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Integrando processos evolutivos e ecológicos no estudo de redes de interações

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Integrando processos evolutivos e ecológicos no estudo de redes de interações

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"Fora da matemática que não tem que ver senão com números mortos e fórmulas vazias, e por isso pode ser perfeitamente lógica, a ciência não é senão um jogo de crianças no crepúsculo, um querer apanhar sombras de aves e parar sombras de ervas ao vento." Fernando Pessoa

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Resumo

Compreender como as espécies interagem e como a topologia de redes ecológicas influencia a dinâmica de populações e comunidades tem sido um dos principais objetivos de estudos ecológicos há mais de um século. Apesar desta longa tradição, o estudo de redes ecológicas tem aumentado drasticamente nas últimas duas décadas. No entanto, só recentemente ecólogos começaram a ir além da descrição de padrões topológicos e passaram a integrar outros dados biológicos importantes, como características funcionais e filogenia. Esta tese teve principalmente dois objetivos: i) desenvolver novas abordagens analíticas capazes de integrar informações funcionais e filogenéticas, a fim de descrever padrões estruturais em redes ecológicas, e ii) compreender a influência de dinâmicas eco-evolutivas na robustez de redes mutualísticas. No Capítulo I, desenvolvi uma abordagem analítica integradora para particionar os efeitos da filogenia e de características funcionais sobre a estrutura de redes de interação biótica. O método combina Teoria de Conjuntos Difusos e correlação matricial. Eu também desenvolvi um estudo de simulação para testar a acurácia da metodologia proposta em termos de Erro Tipo I. As simulações demonstram que o método é acurado, ou seja, rejeita incorretamente uma hipótese nula verdadeira em ~ 5% dos casos. No Capítulo II, investiguei como diferentes cenários de extinção afetam a robustez de uma rede de dispersão de sementes do sul do Brasil, incluindo cenários onde as espécies são eliminadas com base em sua distinção evolutiva e funcional. Os resultados indicam que a perda de espécies generalistas e diversidade funcional faz com que rede seja mais propensa a colapsar. No Capítulo III, desenvolvi uma investigação teórica sobre a influencia de diferentes modos de evolução de atributos sobre a robustez de redes mutualísticas que estão sofrendo um ataque funcional. Os resultados mostram que, apesar da pequena faixa de variação na robustez das redes, o modo de evolução dos atributos, e a interação entre modos de evolução de cada conjunto de espécies que interagem, influenciam a robustez de redes ecológicas, especialmente em casos extremos, onde os atributos das espécies apresentam sinal filogenético muito baixo ou muito forte.

Palavras-chave: Atributos funcionais, coextinção, Dinâmica eco-evolutiva, ecologia filogenética, ecologia quantitativa, mutualismo.

Abstract

Understanding how species interact and how the topology of ecological networks influences the dynamics of populations and communities has been mind-boggling ecologists for over a century now. Despite this long tradition, the study of complex ecological networks has increased dramatically in the past two decades. Nonetheless, only recently ecologists have started to move beyond the description of topological patterns and started to integrate other important biological data, such as functional traits and phylogenies. Therefore, the aim of this dissertation was mainly two-fold: develop new analytical approaches capable to integrate functional and phylogenetic information in order to describe structural patterns in ecological networks, and to understand the effects of eco-evolutionary dynamics on network robustness. In Chapter I, I developed a new and integrative analytical framework to partition the effects of phylogenies and functional traits on the structure of ecological networks. The method combines fuzzy set theory and matrix correlation. I also developed a simulation study to test the accuracy of the framework I proposed. My simulation study demonstrates that the method is accurate, i.e., incorrectly rejecting a true null hypothesis in ~ 5%. In Chapter II, I investigated how different extinction scenarios affect the robustness of a seed dispersal network, from southern Brazil, including scenarios where species are eliminated based on their evolutionary and functional distinctiveness. The results indicate that loss of generalist species and functional diversity makes the system more likely to collapse. In Chapter III, I developed a theoretical investigation of the role of distinct trait evolution modes on the robustness of mutualistic networks undergoing functional trait extinctions. My results show that, despite the small range of variation in network robustness, the mode of trait evolution alone and the interaction between modes of evolution of each set of interacting species matter for network robustness, especially in extreme cases, where species traits present either very low or very strong phylogenetic signal.

Key-words: Coextinction, eco-evolutionary dynamics, ecophylogenetics, functional traits, mutualism, quantitative ecology.

Introdução Geral

Compreender como sistemas ecológicos respondem a perturbações é um dos temas mais centrais em Ecologia (Camerano 1880, May 1972, 2001, Pimm 1984, 1991, Neutel et al, 2002, Allesina & Tang 2012, Myers et al. 2015, Pires et al. 2015). O crescimento da população humana e de suas atividades socioeconômicas tem ocasionado uma nova onda de extinções em massa, sendo que algumas estimativas demonstram que as taxas atuais de extinção estão centenas ou milhares de vezes acima das taxas de fundo, inferida a partir do registro fóssil (Pimm et al. 2014, Ceballos et al. 2015). Dessa maneira, esta necessidade de compreender e prever a consequência de perturbações em sistemas ecológicos, e em especial a perda de diversidade biológica, tornou-se uma tarefa ainda mais importante para que possamos criar politicas e ações de conservação efetivas (Vieira & Almeida-Neto 2014, Ceballos et al. 2015). Modelos teóricos e evidências empíricas têm demonstrado que a perda de uma única espécie pode gerar um "efeito cascata", desencadeando extinções subsequentes ou secundárias (Jackson et al. 2001, Dobson et al. 2006, Colwell et al. 2012, Säterberg et al. 2013, Vieira et al. 2013, Brodie et al. 2014, Vieira & Almeida-Neto 2014) e a perda de diversidade funcional, comprometendo de forma irreversível o funcionamento dos ecossistemas naturais (Dobson et al. 2006, Duffy et al. 2007, Fonseca & Ganade 2001, Petchey & Gaston 2002). No entanto, a maioria dos estudos empíricos que examinaram a magnitude de perda de diversidade biológica geralmente ignora processos de coextinção (Dunn et al. 2009, Vieira & Almeida-Neto 2014).

O estudo de interações ecológicas é um dos tópicos mais relevantes para se compreender processos e padrões ecológicos e evolutivos (Post & Palkovacs 2009, Drossel et al. 2001, Gómez et al. 2010, Guimarães et al. 2011, Ferriere & Legendre 2013, Bascompte & Jordano 2014, Scheuning et al. 2015). Intuitivamente, espera-se que os efeitos em cascata causados pela perda de diversidade biológica sejam ainda mais pervasivos e severos em alguns tipos especiais de interações ecológicas, como por exemplo em interações hospedeiro-parasito e mutualísticas (Dunn et al. 2009). Devido aos efeitos em cascata, o estudo de interações ecológicas também é fundamental para aumentar a eficácia de ações de conservação (Bascompte 2009, Kiers et al. 2010, Brodie et al. 2014). Apesar desta importância, interações ecológicas continuam sendo um dos atributos mais difíceis de se quantificar e de se relacionar com outros aspectos da biodiversidade (Strauss & Irwin 2004, Pascual & Dunne 2006, Bersier & Kehrli 2008, Gómez et al. 2010). Darwin (1859) descreveu a complexa e intricada rede de organismos que interagem como sendo um banco “emaranhado", que segue “leis” que determinam suas associações. Atualmente, reconhecemos que essas “leis” refletem diferentes processos ecológicos e evolutivos (Strauss & Irwin, 2004) e apesar dos grandes avanços teóricos e empíricos, desde a publicação de o “origem das espécies”, sobre os processos que geram os padrões de diversidade biológica e suas interações, o nosso entendimento sobre estes processos está longe de ser suficiente para explicar a complexidade do “emaranhado darwiniano” e de ser capaz de prever o resultado de dinâmicas ecológicas e evolutivas.

Nas últimas décadas, ecólogos têm observado a rápida ascensão de um novo e prolífico campo de conhecimento, que tem sido denominado como Ecologia de Redes (Prouxl et al. 2005, Bersier 2007, Pascual & Dunne 2007, Bascompte 2009, Miranda et al. 2013). Apropriando-se do desenvolvimento teórico e do ferramental analítico desenvolvido em Teoria de Redes Complexas, ecólogos têm tentado descrever as intricadas relações bióticas em diferentes escalas de organização biológica, quantificando o comportamento e a estrutura de ligações, e.g., interações tróficas ou não-tróficas, entre um conjunto de nós, e.g., indivíduos e/ou espécies (Prouxl et al. 2005, Bersier 2007, Pascual & Dunne 2007, Bascompte 2009, Guimarães Jr 2009, Miranda et al. 2013). Estas redes são assim chamadas em referência à Ciência da Complexidade que estuda sistemas caracterizados por propriedades emergentes, ou seja, as propriedades do sistema não são uma consequência natural de seus elementos constituintes individuais, mas sim resultante de suas interações (Guimarães Jr 2009). A utilização da abordagem de redes complexas permitiu que ecólogos pudessem ir além da descrição de interações entre pares de espécies, ou conjuntos pequenos de espécies, e começassem a descrever e quantificar as interações interespecíficas em nível de comunidades (Bascompte 2009, Guimarães Jr 2009). Apesar dos avanços recentes e do grande número de trabalhos publicados em pouco mais de duas décadas (Miranda et al. 2013), o estudo de redes ecológicas ainda se encontra na sua fase de "história natural" (Proulx et al. 2005). Boa parte dos trabalhos empíricos de redes ecológicas são estudos locais, que tentam descrever padrões topológicos de redes (Pascual & Dunne 2007, Miranda et al. 2013). Em uma revisão bibliográfica recente, Miranda et al. (2013) demonstram que o estudo de redes ecológicas tem focado principalmente na descrição topológica de redes mutualísticas, em especial redes formadas por planta e seus polinizadores. As principais características analisadas nestes estudos são a dimensão (i.e., número de espécies), conectância, distribuição das interações e padrões de agrupamento dentro das redes (Miranda et al. 2013). Estudos integrativos que abordem fatores responsáveis pelas interações e outras dimensões da biodiversidade (e.g., filogenética e funcional) ainda são escassos. Na maior parte dos estudos realizados até o momento, a única informação ecológica incorporada é a associação entre espécies e os dados de abundância. Variáveis ecológicas importantes, como a estrutura de habitat (mas ver, por exemplo, Danielson 1991 e Tylianakis et al. 2007), atributos funcionais das espécies (mas ver, por exemplo, Rossberg et al. 2008, Ibañez 2012 e Vizentin-Bugoni et al. 2014) e semelhança filogenética (Legendre et al. 2002, Ives & Godfray 2006, Hommola et al. 2009 e Hadfield et al. 2014) são normalmente omitidas. Assim sendo, mesmo com os avanços trazidos pela abordagem de redes complexas, o conhecimento do papel independente de processos ecológicos e evolutivos sobre redes de interações em nível de comunidades é ainda incipiente (Mitter et al. 1991, Lewinsohn et al. 2005, Novotny & Basset 2005).

O estudo de atributos funcionais, i.e., características comportamentais ou morfológicas associadas a uma interação biótica ou uma função de ecossistema de interesse (Naeem & Wright 2003, Lefcheck et al. 2015, Schmitz et al. 2015) tem um papel unificador dentro da Ecologia, pois permite integrar padrões e processos em distintos níveis de organização hierárquica, permitindo compreender, não só os padrões de interação intra- e interespecífico em redes de interações como também a influência da biodiversidade no funcionamento dos ecossistemas (Naeem & Wright 2003, Duffy et al. 2007, Lefcheck et al. 2015, Schmitz et al. 2015) e também de integrar dinâmicas ecológicas e evolutivas (Webb et al. 2002, Marks et al. 2006, Johnson & Stinchcombe 2007, Ferriere & Legendre 2013). O papel de atributos funcionais como processo gerador dos padrões observado em redes ecológicas tem sido o foco de estudos recentes (Cohen 1977, Rezende et al. 2007, Santamaría & Rodríguez-Gironés 2007, Rossberg et al. 2008, Ibanéz 2012, Vizentin-Bugoni et al. 2014). Os atributos das espécies podem mediar as interações principalmente com base em mecanismos de complementariedade, quando as similaridades nos valores dos atributos aumentam a probabilidade de dois indivíduos interagirem, ou de barreira, no qual a não correlação entre os atributos gera interações proibidas entre as espécies (Santamaría & Rodríguez-Gironés 2007). Talvez, uma das razões para a escassez de estudos que investiguem a relação entre outras dimensões da biodiversidade e a arquitetura de redes ecológicas é a falta de métodos adequados. Até o momento, poucas abordagens analíticas foram desenvolvidas para integrar dados funcionais e filogenéticos no estudo de redes de interação. Métodos baseados em permutações foram desenvolvidos para averiguar a influência da filogenia sobre o arranjo de redes ecológicas (Legendre et al. 2002, Hommola et al. 2009, Cruz et al. 2012). Abordagens baseadas em modelos, tais como modelos lineares generalizados mistos também foram desenvolvidos para descrever padrões filogenéticos em redes (Ives & Godfray 2006, Hadfield et al. 2014), e recentemente foram ampliados para incluir dados de atributos funcionais (Rafferty & Ives 2013).

A crise de biodiversidade supramencionada tem levantado questões fundamentais não só para podermos conservar a biodiversidade *per se*, mas também para mantermos processos ecossistêmicos importantes para as atividades humanas, como por exemplo mensurar as consequências da perda da biodiversidade e das interações biológicas em nível de comunidades e de ecossistemas e como extinções irão afetar processos ecossistêmicos básicos (Duffy et al. 2007). Para lidar com essas questões, é necessário estudos integrativos que vão além da descrição de padrões de diversidade biológica e de suas interações e que contemplem diferentes dimensões da biodiversidade e sua relação com o funcionamento dos ecossistemas. Assim, o objetivo geral desta tese foi avaliar o efeito de aspectos funcionais e evolutivos sobre a estrutura e a robustez de redes ecológicas. A tese encontra-se estruturada em três capítulos independentes, cada um correspondente a um artigo científico, formatado de acordo com as normas dos periódicos para onde foram ou serão encaminhados. Portanto, cada capítulo possui uma formatação distinta. A seguir, segue uma breve descrição de cada capítulo:

Capítulo 1 - Untangling the tangled bank: a novel method for partitioning the effects of phylogenies and traits on ecological networks

Neste capítulo, realizado em parceria com Pedro M. A. Ferreira, Bethânia O. Azambuja, Grasiela Casas e Vanderlei J. Debastiani Jr., o objetivo foi desenvolver uma nova abordagem analítica capaz de integrar dados filogenéticos e de atributos funcionais, separando seus efeitos sobre a estrutura de redes ecológicas. Neste capítulo, apresento também um estudo de simulação para estimar a acurácia (i.e., probabilidade de se incorrer em Erro Tipo I) dos testes propostos. Uma análise de um rede de dispersão de sementes é apresentada como exemplo da abordagem metodológica. Este artigo encontra-se submetido para a revista *Oikos*.

Capítulo 2 - Distinct plant extinction scenarios affect the robustness of a seed dispersal network

Neste capítulo, desenvolvido em parceria com Vanderlei J. Debastiani Jr. e Bethânia O. Azambuja, avaliei o efeito de distintos cenários de extinção sobre a robustez de uma rede de dispersão de sementes, incluindo cenários no qual as extinções primárias ocorrem em função da "distinção filogenética ou funcional" das espécies, além dos cenários clássicos usados em estudos deste tipo, que simulam extinções aleatórias e com base na especificidade (i.e., número de interações) das espécies. Este artigo será submetido para a *Journal of Vegetation Science*.

Capítulo 3 - The role of evolutionary modes for trait driven coextinctions in mutualistic networks

Neste capítulo, realizado em parceria conjuntamente com Vanderlei J. Debastiani Jr., desenvolvi um estudo teórico para avaliar como diferentes modos de evolução determinam a robustez de redes mutualistas, que estão sofrendo um ataque funcional (i.e., as extinções primárias ocorrem em função das características funcionais das espécies). Este artigo será submetido para a *Journal of Theorethical Ecology*.

Desde o início da minha formação, sempre tive um interesse muito amplo por vários tópicos dentro das áreas de Ecologia e Biologia Evolutiva. Ao longo destes anos, tive a oportunidade de trabalhar em questões aplicadas nas áreas de ecologia populacional e de comunidades, biogeografia e biologia da conservação. Porém, apesar de adorar passar horas e meses em campo, como fiz durante o mestrado, contemplando *in situ* as belezas e as curiosidades do “emaranhado darwiniano”, os tópicos que mais despertavam meus interesses nas horas de “leitura de lazer” eram artigos e livros relacionados com métodos estatísticos e teoria, e em especial, modelos que conciliavam dinâmicas ecológicas e evolutivas, apesar das limitações do meu conhecimento matemático. Até o inicio do doutorado, nunca tinha tido a oportunidade de conciliar estas questões que tanto me interessavam diretamente com meu trabalho. Acredito que finalmente tenha conseguido conciliar estes meus interesses durante o desenvolvimento desta tese, mesclando abordagens teóricas e empíricas, sem perder muito o “contato com a realidade biológica”. Espero que, apesar das limitações das abordagens propostas aqui, elas possam contribuir para o entendimento do fascinante “emaranhado darwiniano”.

Boa leitura!

Referências Bibliográficas

Albert, R. & Barabási, A. 2002. Statistical mechanics of complex networks. *Reviews of Modern Physics* 74: 47–74.

Allesina, S. & Tang, S. 2012. Stability criteria for complex ecosystems. Nature 483:

205-208

Bascompte, J. 2009. Disentangling the web of life*.* *Science* 325: 416-419.

Bascompte, J. & Jordano, P. 2014. Mutualistic networks. Princeton University Press, Princeton.

Bersier, L.F. 2007. A history of the study of ecological networks. In: *Biological networks* (pp. 365-421). World Scientific. Singapore.

Bersier, L.F. & Kehrli, P. 2008. The signature of phylogenetic constraints on food-web structure. *Ecological Complexity* 5: 132-139.

Brodie, J.F., Aslan, C.E., Rogers, H.S., Redford, K.H., Maron, J.L., Bronstein, J.L. & Groves, C.R. 2014. Secondary extinctions of biodiversity. Trends in Ecology and Evolution 29: 664-672.

Camerano, L. 1880. Dell'equilibrio dei viventi merc la reciproca distruzione. *Atti della Reale Accademia delle Scienze di Torino* 15: 393-414.

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle R.M., Palmer, T.M. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances 1: e1400253

Cohen, J.E. 1977. Food webs and the dimensionality of trophic niche space. *Proceedings of The National Academy of Science* 74: 4533-4536.

Colwell, R.K., Dunn, R.R. & Harris, N.C. 2012. Coextinction and persistence of dependent species in a changing world. Annual Review of Ecology, Evolution and Systematics 43: 183–203.

Cruz, C.P., Fonseca, C.R. & Corso, G. 2012. Ecological interaction and phylogeny, studying functionality on composed networks. *Physica A: Statistical Mechanics and its Applications* 391: 673-679.

Danielson, B.J. 1991. Communities in a landscape: the influence of habitat heterogeneity on the interactions between species. *The American Naturalist*, 138: 1105-1120.

Darwin, C. 1859. *On the origin of species by means of natural selection*. J. Murray. London.

Dobson, A., Lodge, D., Alder, J., Cumming, G.S., Keymer, J., McGlade, J., Mooney, H., Rusak, J.A., Sala, O., Wolters, V., Wall, D., Winfree, R. & Xenopoulos M. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. *Ecology* 87: 1915-1954.

Drossel, B., Higgs, P.G., & McKane, A.J. 2001. The influence of predator–prey population dynamics on the long-term evolution of food web structure. *Journal of Theoretical Biology* 208: 91-107.

Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E. & Loreau, M. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10: 522-538.

Dunn, R.R., Harris, N.C., Colwell, R.K., Koh, L.P. & Sodhi, N.S. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists?

Proceedings of the Royal Society of London B: Biological Sciences 276: 3037-3045.

Ferriere, R. & Legendre, S. 2013. Eco-evolutionary feedbacks adaptive dynamics and evolutionary rescue theory. Philosophical Transactions of the Royal Society *B: Biological Sciences*, 368: 20120081.

Fonseca, C.R. & Ganade, G. 2001. Species functional redundancy, random extinctions and the stability of ecosystems. *Journal of Ecology* 89: 118 – 125.

Gómez, J.M., Verdú, M. & Perfectti, F. 2010. Ecological interactions are evolutionarily conserved across the entire tree of life. *Nature* 465: 918-921.

Guimarães Jr. P.G. 2009. A estrutura e a dinâmica evolutiva de redes mutualísticas. *Ciência & Ambiente* 39: 137-148.

Guimarães Jr, P.R., Jordano, P. & Thompson, J.N. 2011. Evolution and coevolution in mutualistic networks. *Ecology Letters* 14: 877-885.

Hadfield, J.D., Krasnov, B.R., Poulin, R. & Nakagawa, S. 2014. A tale of two phylogenies: comparative analyses of ecological interactions. *The American Naturalist* 183: 174-187.

Hommola, K., Smith, J.E., Qiu, Y. & Gilks, W.R. 2009. A permutation test of host–parasite cospeciation. *Molecular Biology and Evolution* 26: 1457-1468.

Ibanéz, S. 2012. Optimizing size thresholds in a plant–pollinator interaction web: towards a mechanistic understanding of ecological networks. *Oecologia* 170: 233-242.

Ives, A.R. & Godfray, H.C.J. 2006. Phylogenetic Analysis of Trophic Associations. *The American Naturalist* 168: E1-E14.

Jackson, J.B., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J. & Warner, R.R. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293: 629-637

Johnson, M.T.J. & Stinchcombe, J.R. 2007. An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology & Evolution* 22: 250-257.

Kiers, E.T., Palmer, T.M., Ives, A.R., Bruno, J.F. & Bronstein, J.L. 2010. Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters* 13: 1459-1474.

Lefcheck, J.S., Bastazini, V.A.G. & Griffin, J.N. 2015. Choosing and using multiple traits in functional diversity research. *Environmental Conservation* 42: 104-107.

Legendre, P., Desdevises, Y. & Bazin, E. 2002. A statistical test for host–parasite coevolution. *Systematic Biology* 51: 217-234.

Lewinsohn, T.M., Novotny, V. & Basset, Y. 2005. Insects on plants: diversity of herbivore assemblages revisited. *Annual Review of Ecology, Evolution, and Systematics* 36: 597-620.

Marks, C.O. & Lechowicz, M.J. 2006. Alternative designs and the evolution of functional diversity. *The American Naturalist* 167: 55-66.

May, R.M. 1972. Will a large complex system be stable? *Nature* 238: 413–414

May, R. M. 2001. *Stability and complexity in model ecosystems* (Vol. 6). Princeton University Press. Princeton.

Miranda, M., Parrini, F. & Dalerum, F. 2013. A categorization of recent network approaches to analyse trophic interactions. *Methods in Ecology and Evolution* 4: 897-905.

Mitter, C., Farrell, B. & Futuyma, D.J. 1991. Phylogenetic studies of insect-plant interactions: insights into the genesis of diversity. *Trends in Ecology & Evolution* 6: 290-293.

Myers, J.A., Chase, J.M., Crandall, R.M. & Jiménez, I. 2015. Disturbance alters beta-diversity but not the relative importance of community assembly mechanisms. Journal of Ecology.

Naeem, S. & Wright, J.P. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecology Letters 6*6: 567-579.

Neutel, A.M., Heesterbeek, J.A. & de Ruiter, P.C. 2002. Stability in real food webs: weak links in long loops. *Science* 296: 1120-1123.

Novotny, V. & Basset, Y. 2005. Host specificity of insect herbivores in tropical forests. *Proceedings of The Royal Society B: Biological Sciences* 272: 1083-1090.

Nuismer, S.L. & Harmon, L.J. 2015. Predicting rates of interspecific interaction from phylogenetic trees. *Ecology Letters* 18: 17-27.

Pascual, M. & Dunne, J.A. 2006. Ecological Networks: Linking structure to Dynamic in food webs. Oxford University press, Oxford.

Pearse, I.S. & Hipp, A.L. 2009. Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences* 106: 18097-18102.

Petchey, O.L. & Gaston, K.J. 2002. Extinction and the loss of functional diversity. *Proceedings of the Royal Society of London B: Biological Sciences* 269: 1721 – 1727.

Pimm, S.L. 1984. The complexity and stability of ecosystems. *Nature* 307: 321-326.

Pimm, S.L. 1991. *The balance of nature? Ecological issues in the conservation of species and communities*. University of Chicago Press. Chicago.

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N. & Sexton, J.O. 2014. The biodiversity of species and their rates of extinction distribution and protection. *Science* 344: 1246752

Pires, M.M., Koch, P.L., Farinã, R.A., de Aguiar, M.A.M., dos Reis, S.F. & Guimarães Jr, P.R. 2015. Pleistocene megafaunal interaction networks became more vulnerable after human arrival. *Proceedings of The Royal Society B: Biological Sciences* 282: 20151367.

Post, D.M. & Palkovacs, E.P. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364: 1629-1640.

Proulx, S.R., Promislow, D.E., & Phillips, P.C. 2005. Network thinking in ecology and evolution. *Trends in Ecology & Evolution* 20: 345-353.

Rafferty, N.E. & Ives, A.R. 2013. Phylogenetic trait-based analyses of ecological networks. *Ecology* 94: 2321-2333.

Rezende, E.L., Lavabre, J.E., Guimarães Jr, P.R., Jordano P. & Bascompte, J. 2007. Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature* 448: 925-928.

Rossberg, A.G., Brännström, Å. & Dieckmann, U. 2010. How trophic interaction strength depends on traits. *Theoretical Ecology* 3: 13-24.

Santamaría, L. & Rodríguez-Gironés, M.A. 2007. Linkage rules for plant–pollinator networks: trait complementarity or exploitation barriers? *PLoS Bio* 5: e31

Säterberg, T., Sellman, S. & Ebenman, B 2013. High frequency of functional extinctions in ecological networks. *Nature* 499: 468-470

Schleuning, M., Fründ, J. & García, D. 2015. Predicting ecosystem functions from biodiversity and mutualistic networks: an extension of trait-based concepts to plant–animal interactions. *Ecography* 38: 380-392

Schmitz, O.J., Buchkowski, R.W., Burghardt, K.T. & Donihue, C.M. 2015. Functional traits and trait-mediated interactions: connecting community-level interactions with ecosystem functioning. Advances in Ecological Research.

Strauss, S.Y. & Irwin, R.E. 2004. Ecological and Evolutionary Consequences of Multispecies Plant-Animal Interactions. *Annual Review of Ecology, Evolution, and Systematics* 35: 435-466.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature* 445: 202-205.

Vázquez, D.P., Blüthgen, N., Cagnolo, L. & Chacoff, N.P. 2009. Uniting pattern and process in plant–animal mutualistic networks: a review. *Annals of Botany* 103: 1445-1457.

Vieira, M.C., Cianciaruso, M.V. & Almeida-Neto, M. 2013. Plant-Pollinator coextinctions and the loss of plant functional and phylogenetic diversity. *Plos One* 8: e81242

Vieira, M.C. & Almeida-Neto, M. 2014. 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: Biological Sciences* 281: 20132397-20132397.

Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M. J. 2002. Phylogenies and Community Ecology. *Annual Review of Ecology and Systematics* 33: 475-505.

Capítulo 1 - Untangling the tangled bank: a novel method for partitioning the effects of phylogenies and traits on ecological networks

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Summary

1. Understanding how evolutionary and ecological processes shape species interaction networks remains as one of the main challenges in eco-evolutionary studies.

2. Here, we present an integrative analytical framework to partition the effects of phylogenies and functional traits on the structure of ecological networks. The method combines fuzzy set theory and matrix correlation, implemented under a Monte Carlo framework.

3. We designed a simulation study in order to estimate the accuracy of the methods proposed here, measuring type I error rates. The simulation study

shows that the method is accurate, i.e., incorrectly rejecting a true null hypothesis in ~ 5% of the cases and falling within the confidence interval.

4. We illustrate our framework, using data from a seed dispersal network from southern Brazil. Our analyses suggest that birds must have specific traits in order to consume their plant resources, and that phylogenetic resemblance has no explanatory power in order to explain species traits and species interactions in this seed-dispersal network.

Key words: eco-evolutionary dynamics, ecophylogenetics, fuzzy sets, trophic association, species interaction

Introduction

Species interactions are central to understanding ecological and evolutionary dynamics, yet one of the most challenging attributes to quantify and relate to other aspects of biodiversity (Strauss & Irwin 2004; Pascual & Dunne 2006; Bersier & Kehrli 2008; Gómez et al. 2010). Darwin (1859) envisaged the complex network of interacting organisms as an “entangled bank” shaped by deterministic “laws” that drive the associations among species. We have now come to recognize that these “laws” likely reflect different ecological and evolutionary processes (Strauss & Irwin 2004). Some of the well-recognized factors shaping the architecture of ecological networks are environmental gradients (Danielson 1991; Tylianakis et al. 2007), species abundance (Krishna et al. 2008; Vázquez et al. 2009; Verdú et al. 2011; Ibanéz 2012), sequential evolution (Jermy 1976), cocladogenesis or cospeciation (Page & Charleston 1998; Page 2003; Thompson 2014), coevolution (Thompson 1994; Gross et al.

2008; Guimarães Jr. 2011) and species functional traits (Cohen 1977, Rezende et al. 2007; Rossberg et al. 2008; Ibanéz 2012; Vizentin-Bugoni et al. 2014), defined here as any measurable organismal eto-ecological characteristic or phenotype associated with a biotic interaction or an ecosystem function of interest (Naeem & Wright 2003; Lefcheck et al. 2015; Schmitz et al 2015). Nonetheless, knowledge of the independent role of these factors on species interactions at the community level is still poorly appreciated (Mitter et al. 1991; Lewinsohn et al. 2005; Novotny & Basset 2005), especially when it comes to measuring the importance of functional traits. As closely related species are likely to retain their ancestral traits, discriminating the effects of species traits and long-term evolutionary history is troublesome (Felsenstein 1985; Losos 2008).

Over the past recent decades, ecologists have started to incorporate phylogenetic-based methods into ecological research, which gave rise to a novel and prolific field of inquiry, the so-called Ecophylogenetics (Webb 2000; Webb et al. 2002; Mouquet et al. 2012). These methods have allowed ecologists to overcome some of the difficulties inherent to the study of evolutionary phenomena, and have been very useful in studies trying to understand how historical and evolutionary contingencies affect patterns at different ecological scales (Mouquet et al. 2012). With the ready availability of extensive phylogenies, community ecologists have started to integrate ecological and evolutionary processes in order to describe patterns of ecological community assembly and species diversity (Webb 2000; Webb et al. 2002; Johnson & Stinchcombe 2007; Cavender-Bares et al. 2009).

Despite the advances that phylogenetic data have brought to our understanding of patterns of community structure, the same has not been achieved for the ecological knowledge on interacting species yet. In this respect, approaches that relate phylogenetic data to species interactions have been used in order to verify the degree of phylogenetic clustering or over dispersion in the specificity of plant-herbivore interactions, especially in tropical forests (e.g., Novotny & Basset 2005; Weiblen et al. 2006). Such an approach has also been used to study coevolution within small clades (e.g., Pedron et al. 2012), to investigate the importance of traits and phylogenies within a single trophic level (e.g., Novotny & Basset 2005; Weiblen et al. 2006) and to evaluate the effect of phylogenetic structure on coextinction cascades within networks (Rezende et al. 2007). However, the only ecological information incorporated in these models is the relationship between abundance and each taxa, and important ecological factors such as habitat structure and species traits are usually omitted (but see, for instance, Pearse & Hipp 2009; Vázquez 2009; Rafferty & Ives 2013). The interplay between phylogenetic history and the degree of phylogenetic conservatism in species traits are likely to affect some of the patterns found in empirical ecological networks (Figure 1).

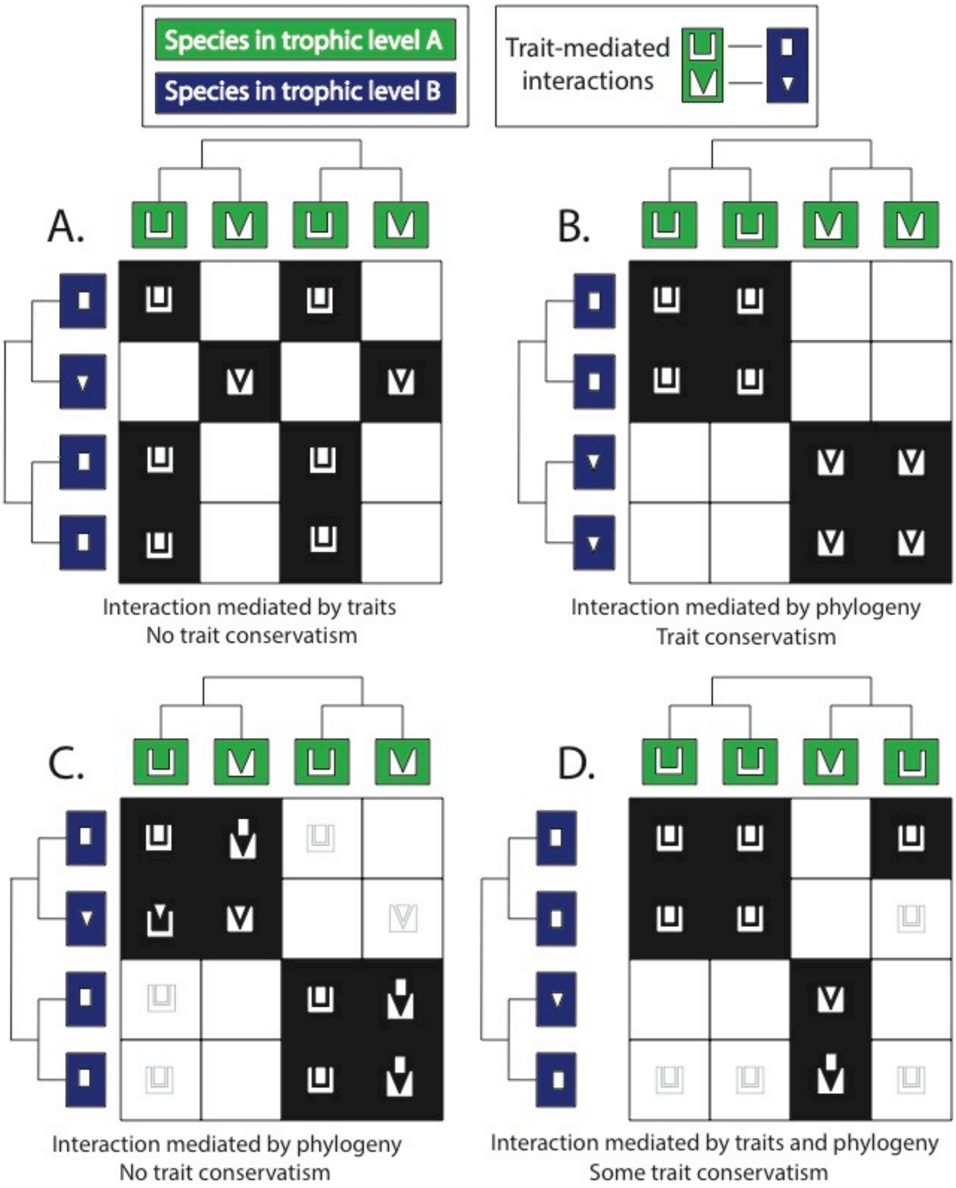

Fig. 1. Patterns of ecological interactions arising from the interplay between species functional traits and their evolutionary history. Black squares denote realized species interactions. A) Species interactions are a result of labile species traits that are not phylogenetic conserved; B) Traits accounting for species interaction are phylogenetic conserved; C) Interactions are mediated by shared phylogenetic history, independent of species traits; D) Functional traits are partially phylogenetic conserved.

Insofar, few integrative methods have been proposed and evaluated in detail. For instance, Legendre et al. (2002) and Hommola et al. (2009) developed permutation methods to measure coevolution and cospeciation between hosts and their parasites. Cruz et al. (2012) developed a method to test the influence of phylogenies in the arrangement of bipartite networks (i.e., a network which has two sets of species, such as plants and pollinators, with edges between and not within sets). These methods use only phylogenetic data, and recent analyses have demonstrated that some of them are likely to result in high rates of Type I error, i.e., incorrectly rejecting a true null hypothesis (Hadfield et al. 2014). Model based approaches, such as generalized linear mixed models or phylogenetic regressions (Ives & Godfray 2006; Hadfield et al. 2014) have also been developed in order to describe phylogenetic patterns in bipartite networks, and have recently been extended to include trait data (Rafferty & Ives 2013). However, these model-based approaches rely on distribution assumptions, which may be unrealistic and complicated to parameterize. Randomization tests offer a simpler solution and are much less restricted by assumptions, as they rely on distribution free statistics and on algorithms that use systematic or random data permutations to generate alternative outcomes for the chosen test statistic under a true null hypothesis (Pillar 2013).

Here, we develop a statistical framework that integrates phylogenetic and trait data from bipartite networks, although the method can be extended to unipartite networks (networks which has only one set of species, such as a co-occurrence network), based on fuzzy set theory (Zadeh 1965) and randomization tests (Manly 1997). Our approach enables the detection of: (1)

phylogenetic signal in traits accounting for species interactions; (2) correlation among traits accounting for species interactions, removing the effect of phylogenies; and (3) correlation between phylogenies, which is a necessary but not sufficient condition to be considered a sign of cospeciation or cocladogenesis. We provide a simulation study that shows that our methodology provides adequate rates of Type I error. We also showcase our framework with an analysis of a mutualistic network from southern Brazil.

Input Data

Our framework requires data organized into, at least, five matrices (Fig. 2A): (i) A matrix containing the observed pairwise species interactions (matrix $\mathbf{N_{AB}}$; containing either binary or frequency data). For simplicity we will assume that this matrix contains two different trophic levels, with prey (A) and predators (B), but it can accommodate any kind of antagonist or mutualistic interaction, and it can also contain data from unipartite networks. The phylogenetic pairwise resemblances between species: matrix $\mathbf{A_A}$ for the lower trophic level and $\mathbf{A_B}$ for the higher trophic level (ii-iii). Species trait data: matrices $\mathbf{B_A}$ (*traits x species*) for the lower trophic level and $\mathbf{B_B}$ for the higher trophic level (iv-v), with traits of any type. An additional binary matrix (**F**), containing species pairwise co-occurrence (either temporal or spatial), coded as 0 (the pair of species do not co-occur) or 1 (the pair of species co-occur), can also be used (Fig. 2C). Matrix **F** is not required for the analysis, but may provide additional information and control for situations where phenological or spatial decoupling, for instance, is common.

Scaling Up of Traits and Phylogenies to the Network Level

Trait data in matrices $\mathbf{B_A}$ and $\mathbf{B_B}$, and phylogenetic data in matrices $\mathbf{A_A}$ and $\mathbf{A_B}$ (Fig. 2A) are scaled up to the network level using similar methods previously described in Pillar & Orlóci (1991), Pillar et al. (2009) and Pillar & Duarte (2010), which are extended and explained here in detail. The first step is to transform trait matrices ($\mathbf{B_A}$ and $\mathbf{B_B}$), into a symmetric similarity matrix (Matrices $\mathbf{S_A}$ and $\mathbf{S_B}$; Fig. 2B), using an appropriate measure. For this, we have used Gower's distance (D), which is then converted into a similarity in the range 0-1. Gower's distance (D) is defined as:

$$D = \sqrt[2]{1 - G} \qquad \text{eqn 1}$$

where G is the Gower's general similarity coefficient, computed as follows:

$$G_{12} = \frac{\sum_{j=1}^{p} w_{12j} s_{12j}}{\sum_{j=1}^{p} w_{12j}} \qquad \text{eqn 2}$$

where s_{12j} is the partial similarity in the range 0-1 between species **1** and **2** of the same trophic level. This partial similarity can be applied to different types of traits (binary, qualitative, semiquantitative, quantitative and mixed variables) each one according to its own mathematical type (Podani 2000; Legendre & Legendre 2012). w_j is the Kronecker's delta, describing the presence of information: $w_j = 0$ when the information about y_j is missing for one or the other species, or both; $w_j = 1$ when information is present for both species (Podani 2000; Legendre & Legendre 2012).

Gower's distance (D) is then converted to a similarity (s) as already explained. Based on their functional similarity, every species i among s species in each trophic level specifies a fuzzy set to which every species *j* (*j* = 1 to *s* species, including species *i*) in the same trophic level belongs with a certain degree of belonging (u_{ij}), taking values in a unit interval. The degrees of belonging of each species are the elements comprised in the new matrices $\mathbf{U_A}$ or $\mathbf{U_B}$ (Fig. 2B) and are standardized to column unit total by

$$u_{ij} = \frac{s_{ij}}{\sum_{k=1}^{s} s_{kj}} \qquad \text{eqn 3}$$

where s_{ij} is the pairwise similarity in the range 0-1, in our definition, Gower's similarity.

Using matrix multiplication, $\mathbf{Y_A} = \mathbf{U_A N_{AB}}$ and $\mathbf{Y_B} = \mathbf{U_B N_{AB}'}$, we can estimate the probability of a species in one trophic level interacting with a species in the other trophic level, weighted by their trait similarities (Fig. 2B). Using the same procedure, matrices of phylogenetic distances $\mathbf{A_A}$ and $\mathbf{A_B}$ are transformed into similarity matrices ($\mathbf{Q_A}$ or $\mathbf{Q_B}$; Fig. 1A), and then scaled up to the network level, generating matrices $\mathbf{P_B}$ and $\mathbf{P_A}$, corresponding to the probability of species interactions weighted by species phylogenetic resemblance, in each trophic level (Fig. 2B). Matrices $\mathbf{Y_A}$, $\mathbf{Y_B}$, $\mathbf{P_A}$ and $\mathbf{P_B}$ contain the probability of interaction between species that are originally described in matrix $\mathbf{N_{AB}}$. However, the probability value for the same interaction will differ between matrices, since in each matrix the values are constrained by a different subset of traits (matrices $\mathbf{Y_A}$ and $\mathbf{Y_B}$) and phylogenetic distances (matrices $\mathbf{P_A}$ and $\mathbf{P_B}$).

Dealing With Co-Occurrence

Ecologists usually assemble networks that result from sampling schemes that encompass different spatial (Winemiller 1990; Dáttilo et al. 2013) and temporal scales (Winemiller 1990; Díaz-Castelazo et al. 2010; Johansson et al. 2015). Nonetheless, in the resulting network some interactions may never happen because a pair of species may not co-occur either in time (e.g., phenological differences) or space (e.g., species inhabit different habitat patches), despite their phylogenetic- or trait-similarity. To illustrate the point, imagine two predator species that have similar probabilities of interaction with a specific prey, either because they are functionally or phylogenetically similar, but one of them may not consume the same resource species because they never occur at the same place and time (e.g., spatial and phenological decoupling), either by chance or by biological reasons. Matrix **F** may be used to take this into account or to reveal spatial and temporal patterns. Using Hadamard element-wise multiplier, we can multiply matrices $\mathbf{Y_A}$, $\mathbf{Y_B}$, $\mathbf{P_A}$ and $\mathbf{P_B}$ by matrix **F** (Fig. 2C). This multiplication will result in four new matrices (Fig. 2C), which contain interaction probabilities, either weighted by trait- or phylogenetic resemblance, but corrected by either spatial or temporal mismatch between species from different trophic levels.

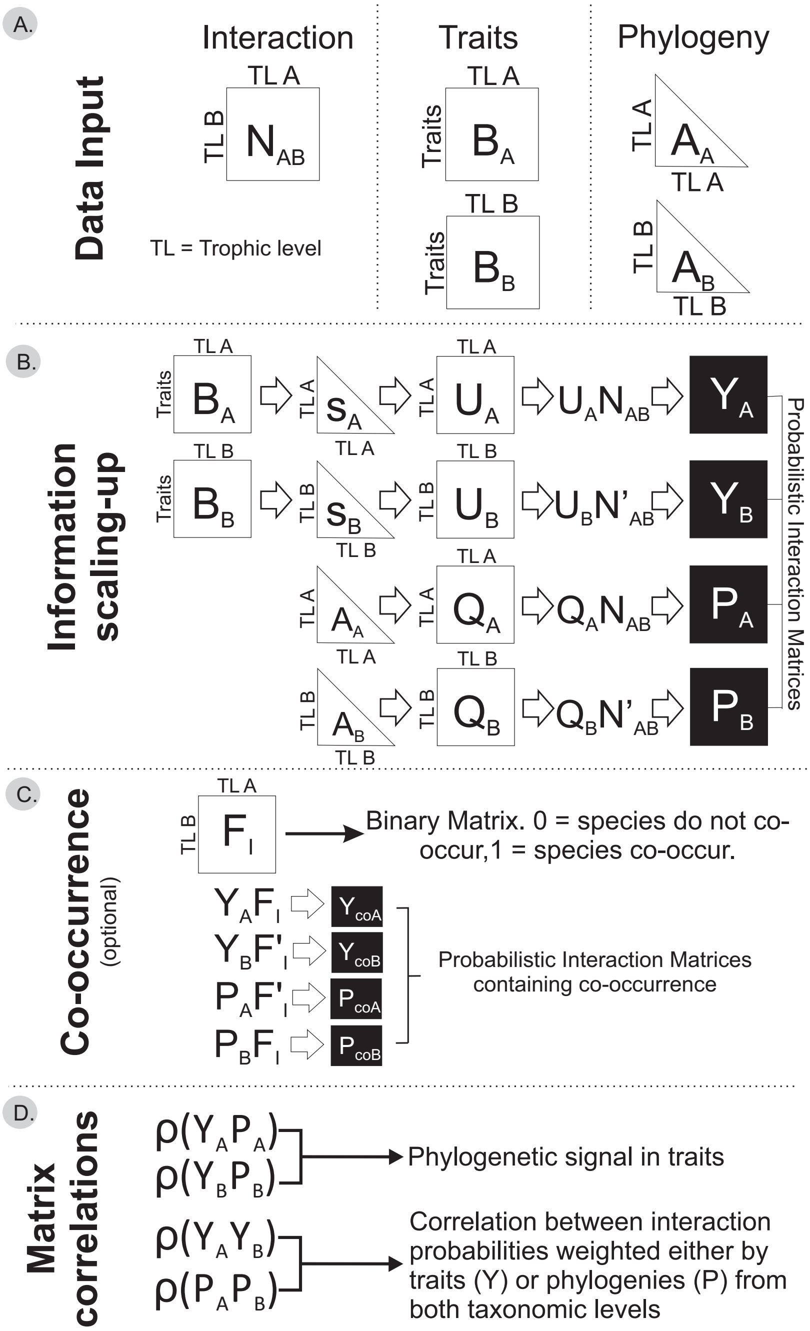

Fig. 2. An overall scheme of our analytical framework. A) Input data; B) Scaling-up of phylogeny and trait-based data to the network level; C) Fuzzy-weighted interaction matrices are multiplied, using Hadamard element-wise multiplier, by a (temporal or spatial) co-ocurrence matrix; D) Eco-evolutionary patterns revealed by matrix correlations.

Matrix Correlations and Null Model

The probabilistic matrices defined before can be used in a series of matrix correlation tests in order to detect i) the phylogenetic component of interactions, ii) phylogenetic signal in traits in both trophic levels and iii) the functional component of interactions (Fig. 2D). The statistical significance of each matrix correlation is evaluated by permutation against null models, which are explained in details bellow. The correlation $\rho(\mathbf{P_A P_B})$ reveals patterns of the phylogenetic component of interactions which might be an evidence of cospeciation or, more generally, cocladogenesis (Fig. 2D), in which two or more interacting lineages undergo matched branching events during their phylogenetic history (Thompson 2014). Phylogenetic signal in traits are evaluated through the correlations $\rho(\mathbf{Y_A P_A})$ and $\rho(\mathbf{Y_B P_B})$ (Fig. 2D). When the correlation $\rho(\mathbf{Y_A Y_B})$ is high, we may infer that the observed interactions are largely due to functional traits of species in both trophic levels, e.g. plant and animal species. However, as we pointed out earlier, traits may present a significant degree of phylogenetic autocorrelation (Felsenstein 1985; Losos 2008), which makes necessary to consider the influence of phylogeny into this trait-based analysis in order to appreciate the independent effect of traits on species interaction. At first, we used higher-order partial matrix correlation, in order to remove the effect of phylogenies and estimate the independent effect of traits, but this approach resulted in tests that were inaccurate in terms of Type I error rates (see section “Simulation Study to Assess Type I Error Rates” for more details). Thus, to assess the independent trait component of interactions, we used a slightly different approach removing the phylogenetic component of interactions by computing linear regressions using the vector that contains species pairwise phylogenetic distances as the predictor variable and the vector containing species pairwise functional distance as response variable. These vectors are obtained from the original phylogenetic and trait matrices from each trophic level (Fig. 3). The residuals of these regressions are then used in the scaling up process, resulting in two new matrices, $\mathbf{U}_{\mathbf{resA}}$ and $\mathbf{U}_{\mathbf{resb}}$ (Fig. 3). By matrix multiplication, we obtain new trait-weighted interaction matrices removing the effect of phylogenies: $\mathbf{R}_{\mathbf{A}} = \mathbf{U}_{\mathbf{resb}} \mathbf{N}_{\mathbf{AB}}$ and $\mathbf{R}_{\mathbf{B}} = \mathbf{U}_{\mathbf{resb}}\mathbf{N}_{\mathbf{AB}}'$. The correlation $\rho(\mathbf{R}_{\mathbf{A}}\mathbf{R}_{\mathbf{B}})$ will demonstrate the correlation between these trait-weighted interaction matrices independent of the effect of phylogenies (Fig. 3)

The statistical significance of each zero-order matrix correlation, based on distance matrices, is then evaluated by permutation against null models, where in each random permutation the columns in one of the fuzzy weighted matrices ($\mathbf{U}_{\mathbf{A}}$, $\mathbf{U}_{\mathbf{B}}$, $\mathbf{Q}_{\mathbf{A}}$ or $\mathbf{Q}_{\mathbf{B}}$ and related matrices; Figs. 2A and 3) are randomly permuted. This random rearrangement is repeated many times and new values of matrix correlations are computed and compared to the observed value; P-values for the test will be the proportion of matrix correlations statistics (ρ) larger than or equal to the observed ρ in a large number of random permutations ($P[\rho^{null} \geq \rho^{observed}]$).

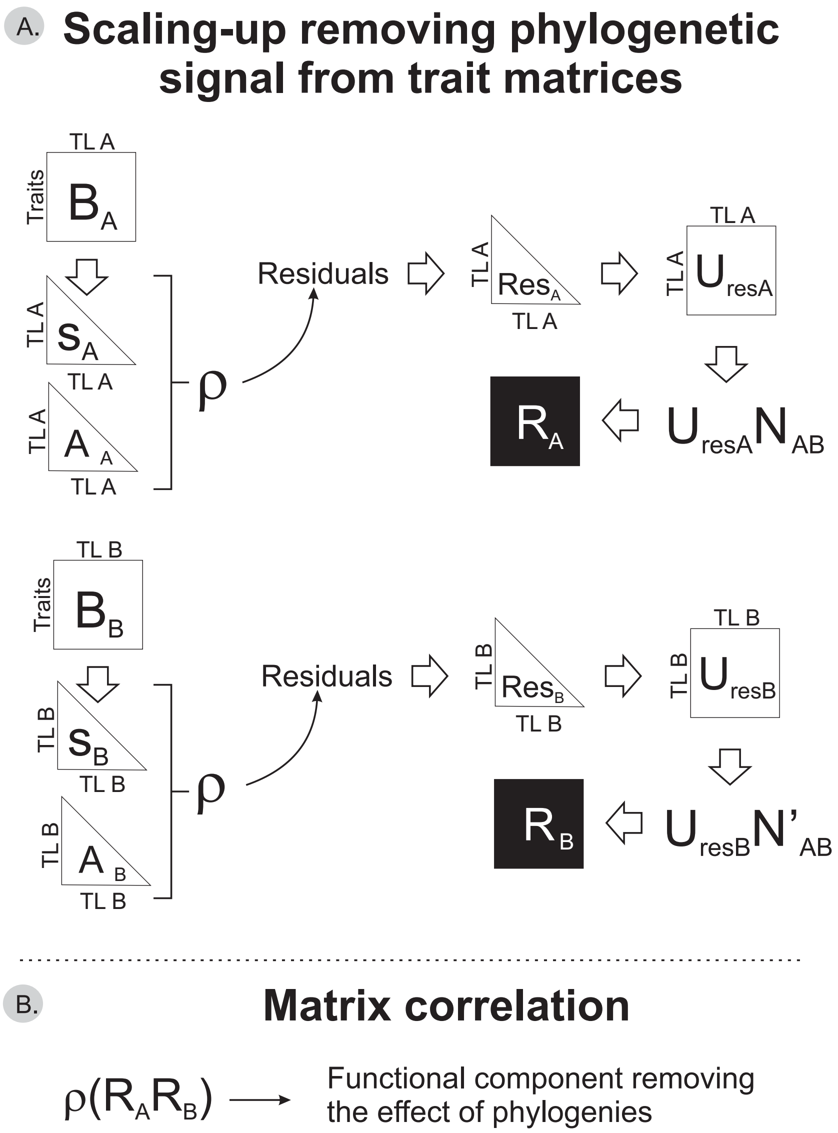

Fig 3. Method used to assess the functional component of interactions removing the effect of phylogeny A) Scaling up of traits to the network level, removing the phylogenetic signal. B) Matrix correlation model used to test the effect of traits in species interactions.

Computer Software

The methods described here have been implemented in the R (R Core Team 2012) package SYNCSA (Debastiani & Pillar 2012) and the code is available as supplementary material (supplementary material 1).

Simulation Study to Assess Type I Error Rates

We evaluated the accuracy of the test by investigating the rates of Type I Error (the incorrect rejection of a true null hypothesis) using large sets of simulated data (varying between 29,000 and 30,164 iterations). Simulations were implemented as to represent realistic biological scenarios, with interaction networks varying in size, number of functional traits and the number of interactions, and with species traits evolving according with distinct evolutionary models. But as we were interested in the accuracy of the test, a pure stochastic process determines species interactions. Simulations were implemented in several steps, which are described in detail below:

a) We first produced simulated phylogenetic trees of different sizes, from 5 to 20 species for each trophic level, which gave us networks (see step *d*) that varied in size, from 10 to 40 species. The size of the networks used in our simulations is consistent with the size of sampled ecological networks, which are usually small, comprising, on average, 23 species as suggested by Jonsson (2014; see also Chapter 3 for an estimate for mutualistic networks);

b) We simulated the evolution of a small number of continuous characters (1-8 traits) along with their phylogenetic trees, under an Ornstein-Uhlenbeck model, which simulates different levels of constraints, such as stabilizing selection towards an optimum trait value for all species in each phylogenetic lineage (Hansen 1997; Blomberg et al. 2003). The choice of the number of traits is also congruent with evidence that suggests that very few traits are necessary to account for species interaction (Cohen 1977; Allesina et al. 2008; Eklöff et al. 2013). The Ornstein-Uhlenbeck model is governed by one special parameter (α), which directs the strength of the stabilizing selection towards an optimum trait value; larger values of α indicate that trait values will be closer to the optimum value, thus showing less phylogenetic signal (Blomberg et al. 2003; Harmon et al. 2010). We simulated different set of parameter values, in order to determine the strength of character evolution towards the optimum trait value. The range of parameter choices used in the simulations allowed trait evolution to either follow a Brownian process ($\alpha \rightarrow 0$), as if evolution of traits followed a random walk through evolutionary time, or to follow a strong stabilizing selection towards an optimum trait value ($\alpha \rightarrow 1$).

c) As we were interested in estimating Type I error rates, we simulated qualitative interaction matrices based on a random process, using a binomial distribution, where each cell of each simulated network has a probability of success, i.e., that a pair of species from different trophic levels interact, equal to 0.5. Therefore, the resulting network is determined solely by chance, without any regard to traits or phylogenies.

d) We then tested three sets of matrix correlations, in order to test different eco-evo patterns (see Fig. 2): phylogenetic component, i.e., the association between phylogenies; the functional component of interactions removing the effect of phylogeny and without removing it. The significance level to reject the null hypothesis was set a priori at α = 0.05. Each test was deemed accurate if Type I Error was within 99.9% confidence limits (0.05 ± 0.004 for both 29,000 or 30,164 simulated datasets).

The results of our simulation study show that our framework is accurate, with Type I Error within 99.9% confidence limits for $P = 0.05$ (Table 1). All tests behave adequately in terms of Type I Error, rejecting a true null hypothesis in ~ 5% of the simulated datasets, independent of network size, number of traits and the strength of stabilizing selection. We do not show the results for each subset of simulation here, because they behaved exactly the same. In a first simulation study, we tried to estimate the functional component of interactions, removing the phylogenetic signal using simple first order matrix correlations (akin to partial Mantel test) using the phylogenetic and trait matrices after they have been scaled up to the network level (matrices $\mathbf{Y_A}$, $\mathbf{Y_B}$, $\mathbf{P_A}$ and $\mathbf{P_B}$ in Fig. 2). However, the results of this first simulation study showed these higher order matrix correlations were inaccurate, with very low rates of Type I Error, rejecting a true null hypothesis in 0.3% of the simulated datasets. In order to circumvent this problem, we used the procedure described above (see section "Matrix Correlations and Null Model" and Fig. 2), scaling up the residual matrices from phylogenetic and trait matrices. This procedure proved to be accurate in terms of Type I Error (Table 1).

Table 1. Simulation results for Type I Error of the matrix correlation tests proposed in this paper. Number of simulated data sets varied between 29,000-30,164. Tests were considered accurate in terms of Type I Error if the rejection rates fell within the 99.9% confidence limits (0.05 ± 0.004).

	Proportion of tests
Phylogenetic component	0.053
Functional component removing phylogenetic signal	0.046
Functional component without removing phylogenetic signal	0.051

An Example from a Mutualistic Network

In order to illustrate our approach, we used data from a seed dispersal network from a grassland-forest mosaic from southern Brazil (30°25'S; 52°21'W). Birds were captured with mist-nets, which were placed in grasslands near to forest patches. Mist nets were set up monthly for eight consecutive days, between July 2007 and June 2008. Birds captured in mist nets were then placed into fabric bags for 20 minutes, and their feces were collected from bags. The seeds found in fecal samples were identified to species, and with this information at hand, it was possible to build an interaction matrix between birds and the plant they consumed, with the frequencies of interaction (i.e., the number of times a specific bird species consumed a specific plant species). This interaction matrix consists of 128 interactions between 22 plant species, and 12 bird species (see Fig. 4). For further information on sampling please refer to Azambuja & Pillar (in prep.).

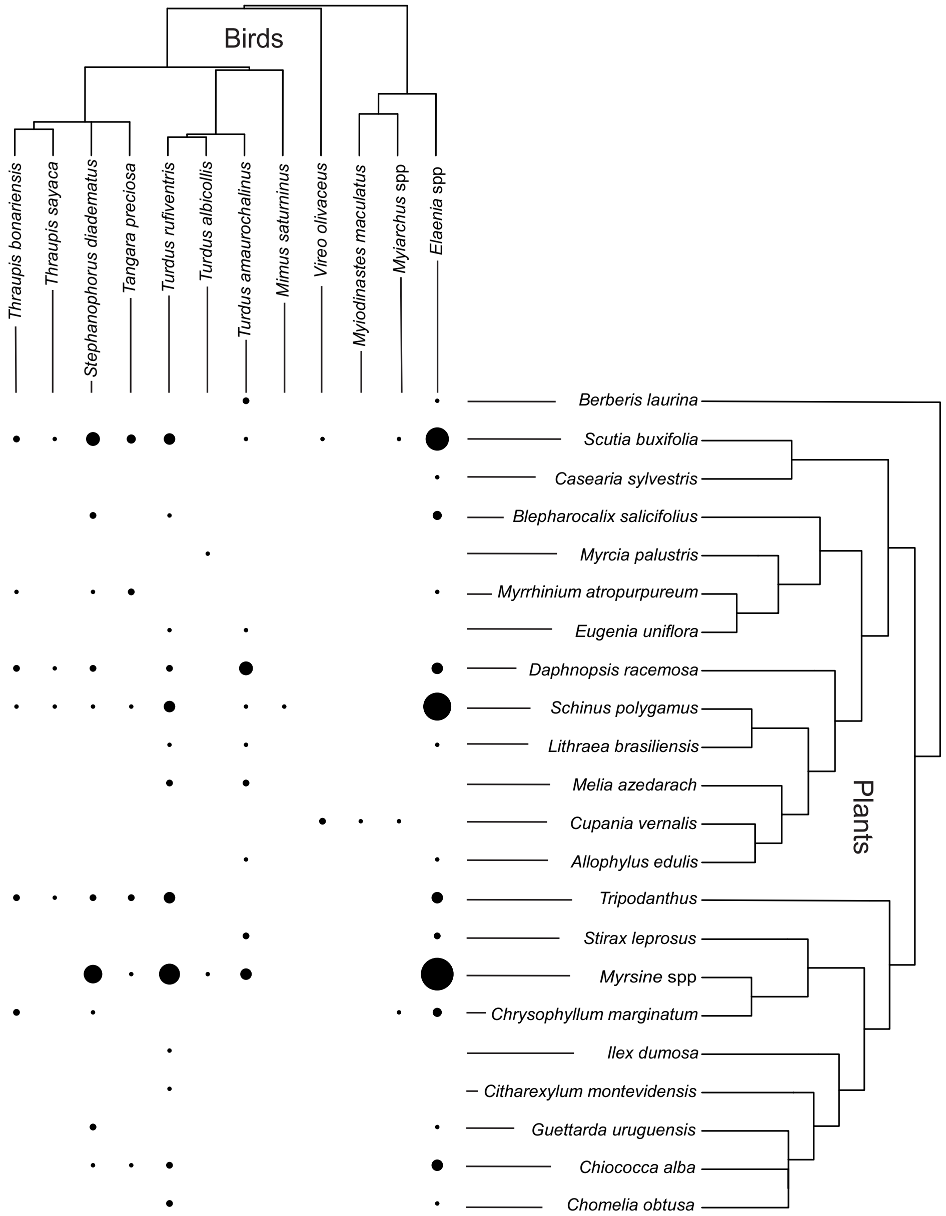

Fig. 4. Seed-dispersal interactions in southern Brazil, with the phylogeny of each trophic level represented in the margins. Circle sizes are proportional to the frequency of the interaction.

We measured five plant traits (dispersule diameter, maximum plant height, aril presence, diaspore shape and color) and four bird traits (body length, beak length, beak height and the hand-wing index; see supplementary material 2 for information on data acquisition). Using the APG III megatree (R20100701.new; Angiosperm Phylogeny Group 2009), we constructed a base family-level phylogeny. Due to the lack of within-family resolution, species and genera were represented as soft polytomies within genera and families, respectively. Branch lengths were added to the phylogeny using the BLADJ algorithm (Webb et al. 2008), which constrains the age of nodes included in the sample according to the dated molecular phylogeny of Wikström et al. (2001). For birds, we used the phylogenetic trees available at birdtree.org (Jetz et al. 2012). We then built a strict consensus tree using Mesquite 2.75 (Maddison & Maddison 2011). As for plant species, polytomies were also represented as soft polytomies. Branch lengths of terminal taxa were arbitrary lengthened in the consensus tree using the function “ultrametricize” in Mesquite. Phylogenetic distances between all pairs of species were then calculated using the PHYDIST procedure of Phylocom (Webb et al. 2008).

We employed our framework to scale phylogenetic and trait data to the network level. As sampling lasted nearly one year, we used Hadamard multiplier to multiply all four probabilistic matrices by the F matrix, which is an incidence matrix, where 1s mean that at least once, during the sampling period, a bird species occurred in the study site when a specific plant species was fructifying, and 0s mean otherwise. We then tested four matrix correlation models, using

Euclidian distance as a resemblance measure: phylogenetic signal in traits in each trophic level; correlation among traits accounting for species interactions, removing the effect of phylogenies and; the correlation between phylogenies, in order to detect patterns of cocladogenesis.

The results are shown in Table 2. Among the four tested models, the only significant correlation was the one between bird and plant traits, showing that birds must have specific traits in order to consume their plant resources, and that phylogenetic resemblance has no explanatory power in order to explain the evolution of species traits and species interactions in this seed-dispersal network. As the benefits of plant-animal disperser interactions are mutual, ecologists and evolutionary biologists have long pondered whether this sort of mutualistic interaction is constrained by strong evolutionary forces (Snow 1971; Morton 1978; Howe 1993; Muller-Landau & Hardesty 2005). Theoretical models and empirical data suggest that plant-seed disperser interactions are a system of low specialization (Herrera 1995; Muller-Landau & Hardesty 2005), in which a species from one trophic level can interact with many species of the other level. Moreover, it has been suggested that frugivory is a foraging mode that does not require special adaptation in traits involved in the interaction (Jordano 2000). Our results seem to corroborate the idea that plant-seed disperser networks exhibit low specialization, in terms of interacting species, and are not governed by strong evolutionary constraints. However, our results also demonstrate that some level of trait complementarity is relevant for determining interaction patterns, despite the high plasticity of the foraging spectrum of birds. Although one might expect trait evolution to be influenced by the phylogenetic relationship among species, it is now widely recognized that traits vary tremendously in their phylogenetic pattern (Blomberg et al. 2003), and recent evidence suggest that this relationship should be expected only under some very specific conditions. Nuismer & Harmon (2015) have demonstrated that mutualistic networks, such as our example, should exhibit less phylogenetic signal in rates of interactions than competitive ones, and that if interactions within a network depend on a mechanism of phenotype differences, phylogenetic information has little predictive power for trait evolution and interaction rates.

Table 2. Summary statistics for four models for the seed dispersal network from southern Brazil.

	ρ	**p-value**
Phylogenetic component	0.06	0.84
Functional component, removing phylogenetic signal	0.49	<0.001*
Phylogenetic signal in plant species	0.28	0.99
Phylogenetic signal in bird species	0.25	0.94

*Results deemed significant at α=0.05.

Discussion and Final Remarks

Understanding how species interact and how the topology of ecological networks influences the dynamics of populations and communities is at the core of ecological inquiry and has been mind-boggling ecologists for over a century now (Camerano 1880; Paine 1980; Polis & Strong 1996; Ings et al. 2009; Schleuning et al. in press.). Despite the long tradition, the study of complex ecological networks has dramatically increased in the past two decades (Miranda et al. 2013). Nonetheless, most empirical studies still focus on the description of network properties, such as size (i.e., species richness within the network), connectivity and degree distribution (Miranda et al. 2013). Only recently, there has been a growing interest of moving beyond the description of topological patterns and the integration of other types of data, such as phylogenetic and trait data, to reveal patterns of species interactions (e.g., Rezende et al. 2007; Rafferty & Ives 2013; Hadfield et al. 2014; Vizentin-Bugoni et al. 2014). Here, we developed a statistical framework based on fuzzy set theory and Monte Carlo statistics capable of identifying eco-evolutionary patterns that might suit some of this interest. More specifically, our method is intended to answer the following ecological questions: Is there phylogenetic signal in traits accounting for species interaction? Are species interactions driven by trait resemblance? Are species interactions driven by phylogenetic resemblance? (i.e., are there evidences of cocladogenesis?)? At this point, however, it is important to note that interpreting cophylogenetic analyses can be extremely difficult and finding correlated phylogenies does not necessarily indicate a history of coevolution or cospeciation (Banks & Paterson 2005). For instance, in host-parasites networks, gradual host-switching by parasite species, followed by speciation events may lead to “false” congruent phylogenies (Banks & Paterson 2005). Thus, results should be interpreted with caution. However, our cophylogenetic analysis can help ecologists to understand network patterns and bring important information to applied matters, such as choosing target species to conservation or restoration plans, as it allows us to identify pair of species with higher chance of interacting, and the role of phylogenetic- and/or trait- resemblance, even if different processes may lead to the same apparent pattern.

Our method is less restricted by statistical assumptions than model-based approaches, it is relatively easy to apply and can be used with both qualitative and quantitative species interaction matrices. Other advantages of the method are that it is suitable for a range of data types, allowing investigators to use qualitative (expanded as dummy variables) and/or quantitative traits. Also it does not require the availability of dated phylogenetic trees, as the method is based on resemblance matrices. In situations where phylogenetic trees are not available, distances can be computed from raw data such as sequences in databases. Moreover, our method could be easily expanded to include other kind of ecological information, such as environmental variables. Using higher order matrix correlations, one could access the effects of phylogeny, traits and the underlying environmental gradient on the architecture of networks.

In a recent contribution, Hadfield et al. (2014) evaluated the accuracy of the permutations cophylogenetic test proposed by Legendre et al. (2002), Homola et al. (2009) and the model-based approach proposed by Ives & Godfray (2006). Their results show that in the presence of phylogenetic signal, and when evolutionary interactions effects are present, these three methods give severely inflated Type I error rates when trying to estimate the correlation between phylogenies in a bipartite network. Contrary to these methods, our statistical framework seems to give appropriate rejection rates, rejecting a true null hypothesis in ~5% of the simulated datasets, even in the presence of strong phylogenetic signal, as show in our simulation study.

We additionally point out another possible application of the framework developed here. The method could be used to construct an interaction probability matrix for unobserved realizations, and predict possible interactions. Using the scaling up process we presented here, one could predict the interaction probability of species that are known to occur in the study site, but were not observed interacting with other species. These species could be included in the interaction matrix, with zero values across their rows or columns. Then, one could estimate the probability of interaction with each and every species in the other trophic level, based on species trait- of phylogenetic- resemblance. Although this possibility has yet to be tested, it is a potential feature of the method, which can account for sampling problems or be used in other applied matters, when one has to estimate the probability of interactions that were not observed directly in the field.

To conclude we stress that the statistical method we developed here and its extension can de used to tackle a wide range of eco-evolutionary questions. We hope that further developments broaden the range of possible applications and our understanding of underlying mechanisms driving patterns in ecological networks.

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References

Allesina, S., Alonso, D. & Pascual, M. (2008). A general model for food web structure. *Science*, 320, 658-661.

Angiosperm Phylogeny Group (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, 161, 105–121.

Azambuja, B. & Pillar, V.D.P. in prep. Seed dispersal by birds to forest patches in South Brazilian grassland-forest mosaic.

Banks, J. C. & Paterson, A.M. (2005). Multi-host parasite species in cophylogenetic studies. *International Journal for Parasitology*, *35*, 741-746.

Bersier, L.F. & Kehrli, P. (2008). The signature of phylogenetic constraints on food-web structure. *Ecological Complexity*, 5, 132-139.

Blomberg, S.P., Garland, T. & Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745.

Camerano, L. (1880). Dell'equilibrio dei viventi merc la reciproca distruzione. *Atti della Reale Accademia delle Scienze di Torino,* 15, 393-414.

Cavender-Bares, J., Kozak, K.H., Fine, P.V. & Kembel, S.W. (2009). The merging of community ecology and phylogenetic biology. *Ecology Letters*, 12,693-715.

Cohen, J.E. (1977). Food webs and the dimensionality of trophic niche space. *Proceedings of The National Academy of Science*, 74, 4533-4536.

Cruz, C.P., Fonseca, C.R. & Corso, G. (2012). Ecological interaction and phylogeny, studying functionality on composed networks. *Physica A: Statistical Mechanics and its Applications*, 391, 673-679.

Danielson, B.J. (1991). Communities in a landscape: the influence of habitat heterogeneity on the interactions between species. *American Naturalist*, 138, 1105-1120.

Darwin, C. (1859). *On the origin of species by means of natural selection*. J. Murray, London.

Dáttilo, W., Guimarães Jr, P.R. & Izzo, T.J. (2013). Spatial structure of ant–plant mutualistic networks. *Oikos*, 122, 1643-1648.

Debastiani, V.J. & Pillar, V.D.P. (2012). SYNCSA – R tool for analysis of metacommunities based on functional traits and phylogeny of the community components. *Bioinformatics* 28, 2067-2068.

Díaz-Castelazo, C., Guimarães Jr, P.R., Jordano, P., Thompson, J. N., Marquis, R. J. & Rico-Gray, V. (2010). Changes of a mutualistic network over time: reanalysis over a 10-year period. *Ecology*, 91, 793-801.

Eklöf, A., Jacob, U., Kopp, J., Bosch, J., Castro-Urgal, R., Chacoff, N.P., Dalsgaard, B., Sassi, C., Galetti, M., Guimarães, P.R., Lomáscolo, S.B., González, A.M.M, Pizo, M.A., Rader, R., Rodrigo, A., Tylianakis, J.M., Vázquez, D.P. & Allesina, S. (2013). The dimensionality of ecological networks. *Ecology Letters*, 16, 577-583.

Felsenstein, J. (1985). Phylogenies and the comparative method. The *American Naturalist*, 125, 1-15.

Gómez, J.M., Verdú, M. & Perfectti, F. (2010). Ecological interactions are evolutionarily conserved across the entire tree of life. *Nature*, 465, 918-921.

Gross, T. & Blasius, B. (2008). Adaptive coevolutionary networks: a review. *Journal of The Royal Society Interface*, 5, 259-271.

Guimarães Jr, P.R., Jordano, P. & Thompson, J.N. (2011). Evolution and coevolution in mutualistic networks. *Ecology Letters*, 14, 877-885.

Hadfield, J.D., Krasnov, B.R., Poulin, R. & Nakagawa, S. (2014). A tale of two phylogenies: comparative analyses of ecological interactions. *The American Naturalist*, 183, 174-187.

Hansen, T.F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution,* 51, 1341–1351

Harmon, L.J., Losos, J.B., Davies, J., Gillespie, R.G., Gittleman, J.L., Jennings, W.B., Kozak, K., McPeek, M.A., Moreno-Roark, F., Near, T. J., Purvis, A., Ricklefs, R.E., Schluter, D., Schulte II, J.A., Seehausen, O., Sidlauskas, B., Torres-

Carvajal, O., Weir, J.T. & Mooers, A.Ø. (2010). Early bursts of body size and shape evolution are rare in comparative data. *Evolution,* 64, 2385-2396.

Herrera, C.M. (1995). Plant-vertebrate seed dispersal systems in the Mediterranean: ecological, evolutionary, and historical determinants. *Annual Review of Ecology and Systematics*, 26, 705–727.

Hommola, K., Smith, J.E., Qiu, Y. & Gilks, W.R. (2009). A permutation test of host–parasite cospeciation. *Molecular Biology and Evolution*, 26, 1457-1468.

Howe, H.F. (1993). Specialized and generalized dispersal systems: where does the paradigm stand? *Vegetatio*, 107,3-13

Ibanez, S. (2012). Optimizing size thresholds in a plant–pollinator interaction web: towards a mechanistic understanding of ecological networks. *Oecologia*, 170, 233-242.

Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C.F., Edwards, F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E., Lewis, H.M., Olesen, J.M., Van Veen, F.J.F., Warren, P.H. & Woodward, G. (2009). Review: Ecological networks–beyond food webs. *Journal of Animal Ecology*, 78, 253-269.

Ives, A.R. & Godfray, H.C.J. (2006). Phylogenetic Analysis of Trophic Associations. *The American Naturalist*, 168, E1-E14.

Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K. & Mooers, A.O. (2012). The global diversity of birds in space and time. *Nature*, 49, 444-448.

Jermy, T. (1976). Insect—Host-plant Relationship—Co-evolution or Sequential Evolution? *The Host-Plant in Relation to Insect Behaviour and Reproduction* (ed T. Jermy) pp 109-113. Springer, US.

Johansson, J., Nilsson, J. Å. & Jonzén, N. (2015). Phenological change and ecological interactions: an introduction. *Oikos*, 124, 1-3.

Johnson, M.T.J. & Stinchcombe, J.R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology & Evolution*, 22, 250-257.

Jonsson, T. (2014). Trophic links and the relationship between predator and prey body sizes in food webs. *Community Ecology*, 15, 54-64.

Jordano, P. (2000). Fruits and frugivory. *Seeds: the ecology of regeneration in plant communities* (ed P. Fenner). CABI Publ., Wallingford.

Lefcheck, J. S., Bastazini, V. A. G. & Griffin, J. N. (2015). Choosing and using multiple traits in functional diversity research. *Environmental Conservation*, 42,104-107.

Krishna, A., Guimaraes Jr, P.R., Jordano, P. & Bascompte, J. (2008). A neutral-niche theory of nestedness in mutualistic networks. *Oikos*, *117*(11), 1609-1618.

Legendre, P., Desdevises, Y. & Bazin, E. (2002). A statistical test for host–parasite coevolution. *Systematic Biology*, 51, 217-234.

Legendre, P. & Legendre, L.F. (2012). *Numerical ecology*. Elsevier.

Lewinsohn, T.M., Novotny, V. & Basset, Y. (2005). Insects on plants: diversity of herbivore assemblages revisited. *Annual Review of Ecology, Evolution, and Systematics*, 36, 597-620.

Losos, B.J. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, 11, 995–1007.

Maddison, W.P. & Maddison, D.R. (2007). Mesquite: a modular system for evolutionary analysis. Version 2.75. 2011. URL *http://mesquiteproject. org*.

Miranda, M., Parrini, F., & Dalerum, F. (2013). A categorization of recent network approaches to analyse trophic interactions. *Methods in Ecology and Evolution*, 4, 897-905.

Mitter, C., Farrell, B. & Futuyma, D. J. (1991). Phylogenetic studies of insect-plant interactions: insights into the genesis of diversity. *Trends in Ecology & Evolution*, 6, 290-293.

Morton, E.S. (1973). On the evolutionary advantages and disadvantages of fruit eating in tropical birds. *The American Naturalist*, 107, 8-22.

Mouquet, N., Devictor, V., Meynard, C.N., Munoz, F., Bersier, L., Chave, J., Couteron, P., Dalecky, A., Fontaine, C., Gravel, D., Hardy, O.J., Jabot, F. Lavergne, S., Leibold, M., Mouillot, D., Münkemüller, T., Pavoine, S., Prinzing, A., Rodrigues, A.S.L., Rohr, R.P., Thébault, E. & Thuiller, W. (2012).

Ecophylogenetics : advances and perspectives, Biological Reviews, 87,769-785.

Muller-Landau, H.C. & Hardesty, B.D. (2005). Seed dispersal of woody plants in tropical forests: concepts, examples and future directions (eds D.F. Burslem, M.A. Pinard & S.E. Hartley). *Biotic interactions in the tropics: Their role in the maintenance of species diversity*, pp. 267-309. Cambridge University Press, Cambridge.

Naeem, S. & Wright, J. P. (2003). Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecology Letters*, *6*6, 567-579.

Novotny, V. & Basset, Y. (2005). Host specificity of insect herbivores in tropical forests. *Proceedings of The Royal Society B*, 272, 1083-1090.

Nuismer, S. L. & Harmon, L. J. (2015). Predicting rates of interspecific interaction from phylogenetic trees. *Ecology Letters*, 18, 17-27.

Page, R.D. (2003). *Tangled trees: phylogeny, cospeciation, and coevolution*. University of Chicago Press, Chicago.

Page, R. D., & Charleston, M.A. (1998). Trees within trees: phylogeny and historical associations. *Trends in Ecology & Evolution*, 13, 356-359.

Paine, R.T. (1980). Food webs: linkage, interaction strength and community infrastructure. *The Journal of Animal Ecology*, 49, 667-685.

Pascual, M. & Dunne, J. A. (2006). *Ecological networks: linking structure to dynamics in food webs*. Oxford University Press, Oxford.

Pearse, I. S, & Hipp, A. L. (2009). Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proceedings of the National Academy of Sciences*, 106, 18097-18102.

Pedron, M., Buzatto, C.R., Singer, R.B., Batista, J. A. & Moser, A. (2012). Pollination biology of four sympatric species of Habenaria (Orchidaceae: Orchidinae) from southern Brazil. *Botanical Journal of the Linnean Society*, 170, 141-156.

Pillar, V.D. (2013). How accurate and powerful are randomization tests in multivariate analysis of variance? *Community Ecology*, 14, 153-163.

Pillar, V.D.P., Duarte, L.D.S., Sosinski, E.E. & Joner, F. (2009). Discriminating trait-convergence and trait-divergence assembly patterns in ecological community gradients. *Journal of Vegetation Science*, 20, 334-348.

Pillar, V.D.P. & Orlóci, L. (1991). Fuzzy components in community level comparisons. *Computer assisted vegetation analysis* (eds E. Feoli & L. Orlóci) pp. 87-93. Kluwer Academic Publishers, Dordrecht.

Podani, J. (2000). *Introduction to the exploration of multivariate biological data*. Backhuys Publishers.

Polis, G.A. & Strong, D.R. (1996). Food web complexity and community dynamics. *The American Naturalist*, 147, 813-846.

Rafferty, N.E. & Ives, A.R. (2013). Phylogenetic trait-based analyses of ecological networks. *Ecology*, *94*, 2321-2333.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, URL http://www.R-project.org/.

Rezende, E.L., Lavabre, J.E., Guimarães Jr, P.R., Jordano, P. & Bascompte, J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature*, 448, 925-928.

Rossberg, A. G., Brännström, Å. & Dieckmann, U. (2010). How trophic interaction strength depends on traits. *Theoretical Ecology*, 3, 13-24.

Schleuning, M., Fründ, J. & García, D. In press. Predicting ecosystem functions from biodiversity and mutualistic networks: an extension of trait-based concepts to plant–animal interactions. *Ecography*.

Schmitz, O. J., Buchkowski, R. W., Burghardt, K. T., & Donihue, C. M. (2015). Functional traits and trait-mediated interactions: connecting community-level interactions with ecosystem functioning. *Advances in Ecological Research*.

Snow, D.W. (1971). Evolutionary aspects of fruit-eating by birds. *Ibis*, 113, 194-202.

Strauss, S.Y. & Irwin, R.E. (2004). Ecological and Evolutionary Consequences of Multispecies Plant-Animal Interactions. *Annual Review of Ecology, Evolution, and Systematics*, 35, 435-466.

Thompson, J.N. (1994). *The coevolutionary process*. University of Chicago Press, Chicago.

Thompson, J.N. (2014). Coevolution and speciation. *The Princeton guide to evolution* (ed J. Losos), pp.535-548. Princeton University Press, Princeton.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007). Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, 445, 202-205.

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

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

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,. 20132397-20132397.

Webb, C.O. (2000). Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. *The American Naturalist*, 156, 145-155.

Webb, C.O., Ackerly, D.D. & Kembel, S.W. (2008). Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, 24, 2098-2100

Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M. J. (2002). Phylogenies and Community Ecology. *Annual Review of Ecology and Systematics*, 33, 475-505.

Webb, C.O. & Donoghue, M.J. (2005). Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes*, 5, 181-183.

Weiblen, G.D., Webb, C.O., Novotny, V., Basset, Y. & Miller, S.E. (2006). Phylogenetic dispersion of host use in a tropical insect herbivore community. *Ecology*, 87, S62-S75.

Wikström, N., Savolainen, V. & Chase, M.W. (2001). Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London B*, 268, 2211-2220.

Winemiller, K.O. (1990). Spatial and temporal variation in tropical fish trophic networks. *Ecological monographs*, 60, 331-367.

Zadeh, L.A. (1965). Fuzzy sets. Information Control, 8, 338-353

Supplementary Material 1: Functional data

Data and methods described here refer to the trait data collected for all the species in the seed dispersal network from a grassland-forest mosaic (30°25'S; 52°21'W) that illustrates our analytical framework.

We used five plant traits in our analyses, two continuous (dispersule diameter and maximum plant height), and three categorical traits (aril presence, diaspore shape and color). We collected 10 dispersules from each species in order to estimate their average diameter (mm). For the species we were not able to collect at least 10 dispersules in the study site, we complemented our sample using specimens from the ICN UFRGS Herbarium. Measurements were taken using a caliper to the nearest 0.1 mm. Data on maximum plant height (m) were acquired from the literature (Lorenzi 2008). Color and shape were treated as dummy variables (five for colors and three for shape [spherical, ellipsoidal and triangular]). Data on dispersule shape and color were also attained from the literature (Lorenzi 2008) and based on our own field observations. For further details on measuring plant traits see Cornelissen et al. (2003) and Pérez-Harguindeguy et al. (2013).

For birds we measured four continuous traits of animals captured in the study site: body length (cm), beak length (cm), beak height (cm) and the hand-wing index (HW), defined as:

$$\text{HW} = 100 \text{ x } WL - SL/WL \qquad \text{eqn 1}$$

where, WL is the length of the closed wing and SL is the distance from the carpal joint to the tip of the first secondary feather (Claramunt et al., 2011). It has been empirically demonstrated that the HW, a wing shape index, is a good surrogate of flight ability (Claramunt et al., 2011). Measurements were taken using a caliper to the nearest 0.1 mm, and for each species we measure from 1 to 34 individuals in order to estimate their average trait value. We also measured the ratio between wing breadth and length and collected some complementary data on the other aforementioned traits from specimens of bird collections of the Science and Technology Museum - PUCRS (Museu de Ciência e Teconologia da PUCRS) and the Museum of Natural Sciences of the Rio Grande do Sul Zoobotanical Foundation (Fundação Zoobotânica do Rio Grande do Sul). The number of each species that we measured varied according to their availability in bird collections (from 1 to 10 specimens). For further details on measuring avian morphological traits see Winker (1998).

References

Claramunt, S., Derryberry, E.P., Remsen, J.V., Brumfield, R.T., Claramunt, S. & Elizabeth, P. (2011) High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proceedings of the Royal Society B,* 279, 1567-1574.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G. & Poorter, H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian journal of Botany*, 51, 335-380.

Lorenzi, H. (2008) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil, 5 edn. Instituto Plantarum, Nova Odessa.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., Vos, A.C, Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti M. V., Conti, G., Staver, A. C., Aquino, S., & Cornelissen, J.H.C. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61, 167-234.

Winker, K. (1998) Suggestions for measuring external characters of birds. *Ornitología Neotropical*, 9, 23-30.

Supplementary Material 2: R code.

```
#######################################
#Untangling the tangled bank: a novel method for partitioning the effects of phylogenies and traits on ecological networks
#Author: Bastazini et al.
#Last updated: 2015-04-15
#Contacts:
#bastazini.vinicius@gmail.com
#vanderleidebastiani@yahoo.com.br
#######################################
###Packages
require(vegan)
require(SYNCSA)
#######################################
### Function mantel.residuals
# it extracts residuals from a mantel matrix correlation (see Fig. 2)
#######################################
```

```
mantel.residuals<-function(A,B){
 a<-as.vector(as.dist(A))
 b<-as.vector(as.dist(B))
 modelo<-lm(a~b)
 RESIDUOS<-residuals(modelo)
 N<-dim(as.matrix(A))[1]
 RES<-matrix(NA,N,N)
 OR<-0
 for(j in 1:N){
  for(i in 1:N){
   if(i==j){
    RES[i,j]=0
   }
   if(i>j){
    OR<-OR+1
    RES[i,j]=RESIDUOS[OR]
   }
  }
 }
 R<-sqrt(summary(modelo)$r.squared)
 Dis_RES<-as.dist(RES,diag=T)
 return(list(R=R,Residuals=Dis_RES))
}
#####################################
###  Function MATRIX.P1
#this is a modification of the function matrix.P from SYNCSA
#which allows the computation of matrices with some rows that sum to 0
#####################################
matrix.p1<-function (comm, dist.spp) {
 matrix.w <- sweep(comm, 1, rowSums(comm), "/")
 for(i in 1:dim(comm)[1]){
  for(j in 1:dim(comm)[2]){
   if(is.nan(matrix.w[i,j])==TRUE){
    matrix.w[i,j]=as.numeric(0)
   }
  }
 }
 similar.phy <- 1 - (dist.spp/max(dist.spp))
 matrix.phy <- 1/colSums(similar.phy)
 matrix.q <- sweep(similar.phy, 1, matrix.phy, "*")
 matrix.P <- matrix.w %*% matrix.q
 return(list(matrix.w = matrix.w, matrix.q = matrix.q, matrix.P = matrix.P))
}
#####################################
######### Data entry #########
#####################################
birds_traits #Bird trait data with traits as rows and species as colums
plants_traits #Plant trait data with traits as rows and species as colums
```

```
interactions #Interaction matrix, with plants as rows and birds as colums
birds_phylogeny #A species by species phylogenetic distance matrix
plants_phylogeny #A species by species phylogenetic distance matrix
temporal # A co-occurrence matrix, with plants as rows and birds as colums

#######################################################
#####################
######### REMOVING PHYLOGENETIC EFFECT FROM TRAITS #########
#######################################################
#####################
sa1<-vegdist(birds_traits,method="gower",na.rm=TRUE);sa1
pa1<-vegdist(birds_phylogeny,method="gower",na.rm=TRUE);pa1
sp1<-vegdist(plants_traits,method="gower",na.rm=TRUE);sp1
pp1<-vegdist(plants_phylogeny,method="gower",na.rm=TRUE);pp1

mant1<-mantel.residuals(sa1,pa1);mant1
mant2<-mantel.residuals(sp1,pp1);mant2
sa<-as.matrix(mant1$Residuals);sa # distance matriz between species described
by their traits, removing the effect of the phylogeny
sb<-as.matrix(mant2$Residuals);sb

#######################################################
#######################################################
####################
#### PROBABILISTIC INTERACTION MATRICES WEIGHTED BY TRAITS AFTER
REMOVING PHYLOGENETIC SIGNAL and accounting for temporal variation
#######################################################
#######################################################
####################
ua<-matrix.p1(t(interactions),sa)
ya<-ua$matrix.P;ya
up<-matrix.p1(interactions,sb)
yp<-up$matrix.P;yp
ya<-t(ya)*temporal
yp<-yp*temporal
ya<-t(ya)
yp<-t(yp)

#######################################################
#######################################################
####################
#### PROBABILISTIC INTERACTION MATRICES WEIGHTED BY PHYLOGENETIC
RESEMBLANCE and accounting for temporal variation
#######################################################
#######################################################
####################

dpa<-matrix.p1(t(interactions), birds_phylogeny)
```

```
pa<-dpa$matrix.P
dpp<-matrix.p1(interactions, plants_phylogeny)
pp<-dpp$matrix.P
pat<-t(pa)*temporal
pat<-t(pat)
ppt<-(pp)*temporal
ppt<-t(ppt)

###############################
#### MODELS TESTED IN TABLE 2
###############################
#Functional component, removing phylogenetic signal
ya.yp<cor.matrix(ua$matrix.w,ua$matrix.q,ya,yp,method="pearson",dist="euclidean",permutations=9999,norm=FALSE);ya.yp
#Phylogenetic signal in bird species
ya.pa<-cor.matrix(ua$matrix.w,ua$matrix.q,ya,pa,method="pearson",dist="euclidean",permutations=9999,norm=FALSE);ya.pa
#Phylogenetic signal in plant species
yp.pp<-cor.matrix(up$matrix.w,up$matrix.q,t(yp),pp,method="pearson",dist="euclidean",permutations=9999,norm=T);yp.pp
#Phylogenetic component
pa.pp<-cor.matrix(ua$matrix.w,ua$matrix.q,pat,ppt,method="pearson",dist="euclidean",permutations=9999,norm=FALSE);pa.pp
.
```

Capítulo 2 - Distinct plant extinction scenarios affect the robustness of a seed dispersal network

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Abstract: Understanding cascading effects of species loss has become a major challenge for ecologists. Traditionally, the robustness of ecological networks has been evaluated based on scenarios where primary extinctions occur at random or as a function of species specialization, ignoring other important biological factors. Here, we estimated the robustness of a seed dispersal network from southern Brazil simulating distinct scenarios of plant species extinction, including scenarios where species are eliminated based on their evolutionary and functional distinctiveness. Our results suggest that the network is more robust when species are eliminated based on their evolutionary uniqueness, followed by random extinctions, the extinction of the most specialist species, functional distinctiveness and, at last, when the most generalist species are sequentially eliminated. Despite its simplicity, our results provide important information for grassland-forest management in southern Brazil, as they indicate that loss of generalist species and functional diversity makes the system more likely to collapse.

Keywords: Coextinctions, Ecophylogenetics, Functional Ecology, Mutualistic Interactions, Network Disassembly

Introduction

As we face the prospect of an unpreceded anthropogenic-induced mass extinction, with species being lost at rates that are about three order of magnitude greater than the background rates from the geological record (Pimm et al. 2014; Ceballos et al. 2015), understanding and predicting species extinction has become an urgent task for conservation scientists and practitioners (Vieira & Almeida-Neto 2014; Ceballos et al. 2015). Even though we have long recognized that the loss of some species may unleash a cascade of secondary extinctions that may bring entire communities and ecosystems to collapse (Jackson et al. 2001; Colwell et al. 2012; Säterberg et al. 2013; Brodie et al. 2014), up to date, empirical studies that have examined the magnitude of biodiversity loss have usually ignore coextinction scenarios (Vieira & Almeida-Neto 2014).

Plants-animal seed disperser interactions are an important mutualism that shapes both, ecological and evolutionary dynamics, through demographic processes that affect the fitness, reproduction success and the distribution of partner species (Snow 1971; Morton 1973; Herrera 1985; Jordano 2000; Fragoso 2005; Correa et al. 2015). Its importance as a key driver behind biodiversity patterns is supported by the fact that only in the Tropics, up to 90% of all plant species rely on animals to disperse their seeds (Jordano 2000). Given their significance to maintain biodiversity and support ecosystem functioning, plant-disperser interactions are considered a crucial component in conservation and restoration strategies (Wunderle Jr. et al. 1997; Trakhtenbrot et al. 2005).

Over the recent decades, graph theory - a branch of discrete mathematics that provides a formalism to describe and to analyze the structure of links (e.g., trophic or non-trophic ecological interactions) between a set of nodes (e.g., individuals or species) - has emerged as a powerful tool to describe the complexity of species interactions (Proulx et al. 2005; Miranda et al. 2013), including their response to disturbance and cascading effects. Despite the importance of seed dispersal networks, this type of interaction has received less attention than other types of ecological networks (Miranda et al. 2013). The robustness of ecological networks, i.e., the system's tolerance to secondary extinctions, is also a subject within network ecology, that has been more explored in other trophic interactions (Dunne et al. 2002; Memmot et al. 2004; Kaiser-Bunbury et al. 2010; Vieira et al. 2013; Astegiano et al. 2015) than in plant-seed disperses (but see Mello et al. 2011 a, b). Moreover, studies of network robustness traditionally evaluate simplistic scenarios that ignore important ecological and evolutionary factors associated to the likelihood of a species becoming extinct (but see Curtsdotter et al. 2011; Srinivasan et al. 2007; Vieira et al. 2013; Astegiano et al. 2015). In these studies, species extinctions usually are supposed to occur at random or as a consequence of species specialization (Solé & Montoya 2001; Dunne et al. 2002; Memmot et al. 2004; Burgos et al. 2007; Rezende et al. 2007; Mello et al. 2011a, b; Pocock et al. 2012).

Grasslands are one of the major biomes of the planet, occupying an area equivalent to 31-43% of the land surface (Coupland 1979; White et al. 2000). Grasslands are characterized by high levels of biological diversity and endemism (White et al. 2000; Bond & Parr 2010) and are responsible for maintaining important ecosystem processes and economic activities (Coupland 1979; Breymer & Van Dyne 1980; White et al. 2000). Nonetheless, their conservation has been historically neglected by environmental policies (Overbeck et al. 2007; Bond & Parr 2010). Despite its large territory and economic and environmental value, grasslands are among the most altered and threatened ecosystems of the planet (White et al. 2000; Bond & Parr 2010; Henwood et al. 2010). In addition to anthropogenic threats, temperate grasslands and savannas suffer from a natural process of forest expansion (Schwartz et al. 1996; Bowman et al. 2001; Bond & Parr 2010; Overbeck et al. 2007; Müller et al. 2012). Although forest expansion is considered a threat to grassland conservation, this process creates a unique and interesting scenario for ecological studies, as it encompasses a "mosaic of life forms" that share very distinct evolutionary origins and life histories (Luza et al. 2015) that, compete for space and other resources. Seed dispersal networks, formed by woody plant and frugivores, are likely to be an important driver of spatial and temporal dynamics in grassland-forest mosaics, as woody seed dispersal may accelerate the expansion of forest ecosystems.

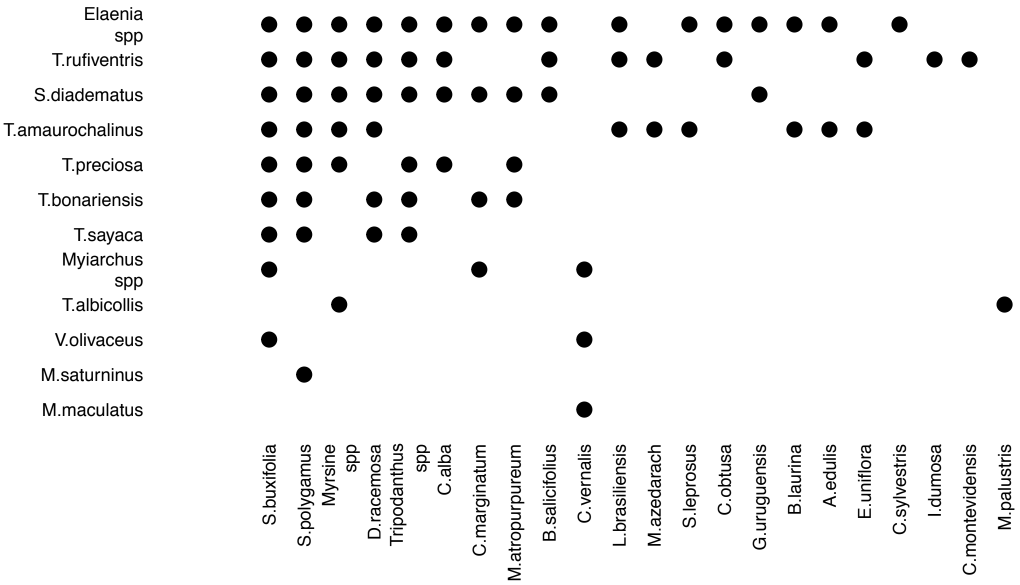
Here, we estimated the robustness of a seed dispersal network formed by woody plants and birds, from a grassland-forest mosaic from southern Brazil. We simulated distinct scenarios of plant species extinction where species are eliminated based on their evolutionary and functional distinctiveness. Although the role of bottom-up and top-down ecosystem regulation has been a much debated topic for decades now (see for instance Hanley & La Pierre 2015 and references therein), both theoretical and empirical evidence suggest that bottom-up dynamics are more prominent in shaping community dynamics in multitrophic scenarios (Goudard & Loreau 2008; Scherber et al. 2010). Thus, we assumed a bottom-up regulation and simulated distinct scenarios of woody plant extinction rather than bird extinctions.

Material and Methods

Dataset

We applied our framework to a qualitative mutualistic network, composed of 22 woody plants and 12 frugivorous birds (Fig. 1), from a forest-grassland mosaic located in southern Brazil (between 30°25’03”S 52°21’37”W and 30°25’54”S 52°22’40”W). A grassland matrix characterizes the region. Large forests patches occupy hill slopes and riparian areas and smaller shrubby and forests patches are sparsely distributed across the grassy landscape.

The data was collected during a bird trapping study that last a year, between July 2007 and June 2008 (Azambuja 2009). Mist-nets were set up monthly for eight consecutive days in grasslands close to forest patches. Birds captured in mist nets were then placed into fabric bags for 20 minutes, and their feces were collected from bags. The seeds found in fecal samples were identified to the lowest taxonomic level possible. Based on this information, we built a qualitative interaction matrix between birds and the plant they consumed (Fig. 1). For further data information and a detail characterization of the region, please refer to Azambuja (2009; see also Chapter 1).



	S.buxifolia	S.polygamus	Myrsine spp	D.racemosa	Tripodanthus spp	C.alba	C.marginatum	M.atropurpureum	B.salicifolius	C.vernalis	L.brasiliensis	M.azedarach	S.leprosus	C.obtusa	G.uruguensis	B.laurina	A.edulis	E.uniflora	C.sylvestris	I.dumosa	C.montevidensis	M.palustris
Elaenia spp	●	●	●	●	●	●	●	●	●		●		●	●	●	●	●		●			
T.rufiventris	●	●	●	●	●	●			●		●	●		●				●		●	●	
S.diadematus	●	●	●	●	●	●	●	●	●						●							
T.amaurochalinus	●	●	●	●							●	●	●			●	●	●				
T.preciosa	●	●	●		●	●		●														
T.bonariensis	●	●		●	●		●	●														
T.sayaca	●	●		●	●																	
Myiarchus spp	●						●			●												
T.albicollis			●																			●
V.olivaceus	●									●												
M.saturninus		●																				
M.maculatus										●												

Fig. 1. Incidence matrix representing the seed dispersal network from a forest-grassland mosaic, southernmost Brazil (for further detail see Azambuja 2009; Full species names are depicted in the Supplementary Material 1).

Functional traits and phylogeny

We used five plant traits in our analyses, two continuous (diaspore diameter and maximum plant height) and three categorical traits (aril presence, diaspore shape and color). We collected 10 diaspores from each species in order to estimate their average diameter (mm). For the species we were not able to collect at least 10 diaspores in the study site, we complemented our sample using specimens from the ICN UFRGS Herbarium. Measurements were taken using a caliper to the nearest 0.1 mm. Data on maximum plant height (m) were acquired from the literature (Lorenzi 2008). Color and shape were treated as dummy variables (five for colors and three for shape [spherical, ellipsoidal and triangular]). Data on diaspore shape and color were also attained from the literature (Lorenzi 2008) and based on field observations (see Azambuja 2009).

We used dated phylogenies for all species in our dataset. Using the APG III megatree (R20100701.new; Angiosperm Phylogeny Group 2009), we constructed a base family-level phylogeny. Due to the lack of within-family resolution, species and genera were represented as soft polytomies within genera and families, respectively. Branch lengths were added to the phylogeny using the BLADJ algorithm (Webb et al. 2008), which constrains the age of nodes included in the sample according to the dated molecular phylogeny of Wikström et al. (2001).

We estimated functional and phylogenetic distinctiveness of each plant species using the Fair Proportion metric, defined as the sum of all edge lengths between the species and the root of the functional dendrogram or phylogenetic tree, with each edge length being divided by the number of species in the cluster it subtends (Redding et al. 2008). The functional dendrogram was built based on plant traits using a Euclidean distance matrix and Ward's minimum variance clustering procedure (Legendre & Legendre 2012).

Numerical analysis

Secondary extinctions of woody plants were simulated based on five distinct elimination schemes. The first one, based on **i)** random extinctions (using 1,000 simulations) provide a baseline scenario to compare the effects of the other scenarios (Dunne et al. 2002; Memmot et al. 2004; Astegiano et al. 2015). The other scenarios were based on species specialization, either with **ii)** the most generalist (most-linked) or **iii)** the most specialist (least-linked) species disappearing first; **iv)** species are eliminated based on their evolutionary distinctiveness (how isolated a species is on the phylogenetic tree); and **v)** based on species' functional distinctiveness (how distinct a species is in terms of its functional traits). For each scenario, we calculated network robustness (*R*), defined as the area below the Attack Tolerance Curve (ATC; Albert & Barabási 2002; Burgos et al. 2007). *R* varies between 0 and 1, and values closer to 1 indicate higher network robustness (Burgos et al. 2007). All numerical simulations and analyses were performed in the R environment (R Core Team 2012) and the code is available in the Supplementary Material 2.

Results

The values of functional and phylogenetic distinctiveness are depicted in Figure 2. There was no correlation between indices of distinctiveness (r= -0.015; p-value = 0.94), which indicates that most unique species in the phylogenetic tree are not the most unique species in terms of their functional traits (Fig. 2).

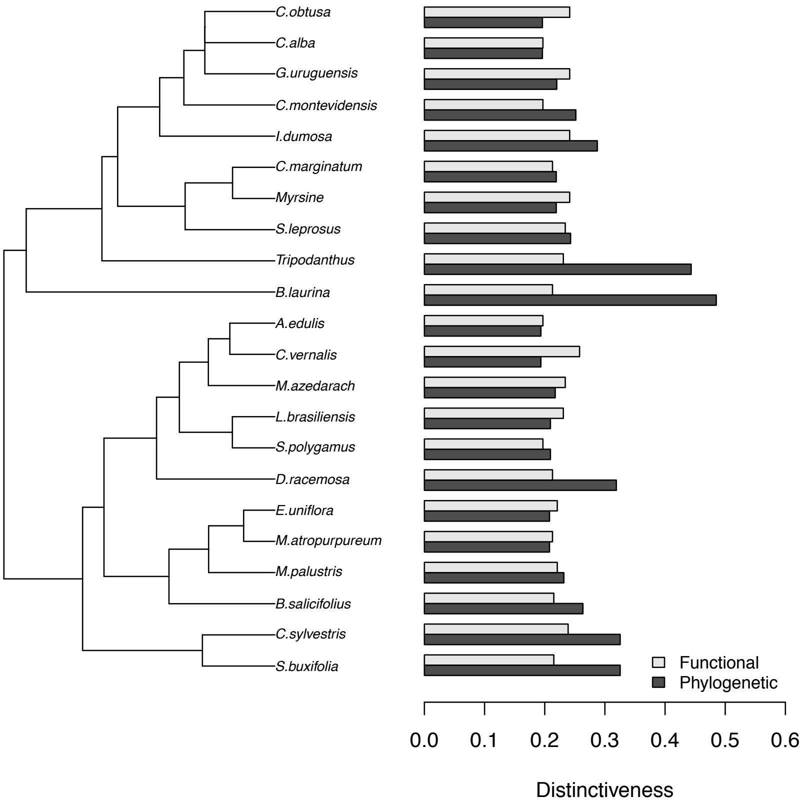


Fig. 2. Functional and phylogenetic distinctiveness of plant species, with the phylogeny plotted alongside, in a seed dispersal network from southern Brazil.

Overall, our seed dispersal network is likely to be very robust to the loss of woody plant species (mean R = 0.74 ± SD = 11.85). However, our simulations show that distinct extinction scenarios have distinct effects on network robustness (Fig. 3). The loss of generalist species seems to take a bigger toll, as the ATC of this scenario exhibits a very abrupt decline (Fig. 3). The other scenarios show a less steep response to the loss of plant species. Network robustness was higher when species were eliminated based on their evolutionary uniqueness, followed by random extinctions, the extinction of the most specialist species and functional distinctiveness (Fig. 3).

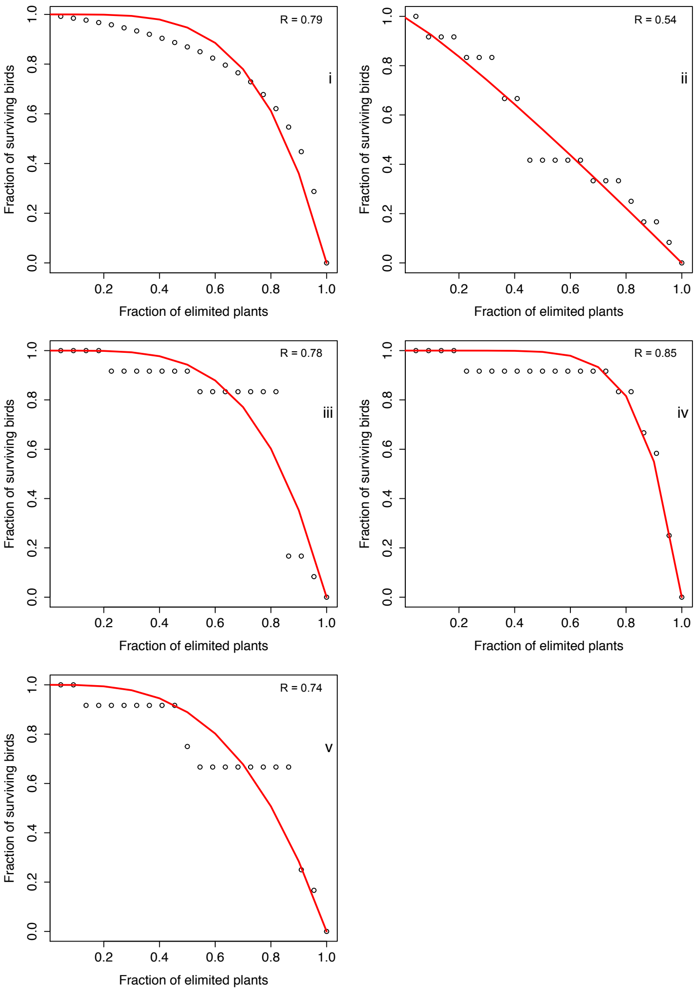


Fig. 3. Attack Tolerance Curves under different plant extinction sequences: i) random; extinctions based on species specialization either with ii) the most generalist (most-linked) or iii) the most specialist (least-linked) species disappearing first; iv) species evolutionary distinctiveness; v) species functional distinctiveness.

Discussion

Over the past years, there has been a growing interest in trying to understand and estimate the consequences of coextinctions in ecological networks (e.g., Solé & Montoya 2001; Dunne et al. 2002; Memmot et al. 2004; Burgos et al. 2007; Rezende et al. 2007; Mello et al. 2011a, b; Pocock et al. 2012). Insofar, most empirical evaluations have been based on scenarios where primary extinctions occur as a function of species specialization or as random events (Solé & Montoya 2001; Dunne et al. 2002; Memmot et al. 2004; Burgos et al. 2007; Pocock et al 2012; Vieira et al. 2013), which are likely to be unrealistic scenarios, as it is well known that extinctions are unlikely to occur at random and that other ecological and evolutionary factors, besides specialization, such as body size and geographical range, influence the probability of a species becoming extinct (Purvis et al. 2000; Cardillo et al. 2005, 2008; Reynolds et al. 2005). Here, we provide further developements to coextinction analyses based on attack tolerance curves, combining functional and phylogenetic information, and show that the loss of generalist species and functional diversity has a large effect on network robustness, when compared to other extinction scenarios. Vieira et al. (2013) investigated the functional and phylogenetic consequences of random pollinator extinction in several pollination networks using a different metric to quantify functional and phylogenetic uniqueness. Their results suggest that there is an uncoupled response between functional and phylogenetic loss in mutualistic systems. Our study corroborates their findings, since our extinction scenarios based on functional and phylogenetic distinctiveness showed very different responses in terms of network robustness. In another contribution (see chapter 1) we demonstrated that there is no phylogenetic signal in plant traits in this seed dispersal network. Thus, we already expected an uncoupled functional and phylogenetic response.

Despite this growing interest in coextinction and robustness analyses, studies focusing on seed dispersal networks are still scarce. Mello et al. (2011b) analyzed the robustness of 21 seed dispersal networks by simulating random extinctions of plants and frugivores (bats and birds). For plant-bird networks, they found that networks should be very robust to the loss of plant species, with a mean value of robustness (R = 0.75) close to what we presented here. Future studies are necessary to demonstrate whether seed dispersal networks are as robust as demonstrated by Mello et al. (2011b) and our own analyses. But as plant-seed disperser interactions are a system of low specialization (Herrera 1995, Muller-Landau & Hardesty 2005), one should expect a high degree of robustness. Mello et al. (2011b) also suggest that networks should be less robust when animal species are lost. Thus, an obvious further step to our study is to expand our framework and investigate the effects of the loss of consumer species.

It is well recognized that generalist species, i.e., species with larger number of connections, play an important role in ecosystem functioning and stability (Memmot et al. 2004; Richmond et al. 2005; Poisot et al. 2013; Valiente-Banuet et al. 2015). González et al. (2010) suggest that generalist species have, at least, two important roles in pollination networks; the first obvious and intuitive role is that generalist species are able to interact with more species than specialist ones and the second role is the ability to connect otherwise unconnected sub-networks. Together, these two roles implicate that generalist species are responsible for creating a more cohesive network. This cohesive pattern is likely to increment the ability of the system to respond to perturbations, and consequently increase its stability (Bascompte et al. 2003). Our seed dispersal network comprises a very heterogeneous set of plant species, in terms of phylogenetic history (15 taxonomic families), life history and functional groups, including trees (e.g., *Scutia buxifolia*, *Cupania vernalis* and *Styrax leprosus*), shrubs (*Berberis laurina*, *Chomelia obtusa* and *Daphnopsis racemosa*), vines (*Chiococca* alba) and hemiparasite species (*Tripodanthus* spp.). Among this diverse set of species, five taxa were responsible for approximately 50% of all pairwise interactions, showing larger number of connections: *Scutia buxifolia*, *Schinus polygamus*, *Myrsine* spp., *Daphnopsis racemosa*, *Tripodanthus* spp. Overall, these five species are characterized by low functional distinctiveness, with values lower than the median (except for *Tripodanthus* spp. and *Myrsine* spp.) and, except for *Schinus polygamus,* high phylogenetic distinctiveness (with values above the median). A common trait shared by these species is the size of their fruits, which is relatively small compared to the other species. As in other trophic interactions where predators have to swallow food items whole, fruit size is an important constraint in seed dispersal networks, and as a general rule, plant with large seeds and fruits tend to attract fewer animal species than plants with small fruits (Wheelwright 1985; Jordano 2000). Temporal availability and co-ocurrence with its seed dispersers is also another important factor that increments the chance of a plant being consumed and dispersed by an animal (Jordano 2000). Among these five taxa, *Myrsine* spp. is the only one that produces fruits almost year round (Azambuja 2009). Nonetheless, these well-connected species are among the taxa with larger temporal co-ocurrence with the most bird species (see chapter 1). Thus, small fruits and phenological patterns seem to be a possible explanation to some structural patterns and robustness of this seed dispersal network.

At last, we would like to underline the implications of our study to conservation policies, especially in southern Brazil. The conservation of grassland-forest mosaics of southern Brazil has been the focus of much debate in the recent years (Overbeck et al., 2007, 2013; Luza et al. 2014). As in other grassland ecosystem around the World, the grasslands of southern Brazil have experienced an increase in density of woody species that is drastically changing its physiognomy (Pillar 2003; Overbeck et al 2007; Müller et al. 2012). Forest expansion, seems to be influenced by nucleation processes influenced by the presence of facilitating structures, such as rocky outcrops or isolated trees established in the grassland matrix, that acts as perches for vertebrate dispersers and as safe sites for woody plants (Pillar 2003; Carlucci et al., 2011; Müller et al. 2012). Most of the generalist tree species that we found in our study seem to be important species for woody plant encroachment into grasslands of southern Brazil (Carlucci et al., 2011; Müller et al. 2012). As our results indicate that loss of generalist species and functional uniqueness are more detrimental to the robustness of seed dispersal networks of southern Brazil, our results could help to develop sounder strategies in species management and restoration of grassland-forest mosaics in southern Brazil. For instance, in situations where the maintenance of grasslands is the goal of conservation initiatives, the removal of generalist plant species, in terms of their seed dispersers, or functionally "unique" species may help to decelerate processes of forest expansion.

In summary, our findings provide important information for forest and grassland management in southern Brazil, as they indicate that the sequential extinction of generalist woody plant species and functional plant diversity makes the system more likely to collapse, whereas the loss of the most "unique" species, in terms of their evolutionary history, and specialist species has a small effect on network robustness. Moreover, despite its simplicity, our framework stresses the importance of considering distinct extinction scenarios and can help ecologists to understand and predict cascading effects in ecological systems.

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References:

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

Albert, R. & Barabási, A. 2002. Statistical mechanics of complex networks. *Reviews of Modern Physics* 74: 47–74.

Astegiano, J., Massol, F., Vidal, M.M., Cheptou, P.O. & Guimarães Jr, P.R. 2015. The Robustness of Plant-Pollinator Assemblages: Linking Plant Interaction Patterns and Sensitivity to Pollinator Loss. *PloS one 10*(2): e0117243.

Azambuja, B.O. 2009. *Relações entre aves dispersoras de sementes e manchas florestais em matriz campestre na Serra do Sudeste, RS*. Ph. D. Thesis, UFRGS, Rios Grande do Sul, BRA

Bascompte, J., P. Jordano, C. J. Melián & J.M. Olesen. 2003. The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences* 100: 9383–9387.

Bond W.J. & Parr, C.L. 2010. Beyond the forest edge: ecology, diversity and conservation of the grassy biomes. *Biological Conservation* 143: 2395–2404.

Bowman D. M. J. S., A. Walsh & Milne, D.J. 2001. Forest expansion and grassland contraction within a Eucalyptus savanna matrix between 1941 and 1994 at Litchfield National Park in the Australian monsoon tropics. *Global Ecology & Biogeography* 10: 535–548.

Breymeyer, A.I. & van Dyne, G.M. 1980. Grasslands, systems analysis and man. Cambridge University Press, Cambridge, UK.

Brodie, J.F., Aslan, C. E., Rogers, H.S., Redford, K.H., Maron, J.L., Bronstein, J.L. & Groves, C.R. 2014. Secondary extinctions of biodiversity. *Trends in Ecology & Evolution 29*(12): 664-672.

Burgos, E., Ceva, H. Perazzo, R.P.J., Devoto, M., Medan, D., Zimmermann, M. & Delbue, A.M. 2007. Why nestedness in mutualistic networks? *Journal of Theoretical Biology* 249: 307–313.

Cardillo, M., Mace, G.M., Jones, K.E., Bielby, J., Bininda-Emonds, O.R., Sechrest, W., Orme, C.D.L. & Purvis, A. 2005. Multiple causes of high extinction risk in large mammal species. *Science 309*(5738): 1239-1241.

Cardillo, M., Mace, G. M., Gittleman, J. L., Jones, K. E., Bielby, J., & Purvis, A. 2008. The predictability of extinction: biological and external correlates of decline in mammals. *Proceedings of the Royal Society of London B: Biological Sciences 275*(1641): 1441-1448.

Carlucci, M.B., Duarte, L.D.S. & Pillar, V.D. 2011. Nurse rocks influence forest expansion over native grassland in southern Brazil. *Journal of Vegetation Science 22*(1): 111-119.

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M., & Palmer, T.M. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances* 1(5): e1400253.

Colwell, R.K., Dunn, R.R. & Harris, N.C. 2012. Coextinction and persistence of dependent species in a changing world. Annual Review in Ecology, Evolution and Systematic 43: 183–203.

Correa, S. B., Costa-Pereira, R., Fleming, T., Goulding, M. & Anderson, J.T. 2015. Neotropical fish–fruit interactions: eco-evolutionary dynamics and conservation. *Biological Reviews*.

Coupland R.T. (Ed.). 1979. Grasslands ecosystems of the world: analysis of grasslands and their uses. Cambridge University Press, Cambridge, UK.

Curtsdotter, A., Binzer, A., Brose, U., de Castro, F., Ebenman, B., Eklöf, A., Riede, J.O., Thierry, A. & Rall, B. C. (2011). Robustness to secondary extinctions: comparing trait-based sequential deletions in static and dynamic food webs. *Basic and Applied Ecology 12*(7): 571-580.

Dunne, J.A., Williams, R.J. & Martinez, N.D. 2002 Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters* 5(4): 558-567.

Fragoso, J. M. V. 2005. The role of trophic interactions in community initiation, maintenance and degradation. *Biotic Interactions in the Tropics: Their Role in the Maintenance of Species Diversity." Burslem, DFRP, MA Pinard and SE Hartley. Cambridge University Press, Cambridge*, UK.

Goudard, A. & Loreau, M. 2008. Nontrophic interactions, biodiversity, and ecosystem functioning: an interaction web model. *The American Naturalist*, 171(1): 91-106.

González, A.M.M., Dalsgaard, B. & Olesen, J.M. 2010. Centrality measures and the importance of generalist species in pollination networks. Ecological Complexity 7(1): 36-43.

Hanley, T.C. & La Pierre, K.J. (Eds.). 2015. *Trophic Ecology: Bottom-up and Top-down Interactions across Aquatic and Terrestrial Systems*. Cambridge University Press, Cambridge, UK.

Henwood, W.D. 2010. Toward a strategy for the conservation and protection of the world’s temperate grasslands. *Great Plains Research* 20:121–134.

Herrera, C.M. 1985. Determinants of plant-animal coevolution: the case of mutualistic dispersal of seeds by vertebrates. *Oikos*: 132-141.

Jackson, J.B., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B. J., Bradbury,R.H., Cooke, R., Erlandson, J. Estes, J.A., Hughes,T.P. (...) & Warner, R.R. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science*: 293(5530): 629-637.

Jordano P. 2000. Fruits and frugivory. In: M. Fenner (ed.). *Seeds: The Ecology of Regeneration in Natural Plant Communities*. Commonw. Agric. Bur. Int., Wallingford, UK.

Kaiser-Bunbury, C.N., Muff, S., Memmott, J., Müller, C.B., & Caflisch, A. 2010. The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. *Ecology Letters* 13(4): 442-452.

Legendre, P. & Legendre, L.F. 2012. *Numerical ecology*. Elsevier, Amsterdam, Netherlands.

Lorenzi, H. 2008. *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil, 5 edn*. Instituto Plantarum, Nova Odessa, BRA.

Luza, A.L., Carlucci, M.B., Hartz, S.M. & Duarte, L. D. 2014. Moving from forest vs. grassland perspectives to an integrated view towards the conservation of forest–grassland mosaics. *Natureza & Conservação* 12(2): 166-169.

Luza, A.L., Gonçalves, G.L., & Hartz, S.M. 2015. Phylogenetic and morphological relationships between nonvolant small mammals reveal assembly processes at different spatial scales. *Ecology and Evolution 5*(4): 889-902.

May, R.M. 1972. Will a large complex system be stable? *Nature* 238: 413–414.

Mello, M.A.R., Marquitti, F.M.D., Guimarães Jr, P.R., Kalko, E.K.V., Jordano, P. & de Aguiar, M.M. 2011a. The missing part of seed dispersal networks: structure and robustness of bat-fruit interactions. *PLoS One* 6(2): e17395-e17395.

Mello, M.A.R., Marquitti, F.M. D., Guimaraes Jr, P.R., Kalko, E.K. V., Jordano, P. & de Aguiar, M.A.M. 2011b. The modularity of seed dispersal: differences in structure and robustness between bat–and bird–fruit networks. *Oecologia*, 167(1): 131-140.

Memmott, J., Waser, N.M. & Price, M.V. 2004. Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society* B 271: 2605–2611.

Miranda, M., Parrini, F. & Dalerum, F. 2013. A categorization of recent network approaches to analyse trophic interactions. *Methods in Ecology and Evolution* 4: 897-905.

Morton, E.S. 1973. On the evolutionary advantages and disadvantages of fruit eating in tropical birds. *The American Naturalist* 107: 8-22.

Muller-Landau, H.C. & Hardesty, B.D. 2005. Seed dispersal of woody plants in tropical forests: concepts, examples and future directions. In: D.F. Burslem, Pinard, M.A. & Hartley, S.E. (eds.). *Biotic interactions in the tropics: Their role* *in the maintenance of species diversity*. Cambridge University Press, Cambridge, UK.

Müller, S.C., Overbeck, G.E., Pfadenhauer, J. & Pillar, V.D. 2012. Woody species patterns at forest–grassland boundaries in southern Brazil. *Flora-Morphology, Distribution, Functional Ecology of Plants* 207(8): 586-598.

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* 9**:**101–116.

Overbeck, G.E., Hermann, J.M., Andrade, B.O., Boldrini, I.I., Kiehl, K., Kirmer, A., Koch, C., Kollmann, J., Meyer, S.T., Müller, S.C., (...) & Pillar, V.D. 2013. Restoration ecology in Brazil–time to step out of the forest. *Natureza & Conservação*, 11: 92-95.

Pillar, V.D. 2003. Dinâmica da expansão florestal em mosaicos de floresta e campos no sul do Brasil. In: V. Claudino-Sales (org.) *Ecossistemas brasileiros: manejo e conservação*. Expressão Gráfica e Editora. Fortaleza. BRA

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M & Sexton, J.O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344(6187): 1246752.

Pocock, M.J., Evans, D.M. & Memmott, J. 2012. The robustness and restoration of a network of ecological networks. *Science* 335(6071): 973-977.

Poisot, T., Mouquet, N. & Gravel, D. 2013. Trophic complementarity drives the biodiversity–ecosystem functioning relationship in food webs. *Ecology Letters* 16(7): 853-861.

Proulx, S.R., Promislow, D. E. & Phillips, P. C. 2005. Network thinking in ecology and evolution. *Trends in Ecology & Evolution* 20(6): 345-353.

Purvis, A., Gittleman, J.L., Cowlishaw, G. & Mace, G.M. 2000. Predicting extinction risk in declining species. *Proceedings of the Royal Society of London B: Biological Sciences* 267(1456): 1947-1952.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, URL http://www.R-project.org/.

Redding, D.W., Hartmann, K., Mimoto, A., Bokal, D., DeVos, M. & Mooers, A. Ø. 2008. Evolutionarily distinctive species often capture more phylogenetic diversity than expected. *Journal of Theoretical Biology* 251(4): 606-615.

Rezende, E.L., Lavabre, J.E., Guimarães, P.R., Jordano,P. & Bascompte, J. 2007. Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156): 925-928.

Reynolds, J.D., Dulvy, N.K., Goodwin, N.B. & Hutchings, J.A. 2005. Biology of extinction risk in marine fishes. *Proceedings of the Royal Society of London B: Biological Sciences* 272(1579): 2337-2344.

Richmond, C.E., Breitburg, D.L., & Rose, K.A. 2005. The role of environmental generalist species in ecosystem function. *Ecological Modelling* 188(2): 279-295.

Säterberg, T., Sellman, S. & Ebenman, B. 2013. High frequency of functional extinctions in ecological networks. *Nature* 499(7459): 468-470.

Scherber, C., Eisenhauer, N., Weisser, W.W., Schmid, B., Voigt, W., Fischer, M., Schulze, E., Roscher, C., Weigelt, A., Allan, E., Beßler, H. (…) & Tscharntke, T. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* 468(7323): 553-556

Schwartz D., Foresta, H., Mariotti, X, Balesdent, J., Massimba, J.P. & Girardin, C. 1996. Present dynamics of the savanna-forest boundary in the Congolese Mayombe: a pedological, botanical and isotopic (13C and 14C) study. Oecologia 106(4): 516-524.

Snow, D.W. 1971. Evolutionary aspects of fruit-eating by birds. *Ibis* 113: 194-202.

Solé, R.V. & Montoya, M. 2001. Complexity and fragility in ecological networks. *Proceedings of the Royal Society of London B: Biological Sciences* 268(1480): 2039-2045.

Srinivasan, U.T., Dunne, J.A., Harte, J. & Martinez, N.D. 2007. Response of complex food webs to realistic extinction sequences. *Ecology* 88(3): 671-682.

Trakhtenbrot, A., Nathan, R., Perry, G. & Richardson, D.M. 2005. The importance of long-distance dispersal in biodiversity conservation. *Diversity and Distributions* 11(2): 173-181.

Valiente-Banuet, A., Aizen, M. A., Alcántara, J.M., Arroyo, J., Cocucci, A., Galetti, M., García, M.B., García, D., Gómez, J.M., (...) & Zamora, R. (2015). Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology* 29(3): 299-307.

Vieira, M.C., Cianciaruso, M.V. & Almeida-Neto, M. 2013. Plant-Pollinator coextinctions and the loss of plant functional and phylogenetic diversity. *PlosOne* e81242.

Vieira, M.C. & Almeida-Neto, M. 2014. A simple stochastic model for complex coextinctions in mutualistic networks: robustness decreases with connectance. *Ecology Letters* 18(2): 144-152.

Webb, C.O., Ackerly, D.D. & Kembel, S.W. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24: 2098-2100.

Wheelwright, N.T. 1985. Fruit-size, gape width, and the diets of fruit-eating birds. *Ecology* 66(3): 808-818.

White R., Murray, S. & Rohweder, M. 2000. Pilot Analysis of Global Ecosystems: Grasslands Ecosystems. World resource Initute. Washington. USA.

Wikström, N., Savolainen, V. & Chase, M.W. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London B* 268: 2211-2220.

Wunderle Jr, J. M. 1997. The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. *Forest Ecology and Management 99*(1-2): 223-235.

Supplementary Material 1: Species names.

Plant species

Chomelia obtusa

Chiococca alba

Guettarda uruguensis

Citharexylum montevidensis

Ilex dumosa

Chrysophyllum marginatum

Myrsine spp.

Stirax leprosus

Tripodanthus spp.

Allophylus edulis

Cupania vernalis

Melia azedarach

Lithraea brasiliensis

Schinus polygamus

Daphnopsis racemosa

Eugenia uniflora

Myrrhinium atropurpureum

Myrcia palustris

Blepharocalix salicifolius

Casearia sylvestris

Scutia buxifolia

Berberis laurina

Bird species

Thraupis bonariensis

Thraupis sayaca

Stephanophorus diadematus

Tangara preciosa

Turdus rufiventris

Turdus albicollis

Turdus amaurochalinus

Mimus saturninus

Vireo olivaceus

Myiodinastes maculatus

Myiarchus spp.

Elaenia spp.

Supplementary Material 2: R code to reproduce the analysis

```
######################################
#Distinct plant extinction scenarios affect the robustness of a seed dispersal network
#Author: Vinicius Bastazini et al.
#Last updated: 2015-11-03
#Contact: bastazini.vinicius@gmail.com
######################################
#packages
require(bipartite)
#data entry
plants_traits #Plant trait data with traits as rows and species as colums
interactions #Interaction matrix, with plants as rows and birds as colums

plants_phylogeny #A species by species phylogenetic distance matrix

##function to fit hyperbolic curves to ATC
fit.hyperbolica=function (object, plot.it = TRUE, ...)
{
 if (class(object) != "bipartite")
   stop("This function cannot be meaningfully applied to objects of this class.")
 N <- colSums(object)
 if (all(object[-nrow(object), 2] == 1))
   y <- -object[, 3]
 else y <- -object[, 2]
 y <- (sum(y) - cumsum(y))/sum(y)
 x <- (object[, "no"]/max(object[, "no"]))
 fit <- try(nls(y ~ 1 - x^a, start = list(a = 1)))
 if (class(fit) == "try-error")
   fit <- nls((y + rnorm(length(y), s = 0.01)) ~ 1 - x^a,
           start = list(a = 1))
 if (plot.it) {
   par(mar = c(5, 5, 1, 1))
   plot(x, y, xlab = "Fraction of elimited plants",
       ylab = "Fraction of surviving birds",
       axes = TRUE, type = "n", cex.lab = 1)



   points(x, y, ...)
   lines(seq(0, 1, 0.1), predict(fit, newdata = data.frame(x = seq(0,
                                              1, 0.1))), col = "red", lwd = 2)
 }
 return(c(exponent = as.numeric(coef(fit)[1])))
}
##Species elimination based on functional distinctiveness
functional=(hclust(dist(traits)))
tree<-compute.brlen(as.phylo(functional))
```

```
plot(tree)
distinc<-evol.distinct(tree,type ="fair.proportion")
distinc
distinc.order<-distinc[order(distinc[,2], decreasing = TRUE), ]
distinc.order$Order<-1:length(tree$tip.label)
distinc.order
rownames(network)==distinc.order[,1]
extinct.row<-distinc.order[match(rownames(network),distinc.order[,1]),3]
extinct.row
rob.funcional=second.extinct(network, participant="lower", method="external",
nrep=50,details=FALSE,ext.row=extinct.row)
robustness(rob.functional)
fit.hyperbolica(rob.funtctional)

## Species elimination based on phylogenetic distinctiveness
tree<-rcoal(9)
tree$tip.label=sample(rownames(network))
filo=(hclust(filo))
tree<-compute.brlen(as.phylo(phylogeny))
plot(tree)
distinc<-evol.distinct(tree,type ="fair.proportion")
distinc
distinc.order<-distinc[order(distinc[,2], decreasing = TRUE), ]
distinc.order$Order<-1:length(tree$tip.label)
distinc.order
rownames(network)==distinc.order[,1]
extinct.row<-distinc.order[match(rownames(network),distinc.order[,1]),3]
extinct.row
rob.phylogenetic=second.extinct(rede, participant="lower", method="external",
nrep=50,details=FALSE,ext.row=extinct.row)
robustness(rob.phylogenetic)

##Random species elimination

rob.aleat=second.extinct(network, participant="lower", method="random",
nrep=1000,details=FALSE)
robustness(rob.aleat)
fit.hyperbolica(rob.aleat)

###degree
# Elimination based on specialists species
rob.grau=second.extinct(network, participant="lower", method="degree",
nrep=1000,details=FALSE)
robustness(rob.grau)
fit.hyperbolica(rob.grau)
# Elimination based on generalists
rob.abun=second.extinct(network, participant="lower", method="abun",
nrep=1000,details=FALSE)
```

```
robustness(rob.abun)
fit.hyperbolica(rob.abun)

#Graphs
par(mfrow=c(3,2))
fit.hyperbolica(rob.aleat)
legend("topright", legend = "i", cex=1.2, bty = "n")
fit.hyperbolica(rob.grau)
legend("topright", legend = "ii", cex=1.2,bty = "n")
fit.hyperbolica(rob.filogenetica)
legend("topright", legend = "iii",cex=1.2, bty = "n")
fit.hyperbolica(rob.funcional)
legend("topright", legend = "iv", cex=1.2,bty = "n")
fit.hyperbolica(rob.abun)
legend("topright", legend = "v", cex=1.2,bty = "n")
```

Capítulo 3- The role of evolutionary modes for trait driven coextinctions in mutualistic networks

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Abstract

Recent evidence suggests that ecological systems loosing functional diversity are more likely to collapse. As species traits are a legacy of their evolutionary history, one might expect that the mode of trait evolution may play an important part in determining community resistance under the loss of functional diversity. In this paper, we provide a theoretical investigation of the role of distinct trait evolution modes on the robustness of mutualistic networks, with strong trait coupling. We simulated evolutionary modes where traits can arise in recent diversification events with weak phylogenetic signal, in early diversification events with strong phylogenetic signal or as a random walk through evolutionary time in different interaction scenarios. Extinctions followed a sequential process, where species were eliminated as a function of their functional trait uniqueness. Our results show that, despite the small range of variation in network robustness, the mode of trait evolution alone and the interaction between modes of evolution of each set of interacting species matter for network robustness undergoing functional trait extinction, especially in extreme cases, where species traits present either very low or very strong phylogenetic signal.

Key-words: Coextinctions, Eco-evolutionary Dynamics, Ecophylogenetics, Ecological Stability, Functional Ecology

Introduction

Understanding how ecological systems respond to disturbances is a central and long-standing issue in both, theoretical and applied ecology (May 1972, 2001; Pimm 1984; Neutel et al. 2002; Allesina & Tang 2012; Myers et al. 2015; Pires et al. 2015). Given the pace of current anthropogenic induced mass extinction, with species being lost at rates that are about three order of magnitude faster than the background rate inferred from fossil record (Pimm et al. 2014; Ceballos et al. 2015), the need to understand and predict species disappearance has become an important task in order to mitigate and reduce human impact on natural ecosystems (Vieira & Almeida-Neto 2014; Ceballos et al. 2015). Ecologists have long acknowledged that the loss of some species may trigger cascading effects in ecological communities, which might bring other species, and even entire ecosystems to extinction (Estes et al. 1998; Jackson et al. 2001; Colwell et al. 2012; Säterberg et al, 2013; Vieira et al. 2013; Brodie et al. 2014; Vieira & Almeida-Neto 2014). However, up to date, most studies that have examined the magnitude of biodiversity loss usually ignore coextinction processes (Dunn et al. 2009; Vieira & Almeida-Neto 2014).

Mutualistic networks are formed by sets of interacting species, which exploit each other for mutual net benefits (Bronstein 2001; Bascompte & Jordano 2007, 2014) and include a wide range of taxonomic groups and interaction types, such as interactions between flowering plants and their animal pollinators and seed dispersers (e.g., Bascompte & Jordano 2007; Muller-Landau & Hardesty 2005; Vizentin-Bugoni et al. 2014), animal cleaning associations (e.g., Wicksten 1998; Guimarães et al. 2007; Sazima et al. 2010) and many mutual benefic human-microbes interactions (Dethlefsen et al. 2007). Mutualistic interactions represent an important model system in order to understand properties of ecological communities given their paramount role in shaping eco-evolutionary dynamics, biodiversity patterns, ecosystem functioning (Guimarães et al. 2011; Ferriere & Legendre 2013; Bascompte & Jordano 2014; Scheuning et al. 2015; Raimundo et al. submitted) and, consequently, for their importance to the development of sound conservation strategies (Kiers et al. 2010; Brodie et al. 2014). Among the many factors that have been recognized as important drivers of mutualistic network organization, species functional traits, i.e., behavioral or ecological characteristic or phenotype associated with biotic interactions and/or an ecosystem function of interest (see Schmitz et al. 2015; Lefcheck et al. 2015 and references herein), seem to play a crucial role, as it can directly constrain or enable the likelihood of an interaction among two or more individuals, imposing threshold on trait values for feasible interactions (Santamaría & Rodríguez-Gironés 2007; Vizentin-Bugoni et al. 2014; Minoarivelo & Hui 2015). Although mutualistic organisms can be flexible with regards to their partners (Bascompte and Jordano 2014), extinctions in mutualistic systems have the potential to accelerate biodiversity loss and ecosystem disruption (Kiers et al. 2010).

Species traits may also affect extinction probability, as some taxa with some specific bio-ecological characteristics, such as, small populations, large body size and narrow niche breadth, are especially more extinction prone (Purvis et al. 2000; Cardillo et al. 2005; Reynolds et al. 2005). The robustness of ecological networks, i.e., the system's tolerance to species loss, has been traditionally evaluated based on scenarios where secondary extinctions are driven by species specialization (i.e., number of interaction links within the foodweb) and/or on stochastic processes (Solé & Montoya 2001; Dunne et al. 2002; Memmot et al. 2004; Burgos et al. 2007; Rezende et al. 2007; Pocock et al 2012). However, these simplistic scenarios usually ignore other important ecological and evolutionary factors that may affect the likelihood of species becoming extinct (but see Curtsdotter et al. 2011; Vieira et al. 2013; Astegiano et al. 2015 and Chapter 2).

Recent evidence suggests that the loss of functional trait diversity takes even a bigger toll in ecological communities, making them more likely to collapse (Galleti et al. 2013; Brodie et al. 2014; Valiente-Banuet et al. 2015; Chapter 2). As species traits are largely a legacy of their evolutionary history (Grafen 1989; Diniz-Filho et al. 2012; Mouquet et al. 2012), one might expect that the mode of evolution, i.e., how traits arise along the phylogenetic history of a clade, may play a pivotal role in ecological dynamics, and consequently, network robustness.

Here, we present a theoretical investigation of how different modes of trait evolution affect the robustness of bipartite mutualistic networks (networks which have two sets of species, such as plants and pollinators, with edges between and not within them) undergoing an extinction process where species are sequentially lost as a consequence of their functional "distinctiveness", i.e., a measure of how distinct is a species in terms of its traits, using simulated data. As evolution is likely to generate functional complementarity and redundancy, which consequently affect ecosystem resilience and resistance (Scheffer et al. 2015), we predicted that networks with high phylogenetic signal would be more robust, as a result of higher trait similarity among species.

Modeling Approach and Statistical Analysis

We developed a modeling framework describing the evolutionary dynamics of bipartite mutualistic networks, formed by two sets of interacting species (Fig.1). We first produced simulated ultrametric phylogenetic trees of different sizes for each set of species, resulting from a uniform birth-death process (Nee et al. 1994). The size of simulated phylogenetic trees ranged from 10 to 20 species, which generated networks that varied in size, ranging from 20 to 40 species. The size of our simulated networks seems to be in agreement with published data, which indicates that sampled mutualistic networks are usually small (mode = 26 species, range: 6-997, based on 66 mutualistic networks acquired in the Interaction Web Database [Accessed in 09/29/2015] and the Web of Life [Accessed in 09/29/2015]).

Secondly, we simulated the evolution of a single trait using a family of power transformations to the branch lengths of simulated phylogenetic trees (Grafen 1989). These transformations were achieved by raising the height of each phylogenetic tree to a different power, denoted by ρ (Grafen 1989). The range of powers used in these transformations simulates different evolutionary models. When the height is raised to the power of 1, it simulates trait evolution under Brownian motion, as if evolution of traits followed a random walk through evolutionary time; power values smaller than 1 compress the branch lengths near the root of the tree, and expand them near the tips of the tree, simulating a recent diversification of traits with low phylogenetic signal, while powers larger than 1 compress the branch lengths near the tips of the tree, and expand them near the root of the tree, simulating an early diversification of traits with high phylogenetic signal.

Networks were then generated using the single-trait complementarity model proposed by Santamaría & Rodríguez-Gironés (2007), which assumes that interactions between species can be described by a specific trait. Following their approach, a mean trait value and its variability characterize each and every species in the network and a pair of species is more likely to interact if their trait values overlap. In their definition, V_i and W_j is the central trait value for species *i* in one partite and species *j* in the other partite, respectively, and δV_i and δW_j are their range of variability. Then, the value of each cell in the incidence matrix, corresponding to this pair of species I_{ij} will be

$$I_{ij} = 1 \; if \; \left|V_i - W_j\right| < 0.5 \text{ x } (\delta V_i + \delta W_j)$$

$$I_{ij} = 0 \text{ otherwise}$$

which means that, a pair of species interact if the cell value is equal to one and they do not interact if it is equal to zero. The variability represented by δV_i and δW_j were defined as random variables with uniform distributions in the intervals 0 – 0.25 (Santamaría & Rodríguez-Gironés 2007). To ensure all species within the network interacted at least with one species from the other set of species, we assigned a random interaction to species that did not have any overlapping trait. Thus, the simulated networks are characterized by strong trait coupling, but with some room for stochastic interactions (Figure 1).

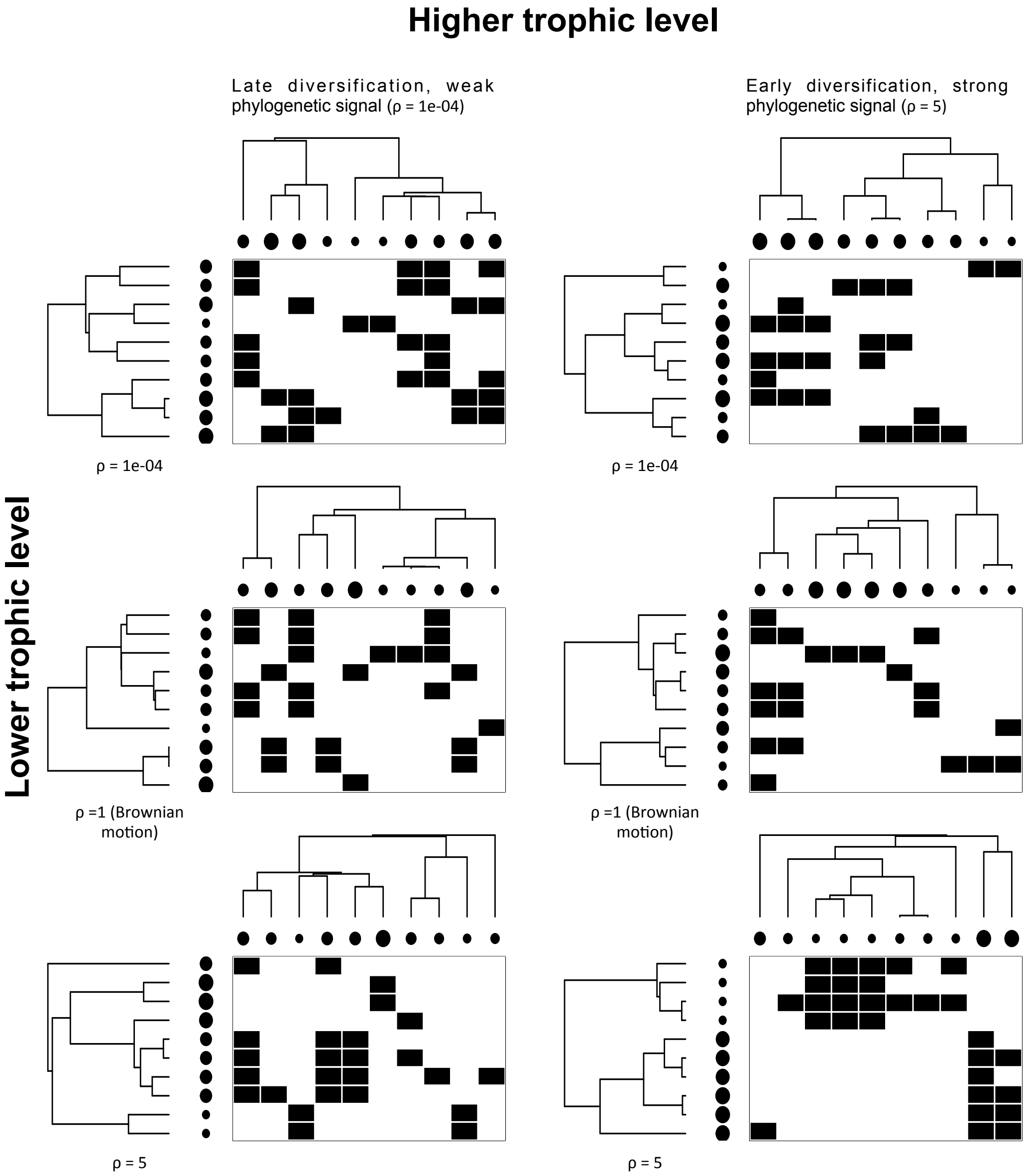

Fig. 1. Examples of the eco-evolutionary dynamics of bipartite mutualistic networks we adopted in our simulations. Species interactions are denoted in the biadjacency matrices along with their evolutionary trees and traits (black circles). The size of each circle corresponds to trait values. Graphen's ρ define the tempo and mode of trait evolution. A single-trait complementarity model defines the probability of interaction of two species. We assigned a random interaction to species that did not have any overlapping trait, to ensure all species interacted with at least with one species from the other set of species.

We estimated the functional distinctiveness of each species using an analogous metric used in phylogenetic studies (Redding et al. 2008). We built a functional dendrogram based on species trait resemblance and then calculated the functional distinctiveness of each species, defined as the sum of all edge lengths between the species and the root of the dendrogram, with each edge length being divided by the number of species in the cluster it subtends.

We calculated network robustness (*R*), defined as the area below of the Attack Tolerance Curve (ATC; Albert & Barabási 2002; Burgos et al. 2007), using an elimination algorithm based on trait distinctiveness; at each time step, the most distinct species, in terms of its functional trait, is removed from the higher trophic level and the fraction of species in the lower trophic level, which still have at least one interaction, is calculated. The ATC is contained in the unit square and starts at a value 1 in the *y*-axis, when no species in one set of species are eliminated and all the species in the other set survive. As species are eliminated, the curve decreases monotonically to 1 in the *x*-axis as no species in one set survive because all the species in the other set went extinct (for further details see Burgos et al. 2007). *R* values closer to 1 indicate higher network robustness, i.e., the system is more tolerant to species extinctions.

At last, we compared four evolutionary modes under four distinct scenarios. The four models compared are related to the evolutionary mode generating traits in the set species belonging to the higher trophic level: i) Traits of species diversified recently, presenting low phylogenetic signal (ρ = 1e-04); ii) Trait evolution follows a Brownian process, (ρ = 1.0); and two models, where traits diversified in the beginning of the evolutionary process, with strong phylogenetic signal (ρ = 2.0 and 5.0, modes iii and iv, respectively). These models were compared in four distinct scenarios according with the evolution of traits in species in the set of species belonging to the lower trophic level: A) a random combination of evolutionary modes, where phylogenetic signal varies from low (ρ = 1e-04) to high phylogenetic signal (ρ = 5.0); B) late diversification of traits (ρ = 1e-04); C) trait evolution following a Brownian process, (ρ = 1.0); and D) early diversification of traits (ρ = 5.0).

We compared these evolutionary modes, within each scenario, using a Bayesian analysis of variance, based on Jeffreys non-informative priors (Kinas & Andrade 2010). The posterior distributions of parameters are defined as:

$$p(\mu_g|Data) \sim St\left(n-G, \bar{y}_g, \frac{S_e}{\sqrt{n_g}}\right)$$

$$p(\sigma^2|Data) \sim GInv\left(\frac{n-G}{2}, \frac{n-G}{2}\, S_e^2\right)$$

where G is the fixed factor representing the evolutionary modes, μ_g is the mean robustness response for each scenario, and σ^2 is the variance of it (for further details see Kinas & Andrade 2010).

Due to the pervasive association between network structure and robustness (see for instance, Bascompte 2009, Bascompte & Jordano 2007, 2014 and references therein), we also evaluated the correlation between network robustness and nestedness and modularity. To do so, we ran another set of simulations (with 1,000 iteractions), across four scenarios, depending on the strength of phylogenetic signal of the species in the higher trophic level (ρ = 1e-

04, 1, 2 and 5). Grafen's ρ varied randomly across the lower trophic level, in each scenario (from 1e-04 to 5). We estimated nestedness using the nested overlap and decreasing fill (NODF) index proposed by Almeida-Neto et al. (2008), and modularity using QuaBiMo algorithm that computes modules, based on a hierarchical representation of species link weights (as in our case, we are simulating qualitative networks, all species have the same weight) and optimal allocation to modules (Dormann and Strauss 2013).

We evaluated the correlation between network structure and robustness under a Bayesian framework (Lee and Wagenmakers 2014), using a multivariate normal distribution with non-informative priors. Monte Carlo Markov Chain was used to obtain the posterior distribution of parameters and correlation coefficient (number of chains= 3; iterations=5,000; burn-in=500). The median of the simulated values from the posterior distributions of the model parameters were used as estimates, and the 2.5% and 97.5% quantiles as lower and upper limits of the 95% credible intervals (95% CrI). The model we used is defined as follows:

$$[x_{i,1}, x_{i,2}] \sim Multivariate\ normal\ ([\mu_1, \mu_2], \Sigma)$$

$$\varSigma = \begin{pmatrix} \sigma_1^2 & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2 & \sigma_1^2 \end{pmatrix}$$

Non-informative priors:

$$\mu_1\mu_2 \sim Normal\ (0,0.001)$$

$$\sigma_1\sigma_2 \sim Uniform\ (0,1000)$$

$$\rho \sim Uniform(-1,1)$$

where ρ is the correlation index, calculated based on the covariance matrix (Σ). The means of the two marginal distributions (μ_1 and μ_2) and the variances (σ_1 and σ_2) are used to parametrize the bivariate normal distribution.

All numerical simulations and statistical analyses were performed in the R environment (R Core Team 2012) and the code is available as a supplementary material.

Results

Our simulations show that the mode of trait evolution alone and that the interactions between the modes of trait evolution of each mutualistic partite matter for network robustness loosing functional diversity (Figure 2). Despite the small range of variation of mean robustness values of each evolutionary mode (Figure 2), they are stochastic different even when some of the Bayesian posterior distributions overlap (Table 1), as in scenarios A and C (Figure 2). One evident pattern is that in extreme cases, either low or high phylogenetic signal, networks are less robust (Figure 2, Table 1). Contrary to our expectation, networks exhibiting strong phylogenetic signal are usually less robust, especially when phylogenetic signal is very low (Figure 2B, Table 1) and when traits of the species in the other partite evolve under Brownian motion (Figure 2C, Table 1). The only scenario that shows that the larger the phylogenetic signal the more robust is the network was scenario D, where both set of species undergo an early diversification of traits (Figure 2D, Table 1). Species traits evolving under

Brownian motion seems to lead to intermediate levels of robustness, except in scenario A, which is a combination of evolutionary modes. In this case, trait evolution under a Brownian motion seems to confer higher robustness (Figure 2A).

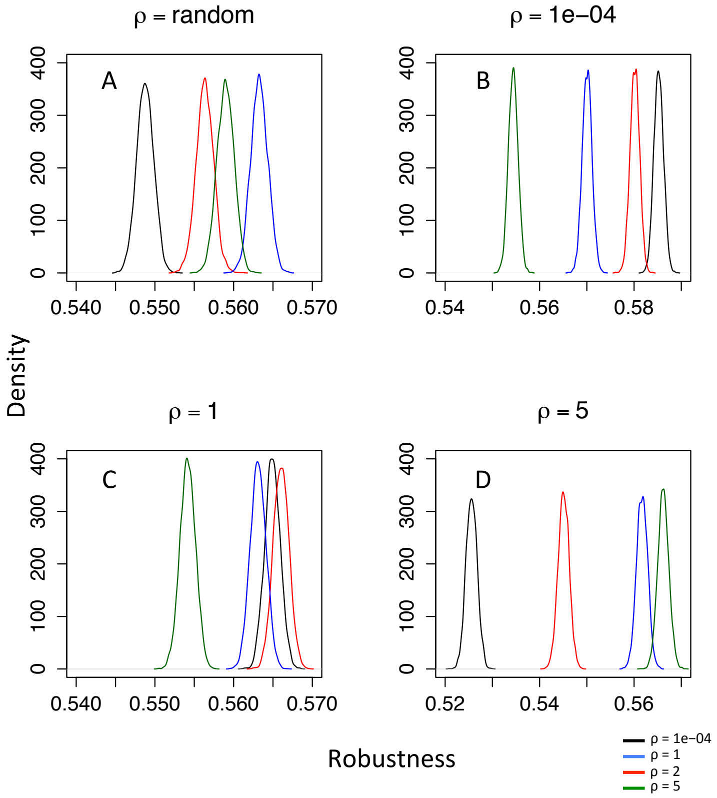

Fig. 2. Posterior distribution of mean robustness for different evolutionary modes under distinct extinction scenarios. A) A random combination of evolutionary modes for the species in the lower trophic level, with phylogenetic signal in trait varying from low to high; B) Species traits in the other partite underwent recent diversification (low phylogenetic signal); C) Trait evolution followed a Brownian process; and D) Species traits in the other partite underwent an early diversification in traits (high phylogenetic signal).

Table 1. Pairwise comparison of the probability of the mean robustness for each evolutionary mode being stochastic larger in each simulated scenario. Probabilities were computed based on the overlap of the posterior distributions.

	ρ = 1e-04	**ρ = 1**	**ρ = 2**	**ρ = 5**
Scenario A				
ρ = 1e-04	-	1	1	1
ρ = 1	0	-	1	0.9528
ρ = 2	0	0	-	0.0022
ρ = 5	0	0.0472	0.9978	-
Scenario B				
ρ = 1e-04	-	0.0004	0	0
ρ = 1	0.9996	-	0	0
ρ = 2	1	1	-	0
ρ = 5	1	1	1	-
Scenario C				
ρ = 1e-04	-	0.7692	0.095	0
ρ = 1	0.2308	-	0.0208	0
ρ = 2	0.905	0.9792	-	0
ρ = 5	1	1	1	-
Scenario D				
ρ = 1e-04	-	1	1	1
ρ = 1	0	-	1	1
ρ = 2	0	0	-	1
ρ = 5	0	0	0	-

The association between network robustness and structure behaved similarly across all scenarios, independent of the strength of phylogenetic signal (Figure 3 and Table 2). Robustness was positively associated with nestedness, with a correlation of approximately 38%, and negatively correlated with modularity (r~ 0.50; Figure 3 and Table 2).

Table 2. Correlation estimates between network robustness and network structure (nestedness and modularity) and the 95% credible intervals.

	Lower bound	**Median**	**Upper bound**
Modularity			
ρ = 1e-04	-0.52	-0.48	-0.43
ρ = 1	-0.57	-0.53	-0.48
ρ = 2	-0.50	-0.46	-0.41
ρ = 5	-0.48	-0.43	-0.38
Nestedness			
ρ = 1e-04	0.32	0.38	0.43
ρ = 1	0.32	0.38	0.43
ρ = 2	0.28	0.34	0.39
ρ = 5	0.32	0.37	0.42

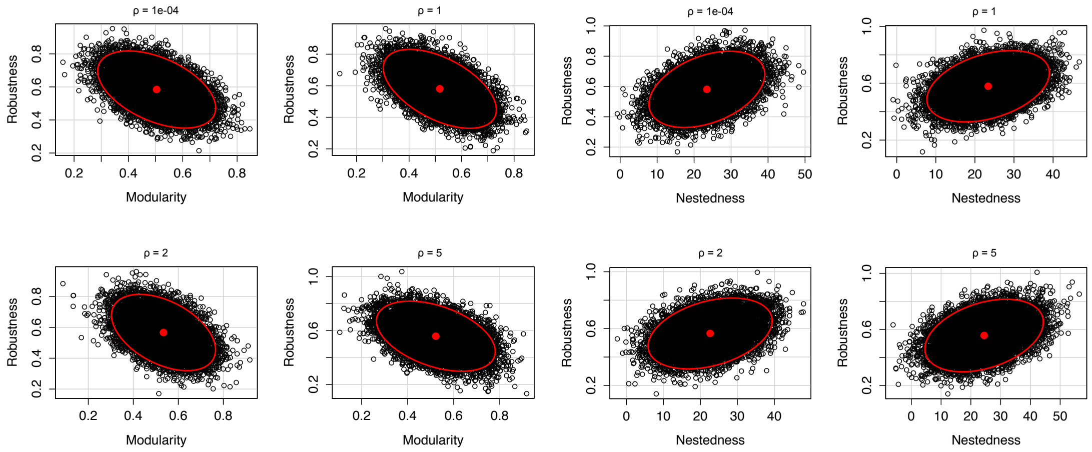

Fig. 3. Bayesian correlations between network robustness and modularity and nestedness. Red ellipse shows 95% density.

Discussion

The recent merging of functional trait and phylogenetic ecology has already contributed to broaden our understanding of the impact of environmental changes upon natural communities (e.g., Díaz et al 2013, Astegiano et al. 2015). Here we developed simplistic model systems that suggest that the mode of trait evolution matter in the study of coextinction in mutualistic networks under functional attack. Over the past years, ecologists have tried to understand how mutualistic network robustness is affected by phylogenetic patterns (Rezende et al. 2007, Vieria & Almeida-Neto 2013, Chapter 2). However these studies are usually concerned with primary extinctions and the phylogenetic information of just one trophic level, or set of species in non-trophic interactions (Vieria & Almeida-Neto 2013, Chapter 2). Our simulations indicate that the interaction between phylogenies of each partite of interacting species seem to influence network robustness and thus should be considered in studies investigating the association between phylogeny and network robustness.

Trait redundancy is thought to promote ecosystem resilience and resistance trough the so–called insurance effect of biodiversity, which states that biodiversity is likely to preclude declines in ecosystem functioning because some species provide greater guarantees, due to their trait similarity, maintaining functioning even with the loss of some species (Yachi et al. 1999, Scheffer et al. 2015). As trait redundancy is likely to be promoted by evolutionary processes (Scheffer et al. 2015), we expected that networks exhibiting strong phylogenetic signal should be more robust, as a result of higher trait similarity among species. However, contrary to our expectations, our results show this is not the case, and in some situations, strong phylogenetic signal will be even associated with reduced robustness, especially when phylogenetic signal in the other set of species is low or when the evolution of traits follows a random walk through evolutionary time. Scenarios B and D suggest that there is a strong coupled phylogenetic response between set of interacting species, as both scenarios show a precise sequential response of network robustness as a consequence of extreme values; in scenario B, when species in the lower trophic level present low phylogenetic signal, network robustness is inverse related to phylogenetic signal, whereas in scenario D, with strong phylogenetic signal, robustness increases with phylogenetic signal.

Previous studies have demonstrated that network structure seem to affect its dynamics. Nestedness is believed to make mutualistic networks more robust to the loss of species or connections (Bascompte 2009, Bascompte & Jordano

2007, 2014). The more cohesive structure of nested networks is more redundant, has more alternative states and provides pathways for the persistence of rare species, when compared to modular ones, which means that it will not collapse as easy (Bascompte 2009, Bascompte & Jordano 2007, 2014). Our analyses seem to corroborate this notion that robustness should increase with nestedness and decrease with modularity.

There are several shortcomings to our simulations. First of all, single trait models may show a poor fit to empirical data (Santamaría & Rodríguez-Gironés 2007). However, trait matching seems to be common in many mutualistic interactions such as plant-pollinator (Garibaldi et al. 2015, Stang et al. 2009, Vizentin-Bugoni et al. 2014) and plant-seed disperser (Chapter 1). Moreover, empirical data also suggest that in some networks, such as predator-prey, trait driven extinction may follow a different dynamic from the one we propose here. For instance, it is largely recognized that large body sized species are more prone to extinction (Cardillo et al. 2005, Reynolds et al. 2005), even if that trait is not so "unique" in the taxonomic group under attack. Thus, testing the role of distinct modes of trait evolution on the robustness of theoretical networks formed by other linkage rules and under different scenarios of functional trait extinctions should provide deeper insights in our understanding of coextinction patterns in real networks. Also, in our simulations, phylogenetic signal is associated with evolutionary process and rate. However, it is important to note that in some situations, this may not be the case, or that this association may be very complex (Revell et al. 2008). Other scenarios involving more complex relationship between phylogenetic signal and evolutionary process and rate could bring further insights. At last, we stress that our framework shares a common shortcoming with similar studies, that is, assuming that species cannot establish new interactions ("rewire") in the absence of original mutualistic partners, thus secondary extinctions should take place every time a species has no surviving partner (Dunne et al. 2002, Memmot et al. 2004, Burgos et al. 2007, Vieira et al. 2013, Astegiano et al. 2015, Chapter 2). However, rewiring should not be common in mutualistic networks with strong trait coupling, as the ones we simulated here, as traits should impose a barrier to new interactions, creating forbidden links due to trait mismatch (Santamaría & Rodríguez-Gironés 2007, Bascompte 2009, Vizentin-Bugoni et al. 2014)

In conclusion, our model networks suggest that the mode of trait evolution alone and the interaction between modes of evolution of each set of interacting species matter for network robustness undergoing functional trait extinction, especially in extreme cases, where species traits present either very low or very strong phylogenetic signal. Despite its simplicity, we hope that our theoretical approach can further our understanding of cascading effects in natural communities, and help to develop better conservation strategies, as it suggests that comprehending phylogenetic patterns of interacting species may help to predict species coextinctions.

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References

Albert R, Barabási AL (2002) Statistical mechanics of complex networks. Rev Mod Phys 74(1): 47-74

Allesina S, Alonso D, Pascual M (2008) A general model for food web structure. Science 320: 658-661

Allesina S, Tang S (2012) Stability criteria for complex ecosystems. Nature 483(7388): 205-208

Almeida-Neto M, Guimarães P, Guimarães Jr PR, Loyola R D, Ulrich W (2008) A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. Oikos 117:1227–1239

Astegiano J, Massol F, Vidal MM, Cheptou PO, & Guimarães Jr PR (2015) The Robustness of Plant-Pollinator Assemblages: Linking Plant Interaction Patterns and Sensitivity to Pollinator Loss. Plos One 10(2): e0117243

Bascompte J (2009) Mutualistic networks. Front Ecol Environ 7: 1-8

Bascompte J, Jordano P (2007) Plant-animal mutualistic networks: the architecture of biodiversity. Annu Rev Ecol Evol Syst 38: 567-593

Bascompte J, Jordano P (2014) Mutualistic networks. Princeton University Press, Princeton

Brodie JF, Aslan CE, Rogers HS, Redford KH, Maron JL, Bronstein JL, Groves C R (2014) Secondary extinctions of biodiversity. Trends Ecol Evo 29(12): 664-

672

Bronstein JF (2001) The exploitation of mutualism. Ecol Let 4:277–287

Burgos E, Ceva H, Perazzo R P, Devoto M, Medan D, Zimmermann M, Delbue AM (2007) Why nestedness in mutualistic networks? J Theor Bio 249(2): 307-313

Cardillo M, Mace GM, Jones KE, Bielby J, Bininda-Emonds OR, Sechrest W, Purvis A (2005) Multiple causes of high extinction risk in large mammal species. Science 309(5738): 1239-1241

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction. Sci Adv 1(5): e1400253

Cohen JE (1977) Food webs and the dimensionality of trophic niche space. P Natl Acad Sci 74: 4533-4536

Colwell RK, Dunn RR, Harris NC (2012) Coextinction and persistence of dependent species in a changing world. Annu Rev Ecol Evol Syst 43: 183–203

Dethlefsen L, McFall-Ngai M, Relman DA(2007) An ecological and evolutionary perspective on human–microbe mutualism and disease. Nature 449(7164): 811-818

Díaz S, Purvis A, Cornelissen JH, Mace GM, Donoghue MJ, Ewers RM, Pearse W D (2013) Functional traits the phylogeny of function and ecosystem service vulnerability. Ecol Evo 3(9): 2958-2975

Diniz-Filho F, Alexandre J, Rangel TF, Santos T, Mauricio Bini L, (2012) Exploring patterns of interspecific variation in quantitative traits using sequential phylogenetic eigenvector regressions. Evolution 66(4): 1079-1090

Dormann C, Strauss R (2013) Detecting modules in quantitative bipartite networks: the QuaBiMo algorithm. arXiv 1304.3218

Dunn RR, Harris NC, Colwell RK, Koh LP, Sodhi NS (2009) The sixth mass coextinction: are most endangered species parasites and mutualists? P Roy Soc B-Biol Sci 276: 3037-3045

Dunne J, Richard A Williams J, Martinez ND (2002) Network structure and biodiversity loss in food webs: robustness increases with connectance. Ecol Lett 5 (4): 558-567

Eklöf A, Jacob U, Kopp J, Bosch J, Castro-Urgal R, Chacoff NP, Dalsgaard B, Sassi C, Galetti M, Guimarães Jr PR, Lomáscolo SB, González AMM, Pizo MA, Rader R, Rodrigo A, Tylianakis JM, Vázquez DP, Allesina S (2013) The dimensionality of ecological networks. Ecol Lett 16: 577-583

Estes, JA, Tinker, MT, Williams, TM, & Doak, DF (1998) Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* 282: 473-476

Ferriere R, Legendre S (2013) Eco-evolutionary feedbacks adaptive dynamics and evolutionary rescue theory. Philos T R Soc B 368(1610): 20120081

Galetti M, Guevara R, Côrtes MC, Fadini R, von Matter S, Leite AB, Jordano P (2013). Functional extinction of birds drives rapid evolutionary changes in seed size. Science 340(6136): 1086-1090

Garibaldi LA, Bartomeus I, Bommarco R, Klein AM, Cunningham SA, Aizen MA, Woyciechowski M (2015) Trait matching of flower visitors and crops predicts fruit set better than trait diversity. J Appl Ecol

Grafen A (1989) The phylogenetic regression. Philos T R Soc B 326(1233): 119-157

Guimarães Jr PR, Sazima C, dos Reis S F, Sazima I (2007) The nested structure of marine cleaning symbiosis: is it like flowers and bees? Bio Lett 3:51–54

Guimaraes Jr PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic networks. Ecol Lett 14(9): 877-885

Interaction Web DataBase. http://www nceas ucsb edu/interactionweb/index html.Accessed in 09/29/2015

Jackson JB, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293(5530): 629-637

Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL (2010) Mutualisms in a changing world: an evolutionary perspective Ecol Let 13(12) 1459-1474

Lee MD, Wagenmakers EJ (2014) *Bayesian cognitive modeling: A practical course*. Cambridge University Press, Cambridge

Lefcheck JS, Bastazini VAG, Griffin JN (2015) Choosing and using multiple traits in functional diversity research. Environ Conserv 42: 104-107

May RM (1972) Will a large complex system be stable? Nature 238: 413–414

May RM (2001) *Stability and complexity in model ecosystems* (Vol. 6). Princeton University Press. Princeton

Memmott J, Waser NM, Price M V (2004) Tolerance of pollination networks to species extinctions. P Roy Soc B-Biol Sci 271(1557): 2605-2611

Minoarivelo HO, Hui C (2015) Trait-mediated interaction leads to structural emergence in mutualistic networks. Evol Ecol 1-17

Mouquet N, Devictor V, Meynard CN, Munoz F, Bersier L Chave J, Couteron P, Dalecky A, Fontaine C, Gravel D, Hardy OJ, Jabot F, Lavergne S, Leibold M, Mouillot D, Münkemüller T, Pavoine S, Prinzing A, Rodrigues ASL, Rohr RP, Thébault E, Thuiller W (2012) Ecophylogenetics : advances and perspectives. Biol Rev 87: 769-785

Muller-Landau HC, Hardesty BD (2005) Seed dispersal of woody plants in tropical forests: concepts examples and future directions. Cambridge University Press, Cambridge. Book Chapter

Myers JA, Chase JM, Crandall RM, Jiménez I (2015) Disturbance alters beta-diversity but not the relative importance of community assembly mechanisms. J Ecol

Nee S, May RM, Harvey PH (1994) The reconstructed evolutionary process. Philos T R Soc B 344(1309): 305-311

Neutel AM, Heesterbeek, JA, de Ruite, PC (2002) Stability in real food webs: weak links in long loops. *Science* 296: 1120-1123

Pimm SL (1984) The complexity and stability of ecosystems. *Nature* 307: 321-326

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Sexton JO (2014) The biodiversity of species and their rates of extinction distribution and protection. Science 344(6187): 1246752

Pires MM, Koch PL, Farinã RA, de Aguiar MAM, dos Reis SF, Guimaraães Jr PR (2015) Pleistocene megafaunal interaction networks became more vulnerable after human arrival. P Roy Soc B-Biol Sci 282: 20151367

Pocock MJ, Evans DM, Memmott J (2012) The robustness and restoration of a network of ecological networks. Science 335(6071): 973-977

Purvis A, Gittleman JL, Cowlishaw G, Mace G M (2000) Predicting extinction risk in declining species P Roy Soc B-Biol Sci 267(1456): 1947-1952

R Core Team (2012) R: A language and environment for statistical computing R Foundation for Statistical Computing Vienna. URL http://www R-project org/

Redding DW, Hartmann K, Mimoto A, Bokal D, DeVos M, Mooers AØ (2008) Evolutionarily distinctive species often capture more phylogenetic diversity than expected. J Theor Bio 251(4): 606-615

Rezende EL, Lavabre JE, Guimarães Jr P R, Jordano P, Bascompte J (2007) Non-random coextinctions in phylogenetically structured mutualistic networks Nature 448(7156): 925-928

Revell LJ, Harmon LJ, Collar DC (2008) Phylogenetic signal evolutionary process and rate. Syst Bio 57(4): 591-601

Reynolds JD, Dulvy NK, Goodwin NB, Hutchings J A (2005) Biology of extinction risk in marine fishes. P Roy Soc B-Biol Sci 272(1579): 2337-2344

Santamaría L, Rodríguez-Gironés MA (2007) Linkage rules for plant–pollinator networks: trait complementarity or exploitation barriers? PLoS Bio 5(2): e31

Stang M, Klinkhamer PG, Waser NM, Stang I, van der Meijden E (2009) Size-specific interaction patterns and size matching in a plant–pollinator interaction web. Ann Bot 103(9): 1459-1469

Säterberg T, Sellman S, Ebenman B (2013) High frequency of functional extinctions in ecological networks. Nature 499(7459): 468-470

Sazima C, Guimarães Jr PR, dos Reis SF, Sazima I (2010) What makes a species central in a cleaning mutualism network? Oikos 119(8): 1319-1325

Scheffer M, Vergnon R, van Nes EH, Cuppen JG, Peeters ET, Leijs R, Nilsson AN (2015) The Evolution of Functionally Redundant Species; Evidence from Beetles. PloS one 10(10): e0137974

Schleuning M, Fründ J, García D (2015) Predicting ecosystem functions from biodiversity and mutualistic networks: an extension of trait-based concepts to plant–animal interactions. Ecography 38(4): 380-392

Schmitz OJ, Buchkowski RW, Burghardt KT, Donihue CM (2015) Functional traits and trait-mediated interactions: connecting community-level interactions with ecosystem functioning. Adv Ecol Res

Solé RV, Montoya M (2001) Complexity and fragility in ecological networks. P Roy Soc B-Biol Sci 268(1480): 2039-2045

Valiente-Banuet A, Aizen MA, Alcántara JM, Arroyo J, Cocucci A, Galetti M, García MB, García D, Gómez JM, Jordano P (2015) Beyond species loss: the extinction of ecological interactions in a changing world. Funct Ecol 29: 299-307

Vieira MC, Cianciaruso MV, Almeida-Neto M (2013) Plant-Pollinator coextinctions and the loss of plant functional and phylogenetic diversity. Plos One 8(11): e81242

Vieira MC, Almeida-Neto M (2014) A simple stochastic model for complex coextinctions in mutualistic networks: robustness decreases with connectance. Ecol Let 18(2): 144-152

Vizentin-Bugoni J, Maruyama PK, Sazima M (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird–plant network. P Roy Soc B-Biol Sci 281(1780): 20132397

Web of Life. http://www web-of-life es. Accessed in 09/29/2015

Wicksten MK (1998) Behaviour of cleaners and their client fishes at Bonaire Netherlands Antilles. J Nat Hist 32:13-30

Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. P Natl Acad Sci 96(4): 1463-1468

Supplementary Material: R code.

```
######################################
# The role of evolutionary modes for trait driven coextinctions in mutualistic
networks network
#Author: Vinicius Bastazini et al.
#Last updated: 2015-11-03
#Contact: bastazini.vinicius@gmail.com
######################################


#Run the main simulation for each level of phylogenetic signal.

require(geiger)

require(plotrix)

require(picante)

require(bipartite)


#rm(list=ls())


runs=10000

n_niveis<-4


power_H=c(0.0001,1,2,5)

#power_L=1


RESULTADOS<-matrix(NA,runs,n_niveis)
```

```
for(o in 1:n_niveis){
    for(m in 1:runs){
        n_spe_H<-sample(10:20,1)
        n_spe_H
        n_spe_L<-sample(10:20,1)
        n_spe_L
        tree_H<-sim.bdtree(b=0.1,d=0,stop="taxa",n=n_spe_H,
extinct=FALSE)
        for (y in 1: length(tree_H$edge.length)) {
            if (tree_H$edge.length[y] == 0.00000000)
                tree_H$edge.length[y] = 0.01
        }
#   plot(tree_H)
        tree_L<-sim.bdtree(b=0.1,d=0,stop="taxa",n=n_spe_L,
extinct=FALSE)
        for (y in 1: length(tree_L$edge.length)) {
            if (tree_L$edge.length[y] == 0.00000000)
                tree_L$edge.length[y] = 0.01
        }
#   plot(tree_L)
        tree_H$tip.label=sprintf("H_%.3d",1:length(tree_H$tip.label))
        tree_L$tip.label=sprintf("L_%.3d",1:length(tree_L$tip.label))
        trait_H<-matrix(NA,n_spe_H,1)
        for(n in 1:1){
            trait_H[,n]<-
rTraitCont(compute.brlen(tree_H,power=power_H[o]),model="BM")
        }
        trait_H[,1]<-rescale(trait_H[,1],c(0,1))
        rownames(trait_H)<-tree_H$tip.label
power_L<-sample(c(0.0001,1,2,3,4,5),1)
```

```
#power_L<-5
        trait_L<-matrix(NA,n_spe_L,1)
        for(n in 1:1){
            trait_L[,n]<-
rTraitCont(compute.brlen(tree_L,power=power_L),model="BM")
        }
        trait_L[,1]<-rescale(trait_L[,1],c(0,1))
        rownames(trait_L)<-tree_L$tip.label
        d_H<-matrix(runif(n_spe_H,max=0.25), n_spe_H,1)
        d_L<-matrix(runif(n_spe_L,max=0.25), n_spe_L,1)
        web<-matrix(NA,n_spe_L,n_spe_H)
        for(i in 1: n_spe_L){
            for(j in 1: n_spe_H){
                II<-abs(trait_H[j]-trait_L[i])
                III<-0.5*(d_H[j]+d_L[i])
                web[i,j]<-ifelse(II<III,1,0)
            }
        }
        colnames(web)<-tree_H$tip.label
        rownames(web)<-tree_L$tip.label
par(mar=c(0.4,0.4,0.4,0.4))
layout(matrix(c(0,0,1,0,0,2,3,4,5),3,3,byrow=T),
widths=c(0.4,0.4,1),heights=c(0.4,0.4,1))
plot(tree_H,show.tip.label=F, direction="downwards")
barplot(trait_H[,1],beside=T, axes=F)
plot(tree_L,show.tip.label=F)
barplot(trait_L[,1],beside=T,horiz=T,axes=F)
color2D.matplot(web,ylab="",xlab="",
yrev=F,cs1=c(1,0),cs2=c(1,0),cs3=c(1,0),border="white",xaxt = "n", yaxt =
"n",axes=F)
```

```
		z_H<-which(colSums(web)==0)
		if(length(z_H)>0){
			for(i in 1:length(z_H)){
				web[sample(1:n_spe_L,1),z_H[i]]<-1
			}
		}

		z_L<-which(rowSums(web)==0)
		if(length(z_L)>0){
			for(i in 1:length(z_L)){
				web[z_L[i],sample(1:n_spe_H,1)]<-1
			}
		}
#par(mar=c(0.4,0.4,0.4,0.4))
#layout(matrix(c(0,0,1,0,0,2,3,4,5),3,3,byrow=T),
widths=c(0.4,0.4,1),heights=c(0.4,0.4,1))
#plot(tree_H,show.tip.label=F, direction="downwards")
#barplot(trait_H[,1],beside=T, axes=F)
#plot(tree_L,show.tip.label=F)
#barplot(trait_L[,1],beside=T,horiz=T,axes=F)
#color2D.matplot(web,ylab="",xlab="", yrev=F,cs1=c(1,0),cs2=c(1,0),cs3=c(1,0),
border="white",xaxt = "n", yaxt = "n",axes=F)

		dis_trait_H<-vegdist(trait_H,method="euclidean")
		clu_H<-hclust(dis_trait_H,method="ward")
		distinc<-evol.distinct(as.phylo(clu_H),type ="fair.proportion")
		distinc
distinc.order<-distinc[order(distinc[,2], decreasing = TRUE), ]
#distinc.order$Order<-1:length(tree_H$tip.label)
```

```
distinc.order
extinct.col<-match(as.character(distinc.order[,1]),colnames(web))
#extinct.col<-
distinc.order[match(colnames(web),as.character(distinc.order[,1]))],3]
extinct.col
test_hig<-second.extinct(web, participant="higher", method="external",
nrep=1,details=FALSE,ext.col=extinct.col)
test_hig
#layout(1)
#par(mar=c(4.5,4.5,3,3))
#slope.bipartite(test_hig)
RESULTADOS[m,o]<-robustness(test_hig
}
}
##save each set of simulations
#RESULTADOS_NS<-RESULTADOS
#RESULTADOS_BM<-RESULTADOS
#RESULTADOS_FS<-RESULTADOS
#RESULTADOS_FS5<-RESULTADOS

## Code for the second set of simulations, comparing network robustness, modularity and nestedeness
runs=1000
n_niveis<-4
power_H=c(0.0001,1,2,5)
power_L=sample(c(0.0001,1,2,5),1)
aninhamento<-matrix(NA,runs,n_niveis)
modularidade<-matrix(NA,runs,n_niveis)
robustez<-matrix(NA,runs,n_niveis)
for(o in 1:n_niveis){
```

```
  for(m in 1:runs){
    n_spe_H<-sample(10:20,1)
    n_spe_H
    n_spe_L<-sample(10:20,1)
    n_spe_L
tree_H<-sim.bdtree(b=0.1,d=0,stop="taxa",n=n_spe_H, extinct=FALSE)
    for (y in 1: length(tree_H$edge.length)) {
      if (tree_H$edge.length[y] == 0.00000000)
        tree_H$edge.length[y] = 0.01
    }
     tree_L<-sim.bdtree(b=0.1,d=0,stop="taxa",n=n_spe_L, extinct=FALSE)
    for (y in 1: length(tree_L$edge.length)) {
      if (tree_L$edge.length[y] == 0.00000000)
        tree_L$edge.length[y] = 0.01
    }
tree_H$tip.label=sprintf("H_%.3d",1:length(tree_H$tip.label))
    tree_L$tip.label=sprintf("L_%.3d",1:length(tree_L$tip.label))
    trait_H<-matrix(NA,n_spe_H,1)
    for(n in 1:1){
      trait_H[,n]<-
        rTraitCont(compute.brlen(tree_H,power=power_H[o]),model="BM")
    }
    trait_H[,1]<-rescale(trait_H[,1],c(0,1))
    rownames(trait_H)<-tree_H$tip.label
    trait_L<-matrix(NA,n_spe_L,1)
    for(n in 1:1){
      trait_L[,n]<-rTraitCont(compute.brlen(tree_L,power=power_L),model="BM")
    }
    trait_L[,1]<-rescale(trait_L[,1],c(0,1))
```

```
  rownames(trait_L)<-tree_L$tip.label
d_H<-matrix(runif(n_spe_H,max=0.25), n_spe_H,1)
  d_L<-matrix(runif(n_spe_L,max=0.25), n_spe_L,1)
web<-matrix(NA,n_spe_L,n_spe_H)
  for(i in 1: n_spe_L){
   for(j in 1: n_spe_H){
    II<-abs(trait_H[j]-trait_L[i])
    III<-0.5*(d_H[j]+d_L[i])
    web[i,j]<-ifelse(II<III,1,0)
   }
  }
  colnames(web)<-tree_H$tip.label
  rownames(web)<-tree_L$tip.label

    z_H<-which(colSums(web)==0)
  if(length(z_H)>0){
   for(i in 1:length(z_H)){
    web[sample(1:n_spe_L,1),z_H[i]]<-1
   }
  }
z_L<-which(rowSums(web)==0)
  if(length(z_L)>0){
   for(i in 1:length(z_L)){
    web[z_L[i],sample(1:n_spe_H,1)]<-1
   }
  }
  dis_trait_H<-vegdist(trait_H,method="euclidean")
  clu_H<-hclust(dis_trait_H,method="ward")
```

```
  distinc<-evol.distinct(as.phylo(clu_H),type ="fair.proportion")
  distinc

  distinc.order<-distinc[order(distinc[,2], decreasing = TRUE), ]
  #distinc.order$Order<-1:length(tree_H$tip.label)
  distinc.order
extinct.col<-match(as.character(distinc.order[,1]),colnames(web))
  #extinct.col<-distinc.order[match(colnames(web),as.character(distinc.order[,1])),3]
  extinct.col
  test_hig<-second.extinct(web, participant="higher", method="external", nrep=1,details=FALSE,ext.col=extinct.col)
  test_hig
  robustez[m,o]<-robustness(test_hig)
  aninhamento[m,o]<-nested(web,method = "NODF2")
  mod=computeModules(web, steps=1E6)
  if(!is.null(mod)){
  modularidade[m,o]<-mod@likelihood
  }else{
  i=i-1
  l=l-1
   }
 }
```

Considerações Finais

Apesar da longa tradição no estudo de interações ecológicas e o extenso material teórico e empírico desenvolvido principalmente desde o final da década de 60, o estudo de interações ecológicas continua na sua fase de "história natural". Ainda estamos descrevendo padrões estruturais de redes de interações bióticas, sendo que abordagens integrativas ainda são escassas. Nesta tese tentei integrar três subdisciplinas que tem atraído a atenção de ecólogos e biólogos evolutivos na última década: Ecologia de Redes, Ecologia Funcional e Ecologia Filogenética de Comunidades.

No primeiro capítulo desenvolvi uma nova abordagem analítica capaz de integrar dados filogenéticos e de atributos funcionais, separando seus efeitos sobre a estrutura das redes ecológicas. O método combina Teoria de Conjuntos Difusos e correlação matricial. A análise de acurácia demonstrou que a abordagem proposta é robusta a erros do Tipo I. Para ilustrar o método utilizei dados de uma rede de frugivoria do sul do Brasil. Apesar de ser apenas um caso ilustrativo, as análises demonstram que, ao contrario do que é defendido na literatura, interação planta - aves frugivoras tem um componente funcional importante, ou seja, mecanismos de complementariedade de atributos parecem determinar as interações.

No capítulo 2, quis integrar a abordagem funcional e filogenética, em um problema que é um dos pilares dos estudos ecológicos teóricos; a estabilidade e resistência de sistemas ecológicos perante a perturbações. Para tal, estimei a robustez, ou resistência, de redes ecológicas com base em cenários de extinções sequenciais onde as espécies são eliminadas conforme a sua distinção evolutiva e funcional, especificidade e ao acaso. Esta análise sugere que a perda de espécies generalistas e de diversidade funcional torna o sistema mais propenso a colapsar, tendo importantes implicações para o manejo do mosaico campo – floresta do sul do Brasil .

Os resultados do capítulo 2 e outras evidências da literatura sugerem que sistemas ecológicos são mais propensos a entrar em colapso, quando perdem diversidade funcional. Assim, no capítulo 3 desenvolvi um estudo teórico para avaliar como diferentes modos de evolução determinam a robustez de redes mutualistas, que estão sofrendo um ataque funcional (i.e., as extinções primárias ocorrem em função das características funcionais das espécies). Os resultados mostram que, apesar da pequena faixa de variação na robustez da rede, o modo de evolução dos atributos por si só, e a interação entre modos de evolução de cada conjunto de espécies mutualísticas influenciam a robustez de redes mutualísticas, especialmente em casos extremos, onde os atributos das espécies possuem um sinal filogenético muito forte ou muito fraco.

Desemaranhar o "emaranhado darwiniano" será por muito tempo, uma tarefa árdua, que provavelmente nunca será completada. Espero que os desenvolvimentos metodológicos e os resultados obtidos nesta tese possam contribuir com esta tarefa.