

# The disruption of a keystone interaction erodes pollination and seed dispersal networks

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## Abstract

Understanding the impacts of global change on mutualistic networks is a major challenge in modern ecology. The gain or loss of particular species and the disruption of key interactions are both consequences and drivers of global change that can lead to the disassembly of complete mutualistic webs. We explored whether the disruption of a hummingbird-mistletoe-marsupial keystone interaction by the invasion of non-native species can have cascading effects on both pollination and seed dispersal networks in the temperate forest of Patagonia. We found that the disruption of the keystone interaction resulted in diverse indirect effects that led to less complex pollination and seed dispersal networks. Similarly, the disruption of the keystone interaction decreased the functional redundancy among generalist plants and pollinators. Our results demonstrate how the disruption of a keystone interaction can have cascading effects through the community, reducing the complexity and stability of ecological networks.

## Introduction

A central attribute of ecological communities is that only few species and interactions may be essential to maintain community structure and ecosystem processes, even though many species might coexist and interact (Ellison *et al.* 2005; Valiente-Banuet *et al.* 2015). The loss of essential species and the disruption of essential interactions may have cascading effects in the community and increasing species extinction rates (Gilbert 1980; Koh *et al.* 2004; Dunn *et al.* 2009; Rodriguez-Cabal *et al.* 2013). Thus, studying the loss of native species and gains of non-native invasive species is crucial to understanding the structure and functionality of communities in a changing world.

In particular, mutualistic interactions such as pollination and seed dispersal are essential in supporting terrestrial ecosystems because most of the plant reproduction success relies on these interactions (Bond 1994; Ashman *et al.* 2004). Ecological networks provide a systematic way of representing, characterizing and comparing the complexity of ecological communities, in which species are represented by nodes and interactions by links between nodes (Pimm 1982; van Veen *et al.* 2008). In the last two decades, the study of mutualistic networks has been critical to understand the structure and functionality of communities (Bascompte *et al.* 2003; Bascompte & Jordano 2007; Rohr *et al.* 2014). However, although several studies have focused on the impacts of human-caused environmental change on mutualistic interactions (Traveset & Richardson 2006; Rodriguez-Cabal *et al.* 2007; Tylianakis *et al.* 2008; Traveset & Richardson 2014), few studies have focused on how such change triggers the disassembly of mutualistic webs (Fortuna *et al.* 2013; Rader *et al.* 2014). Moreover, the indirect effects on interaction webs are not well understood because indirect effects are complex to study and because perturbations can be attenuated by functional redundancy (Lawton & Brown 1993; Terborgh & Feeley 2010).

The temperate forest of Patagonia is known for its highly endemic flora and high proportion of plant species

requiring animal mutualists for pollination and seed dispersal (Aizen & Ezcurra 1998). Almost 60% of plant species require at least one animal species for pollination (Riveros 1991) and nearly 50% of plants produce fleshy fruits suggesting animal seed dispersal (Armesto *et al.* 1987). In the northern portion of this temperate forest occurs a unique mutualistic interaction involving a hummingbird (*Sephanoides sephanioides*), a marsupial (*Dromiciops gliroides*), a mistletoe (*Tristerix corymbosus*), and its main host (*Aristotelia chilensis*), a common understory shrub. The nectar produced by the mistletoe is the primary food resource for the hummingbird during winter (Smith-Ramírez 1993; Aizen *et al.* 2002). In spring and summer the hummingbird pollinates almost 20% of the endemic woody genera in this region (Aizen & Ezcurra 1998; Aizen 2003). The marsupial provides an efficient seed dispersal service by dispersing at least 16 fleshy-fruited species, including large fruits (>11 mm) not dispersed by the native birds (Amico *et al.* 2009), and is the only seed disperser of the mistletoe (Amico *et al.* 2011), which thus depends fully on the marsupial for its persistence. Previous studies found that herbivory on *A. chilensis* by non-native ungulates leads to the disassembly of the hummingbird-mistletoe-marsupial interaction (Rodríguez-Caballero *et al.* 2013). Owing to the high degree of asymmetry of plant-animal mutualisms in the temperate forest of Patagonia, where most of the plants depend only on a few mutualistic partners (Armesto *et al.* 1987; Riveros 1991; Aizen & Ezcurra 1998), the disruption of this interaction could trigger a cascade of linked extinction events throughout the community.

In this study, we evaluate whether the disruption of a hummingbird-mistletoe-marsupial interaction by non-native ungulates can have cascading effects on pollination and seed dispersal networks in the temperate forest of Patagonia, Argentina. Specifically, we address the following questions: (1) Is the hummingbird-mistletoe-marsupial mutualism a keystone interaction? We expect that the complexity and functional redundancy among generalist species of pollination and seed dispersal networks will be greater in sites with this interaction than in sites without the interaction. We also expect that the ecological importance of the species involved in this proposed keystone interaction will be greater in sites with this interaction, occupying a high number of positions in the networks. (2) Does the disruption of this proposed keystone interaction produce cascading effects on the community? We expect that the complexity and functional redundancy among generalist species of pollination and seed dispersal networks will be lower in sites invaded by non-native ungulates than in intact forest sites.

## Material and methods

### Study Area and Natural History

The study was conducted in Nahuel Huapi National Park and Llao Llao Municipal Reserve in northwestern Patagonia, Argentina. The climate in this region has a dry season in spring-summer and a humid season in autumn-winter, with 1800 mm of average annual precipitation and 9°C of average annual temperature. The forest is dominated by the evergreen southern beech (*Nothofagus dombeyi*) and cordilleran cypress (*Austrocedrus chilensis*), with an understory layer dominated by the shrub *Aristotelia chilensis* and bamboo *Chusquea culeou* (Mermoz & Martín 1986). Currently the non-native ungulates red deer (*Cervus elaphus*), dama deer (*Dama dama*), and domestic cattle (*Bos taurus*) are the most abundant ungulates in these forests (Jaksic 1998; Jaksic *et al.* 2002; Merino *et al.* 2009) and occupy 56% of the area of Nahuel Huapi National Park (Lauría-Sorge & Romero 1999).

In this study we took advantage of the contagious distribution of the mistletoe populations (García *et al.* 2009) that allows us to compare sites with high and low mistletoe density. We selected six 1-ha native forest sites, separated by more than 2 km. Four sites were located in intact forest lacking introduced ungulates: two sites with high density of mistletoes (>400 reproductive individuals per hectare) and the presence of *A. chilensis*, and two sites with low density of mistletoes (<20 reproductive individuals per hectare) and the presence of *A. chilensis*. Two additional sites were located in forests that have been invaded by non-native ungulates. We considered invaded sites those with records of non-native ungulates over the last 100 years. Due to the herbivory pressure by non-native ungulates, the abundance of the mistletoe and its host *A. chilensis* is low and the hummingbird-mistletoe-marsupial interaction is ecologically extinct (Rodríguez-Cabal *et al.* 2013). Each site was chosen haphazardly from a pool of possible sites. Fieldwork and data collection were

carried out during two consecutive austral springs and summers (2017-2018 and 2018-2019).

## Pollination networks

During the flowering season (October–February) we identified all plant species pollinated by animals in each site and estimated the rate of flower visits. To this end, we conducted 10 min censuses per plant where we recorded the identity, number of pollinator visits, and number of flowers per individual or branch visited (when the sample plant has a large number of flowers). A visit was recorded when the visitor touched a reproductive structure of the flower. Pollinator species that could not be identified in the field were collected and identified in the laboratory. We performed the censuses from 11:00 to 17:00 h in sunny, non-windy days. We carried out at least 12 censuses per plant species. We conducted 2277 observation periods for all sites and plant species in 2017-2018, and 2269 observation periods in 2018-2019. These numbers varied across species and sites because species differed in their abundance, duration of flowering period and flower production (See Table S1 in Supporting Information).

## Seed dispersal networks

During the fruiting season (January–April) we identified all fleshy-fruited plant species in each site. To quantify seed dispersal by birds, we observed one hour per plant and recorded the number of fruits consumed by birds. We discarded the records of seed predators and pulp pickers. Censuses were performed from 6:30 to 10:30 h. In addition, to evaluate seed dispersal by the endemic marsupial, we collected and analyzed their feces. The feces were collected with a mesh on the floor of Tomahawk traps (10 cm × 10 cm × 30 cm). We placed 17 Tomahawk traps at a height of 1-2 m above the ground on shrubs or trees. Traps were arranged in a star grid and separated from each other by 15 m to cover an effective area of one hectare. We controlled the traps daily at dawn during four consecutive days in February and March. To determine the number of fruits dispersed by *D. gliroides*, we divided the number of seeds of each plant species found in the sample (feces) by the average number of seeds per fruit of the same species. Moreover, to accumulate more observation hours per plant species we used two infrared camera traps (Bushnell trophy cam) per site, programmed to record during day and night. Cameras were placed focusing branches bearing fruits of different plant species and relocated periodically to obtain records of all species present in the site. From the videos, we documented the same variables recorded with direct observations, considering a dispersal event when we visually recorded the consumption of fruit by the seed disperser. Overall, 132 observation periods were conducted for all sites and plant species in 2017–2018, and 158 observation periods in 2018–2019. These numbers varied across species and sites because species differed in their abundance, duration of fruiting period and fruit production (Table S2).

## Data analysis

For each site we built one pollinator and one seed dispersal network per season, resulting in a total of six plant-pollinator and six plant-seed disperser networks (i.e. each site-year combination has a corresponding network). The plant-seed disperser network in 2017-2018 in one of the sites without the keystone interaction was excluded from the analysis because several plant species produced no fruits.

Our analysis focused on network “motifs”, which may be regarded as the building blocks of networks, and consist in sub-networks composed of a small number of species exhibiting particular patterns of interactions (Milo *et al.* 2002). Motifs are valuable tools to assess the structure and ecological importance of species in networks because they have two structural levels of organization. In one structural level, a motif represents a unique pattern of interactions among a subset of species within a community (Simmons *et al.* 2018), while in the other level a motif is composed by two or more unique positions that can be occupied by different species simultaneously (Fig. S1). Each of these positions represents a different ecological pattern with direct and indirect interactions (Stouffer *et al.* 2012; Baker *et al.* 2015; Cirtwill & Stouffer 2015; Simmons *et al.* 2019). Therefore, the frequency of positions that a particular species occupies defines its ecological importance in the community (Simmons *et al.* 2019). Even if different species occupy the same position, the motif still conserves its ecological function. For example, in the motif integrated by two plant species and a pollinator species, plant species could be “A and B” or “C and D” but the motif would still indicate competition or facilitation

between the two plant species. The advantage of motifs is that they are significantly more sensitive to changes in network structure than the network indices commonly used (i.e. degree distribution, nestedness) (Simmons *et al.* 2019). In addition, the meso-scale level motif analysis incorporates indirect interactions undetected by macro-scale network indices (i.e. nestedness, connectance) and lost at species-level indices such as species strength (Simmons *et al.* 2018).

Is the hummingbird-mistletoe-marsupial mutualism a keystone interaction?

To test if the hummingbird-mistletoe-marsupial mutualism is a keystone interaction we compared network complexity, functional redundancy among generalist species, and the ecological importance of keystone interaction members between intact forest sites with and without the keystone interaction.

*Network Complexity* - Network structure is influenced by the number of motifs and their frequency (Simmons *et al.* 2018). Networks composed by a greater number of different motifs are more complex structures because they harbor more different direct and indirect ecological interactions (Milo *et al.* 2002; Simmons *et al.* 2018). Likewise, network complexity increases with the frequency of the largest motifs (those composed of six species; Fig. S1). To estimate network complexity, network structure was described using the frequencies of motifs containing between two and six species, resulting in 44 possible motif combinations. Motif frequencies were calculated for each pollination and seed dispersal network using the “bmotif” R package (Simmons *et al.* 2018). We then normalized motif counts to control for network size. We calculated motif frequency using the method “normalize\_sum”, which expresses counts as the proportion of motifs in the network and considers whether species are more involved in smaller or larger motifs (Simmons *et al.* 2018).

To assess dissimilarity in motif frequencies between intact forest sites with or without the hummingbird-mistletoe-marsupial mutualism interaction, here after keystone treatments, we used a non-parametric permutational multivariate analysis of variance (PERMANOVA). This method allows comparing dissimilarity among and within groups using a pseudo F-statistic (Anderson 2001). We used Bray-Curtis dissimilarity to quantify differences among network structures because it is a robust measure of dissimilarity for multiple ecological properties, including motifs (Anderson 2001; Baker *et al.* 2015; Simmons *et al.* 2019). We conducted a PERMANOVA test with keystone treatments as fixed factor and considered networks from different years built in the same site as replicates. We performed PERMANOVA with the `adonis2` function of the `vegan` package (Oksanen *et al.* 2012) of R (R Core Team), and using 9999 permutations to generate the null distribution of test statics. Finally, we validated the results of PERMANOVA test by estimating dispersion in the data using the `betadisp` function of `vegan` package and testing with one-way ANOVA whether dispersion varied between treatments using `lme4` package (Bates *et al.* 2015). We found no significant differences between dispersion values, which indicates that PERMANOVA results are not caused by heterogeneous dispersion of the data.

*Functional redundancy among generalist species* - In mutualistic networks, nestedness is associated with stability and robustness of species extinction (Memmot *et al.* 2004; Burgos *et al.* 2007; Thébault & Fontaine 2010). In nested networks, a core of generalist plants and animals interacts with each other while specialist species interact only with generalist species, enhancing robustness to species extinction. In addition, functional redundancy among generalist species can increase stability by replacing the position of other generalist after its extinction and reducing the cascading effect on other species (Memmott *et al.* 2004; Kaiser-Bunbury *et al.* 2010).

We estimated the functional redundancy of generalist species and compared between keystone treatments to assess community stability on pollination and seed dispersal networks. First, for each network we calculated the number of species that occupy generalist positions in motifs using `bmotif` package in R. When we disassemble a network in subnetworks, we obtain 148 different species positions across the 44 motifs (Fig. S1). For pollinator and seed disperser species, we selected the positions number 16 and 148, which represent generalist species, as these positions have the highest number of direct interactions with plants (four and five respectively; Fig. S2). For plants, we selected as generalist positions the number 17 and 47, with four and five interactions respectively (Fig. S2). Functional redundancy among generalist species will be higher when

more species occupy these positions. Second, we built regression models for each generalist position (16, 17, 48, and 148) with number of species as the response variable to check differences of functional redundancy between keystone treatments. We selected keystone treatments as fixed factor and considered networks from different years built in the same site as replicates to test this difference. We used the Poisson distribution in our GLMs with a log link function because the response variable is positive and consists in count data (Zuur *et al.* 2009). Regression models were conducted using the lme4 package of R.

*Ecological importance of the keystone interaction members* - As a consequence of the constrained relationship among hummingbird-mistletoe-marsupial, the reduction in the abundance or alteration of behavior of any of them could have the potential to disrupt this keystone interaction. Therefore, we expect that the ecological importance of these species change between intact forests sites with and without the keystone interaction. Because motif positions represent different direct and indirect effects and have different ecological meanings (Stouffer *et al.* 2012; Baker *et al.* 2015; Cirtwill & Stouffer 2015); hence, the greater the variety of positions a species occupies, the greater its ecological importance.

To assess the difference in the ecological importance of the mistletoe, hummingbird, and marsupial, we first calculated the number of positions occupied by these species using bmotif package in R. The mistletoe and hummingbird importance were calculated from pollination networks, while the importance of the marsupial was calculated from seed dispersal networks. We normalized the data using the method “sum” to control the tendency that nodes (e.g. species) with more interactions will occupy more positions than nodes with fewer interactions. This method expresses position counts as the proportion of total occurrences a node occurs at any position. Additionally, to check the difference between keystone treatments we built regression models for each species with number of positions as response variable and keystone treatments as fixed factor. We considered networks from different years built in the same site as replicates to test for this difference. We used the negative binomial family distribution because we found overdispersion in the data and this distribution is a good alternative to deal with it in count data (Zuur *et al.* 2009). Regression models were conducted using the R package lme4.

Does the disruption of the keystone interaction by non-native ungulates produce cascading effects on the community?

To test if non-native ungulates produce cascading effects on the community by disrupting the keystone interaction we compared network complexity, functional redundancy among generalist species, and the ecological importance of keystone interaction members between intact forest sites with the keystone interaction and sites invaded by non-native ungulates. Network complexity, functional redundancy among generalist species, and the ecological importance of keystone interaction members were estimated using the same methodology explained above.

## Results

Overall, we recorded 5151 pollination individual interaction events and 2487 seed dispersal events (Fig. 1). For pollination networks, most of these interactions (56%) were recorded in intact forest sites with the keystone interaction, while 29% of interactions were recorded in sites invaded by non-native ungulates and only 15% in intact forest sites without the keystone interaction. Similarly, most seed dispersal events (50%) were recorded also in sites with the keystone interaction, followed by 27% in invaded sites and 23% in sites without the keystone interaction.

### *Is the hummingbird-mistletoe-marsupial mutualism a keystone interaction?*

*Network complexity* - We found different complexity for both pollination ( $F_{1,7} = 7.021$ ,  $P = 0.031$ ) and seed dispersal ( $F_{1,6} = 4.794$ ,  $P = 0.028$ ) networks between intact forest sites with and without the keystone interaction. We found that in sites with the keystone interaction both pollination and seed dispersal networks were supported by 35% and 47% more different motifs respectively, and hence by greater direct and indirect ecological interactions, than networks in sites without this interaction (Fig. 2a and 2c). In addition, both pollination and seed dispersal networks had 29% and 12% greater frequency of largest motifs integrated by

six species in sites with the keystone interaction than in sites without it (Fig. 2b and 2d), which suggests a greater proportion of complex interactions supporting these networks.

*Functional redundancy among generalist species* - The number of pollinator and plant species that occupied generalist positions was over three times higher in intact forest sites with the keystone interaction than in forests without it (Fig. 3a; Table S3). Moreover, in sites without the keystone interaction no pollinator species occupied the most generalist position and they barely occupied the second one. This analysis was not possible in seed dispersal networks because of the reduced number of frugivorous species.

*Ecological importance of the keystone interaction members* - We found differences in the ecological importance of the mistletoe ( $Z_{1,7} = 3.452$ ,  $P < 0.001$ ) and the hummingbird ( $Z_{1,7} = 2.579$ ,  $P < 0.001$ ) but not on the ecological importance of the marsupial ( $Z_{1,7} = 0.941$ ,  $P = 0.146$ ) between intact forest sites with and without the keystone interaction (Fig. 3b). In sites with the keystone interaction, the mistletoe and the hummingbird occupied 10x and 29x more positions than in sites without this interaction. These results suggest a greater ecological importance of these species in pollination network because they exert more direct and indirect ecological interactions.

Does the disruption of the keystone interaction by non-native ungulates produce cascading effects on the community?

*Network complexity* - We found differences on network complexity in pollination networks ( $F_{1,7} = 5.032$ ,  $P = 0.029$ ) but only marginal in seed dispersal networks ( $F_{1,7} = 3.849$ ,  $P = 0.087$ ) between intact forest sites with the keystone interaction and sites invaded by non-native ungulates. The complexity on both pollination and seed dispersal networks was lower in invaded sites than in sites with the keystone interaction, exhibiting 23% and 59% fewer motifs respectively, and hence, fewer direct and indirect ecological interactions supporting the community (Fig. 4a and 4c). In addition, both pollination and seed dispersal networks exhibit 12% and 11% fewer frequency of largest motifs integrated by six species in invaded sites than in sites with the keystone interaction (Fig. 4b and 4d).

*Functional redundancy among generalist species* - The number of pollinator and plant species that occupied generalist positions was twice as high in intact forest sites with the keystone interaction than in invaded sites (Fig. 5a; Table S3). Moreover, in presence of the keystone interaction a great number of pollinator species occupied the most generalist position providing more network stability. On the other hand, the low functional redundancy among generalist species found in invaded sites suggests low stability in pollinator networks. This analysis was not possible in seed dispersal networks because of the reduced number of frugivorous species.

*Ecological importance of the keystone interaction members* - We found differences in the ecological importance of the members of the keystone interaction between intact forest sites with the keystone interaction and invaded sites (Fig. 5b). In sites with the keystone interaction, the number of positions occupied by the mistletoe was at least three times higher than in invaded sites ( $Z_{1,7} = -2.998$ ,  $P = 0.002$ ), suggesting a greater ecological importance of the mistletoe in the pollination network. In addition, we caught no individuals of *D. gliroides* in any field season in the invaded sites, which indicates that the marsupial may be ecologically extinct in these sites (Rodríguez-Cabal & Branch 2011). Consequently, we were unable to assess statistically the ecological importance of the marsupial between treatments. Furthermore, we found no differences in the ecological importance of the hummingbird between treatments ( $Z_{1,7} = -0.715$ ,  $P = 0.475$ ).

## Discussion

Our results indicate that the hummingbird-mistletoe-marsupial mutualism has the potential to promote the complexity and stability of pollination and seed dispersal networks in the Patagonian temperate forest due the ecological importance of its members. Moreover, our results show that the disruption of this keystone interaction by non-native ungulates has cascading effects on the rest of the community by eroding pollination and seed dispersal networks in invaded sites.

*Is the hummingbird-mistletoe-marsupial mutualism a keystone interaction?*

Complexity of both pollination and seed dispersal networks were different between intact forest sites with and without the keystone interaction. The diversity of motifs and the frequency of largest motifs suggest that networks are more complex in the presence of the hummingbird-mistletoe-marsupial interaction, with a wider range of direct and indirect interactions (Milo *et al.* 2002; Simmon *et al.* 2018). The high diversity of interaction types found may increase persistence and stability of communities due to the influence of direct and indirect interactions on the structure and organization of communities (Wootton 1994; Mougi & Kondoh 2012; Aschehoug & Callaway 2015). In addition to affect persistence and stability of communities, the diversity of interactions may influence ecological processes on a larger time scale. Ecological interactions play an essential role on driving the selection of traits, mainly in multiple partner mutualism such as pollination and seed dispersal interactions (Strauss & Irwin 2004; Guimarães Jr *et al.* 2011). Under normal disturbance conditions, the high number of direct and indirect pathways in sites with the hummingbird-mistletoe-marsupial interaction may allow to reshape continuously and slowly the direction of trait selection, promoting the emergence of species traits (Guimarães Jr *et al.* 2011; Guimarães Jr *et al.* 2017). However, this high number of pathways also may slow down the response of species when rapid environmental changes occur, increasing its vulnerability to strong disturbances (Guimarães Jr *et al.* 2017).

The higher number of plant and pollinator species at generalist positions suggests greater network stability in intact forest sites with the keystone interaction than in sites without it. Several studies have demonstrated that the loss of generalist species produces stronger cascade effects and faster network collapse than the loss of specialized ones (Memmot *et al.* 2004; Kaiser-Bunbury *et al.* 2010). However, the impact of generalist species loss may be attenuated by functional redundancy among species when they perform similar roles in the community (Lawton & Brown 1993). The great number of pollinator and plant species that occupied positions with a high number of direct interactions indicates low network susceptibility to generalist species extinction by partial functional redundancy among them. Thus, the hummingbird-mistletoe-marsupial mutualism can be considered as a keystone interaction that provides a strong robustness to species extinction on pollination networks by promoting the presence of multiple generalist species that support specialized ones (Okuyama & Holland 2008; Thébault & Fontaine 2010).

When the keystone interaction is present, the mistletoe and hummingbird are key members of the community by occupying high number of positions and exerting great direct and indirect interactions. The increase in their ecological importance may be induced by the spatial distribution of the mistletoe and the co-evolutionary history of both species. Since mistletoe population has a contagious distribution (García *et al.* 2009), the high mistletoe abundance in may increase its probability of interacting directly and indirectly with other species (Vázquez *et al.* 2009). Moreover, the mistletoe-hummingbird interaction become specialist in winter because mistletoe flowers are the only nectar resource available for the hummingbird, promoting its permanence in patches with high mistletoe abundance (Smith-Ramírez 1993). In contrast with the mistletoe and hummingbird, the presence of the keystone interaction did not affect the dispersal role of the marsupial. Its ample diet of insects, bird eggs, insects and fleshy fruits (Fontúrbel *et al.* 2012) may allow the marsupial to sustain a stable population without consuming large amount of mistletoe fruits in sites with low mistletoe density. Indeed, the presence of the marsupial and mistletoe in these sites may be a potential source of the mistletoe contagious distribution and become sites with high density of the keystone interaction in the future due to the full dependence of the mistletoe on the marsupial for its seed dispersal (García *et al.* 2009; Amico *et al.* 2011).

Does the disruption of the keystone interaction by non-native ungulates produce cascading effects on the community?

Non-native ungulates produce cascading effects on pollination networks by disrupting the keystone interaction but produce only marginal impacts on seed dispersal networks. Non-native ungulates caused the ecological extinction of the marsupial and reduced the ecological importance of the mistletoe, preventing its recruitment and leading currently its population to extinction. Pollination networks are much simpler and less stable in invaded sites than in intact forest sites with the keystone interaction, presumably because these sites support fewer direct and indirect interactions and fewer generalist plants and pollinators. The low interaction diversity

in invaded sites concentrates the energy flow in few pathways, reducing the persistence and stability of pollination networks (Mougi & Kondoh 2012). Similarly, the simplification of ecological networks has been demonstrated for others human disturbances (Albrecht *et al.* 2007; Tylianakis *et al.* 2007; Galiana *et al.* 2014). Moreover, the low number of plants and pollinators that occupied positions with high number of direct interactions makes the network susceptible to generalist species extinction when non-native ungulates are present.

On the other hand, we found no cascading effects on seed dispersal network despite the high vulnerability of this forest to the loss of mutualistic interactions. The impact of non-native ungulates on seed dispersal networks may be attenuated by the strong presence of the most generalist bird seed disperser of this forest, *Elaenia albiceps* (Amico & Aizen 2005). This migratory bird is abundant in summer, overlapping with fruit ripening and playing a critical role forest regeneration (Bravo *et al.* 2015).

## Conclusion

One or a few species and their direct and indirect interactions can be critical to maintain the structure of communities and ecosystem processes. Thus, identifying which species or interaction to conserve is critical to prevent cascading effects in the community. Here, we have demonstrated the role of a hummingbird-mistletoe-marsupial mutualism as a keystone interaction in Patagonian temperate forest and the cascading impacts of the disruption of this interaction by non-native ungulates. We analyzed interaction motifs as the building blocks of pollination and seed dispersal networks, and found that the hummingbird-mistletoe-marsupial mutualism increases complexity on pollination and seed dispersal networks, supporting a high diversity and frequency of complex motifs and improving the persistence of the community. In addition, the keystone interaction can increase the robustness of pollination networks to generalist species extinction due the high number of plant and pollinator species at generalist motif positions. Moreover, we found that non-native ungulates disrupt this keystone interaction, affecting the ecological importance of the mistletoe and marsupial and reducing the complexity and stability of pollination networks and, marginally, of seed dispersal networks. Recognizing the keystone interactions is essential to achieve the conservation of communities due its role to maintain the structure and functionality of them, especially in the current scenario of global biodiversity loss and spread of invasive species. Moreover, incorporate indirect effects in ecological studies is necessary to increase the realism of global changes impacts on communities, widely underestimated when direct effects are only considered.

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## Figures

**Figure 1. Sites with the keystone interaction recorded more individual interactions in pollination and seed dispersal networks .** Treatments from top to bottom: intact forest sites with the keystone interaction (**a-b** ), intact forest sites without the keystone interaction (**c-d** ), and sites invaded by non-native ungulates (**e-f** ). Left and right panels correspond to pollination and seed dispersal networks, respectively. In each panel, boxes represent species of plants (green), pollinators (red), and seed dispersers (blue). Lines represent ecological interactions between species. Box width is proportional to number of interactions.

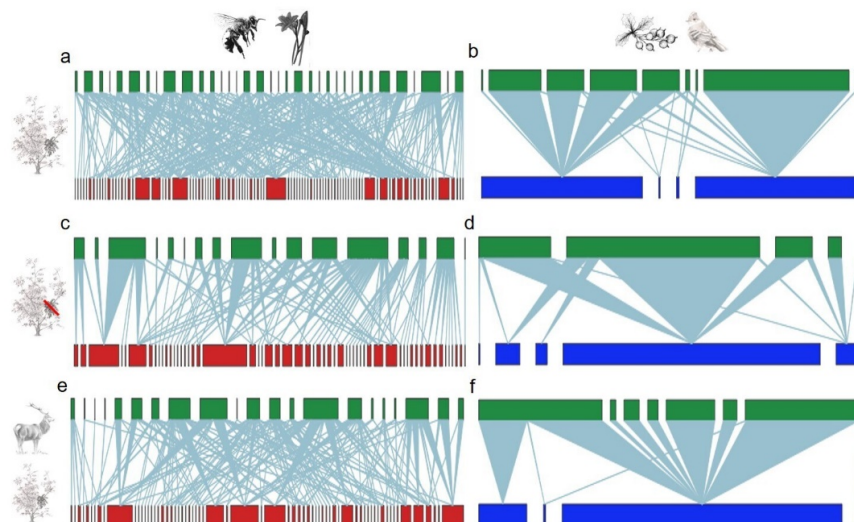
**Figure 2. The complexity of pollination and seed dispersal networks was greater in intact forest sites with the keystone interaction .** Number and relative frequency of motifs related to their complexity (number of nodes-species- per motif) for both pollination (**a** and **b** ) and seed dispersal (**c** and **d** ) networks. Bar color represents treatment: intact forest sites with (violet) and without (white) the keystone interaction. Bars represent means  $\pm$  standard error.

**Figure 3. The functional redundancy among generalist species and the ecological importance of the mistletoe, hummingbird and marsupial were greater in intact forest sites with the keystone interaction than in sites without it .** **a)** Number of pollinator and plant species occupying generalist positions in pollination networks. Number within the circles represents the generalist position occupied by the species: the positions number 46 and 17 indicate four direct interactions, and the positions number 148 and 47 indicate five direct interactions. Circle color represents the trophic group occupying the generalist position: pollinators (red) and plants (green). **b)** Ecological importance -number of motif positions occupied in the network- of the keystone mutualism members. From left to right: mistletoe, hummingbird, and marsupial. Letters mean significant difference between treatments. Bars represent means  $\pm$  standard error.

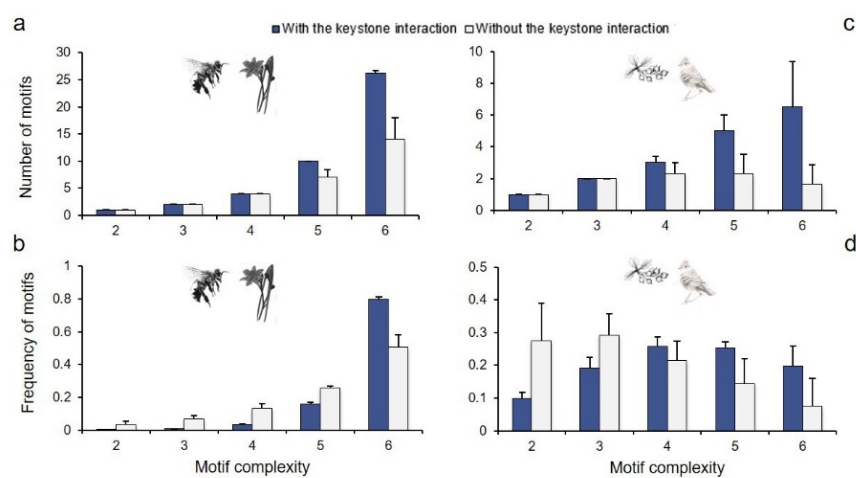
**Figure 4. Complexity of pollination and seed dispersal networks was greater in intact forest sites with the keystone interaction than in sites invaded by non-native ungulates.** Number and relative frequency of motifs related to their complexity (number of nodes -species- per motif) for both pollination (**a** and **b** ) and seed dispersal (**c** and **d** ) networks. Bar color represents treatment: intact forest sites with the keystone interaction (violet) and sites invaded by non-native ungulates (orange). Bar represent means  $\pm$  standard error.

**Figure 5. The functional redundancy among generalist species and the ecological importance of the mistletoe, hummingbird, and marsupial were greater in intact forest sites with the keystone interaction than in sites invaded by non-native ungulates.** **a)** Number of pollinator and plant species occupying generalist positions in pollination networks. Number within the circles represents the generalist position occupied by the species: the positions number 46 and 17 indicate four direct interactions, and the positions number 148 and 47 indicate five direct interactions. Circle color represents the trophic group occupying the generalist position: pollinators (red) and plants (green). **b)** Ecological importance -number of motif positions occupied in the network- of the keystone mutualism members. From left to right: mistletoe, hummingbird, and marsupial. Letters mean significant difference between treatments. Bars represent means  $\pm$  standard error.

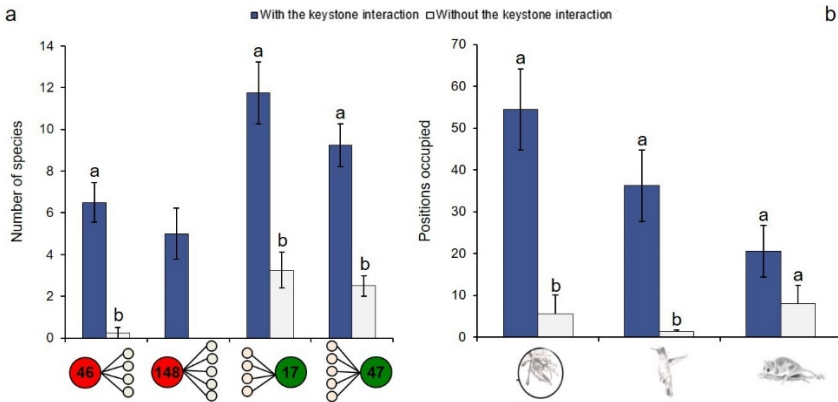
## FIG. 1



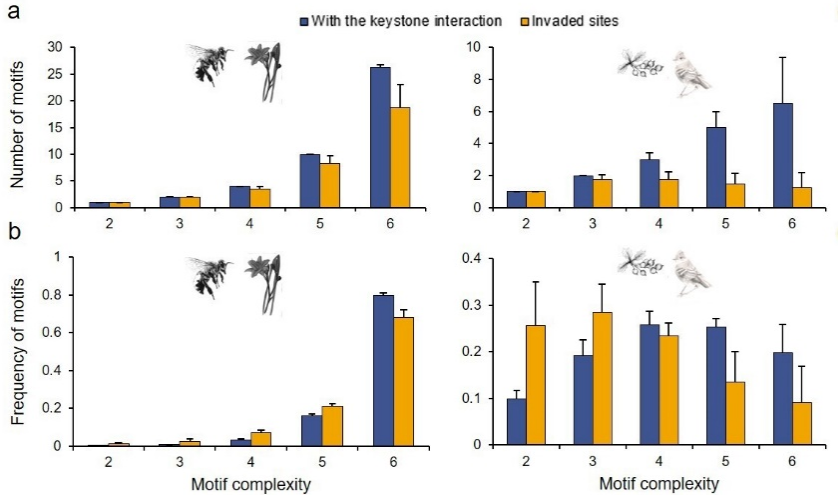
**FIG. 2**



**FIG. 3**



**FIG. 4**



**FIG. 5**

