouth apparatus (1 to 5 mm) and 3) large mouth apparatus (more than 5 mm). Based on this, we classified interactions into the following morphology match categories: 1) possible, but improbable (plants with large corolla and pollinator without proboscis or tongue), 2) possible (plants with small corolla and pollinator without proboscis or tongue, or plants with large corolla and pollinator with small proboscis or tongue), and 3) adequate match (plants and pollinator of corresponding classes).

Analyses of the plant-pollinator network structure and structuring processes

All the statistical analyses reported here were conducted in R 2.15.3 (R Development Core Team 2008). In order to test if grazing changes the structure propriety of the networks, we calculated: nestedness, connectance (C), modularity (QuabiMo), specialization (H'2), interaction evenness (IE), besides the number of species and links (See more details in Vázquez *et al.* 2009; Vizentin-Bugoni *et al.* 2014). At the species level, we calculated species strength (s strength) to identify the keystone species within each networks. The observed metric values were related to vegetation height which we used as a proxy for ‘grazing intensities’, in order to test their distributions along to disturbance gradient. We used Akaike criteria to test linear and quadratic curve fitting models (AICc, corrected AIC for small sample sizes), selecting models with the lowest AICc values.

For each sample plot, we created a quantitative matrix containing all observed pair-wise insect–plant interactions, containing potential pollinator species in lines (*i*), plants species in columns (*j*) and number of visits in cells (*ij*). Also, three probabilistic matrices were created: a matrix where the abundance of each plant species was multiplied by the abundance of each flower visitor (A), another matrix based on phenological overlap, where the cell was filled by the number of months that each plant and pollinator co-occurred along the study (P) and another matrix based on the morphological match, considering the three coupling categories cited (M). All matrices were created for each sample plot representing different grazing intensity along the vegetation height gradient.

In order to evaluate whether abundances, phenological overlap or morphological match were able to predict the observed frequencies of interactions change along the grazing gradient, we used the framework proposed by Vázquez *et al.* (2009) and adapted by Vizentin-Bugoni *et al.* (2014). First, the probabilistic matrices based on abundances (A), phenological overlap (P) and morphology coupling (M) and all possible combinations among them (AM, AP, PM and APM) were produced and then normalized, i.e. division of cells values by the matrix sum (total number of observed interactions). Then, we used a likelihood approach to relate the observed matrix of each plot with each model, i.e. probability matrices. Finally, we used the Akaike information criteria (AIC) to evaluate the prediction ability of each model and then ΔAIC to find the model with the best prediction ability in each plot along the gradient.

Results

Plants and pollinator diversity

A total of 3443 links were observed among 165 morphospecies of potential pollinators and 64 flowering plant species (Fig.1 and supplements): Hymenoptera (bees: 22 morphospecies; wasps: 17 spp. and ants: 11 spp.), Diptera (61 morphospecies), Coleoptera (26 morphospecies), Lepidoptera (26 spp.), Blatodea and (1 sp.) Orthoptera (1 sp.). The plant families with highest numbers of species were: Asteraceae (22 spp.), Fabaceae (7 spp.), Rubiaceae (6 spp.) and Verbenaceae (4 spp.). The plant species with the largest pollinator richness were *Vernonanthura nudiflora* (38 spp.), *Aspilia* *montevidensis* (35 spp.), *Senecio leptolobus* (34 spp.), *Baccharis crispa* (33 spp.) and *Baccharis articulata* (30 spp.), all belonging to Asteraceae family. *A. montevidensis* was the most important species, based on the s strength values, for seven plots (Table I) and *B. crispa*, *B. articulata* and *V. nudiflora* were the most important in the other five plots. With exception of *Eryngium horridum* (Apiaceae), all the key-species recorded belonged to Asteraceae family.

Flowering period was relatively similar for the majority of plants (± 2.0 months), with only six species (9%) flowering for more than three months and 10 species (15.6%) flowering only one month. Flower abundance varied among species with 23 species (35.9%) produced more than 100 flowers, and only three species (4.68%) produced more than 1000 flowers (*B. articulata*- 1100 flowers, *B. crispa* -1824 flowers, *V. nudiflora* -1117 flowers). From the 165 pollinators recorded, only 25 (14.8%) had more than 10 individuals and 12 had more than 50 individuals in total. The most abundant insects were *Apis mellifera* (224 individuals) and *Camponotus blandus* (172 individuals). The most abundant plant species and pollinators changed along the gradient of disturbance (Table II). Most pollinators were rare with occurrence in only one month and only 30 morphospecies (17.85%) occurred more than two months. With regards to flower morphology, 27 plant species (42%) did not present a corolla tube. Only few plant species had a mean corolla length longer than 10 mm (*Ruellia hypericoides* - 20 mm, *Ruellia morongii* - 14 mm, *Stenachaenium megapotamicum* - 12.15 mm, *Glandularia morrubioides*- 11.9 mm, *Senecio heterotrichius* - 10.43 mm, *Richardia grandiflora* - 10.25 mm). Among pollinators, only seven species had proboscis greater than 10 mm: *Heraclides austrialis* – Nymphalidae (20 mm), *Euryades coretrhus* - Papilionidae(16.5 mm), *Agraulis vanillae* - Nymphalidae (15 mm), *Hylephyla phyteus* - Hesperiidae (15 mm), *Polistes vibex catilina* - Hesperiidae (13.6

mm), *Urbanus sp.* – Hesperiidae (12 mm) e *Vanessa braziliensis*- Nymphalidae (11 mm).

Grazing gradient and networks structure

In general, networks showed low values of connectance (0.10 ± 0.01; mean ± standard error, respectively), specialization (0.62 ± 0.03) and interaction evenness (0.52 ± 0.05). The nestedness values present a larger variation (from 6.3 to 17.4) than modularity (from 0.14 to 0.59). The largest networks had 88 species (26 plant species and 61 pollinators) and the smallest had 36 species (12 plant species and 26 pollinators). The largest numbers of links were found in a network at an intermediate position along the grazing gradient (565 links) and in a network with absence of grazing (404 links; Table III).

Most networks attributes, such as number of links and species, modularity, specialization (H’2) and nestedness did not change in response to the grazing gradient. However, connectance was highest at the extremes of the gradient (R= 0.42, p=0.03, Fig.2) and interaction evenness decreased at low grazing intensities (R=0.41, p= 0.02; Fig. 3) (Table IV).

Processes affecting network proprieties

Regardless grazing intensity, species abundance was the best predictor (lower values of Aic) of the frequency of interaction among plants and pollinators, with all models including abundance alone performing better than the ones including phenological overlap and morphological matching (Table V). Indeed, there was a significant association between the probability of interaction within the abundance matrix and the frequency of observed interactions (Fig.4), indicating that abundance was also a good predictor of the interaction frequency between plants and pollinators.

Discussion

According to our predictions, some network attributes changed in response to grazing intensities indicating more complexity at the extremes of gradient. However, only connectance and nestedness, but not specialization, modularity, interaction evenness, number of links and species were significantly affected. This indicates that most network attributes were preserved along the disturbance gradient in our system and could indicate the high resilience of plant-pollinator interactions related to grazing disturbance. We also showed that the most important determinants of the network structure remains unaffected by grazing and that species abundances played a fundamental structuring role in these plant-pollinator networks. Finally, we clarify the role of some plant groups that are avoided by cattle and thereby contribute to the maintenance and structure of plant pollinator interactions in grazing communities.

A steraceae was the richest plant family in our grassland, corresponding to the importance of this family in South Brazilian grasslands in general. Asteraceaen species were visited by a wide range of pollinators, and, additionally, most of the species with high ‘s strength values’ belonged to the Asteraceae. Several studies have shown the importance of Asteracaeae for resource availability to pollinators, and consequently with the highest number of interactions (Torres & Galleto 2002; Antonini & Martins 2003; Pinheiro *et al.* 2008). Therefore, Asteraceae, besides being one of most abundant families of angiosperms in grassland communities (Boldrini 1997; Matzenbacher 2003), represent an important group regarding resource availability for pollinators in South Brazilian grassland. Attractiveness of Asteraceaen flowers for floral visitors are well known: they have inflorescences with a large number of flowers, small floral tube size and presentation of secondary pollen, i.e., they allow free access to the resources (nectar and pollen) to a wide range of floral visitors (Proctor *et al.* 1996; Torres & Galleto 2002; Pinheiro *et al.* 2008).

Considering the potential pollinators, *Apis mellifera* (non-native honeybee) and *Camponotus blandus* (ant) were the most abundant species in the community. Honeybees are known for their high abundance because they form extremely long lived-societies with activity along all seasons (Westernkamp 1991). They also present a very generalist foraging and extremely competitive behavior that could lead to the displacement of native bee populations (Aizen & Feisinger 1994); the fact that they here were the most abundant species of pollinators is emblematic for the risks posed by this exotic species. Ants were, in addition to bees and wasps, another important group of animals. There is a high number of plant species whose flowers are visited by ants (García *et al.* 1996), including in grassland communities (Herreta *et al*. 1984). The role of ants as effective pollinators is contradictory and poorly understudied. Some studies have showed that ants present antibiotics which may be detrimental to pollen and that they also could steal nectar from flowers, causing damage and reducing the likelihood of later pollinators' visits (Dutton & Frederickson 2012). On the other hand, ants could act with effective pollinators of some ant-pollinated species and contribute to increase fruit set and enhance the reproductive success (Gómez 2000; Ashman & King, 2005; Blancafort & Gómez 2005). In our study system, ant species were related mainly to *Baccharis* species and further studies should be conducted in order to test the effectiveness of them as pollinator. Altogether, the most important pollinator species, based on their ‘s strength’ values, varied along the gradient of disturbance, but all species are associated with Asteraceae species.

Grazing gradient and network structure

n general, the studied grassland showed intermediated values of nestedness and specialization and low values of modularity and connectance. Interaction evenness reflects the homogeneity of interaction frequencies across all links in the network. Then, the highest interaction evenness values found at high grazing areas reflected more uniform spread of interaction among the species in the community. (Kaiser-Bunbury & Blüthgen 2015). At the other extremes of the gradient, a small number of dominant species concentrate a high number of interactions while the other species are less abundant and visited decreasing the interaction evenness values. The intermediated values of specialization associated with low modularity and connectance suggests a high generalization within the network, where the species are less dependent of a particular pollinator. This makes the network less vulnerable to species losses (Kaiser-Bunbury & Blüthgen 2015).

The higher values of connectance at the extremes of the gradient suggest that the high dominance of ruderal, unpalatable species of the Asteraceae, such as *Senecio* and *Vernonanthura*, both genera that are rejected by cattle even at high grazing intensities (Mabry *et al.* 1977), together with the occurrence of small pollinated dependent herbs from other plant families enhance the plant pollinator network at high grazing levels. Additionally, high dominance of *Baccharis* species that are highly abundant, generalist and also rejected by cattle (Pillar and Focht 2003), promotes higher network generalization and pollinator diversity at low grazing levels.

Thus, in our study, intermediate livestock grazing by cattle does not increase the generalization and complexity of plant-pollinator network, contrasting the IDH (Intermediated Disturbance Hypothesis - Connel, 1978) and the findings by Lazaro *et al.* (2015). Instead, we observed a decrease of network connectance at intermediated grazing levels and an increase in interaction evenness at higher grazing intensities. The lower values of interaction evenness found at lower grazing intensities are an indicative that most plant species had a small number of interactions and that a small core of species and their links dominate the community (Nielsen & Bascompte 2007). This is because the lower grazing areas are dominated by unpalatable shrubs, such as *Baccharis* species that are among the most abundant species within the community, producing a large number of flowers and thereby concentrating most network interaction present at these sites promoting a decrease in interaction evenness. Therefore we corroborate the hypothesis that the dominance of unpalatable, abundant and generalist shrubs species of Asteraceae could define the principal network proprieties in these grazing areas. The role of shrubs for plant pollinator interactions in grassland community was evidenced by Lara-Romero *et al.* (2015) that showed highest flower visitors and visits in grassland encroached by shrubs, besides their clear contribution to higher linkage and strength of networks.

Several studies have showed an increase of plant diversity in intermediated levels of disturbance in our study region (Boldrini & Eggers, 1996; Isselstein, Jeangros, & Pavlu, 2005; Nabinger, Moraes & Maraschin, 2000), but our findings suggest that this does not translate into a greater complexity of pollination networks. The increase in plant diversity related to higher structural heterogeneity in consequence of the co-existance of grasses related to different disturbance intensities results in an increase of spider and other arthropods functional diversity that are more susceptible and dependent to changes in structure of vegetation (Podgaiski *et al.* 2013; Ferreira-Abreu 2014). However, changes in structure of vegetation did not substantially affect plant-pollinator interactions, as those plant species that respond mostly clearly to the grazing intensities – grasses – are of no importance for potential pollinators.

There are plausible reasons why our results differ from that of Lázaro *et al*. (2015) whose study supported the IDH. First, there are pronounced differences in functional groups of plants in studied system: robust flowering shrubs dominate the Mediteranean garrigue vegetation (Hadar *et al.* 2009), and this functional group of plants has an important role for pollination interaction. In South Brazilian grasslands, in contrast, we find distinct groups of flowering plants – albeit predominantly from the same plant family, Asteraceae – in high abundance at both ends of the gradient, while flower abundances were lower at intermediate grazing intensities, where we observed a high richness of typical grasses species (Boldrini & Eggers 1996; Pillar & Focht 2003). Second, goats and sheeps present forb-biased foraging and reduce the floral diversity of insect-pollinated species at high grazing levels. On the contrary, cattle present grass-biased foraging and are more selective (Rutter 2006; Vavra *et al.* 2007); nonetheless, they avoid especially the unpalatable insect-pollinated plants from the Asteraceae (Mabry *et al.* 1977; Cruz *et al.* 2010).

Only two of six network metrics measured changed in response to grazing intensities. Two factors could be responsible for this: First, species of Asteraceae were the key-species, based on s strength values, of networks along the entire disturbance gradient. The constant presence of Asteraceae species along the gradient demonstrates that this group of plants plays an important role in structuring network. The differences in connectance and interaction evenness result from the increase in some Asteraceae species dominance in extremes levels of gradient, yet this does not bring about changes in other measured network attributes. Second, as mentioned previously, our data suggest that the differences in grazing intensities – with exception of the extremes of the gradient, as already discussed – are more important for the structural diversity of the grass component than that of the other species. Grazing acts directly on the balance of rhizomateous and tussock grasses, with the latter increasing in cover at lower grazing pressures (Boldrini & Eggers 1996). However, these changes, associated to increases of diversity of grasses at intermediate disturbance levels, do not have effects on the pollination network, as these species are not pollinator dependent.

Ferreira-Abreu (2014), working in the same region as we on the effect of different grazing management, found a positive relationship between plant functional diversity and arthropod diversity orders, with highest plant functional and arthropod diversity at higher grazing level. However, only spiders and beetles responded to grazing intensity and plant functional diversity, because these groups are more susceptible to changes in structure of grasses species promote by livestock grazing (i.e cover of tussock and prostate grasses). As Ferreira-Abreu (2014) worked with order diversity and structural characters of vegetation, we can expect different results in our study that investigated specific insect guilds and reproductive aspects of plants. Therefore, we observed that plant-pollinator interactions are very resilient to grazing disturbance in our studied system.

Process affecting network proprieties

Abundance was the best predictor of the interaction frequency between plants and pollinators, independent of grazing intensities. Previous studies have showed that neutral processes, such as abundance have an important role in determining network interactions structure (Vázquez *et al.* 2007; Vázquez *et al.* 2009; Verdú & Valiente-Banuet 2011). This means that individuals interact randomly, independent of morphological traits or specializations. Thus, the most abundant species interact with a great number of partners and with larger frequency than other. In our study system, Asteraceae species was the most abundant; as mentioned above, they are also known for their generalist floral traits that attract a large number of pollinators. Hence, changes in abundance of this species along the grazing gradient could affect directly the abundance of pollinators within the plots. The gazing activity affects plant population density and abundance and consequently pollinator abundance, strengthening the role of abundance for plant-pollinator network in the community studied (Vázquez & Simberloff 2004). Although the differences in abundance of species were not be enough to change most of the metrics measured, we can appoint abundance as determining process for network pollination in the grazing grasslands communities.

Processes such as morphology traits and phenology can have important role in plant-pollination network as evidenced by Vizentin-Bugoni *et al*. (2014), but these processes could be more determinant for specialized system, such as plant-hummingbird interactions where morphological traits could restrict plant-pollinator interactions or when the community presents a flowering pattern along the year. The community studied here presented very generalist traits with little variation of measured attributes. This indicates that morphology does not have an effect on plant-pollinator interaction in the studied system. Although the studied community present flowering seasonality (Pinheiro *et al.* 2008) and a segregated flowering pattern (Oleques et. al 2016) the model that include phenology was no good predictor of observed interactions.

In summary, this study reports that the high structural diversity of the plant community at intermediated grazing levels did not lead to changes or more complex plant-pollinator networks in subtropical grassland communities. We provide evidences that abundance play a fundamental role in network organization in our study system. Intermediate grazing has been pointed out to be compatible with the conservation of plant diversity (Boldrini & Eggers 1996; Pillar & Focht 2003) and also promotes habitat heterogeneity that increases diversity of some groups of animals, such as spiders and beetles (Ferreira-Abreu 2014). Pollinator richness and diversity, in contrast, is not enhanced at intermediate grazing levels, because the most generalist plants have especially high abundances at the opposite extremes of the gradient, promoting large and complex networks. Altogether, in our system, plant-pollinator networks change little along the gradient of grazing intensity, and thus – demonstrated an important attribute of these plant-pollinator communities: the high resilience to grazing disturbance.

Acknowledgements

We thank to G. Minervini for field work support, M. Pinheiro (bees), B.R Garcete-Barret (wasps), J. Duarte (flies), M. Morales (flies-Syrphidae), M. S. Pires (ants), N. Mega (butterflies) and D. Lucas (plants) for their help in the species identification. SSO received a Master scholarship from the Brazilian CNPq.This study was conducted in the context of the LTER/PELD Campos Sulinos, funded by CNPq (grant 403750/2012-1 to VDP) and received additional funding by CNPq (477618/2013-8 to GEO). GEO is supported by a CNPq grant.

References

Aizen, A.M. & Feisinger, P. (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*, **75**, 330-351.

Antonini, Y. & Martins, R.P. (2003) The flowering- visiting bees at the Ecological Station of the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. *Neotropical Entomology,* **32**, 565-575.

Ashman, T.L. & King, E.A. (2005) Are flower-visiting ants mutualists or antagonists? A study in a gynodioecious wild strawberry. *American Journal of Botany,* **92,** 891-895

Baldissera, R., Fritz, L., Rauber, R. & Muller, S.C. (2010) Comparison between grassland communities with and without disturbances. *Neotropical Biology and Conservation*, **5**, 3– 10.

Bascompte, J. & Jordano, P. (2007) Plant-Animal Mutualistic Networks: The Architecture of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics,* **38**, 567–593.

Bascompte, J., Jordano, P. & Melia, C.J. (2003) The nested assembly of plant – animal mutualistic networks. *PNAS*, **100**, 9383–9387.

Basilio, A.M., Medan, D., Torreta, J.P. & Bartoloni, N.J. (2006) A year-long plant-pollinator network. *Austral Ecology*, **31**, 975–983.

Bastolla, U., Fortuna, M.A., Pascual-García, A., Ferrera, A., Luque, B. & Bascompte, J. (2009) The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature*, **458**, 1018–1020.

Blancafort, X. & Gómez, C. (2005) Consequences of the Argentine ant, Linepithema humile(Mayr), invasion on pollination of Euphorbia characias (L.) (Euphorbiaceae). *Oecologia*, **28**, 49-55.

Blüthgen, N., Fründ, J., Vásquez, D.P. & Menzel, F. (2008) What do interaction net-works metrics tell us about specialization and biological traits? *Ecology*, **89**, 3387–3399.

Boldrini, I.I. & Eggers, L. (1996) Vegetação campestre do sul do Brasil: resposta e dinâmica de espécies à exclusão. *Acta Botanica Brasilica*, **10**, 37–50.

Connel, J.H. (1978) Diversity in Tropical Rain Forest and Coral Reefs. *Science*, **199**, 1302–1310.

Costanza, R. *et al.* (1997) The value of the world's ecosystem services and natural capital. *Nature*, **387**, 253–260.

Cruz, C. E. F., Karam, F.C., Dalto, A. C., Pavarini, S. P., Bandarra, P. M. & Driemeier, D. (2010) Fireweed (*Senecio madagascariensis*) poisoning in cattle. Pesquisa Veterinária Brasileira, doi:org/10.1590/S0100-736X2010000100002.

Develey, P.F. *et al.*, (2008) Conservação das aves e da biodiversidade no bioma pampa aliada a sistemas de produção animal. *Revista Brasileira de Ornitologia*, **16**, 308–315.

Dutton, E. M. & Frederinckson, M. E. (2012). Why ant pollination is rare: new evidence and implications of the antibiotic hypothesis. *Arthropod -plant interaction*, **6**, 569- 661.

Ferreira- Abreu, P. M. (2014) Abordagem quali-quantitativa e funcional de vegetação campestre nos biomas Pampa e Mata Atlântica. PhD thesis. Universidade Federal do Rio Grande do Sul, Brazil.

Fontaine, C., Dajoz, I., Meriguet, J. & Loreau, M. (2005x) Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities. *PLoS biology*, **4**, doi:10.1371/journal.pbio.0040001.

Fortuna, M.A., Stouffer, D.B., Olesen, J.M., Jordano, P., Mouillot, D., Krasnov, B.R., Poulin, R. & Bascompte, J. (2010) Nestedness versus modularity in ecological networks: Two sides of the same coin? *Journal of Animal Ecology*, **79**, 811–817.

Hadar, L., Noy-Meir, I. & Perevolotsky, A. (2009) The effect of shrub clearing and grazing on the composition of a Mediterranean plant community: functional groups versus species. *Journal of Vegetation Science*, **10**, 673-682.

Isselstein, J., Jeangros, B. & Pavlu, V. (2005) Agronomic aspect of biodiversity targeted management of temperate grassland in Europe – A review. *Agronomic Research*, **3**,139-151.

García, D., Hódar, J. A., Zamora, R. and Gómez, J. M. (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia*, **105**, 236-242.

Gómez, J. M. (2000) Effectiveness of ants as pollinators ofLobularia maritima: effects on main sequential fitness components of the host plant. *Oecologia*, 122, 90 – 97.

Heleno, R., Devoto, M. & Pocock, M. (2012) Connectance of species interaction networks and conservation value: Is it any good to be well connected? *Ecological Indicators*, **14**, 7–10.

Jordano, P., Bascompte, J. & Olesen, J.M. (2003) Invariant properties in co-evolutionary networks of plant-animal interactions. *Ecology Letters*, **6**, 69–81.

Kaiser-Bunbury, C.N. & Blüthgen, N. (2015) Integrating network ecology with applied conservation: A synthesis and guide to implementation. *AoB Plants*, **7**, doi: 10.1093/aobpla/plv076 .

Klein, A-M., Vaissiére, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of Royal Society B*, **274**, 303–313.

Lázaro, A. , Tscheulin, T., Devalez, J., Nakas, G., Stefanaki, A., Hanlidou, E. & Petanidou, T. (2015) Moderation is best: effects of grazing intensity on plant–flower visitor networks in Mediterranean communities. *Ecological Applications*, doi:10.1890/15-0202.1.

Lara-Romero, C., García, C. & Iriondo, J. M. (2015) Direct and indirect effects of shrub encroachment on alpine grasslands mediated by plant-flower-visitor interactions. *Functional Ecology*, doi: 10.1111/1365-2435.12637.

Mabry, T. M., Jones S. B. & Burnett, W. C. (1977) Evolutionary Implications of Sesquiterpene Lactones in *Vernonia* (Compositae) and Mammalian Herbivores. *Taxon*, **26**, 203-207.

Mc Laughlin, Ó.B., Emmerson, M.C. & O'Gorman, E.J. (2013) *Global Change in Multispecies Systems* (eds. Ute Jacob & Guy Woodward), pp. 99-110, Elsevier, London.

Milchunas, D. G., Sala, O. E. & Laurenroth W. K. (1988) A Generalized model of the effects of grazing by large herbivores on grassland community structure. The *American Naturalist*, **132,** 87-106.

Moeller, D.A. *et al.* (2012) Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. *Ecology*, **93**,1036–1048.

Nabinger, C., Moraes, A. De & Maraschin, G.E. (2000) Campos in Southern Brazil (eds In C. Lemaire, G. Hodgson, J. de Moraes, A., Carvalho, P.C de F, Nabinger) *Grassland Ecophysiology and Grazing Ecology*, pp. 355–376, Wallingford.

Nielsen, A. & Bascompte, J. (2007) Ecological networks, nestedness and sampling effort. *Journal of Ecology*, **95** ,1134–1141.

Nielsen, A. &Totland, Ø. (2014) Structural properties of mutualistic networks withstand habitat degradation while species functional roles might change. *Oikos*, **123**, 323–333.

Nabinger, C. *et al.* 2009. Produção animal com base no campo native: aplicações de resultados de pesquisas. (Eds Pillar, V.P. et al). Campos Sulinos, conservação e uso sustentável da biodiversidade. MMA, Brasília/DF PP 175-198.

Okuyama, T. & Holland, J.N. (2008) Network structural properties mediate the stability of mutualistic communities. *Ecology Letters*, **11**, 208–216.

Olito, C. & Fox, J.W. (2015) Species traits and abundances predict metrics of plant-pollinator network structure, but not pairwise interactions. *Oikos*, **124**, 428–436.

Oleques, S. S., Overbeck, G.E. & Avila Jr, R. S. (2016). Temporal segregation in flowering time and plant-pollinator interactions in a grassland community of Southern Brazil. Accepted for publication. *Plant Biology*.

Overbeck, G. *et al.* (2007) Brazil’s neglected biome: The South Brazilian Campos.*Perspectives in Plant Ecology, Evolution and Systematics*, **9**,101–116.

Overbeck, G.E., Muller, S.C., Pillar, V.D. & Pfandenhauer, J. (2005) Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *Journal of Vegetation Science*, **16**, 655–664.

Pillar, V.D & Focht, T. (2003) Spatial patterns and relations with site factors in a campos grassland under grazing. *Brazilian Journal of Biology*, **63**.

Podgaiski, L.R. *et al.* (2013) Spider Trait Assembly Patterns and Resilience under Fire-Induced Vegetation Change in South Brazilian Grasslands. *PLoS ONE,* **8**.

Potts, S.G. *et al.*, 2010. Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*, **25**,345–353.

Proctor, M., Yeo, P. & Lack, A. (1996) *The natural history of pollination*. Harper Collins Publishers, London.

Rutter, S. M. (2006) Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Applied Animal Behavior *Science*, **97**, 17-35.

Soares, A.B. *et al.* (2005) Produção animal e de forragem em pastagem nativa submetida a distintas ofertas de forragem. *Ciência Rural*, **35**,1148-1153.

Spieman, B.J. & Inouye, B.D. (2013) Habitat loss alters the architecture of plant – pollinator interaction networks. , **94**, 2688–2696.

Stang, M., Klinkhamer, P.G.L. & Van Der Meijden, E. (2006) Size constraints and flower abundance determine the number of interactions in a plant–flower visitor web. *Oikos*, **112**,111–121.

Torres, C. & Galleto, L. 2002. Are nectar-sugar composition and corolla-tube length related to the diversity of insects that visit Asteraceae flowers? *Plant Biology*, **4,** 360-366.

Thébault, E. & Fontaine, C. (2010) Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, **329**, 853–856.

Tscharntke, T. &Tylianakis, J. (2010) Conserving complexity: Global change and community-scale interactions.*Biological Conservation*, **143**, 2249–2250.

Vázquez, D.P. *et al.*, 2007. Species abundance and asymmetric interaction strength in ecological networks.*Oikos*, 116(7), pp.1120–1127.

Vázquez, D.P. *et al.*, 2009. Uniting pattern and process in plant-animal mutualistic networks: a review. *Annals of botany*, 103(9), pp.1445–57.

Vázquez, D.P. & Aizen, M. 2004. Asymmetric Specialization: a Pervasive Feature of Plant–Pollinator Interactions. *Ecology*, 85(5), pp.1251–1257.

Vázquez, D.P. & Simberloff, D. (2004) Indirect effects of an introduced ungulate on pollination and plant reproduction. *Ecological Monographs*, **74**, 281–308.

Verdú, M. &Valiente-Banuet, A. (2011) The relative contribution of abundance and phylogeny to the structure of plant facilitation networks.*Oikos*, **120**, 1351–1356.

Vieira, M.C. & Almeida-Neto, M. (2015) A simple stochastic model for complex coextinctions in mutualistic networks: robustness decreases with connectance. *Ecology Letters*, **18**,144–152.

Vizentin-Bugoni, J.,Maruyama, P.K. & Sazima, M. (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird-plant network. *Proceedings of the Royal Society B,* **281**.

Vavra, M., Parks, C. G. & Wisdom, M. J. (2007) Biodiversity, exotic plant species, and herbivory: The good, the bad, and the ungulate. *Forest Ecology and Management*, **246**, 66-72.

Westerkamp, C. (1991) Honeybees are poor pollinators – Why? Plant Systematics and *Evolution*, **177**, 71-75.

Yoshihara, Y. *et al.* (2008) Effects of livestock grazing on pollination on a steppe in eastern Mongolia. *Biological Conservation*, **1419**, 2376–2386.

Tables

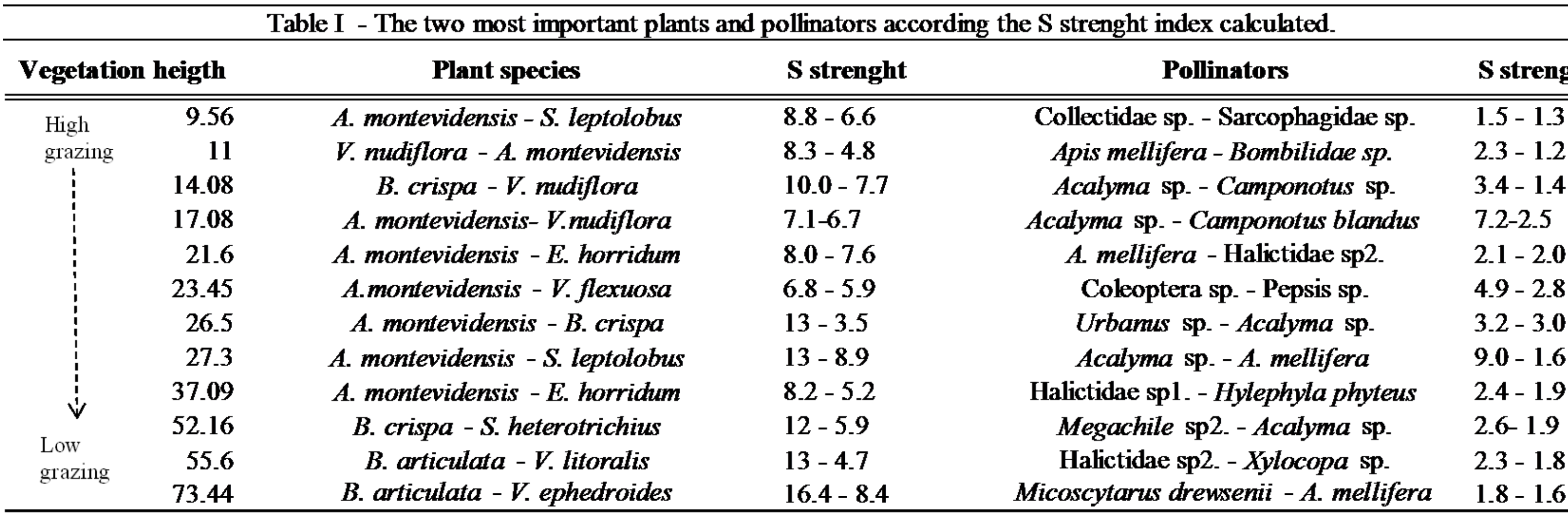


Table I - The two most important plants and pollinators according the S strenght index calculated.

Vegetation heigth		Plant species	S strenght	Pollinators	S streng
High grazing	9.56	*A. montevidensis - S. leptolobus*	8.8 - 6.6	Collectidae sp. - Sarcophagidae sp.	1.5 - 1.3
	11	*V. nudiflora - A. montevidensis*	8.3 - 4.8	*Apis mellifera - Bombilidae sp.*	2.3 - 1.2
	14.08	*B. crispa - V. nudiflora*	10.0 - 7.7	*Acalyma* sp. - *Camponotus* sp.	3.4 - 1.4
	17.08	*A. montevidensis- V.nudiflora*	7.1-6.7	*Acalyma* sp. - *Camponotus blandus*	7.2-2.5
	21.6	*A. montevidensis - E. horridum*	8.0 - 7.6	*A. mellifera* - Halictidae sp2.	2.1 - 2.0
	23.45	*A.montevidensis - V. flexuosa*	6.8 - 5.9	Coleoptera sp. - Pepsis sp.	4.9 - 2.8
	26.5	*A. montevidensis - B. crispa*	13 - 3.5	*Urbanus* sp. - *Acalyma* sp.	3.2 - 3.0
	27.3	*A. montevidensis - S. leptolobus*	13 - 8.9	*Acalyma* sp. - *A. mellifera*	9.0 - 1.6
	37.09	*A. montevidensis - E. horridum*	8.2 - 5.2	Halictidae sp1. - *Hylephyla phyteus*	2.4 - 1.9
	52.16	*B. crispa - S. heterotrichius*	12 - 5.9	*Megachile* sp2. - *Acalyma* sp.	2.6- 1.9
Low grazing	55.6	*B. articulata - V. litoralis*	13 - 4.7	Halictidae sp2. - *Xylocopa* sp.	2.3 - 1.8
	73.44	*B. articulata - V. ephedroides*	16.4 - 8.4	*Micoscytarus drewsenii - A. mellifera*	1.8 - 1.6

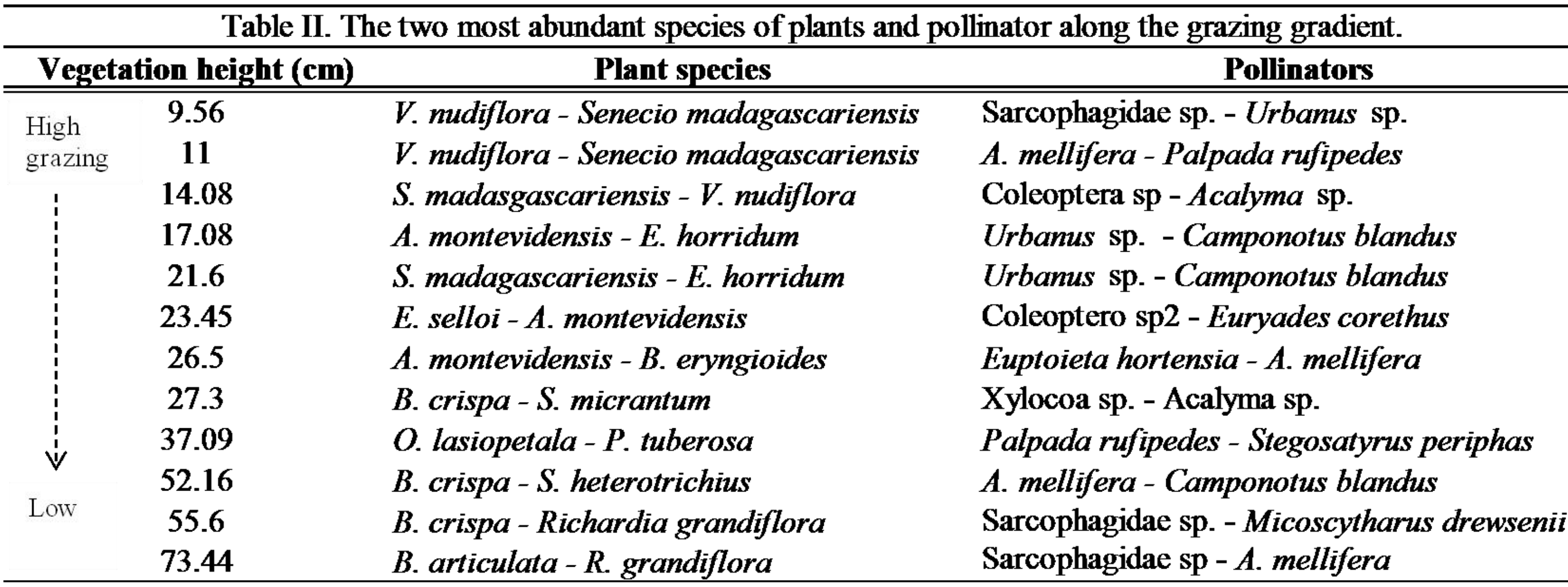


Table II. The two most abundant species of plants and pollinator along the grazing gradient.

Vegetation height (cm)		Plant species	Pollinators
High grazing	9.56	*V. nudiflora - Senecio madagascariensis*	Sarcophagidae sp. - *Urbanus* sp.
	11	*V. nudiflora - Senecio madagascariensis*	*A. mellifera - Palpada rufipedes*
	14.08	*S. madasgascariensis - V. nudiflora*	Coleoptera sp - *Acalyma* sp.
	17.08	*A. montevidensis - E. horridum*	*Urbanus* sp. - *Camponotus blandus*
	21.6	*S. madagascariensis - E. horridum*	*Urbanus* sp. - *Camponotus blandus*
	23.45	*E. selloi - A. montevidensis*	Coleoptero sp2 - *Euryades corethus*
	26.5	*A. montevidensis - B. eryngioides*	*Euptoieta hortensia - A. mellifera*
	27.3	*B. crispa - S. micrantum*	Xylocoa sp. - Acalyma sp.
	37.09	*O. lasiopetala - P. tuberosa*	*Palpada rufipedes - Stegosatyrus periphas*
	52.16	*B. crispa - S. heterotrichius*	*A. mellifera - Camponotus blandus*
Low	55.6	*B. crispa - Richardia grandiflora*	Sarcophagidae sp. - *Micoscytharus drewsenii*
	73.44	*B. articulata - R. grandiflora*	Sarcophagidae sp - *A. mellifera*

Table III - Transformed metrics calculated from each network interaction along the grazing gradient.

	Vegetation height (cm)	C*	H2'	NE	M	IE	W.NODF	NS	NL
High grazing	9.56	0.115	0.121	3.283	0.103	0.000	-2.280	51	175
	11	0.107	0.266	6.974	-0.017	-0.002	-9.070	55	241
	14.08	0.106	0.286	3.086	-0.016	-0.006	-9.461	55	304
	17.08	0.106	0.102	-4.494	0.014	0.033	-3.551	54	130
	21.6	0.100	0.269	5.363	-0.006	-0.031	-14.838	68	363
	23.45	0.080	0.284	3.897	-0.009	0.001	-11.887	57	253
	26.5	0.076	0.398	2.561	0.000	-0.025	-15.487	60	318
	27.3	0.068	0.329	1.506	0.008	-0.016	-6.872	88	565
	37.09	0.090	-0.311	4.010	0.002	-0.010	-4.775	56	170
	52.16	0.118	0.362	2.281	0.028	-0.017	-9.389	38	262
Low grazing	55.6	0.116	0.353	3.193	-0.019	-0.024	-15.736	46	258
	73.44	0.125	0.405	5.703	-0.014	-0.045	-12.135	55	404

* Connectance (C), Specialization (H2'), Nestedness (NE), Modularity (M), Number of links (NL), Interaction eveness(IE), Number of species(NS).

Table IV - Regression analisys and test of linear models from each null model correccted network metric and mean of plant height.

? network metric

Specialization (H2')	Intercept (a)	Coeficient (b1,b2)	R square	F statistics	p value	Aicc	? Aicc
Linear (Y' = a + bX)			0.058	0.621	0.448	-11.661	13.021
Quadratic (Y' = a + (b1)X + (b2)X^2)			0.226	1.314	0.315	-24.682	0
Modularity							
Linear (Y' = a + bX)	0.021	0.000	0.080	0.900	0.363	-15.980	0.000
Quadratic (Y' = a + (b1)X + (b2)X^2)	0.039	0.001,0	0.110	0.575	0.585	-15.830	0.150
Nestedness							
Linear (Y' = a + bX)	2.370	0.024	0.028	0.296	0.598	14.324	0.000
Quadratic (Y' = a + (b1)X + (b2)X^2)	4.680	0.139, 0.002	0.095	0.475	0.636	15.952	1.628
Weighted NODF							
Linear (Y' = a + bX)	-6.863	-0.089	0.158	1.804	0.208	20.585	0.170
Quadratic (Y' = a + (b1)X + (b2)X^2)	-4.489	0.257, 0.002	0.180	0.987	0.409	20.415	0
Interaction eveness							
Linear (Y' = a + bX)	0.007	0.000	**0.422**	7.304	**0.022***	-40.22	**0***
Quadratic (Y' = a + (b1)X + (b2)X^2)	5.806 e 03	4.945 e04,-1.847 e04	0.423	3.302	0.084	-38.232	1.988
Connectance							
Linear (Y' = a + bX)	0.090	0.000	0.080	0.880	0.360	-69.750	5.000
Quadratic (Y' = a + (b1)X + (b2)X^2)	0.120	0.002, 0	**0.420**	5.050	**0.030***	-74.750	0.000
Number of species							
Linear (Y' = a + bX)	62.150	0.170	0.070	0.850	0.370	87.780	0.460
Quadratic (Y' = a + (b1)X + (b2)X^2)	55.100	0.32, - 0.00	0.110	0.570	0.580	87.320	0.000
Number of links							
Linear (Y' = a + bX)	249.800	0.930	0.020	0.230	0.630	144.144	0.001
Quadratic (Y' = a + (b1)X + (b2)X^2)	247.900	1.06, - 0.001	0.020	0.100	0.900	144.143	0.000

* Significant results ($p<0.05$) are indiccating in bold.

Tabela V - Results from Akaike information criteria analysis..

		?AIC calculated from each model							
Vegetation height (cm)		**Null**	**A**	**F**	**M**	**AF**	**AM**	**FM**	**AFM**
High grazing	9.56	393.18	**0***	358.15	516.15	124.52	100.13	471.9	255.32
	11	813.55	**0***	594.9	914.06	175.19	76.5	680.63	266.03
	14.08	633.86	**0***	394.6	813.06	327.75	314.3	568.02	647.04
	17.08	241.89	**0***	159.74	341.05	169.77	158.4	263.62	332
	21.6	1152.37	**0***	839.45	1354.95	171.55	213.9	1032.17	373.62
	23.45	1154.9	**0***	849.25	1523.9	164.5	109.8	951.2	276.2
	26.5	1889.19	**0***	1255.91	1914.92	181.56	69.46	1300.98	248.82
	27.3	2907.58	**0***	1884.48	2857.68	412.02	32.59	1806.33	484.82
	37.09	618.97	**0***	419.5	756.18	124.85	135	555.3	259.51
	52.16	1316.12	**0***	1108.79	1347.46	112.41	136.47	1171.55	253.98
Low grazing	55.6	1127.11	**0***	997.17	1227.81	153.4	228.82	1093.6	366.44
	73.44	1947.44	**0***	1947.44	2173.06	165.04	300.7	1704.9	409.2

*** Best predictor model - ? = delta symbol**

Figures

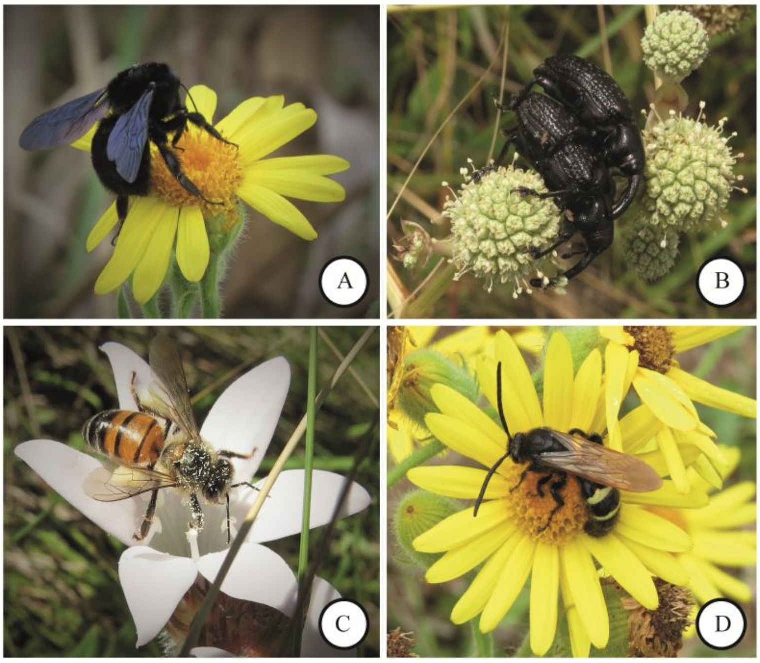


Figure 1 – Plant-pollinators interactions registred: A) *Xylocopa* sp. visiting *Senecio heterotrichius*. B) Curculionidae sp. in *Eryngium horridum*. C) *Apis mellifera* visiting *Richardia grandiflora* flower and D) *Campsomeris* sp. in *S. heterotrichius.*

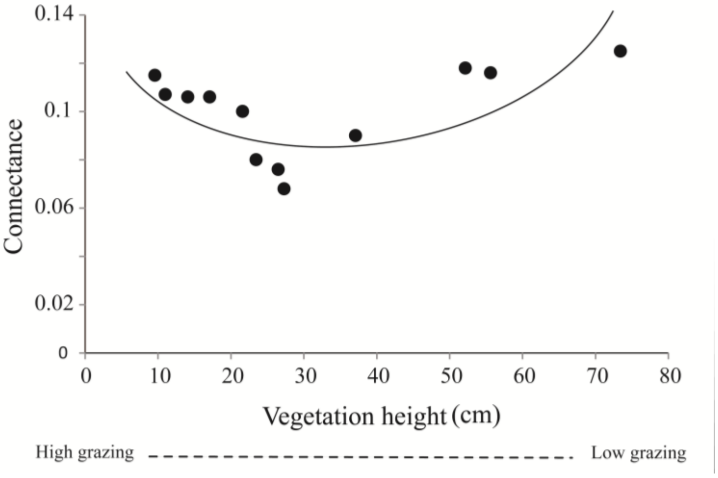


Figure 2 – Quadratic regression between the variables vegetation height (grazing intensities) and vonnectance. The highest values of connectance were registred in the extremes of grazing gradient.

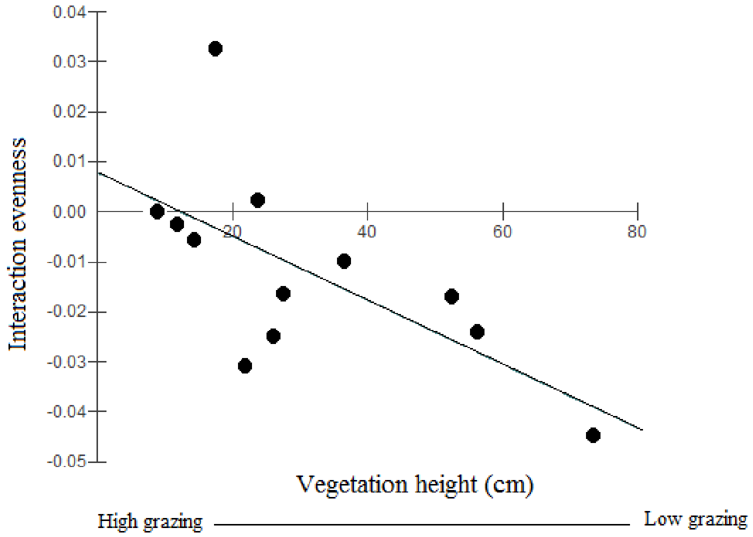


Figure 3 – Linear regression between vegetation height (grazing intensities) and interaction eveness. The interaction eveness decreaseat low grazing intensities.

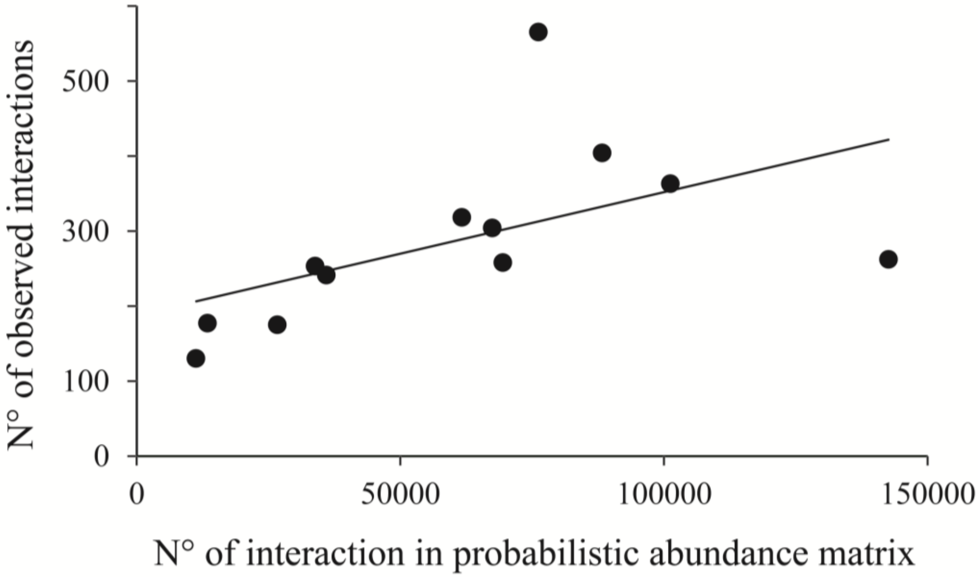


Figure 4 – Linear and positive association between number of observed interactions and number of interactions predict by abundance matrix. This relationship between variables showed that abundance of species is a good predictor of number of interactions observed.

CONCLUSÕES GERAIS

Foto: *Pepsis* sp. em *Pterocaulon polypterum*

CONCLUSÕES GERAIS

Este trabalho foi o primeiro a avaliar aspectos da fenologia reprodutiva e redes de interações planta-polinizador de comunidades campestres do Rio Grande do Sul. Em relação aos aspectos fenológicos a comunidade campestre apresentou um padrão de floração segregado no tempo, com baixa sobreposição dos picos de floração entre as espécies. Padrões fenológicos segregados podem estar associados a estratégias evolutivas das plantas para minimizar os possíveis negativos da competição interespecífica por polinizadores. Desta forma, este estudo aponta, pela primeira vez a relevância das interações planta-polinizador na estruturação do padrão fenológico reprodutivo em nível de uma comunidade vegetal. Além disso, este estudo demonstrou que não há vantagem para espécies que florescem intensivamente e por longos períodos, pois as mesmas não apresentaram maior número de polinizadores ou maior similaridade de polinizadores com o restante da comunidade. Da mesma forma, demonstramos que espécies com flores de distintas cores não diferem quanto ao padrão fenológico, sendo aleatoriamente distribuídas ao longo do período de floração na comunidade. Nosso estudo evidencia que embora a comunidade apresente um padrão fenológico que possa ter sido gerado por eventos de competição, atualmente outros fatores, como a cor, podem determinar as interações planta-polinizador na comunidade estudada.

Em relação às redes de interações e o gradiente de distúrbio este estudo evidenciou que a alta diversidade estrutural de plantas em níveis intermediários de pastejo não resulta em redes de interações planta-polinizador mais complexas. Aqui, provemos evidências de que a abundância das espécies desempenha um papel fundamental na organização das interações em nosso sistema de estudo mais do que restrições morfológicas e aspectos fenológicos. Níveis intermediários de pastejo têm sido associados com maior diversidade de plantas e maior heterogeneidade de habitat, resultando em aumento da diversidade alguns grupos de animais, como aranhas e besouros. No entanto, isto não é aplicável para interações planta-polinizador, pois as espécies mais generalistas e que atraem um maior número de polinizadores são mais dominantes nos extremos do gradiente, como por exemplo, espécies impalatáveis para o gado como *Senecio* e *Baccharis*. Sendo assim, áreas altamente pastejas e abandonadas promovem redes mais conectadas e genetalistas em nosso sistema de estudo muito embora a maioria dos atributos de rede não tenham respondido as diferentes intensidades de pastejo. As espécies da família Asteraceae, conforme já evidenciado por outros estudos, compõem uma importante fonte de recursos aos polinizadores. Além disso, por serem espécies altamente generalistas e atraírem uma ampla diversidade de polinizadores as espécies de Asteraceae influenciaram a estrutura das redes, principalmente nos extremos do gradiente. De forma geral, as redes planta-polinizador variaram pouco ao longo do gradiente de pastejo, uma vez que o pastejo influencia principalmente na diversidade estrutural e composição de espécies de gramíneas que não dependem de polinizadores. Desta forma, embora a polinização tenha um papel fundamental para as comunidades de plantas, não apresenta um potencial para ser utilizado como processo indicador para reforçar a idéia de níveis intermediários de pastejo como manejo adequado aos campos. Isto porque as interações planta-polinizador são altamente resilientes a intensidade de pastejo. Sendo assim, a ideia de que níveis intermediários de distúrbio garantem altos níveis de diversidade (Hipótese do Distúrbio Intermediário Connel, 1978), podem não ser aplicável em relação as interações planta-polinizador em nosso sistema de estudo.

Este trabalho reforça a necessidade de mais estudos sobre a influência do manejo sobre as interações animal-planta. Além disso, estudos sobre redes de interação planta-polinizador fornecem suporte para futuros trabalhos sobre aspectos reprodutivos de alguns grupos de espécies que compõem a comunidade campestre do Rio Grande do Sul, uma vez que poucos estudos vêm sendo realizados neste contexto.

Apêndices

List of plant species from Eldorado do Sul, Brazil, 2015.	
Acanthaceae	
Ruellia hypericoides	(Nees) Lindau
Ruellia morongii	Britton
Alliaceae	
Nothoscordum bonariense	Beauverd
Nothoscordum montevidense	(Pers.) Beauverd
Amaranthaceae	
Pfaffia tuberosa	(Spreng.) Hicken
Apiaceae	(Spreng.) Hicken
Eryngium horridum	Malme
Eryngium ciliatum	Cham. & Schltdl.
Asteraceae	
Achyrocline satureioides	(Lam.) DC.
Aspilia montevidensis	(Spreng.) Kuntze
Baccharis articulata	(Lam.) Pers.
Baccharis crispa	Spreng.
Baccharis dracunculifolia	DC.
Chaptalia integerrima	(Vell.) Burk.
Elephantopus mollis	Kunth.
Chromolaena squarrulosa	(Hook. & Arn.) R.M.King & H.Rob
Eupatorium subhastatum	Hook. & Arn.
Hieracium commersonii	Monnier
Hypochaeris chillensis	(H.B.K.) Hieron
Hypochaeris megapotamica	Cabr.
Orthopappus angustifolius	Gleason
Pterocaulon polypterum	(DC.) Cabrera
Senecio heterotrichius	DC.
Senecio leptolobus	DC.
Senecio madagascariensis	Poir.
Stenachaenium megapotamicum	(Spreng.) Baker
Verbena ephedroides	Cham.
Chrysolaena flexuosa	(Sims) H. Rob.
Stenocephalum megapotamicum	(Spreng.) Sch. Bip
Vernonanthura nudiflora	(Less.) H.Rob.
Cistaceae	

Helianthemum brasiliense	(Lam.) Pers.
Convovulaceae	
Evolvulus sericeus	Sw.
Euphorbiaceae	
Croton gnaphalii	Baill.
Euphorbia selloi	(Klotzsch & Garcke) Boiss.
Fabaceae	
Aeschynomene falcata	(Poir.) DC.
Chamaecrista repens	(Vogel) H.S. Irwin & Barneby
Crotalaria twediana	Benth.
Desmodium incanum	DC.
Macroptilium prostatum	(Benth.) Urb.
Stylosanthes leiocarpa	Vogel
Stylosanthes montevidensis	Vogel
Hypoxidaceae	
Hypoxis decumbens	L.
Iridaceae	
Herbertia lahue	(Molina) Goldblatt
Sisyrinchium micrantum	Cav.
Sisyrinchium sellowianum	Klatt
Lamiaceae	
Peltodon longipes	Kunth. ex Benth.
Lythraceae	
Cuphea glutinosa	Cham. & Schltdl.
Malvaceae	
Waltheria douradinha	A. St.-Hil.
Oxalidaceae	
Oxalis conorrhiza	Jacq.
Oxalis eriocarpa	DC.
Oxalis lasiopetala	Zuccarini
Plantaginaceae	
Mecardônia tenella	(Cham. & Schltdl.) Pennell
Polygalaceae	
Polygala pulchela	A. St.-Hil.
Rubiaceae	
Borreria capitata	(Ruiz & Pav.) DC.
Borreria eryngioides	Cham. & Schltdl.
Borreria tenella	(Kunth) Cham. & Schltdl.
Borreria verticillata	L.
Galianthe fastigiata	Griseb.
Richardia grandiflora	(Cham. & Schltdl.) Steud.
Scrophulariaceae	
Linaria texana	Scheele
Verbenaceae	
Glandularia morrubioides	(Cham.) Tronc.

Lippia turnerifolia	Cham.
Verbena ephedroides	Cham.
Verbena litoralis	Kunth
Violaceae	
Hybanthus bicolor	(Saint-Hilaire) Baill.

List of Pollinators collected from Eldorado do Sul, Brazil, 2015.

HYMENOPTERA

- **Apidae**
  - **Apinae**
    - *Apis mellifera* (Linnaeus, 1758)
    - *Bombus* sp.
  - **Adreninae**
    - *Psaenythia* sp.1
    - *Psaenythia* sp.2
  - **Anthophorinae**
    - *Xylocopa* sp.
  - **Colletinae**
    - Colletinae sp.1
    - Colletinae sp.2
    - Colletinae sp.3
    - Colletinae sp.4
  - **Megachiliinae**
    - *Hypanthidium* sp.
    - *Megachile* sp.1
    - *Megachile* sp.2
  - **Halictinae**
    - Halictinae sp.1
    - Halictinae sp.2
    - Halictinae sp.3
    - Halictinae sp.4
    - Halictinae sp.5
    - Halictinae sp.6
    - Halictinae sp.7
    - Halictinae sp.8
    - Halictinae sp.9
    - Halictinae sp.10
- **Formicidae**
  - *Camponotus* sp.
  - *Camponotus crassus*
  - *Camponotus blandus*

Pheidole sp.1
Acromyrmex sp.
Pheidole sp.2
Gnamptogenys sp.
Tapinoma sp.1
Tapinoma sp.2
Neivamyrmex sp.
Pseudomyrmex sp.

Cabronidae

Cerceris sp.

Pompilidae

Pepsis sp.

Scoliidae

Campsomeris sp. 1
Campsomeris sp.2
Campsomeris sp.3
Campsomeris sp.4

Tiphiidae

Myzinum sp.

Vespidae

Brachygastra augusti (Saussure, 1854)
Mischocyttarus drewseni (Saussure, 1857)
Montezumia nigriceps (Spinosa 1841)
Pachodynerus sp.
Polistes billardieri (Saussure, 1853)
Polistes cinerascens (Saussure, 1854)
Polybia ignobilis (Haliday, 1836)
Polybia scutellaris (White, 1841)
Polybia sericea (Oliver, 1922)
Vespidae sp.

DIPTERA

Diptera sp.1
Diptera sp.2
Diptera sp.3
Diptera sp.4

Bombilidae

Bombilidae sp.1
Bombilidae sp.2
Bombilidae sp.3

Bibionidae

Bibionidae sp.1
Bibionidae sp.2

Chloropidae

Chloropidae sp.1
Chloropidae sp.2

Chloropidae sp.3

Ephydridae

Ephydridae sp.

Muscidae

Stomopogon argentina (Snyder, 1957)
Muscidae sp.1
Muscidae sp.2
Muscidae sp.3
Muscidae sp.4
Muscidae sp.5
Muscidae sp.6

Michilidae

Michilidae sp.

Syrphidae

Allograpta exótica (Wiedemann, 1830)
Ocyptamus sp.1
Palpada sp.
Palpada distinguenda (Wiedemann, 1830)
Palpada furcata (Wiedemann, 1819)
Palpada rufipedes (Thompson, 1976)
Pseudodoros clavatus (Fabricius, 1794)
Syrphidae sp.1
Syrphidae sp.2
Syrphidae sp.3
Syrphidae sp.4
Syrphidae sp.5
Syrphidae sp.6
Toxomerus sp.1
Toxomerus sp.2
Toxomerus sp.3
Toxomerus duplicatus (Wiedemann, 1830)
Toxomerus politus (Say, 1823)
Toxomerus lacrymosus (Bigot, 1884)
Toxomerus norma (Curran, 1930)
Toxomerus productus (Curran, 1930)

Sarcophagidae

Sarcophagidae sp.1
Sarcophagidae sp.2
Sarcophagidae sp.3
Sarcophagidae sp.4
Sarcophagidae sp.5
Sarcophagidae sp.6

Sepsidae

Sepsidae sp.

Tachinidae

Tachinidae sp.1
Tachinidae sp.2
Tachinidae sp.3
Tachinidae sp.4

Tephritidae

Tephritidae sp.1
Tephritidae sp.2
Tephritidae sp.3
Tephritidae sp.4
Tephritidae sp.5

Tabanidae

Dichelacera sp.

COLEOPTERA

Buprestidae

Buprestidae sp.1

Cantharidae

Cantharidae sp. 1
Cantharidae sp. 2

Chrysomelidae

Gastrophysa sp.
Oulema sp.
Acalyma sp.
Chrysomelidae sp. 1
Chrysomelidae sp. 2
Chrysomelidae sp. 3
Chrysomelidae sp. 4
Chrysomelidae sp. 5
Chrysomelidae sp. 6
Chrysomelidae sp. 7
Chrysomelidae sp. 8
Chrysomelidae sp. 9
Chrysomelidae sp. 10
Chrysomelidae sp. 11
Chrysomelidae sp. 12
Chrysomelidae sp. 13
Chrysomelidae sp. 14
Chrysomelidae sp. 15

Curculionidae

Curculionidae sp. 1
Curculionidae sp.2
Curculionidae sp. 3

Elateridae

Elateridae sp. 1
Elateridae sp. 2

LEPIDOPTERA

Nymphalidae

Agraulis vanillae (Linnaeus, 1758)
Vanessa braziliensis (Moore, 1883)
Tegosa orobia (Hewitson, 1864)
*Jwnonia evarete (*Cramer, 1779)
Euptoieta hortensia (Blanchard, 185*2)*
Stegosatyrus periphas (Godart, 1824)
Anartia amathea (Linnaeus, 1758)
Heliconius erato phyllis (Fabricius, 1775)

Lycaenidae

Strymon rana (Schaus, 1902)
Strymon bazochii (Hübner, 1818)

Hesperidae

Hylephila phyleus (Drury, 1773)
Polites vibex catilina (Plötz, 1886)
Heliopetes omrina (A. Butler, 1870)
Urbanus sp.
Urbanus evenus (Ménétriés, 1855)
Hesperidae sp.1
Hesperidae sp.2
Hesperidae sp.3
Hesperidae sp.4
Hesperidae sp.5

Papilionidae

Heraclides astyalus (Godart, 1819)
Euryades corethrus (Boisduval, 1836)

Pieridae

Phoebis sennae (Linnaeus, 1758)
Eurema elathea (Cramer, 1777)
Pieridae sp.1
Pieridae sp.2

ORTHOPTERA

Proscopidae sp.

BLATODEA

Blatidae sp.