BIOTIC CONTROLS OF PLANT COEXISTENCE

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Frequency dependence of pollinator visitation rates suggests that pollination niches can allow plant species coexistence

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Abstract

1. How do many species coexist within a trophic level? Resource niches are the classical answer, but in plants which share a small set of abiotic resources, the possibilities for resource partitioning are limited. One possible explanation is that plant species have different pollination niches, with each species specialized to a subset of the available animal species. If this pollinator partitioning results in negative frequency dependence such that each plant species' reproduction is reduced when it becomes abundant, pollination niches could maintain plant diversity, provided that the strength of negative frequency dependence is sufficient to overcome fitness inequalities between species.

2. We tested this idea by quantifying the effect of species relative abundance on pollinator visitation rate in a 7,000 m^2 plot of South African Fynbos vegetation. In addition, we quantified the effect of intraspecific abundance variation at a smaller spatial scale (9 m^2 plots), documented species' pollination niches, and tested the importance of pollinators for seed set in a subset of the plant species.

3. We found that visitation rate indeed declined sharply across the 33 plant species with increasing abundance, but visitation rate was also somewhat depressed in very rare species such that the resulting relationship between visitation rate and relative abundance was hump-shaped. Pollinator niche partitioning among plant species was evident, but less pronounced than in many other studies. Visitation rate was slightly higher in more generalized species, suggesting that they have access to a larger pollination resource. At the intraspecific level and smaller spatial scale, results were less clear and varied among species. Pollinators enhanced seed set in most species.

4. *Synthesis*. The results imply that, above an abundance threshold, intraspecific competition for pollination could limit the reproduction of common species, thus promoting plant species coexistence. However, the rarest plant species could become extinct due to pollen limitation, that is, an Allee effect. In addition, interactions with pollinators may introduce frequency-independent fitness differences between plant species, thereby increasing the strength of negative frequency dependence required for stable coexistence. These findings shed new light on the role of the pollination niche in plant coexistence.

1 | INTRODUCTION

How the average fitness of plant species varies with their frequency (relative abundance) has important implications for the maintenance of diversity in plant communities. A negative relationship between frequency and fitness (negative frequency dependence) can stabilize species coexistence by preventing any one species from reaching complete dominance (Chesson, 2000; Vellend, 2010), provided that the reduction in fitness with increasing frequency is sufficient to overcome average fitness differences between species (Adler, HilleRisLambers, & Levine, 2007). Positive frequency dependence, on the other hand, tends to lead to species loss (but see Molofsky & Bever, 2002).

Since the reproductive success of most plant species depends at least to some extent on pollination by animals (Ollerton, Winfree, & Tarrant, 2011), variation in pollination success with relative abundance could potentially promote or impede the maintenance of plant diversity. Moreover, frequency-independent differences in pollination success (e.g., due to species-specific traits which affect attractivity to pollinators) could amplify or reduce fitness inequalities between species, thereby affecting the strength of stabilization required for long-term coexistence. The effect of plant species frequency on pollination success has rarely been studied until now, and most previous studies only considered one or a few co-occurring plant species (e.g., Kunin, 1993; Runquist & Stanton, 2013; but see Lanuza, Bartomeus, Godoy, & Gómez, 2018). By contrast, in the current study, we investigate the frequency dependence of pollination in a species-rich plant community. We quantify effects of abundance variation within and across species at the spatial scale of the study site ($c.$ 7,000 m^2) as well as within-species abundance variation at a smaller spatial scale (9 m^2 plots). In addition, we test for a frequency-independent effect of plant species generalization on pollination success. Like many community-level studies of plant-pollinator interactions (e.g., Aizen, Morales, & Morales, 2008; Kaiser-Bunbury, Memmott, & Müller, 2009; Lazaro, Lundgren, & Totland, 2009), our study uses flower visitation rate as a surrogate for pollination success. This approach is reasonable as long as the variation in visitation rate is large relative to variation in per-visit effectiveness (Rader et al., 2016; Sahli & Conner, 2006; Vazquez, Morris, & Jordano, 2005) and observed patterns are interpreted with appropriate caution. To test whether the species' reproductive success is positively affected by animal pollination, we compare seed set of open-pollinated and bagged flowers of a subset of species.

How flower visitation rates vary with plant species abundance should depend on degrees of specialization, adaptive foraging behaviour, and abundance of flower-visiting animals. We expected that these factors should result in a hump-shaped relationship between floral abundance and flower visitation rate at both the site and plot scale (hypothesis 1; see also Rathcke, 1983). This hypothesis is based on the following reasoning: Rare plant species can be expected to receive relatively few visits per flower because specialized animals have long search and travel times between rare flowers (Kunin, 1993) and generalists should prefer abundant flower types over rarer ones to maximize their foraging efficiency (Smithson, 2001). Moreover, at small spatial scales, pollinators typically concentrate their foraging efforts in areas with high density of their preferred flower types (e.g., Rathcke, 1983; Thomson, 1981; Totland, 1994). For these reasons, flower visitation rates should initially increase with increasing floral abundance (positive frequency dependence, i.e., an Allee effect). At high abundance, the number of visits per flower may decrease again due to intraspecific competition for flower visits. This negative frequency dependence requires a certain degree of pollination niche partitioning between plant species, such that each species' visitation rate is limited by the abundance of its pollinator assemblage (Pauw, 2013). Without pollinator partitioning, all plant species would receive equal numbers of visits per flower, regardless of their frequency. Since the behaviour of generalist pollinators should be influenced both by the absolute and relative abundance of flowering plant species, while specialized pollinators should only respond to the absolute abundance of their preferred flower type, we study both the effects of relative and absolute abundance at the plot scale on flower visitation rates (i.e., frequency dependence and density dependence). At the scale of the whole study site, this distinction is not necessary because absolute and relative abundance of each species are equivalent.

In addition to the influence of abundance, we expected to find a positive effect of plant species generalization on flower visitation rate in the comparison across species at the site scale (hypothesis 2). As specialized plant species are visited by few pollinator species, the total number of visitors of a specialized plant should on average be lower than the size of the visitor assemblage of a generalist plant. Assuming that flower visitation rate shows a hump-shaped relationship with abundance, with a peak at intermediate abundance (hypothesis 1), we thus expected an increase of peak visitation rate and a shift of the peak towards higher abundance with increasing plant species generalization.

2 | MATERIALS AND METHODS

2.1 | Data collection

The data for this study (Benadi & Pauw, 2018) were collected in the Cape Floristic Region of South Africa, which is known for its

exceptional plant diversity (Linder, 2003; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). The study site was located in Banhoek Valley, *c.* 9 km east of Stellenbosch (site coordinates: 33.943343S, 18.967784E, elevation 440 m). It was covered by Fynbos, a fire-adapted shrubland native to this region with its Mediterranean climate. As the last fire had swept the valley approximately 9 months prior to the study period, at the time of data collection, the vegetation was at an early successional stage dominated by geophytes and resprouting shrubs. We chose this early successional vegetation for its high number of simultaneously flowering plant species, since later successional stages tend to have lower alpha diversity and a longer flowering season with fewer simultaneously flowering species. For the tests of frequency dependence of flower visitation rates at two spatial scales and the effect of plant species generalization, data on flower abundances, floral traits, flower visitation rates, and plant species generalization were collected by four observers during the peak flowering period, from 14 September to 21 October 2017. To assess the effect of animal pollination on plant reproduction, fruit and seed set of selected bagged and open-pollinated plants were recorded by a fifth person in the following weeks, as soon as the fruits had matured.

For the study, 120 square plots of 3 x 3 m were set up on a south-facing slope on the northern side of the valley. The plots were arranged in groups of four, each forming a larger square of 6 x 6 m. Three rows of 10 of these larger squares were set up in north–south direction to cover a vegetation gradient presumably driven by changing humidity and nutrient richness along the slope. Squares were spaced 12 m apart both within each row and between neighbouring rows. One of the squares had to be shifted due to the presence of a large boulder at its designated position. This square was placed at the bottom of its row, 12 m further down from the nearest square. Data on plant species generalization, floral traits, and fruit and seed set were collected both in the plots and the surrounding area, but no further than 10 m from the nearest plot. Flower abundances and visitation rates were exclusively recorded inside plots.

To determine floral abundances of all plant species at the plot and site scale, we performed a full census on 14 September, counting flowers of all species in each plot. The census was repeated twice (on 27 September and 12 October) to account for turnover in abundances. On 18 October, we additionally counted flowers of a newly blooming and already fairly abundant species (*Asphalathus* sp., Fabaceae) and added these counts to the ones of the previous census. To facilitate both rapid and reasonably accurate counting, we chose a unit of counting for each plant species depending on a rough estimate of its overall abundance and the number of flowers per inflorescence and individual. For highly abundant plant species with multiple flowers per individual, we used the individual as a counting unit, while inflorescences or branches were used for moderately abundant species and single flowers for all other species. Composite flowers of Asteraceae were treated as single flowers. We calculated the total number of flowers of each species per plot by multiplying the number of counting units by the average number of flowers per unit based on counts from five individuals. Measurements of flower

size (largest diameter in two perpendicular directions) and height from the ground were taken from a haphazardly chosen flower of each of the same five individuals and averaged across individuals. Flower area was calculated assuming a circular or oval shape, depending on whether a species had radially symmetric or zygomorphic flowers, respectively.

Flower visitation rates were recorded on nine warm and sunny days with no more than a light breeze. Observations were made during the hours of direct sunlight on the study site, between 10:00 and 17:00 hr. We conducted plot-based flower visitor observations in units of 30 min. Plots were selected for observations based on the most recent flower census with the aim of covering all species and their intraspecific variation in flower density at the plot scale. During a 30-min observation period, the observer stayed quietly at the edge of the plot and recorded numbers of visits to each of the flowering plant species within their view. As it was often not possible to see whether a visitor made contact with the reproductive parts of the flower, a flower visit was defined as any behaviour which could potentially involve contact with the stigma or anthers. Insects from taxonomic groups known not to be legitimate pollinators (e.g., spiders, ants) were excluded from visitation counts.

If possible, all flowers in a plot were observed simultaneously. When this was not possible, for example, due to dense vegetation or rocks obstructing the view, we observed a subset of the open flowers in the plot. Total and observed flower numbers of all species in the plot were counted before each observation period. On each observation day, we recorded air temperature at approximately 50 cm above the ground in the shade of a large rock using a mobile weather station. Three temperature readings were taken per day (at the beginning, after the first half and at the end of the observation day) and daily averages calculated for the analysis.

To determine the degree of generalization of plant–flower visitor interactions, we caught insects from flowers between 10:00 and 17:00 hr on 8 days during the sampling period. Visitation counts and insect collection were performed on separate days to minimize effects of insect removal on flower visitation rates. Flower visitors were caught with nets and killed with ethyl acetate (Hymenoptera, Lepidoptera) or 70% ethanol (Diptera, Coleoptera). We selected flowering plant species for insect collection with the aim of achieving equal information per plant species. Thus, plant species with low flower abundance and low visitation rates were observed for the longest total amount of time. Observers moved up and down the slope, staying close to a patch of flowers for 5–10 min or until they had caught five individuals, then moved on to the next patch. All insects were sorted into morphospecies under a dissecting microscope in the laboratory.

For a subset of 12 plant species, we assessed to what extent their reproduction benefited from animal pollination by comparing fruit and seed set of open-pollinated and bagged flowers. Fourteen individuals of each species were selected before the onset of flowering. If possible, we selected pairs of individuals no more than a few metres apart and assigned one individual to each treatment.

On seven individuals, flower visitors were excluded by covering one bud-bearing branch with a Crispac bag secured with a cable tie. We marked one branch with buds on the remaining seven individuals and counted the number of buds on all selected branches. Branches of the open-pollinated treatment were bagged after all flowers had decayed to prevent loss of fruits before they could be counted. Fruits of all individuals were harvested when they were ripe. Seeds were extracted and counted in the laboratory.

2.2 | Data analysis

We fitted a generalized linear mixed model (GLMM) in R (R Core Team, 2017) to the flower visitation data to test the hypothesis of a hump-shaped relationship between flower abundance at the site scale and visitation rate of plant species (hypothesis 1), with the height and position of the maximum depending on the degree of generalization (hypothesis 2). As response variable, we used the number of flower visits observed on all flowers of a species in a 30-min period, with log(number of observed flowers) as offset. We included flower abundance and generalization as single and quadratic terms and their interaction in the model. To assign a site-scale flower abundance to each observation of a plant species' visitation rate, we used the sum of flower numbers of this species counted in all 120 plots in the most recent flower census. Since flower abundances of plant species at the plot and site scale were highly correlated, we only included site-scale abundance in the statistical model of data from all species. The degree of generalization of interactions with pollinators was quantified as the effective number of interaction partners e^H of each plant species, where H is the Shannon diversity of insect morphospecies caught on flowers of this plant species (Bersier, Banasek-Richter, & Cattin, 2002; Dormann, 2011). In addition to this measure of species-level generalization, we calculated H'_2 (Blüthgen, Menzel, & Blüthgen, 2006) and $\Delta H'_2$ (Schleuning et al., 2012) to assess the overall degree of specialization of the plant–flower visitor interaction network. Both species-level and network-level specialization indices were computed using R package "bipartite" (Dormann, Gruber, & Fründ, 2008). Beside flower abundance and plant species generalization, we included flower area and flower height of each plant species as predictors in the site-scale regression model. We expected that both factors should have a positive effect on flower visitation rates, as they presumably increase the flowers' conspicuousness to a pollinator. Since the overall flower visitor activity may vary depending on weather conditions and over the course of a day (e.g., McCall & Primack, 1992; Totland, 1994; Vicens & Bosch, 2000), we also included average daily temperature and time of day in the model. Observer and census period were included as categorical predictors. To account for the possible nonindependence of observations of the same species and of different species which were observed simultaneously, we included plant species and observation period as random factors in the model.

In addition to the model of site-scale abundance effects, we analysed the effects of intraspecific variation in plot-scale abundance and frequency on flower visitation rate using data from seven species which showed sufficient variation in flower numbers at the plot level. As plot-scale flower abundance, we used the flower count performed immediately before the observation period. Flower frequency (relative abundance) was calculated by dividing the focal species' flower abundance by the total abundance of all plant species flowering in the plot. Because of the limited sample size and strong correlation between flower frequency and absolute abundance of the seven selected species, we ran separate models with flower abundance and frequency as predictor. Beside plot-scale flower abundance or frequency (as linear and quadratic term), plant species and their interaction, we included observer, temperature and time of day as predictors in these models. Observation period was included as a random effect.

Prior to the analyses, we checked for collinearity of predictors and log-transformed values of predictors with a strongly skewed distribution. To facilitate model convergence, values of all continuous-scale predictors were z-transformed (scaled to a mean of 0 and standard deviation of 1) before the analyses. In the site-scale analysis, the dataset consisted of 786 observations of 33 plant species made during 250 30-min observation periods. The plot-scale models used a subset of 459 observations of seven plant species from 215 observation periods. For the dioecious species *Montinia caryophyllacea* (Montiniaceae), we only used observations of visits to female individuals, but included counts of male flowers in the total abundance for the calculation of relative abundance at the plot scale. We initially ran GLMM with Poisson errors and log link, but since diagnostic tests indicated significant overdispersion, we subsequently used negative binomial GLMMs (function "glmer.nb" in R package "lme4"; Bates, Mächler, Bolker, & Walker, 2015) to account for the greater mean/variance ratio in the data. We checked for patterns in the residuals plotted against fitted values and against all predictors using simulation-based standardized residuals provided by R package "DHARMa" (Hartig, 2017). Since the purpose of the analyses was to explain observed patterns in the data rather than prediction to new data, we did not perform model selection to avoid biasing parameter estimates and their standard errors (Harrell, 2015).

To assess whether plant species' reproduction benefited from pollination by animals, we compared numbers of seeds per marked bud of the bagged and open-pollinated treatments using nonparametric Mann–Whitney U tests.

3 | RESULTS

In accordance with hypothesis 1, we found a significant negative quadratic effect of site-scale flower abundance on flower visitation rate (Table 1, Figure 1). The predicted number of visits per flower initially increased slightly with increasing abundance. For high abundances, the model predicted a decline in visitation rate by three orders of magnitude. As expected from hypothesis 2, plant species generalization (effective number of partners, e^H) had a positive effect on flower visitation rate, but the effect of generalization was

TABLE 1 Results of a generalized linear mixed model with negative binomial error distribution testing the effects of site-scale flower abundance and generalization (e^H) of plant species on visitation rate (visits per flower in 30 min, N_{obs} = 786). The table presents the estimated value of each fixed effect at the link scale together with its standard error and the p-value of a Wald z test against the null hypothesis of the effect being zero. Significant results at the 5% level are in bold. For the categorical predictors, flower census 1 and observer AL were used as a reference level. The estimated among-group variance of random effects was 0.293 for observation period (250 levels) and 0.584 for plant species (33 levels)

Term	Estimate	SE	p-value
Intercept	−2.2311	0.3519	**<0.001**
log(Flower abundance)	−1.2099	0.2426	**<0.001**
log(Flower abundance)^2	−0.5297	0.1646	**0.0013**
e^H	0.6229	0.2813	**0.0268**
$(e^H)^2$	−0.1450	0.2400	0.5456
log(Flower area)	0.6208	0.1936	**0.0013**
log(Flower height)	0.1292	0.2042	0.5270
Flower census 2	−0.4032	0.2017	**0.0456**
Flower census 3	−0.3241	0.3050	0.2880
Flower census 4	−0.5437	0.4223	0.1979
Temperature	0.0544	0.0824	0.5089
Observer GB	−0.5557	0.2117	**0.0087**
Observer KP	−0.0138	0.1854	0.9405
Observer MJ	−0.3301	0.3015	0.2736
Time of day	0.0104	0.0804	0.8972
log(Flower abundance):e^H	0.1231	0.2015	0.5414

FIGURE 1 Effects of plant species frequency in the community (site-scale flower abundance) and generalization (effective number of partners e^H) of plant species on flower visitation rate. Lines show the effect of log(flower abundance) predicted by a negative binomial GLMM (Table 1) for two levels of generalization (specialized: e^H = 1, generalized: e^H = 13). These levels of generalization were chosen to show the extremes of the range of plant species generalization in the data (Table S2). Points are partial residuals, jittered slightly for clarity. Residuals were assigned to the prediction with the closest e^H value. All other continuous-scale predictors were set to their median values for the prediction, categorical predictors to the most common category

relatively small compared to that of flower abundance. The model predicted an increase in peak visitation rate from 0.07 visits per flower in 30 min for specialized plant species (e^H = 1) to 0.24 visits for generalists (e^H = 13). While the position of the peak shifted slightly towards higher abundances with increasing generalization (from 19.4 to 46.07 at the untransformed abundance scale) in accordance with our hypothesis, the shift was not statistically significant. We found significant differences in visitation rates between flower census periods and observers (Table 1). Flower area had the expected positive effect, whereas the effects of flower height, temperature, and time of day were not significantly different from zero.

Since one of the plant species (*Muraltia* sp., Polygalaceae) far exceeded all other species in abundance, we repeated the site-scale analysis without the data of this species to assess its influence on the patterns observed in the full dataset. The reduced dataset showed significant positive effects of plant species generalization (e^H) and flower area, and a significant negative effect of flower abundance (Table S1), similar to the results of the full dataset. However, the estimated effect of floral abundance was weaker without *Muraltia* sp., and the quadratic term was no longer significant.

While both interspecific abundance variation and intraspecific variation between census periods contributed to the abundance
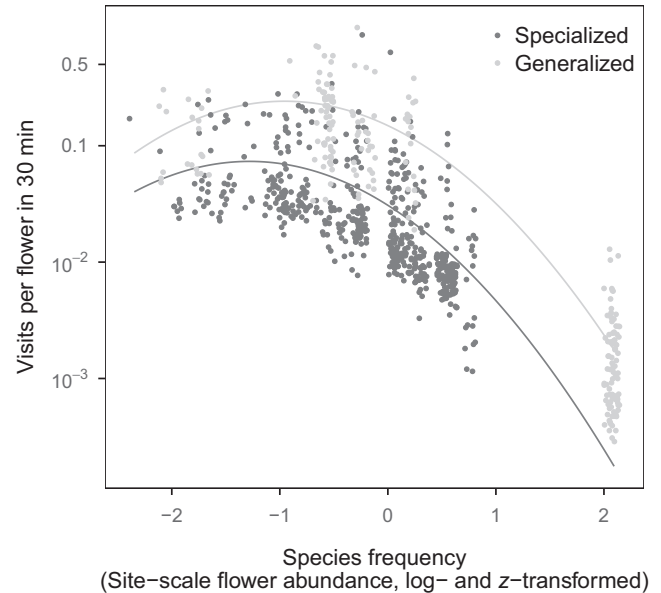
effect of the site-scale model, interspecific differences accounted for most of the variation. The variation in mean flower abundance between species was several orders of magnitude larger than variation within species (variance 1.262×10^{11} and 1.692×10^{7}, respectively; $F_{33,753}$ = 7,459.6, p < 0.001). Overall, flower abundances at the site scale varied from 1 to 232,216 flowers (mean 27,131, median 784, SD 71,839; see also Table S2 for a list of summary statistics of each plant species). Plant species generalization (e^H) varied from 1 to 13.78 effective interaction partners (mean 6.1, median 5.15, SD 4.28). At the community level, interactions were fairly generalized (H'_2 = 0.410, $\Delta H'_2$ = 0.266, Figure S1) compared to other published plant–flower visitor networks (Pauw & Stanway, 2015; Schleuning et al., 2012).

At the plot scale, the effect of flower abundance differed between plant species (Figure 2, Table S3). The model predicted a significant increase of visitation rate with floral abundance for *Geissorhiza aspera*, and a hump-shaped relationship for *Pelargonium myrrhifolium*. *Montinia caryophyllacea* and *Lachenalia orchioides* showed similar increasing and unimodal relationships, respectively, but for these species, the effect of floral abundance was not significantly different from zero. Visitation rates to *Diosma hirsuta*, *Muraltia* sp. and *Sebaea exacoides* showed a slight, nonsignificant decline with increasing abundance. Plot-scale flower frequency did not have a
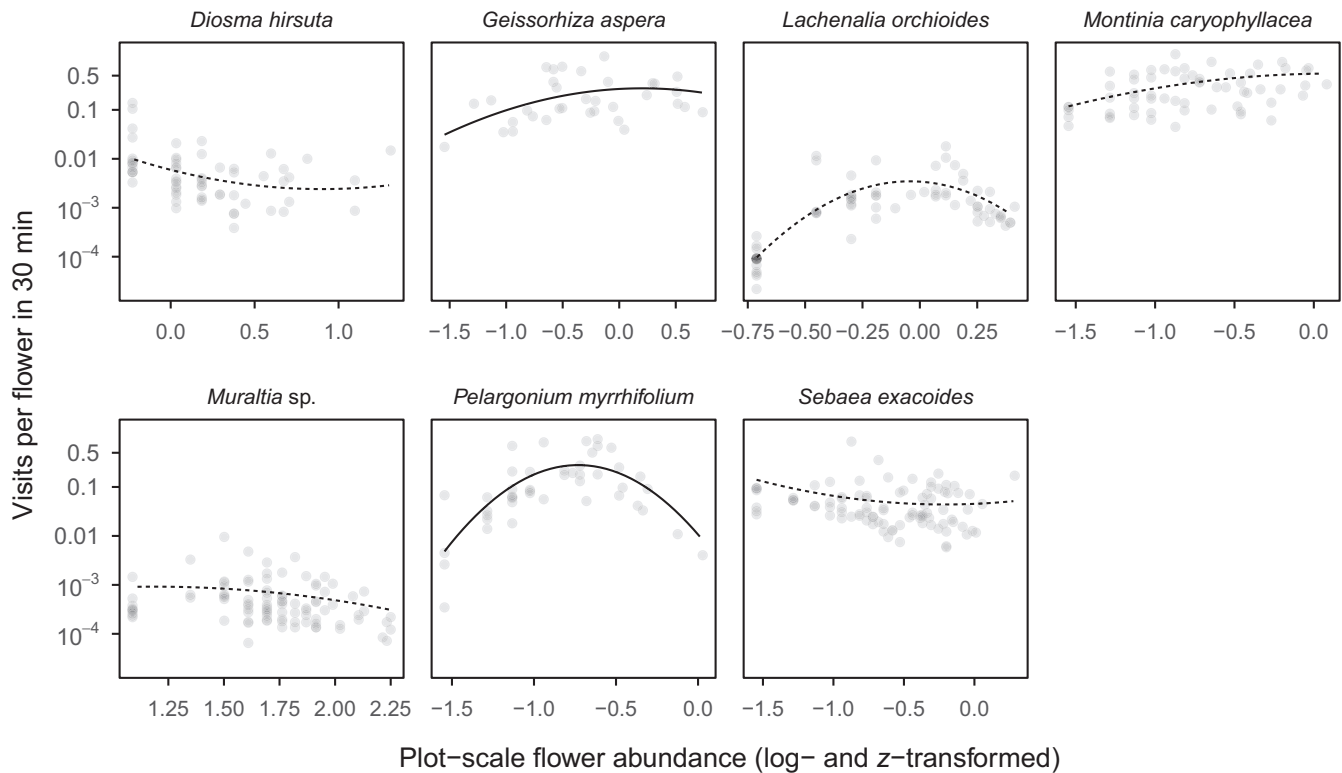
FIGURE 2   Effect of flower abundance at the plot scale on flower visitation rates of selected plant species. Lines show the effect of log(flower abundance) predicted by a negative binomial GLMM (Table S3); points are partial residuals. Solid lines indicate significant effects of flower abundance at the 5% level, dashed lines nonsignificant relationships. All other continuous-scale predictors were set to their median values for the prediction, categorical predictors to the most common category

significant effect on flower visitation rate for any of the seven plant species (Table S4).

For 11 of 12 plant species tested, median seed set per bud was higher in open-pollinated individuals than in bagged ones (Figure 3). The remaining species, *Muraltia* sp., had a median of zero seeds per bud in both treatments. The difference in seed set between treatments was statistically significant ($p < 0.05$) for five species and marginally significant ($p < 0.1$) for a further three species.

4 | DISCUSSION

In this study, we used data from a species-rich community of South African Fynbos vegetation to investigate how flower visitation rate varies with relative and absolute abundance of flowering plant species at two spatial scales. At the scale of the entire site, we found a hump-shaped relationship between visitation rate and floral abundance, in accordance with hypothesis 1. Since interspecific differences accounted for most of the abundance variation, we can conclude that plant species with intermediate floral abundance received the highest number of visits per flower, while flowers of rarer and more common species were less frequently visited. If flower visitation rate is positively correlated to overall plant fitness, this finding has important consequences for the maintenance of diversity in plant communities. The initial positive frequency dependence of

flower visitation implies a rarity disadvantage (an Allee effect), which could lead to loss of species from the community and present a barrier to the establishment of immigrating species (Taylor & Hastings, 2005). However, the positive frequency dependence of visitation rate at low abundance was relatively weak compared to the strong negative frequency dependence at higher abundances, and the initial increase of visitation rate was no longer statistically significant when the most abundant species was removed from the dataset. The pattern of negative frequency dependence of flower visitation found in this study could promote plant diversity by preventing competitive exclusion, provided that the strength of negative frequency dependence is sufficient to overcome frequency-independent fitness differences between species (Adler et al., 2007). The fact that we found a negative abundance effect across species suggests that in this particular plant community frequency dependence of flower visitation rate is strong relative to frequency-independent differences in visitation rate between species. Nevertheless, since many factors other than flower visitation contribute to plant fitness (see below), it remains uncertain whether the influence of flower visitation is sufficiently strong to allow long-term species coexistence.

Our finding of strong negative frequency dependence of visits per flower is particularly interesting given that the overall degree of specialization of the plant–flower visitor network was comparatively low. As mentioned in the introduction, negative frequency dependence of flower visitation requires partitioning of the pollinator
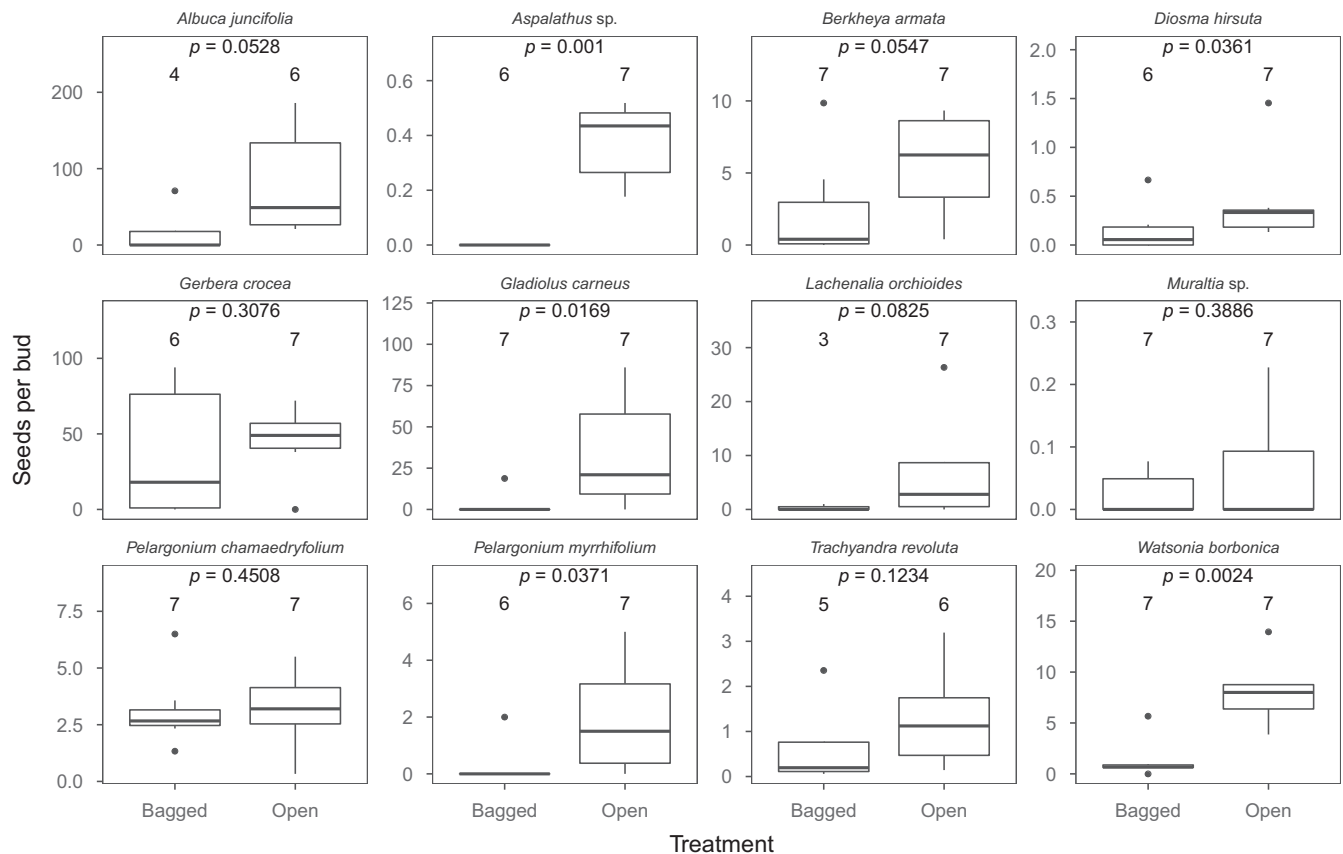
FIGURE 3 Seed set of bagged and open-pollinated flowers of 12 plant species. Box plots show median and quartile seed numbers per bud for each species and treatment, whiskers extend to $1.5 \cdot$ interquartile range. Numbers above the box plots are sample sizes. The p-value at the top of each panel is the result of a Mann–Whitney U test for a difference between treatments

community between plant species, analogous to other types of niche partitioning (Pauw, 2013). If all plant species overlapped entirely in their pollinator fauna, each plant species would receive the same number of visits per flower, regardless of its abundance. Conversely, if co-occurring plant species have different pollinator assemblages, their visitation rates per flower may decrease at high abundance due to increased intraspecific competition for visits. Such intensification of intraspecific competition at high abundance requires that pollinator density is limited by factors other than floral resources (Benadi, 2015; Pauw, 2013). The fact that negative frequency dependence of flower visitation occurred in this study system, even though the degree of pollinator niche partitioning between plant species was relatively low, suggests that the effect could be even stronger in other systems. Positive density or frequency dependence of flower visitation rates and seed set has been previously described in a number of studies (e.g., Dauber et al., 2010; Hackney & McGraw, 2001; Kunin, 1993, 1997; Le Cadre et al., 2008; Roll, Mitchell, Cabin, & Marshall, 1997; Runquist & Stanton, 2013; Thomson, 1981), but few previous studies reported negative density dependence of pollen removal rates (Brys, Jacquemyn, & Hermy, 2008; Heystek & Pauw, 2014), flower visitation rate (Hegland, 2014) or seed set (Brys et al., 2008; Gunton & Kunin, 2009; Lanuza et al., 2018). Moreover, nearly all previous studies only considered intraspecific abundance variation

of a small number of species and therefore did not directly address the question how negative frequency dependence can promote diversity at the community level (but see Lanuza et al., 2018). Further studies of interspecific variation are needed to understand how frequently negative frequency dependence among plant species occurs in natural plant communities.

Beside the effect of flower abundance, we found a positive effect of plant species generalization on flower visitation rate, in accordance with our second hypothesis. If visitation rate is positively related to fitness, this implies a competitive advantage for generalized plant species. In that case, coexistence of generalist and specialist plants requires either strong negative frequency dependence (see above) or equalizing mechanisms which reduce fitness inequalities between species (Adler et al., 2007; Chesson, 2000). For instance, a trade-off between the number of animal species visiting a plant and their average effectiveness as pollinators (reviewed by Armbruster, 2014) could result in similar pollination success of plant species with different degrees of generalization. Since the difference in visitation rates between the most specialized and generalized species in our system was relatively small, a weak trade-off should be sufficient to achieve equal fitness.

Regarding the measurement of plant species generalization, a limitation of our study is that the effective number of visitor species

(e^H, where H is the Shannon diversity of visitors) is necessarily correlated to the number of visitor individuals observed per species. Since observed numbers of interactions varied between plant species despite our effort to achieve equal information per species (Figure S1), it is possible that our study underestimates the degree of generalization, especially for plant species with few observed interactions.

Compared to the effects of inter- and intraspecific abundance variation at the site scale, our analyses of intraspecific density and frequency effects at a smaller spatial scale showed weaker and more inconsistent patterns. By contrast, most previous studies found a clear positive effect of absolute abundance on visitation rate and seed set at comparable spatial scales (e.g., Kunin, 1993; Roll et al., 1997; but see Gunton & Kunin, 2009). Both positive and negative local abundance effects could promote the maintenance of diversity at larger spatial scales, especially if seed dispersal and seedling competition also occur at a local scale (Molofsky & Bever, 2002). A possible explanation for the lack of an effect of local flower frequency on flower visitation could be that the effect depends on the species composition of the floral neighbourhood. For example, coflowering species with similar floral traits and flower visitor assemblages could have a positive (facilitation) or negative (competition) impact on the focal species' visitation rate, while species with nonoverlapping pollinator niches may have little effect (Carvalheiro et al., 2014; Ghazoul, 2006; Hegland, 2014; Moeller, 2004). Alternatively, the spatial scale of 9 m^2 plots used in this study may not be the one at which flower-visiting animals respond to variation in the relative abundance of flower types (see e.g., Thomson, 1981, for a test of the relevant spatial scale).

While the comparison of seed set between bagged and open-pollinated flowers suggests that most species' reproductive success was increased by animal visitation, for several reasons it seems unlikely that flower visitation rate is a direct predictor of plant species' average fitness, even for obligately outcrossing species. First, pollination success does not only depend on the quantity of visits received but also on the amount and quality of conspecific pollen transferred per visit (e.g., Ne'eman, Jürgens, Newstrom-Lloyd, Potts, & Dafni, 2010). Several previous studies found that at the community level differences in visitation rate were considerably more important than variation in pollination effectiveness per visit (Rader et al., 2016; Sahli & Conner, 2006; Vazquez et al., 2005). Nevertheless, variation in pollination effectiveness could affect the frequency dependence of species' reproductive success, particularly if the quality of pollination is itself frequency-dependent. Specifically, rare plant species with generalized pollinators may receive reduced amounts of conspecific pollen because their pollinators typically visit many heterospecific flowers between visits to conspecific flowers (Campbell, 1986; Kunin, 1993; Levin & Anderson, 1970; Runquist & Stanton, 2013). Such an additional rare-species disadvantage should increase the strength of positive frequency dependence of pollination and impede the maintenance of diversity, at least at the scale of the local community.

Even if all plant species received the same quantity and quality of pollinator visits per flower, their reproductive output would vary widely due to interspecific differences in numbers of flowers per individual and ovule numbers per flower. Such interspecific differences are thought to reflect different life-history strategies (e.g., Schoen & Dubuc, 1990). Under given environmental conditions, a particular strategy could provide a fitness advantage to certain species, or several strategies could result in equal fitness due to trade-offs such as the well-studied negative relationship between seed number and seed size (Jakobsson & Eriksson, 2000). In addition, many processes other than pollination contribute to a plant's lifetime reproductive success and population growth rate, including competition for abiotic resources, resistance to stress and interactions with antagonists such as pathogens and herbivores (e.g., Crain, Silliman, Bertness, & Bertness, 2004; Goldberg & Barton, 1992; Olff & Ritchie, 1998; Parker et al., 2015). Each of these factors can potentially affect plant species coexistence through its influence on frequency-dependent and frequency-independent fitness differences between species.

Thus, further studies are needed to quantify the contribution of animal pollination to overall fitness and fully understand the role of plant–pollinator interactions for the maintenance of plant diversity.

5 | CONCLUSIONS

Whereas the influence of certain types of biotic interactions (e.g., resource competition: Goldberg & Barton, 1992; herbivory: Olff & Ritchie, 1998) on plant species coexistence has been intensively studied, the role of plant–pollinator interactions has received comparatively little attention until now. In this study, we found a hump-shaped relationship between pollinator visitation rates and plant species abundance across a species-rich South African plant community and a slight increase of peak visitation rate with plant species generalization. If flower visitation rate is positively related to plant fitness, these findings imply that the rarest plant species could become extinct from the community due to an Allee effect, whereas, above an abundance threshold, intraspecific competition for pollination could limit the reproductive output of common species, thus promoting the maintenance of plant diversity by preventing competitive exclusion. Our findings shed new light on the possible role of the pollination niche in plant community dynamics and call for further studies of the contribution of pollination to plant lifetime reproductive success.

AUTHORS' CONTRIBUTIONS

G.B. and A.P. conceived the study; G.B. collected and analysed the data; G.B. and A.P. wrote the manuscript.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.c8m244m (Benadi & Pauw 2018).

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