A E E T

ASOCIACIÓN ESPAÑOLA DE ECOLOGÍA TERRESTRE

Convocatoria de ayuda a proyectos de investigación liderados por jóvenes investigadores (6ª ed., 2016)

1. Datos de identificación.

Título de la propuesta	Sex-related plant facilitation in harsh Neotropical mountaintops: Implications for the ecology and restoration of highly diverse rupestrian grasslands
Categoría	Consolidando la investigación
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2. Memoria Técnica. Actividades y resultados de investigación

2.1. Introducción (Planteamiento, objetivos y justificación)

Rupestrian grasslands are subtropical montane savannas that represent megadiverse ancient ecosystems characterized by infertile soils, high endemism, and unique species compositions (Fernandes, 2016; Photo 1 and 2 of Annex). However, this natural heritage is currently under threat due to human-induced disturbances and climate change. The role played by key nurse shrubs is particularly important due to their capability to assemble ecological communities and maintain biodiversity, mostly through facilitative interactions (Callaway 1995; Armas and Pugnaire 2005). However, most studies on plant facilitation only explore pairwise plant-plant interactions, lacking a more integrative, community approach (Verdú and Valiente-Banuet, 2008). In addition, very little is known about the role played by dioecious nurse plants, with female and male individuals, in providing facilitative interactions and assembling ecological communities. This neglected aspect of plant facilitation seems crucial to address sex-related plant-plant interactions. We hypothesize that sex-biased facilitation occurs even at different scales (different positions under the shrub), based on some potential sex-related differences such as niche partitioning between sexes (Cox, 1981), sex-biased plant-animal interactions (Verdú and García-Fayos, 2003) or dissimilar sex-related physiology (Boecklen et al. 1990). In addition, rupestrian grasslands are highly invaded ecosystems where a broad spectrum of exotic species are fairly abundant, particularly in the proximity of roads (Barbosa et al. 2010).

This high degree of invasiveness might actually be threatening the high levels of biodiversity and endemism (Barbosa et al. 2010). Therefore, this project aims to explore sex-related plant facilitation in the threatened rupestrian grasslands of Brazil, and search for possible applications to restore and conserve this paramount ecosystem.

The **main objective** is to analyze the possible differential effect between female and male individuals of *Baccharis dracunculifolia* (a potential generalist nurse shrub; Photo 3 of Annex) on facilitating understory herbaceous vegetation. In addition, this proposal aims to provide new management and conservation practices towards the restoration and preservation of rupestrian grasslands by focusing on the use of nurse shrubs to restore the grassland communities. Specifically, we aim to examine the following aspects:

1) To what extent shrubs (regardless its sex) affect dry biomass and composition of the herbaceous communities compared to open microsites (control)

2) Analyze the differential effect of female and male shrubs at a local level (individual scale) by comparing biomass and herbaceous diversity at different positions beneath the shrub (edge vs. interior) and for different shrub sizes.

3) Investigate the possible differential facilitate effects of nurse shrubs on native vs. exotic species.

4) Examine possible applications for conservation and restoration of rupestrian grasslands based on the shrub characteristics (sex, size), the ability to facilitate native vs. exotic species, and the distance to the shrub edge.

2.2. Descripción de la ejecución- Metodología

In the rupestrian grasslands of Serra do Espinhaçao, Minas Gerais (Brazil), and with the help of the local researchers from Universidade Federal de Minas Gerais (UFMG) of Belo Horizonte (Photo 4 of Annex), we first selected three sites where the evergreen shrub *Baccharis dracunculifolia* was predominant. In each site, 12 mature individuals were selected (6 male and 6 female). We followed a paired design where the female and the male individuals within each pair were of similar size and located 5-15 m from each other. To ensure statistical independence individuals we selected individuals that were isolated (no other shrub individual in 5 m around). Morphometric variables of each target shrub such as height, canopy cover and perpendicular diameters were measured to characterize each individual. For each individual we established 6 sampling quadrats of 25 cm x 25 cm (Fig. 1).

The quadrats were distributed along the North-South direction (three at North and 3 at South) and at three different distances: exterior (3 m outside the shrub), ecotone (shrub edge), and interior (30 cm inwards from the ecotone quadrat). See Fig. 1 for details. Quadrats located in the exterior part worked as control samples (no shrub effect).

For each sampling quadrat, the aboveground herbaceous biomass was harvested at ground level. The material was taken to the laboratory for species identification (or morphospecies when species-level identification was not possible) and, then, dried at 70ºC until constant weight. We then obtained dry biomass and plant composition of each quadrat and calculated: (1) alfa diversity (species richness index *sensu* Whittaker et al., 2001) and (2) species turnover through Jaccard similirarity-disimilarity index (Jaccard 1912; Koleff et al. 2003). Beta diversity was calculated for all possible pairwise combinations (i.e., interior-ecotone, interior-exterior, and ecotone-exterior) along each cardinal direction (North-South), and between female and male individuals at the same sampling position.

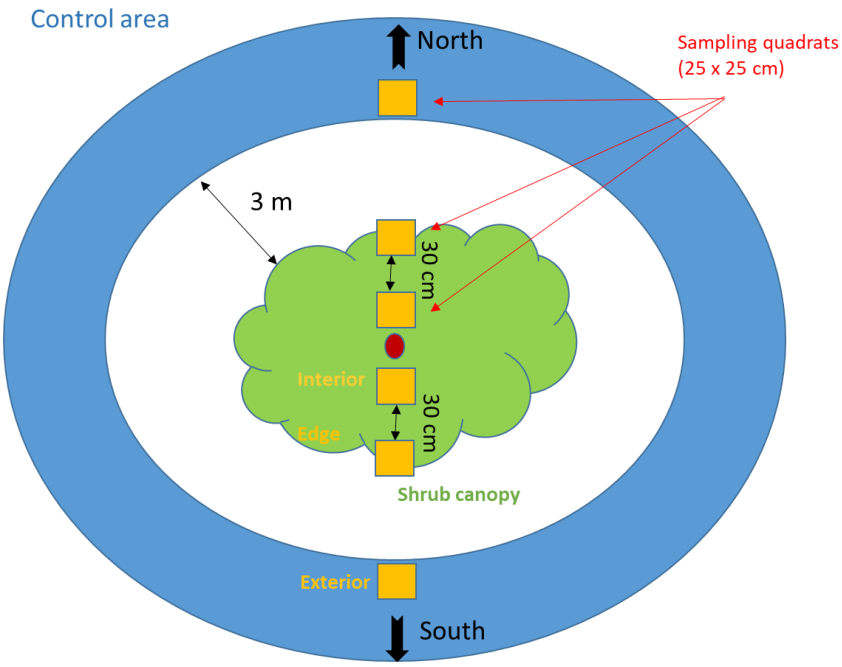

Fig. 1 Diagram with a nurse shrub individual (green color, view from above) and the location of the sampling quadrats along the N-S direction. Quadrats were located in the interior, edge and exterior (control) of the shrub canopy.

Additionally, we established 60 belt transects (10 x 4 m) in 30 pairs. Transects of each pair had one transect with *Baccharis dracunculifolia* as the dominant species and another transect with no presence of *Baccharis dracunculifolia* (Photo 5 and 6 of Annex). Each paired transect was located along a road where exotic species are abundant. Distance between transects of the same pair were 5-20 m apart. We estimated the abundance of each plant species (native and exotic) by measuring the centimeters of each plant species contacting the central transect line, using a measuring tape (Fig. 2). For each plant species, we also measured whether they were growing underneath *Baccharis* (facilitation) or not.

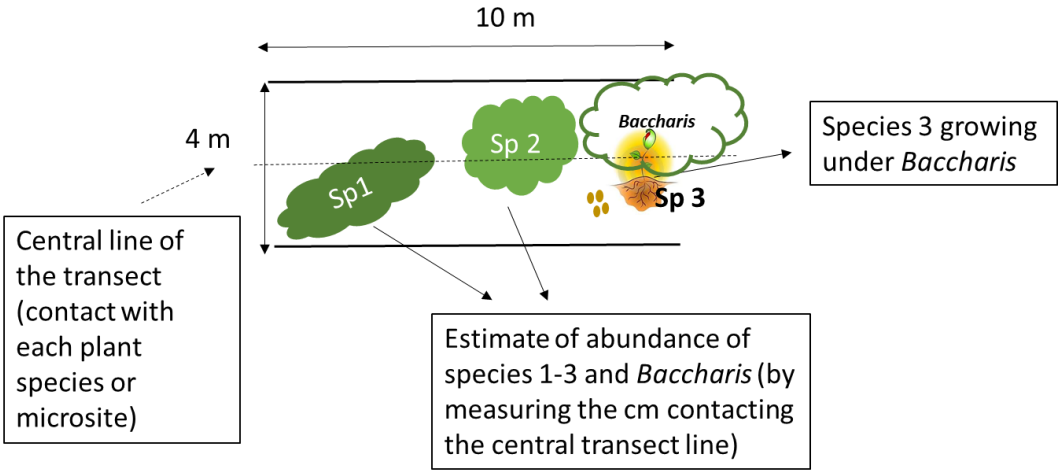

Fig. 2. Example of a transect line with presence of *Baccharis dracunculifolia*. Species 1-3 represent three random species (1-2 growing outside *Baccharis* cover and 3 growing under the cover of *Baccharis dracunculifolia*)

Data Analysis

Generalized Linear Models (GLMM) were used to compare species richness between sexes, aspects (North-South) and along the distance gradient (interior, ecotone, exterior). Models were fitted with a log link function according to our data properties (Bolker et al., 2009), using the ‘‘glmer’’ function within the ‘‘lme4’’ package. Jaccard similarity index (Jaccard 1912; Koleff et al. 2003) was used to analyze species turnover along the distance gradient, with has a minimum value of zero (completely different communities) and a maximum of 1 (identical communities in terms of species presence/absence) using the “vegan” package of R (Oksanen et al, 2013).

Generalized Linear Models (GLMM) were used to analyze species richness in transects with and without *Baccharis* (count data; Poisson distribution) where zone containing each pair of transects was the random structure. Linear Mixed Models (LMM) were used to analyze the proportion of native species in transects with vs. without *Baccharis*. In all LMs we used Shapiro tests to ensure normality of residuals and Levene's tests to check for homoscedasticity. A Facilitation Value was defined for each plant species *i* (FVi) as the proportion (in m of the transect) of plant *i* growing under *Baccharis* divided by the proportion of *Baccharis* within that particular transect. Values of FV>1 indicates occurrence of *Baccharis* facilitation whereas FV<1 indicates no facilitation. FV was calculated only in transects where *Baccharis* was present. We performed a GLM model to analyze the probability of facilitation occurrence as a function of the origin of the species (native vs. exotic) using a logit link function and Laplace approximation with the the ''glmer'' function of the ''lme4'' package. All analyses were performed with R 3.3.1 software.

2.3. Resultados obtenidos (cumplimiento de objetivos)

Results showed no significant differences in species richness between male and female *Baccharis* for either the edge (ecotone) position (χ^2_1=0.043; *P*=0.947) or the interior position (χ^2_1=0.039; *P*=0.844). Similarly, no significant differences in species richness were found between North and South aspects under the *Baccharis* cover for both positions, interior (χ^2_1=0.025; *P*=0.874) and edge (χ^2_1=0.196; *P*=0.658).

Preliminary analysis showed that there was greater species turnover between exterior and edge position of the shrub (Jaccard similarity index of J=0.27 for females and J=0.30 for males) than between interior and edge (J=0.47 for females and J=0.38 for males, respectively; Fig. 3). As shown, female shrubs seem to have greater effect in species turnover with only 0.27 similarity between edge and exterior communities, 20% lower similarity than between interior and edge communities whereas for males this turnover difference is only 8% (Fig. 3)

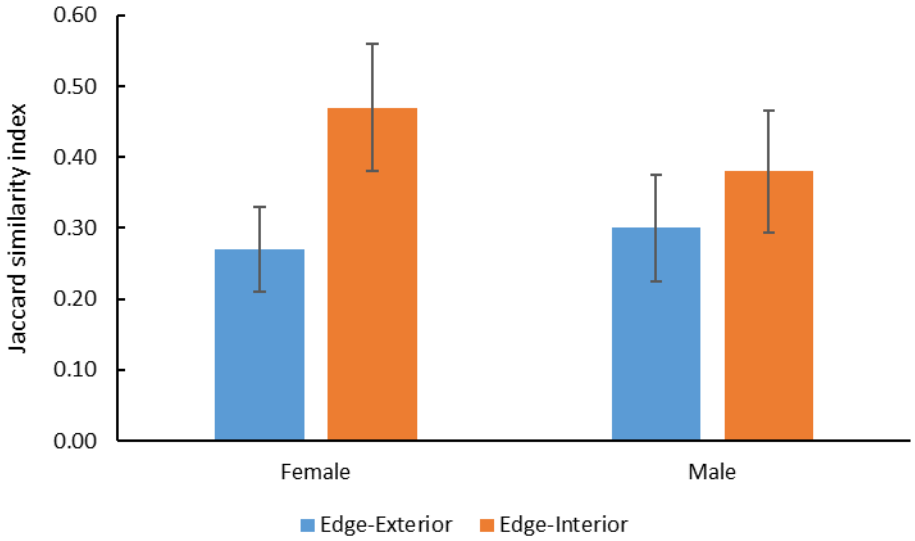

Fig. 3. Jaccard similarity index between communities located at the edge and at the exterior and interior part of the nurse shrub for female and male *Baccharis*. Notice how female individuals caused greater shifts in species turnover (Jaccard index) than male individuals.

Overall, we found 178 plant species or morphospecies in the 60 transects. For those transects with *Baccharis*, the relative abundance of *Baccharis* was 34.5±3.05 % (Mean±SE m in transects). We found significantly greater species richness (57% greater) in transects with *Baccharis* as compared to those without *Baccharis* (χ^2_1=30.58; *P*<0.0001; Fig. 4). However, the proportion of exotic species was significantly higher (27% greater) in transects without *Baccharis* (χ^2_1=4.04; *P*=0.044; Fig. 4).

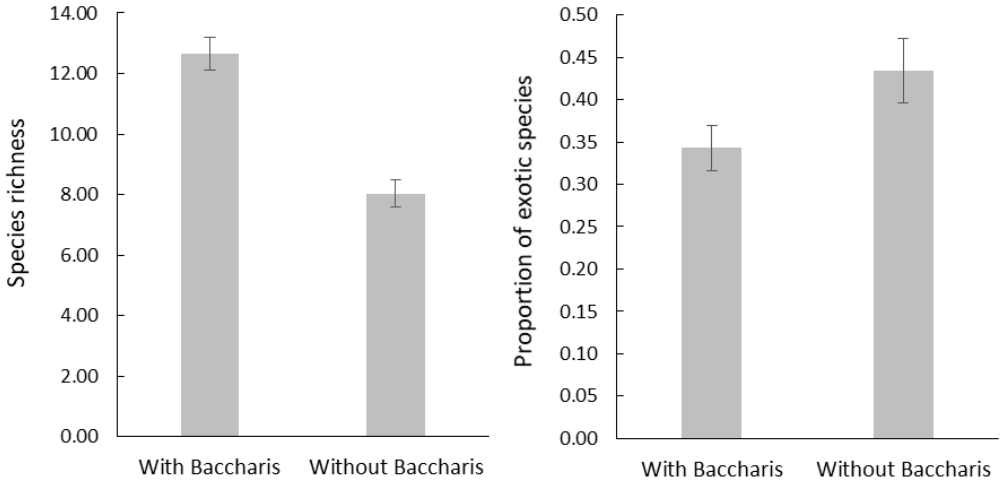

Fig. 4. Species richness (left) and proportion of exotic species (right) in paired transects with and without *Baccharis* (n=60 transects). Error bars indicate standard errors.

Facilitation occurrence by *Baccharis* was significantly greater for native species as compared to exotic species (L-Rχ^2_1=5.07; *P*=0.024). Thus, mean facilitation value of *Baccharis* (FV) for native species was 55% higher than that for exotic species (Fig. 5). Predicted probability of *Baccharis* facilitating a native species was 0.35, 61% greater than that of an exotic species (probability of 0.22).

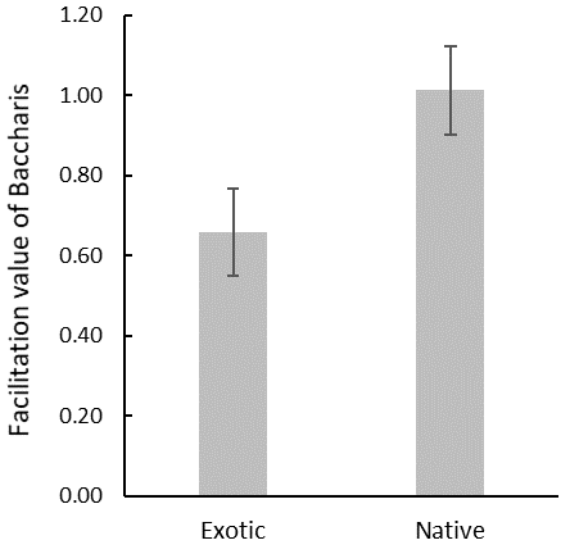

Fig. 5. Mean facilitation Value of *Baccharis* for all exotic and native species. Values of FV>1 indicates occurrence of *Baccharis* facilitation whereas FV<1 indicates no facilitation. Error bars indicate standard errors.

2.4. Conclusiones y valoración de la ejecución

Data collection was successfully carried out in spring 2017 by local students with the support and advice of Dr. Ramón Perea and Prof. Geraldo Fernandes (Universidade Federal of Minas Gerais, Belo Horizonte). Identification of plant species has represented an important drawback given the high diversity of plants species found in the sampled sites of the rupestrian grasslands, some of them belonging to cryptic species that, at least, have been assigned to morphospecies. Abundance of each species, dry biomass of all sampled species and biometric of each *Baccharis* individual have been successfully completed for all pairs of female-male individuals. Additionally, data on 60 transects have been collected and analyzed with very promising results. The use of Dualex Scientific, a leaf-holding device that provides indices of chlorophyll, flavonol and anthocyanin content in leaves (Agati et al., 2016) was used in each Baccharis individual (5 measurement per plant). However, measurements in

each *Baccharis* have not yet been analyzed. Students from the Universidade Federal of Minas Gerais (UFMG) are carrying out the data analysis in the Laboratorio de Ecologia Evolutiva e Biodiversidade (LEEB).

Preliminary analysis on the sex-related plant facilitation showed no effect of sex on species richness under their cover. However, we found differential species turnover for males and females which needs further analysis to corroborate a greater enhancement (facilitation) of plant community heterogeneity by female individuals as compared to male individuals. In addition, we still need to analyse biomass production (data have been collected) for each quadrat as well as possible variation in exotic vs. native biomass along the gradient for male and female individuals. Students from UFMG are building the dataset where all species (>200) need to be identified and assigned to their corresponding dry biomass.

The data from the 60 sampled transects have been successfully collected and analyzed with very interesting findings. The results revealed a differential facilitative effect of native nurse plants (*Baccharis*) on exotic and native plants. These findings also have implications for restoration ecology, particularly in highly invaded ecosystems such as the grasslands of subtropical montane savannas. Since these results are straightforward and highly novel we have first focused on analyzing and publishing these data. One first Manuscript is in preparation regarding the differential facilitative effect of *Baccharis* for exotic vs. native plants.

2.5. Publicaciones resultantes

Part of the results has been presented at the XI Eugene Warming Lectures in Evolutionary Biology at the Universidade Federal de Minas Gerais (UFMG, Belo Horizonte, Brazil) with the following reference:

Cunha da Silveira J, Spadeto C, Matos-Gomes V, Moura AL, Rúbia B, Fernandes GW, Perea R. 2017. *Facilitaçao por Baccharis dracunculifolia no campo rupestre*. XI Eugene Warming Lectures in Evolutionary Biology. Rupestrian Ecosystem: Conservation Status and the Deadly Route to Collapse. November 27th-30th, Belo Horizonte, Brazil.

In addition, the following manuscripts are currently in preparation:

Perea R, Cunha da Silveira J, Spadeto C, Matos-Gomes V, Moura AL, Rúbia B, Fernandes GW. 2018. Native shrubs as differential facilitators of exotic and native species: the use of native shrubs to restore highly invaded ecosystems

Perea R, Cunha da Silveira J, Spadeto C, Matos-Gomes V, Moura AL, Rúbia B, Fernandes GW. 2018. Sex-dependence plant facilitation in subtropical montane savannas.

3. Informe de gastos del proyecto. Relación de partidas de gastos y sus importes. Se deberán aportar justificantes originales de los pagos realizados (tickets, recibos o facturas).

	Brazilian Reales (R$)	Euros (€)
Flight Madrid-Belo Horizonte (Brazil)-Madrid (4 planes)		1847.06
Accommodation Belo Horizonte (5 days)	1098.95	345.58
Van rental for fieldwork (including gas and driver from Universidade Federal Minas Gerais; 8.5 days x 180 R$/day)	1530.00	481.13
PI and students subsistence allowance at Reserva Natural Vellozia, Serra do Cipó (7 days)	1400.00	440.25
Materials (batteries for Dualex+, transect meter, water, flagging tape, paper bags, plastic bags, markers)	38+16+150.68+9=213.68	67.19
TOTAL		**3181.21 €**

R$ to € exchange rate at 3.18 (April 2017).

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Annex I. Supplementary Imaging Material

Photo 1. General landscape of the rupestrian mountaintop grasslands of Minas Gerais (Brazil).

Photo 2. Rupestrian grasslands of Minas Gerais represent megadiverse ancient ecosystems characterized by infertile soils, high endemism, and unique species compositions

Photo 3. Students from the Universidade Federal Minas Gerais (UFMG) marking female and male individuals of *Baccharis dracunculifolia.* Students from UFMG (Laboratorio de Ecologia Evolutiva e Biodiversidade) helped with all the fieldwork.

Photo 4. Female (left) and male (right) individuals of *Baccharis dracunculifolia* at Site 1 during the data collection campaign in April 2017

Photo 5. Establishing a 10 m transect line with *Baccharis* at the mountaintop savannas of Minas Gerais (Brazil).

Photo 6. Transect established in areas with no presence of *Baccharis*. A paired design based on transects with and without the nurse shrub *Baccharis* was used to analyze the effect of nurse plants of facilitating exotic and native plant species.