

Direct and indirect effects of shrub encroachment on alpine grasslands mediated by plant–flower visitor interactions

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Summary

1. Mutualistic interactions structure ecological communities and they are strongly influenced by the combined effect of different drivers of global change. Land-use changes and global warming have elicited rapid shrub encroachment in alpine grasslands in recent decades, which may have detrimental outcomes for native alpine forbs. In spite of the importance of this process, we lack knowledge about how shrub encroachment modifies community-wide patterns of plant–pollinator mutualistic interactions.

2. Based on the functional biodiversity hypothesis (FBH), which predicts higher pollinator biodiversity in species-rich plant communities, we asked whether the increase in nutritional resources available for pollinators due to shrub expansion modifies pollinator niche breadth and species richness, and whether these changes affect plant–plant interactions.

3. For this purpose, we compared quantitative plant–flower visitor interaction network assemblages at replicated plots in two habitat types in dry cryophilic grasslands of Sierra de Guadarrama (Spain): (i) encroached pastures (EP) and (ii) pastures dominated by forb species where shrub species are absent (PA).

4. As predicted by FBH, flower visitor richness increased in EP, but their niche breadth did not vary. Furthermore, shrubs had more interactions with flower visitors and received more visits per plant than forbs in EP in agreement with their significantly higher linkage and strength.

5. Overall, results revealed that moderate levels of shrub encroachment affected the flower visitation patterns of forb species in alpine grasslands as flower visitor diversity increased and plant–plant competition for shared flower visitors became greater. These findings highlight the need to use an integrative approach to study the cascading effects of global change drivers on species interactions and their impact on the structure and functioning of threatened ecological communities.

Key-words: functional biodiversity, invasion ecology, land-use change, native plant communities, pollination networks, specialization

Introduction

Global change drivers cause species extinction, modify species distributions and promote the emergence of novel communities, ultimately affecting the state of ecosystems and their associated services (Tylianakis *et al.* 2008; Algar *et al.* 2009). High-mountain ecosystems are particularly responsive to the action of these drivers (Wookey *et al.* 2009; Pauli *et al.* 2012). Climate warming and the decline

of traditional land management have shifted the distribution ranges of plant species and the composition of plant communities world-wide (Pauli *et al.* 2012; Brandt *et al.* 2013). In many cases, these alterations have caused an increase in the density, cover and biomass of native woody plants to the detriment of herbaceous flowering plants (Sanz-Elorza *et al.* 2003; Wookey *et al.* 2009; Brandt *et al.* 2013). Numerous studies document the effect of shrub encroachment on the abiotic environment (e.g. soil erosion and nutrient cycling), plant–plant interactions and

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vegetation composition (Reviewed in Wookey *et al.* 2009). However, we lack empirical data showing how shrub encroachment modifies plant–animal interactions (e.g. herbivory, parasitism, pollination; Tylianakis *et al.* 2008; Ferreira, Boscolo & Viana 2013).

The harsh environmental conditions of high-mountain ecosystems limit pollinator richness and pollination services for plant reproduction (Inouye & Pyke 1988; Kearns & Inouye 1994; Dupont, Hansen & Olesen 2003). In this context of limited resources, shrub encroachment increases nutritional resources for pollinators (Eldridge *et al.* 2011). According to the functional niche hypothesis (Blüthgen & Klein 2011) (FBH), when the diversity of functionally complementary plants (the producer trophic level) increases, the overall requirements of their pollinator (the consumer trophic level) are better met. This results in a positive feedback at both trophic levels, as more flowering plant species would tend to harbour higher diversity of pollinator species in encroached areas, which in turn might increase the pollination success of local plants (Ebeling *et al.* 2008; Blüthgen & Klein 2011). Furthermore, generalization of pollinators can vary with trophic resources availability (Albrecht, Riesen & Schmid 2010; Fründ, Linsenmair & Blüthgen 2010; Benadi *et al.* 2014). Thus, at high densities, a more productive environment should lead to an increased trophic specialization (MacArthur & Pianka 1966; Fründ, Linsenmair & Blüthgen 2010; Ebeling, Klein & Tscharrntke 2011). Consequently, although the overall niche breadth of the pollinator community may expand, the specific niche breadth of each pollinator could become narrow as plant species richness and flower abundance increased. Indirect plant–plant competitive interactions might also arise between shrubs and co-flowering forb species for shared pollinators. Empirical studies addressing the consequences of shifts in plant communities have obtained mixed results. On the one hand, higher pollinator diversity in encroached pastures (EP) may increase visitation rates to forbs increasing their reproductive output (Johnson *et al.* 2003; Ghazoul 2006). On the other hand, pollinators may shift their preference from forbs to expanding shrubs in encroached areas, thus leading to decreased visitation to forb plants which may reduce their seed set (Reviewed in Morales & Traveset 2009). The strength of plant–plant competition for shared pollinators depends on their flowering phenology and phenotypic similarity in floral resources (Jordano, Bascompte & Olesen 2003; Morales & Traveset 2009). Many shrub species have less accessible flower types (e.g. in *Fabaceae*), and therefore, competition between pasture and shrub flowering plants for pollinators may involve just the subset of pollinators that have access to these flowers, such as bees and bumblebees (Fenster *et al.* 2004; Fontaine *et al.* 2006).

Although previous experimental research has shown that diet breadth is a flexible pollinator trait partially affected by behavioural adaptation to resource availability (Fontaine *et al.* 2006; Fontaine, Collin & Dajoz 2008), variation in diet breadth and its effects at the community scale

remains largely unexplored (Fontaine, Collin & Dajoz 2008). These community-wide patterns of plant–pollinator and plant–plant interactions can be explored through the application of a species interaction network approach (Jordano 1987). Interaction networks summarize the diversity of interactions among species in a community and are widely used to investigate the effect of species composition shifts on ecological communities (e.g. Lopezaraiza-Mikel *et al.* 2007; Aizen, Morales & Morales 2008; Vilá *et al.* 2009). The topological properties of these networks provide information on community organization and help to predict community dynamics in response to ecosystem disturbance (Jordano, Bascompte & Olesen 2003; Tylianakis *et al.* 2008; Blüthgen 2010).

In this study, we investigated the relationship between ecological heterogeneity and niche breadth and evaluated the cascading effects of shrub encroachment on alpine plant–flower visitor networks, using flower visitors as a proxy for pollinators. The study was carried out in Mediterranean alpine communities of Sierra de Guadarrama, a mountain range located in central Spain. In these mountains, the abandonment of traditional pastoral activities and higher winter temperatures have caused an expansion of native woody plants in alpine pastures to the detriment of herbaceous flowering plants (Sanz-Elorza *et al.* 2003; Garcia-Romero *et al.* 2010). Two dominant leguminous species of the shrub belt have showy floral displays and co-flower with the pasture community (Castroviejo *et al.* 1990; Escudero *et al.* 2004). Based on FBH, we expected shrub encroachment to increase pollinator species richness and simultaneously to reduce the niche breadth of each pollinator species, ultimately affecting plant–plant interactions through competition for shared pollinators. Specifically, we asked:

1. Does higher diversity at the producer trophic level (plants) result in increased diversity at the consumers (pollinators) trophic level, as predicted by FBH?
2. Does shrub encroachment reduce the specific niche breadth of pollinators?
3. Do shared pollinators mediate facilitative or competitive indirect interactions between shrubs and forbs?

Materials and methods

STUDY SYSTEM

This study was carried out in Mediterranean alpine communities of Sierra de Guadarrama, a mountain range located in the Central System in Spain (40° 50'N, 3° 57'W; Fig. S1, Supporting information). Dry cryophilic pastures occur on the higher summits above the tree line between 2000 and 2430 m.a.s.l and are characterized by a Mediterranean alpine-type climate (Escudero *et al.* 2004). The dominant vegetation is an extremely short pasture (rarely exceeding 3 cm height) dominated by graminoid *Festuca curvifolia* Lag. ex Lange with several creeping chamaephytes and caespituous grasses (hereafter forb species). The timberline is located between 1900 and 2100 m and is dominated by stunted pines (*Pinus sylvestris*), which appear interspersed in a shrub matrix

characterized by *Cytisus oromediterraneus* (Fabaceae) Rivas Mart. *et al.* *Adenocarpus hispanicus* (Fabaceae) (Lam. DC.) and *Juniperus communis* subsp. *alpina* (Cupressaceae) (Suter) Čelak. The first two shrubs (hereafter shrub species) co-flower with forb species. These species produce showy resource-rich flowers with higher reward levels for flower visitors compared to the small, resource-poor flowers produced by forb species (Castroviejo *et al.* 1990).

SAMPLING DESIGN AND FIELD SURVEY

Study sites

We replicated the study at two localities separated by a distance of about 17 km (Peñalara and Nevero peaks, Fig. S1). At each locality, we selected two sampling sites – a pasture dominated by forb species with no shrub species (hereafter, PA) and an encroached pasture (hereafter, EP) with ~25% of the surface area covered by shrub species. We selected EP sites with a moderate level of shrub encroachment to avoid comparing communities where forb species have already been displaced. The sampling sites were 700 and 900 m apart from each other in Peñalara and Nevero, respectively. Previous studies have shown that this distance allows spatially independent flower visitor communities (e.g. Aizen, Morales & Morales 2008; Kaiser-Bunbury, Memmott & Müller 2009). Differences in altitude between the habitat types (i.e. EP vs. PA) at the two localities were <100 m (Fig. S1). All sampling sites had an NW orientation and similar plant communities (74% of forb species occurred in both habitat types, Table S1).

Monitoring of plant–pollinator interactions

We collected data throughout the flowering period (Fig. S2: 13 June to 28 July 2011) for a total of 10 census days for each sampling site at Nevero and 11 census days for each sampling site at Peñalara. There was one additional census day at Peñalara in order to balance the number of hours surveyed at the two localities (approximately 160 h per site). Weather conditions determined the number of census days, as warm, dry and light wind conditions are unusual in high-mountain environments (Dupont, Hansen & Olesen 2003). Observations were made on sunny days without wind from 10:00 to 18:00 h when the environmental conditions at these altitudes allow visitor activity (Dupont, Hansen & Olesen 2003). To collect visitation data, we established two 60 × 100 m sampling plots at each locality (PA and EP) where we established 10 linear transects across the width of the plots (60 m long and 5 m wide) (Fig. S1). We made successive walks through the line transects, noting the identity and number of contacts between plant and insect species. This methodology was used to observe the complete area of each sampling plot. Transect methods are very effective for monitoring plant–flower visitor interactions in environments where time available for observations is limited (Gibson *et al.* 2011), as in the case of high-mountain environments. We recorded a plant–flower visitor interaction when an insect maintained contact with the reproductive organs of a flower for more than 1 s (Gibson *et al.* 2011). Thus, all flowering visiting insects that feed on flowers were recorded, regardless of the efficacy of their visit. The two sampling sites at each locality were simultaneously surveyed by two work teams. Insects were captured and determined to the lowest taxonomic category possible with the help of experts (Table S1). Voucher specimens were deposited at Rey Juan Carlos University. We grouped the flower visitors into functional groups to facilitate the detection of general patterns. We defined ‘functional group’ as a set of insects that tend to interact with flowers in a similar way (Fenster *et al.* 2004). Following the methodology employed in Gomez *et al.* (2008), we used criteria of similarity in size, proboscis length, foraging behaviour and feeding habits rather than taxonomic relationship. We

established eleven functional groups (Appendix S1 and Table S1). Plant species were determined in the field. For each census day, the number of flowering plants of each species was recorded in 10 transects (60 m long and 2 m wide) per sampling site. The sum of all transects was used as an estimation of the total number of flowering plants per species.

DATA ANALYSIS

Effect of shrub encroachment on species assemblages and visitation patterns

We used linear mixed models (LMMs) to test the effect of shrub encroachment on number of flowering plants per species, number of plant–flower visitor interactions, total number of visits and number of visits per individual per plant species (hereafter visits per plant). All models included habitat type (PA and EP) as a fixed factor, and locality and census day nested in locality as random factors to control for potential temporal and spatial non-independence (Zuur *et al.* 2009). Number of flowering plants per species was included as a continuous predictor variable in models that fitted the number of interactions, total number of visits and number of visits per plant, as this variable affects visitation patterns (Vázquez 2005; Blüthgen, Menzel & Blüthgen 2006). We assumed Gaussian error for all the LMMs, and response variables were ln-transformed to reach normality. QQ-plots and histograms of the residuals were used to verify normality assumption (Zuur *et al.* 2009). We used a variance power function to account for heteroscedasticity in the models; for example, we modelled the variance of the residuals as σ^2 multiplied by the power of the absolute value of number of flowering plants per species (Zuur *et al.* 2009). We fitted the LMMs in the open source software R v 3.0.3 using the package ‘NLME’ (Pinheiro *et al.* 2014).

We then assessed whether the presence of shrub species affected flower visitors and forbs in a similar way, or whether it had differential effects for functional groups of flower visitors or certain plant species. With regard to flower visitors, we tested FBH (i.e. whether the total number of flower visitor species increased at EP) by using rarefaction curves. Rarefaction methods allow the comparison of data sets by standardizing variables by sampling effort (Gotelli & Entsminger 2004). We used ECOSIM ver 7.71 (Gotelli & Entsminger 2004) to compute sample-based rarefaction curves and their 95% confidence interval curves for each habitat type pooling data from all census days and sampling sites. Curves were rescaled by the number of observations (i.e. number of visits) to allow for species richness comparison (Gotelli & Entsminger 2004). Then, we applied LMM to test whether shrub encroachment affected the number of interactions and total number of visits recorded for each functional group of flower visitors. All models included habitat type, functional group and their interaction terms as fixed factors; locality and census day nested within locality as random factors; and number of flowering plants per species as a continuous predictor.

With regard to plant species, we tested whether shrub encroachment affected the number of interactions, total number of visits and number of visits per plant by applying LMMs. These models allowed the detection of shifts in flower visitor preferences and indirect plant–plant interactions mediated by shared flower visitors. We compared whether visitation patterns differed among plant species according to their life form (fixed factor: shrub or forb) in EP, as both life forms only co-occur at EP sites. Once we assessed the effect of life form on shaping flower visitor preference, we estimated the effect of habitat type (fixed factor) by comparing visitation patterns among forb species found in PA and EP. The model also included life form and the interaction term with habitat type as fixed factors. All models fitted for plant species included locality and census day nested in locality as random

factors and flowering plant abundance of each species as a continuous predictor.

Topological network metrics

We built quantitative bipartite networks for full-season data for each habitat type (Jordano 1987). We used visitation frequencies as a surrogate for interaction strength (Vázquez 2005). We defined visitation frequency between flower visitor i and plant species j as the total number of visits of i to j . We calculated eight niche-based topological metrics that capture the degree of specialization, niche breadth and niche overlap, while providing insights into inter-specific competition within trophic levels (Jordano, Bascompte & Olesen 2003; Blüthgen, Menzel & Blüthgen 2006; Blüthgen 2010):

(i) number of plant species (P); (ii) number of flower visitor species (A); (iii) total number of interactions (I); (iv) total number of visits (V); (v) connectance ($C = I/AP$), i.e. the fraction of realized interactions in the network (Jordano 1987); (vi, vii) weighted linkage for plants (lw_p) and flower visitors (lw_a) (also called weighted generality and vulnerability) as the effective mean number of partners per plant and per flower visitor, respectively (Dormann, Gruber & Fruend 2008). Both indices are, therefore, a measure of niche breadth; and (viii) index of network specialization (H'^2), which quantifies the degree of niche divergence of elements within an entire bipartite network (Blüthgen, Menzel & Blüthgen 2006). It ranges from 0 (low specialization, high niche overlap) to 1 (high specialization, low niche overlap).

We also estimated three species-level metrics for each plant (p) and flower visitor (a) species:

(i) Linkage level (L , the number of links or partners that each species had in the network); (ii) index of species specialization (d'), which expresses the level of specialization of each species based on its discrimination from random selection of partners (Blüthgen, Menzel & Blüthgen 2006; Dormann, Gruber & Fruend 2008). It ranges from 0 (no specialization, or species that interact with their partners proportionally to their availability) to 1 (perfect specialist, or species that disproportionately interact with rare partners);

and (iii) species strength (S) as the sum of dependencies of the partners relying on each species (Dormann, Gruber & Fruend 2008). It quantifies the importance of each species from the perspective of their partners. This metric is a quantitative extension of the linkage level.

We finally estimated the modularity (Q) of the networks, which measures the extent to which species interactions are organized into modules. Q was estimated using the *QuaBiMo* algorithm, based on a hierarchical random graph approach adapted for quantitative bipartite networks (Dormann & Strauss 2014). We assessed the significance level of Q against a reference distribution derived from 100 random networks with the same species degree distribution as the empirical network (Dormann & Strauss 2014). Modularity increases with increasing link density within modules and decreasing connectedness between different modules (i.e. as specialization increases) (Albrecht *et al.* 2014; Dormann & Strauss 2014). Thus, we expected a considerable increase in Q values in EP compared to PA due to reduced connectivity among modules.

All these metrics were calculated using the R package 'BIPARTITE' v. 2.0.4 (Dormann, Gruber & Fruend 2008). We used unpaired two-sample t -test to check for differences in species-level metrics between habitats, trophic levels (i.e. plant and flower visitors) and functional groups of flower visitors and one-sample t -tests to test for differences between plant life forms in EP.

Results

EFFECT OF SHRUB ENCROACHMENT ON SPECIES ASSEMBLAGES AND VISITATION PATTERNS

Overall, the number of flowering plants per species, number of interactions and total number of visits recorded per census were higher in EP than in PA, while the number of visits per plant was lower (Fig. 1). Number of flowering plants per species influenced the number of visits per plant

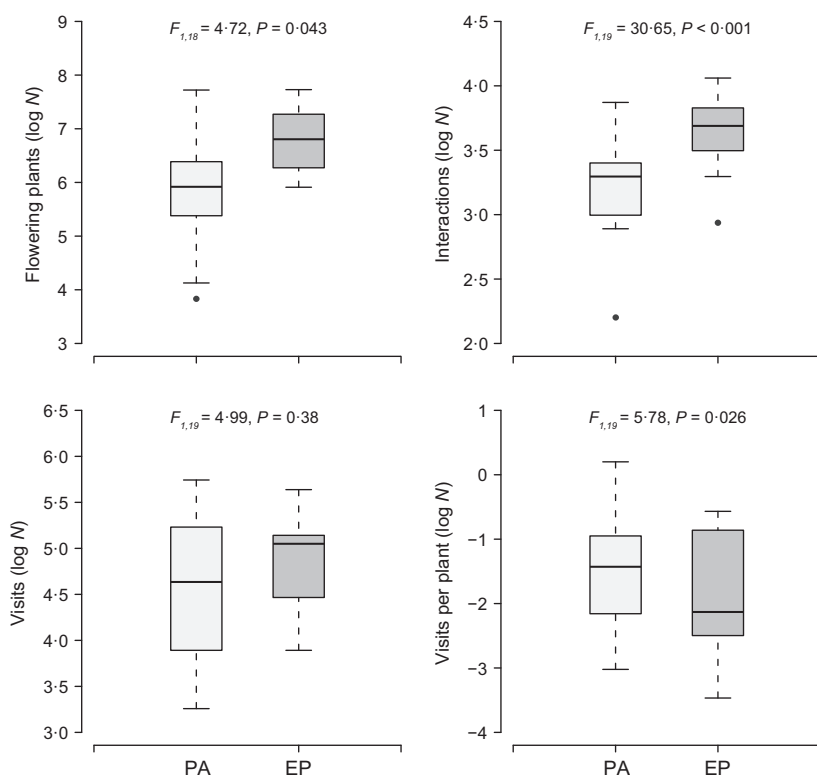


Fig. 1. Number of flowering plants per species, number of interactions, total number of visits and number of visits per plant measured in pastures (PA) and encroached pastures (EP). In boxplots, thick lines represent the median, boxes represent the lower and upper quartile, and whiskers above and below the box indicate the minimum and maximum values excluding outliers. Linear mixed models results for testing the effects of shrub encroachment are displayed above each graph.

($F_{1,19} = 74.57$, $P < 0.001$; $\beta = -0.0012$; $t = -8.64$, $P < 0.001$) and was more constant throughout the flowering season in EP (weekly mean \pm SD: $0.9 \text{ plant m}^{-2} \pm 0.5$) than in PA ($0.6 \text{ plant m}^{-2} \pm 0.7$).

Flower visitor richness was higher in EP than in PA in all functional groups (Fig. 2, Table S1). As a result, sample-based rarefied estimates of flower visitor richness were significantly higher in EP than in PA (Curves rescaled to 2000 visits: $A_{EP} = 108$ species, 95% CI: 102–113; $A_{PA} = 91$ species, 95% CI: 87–94; Fig. S3). The main functional groups of flower visitors did not vary between habitat types (Fig. 2, Table S1): butterflies were the predominant flower visitors followed by flies, small flies and hoverflies (Order: Diptera), large and small solitary bees (Hymenoptera) and beetles (Coleoptera). The greater number of species observed in EP resulted in an increased number of interactions and visits for each functional group (Table 1, Table S2: $\beta = 0.42$, $t = 2.39$, $P = 0.02$ for number of interactions; $\beta = 0.78$, $t = 2.45$, $P = 0.01$ for number of visits). Functional group also had a significant effect on visitation

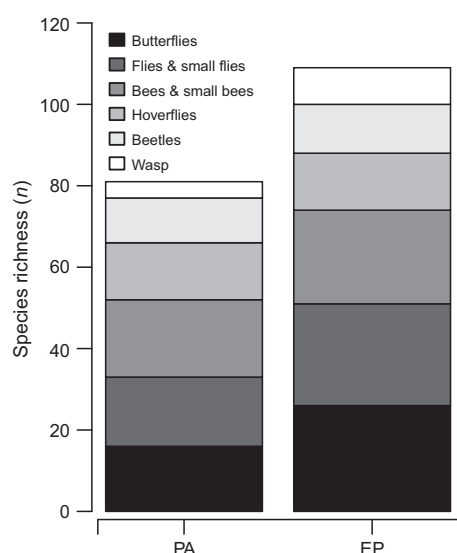


Fig. 2. Species richness of the main functional groups of flower visitors at pastures (PA) and encroached pastures (EP). See Table S1 for details.

patterns (Tables 1 and S2). According to Tukey's *post hoc* tests, this effect was mainly due to the higher number of interactions and visits by butterflies, hoverflies, small solitary bees and beetles compared to the rest of the community (Table S3). Furthermore, the interaction habitat type \times functional group was non-significant (Table 1), suggesting that insects from all eleven functional groups responded to shrub encroachment similarly. Number of flowering plants per species did not affect visitation patterns (Table 1).

Life form (i.e. shrubs and forbs) affected the number of interactions with flower visitors and number of visits per plant in EP (Table 2a). Hence, shrub species had more interactions with flower visitors (Table S4: $\beta = 0.53$, $t = 2.43$, $P = 0.02$) and received more visits per plant (Table S4: $\beta = 0.81$, $t = 2.35$, $P = 0.02$) than forb species. Models fitted for forb species occurring in both habitat types showed that the number of interactions with flower visitors and total number of visits recorded for forb species did not differ between PA and EP (Table 2b). However, plant species had a significant effect on these two variables (Tables 2b and S5), mainly because *Jasione crispa*, *Jurinea humilis* and *Senecio pyrenaicus* had a significantly greater number of interactions and visits compared to most of the forb plant species as indicated by Tukey's *post hoc* tests (Table S6). The interaction habitat type \times plant species was not significant (Table 2b). In contrast, the number of visits received per plant was significantly lower in EP (Tables 2b and S5: $\beta = -1.61$, $t = -3.04$, $P < 0.01$), and habitat type \times plant species interaction had a significant effect on this variable (Table 2b). Although all forb species received fewer visits per plant in EP, shrub encroachment had a greater negative effect on two of them, namely *Biscutella intermedia* ($\beta = -2.30$, $t = -2.39$, $P = 0.02$) and *Solidago virgaurea* ($\beta = -1.63$, $t = -1.98$, $P = 0.04$) (Table S5). Number of flowering plants per species had a significant effect on all models fitted for plant species (Table 2a,b), which may partially explain the visitation patterns to plant species in the two habitat types. Hence, plant species with greater flowering plant abundance had more interactions with flower visitors (Tables S4 and S5) and therefore received more visits (Tables S4 and S5).

Table 1. Effects of habitat type, functional group of flower visitors and number of flowering plants per species on the number of interactions and total number of visits recorded for each functional group of flower visitors. Data were analysed using linear mixed models

	Num. Df	Den. Df	Number of interactions		Number of visits	
			F value	Pr(> F)	F value	Pr(> F)
Intercept	1	419	1109.51	< 0.001	773.74	< 0.001
Habitat type	1	419	20.75	< 0.001	14.16	< 0.001
Flower visitor functional group	10	419	34.96	< 0.001	25.40	< 0.001
Number of flowering plants	1	419	0.07	0.78	0.10	0.76
Habitat type: F. group	10	419	1.42	0.17	1.34	0.21

Num. Df, numerator degrees of freedom of fixed effects; Den. Df, denominator degrees of freedom for fixed effects. Significant effects ($p < 0.05$) are indicated in bold. Parameter estimates fitted for fixed effects and standard deviations fitted for random effects are provided as Supporting information (Table S2).

Table 2. Effects of habitat type, plant species, life form and number of flowering plants per species on number of interactions, total number of visits and number of visits per plant recorded for each plant species. Data were analysed using linear mixed models

			Number of interactions		Number of visits		Visits per plant	
	Num. <i>Df</i>	Den. <i>Df</i>	<i>F</i> value	<i>Pr</i> (> <i>F</i>)	<i>F</i> value	<i>Pr</i> (> <i>F</i>)	<i>F</i> value	<i>Pr</i> (> <i>F</i>)
(a) Species that occurred at encroached pastures								
Intercept	1	115	356.87	< 0.001	423.69	< 0.001	140.69	< 0.001
Life form	1	115	3.91	0.05	1.12	0.29	12.26	< 0.001
Number of flowering plants	1	115	10.88	< 0.01	19.46	< 0.001	53.28	< 0.001
(b) Species that occurred in both habitat types								
Intercept	1	155	580.23	< 0.001	645.22	< 0.001	359.16	< 0.001
Habitat type	1	155	0.019	0.89	0.12	0.73	25.40	< 0.001
Plant species	10	155	4.64	< 0.001	4.70	< 0.001	22.00	< 0.001
Number of flowering plants	1	155	26.98	< 0.001	37.84	< 0.001	47.71	< 0.001
H. Type: P. Species	10	155	0.52	0.87	0.45	0.92	2.36	0.01

Num. Df, numerator degrees of freedom of fixed effects; Den. Df, denominator degrees of freedom for fixed effects. Significant effects ($p < 0.05$) are indicated in bold. Parameter estimates fitted for fixed effects and standard deviations fitted for random effects are provided as Supporting information (Table S3 and S4).

However, number of flowering plants per species was inversely related to the number of visits per plant (Tables S4 and S5).

TOPOLOGICAL NETWORK METRICS

The analysis performed on the full-season data set confirmed that the total number of interactions and visits were higher in EP than in PA (Table 3). Four functional groups of flower visitors (butterflies, hoverflies, small solitary bees and beetles) accounted for the majority of interactions and visits in both habitat types. They added up to 63% of interactions and 74% of visits in PA and up to 60% of interactions and 76% of visits in EP. The forb species *J. crista*, *J. humilis* and *S. pyrenaicus* were involved in 40% of all links and received 59% of all visits in PA, while these species together with the shrub species *C. oromediterraneus* and *A. hispanicus* accounted for 54% of all links and 62% of all visits in EP. Nevertheless, most plant species were highly connected, with 10 species (85%) in PA and 12 species (80%) in EP participating in at least 10 interactions with 37 or more visits (For further details on each plant species, see Table S1). Regarding network-level metrics, similar connectance (C) values were obtained for the two habitat types, while the index of network specialization ($H'2$) was slightly lower in EP than in PA (Table 3). Weighted linkage for plants (lw_p) was four times lower than for flower visitors (lw_a), showing that plants interact with a high number of flower visitor species. Regarding habitat types, lw_p and lw_a in EP increased by 25% and 17%, respectively, compared to PA (Table 3). The plant–flower visitor networks of both habitat types displayed a similar modular structure: $Q = 0.185$ and 0.194 for PA and EP, respectively. In both cases, modularity values were significant (Z -test: all $P < 0.001$).

Means of all species-level metrics namely number of links (L), species specialization (d') and species strength (S) estimated for both plant (p) and flower visitors (a) were

similar between habitat types (t -test: all $P > 0.05$; Table 3). This pattern was constant for all functional groups (t -test: all $P > 0.05$). However, linkage ($L = 31 \pm 9.31$) and strength ($S = 12.68 \pm 7.26$) of shrub species were higher than those of forb species in EP ($L = 21.07 \pm 15.67$, $t = -2.45$, $P < 0.03$; $S = 6.44 \pm 6.45$, $t = -3.74$, $P < 0.002$). Furthermore, mean L , mean S and mean d' were significantly higher in plants than in flower visitors in both PA and EP (t -test: $P < 0.001$).

Discussion

Patterns of flower visitation changed in EP compared to pastures without encroachment and shrub encroachment had distinctive effects on plant and flower visitor species. As predicted by the FBH, the integration of shrub species into the network increased richness and visitation rates of all functional groups of flower visitors, but their niche breadth did not vary, contrary to our expectations. From the perspective of plant species, shrub expansion reduced the number of visits received per plant in forb species. Shrubs had more interactions with flower visitors and received more visits per plant than forb species, which is in agreement with their significantly higher linkage and strength. This suggests increased plant–plant competition for shared pollinators.

EFFECTS OF SHRUB ENCROACHMENT ON FLOWER VISITOR DIVERSITY AND VISITATION PATTERNS

Increased species richness in all functional groups at EP is congruent with our expectations based on FBH which predicts higher pollinator biodiversity in species-rich plant communities (Ebeling *et al.* 2008; Blüthgen & Klein 2011). This relationship also occurs in exotic plant invasions, where invaded communities tend to exhibit greater plant and pollinator richness than non-invaded communities (Stouffer, Cirtwill & Bascompte 2014). The degree and

Table 3. Topological network metrics describing bipartite plant–flower visitor networks obtained for pastures (PA) and encroached pastures (EP)

Metrics	PA	EP
Network-level metrics		
Number of plants (P)	15	17
Number of animals (A)	95	122
Number of interactions (I)	268	367
Number of visits (V)	2423	3113
Connectance (C)	0.19	0.18
Weighted plant linkage (lw_p)	10.60	11.95
Weighted flower visitor linkage (lwa)	3.88	4.25
Network specialization ($H'2$)	0.43	0.40
Species-level metrics (Mean \pm SE)		
Plant linkage (L_p)	18.33 \pm 3.07	22.23 \pm 3.71
Flower visitor linkage (L_a)	2.89 \pm 0.28	3.10 \pm 0.26
Plant species specialization (d'_p)	0.41 \pm 0.03	0.44 \pm 0.03
Flower visitor species specialization (d'_a)	0.23 \pm 0.01	0.24 \pm 0.01
Plant species strength (S_p)	6.33 \pm 1.57	7.18 \pm 1.61
Flower visitor species strength (S_a)	0.16 \pm 0.04	0.14 \pm 0.03

SE, standard error.

Non-significant differences between PA and EP were found in all tested species-level metrics ($P > 0.05$).

duration of snow cover in winter could partly explain the observed trend. This factor restricts the establishment of shrubs species but also affects forb density and distribution (Sanz-Elorza *et al.* 2003; Garcia-Romero *et al.* 2010). Ideally the paired design should have been expanded to include a third treatment which had been invaded by shrubs and subsequently subjected to complete shrub removal (e.g. Lopezaiza-Mikel *et al.* 2007). This way other biotic or abiotic factors would keep constant between encroached and non-encroached experimental plots. In any case, habitat type was significant even after accounting for the variation explained by flower abundance, which suggests that the effect of shrub encroachment on visitation patterns goes beyond flower abundance at the study plots.

We expected the specific niche breadth of flower visitors to become narrower consistent with increased trophic specialization. Yet, network metrics showed that shrub encroachment did not affect flower visitor niche breadth (lw_a , d'_a); in fact, the index of network specialization ($H'2$) decreased and weighted linkage (lwp , lwa) increased suggesting higher generalization (greater niche overlap) with shrub encroachment. Hence, increased flower visitor richness and visitation in EP does not appear to be related to an increase in complementary specialization at the community scale, which would have decreased niche breadth at both trophic levels (Blüthgen & Klein 2011). Rather, it may be related to an increase in the quantity and/or quality of resources over space in EP which would allow flower visitors to obtain a more balanced diet by feeding on different plant species (Blüth-

gen & Klein 2011). Another possible explanation could be temporal partitioning in the flowering period of plant species throughout the growing season. The flowering period of the study species rarely overlapped (Fig. S2), and they show short blooming periods (mean \pm SD: 19 days \pm 8.2 in PA; 20 days \pm 9.5 in EP). Contrarily, flower visitors tended to be active across the growing season exceeding the blooming period of each plant species (J. Morente, C. Lara-Romero and J. M. Iriondo, Unpubl. results). From the perspective of flower visitors, this means that different plant species provided resources at different time-lags, and thus, several plant species with non-overlapping flowering peaks are required to fulfil their overall foraging demands in alpine grasslands. This phenological complementarity (*sensu* Blüthgen & Klein 2011) increases the stability of the flower visitor community, but it may not entail trophic specialization towards particular plant species (Benadi *et al.* 2014), as shown by our results.

PLANT–PLANT INTERACTIONS MEDIATED BY SHARED FLOWER VISITORS

Although generalization in plant pollination systems can improve the stability of plant communities, it can also enhance interspecific competition when resources are limited (Blüthgen & Klein 2011). Shrub encroachment increased plant linkage (i.e. L_p and lw_p) while reducing the number of visits per plant of forb species without affecting their specialization (d'_p). These findings have important ecological consequences because they suggest the existence of competition among plant species mediated by pollinator sharing, which can potentially result in reduced reproductive success of some plant species (Morales & Traveset 2009).

Previous studies have shown that generalists have the greatest potential to compete with other plants because their resources are accessible to a wide range of pollinators for lengthy time intervals (Lopezaiza-Mikel *et al.* 2007; Morales & Traveset 2009; Seifan *et al.* 2014). In EP, results show a low degree of specialization (d'), but high linkage (L) and flower visitation for shrub species that would act as super-generalists. However, interpretations of specialization and generalization can be limited when only visitation is recorded, as it is the effect of pollination on seed set that has the potential to impact plant population dynamics (Fenster *et al.* 2004; Seifan *et al.* 2014). Hence, further research is needed to evaluate the effective outcome of the integration of shrubs into the network (increased stability vs. increased competition). For instance, valuable information could be obtained by evaluating the presence of shrub pollen grains in the pollen transport network (Lopezaiza-Mikel *et al.* 2007) and the joint estimation of the 'quantity' (i.e. visit number) and 'quality' (i.e. visit efficiency) component of the interaction and the subsequent effect on plant fitness (Fenster *et al.* 2004; Seifan *et al.* 2014).

EFFECTS OF SHRUB ENCROACHMENT ON THE TOPOLOGY OF THE PLANT–FLOWER VISITOR NETWORK

In spite of the central role of shrub species, the structure of the networks was very robust to the effects of shrub encroachment (e.g. C , Q and H^2). These results are congruent with previous findings in the literature where strong shifts in network properties were only found in highly invaded habitats, where invader species had already displaced native ones (e.g. Aizen, Morales & Morales 2008). This finding has important implications because the relative invariance of network structure implies that plant–flower visitor interaction networks might be less prone to the detrimental effects of disturbance than previously thought (Vilá *et al.* 2009; Stouffer, Cirtwill & Bascompte 2014), as may be the case of the network studied here. Moreover, a strong dependence of pollinators on a narrow set of plant species would render these species more vulnerable to co-extinction (Tylianakis *et al.* 2010; Svenning *et al.* 2014). In this context, the integration of generalist shrub species would be advantageous, because it might increase network resilience after disruption and the capacity of the network to respond to environmental changes (Elmqvist *et al.* 2003; Blüthgen & Klein 2011). Indeed, recent simulated species extinctions models have shown that integration into species networks with higher generalization levels, but without the displacement of native species, makes networks more robust against random secondary species extinctions (Albrecht *et al.* 2014). Nevertheless, this study focused on mountain sites with a moderate level of shrub encroachment. The integration of shrub species into the network could represent a risk if shrub cover reached a threshold value that led to a significant decline in forb richness (Aizen, Morales & Morales 2008; Seifan *et al.* 2014). In the face of increasing shrub encroachment rates in mountain environments world-wide (Wookey *et al.* 2009), we urgently need further empirical studies to measure the amount of shrub expansion that would disrupt the original properties of plant–flower visitor interaction networks in alpine grasslands.

Conclusions

This study revealed contrasting effects of moderate levels of shrub encroachment on plant and flower visitor in alpine grasslands because shrub encroachment increased species richness and visitation rates, but it also increased indirect plant–plant competition mediated by shared flower visitors, which can result in reduced reproductive success of key native forbs. In spite of the central role of shrub species, the topological structure of the network was robust to the integration of shrub species, although it increased network generalization. FBH explained the direct outcome of shrub encroachment for forb species as flower visitor diversity increased. These findings improve our understanding of the role of shrub encroachment in

high-mountain ecosystems and point out the need for an integrative approach to study the effect of global change drivers on species interactions and associated ecosystem services. Future research should investigate thresholds above which the integration of shrubs in to the community would disrupt the original properties of plant–flower visitor networks in alpine grasslands. From an applied perspective, our results demonstrate that changes in community function can be tracked with metrics of plant and flower visitor networks that capture the structure and stability of networks rather than simply the diversity of pollinators and flower visitors, as is frequently used. Further studies applying concepts and tools of ecological networks should prove useful in developing evidence-based recommendations for habitat restoration and management plans.

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Data accessibility

Plant–flower visitor interaction data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.p869n> (Lara-Romero *et al.* 2016).

References

- Aizen, M.A., Morales, C.L. & Morales, J.M. (2008) Invasive mutualists erode native pollination webs. *PLoS Biology*, **6**, e31.
- Albrecht, M., Riesen, M. & Schmid, B. (2010) Plant–pollinator network assembly along the chronosequence of a glacier foreland. *Oikos*, **119**, 1610–1624.
- Albrecht, M., Padrón, B., Bartomeus, I. & Traveset, A. (2014) Consequences of plant invasions on compartmentalization and species's roles in plant–pollinator networks. *Proceedings of the Royal Society of London B: Biological Sciences*, **281**, 20140773.
- Algar, A.C., Kharouba, H.M., Young, E.R. & Kerr, J.T. (2009) Predicting the future of species diversity: macroecological theory, climate change, and direct tests of alternative forecasting methods. *Ecography*, **32**, 22–33.
- Benadi, G., Hovestadt, T., Poethke, H.-J. & Blüthgen, N. (2014) Specialization and phenological synchrony of plant–pollinator interactions along an altitudinal gradient. *Journal of Animal Ecology*, **83**, 639–650.
- Blüthgen, N. (2010) Why network analysis is often disconnected from community ecology: a critique and an ecologist's guide. *Basic and Applied Ecology*, **11**, 185–195.
- Blüthgen, N. & Klein, A.-M. (2011) Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. *Basic and Applied Ecology*, **12**, 282–291.
- Blüthgen, N., Menzel, F. & Blüthgen, N. (2006) Measuring specialization in species interaction networks. *BMC Ecology*, **6**, 9.
- Brandt, J.S., Haynes, M.A., Kuemmerle, T., Waller, D.M. & Radeloff, V.C. (2013) Regime shift on the roof of the world: Alpine meadows con-

- verting to shrublands in the southern Himalayas. *Biological Conservation*, **158**, 116–127.
- Castroviejo, S., Lainz, M., López González, G., Montserrat, P., Muñoz Garmendia, G., Paiva, J. *et al.* (1990) *Flora ibérica. Plantas vasculares de la Península Ibérica e Islas Baleares*, Vol. II. Real Jardín Botánico, CSIC, Madrid, Spain.
- Dormann, C.F., Gruber, B. & Fruend, J. (2008) Introducing the bipartite Package: analysing Ecological Networks. *R News*, **8**, 8–11.
- Dormann, C.F. & Strauss, R. (2014) Detecting modules in quantitative bipartite networks: the QuaBiMo algorithm. *Methods in Ecology and Evolution*, **5**, 90–98.
- Dupont, Y.L., Hansen, D.M. & Olesen, J.M. (2003) Structure of a plant–flower–visitor network in the high-altitude sub-alpine desert of Tenerife, Canary Islands. *Ecography*, **26**, 301–310.
- Ebeling, A., Klein, A.M. & Tscharrntke, T. (2011) Plant–flower visitor interaction webs: temporal stability and pollinator specialization increases along an experimental plant diversity gradient. *Basic and Applied Ecology*, **12**, 300–309.
- Ebeling, A., Klein, A.-M., Schumacher, J., Weisser, W.W. & Tscharrntke, T. (2008) How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos*, **117**, 1808–1815.
- Eldridge, D.J., Bowker, M.A., Maestre, F.T., Roger, E., Reynolds, J.F. & Whitford, W.G. (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecology Letters*, **14**, 709–722.
- Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B. *et al.* (2003) Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, **1**, 488–494.
- Escudero, A., Gimenez-Benavides, L., Iriondo, J.M. & Rubio, A. (2004) Patch dynamics and islands of fertility in a high mountain Mediterranean community. *Arctic Antarctic and Alpine Research*, **36**, 518–527.
- Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R. & Thomson, J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 375–403.
- Ferreira, P.A., Boscolo, D. & Viana, B.F. (2013) What do we know about the effects of landscape changes on plant–pollinator interaction networks? *Ecological Indicators*, **31**, 35–40.
- Fontaine, C., Collin, C.L. & Dajoz, I. (2008) Generalist foraging of pollinators: diet expansion at high density. *Journal of Ecology*, **96**, 1002–1010.
- Fontaine, C., Dajoz, I., Meriguet, J. & Loreau, M. (2006) Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. *PLoS Biology*, **4**, e1.
- Fründ, J., Linsenmair, K.E. & Blüthgen, N. (2010) Pollinator diversity and specialization in relation to flower diversity. *Oikos*, **119**, 1581–1590.
- García-Romero, A., Muñoz, J., Andres, N. & Palacios, D. (2010) Relationship between climate change and vegetation distribution in the Mediterranean mountains: Manzanares Head Valley, Sierra de Guadarrama (Central Spain). *Climatic Change*, **100**, 645–666.
- Ghazoul, J. (2006) Floral diversity and the facilitation of pollination. *Journal of Ecology*, **94**, 295–304.
- Gibson, R.H., Knott, B., Eberlein, T. & Memmott, J. (2011) Sampling method influences the structure of plant–pollinator networks. *Oikos*, **120**, 822–831.
- Gomez, J.M., Bosch, J., Perfectti, F., Fernández, J.D., Abdelaziz, M. & Camacho, J.P.M. (2008) Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **275**, 2241–2249.
- Gotelli, N.J. & Entsminger, G. (2004) *EcoSim: Null Models Software for Ecology*. Acquired Intelligence & Kesey-Bear, Jericho, VT, USA.
- Inouye, D.W. & Pyke, G.H. (1988) Pollination biology in the Snowy Mountains of Australia: comparisons with Montane Colorado, USA. *Australian Journal of Ecology*, **13**, 191–205.
- Johnson, S.D., Peter, C.I., Nilsson, L.A. & Agren, J. (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology*, **84**, 2919–2927.
- Jordano, P. (1987) Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. *The American Naturalist*, **129**, 657–677.
- Jordano, P., Bascompte, J. & Olesen, J.M. (2003) Invariant properties in coevolutionary networks of plant–animal interactions. *Ecology Letters*, **6**, 69–81.
- Kaiser-Bunbury, C.N., Memmott, J. & Müller, C.B. (2009) Community structure of pollination webs of Mauritian heathland habitats. *Perspectives in Plant Ecology, Evolution and Systematics*, **11**, 241–254.
- Kearns, C.A. & Inouye, D.W. (1994) Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany*, **81**, 1091–1095.
- Lara-Romero, C., García, C., Morente-López, J. & Iriondo, J.M. (2016) Data from: Direct and indirect effects of shrub encroachment on alpine grasslands mediated by plant–flower–visitor interactions. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.p869n>.
- Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R. & Memmott, J. (2007) The impact of an alien plant on a native plant–pollinator network: an experimental approach. *Ecology Letters*, **10**, 539–550.
- MacArthur, R.H. & Pianka, E.R. (1966) On optimal use of a patchy environment. *The American Naturalist*, **100**, 603–609.
- Morales, C.L. & Traveset, A. (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, **12**, 716–728.
- Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J.L.B. *et al.* (2012) Recent plant diversity changes on Europe's mountain summits. *Science*, **336**, 353–355.
- Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. & R Core Team (2014) *nlme: Linear and Nonlinear Mixed Effects Models*. R Package Version 3.1. R Foundation for Statistical Computing, Vienna, Austria.
- Sanz-Elorza, M., Dana, E.D., Gonzalez, A. & Sobrino, E. (2003) Changes in the high-mountain vegetation of the central Iberian Peninsula as a probable sign of global warming. *Annals of Botany*, **92**, 273–280.
- Seifan, M., Hoch, E.-M., Hanoteaux, S. & Tielbörger, K. (2014) The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbour. *Journal of Ecology*, **102**, 953–962.
- Stouffer, D.B., Cirtwill, A.R. & Bascompte, J. (2014) How exotic plants integrate into pollination networks. *Journal of Ecology*, **102**, 1442–1450.
- Svenning, J.-C., Gravel, D., Holt, R.D., Schurr, F.M., Thuiller, W., Münkemüller, T. *et al.* (2014) The influence of interspecific interactions on species range expansion rates. *Ecography*, **37**, 1198–1209.
- Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351–1363.
- Tylianakis, J.M., Laliberté, E., Nielsen, A. & Bascompte, J. (2010) Conservation of species interaction networks. *Biological Conservation*, **143**, 2270–2279.
- Vázquez, D.P. (2005) Degree distribution in plant–animal mutualistic networks: forbidden links or random interactions? *Oikos*, **108**, 421–426.
- Vilá, M., Bartomeus, I., Dietzsch, A.C., Petanidou, T., Steffan-Dewenter, I., Stout, J.C. *et al.* (2009) Invasive plant integration into native plant–pollinator networks across Europe. *Proceedings of the Royal Society of London B: Biological Sciences*, **276**, 3887–3893.
- Wookey, P.A., Aerts, R., Bardgett, R.D., Baptist, F., Brathen, K.A., Cornelissen, J.H.C. *et al.* (2009) Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. *Global Change Biology*, **15**, 1153–1172.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY, USA.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Functional group of flower visitors defined following the criteria of similarity in size, proboscis length, foraging behavior and feeding habits.

Fig. S1. Location of the sampling sites in Sierra de Guadarrama (Spain).

Fig. S2. Flowering phenology of the study species at pastures (PA) and encroached pastures (EP) from 13 June to 28 July 2011.

Fig. S3. Rarefaction curves of species richness and sampling effort.

Fig. S4. Visitation networks from pastures (PA) and encroached pasture (EP).

Table S1. List of species studied in each habitat type.

Table S2. Parameter estimates and standard deviation fitted for fixed and random effects by LMM performed for testing the effects of habitat type, functional group and flowering plants abundance on number of interactions and total number of visits recorded for each functional group.

Table S3. Z ratio values for Tukey's *post-hoc* tests used to detect differences between functional groups of flower visitors in the number of interactions and number of visits recorded.

Table S4. Parameter estimates and standard deviation fitted for fixed and random effects by LMM performed to test the effects of life form and flowering plant abundance on number of interac-

tions, total number of visits and number of visits received per flowering plant.

Table S5. Parameter estimates and standard deviation fitted for fixed and random effects by LMM performed to test the effects of habitat type, plant species and flowering plant abundance number of interactions, total number of visits and number of visits received per flowering plant.

Table S6. Z ratio values for Tukey's *post-hoc* tests used to detect differences between plant species in the number of interactions, total number of visits and number of visits per flowering plant recorded.