

# Experimental simulation of pollinator decline causes community-wide reductions in seedling diversity and abundance

REBEKKA LUNDGREN,<sup>1,3</sup> ØRJAN TOTLAND,<sup>1</sup> AND AMPARO LÁZARO<sup>2</sup>

<sup>1</sup>*Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, P. O. Box, 5003, Ås, Norway*

<sup>2</sup>*Mediterranean Institute for Advanced Studies, C/ Miquel Marqués 21, 07190, Esporles, Balearic Islands, Spain*

**Abstract.** Pollinator decline can disrupt the mutualistic interactions between plants and pollinators and potentially affect the maintenance of plant populations. However, there is still little knowledge on how changes in pollinator abundance can affect seedling recruitment, which is essential for population persistence. We experimentally simulated a community-wide reduction in pollinator availability during four years to examine its effects on seedling recruitment in 10 perennial herbs in a Norwegian hay meadow. Our experimental reduction in pollinator availability significantly reduced community-wide seedling diversity. Overall seedling abundance was also consistently lower under reduced pollinator availability, although this effect was only significant when the most abundant plant species in the community was excluded from the analysis. Despite an overall negative effect on seedling abundance, the experimental reduction in pollinator availability had contrasting effects on individual plant species. This tended to cause a larger change in seedling species composition in the experimental than in the control plots after the four study years. Our study demonstrates for the first time a direct causal link between reduced pollinator availability and reduced plant diversity and abundance.

**Key words:** *abundance; diversity; mutualistic interactions; pollinator loss; recruitment; richness; seedlings; species composition.*

## INTRODUCTION

During the last decade, several studies have documented widespread pollinator declines and warned about their potential consequences (e.g., Biesmeijer et al. 2006, Potts et al. 2010, Burkle et al. 2013, Carvalheiro et al. 2013, González-Varo et al. 2013). Pollinator losses cause great concerns because more than 85% of flowering plant species depend on animals for successful reproduction (Ollerton et al. 2011), and therefore, pollination is regarded as an essential process for maintaining biodiversity and ecosystem functioning (Klein et al. 2007, Kremen et al. 2007). A pollinator decline may disrupt the mutualistic interactions between plants and pollinators, potentially affecting the persistence of plant populations and triggering cascading effects of biodiversity loss (Toby Kiers et al. 2010, Aslan et al. 2013). Parallel declines in pollinators and outcrossing plant species (Biesmeijer et al. 2006, Anderson et al. 2011, Pauw and Hawkins 2011) support this idea and suggest a causal connection between the declines of these two mutualistic groups. Further evidence is given by studies showing that pollinator diversity (Gómez et al. 2007) and composition (Herrera 2000) can affect the reproductive success and seedling recruitment of individual plant species.

Experimental studies at the community level are necessary to establish causal relationships between pollinator decline, plant community persistence, and biodiversity maintenance. Studies at this level are needed because individual species do not act in isolation, but rather interact through processes like competition and facilitation (Callaway and Walker 1997, Martorell and Freckleton 2014), which influence pollinator visitation (Mustajärvi et al. 2001, Moeller 2004), seedling recruitment (Menges 1991, Tilman 1997), and the functional contribution of species in the community (Brosi and Briggs 2013). However, studies in which pollinator abundances are experimentally manipulated are scarce, particularly at the community level. Recent experimental studies at the species (Albrecht et al. 2012, Brittain et al. 2013) and community (Fontaine et al. 2006, Brosi and Briggs 2013, Fründ et al. 2013) levels indicate that pollinator diversity increases the reproductive output of plants. In addition, a few studies have shown that experimental changes in pollinator availability can affect the recruitment of individual plant species (Geib and Galen 2012, Lundgren et al. 2015). Hitherto, no study has established a direct causal link between a decrease in overall pollinator availability and a community-wide change in plant species diversity and the abundance of individuals.

Despite potential overall trends at the community level, plant responses to a pollinator decline may be species-specific. Plant species more dependent on pollinators for seed production may experience stronger pollen limitation and lower reproductive success if pollinator

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<sup>3</sup>E-mail: rebekka.lundgren@nmbu.no

visitation rates and pollination efficiency decline (Ashman et al. 2004, Lundgren et al. 2013). However, this will only affect plant population dynamics if recruitment is also limited by seed production (Crawley 1990, Eriksson and Ehrlén 1992, Bond 1994). If recruitment is more limited by abiotic or extrinsic biotic conditions than by seed production (Clark et al. 1998, Turnbull et al. 2000), plant population density and persistence should not be reduced as a result of pollinator decline (Lundgren et al. 2015). These different responses of plant species might not only affect overall diversity, but also cause changes in the species composition of plant communities over time.

We experimentally simulated a community-wide decline in pollinator abundance to examine its effects on seedling recruitment in 10 perennial herb species during 4 yr. While other studies on the effect of pollination intensity on plant population densities have attempted to obtain pollination saturation through supplemental pollination (e.g., Ehrlén and Eriksson 1995, Ackerman et al. 1996, Hegland and Totland 2007), we used semi-closed cages to experimentally reduce pollinator availability. This approach enables us to study the implications of reduced pollinator availability on plant demography and thereby provide a more realistic assessment of the potential consequences of pollinator decline for plant community properties. Based on our results from previous studies with this experimental design, we expected that species with a high degree of pollinator dependence for seed production (Lundgren et al. 2013), strong seed limitation on population growth (Lundgren et al. 2015) and low local conspecific density (Lázaro et al. 2014a) to be most strongly affected by pollinator decline. If responses to pollinator decline vary among species, this should ultimately change the species composition of the plant community. Here, we first show that our experimental treatment reduced flower visitation and seed

production in the study species. Then, we specifically asked: (1) Does the reduction in pollinator visitation cause an overall reduction in seedling diversity and abundance? (2) Does the pollinator reduction have contrasting effects on seedling recruitment of different species? (3) Does the pollinator reduction change seedling species composition over time?

## METHODS

### *Study area and species*

The study area is located on a species-rich hay meadow at Ryghsetra (59°44'03" N, 10°02'48" E), in Buskerud county, south Norway. The meadow was mown early in July until 2004, and every autumn after seed dispersal in 2005 and during the study years (2006–2009). The blooming season of the plant community begins in early May and ends in mid-late August, and approximately 55 species bloom during this period. We studied the effects of reduced visitation rates on seedling recruitment in 10 perennial plant species (Table 1) with easily identifiable genets (except for *Centaurea jacea* and *Centaurea scabiosa*, for which monocarpic shoots were used to assess recruitment).

### *Experimental manipulation of pollinator visitation*

In May 2006, we placed 30 pairs of permanent plots (2 × 2 m) systematically along two parallel rows (separated by ~5 m) across the study area, and marked an inner square of 1 × 1 m within each plot. Plot pairs were separated by at least 3 m, and plots within a pair by ~2 m. At the onset of the experiment, we randomly selected one plot of each pair for the experimental reduction of pollinator visitation (experimental plots, hereafter), and the

TABLE 1. The 10 study species, their family, clonality (Clonal), their degree of pollinator dependence (DPD), and pollen limitation index (PL) in 2006 (DPD and PL values from Lázaro et al. 2014a), total number of seedlings counted across treatment groups in all four study years (Seedlings), and the number of plot pairs included in the individual analyses (Plot pairs).

Species	Family	Clonal	DPD	PL	Seedlings	Plot pairs
<i>Anthyllis vulneraria</i>	Fabaceae	no†	0.94	0.56	135	19
<i>Centaurea jacea</i>	Asteraceae	yes‡	0.07	0.18	2845	29
<i>Centaurea scabiosa</i>	Asteraceae	yes‡			73	22
<i>Knautia arvensis</i>	Caprifoliaceae	yes§	0.90	0	107	22
<i>Lathyrus linifolius</i>	Fabaceae	no¶	1	0	51	14
<i>Leucanthemum vulgare</i>	Asteraceae	yes‡	0.61	0	504	28
<i>Lotus corniculatus</i>	Fabaceae	yes‡	0.99	0.59	30	8
<i>Potentilla thuringiaca</i>	Rosaceae		0.22	0	1039	19
<i>Prunella vulgaris</i>	Lamiaceae	yes‡	0.64	0.42	230	23
<i>Vicia cracca</i>	Fabaceae	yes‡	1	0.64	144	22

Notes: A value of 0 in DPD and PL indicates that the species is not dependent on pollinators for reproduction or not pollen limited, respectively; and a value of 1 indicates complete dependence of pollinators and pollen limitation, respectively.

†Silvertown et al. (1993).

‡Tamm et al. (2001).

§Vange (2002).

¶Dupré and Ehrlén (2002).

other plot was left unmanipulated and open for natural pollination (control plots, hereafter) for the duration of the experiment (2006–2009). To reduce pollinator visitation in the experimental plots, we placed dome-shaped semi-closed cages ( $2 \times 2 \times 1$  m length, width, height) made of two 4 m long PVC tubes bent diagonally over the plots, and covered the domes with transparent nylon fishnet with a mesh width of  $1.05 \times 1.05$  cm (Appendix S1: Fig. S1). To allow pollinators inside the cages an easy exit, we left an  $\sim 10$  cm opening at the base of the cages and a  $0.5 \times 0.5$  m opening on the top.

The experimental treatment did not affect biotic and abiotic conditions, pollinator composition (insects were not differently excluded depending on their size), or pollinator behavior, such as the number of flowers contacted per individual visitor, visit duration, and number of conspecific vs. heterospecific flowers visited within foraging bouts (Supplemental Material in Lázaro et al. 2014a, Lundgren et al. 2015).

To assess whether the experimental treatment reduced pollinator visitation, we observed flower visitation from 27 May to 19 July, 2006, covering the entire blooming season of the study species. Both experimental and control plots of the same pair were observed simultaneously (or immediately after each other) using 20-min observation periods. We counted the number of open flowers of the study species occurring within the inner  $1 \times 1$  m square of the plot, and recorded pollinator visitation to their open flowers/inflorescences (flowers, hereafter). Total visitation rate per flower and plot was obtained for each study plant species in each observation period, by dividing the total number of pollinator visits to each plant species by the number of open flowers of that plant species in the inner squares of the plots. Further details on the procedure are described in Lundgren et al. (2013).

To assess whether the experimental treatment reduced seed production, we haphazardly selected and marked one flower in up to three individuals per species and plot in 2006. We collected the fruits/inflorescences (fruits, hereafter) of marked flowers, when they were dry and immediately before dispersal. Further details on the procedure are described in Lundgren et al. (2013). For each species we estimated the number of seeds in the inner  $1 \text{ m}^2$  of the plot as the mean number of developed seeds per fruit  $\times$  mean number of fruits per individual  $\times$  total number of reproductive individuals in the inner  $1 \text{ m}^2$  of the plot. Collected seeds were not returned to the plots, but since we collected an equivalent number of fruits per experimental treatment this should not have any bearing on the results. It is also unlikely that our collection procedure affected plant species differently, because the amount of fruits collected was small and proportional to each species' abundance. We assume that seed dispersal was not affected by the experimental treatment because we removed the dome-shaped cages before the main seed dispersal period of the community, and put them back again just before the flowering period each study year.

Thus, our experimental treatment may provide a direct test of the effects of a pollinator reduction on seedling recruitment exclusively through reduction in seed production.

#### *Density and diversity of seedlings*

To quantify how the experimental reduction of pollinator visitation affected seedling establishment (seedling abundance, hereafter), we counted the number of seedlings (i.e., plants up to 1–5 cm tall, depending on the species, with fewer than six leaves, and very often with the cotyledons still attached) of the 10 study species in August each year (2006–2009), in the inner square meter of all 30 control and 30 experimental plots. We recorded the number of seedlings in 2006 to account for the initial variance among plots in seedling diversity and abundance, since the seedlings in 2006 are the result of reproductive events not yet affected by the experiment. In addition, we counted the number of reproductive individuals of each species in each plot and year, in order to account for its potential effects on seedling density the following year.

We used three diversity measurements to assess the effects of the experimental treatment on seedling diversity: (1) seedling richness ( $R$ ), i.e., the number of species in each plot and year; (2) individual-based rarefied seedling richness (RR) to control for differences in overall seedling abundance between experimental treatment groups by rarefying sample sizes to 10 individuals per plot (Gotelli and Colwell 2001); and (3) the exponential of Shannon entropy ( $\exp H'$ ), a entropy that weights all species proportionately to their frequencies without favoring common or rare species (Jost 2006). The exponential of Shannon entropy was calculated as

$$\exp H' = \exp\left(-\sum_{i=1}^R p_i \log p_i\right),$$

where  $p_i$  is the proportion of individuals belonging to the  $i$ th species, and  $R$  is species richness. The exponential transformation converts the Shannon diversity index (Shannon 1948) to a true measure of diversity, expressed in units of effective number of species.

#### *Statistical analysis*

All analyses presented here were conducted in R 3.0.3 (R Development Core Team 2014). We used general or generalized linear mixed models to test whether the experimental treatment reduced total flower visitation rates per plot, and seed production per plot, using data from the 10 study species in 2006. In both analyses, we included plant species and plots nested into plant species as random factors, and the experimental treatment (controls vs. experimental plots) as a fixed categorical factor. Due to the nature of the data, we used (1) normal distribution with link identity for the analysis of visitation rate and (2) negative binomial distribution with log-link function for the analysis of seed production.

To assess the effects of the experimental treatment on seedling diversity and abundance, we analyzed overall effects on the community, as well as species-specific responses, by using Generalized Estimating Equation models (GEEs; Liang and Zeger 1986). In all GEE analyses the experimental treatment (control vs. experimental plots), year and their interaction were included as fixed categorical effects. As an additional continuous predictor variable, we included the number of conspecific reproductive individuals (log-transformed) in the previous year in the models of seedling abundances, because the number of seeds available for recruitment within a patch or population may be related to the density of reproductive individuals (Clark et al. 1998). We first assessed overall effects of the experimental treatment on community-wide seedling diversity ( $R$ ,  $RR$ , and  $\exp H'$ ) and total seedling abundance over time, by means of global analyses that included all the study species. These analyses might indicate whether a reduction in pollinator densities causes an overall reduction in plant densities. An additional analysis for total seedling abundance was conducted by excluding the most abundant species in the community (*Centaurea jacea*) from the data set. This was done because *Centaurea jacea* accounted for >50% of seedlings across all four years (Table 1) and, therefore, the response of this plant species to the experimental treatment could strongly influence the results of the global analysis. For the global analyses of total seedling abundance, we summed up the number of seedlings of each study species in all experimental plots and in all control plots within each year to avoid zero inflation. Thus, we had one estimate of seedling abundance per treatment group per species and year (resulting in six values per study species). Second, we performed separate analyses (GEEs) for each of the 10 study species to examine the species-specific responses to the experimental treatment across the four study years. Zero-inflation was reduced in these analyses for each species by removing plot pairs with no seedlings in any of the years in either experimental or control plots. Thus, we had one estimate per treatment group per plot pair per year (resulting in six values times the number of plot pairs included in the analyses; see Table 1 for number of plot pairs used in each model).

GEEs allow accounting for the correlation within repeated measurements, by incorporating a parameterized within-subject correlation structure (Zuur et al. 2009). Therefore, these models are the most appropriate for our data set, which contains annual measurements on the same species and plots. We used species as the grouping structure (i.e., the subjects within which the measurements are repeated) in the global analyses of total seedling abundance, and plot as the grouping structure in the analyses of seedling diversity ( $R$ ,  $RR$ , and  $\exp H'$ ), and the individual analyses for each plant species. We used a first order autoregressive correlation structure (AR-1) to parameterize an exponentially decaying temporal correlation among within-subject observations

(Zuur et al. 2009). The effect of the experimental treatment on seedling diversity and abundance cannot be properly evaluated without taking the natural among-plot variation into consideration. Therefore, we used the diversity ( $R$ ,  $RR$ , and  $\exp H'$ ) and abundance of seedlings in 2006 (i.e., seedlings germinated from reproductive adults not yet affected by the experiment) as offsets in the models of seedling diversity ( $R$ ,  $RR$ , and  $\exp H'$ ) and abundance, respectively. For count data (global and individual seedling abundance,  $R$  and  $RR$ ) we used Poisson error distributions and log link functions, whereas for continuous data ( $\exp H'$  of seedlings) we used Gamma distribution and identity link function. All GEE analyses were implemented with the `geeglm` function in the `geepack` package in R (Højsgaard et al. 2006). The quasilikelihood-based QIC criterion (Pan 2001) was used for model selection in GEE models, using the `dredge` function in the `MuMIn` R package (Barton 2014). Because our main objective was to test the effect of the experimental treatment, we fixed this variable during automatic model selection, so that the experimental treatment was included in all the models selected, independently of whether it was significant or not. The models within  $\Delta QIC < 2$  of the minimum QIC were considered as the best set of models and the three best alternative models are shown in Appendix S1: Tables S1 and S2.

To test whether the experimental treatment changed seedling species composition over time, we compared seedling assemblages across experimental treatments and years. For that, we used a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), which is a nonparametric multivariate analog of ANOVA that allows constraining the permutations (999) within plots to account for the repeated-measure nature of our data (i.e., strata = plot). In this analysis, we included the experimental treatment (control vs. experimental plots), year, and their interaction, as fixed factors. A significant interaction between the experimental treatment and year may indicate that changes in seedling composition over time differed between the experimental treatments. PERMANOVA partitions the variation among the individual terms in the model and tests whether the group centroids, as defined in the space of a similarity index, are equivalent for all groups (Anderson and Walsh 2013). Our analysis was based on the Morisita-Horn similarity index (Horn 1966), calculated as

$$S_{MH} = 1 - (\sum_{i=1}^R (p_{i1} - p_{i2})^2) / (\sum_{i=1}^R p_{i1}^2 + \sum_{i=1}^R p_{i2}^2),$$

where  $p_i$  is the relative abundance of the  $i$ th species, and  $R$  is species richness. The PERMANOVA was implemented with the `adonis` function in the `vegan` package in R (Oksanen et al. 2013).

## RESULTS

The experimental treatment reduced both visitation rates (261 observation periods;  $F_{1,163} = 10.9$ ,  $P = 0.001$ ;



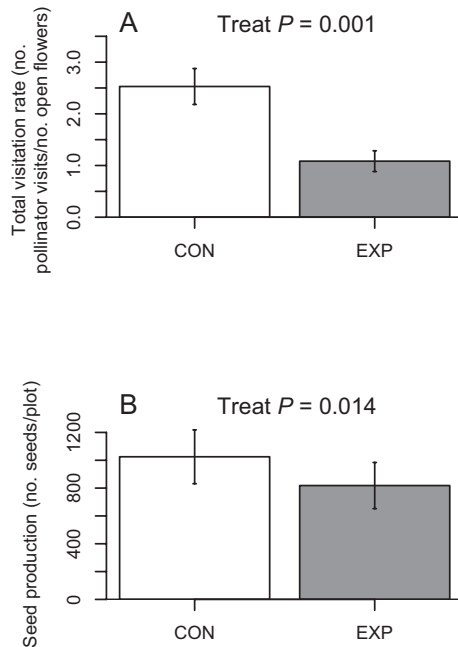


FIG. 1. (A) Total visitation rate and (B) total seed production per plot in control (CON; white bars) and experimental plots (EXP; gray bars). Values are mean  $\pm$  SE. Significance of the experimental treatment (Treat) is also shown.

Fig. 1A) and seed production (304 plots;  $\chi_1^2 = 5.99$ ,  $P = 0.014$ ; Fig. 1B) of the 10 study species by 57% and 22%, respectively, and thus could potentially affect seedling recruitment.

#### Seedling diversity

The experimental reduction of pollinator visitation significantly reduced seedling richness ( $R$ ;  $\chi_1^2 = 8.99$ ,  $P = 0.003$ ; Fig. 2A), rarefied richness (RR;  $\chi_1^2 = 5.8$ ,  $P = 0.016$ ; Fig. 2B) and seedling diversity measured as the exponential of Shannon's diversity index, ( $\exp H'$ ;  $\chi_1^2 = 4.9$ ,  $P = 0.028$ ; Fig. 2C). Rarefied richness also differed among years (RR;  $\chi_2^2 = 9.9$ ,  $P = 0.007$ ). For seedling richness there was also an alternative model with  $\Delta\text{QIC} < 2$ . This model included the same significant variables as the best model, but also a nonsignificant effect of year (Appendix S1: Table S1).

#### Overall seedling abundance

The total number of seedlings was on average 26.6% lower in the experimental than in the control plots, varying from 16% to 35% depending on the study year (Fig. 3). However, the experimental treatment had only a marginally significant negative effect on total seedling abundance (Treatment,  $\chi_1^2 = 3.7$ ,  $P = 0.054$ ). The interaction between the experimental treatment and year was also marginally significant (Year,  $\chi_2^2 = 3.2$ ,  $P = 0.20$ ; Year  $\times$  Treatment,  $\chi_2^2 = 5.7$ ,  $P = 0.058$ ), suggesting that

experimental effect sizes tended to differ among years (Fig. 3A). Total seedling abundance increased significantly with the total density of reproductive individuals in the previous year ( $\chi_1^2 = 6.68$ ,  $P = 0.01$ ; Appendix S1: Fig. S2). Remarkably, when the abundance of the most abundant species in the community (*Centaurea jacea*, see Table 1) was excluded from the analysis, the experimental treatment strongly and significantly reduced seedling abundance (Treatment,  $\chi_1^2 = 6.2$ ,  $P = 0.013$ , Fig. 3B), consistently across years (Year,  $\chi_2^2 = 0.2$ ,  $P = 0.91$ ; Year  $\times$  Treatment,  $\chi_2^2 = 2.2$ ,  $P = 0.338$ ). Moreover, total seedling abundance no longer increased with the total density of reproductive individuals in the previous year when the abundance of *C. jacea* was excluded from the data set ( $\chi_1^2 = 2.8$ ,  $P = 0.09$ ).

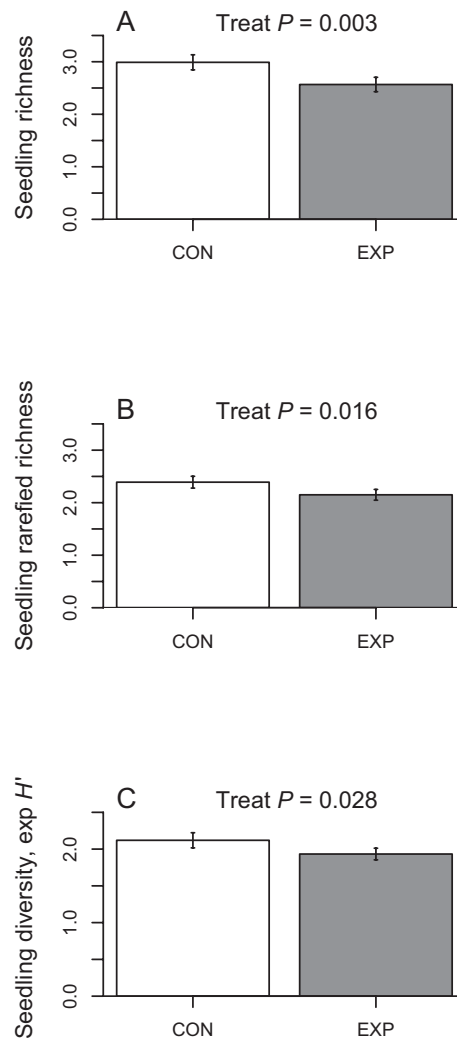


FIG. 2. Seedling diversity. (A) Seedling species richness, (B) species individual-based rarefied seedling richness (RR; see *Methods: Density and diversity of seedlings* for RR calculation), and (C) the exponential Shannon diversity index ( $\exp H'$ ) in control (CON; white bars) and experimental plots (EXP; gray bars). Values are mean  $\pm$  SE. Significance of the experimental treatment (Treat) is also shown.

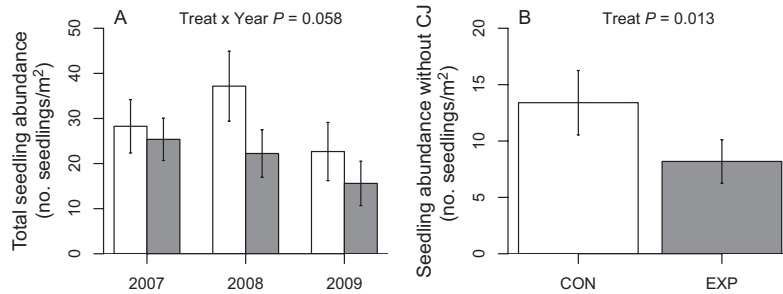


FIG. 3. Overall seedling abundance. (A) Total seedling abundance and (B) total seedling abundance without *Centaurea jacea* (CJ) in control (CON; white bars) and experimental plots (EXP; gray bars). Values are mean  $\pm$  SE. When the best model showed an interaction between experimental treatment and year, seedling abundance in control and experimental plots is shown for each year, and the significance is given for the interaction (treat  $\times$  year); otherwise the significance of the experimental treatment (Treat) is given.

#### Species-specific effects on seedling abundance

Nine of the 10 study species had fewer seedlings in the experimental than in the control plots. In these nine species, the experimental reduction in seedling recruitment ranged from 9% to 54%, and it was on average 29% lower in the experimental than in the control plots across all years, and 34% lower in the last study year. Seedling recruitment was reduced by over 40% in six of the 10 study species (Fig. 4). Despite this consistent response from most of the study species, only two of them were significant (Table 2). The experimental treatment significantly reduced seedling abundance in *Lotus* (Fig. 4G; Table 2). In *Prunella*, the experiment reduced seedling abundance in two of three years, but the reduction was statistically significant only in the last year (Fig. 4I; Table 2). On the contrary, in *Knautia* there was a marginally significant increase in seedling abundance in experimental plots (Fig. 4D; Table 2). Seedling abundance was positively related to the number of conspecific reproductive individuals the previous year in *Anthyllis*, *Lathyrus*, *Prunella*, and *Vicia* (Table 2; Appendix S1; Fig. S3), and differed among years in *Centaurea jacea*, *Leucanthemum*, and *Lotus* (Table 2). For four species (*C. scabiosa*, *Knautia*, *Lathyrus*, and *Potentilla*), we found competing models with  $\Delta\text{QIC} < 2$ . However, all these alternative models showed the same significant variables as the best ones (see Appendix S1; Table S2).

#### Seedling composition

The PERMANOVA showed changes in seedling species composition over time (Year,  $F_{1,230} = 8.8$ ,  $P > 0.001$ ) and a marginal significant interaction between the experimental treatment and year (Year  $\times$  Treatment,  $F_{1,230} = 2.25$ ,  $P = 0.067$ ; Treatment,  $F_{1,230} = 0.1$ ,  $P = 0.96$ ). This marginally significant interaction suggests stronger changes in the composition of seedlings in experimental plots (2006 vs. 2009,  $F_{1,54} = 6.75$ ,  $P > 0.001$ ) than in the control plots (2006 vs. 2009;  $F_{1,56} = 2.23$ ,  $P = 0.07$ ) by the end of the experiment.

#### DISCUSSION

To the best of our knowledge, this is the first study that shows direct causal links between a reduction in pollinator availability and reductions in plant diversity and abundance. Our experimental reduction in pollinator visitation rates caused a community-wide reduction in seedling diversity. Overall seedling abundance was also consistently lower in the experimental plots compared to control plots, although the effect of the experimental treatment was only strongly significant when the most abundant species (*Centaurea jacea*) was excluded from the data set. In addition, the results showed that the experimental treatment affected the recruitment of species differently, which tended to change seedling species composition in the experimental more than in the control plots along the four study years. Due to the positive relationship between the number of reproductive individuals and the number of seedlings, our results also suggests that these changes may accelerate over time, and that feedback loops between floral abundance and pollinator activity could further affect plant and pollinator diversity and abundance.

#### Overall effects on seedling diversity and abundance

In this study, we show that reduced pollinator availability has direct negative effects on seedling diversity measured as richness, rarefied richness, and the exponential Shannon diversity index. Interestingly, the negative effect of the experimental treatment on rarefied richness indicates that a reduction in pollinator visitation has direct effects on diversity independently of its effect on plant abundances. Seedling abundance in the community was also reduced by the experiment every year, but more strongly and consistently for non-dominant species in the community. Our results agree with a number of recent studies suggesting that reduced pollination intensity may negatively affect plant densities or diversities. However, these studies have focused on single species (e.g., Ehrlén and Eriksson 1995, Steffan-Dewenter and Tscharntke 1999, Lennartsson 2002, Hegland and

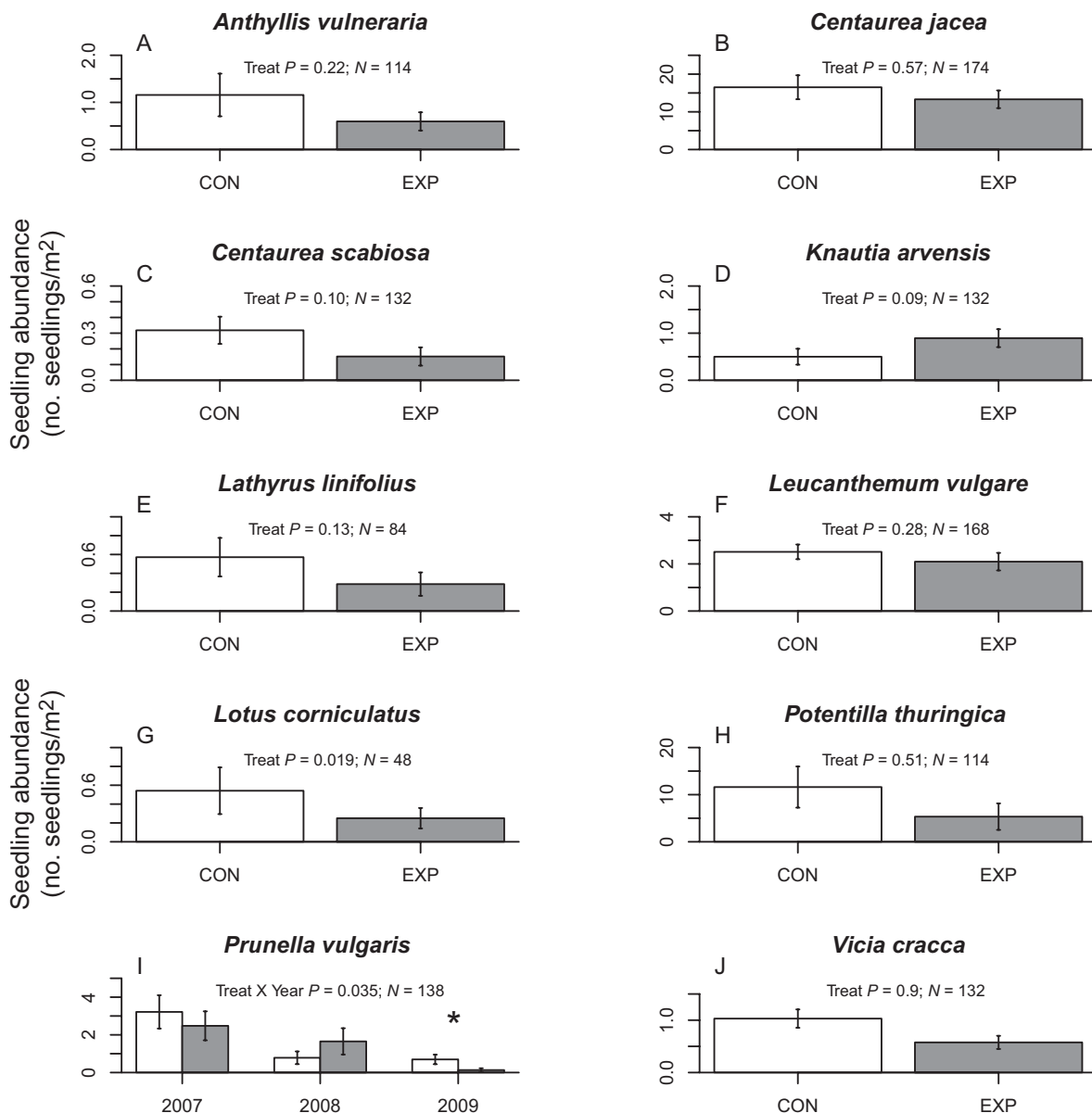


FIG. 4. Species-specific responses. Seedling abundance in (A) *Anthyllis vulneraria*, (B) *Centaurea jacea*, (C) *Centaurea scabiosa*, (D) *Knautia arvensis*, (E) *Lathyrus linifolius*, (F) *Leucanthemum vulgare*, (G) *Lotus corniculatus*, (H) *Potentilla thuringiaca*, (I) *Prunella vulgaris*, and (J) *Vicia cracca* in control (CON) and experimental plots (EXP). Values are mean  $\pm$  SE. The significance of the experimental treatment (Treat) and sample size ( $N$ ) is given in the figure. When the interaction Treatment  $\times$  Year is significant, the average ( $\pm$  SE) seedling abundance in control (white bars) and experimental plots is given for each year and the significant ( $P < 0.05$ ) pairwise comparisons are marked with an asterisk.

Totland 2007, Geib and Galen 2012, Lundgren et al. 2015) and/or were based on correlations (Biesmeijer et al. 2006, Anderson et al. 2011, Pauw and Hawkins 2011). Therefore, our study adds to previous ones because it is a community-wide experimental test of these relationships, and because we document direct causal links between reduced pollinator availability and seedling recruitment at the community level.

Our results also show positive relationships between the number of reproductive individuals and the number

of seedlings produced in the following year, likely because a higher number of the reproductive individuals increase the number of ovules available for producing seeds, but also the attractiveness of the patch for pollinators (Dauber et al. 2010). This is in line with other studies at the landscape level that show a relationship between plant and pollinator densities and suggest that decreased plant densities and diversities may cause declines in pollinator abundances and diversities (Steffan-Dewenter et al. 2002, Potts et al. 2003, Weiner et al. 2014). Indeed,

TABLE 2. Results of the Generalized Estimating Equation models studying the effect of the experimental treatment on seedling abundance for each study species.

Species	Treatment	Year	Treatment × Year	log(reproductive individuals)
<i>Anthyllis vulneraria</i>	$\chi^2_1 = 1.5, P = 0.22$			$\chi^2_1 = 16.4, P < 0.001(+)$
<i>Centaurea jacea</i>	$\chi^2_1 = 0.3, P = 0.57$	$\chi^2_2 = 13.3, P = 0.001$ (2008: +; 2009: -)	$\chi^2_2 = 0.5, P = 0.78$	$\chi^2_1 = 0.7, P = 0.39$
<i>Centaurea scabiosa</i>	$\chi^2_1 = 2.7, P = 0.10(-)$			$\chi^2_1 = 1.2, P = 0.27$
<i>Knautia arvensis</i>	$\chi^2_1 = 2.9, P = 0.09(+)$			
<i>Lathyrus linifolius</i>	$\chi^2_1 = 2.3, P = 0.13$			$\chi^2_1 = 27.3, P < 0.001(+)$
<i>Leucanthemum vulgare</i>	$\chi^2_1 = 0.3, P = 0.28$	$\chi^2_2 = 14.5, P < 0.001$ (2008: -, 2009: -)	$\chi^2_2 = 2.4, P = 0.31$	$\chi^2_1 = 2.0, P = 0.16$
<i>Lotus corniculatus</i>	$\chi^2_1 = 5.5, P = 0.02(-)$	$\chi^2_2 = 14.3, P < 0.001$ (2008: +; 2009: +)		
<i>Potentilla thuringiaca</i>	$\chi^2_1 = 0.4, P = 0.51$	$\chi^2_1 = 1.1, P = 0.57$	$\chi^2_1 = 0.6, P = 0.73$	
<i>Prunella vulgaris</i>	$\chi^2_1 = 1.7, P = 0.2$	$\chi^2_2 = 14.3, P < 0.001$ (2008: -, 2009: -)	$\chi^2_2 = 6.7,$ $P = 0.035(2007: -;$ $2008: +; 2009: -)$	$\chi^2_1 = 26.2, P < 0.001(+)$
<i>Vicia cracca</i>	$\chi^2_1 = 0.02, P = 0.9$			$\chi^2_1 = 5.2, P = 0.023(+)$

Notes: Best models were selected using the dredge function in R, from full models containing the experimental treatment (Treatment), year, and their interaction, and the number of conspecific reproductive individuals in the previous year (log-transformed). The  $\chi^2$  and  $P$  values are given for each significant term included in the best models. The sign of the estimated coefficients is also given in brackets when  $P$  values  $\leq 0.1$ , and refer to the experimental plots compared to the control plots (reference in the analyses), and to years 2008 and 2009 compared to 2007 (reference year).

the abundance and diversity of plants and pollinators are interrelated and may affect each other continuously in both directions through feedback loops.

#### Species-specific responses and changes in seedling community composition

Despite the overall community-wide effects of the experimental treatment, the responses of individual species differed. However, different responses were mostly related to the strength of the effect and not to the direction, since nine of the 10 study species had fewer seedlings in the experimental than in the control plots. Species-specific responses can to some extent be related to species traits. We hypothesized that responses to the experimental treatment should differ among species depending on their dependence of pollinator visitation for seed production, because the strongest effect of our experimental treatment on reproductive success occurred in the species that were most dependent on pollinators for seed production (Lundgren et al. 2013), and because self-incompatible species decline more than self-compatible species with pollinator loss (Biesmeijer et al. 2006). Our results do not clearly support this prediction. On one hand, *Prunella*, one of the species significantly affected by the experimental reduction of pollinators has intermediate pollinator dependence levels (Table 1). On the other hand, although we found a significant reduction in the highly pollinator-dependent and pollen-limited *Lotus*, other species that are strongly dependent on pollinators for reproduction, such as *Anthyllis*, *Lathyrus*, *Vicia*, and *Knautia*, were not significantly affected by the

experimental treatment. Inter-specific correlations did not show any relationship between the extent to which the experiment reduced species' recruitment and the degree of their pollinator dependence or pollen limitation (Appendix S1: Table S3). Nevertheless, we cannot discard the possibility that this absence of significant relationships is due to the low number of species included in these correlations.

Morphological floral traits, such as floral symmetry, could also influence the susceptibility of different species to pollinator declines. Although most species, both zygomorphic and actinomorphic, had fewer seedlings in the experimental plots (Fig. 4), the two species that showed a significant reduction in seedling abundance in the experimental plots were zygomorphic. Zygomorphic flowers are often visited by fewer and more specialized pollinator groups (Fenster et al. 2004), and previous studies have found that species with specialized flowers are more pollen limited than species with unspecialized flowers (Vamosi et al. 2013, Lázaro et al. 2014b). This could explain our results. However, there was no overall significant effect of floral symmetry on the decrease in seedling abundance by the experimental treatment (Appendix S1: Table S3), most likely due to low statistical power, because the parameter estimate of the model indicated a higher reduction in recruitment in species with zygomorphic flowers (Appendix S1: Table S3). Whether species with zygomorphic flowers are more susceptible to pollinator declines than species with actinomorphic flowers must be studied more in detail with a larger number of species.

Apart from species traits, many other factors could explain why a direct link between a reduction in seed



production and a reduction in seedling recruitment does not occur in all species. For example, pollinator availability may simply not affect seedling recruitment because recruitment is not constrained by seed inputs (Bond 1994, Geib and Galen 2012, Lundgren et al. 2015). Indeed, although some studies have found a link between pollination intensity and plant population densities (e.g., Ackerman et al. 1996, Kelly et al. 2007) or population growth (e.g., Price et al. 2008), others have shown that the effect of pollination intensity could be outweighed by stronger effects of demographic costs of reproduction (Ehrlén and Eriksson 1995), competition (Hegland and Totland 2007), herbivory (Knight 2004), or intraspecific density dependence (Waser et al. 2010). The local flowering context might also alter the effect of an overall reduction in pollinator densities on seedling recruitment. Flowering neighbors may influence seedling recruitment not only through facilitation and competition for abiotic resources (Klanderud 2010), but also by increasing pollinator availability through facilitation of pollinator attraction (Moeller 2004) or decreasing pollinator availability through competition for pollinator visitation (Mustajärvi et al. 2001). In fact, Lázaro et al. (2014a) show that a high conspecific density may to some extent buffer the effect of a pollinator decline. The fact that the negative effects of the experiment on seedling abundance were stronger when the most abundant species (*C. jacea*) was removed from the data set, may support the finding that conspecific density buffers negative effects of pollinator decline. However, there was no relationship between the number of reproductive conspecifics and the degree to which the experimental treatment reduced recruitment among the species studied here (Appendix S1: Table S3). Last, seed germination from persistent soil seed banks may mask any effects of difference in seed production on recruitment caused by reduced pollinator availability (Venable and Brown 1988, Hille Ris Lambers et al. 2005), at least in the short term. In fact, since most of the species in this study have persistent seed banks (Bakker et al. 1996, Eriksson and Eriksson 1997), we cannot discard the possibility that germination from seed banks influenced the results by reducing the difference in seedling abundance between treatment groups. Anyway, permanent reductions in seed rain caused by pollinator decline would deplete even persistent seed banks in the long term. Moreover, even if a pollinator decline may not reduce the abundance of some species in the short term, the longer term negative effects of a potential loss of genetic diversity in those species, caused by reduced outcrossing, should not be neglected (Eckert et al. 2010).

Species-specific responses to reduced pollinator availability may change plant community composition over time. Indeed, our composition analysis showed a tendency for seedling species composition to change over the four study years more strongly in the experimental than the control plots. It is possible that a longer duration of the study would have caused a stronger experimental effect on seedling composition due to a cumulative

deficiency in seed input. Changes in seedling species composition may result in permanent changes in plant species composition in the community if environmental filtering and density dependence do not diminish these effects over time (Schupp 1995, Comita et al. 2010, Paine et al. 2012). Experimental manipulation of pollinator abundances over a long time period would be necessary to reveal if the tendency detected here would intensify over time.

#### *Future research directions*

To further improve our understanding of how pollinator decline may affect plant population persistence and plant community composition, future research should link pollination availability to more detailed studies of plant demography. Here, we study the effect of reduced pollinator availability on recruitment, but it will be necessary to follow the seedlings to reproductive stages to clearly identify which characteristics of plants make them particularly vulnerable to pollinator loss, and to predict changes in the composition of plant communities. Our experimental setup could also be used to detect how a pollinator decline may affect resource allocation in plants. In addition, this experimental design could help to gain a better understanding of the evolutionary consequences of a pollinator decline. For instance, by combining this approach with genetic analysis, it should be possible to examine if pollinator loss may reduce genetic diversity of plant populations, whether it could cause mating systems to shift from outcrossing to selfing as a mechanism of reproductive assurance, and whether differences in phenotypic selection due to reduced pollinator availability could cause genetic differentiation among populations. In general, many ecological and evolutionary theories are based on changes in resource availability, and pollinators are a resource for plants. Therefore, our experimental approach, which easily changes pollinator availability, could have a wide range of applications within the fields of pollination ecology, plant reproduction, and evolutionary ecology.

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