Ecology, 99(6), 2018, pp. 1480–1489

Flowering and floral visitation predict changes in community structure provided that mycorrhizas remain intact

Jonathan A. Bennett[1,2] and James F. Cahill, Jr.

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9 Canada

Abstract. Pollination is critical for plant fitness and population dynamics, yet little attention is paid to the role of flowering and plant-pollinator interactions in structuring plant communities, including community responses to environmental change. Changes in arbuscular mycorrhizal fungi (AMF), nutrient abundances, and plant litter all affect plant access to different resources, and are known regulators of community structure. Each factor can also affect flowering and plant-pollinator interactions, potentially contributing to changes in community structure. To test whether AMF, nutrients, and litter influenced the relationship between pollination and community structure, we conducted a 5-yr field experiment applying fungicide, adding fertilizer, and removing plant litter in native grassland. We measured the distribution of flowers and floral visits among species in year three and linked these measures to changes in plant composition and species richness between years three and five. We hypothesized that an uneven distribution of flowers and visits among species would lead to greater community change, but that the treatments would disrupt this relationship by altering sexual allocation and recruitment. Consistent with our hypothesis, communities with uneven flower distributions exhibited greater changes in community composition and richness under ambient conditions. However, AMF suppression neutralized this relationship and regulated the other treatment effects, highlighting the potential importance of AMF for stabilizing recruitment dynamics. Combined, AMF suppression and nutrient addition caused species losses when few species flowered, likely by compounding stresses for those species. The treatment effects on the relationship between flowering and community composition were more nuanced, but were likely driven by increased competition and altered flowering among species. By contrast, community composition was more stable when visitation rates were uneven among species, irrespective of any treatments. This suggests that some species require high visitation rates to maintain their populations due to greater dependence on sexual reproduction. Combined, these results highlight the importance of flowering and floral visitation to the dynamics of grassland communities. They also suggest that altered recruitment dynamics is a major, yet understudied, mechanism by which environmental change affects communities. Consequently, understanding the effects of environmental change on plant communities will require study of both plant growth and sexual reproduction.

Key words: bees; community assembly; diversity; floral visitation; mycorrhiza; nutrients; plant community dynamics; plant litter; pollination; recruitment; regeneration niche; sexual allocation.

INTRODUCTION

Changes in sexual reproduction can have profound effects on plant populations (Potts et al. 2010) and potentially plant communities (Sargent and Ackerly 2008, Burkle and Alarcón 2011). Despite the wealth of studies exploring the complex interactions between plants and pollinators (Traveset et al. 2016), empirical evidence showing that these interactions regulate plant community structure remains limited (Burkle and Alarcón 2011, Brosi and Briggs 2013). However, parallel declines between plants and pollinators (Biesmeijer et al. 2006, Burkle et al. 2013) and the importance of propagule limitation within many communities (Myers and Harms 2009) suggest that sexual reproduction is important for plant communities. Consequently, understanding how flowering and plant-pollinator interactions relate to changes in plant community structure may be integral to understanding long-term community dynamics, including the maintenance of plant diversity (Burkle and Alarcón 2011, Maron et al. 2014, Larson and Funk 2016).

Flowering and plant-pollinator interactions are expected to affect community change by altering the relative extent of seed production and recruitment among species (Burkle and Alarcón 2011, Maron et al. 2014, Larson and Funk 2016). Species that flower less or are less frequently visited by pollinators should produce fewer seeds and decline in abundance relative to species that have more flowers or are less pollen limited. Consequently, if the distribution of flowers or floral visits among species deviates from the distribution of vegetative abundances, the community should change over time. Similarly, if some species do not flower or are not pollinated, then those species may be lost from the community, thereby reducing species richness. This suggests that the distribution of flowers and floral visits among species should be predictive of future community change. However, species differ in their reliance on sexual reproduction for population growth (Herben et al. 2015), which may result in an uneven distribution of flowers and floral visits among species in stable communities. Consequently, uneven distributions of sexual reproduction may also result in community stability.

Manuscript received 8 September 2017; revised 29 January 2018; accepted 19 March 2018. Corresponding Editor: Ken N. Paige.

[1] Present address: Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8 Canada.

[2] E-mail: jon.bennett@usask.ca

Sexual reproduction and recruitment both depend on resource availability, suggesting that resource availability may also alter the relationship among flowering, floral visitation, and community structure (Burkle and Irwin 2010, Foster et al. 2011). To explore such effects, we suppressed arbuscular mycorrhizal fungi (AMF), added nutrients, and removed plant litter: each of which alter the relative availability of light, water, and nutrients. AMF exchange soil nutrients for photosynthate; consequently, AMF enhance nutrient availability when nutrients are scarce, but become parasitic when nutrients are abundant and when carbon is limiting (Johnson et al. 1997, 2015). Nutrient addition increases the availability of essential nutrients, but also reduces light available to some species by increasing the growth of others (Hautier et al. 2009). Litter removal increases light penetration, but can reduce soil moisture by increasing evaporation (Facelli and Pickett 1991). Due to this combination of effects, nutrient addition may increase the parasitic effects of AMF for species that do not respond positively to nutrient addition and become carbon limited. By contrast, litter removal may offset any negative effects associated with carbon limitation by increasing light availability. Moreover, each of these factors can affect the relative abundance of different plant species (Bennett and Cahill 2013) and sexual reproduction (Cahill et al. 2008, Burkle and Irwin 2010, Baude et al. 2011, Masters and Emery 2015), but it is unclear whether these effects are linked.

The effects of AMF, nutrients, and litter on plant community structure are frequently site dependent (Hartnett and Wilson 2002, Hautier et al. 2009, Dickson and Foster 2011, Loydi et al. 2013), and any effect on the relationship among flowering, floral visitation, and community change will likely be as well. This study occurred in a C_3-dominated grassland at the northern edge of the Great Plains (see *Methods*). In this system, AMF promote plant diversity by increasing the establishment of rare species (Bennett and Cahill 2016), while nutrients and litter decrease diversity by reducing light availability (Lamb 2008), which may act to increase competition or reduce germination. If recruitment is important, we may therefore expect AMF suppression and nutrient addition to weaken the relationship among flowering, visitation, and community change, and for litter removal to strengthen the relationship. In the absence of significant recruitment, which may be common in perennial communities, treatment effects on flowering and visitation may still be linked to changes in the community if allocation to flowering and pollinator attraction change in concert with plant growth (Hartnett 1990, Karlsson and Mendez 2005). Nonetheless, it is difficult to predict how resource effects on sexual allocation will affect the relationship among flowering, floral visitation, and community change as species vary greatly in their sexual allocation strategies (Herben et al. 2015). These relationships are further complicated because AMF, nutrients, and litter do not have consistent effects on insect-pollinated species' abundances at the site (Bennett and Cahill 2013) and, at least for AMF, show similarly inconsistent effects on flowering and floral visitation (Cahill et al. 2008). Nevertheless, such interspecific variability in treatment effects on plant growth and sexual reproduction suggests that the treatments will affect the composition and diversity of species flowering and being visited, and potentially their relationship with future changes in community structure.

To determine how AMF suppression, nutrient addition, and litter removal affect the relationship between sexual reproduction and plant community dynamics, we conducted a 5-yr experiment where we applied the treatments factorially. As we were interested in the role of plant-pollinator interactions, we focused on insect-pollinated species. We quantified flowering, floral visitation, and vegetative abundances within the experimental plots after 3 yr. To quantify future changes in the community, we remeasured vegetative abundances after two additional years. Using this data, we tested how the relative distribution of flowers and floral visits among species in year three were related to changes in community composition and species richness between years three and five. We hypothesized that plots in which flowering or floral visitation were disproportionate within the community would exhibit the greatest changes in community composition and richness over time. Further, AMF suppression and nutrient addition were expected to weaken the relationships among flowering, visitation, and community change, while litter removal would strengthen these relationships, with additive interaction effects among treatments.

Materials and Methods

Site description and study design

The study occurred at the University of Alberta Roy Berg Ranch at Kinsella, Alberta, Canada (53°05′ N, 111°33′ W). The field site is a savannah type habitat with mixed grass native prairie interspersed with stands of aspen (*Populus tremuloides* Michx.). Graminoids dominate the community in terms of abundance, but most species are forbs (Bennett et al. 2014*b*). Productivity is water and nitrogen limited (Lamb et al. 2007) and can be an important determinant of floral visitation (Bennett et al. 2014*a*). However, flower abundances, while spatially variable, are the primary driver of floral visitation (Bennett et al. 2014*a*). AMF suppression, nutrient addition, and litter abundance can all reduce plant diversity (Lamb 2008, Bennett and Cahill 2016), although their effects are inconsistent among insect-pollinated species (Cahill et al. 2008, Bennett and Cahill 2013).

In May 2008, we established 20 blocks haphazardly distributed over a 7-ha area. Each block was located within open grassland, and consisted of paired 5 × 7 m plots, with at least 1 m between the two plots in each pair. The outer 0.5 m of each plot was designated as a buffer where no measurements were taken to minimize edge effects, similar to other studies (e.g., Brunbjerg et al. 2014). We applied the fungicide Rovral FLO (Bayer Crop Science, Calgary, Alberta, Canada) to the entire area of one randomly selected 5 × 7 m plot per block, at a rate of 82.3 mg/m^2 iprodione in 7.5 L water. Control plots received only water. The fungicide treatment was applied biweekly from May through September until the end of the experiment in 2012. Iprodione effectively suppresses mycorrhizas, but can also suppress other fungi (West et al. 1993). To maximize effects on mycorrhizas, fungicide was applied just prior to light rain when possible to ensure that the fungicide penetrated the soil. In a previous study at this site, Rovral suppressed AMF colonization for forbs, but not grasses, and had inconsistent effects on non-AM root-associated fungi (Bennett and Cahill 2016). In this study, fungicide reduced AMF infectivity by 40%, but had no effect on non-AM hyphae (see Appendix S1: Fig. S1 and Table S1). Although other fungicides have been shown to have no direct effect on pollinator behavior (Cahill et al. 2008), the direct effects of this fungicide are unknown. Consequently, we applied fungicide only after all observations were completed for each two-week interval in 2010 to minimize direct effects on pollinators, with a minimum of 5 d between fungicide application and insect observation. Fungicide application also had no significant effect on resource availability or plant productivity. See Appendix S1 for methods, and Appendix S1: Table S2 and Fig. S1 for full results.

In April 2009, each plot was divided into four smaller 2.5 × 3.5 m subplots. To minimize interactions among plants in adjacent subplots, we cut the roots along subplot borders to at least 10 cm using an edging shovel: most root biomass and belowground competition occurs in the upper 10–15 cm of grassland soils (Cook and Ratcliff 1984, Gill et al. 1999). Within the subplots, we removed litter and added NPK fertilizer in a restricted randomized design, such that each combination of nutrient and litter removal was replicated within the larger plot. At the beginning of each May between 2009 and 2012, we removed plant litter from one-half of the subplots by raking them until all fallen litter was removed. We also raked the subplots without litter removal to simulate the same level of mechanical disturbance, but the litter was replaced. Immediately following raking, we added a slow release fertilizer (14-14-14 NPK Osmocote Classic; Scotts Professional, Marysville, Ohio, USA) to one-half of the subplots at a rate of 5.22 g NPK/m^2: this rate was chosen to match previous nutrient manipulations at the site (Lamb et al. 2007). In this study, nutrient addition increased nitrogen availability and plant productivity, primarily of graminoids, consequently reducing light availability and soil moisture. Litter removal reduced litter mass, thereby increasing light penetration and reducing soil moisture, although the effects on light were attenuated when nutrients were added. Litter removal also reduced graminoid abundance unless fungicide was applied. Full methods for resource and productivity quantification are found in Appendix S1 with the results found in Appendix S1: Table S2 and Fig. S1.

Plant abundances, flowering, and plant-pollinator interactions

Within each of the 2.5 × 3.5 m subplots, we established a central 0.5 × 0.5 m permanent quadrat at the onset of the experiment. In June, July, and August of 2010 and 2012, we estimated percent cover of all vascular plants within this quadrat. As species abundances vary throughout the growing season, we considered the abundance of each species in a quadrat as the maximum cover recorded. Hereafter, we refer to cover from 2010 as initial cover and 2012 as final cover. As we are focused on the effects of pollination on plant communities, when calculating community composition and species richness, we include only those species where flowers had been visited by insects in this study or a previous study at the site (Bennett et al. 2014*a*). The changes in composition and richness of insect-pollinated species were, however, correlated with the change across the entire plant community (composition $R^2 = 0.32$, $P < 0.001$; richness $R^2 = 0.69$, $P < 0.001$; see Appendix S1: Fig. S2).

Both flowering stem abundance and floral visitation were surveyed from 1 June to 15 September 2010. Flowering stem composition and insect visits to those stems were observed in each subplot for three 4-min intervals every two weeks (12 total minutes per two-week period): once in the morning, early afternoon, and late afternoon per period. We limited our observations to mostly sunny and calm days between 09:00 and 19:00 h, and adjusted observations to avoid cool mornings and evenings in June and September and hot periods near solar noon in July and August when pollinators would be less active. We restricted our measurement of flowering to flowering stems because it was unfeasible to count individual flowers given the time constraints and number of flowering stems (approximately 6,000 stems per two weeks). We recognize that stems can vary greatly in the number and size of flowers, which could impact sexual reproduction. However, the number of flowers per stem and flower size were not strongly affected by fungicide application at this site in a previous study, suggesting that, at the population level, flowering stem densities may be a sufficient measurement of how flowering responds to the treatments (Cahill et al. 2008). As flowering stems persisted across multiple surveys and it was not feasible to track each individual stem, we use the maximum number of flowering stems per species per subplot as a conservative estimate of flower abundance. Flower visitation was monitored by direct observation of the number of visits to each flower species. Each contact between an insect and the reproductive parts of a flowering stem was considered a single visit (Cahill et al. 2008).

Abundance, composition, and diversity

To explore the relationship among flowering, visitation, and changes in community structure, we first calculated measures of these metrics that were independent of the initial community structure and of each other (hereafter “relative” measures). Using only data for insect-pollinated species, we first quantified community composition and species richness using each species’ maximum initial and final percent cover, maximum flowering stem densities, and total number of visits. For each of the plant measures, community composition was represented by the species by subplot matrix and species richness as the number of species present in that subplot. We then calculated the relative measures of composition and richness represented the distribution of flowers and visits among species, and changes in vegetative composition and richness between 2010 and 2012. The relative distribution of flowers was calculated by comparing the composition and number of species of flowering stems to either the composition or species richness of initial cover. Similarly, relative visitation was calculated as visits relative to flowering stems, and community change as final relative to initial cover.

We calculated the compositional measures of relative flowering visitation, and community change using Bray-Curtis dissimilarities. To minimize the influence of absolute abundances and rare species on dissimilarity, we standardized the data using a Hellinger transformation (Legendre and Gallagher 2001). In eight subplots, no insect-pollinated species were observed for at least one of the measurements. These subplots were dropped from subsequent analyses.

We calculated the relative number of species flowering and being visited, and the change in species richness as the standardized residuals of linear regressions. We use residuals rather than ratios as residuals allow for zeroes in the denominator, as would occur if new species colonized a plot between 2010 and 2012. Residuals also give a better estimate of the magnitude of the difference when the numerator is zero: 0/2 and 0/7 species flowering would have the same ratio, but differ in magnitude. We used standardized residuals so that cover, flowering, and visitation were all on the same scale.

Analyses

Flowering, visitation, and community change.—To test whether relative flowering and floral visitation were related to future changes in vegetative abundances, we used separate mixed models for species composition and richness. These models included the change in composition or richness as the response variable and either the relative initial composition or number of species flowering and being visited as fixed effects. We also used these models to test whether these relationships were disrupted by the treatments. Consequently, both models included the factorial interactions among the treatments, relative flowering, and relative visitation as fixed effects, but did not include any interactions between flowering and visitation. To account for the split-plot design, we also included block and the block by fungicide interaction as random effects. We also tested whether these relationships were affected by changes in total grass cover; however, including changes in grass cover greatly complicated the model, but did not alter the results, so we do not present the results here. All mixed models were conducted using the R package lme4 (Bates et al. 2014) with degrees of freedom estimated by the Satterthwaite method in the r package lmerTest (Kuznetsova et al. 2014).

Treatment effects on flowering and visitation.—In addition to testing the relationship among flowering, visitation, and community change, we were also interested in treatment effects on the relative composition and number of species flowering and being visited. We tested for treatment effects on both metrics of relative flowering and visitation using four separate mixed models: one using each combination of plant measure (flowering or visitation) and community metric (composition or species number) as the response variable. Each model included fungicide, nutrient addition, and litter removal as factorial fixed effects, with block and a block by fungicide interaction as random effects to account for the split-plot design.

RESULTS

Across all plots, we recorded 24,753 flowering stems belonging to 51 insect-pollinated species that were observed being visited 7,149 times, primarily by bumblebees (see Appendix S1: Fig. S3). Insect-pollinated species accounted for 39% of the vascular plant cover in 2010 and 36% in 2012, averaged across all treatments. Despite little change in total cover, the average number of species composing these cover estimates declined 29%, from seven to five species per plot, driven by the loss of species in plots where nutrients were applied (see Appendix S1: Fig. S4).

Flowering, visitation, and compositional change

Consistent with our hypothesis, plots in which the composition of flowers was not proportional to vegetative abundances exhibited the greatest future change in cover composition under ambient conditions (positive relationship in Fig. 1A; Table 1). Conversely, when insect visits were unevenly distributed among the available flowers, there was less change in community composition (negative relationship in Fig. 1B; Table 1). Further, the relationship between flowering and compositional change was sensitive to the treatments, whereas the treatments had no effect on the relationship between visitation and changes in community composition (Table 1). Fungal suppression largely eliminated the relationship between relative flower composition and changes in community composition (Fig. 1A; Table 1), but fungicide effects were modified by nutrient addition and litter removal (four-way interaction; Table 1). Nutrient addition caused the relationship between flowering and changes in community composition to become negative in fungicide treated plots (i.e., less change when flowers were unevenly distributed), but only when litter was intact (Fig. 1A).

Flowering, visitation, and changes in species richness

When a greater proportion of species flowered under ambient conditions, future species losses were minimized within the community, mirroring the relationship found for community composition (positive relationship in Fig. 2A; Table 1). However, the relative number of species being visited was unrelated to changes in species richness (Fig. 2B; Table 1). Additionally, there was a significant interaction effect among fungicide, nutrient addition, and the relative number of species flowering (Table 1). Fungicide neutralized the relationship between flowering and changes in species richness when no nutrients had been added (Fig. 2A). When both fungicide and nutrients were added, the slope of the relationship between flowering and species richness remained positive, although this combination of treatments caused large declines in species richness relative to ambient conditions, especially when few species flowered within the plot.

Treatment effects on flowering and visitation

Treatment effects on the relationship between flowering and community change were largely independent of any treatment effects on the relative composition (Fig. 3A; Appendix S1: Table S3) or number of species flowering (Fig. 3B; Appendix S1: Table S3). For the relative composition of species flowering, only litter removal had a significant effect ($F_{1,103} = 4.07$, $P = 0.046$), causing the community floral display to be more proportional to the vegetative composition of the community (i.e., lower dissimilarity; Fig. 3A). For species richness, nutrient addition marginally increased the relative number of species flowering

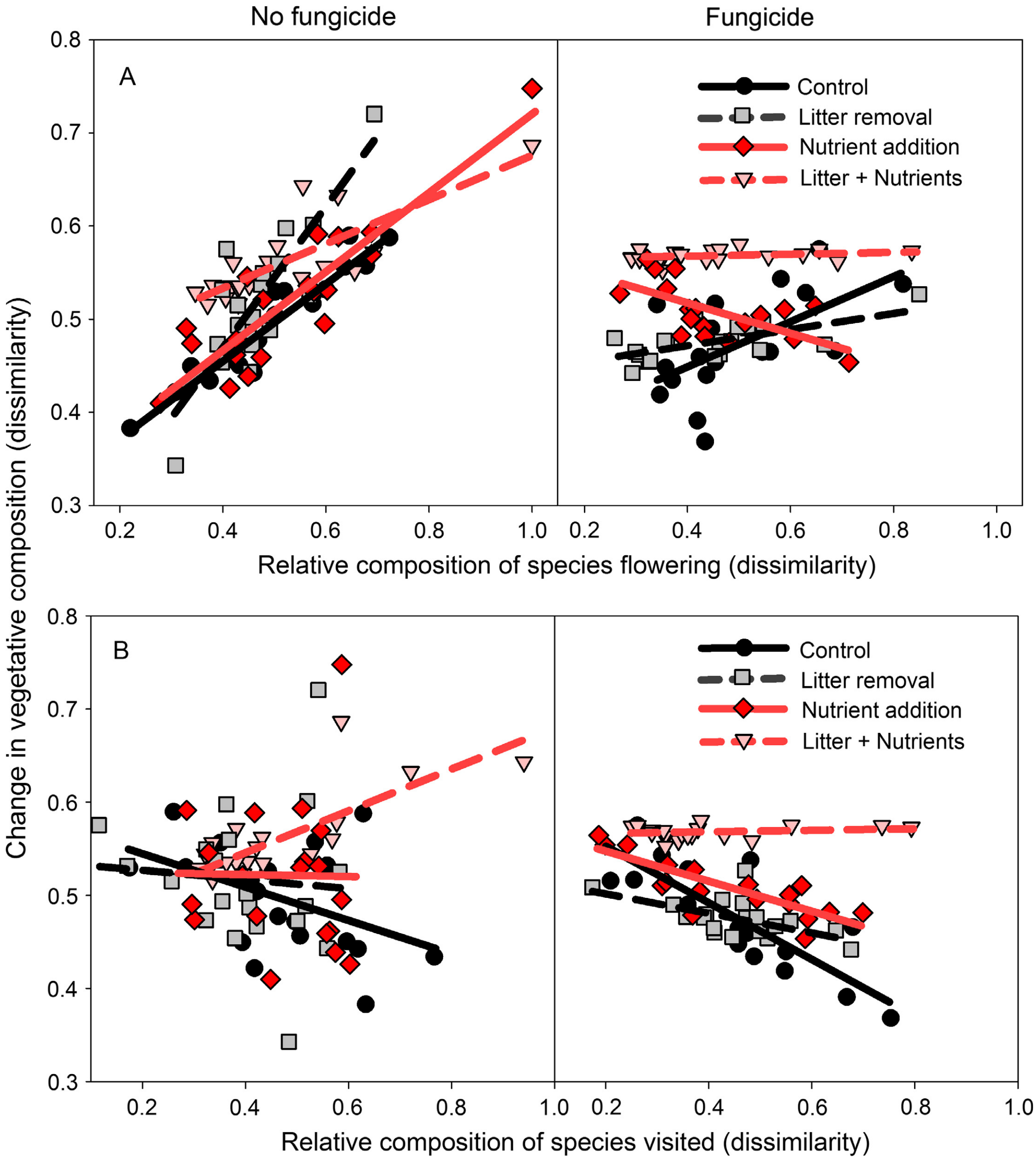

FIG. 1. The change in community composition as a function of (A) the relative composition of species flowering and (B) the relative composition of species visited. Fungicide treated subplots are on the right and subplots without fungicide on the left. Other treatments are as shown in the legend. Relative flowering was measured as dissimilarity between the composition of species as flowering stems and as cover in 2010, and relative visitation as dissimilarity between the composition of species flowering and those being visited. Data points represent model predictions with associated linear regressions.

(Fig. 3B; $F_{1,114} = 3.74$, $P = 0.056$), but no other treatments had any significant effect (see Appendix S1: Table S3). There were also no significant effects of the treatments on the composition or number of species being visited by insects (Fig. 3C, D, Appendix S1: Table S3).

DISCUSSION

Both flowering and visitation were related to future changes in the community, but in opposite ways. Plots in which flowers were not proportional to vegetative abundances exhibited the greatest change in community composition. By contrast, disproportionate visitation among flowers was associated with fewer changes in community composition. These results suggest that allocation toward flowering stem production and pollinator attraction represent different strategies within the community, but that both may be useful indicators of future community change. However, the relationship between flowering and community change was disrupted by the treatments, primarily the suppression of mycorrhizal fungi. The sensitivity of this relationship to changes in the environment could be attributed to either changes in the relationship between flowering and vegetative growth or change in recruitment within the community. Disruption of either of these processes may reduce the utility of flower production as an indicator of long-term community dynamics.

TABLE 1. Results of mixed models testing the effects of the treatments, flowering, and visitation on changes in species richness and community composition.

	Composition			Species richness		
Factor	df	*F*	*P*	df	*F*	*P*
Fungicide	**116**	**4.90**	**0.029**	**19**	**8.31**	**0.010**
Nutrient addition	123	1.61	0.207	**103**	**39.68**	**<0.001**
Litter removal	120	0.60	0.441	103	0.84	0.362
Fungicide × Nutrient	123	0.05	0.830	**103**	**10.03**	**0.002**
Fungicide × Litter	120	0.06	0.806	103	0.16	0.691
Nutrient × Litter	122	0.06	0.811	**104**	**4.41**	**0.038**
Fungicide × Nutrient × Litter	122	0.42	0.520	104	2.84	0.095
Flowering	**106**	**13.80**	**<0.001**	**125**	**9.85**	**0.002**
Fungicide × Flowering	**106**	**9.47**	**0.003**	102	0.01	0.933
Nutrient × Flowering	122	3.78	0.054	122	1.35	0.247
Litter × Flowering	118	0.26	0.612	122	1.63	0.205
Fungicide × Nutrient × Flowering	122	0.35	0.557	**123**	**6.82**	**0.010**
Fungicide × Litter × Flowering	118	0.20	0.654	123	0.16	0.690
Nutrient × Litter × Flowering	120	1.50	0.223	124	1.97	0.163
Fungicide × Nutrient × Litter × Flowering	**120**	**4.30**	**0.040**	123	1.01	0.316
Visitation	**127**	**3.97**	**0.048**	133	1.38	0.242
Fungicide × Visitation	127	0.12	0.732	111	0.06	0.811
Nutrient × Visitation	127	1.39	0.241	125	1.79	0.184
Litter × Visitation	119	1.11	0.294	127	0.75	0.388
Fungicide × Nutrient × Visitation	127	0.01	0.931	123	0.02	0.882
Fungicide × Litter × Visitation	119	0.04	0.837	127	0.05	0.825
Nutrient × Litter × Visitation	124	2.03	0.157	129	0.91	0.342
Fungicide × Nutrient × Litter × Visitation	124	1.83	0.179	129	3.47	0.065

Notes: Degrees of freedom were estimated using the Satterthwaite method. Effects significant at $P < 0.05$ are shown in boldface text.

Flowering, visitation, and community change

Under ambient conditions, we predicted that relative flowering and floral visitation would be positively related to changes in the community because species that produce more seed should become more abundant, whereas species that do not produce seed should decline in abundance and may be excluded from the community (Burkle and Alarcón 2011, Maron et al. 2014, Larson and Funk 2016). For flowering, our results were consistent with this hypothesis for both community composition and species richness, but it is unclear whether this relationship can be attributed to recruitment, as recruitment can take a long time to influence community structure in perennial grasslands (Stampfli and Zeiter 2004). However, individuals in beneficial conditions frequently increase both growth and sexual allocation, while stressed individuals allocate resources to persistence, rather than reproduction (Hartnett 1990, Karlsson and Mendez 2005). A lack of flowers for stressed individuals would also reduce the relative number of species flowering and may forecast their loss from the community. These scenarios could account for the positive relationship between flowering and changes in community structure, irrespective of any changes in recruitment. Future work should measure relative seed production and recruitment to differentiate between these mechanisms. Nevertheless, the distribution of flowering effort among individuals and populations remains a useful indicator of future changes in community composition and species richness in clonal perennial communities, at least in relatively stable conditions.

By contrast, the distribution of visits within the community were negatively associated with changes in the composition of insect-pollinated species. In other words, community composition changed the least in plots where visits were unevenly distributed among species and changed the most in plots where visits were evenly distributed. This relationship may result from differences among species in their allocation toward vegetative and sexual reproduction, which can vary greatly (Herben et al. 2015). Species that rely primarily on sexual reproduction may require disproportionately higher visitation rates to maintain their population, whereas pollinator attraction can be less important for clonal species (Lovett Doust 1989). Consequently, the community will be stable if those species requiring pollination for population growth receive a greater proportion of the visits and are able to produce seeds and new individuals. Even without recruitment, declining visitation rates for these species and more evenly distributed visits among flowers may signal future population declines, if resources are allocated away from pollinator attraction toward persistence (Karlsson and Mendez 2005).

At first glance, the fact that communities change more when flowering is unevenly distributed among species, but less when visits are unevenly distributed among species seems contradictory. These two relationships may represent two subsets of the community: species that rely on vegetative reproduction and those that rely on sexual reproduction. Many of the species at the study site produce extensive clones. Clonal species in beneficial conditions will expand vegetatively (Lovett Doust 1989), but many clonal perennials will also increase sexual allocation (Hartnett 1990). This

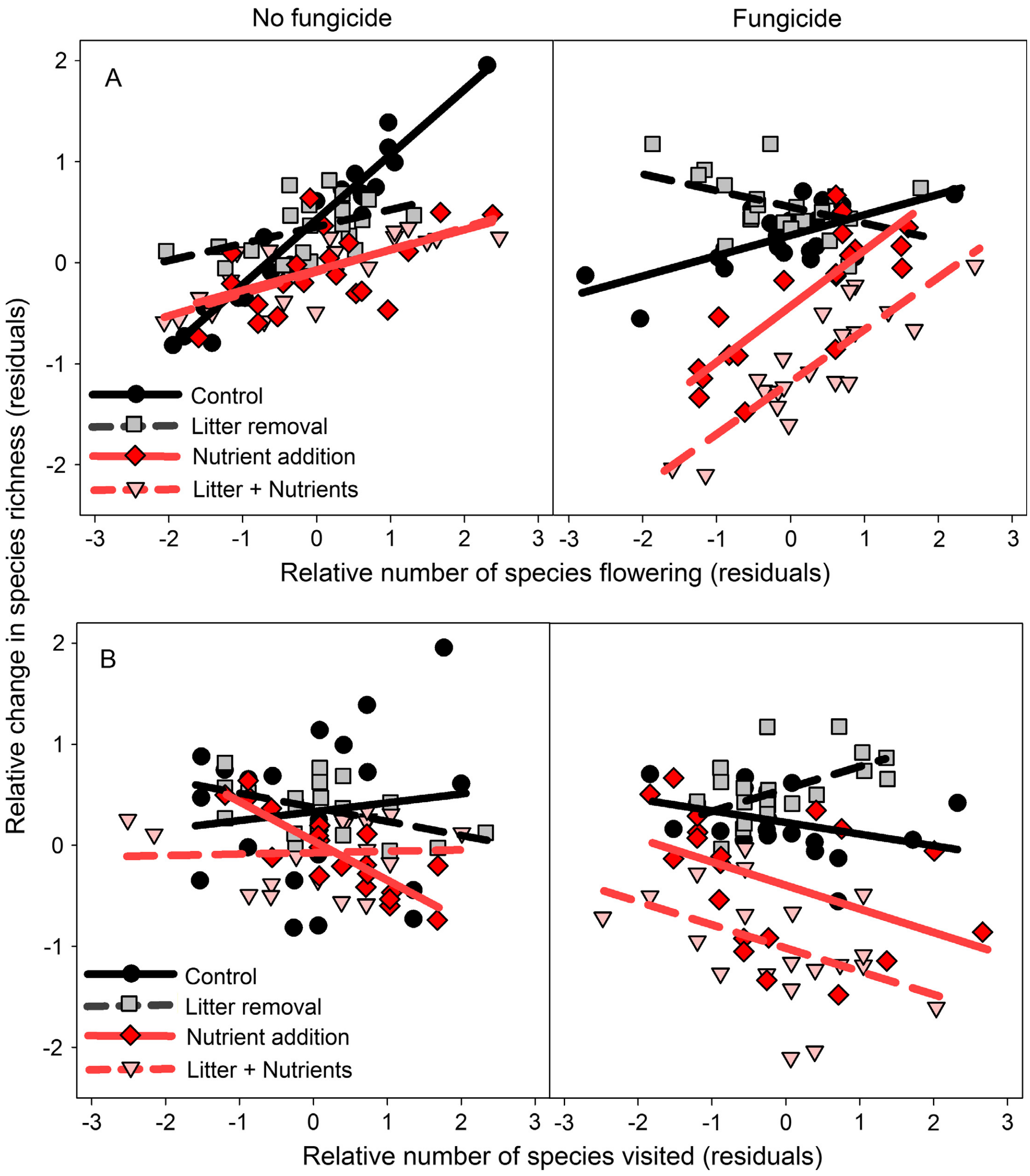

FIG. 2. The relationships between (A) the relative number of species flowering and the change in species richness and (B) the relative number of species visited and the change in species richness. Subplots without fungicide application are on the left and subplots with fungicide application are on the right. Other treatments are as shown in the legend. Here, relative flowering was measured as the standardized residuals between the number of species flowering and those observed vegetatively, and relative visitation as the standardized residuals between the number of species visited and flowering. Data points represent model predicted values and lines represent linear regression based on those values.

would increase dissimilarity between vegetative and flowering stem abundances and may forecast expansion of the genet at the expense of other species that are either non-clonal or expand slowly. Non-clonal or weakly clonal species are likely to rely more heavily on sexual reproduction for population growth (Herben et al. 2015). They are also smaller and may not have the resources to produce more flowering stems, which can impose significant costs on plants (Obeso 2002). These non-clonal or weakly clonal species still require seed production to maintain their populations, so may increase allocation to floral reward to minimize pollen limitation (Knight et al. 2005). By splitting the data set into species that are either aggressively clonal or non-clonal and weakly clonal (Appendix S1: Table S4), we find results that are partially consistent with this hypothesis. Aggressively clonal species drive the relationship between flowering and species richness and only less aggressive species show a negative relationship between visitation and changes in composition (Appendix S1: Table S5). However, neither group exhibited a strong relationship between flowering and changes in composition. It also remains possible that some species allocate resources to flowering in beneficial conditions, and to pollinator attraction when resources are limited. Nevertheless, the observed patterns still indicate that

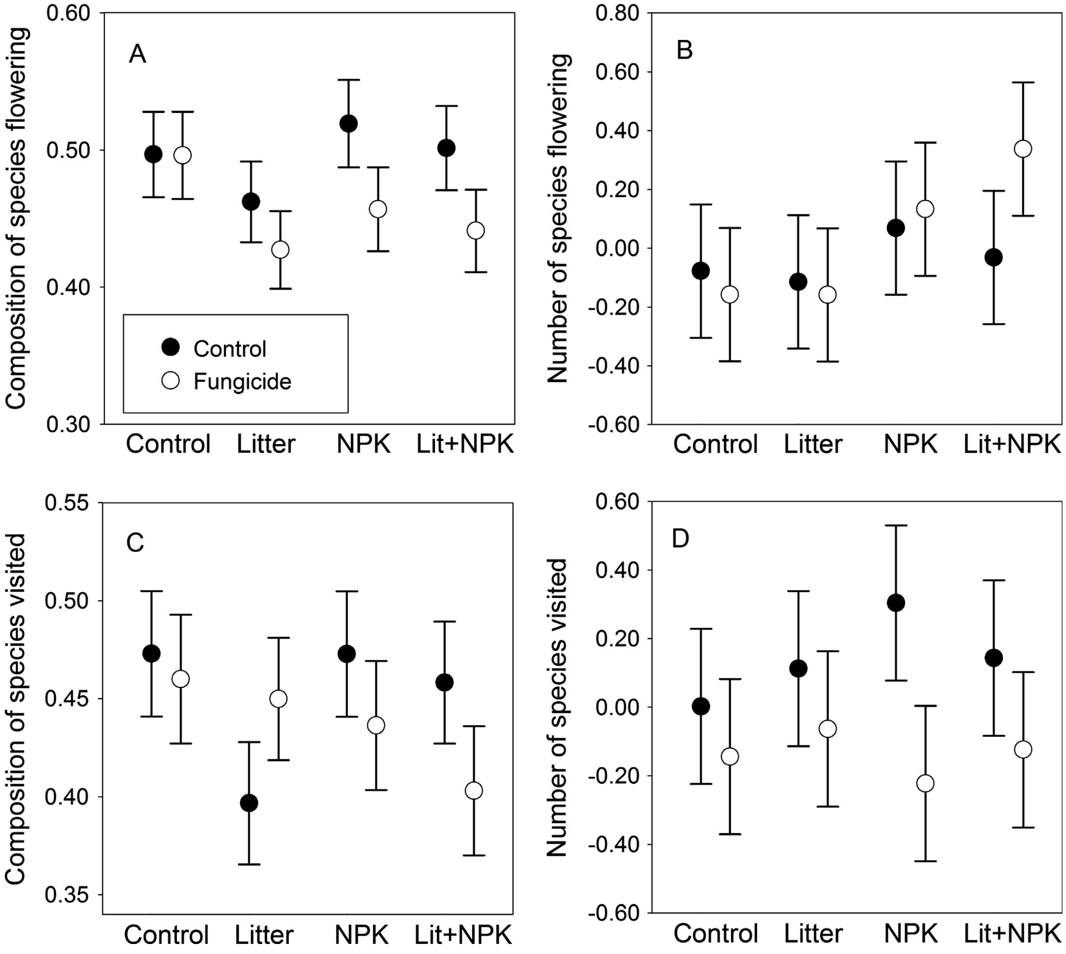

Fig. 3. Fungicide, nutrient addition (NPK), and litter removal effects on relative flowering and floral visitation. Black circles denote conditions without fungicide and white circles with fungicide. In all cases, flowering is relative to cover and visitation relative to flowering. Shown are Bray-Curtis dissimilarity between (A) the composition of flowering stems and cover or (B) between visits and flowering stems and (C) the number of species flowering relative to those observed as cover or (D) the number of species visited relative to the number flowering. For species richness (C, D), the values represent standardized residuals from linear regressions. Means and standard errors are estimated using the mixed models (see *Materials and Methods*).

community stability should result from a proportionate distribution of flowers among species and an uneven distribution of visits among flowers.

Effects of AMF, nutrients, and litter

Although flowering was a reliable indicator of future changes in community composition and species richness under ambient conditions, this was not the case when AMF were suppressed. Further, the effect of the other treatments on the relationship between flowering and community change only occurred when AMF were suppressed, indicating that mycorrhizas play a prominent role in regulating community dynamics. The precise mechanism is unclear. Previous studies have shown that mycorrhizas can alter sexual allocation, seed production, and seedling establishment, but that the effects differ among species (Wolfe et al. 2005, Cahill et al. 2008, van der Heijden and Horton 2009, Bennett and Cahill 2016). By altering sexual allocation for a subset of the species, AMF suppression could eliminate the relationship between flowering and changes in community composition or species richness without any effect on recruitment. However, we found no significant effects of AMF suppression on the relative composition or number of species flowering, suggesting that changes in recruitment dynamics are likely involved. This supports the hypothesis that AMF modulate plant communities through effects on recruitment in addition to effects on adult plants (van der Heijden and Horton 2009, Bennett and Cahill 2016).

Nutrient addition only affected the relationship between flowering and community changes when AMF were suppressed, but the effect differed between composition and richness. Nutrient addition resulted in significant shading within the plots, largely due to an increase in the abundance of graminoids (Appendix S1: Table S2, Fig. S1). Low-light conditions can increase competitive exclusion (Hautier et al. 2009) and inhibit recruitment by reducing microsite availability (Foster and Gross 1998), which likely led to the observed loss of insect-pollinated species (Appendix S1: Fig. S4). Species loss was greatest in plots where relatively few species flowered and required AMF suppression, indicating that nutrient addition had the greatest effect on already stressed plants that had their mycorrhizas disrupted. The loss of mycorrhizas likely increased competitive pressure from the dominant C3 grasses, which increased with nutrient addition and are typically less reliant on mycorrhizas (Reinhart et al. 2012).

When considering composition, the combination of nutrient addition and AMF suppression resulted in fewer changes in the community when flowering was dissimilar to community composition, and greater changes when flowering was more proportionate to abundances. Nutrient addition stimulated flowering and the number of species flowering (Appendix S1: Fig. S4), as found in other studies (Burkle and Irwin 2010), but caused no change in the composition of species flowering. This suggests that nutrients stimulate flowering for both common and rarer species. For rarer species, flowering may represent an attempt to pass their genes on to future generations in poor conditions (Griffiths and Bonser 2013). When these species flower, the composition of species flowering would become more proportional to their vegetative abundances. However, if these species are subsequently lost from the community, due to increased competition and the loss of mycorrhizas, this may cause changes in community composition, despite the floral display being similar to initial vegetative abundances. This relationship was offset once litter was removed. This suggests that increased light reduced competitive exclusion (Hautier et al. 2009) or stimulated recruitment, but species losses were greatest in plots where all three treatments were applied (Fig. 2). Consequently, it is more likely that the combined effects of all three treatments cause changes in the community that overwhelm any relationship between flowering and changes in composition.

CONCLUSIONS

Our results show that flowering and floral visitation are potentially important indicators of future community dynamics, but that these two indicators may be linked to different subsets of the community. For flowering, the relationship with community composition and richness was easily disrupted, indicating that effects on sexual reproduction and recruitment are a potentially critical, yet frequently ignored, pathway by which environmental change may alter plant communities. Over the short term, any disruption of this pathway may be minimized by maintaining intact mycorrhizas. However, it is unclear whether the link between sexual allocation and future community change are indicative of altered recruitment or plant health. Measurements of seed production and recruitment are required to isolate any particular mechanism. Nonetheless, changes in community composition and diversity mediated through altered recruitment will undoubtedly become important over the long-term (Stampfli and Zeiter 2004, Burkle et al. 2013), reinforcing the need for further study on the role of sexual reproduction within perennial plant communities.

ACKNOWLEDGMENTS

We would like to thank G. Singh, N. Mahaffi, K. Higgins, E. Gould, P. Belter, P. Rosa, J. Siren, S. White, and K. Peck for help in the field. We also thank the Kinsella ranch staff for logistical support. This work was funded by an NSERC discovery grant and NSERC DAS to J. F. Cahill and an ACA Biodiversity Grant to J. A. Bennett and J. F. Cahill. J. A. Bennett was supported by NSERC PGS-D and Alberta QEII Scholarships and an NSERC PDF.

LITERATURE CITED

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. http://CRAN.R-project.org/package=lme4

Baude, M., J. Leloup, S. Suchail, B. Allard, D. Benest, J. Mériguet, N. Nunan, I. Dajoz, and X. Raynaud. 2011. Litter inputs and plant interactions affect nectar sugar content. Journal of Ecology 99:828–837.

Bennett, J. A., and J. F. Cahill. 2013. Conservatism of responses to environmental change is rare under natural conditions in a native grassland. Perspectives in Plant Ecology, Evolution and Systematics 15:328–337.

Bennett, J. A., and J. F. Cahill. 2016. Fungal effects on plant-plant interactions contribute to grassland plant abundances: evidence from the field. Journal of Ecology 104:755–764.

Bennett, J. A., G. C. Gensler, and J. F. Jr Cahill. 2014*a*. Small-scale bee patch use is affected equally by flower availability and local habitat configuration. Basic and Applied Ecology 15:260–268.

Bennett, J. A., G. C. Stotz, and J. F. Cahill. 2014*b*. Patterns of phylogenetic diversity are linked to invasion impacts, not invasion resistance, in a native grassland. Journal of Vegetation Science 25:1315–1326.

Biesmeijer, J. C., et al. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313:351–354.

Brosi, B. J., and H. M. Briggs. 2013. Single pollinator species losses reduce floral fidelity and plant reproductive function. Proceedings of the National Academy of Sciences USA 110:13044–13048.

Brunbjerg, A. K., J.-C. Svenning, and R. Ejrnæs. 2014. Experimental evidence for disturbance as key to the conservation of dune grassland. Biological Conservation 174:101–110.

Burkle, L. A., and R. Alarcón. 2011. The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. American Journal of Botany 98:528–538.

Burkle, L. A., and R. E. Irwin. 2010. Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. Journal of Ecology 98:705–717.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. Science 339:1611–1615.

Cahill, J. F., E. Elle, G. R. Smith, and B. H. Shore. 2008. Disruption of a belowground mutualism alters interactions between plants and their floral visitors. Ecology 89:1791–1801.

Cook, S. J., and D. Ratcliff. 1984. A study of the effects of root and shoot competition on the growth of green panic (*Panicum maximum* var. trichoglume) seedlings in an existing grassland using root exclusion tubes. Journal of Applied Ecology 21:971–982.

Dickson, T. L., and B. L. Foster. 2011. Fertilization decreases plant biodiversity even when light is not limiting. Ecology Letters 14:380–388.

Facelli, J. M., and S. T. A. Pickett. 1991. Plant litter—its dynamics and effects on plant community structure. Botanical Review 57:1–32.

Foster, B. L., and K. L. Gross. 1998. Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. Ecology 79:2593–2602.

Foster, B. L., E. J. Questad, C. D. Collins, C. A. Murphy, T. L. Dickson, and V. H. Smith. 2011. Seed availability constrains plant species sorting along a soil fertility gradient. Journal of Ecology 99:473–481.

Gill, R., I. C. Burke, D. G. Milchunas, and W. K. Lauenroth. 1999. Relationship between root biomass and soil organic matter pools in the shortgrass steppe of Eastern Colorado. Ecosystems 2:226–236.

Griffiths, J. G., and S. P. Bonser. 2013. Is sex advantageous in adverse environments? A test of the abandon-ship hypothesis. American Naturalist 182:718–725.

Hartnett, D. 1990. Size-dependent allocation to sexual and vegetative reproduction in four clonal composites. Oecologia 84:254–259.

Hartnett, D. C., and G. W. T. Wilson. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant and Soil 244:319–331.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324:636–638.

Herben, T., B. Šerá, and J. Klimešová. 2015. Clonal growth and sexual reproduction: tradeoffs and environmental constraints. Oikos 124:469–476.

Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135:575–585.

Johnson, N. C., G. W. T. Wilson, J. A. Wilson, R. M. Miller, and M. A. Bowker. 2015. Mycorrhizal phenotypes and the law of the minimum. New Phytologist 205:1473–1484.

Karlsson, P. S., and M. Mendez. 2005. The resource economy of plant reproduction. Pages 1–49 *in* E. G. Reekie, and F. A. Bazzaz, editors. Reproductive allocation in plants. Elsevier, San Francisco, California, USA.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. Annual Review of Ecology Evolution and Systematics 36:467–497.

Kuznetsova, A., P. Brockhoff, and R. Christensen. 2014. LmerTest: Tests for random and fixed effects for linear mixed effect models.

Lamb, E. G. 2008. Direct and indirect control of grassland community structure by litter, resources, and biomass. Ecology 89:216–225.

Lamb, E. G., B. H. Shore, and J. F. Cahill. 2007. Water and nitrogen addition differentially impact plant competition in a native rough fescue grassland. Plant Ecology 192:21–33.

Larson, J. E., and J. L. Funk. 2016. Regeneration: an overlooked aspect of trait-based plant community assembly models. Journal of Ecology 104:1284–1298.

Legendre, P., and E. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.

Lovett Doust, J. 1989. Plant reproductive strategies and resource allocation. Trends in Ecology and Evolution 4:230–234.

Loydi, A., R. L. Eckstein, A. Otte, and T. W. Donath. 2013. Effects of litter on seedling establishment in natural and semi-natural grasslands: a meta-analysis. Journal of Ecology 101:454–464.

Maron, J. L., K. C. Baer, and A. L. Angert. 2014. Disentangling the drivers of context-dependent plant–animal interactions. Journal of Ecology 102:1485–1496.

Masters, J. A., and S. M. Emery. 2015. Leaf litter depth has only a small influence on *Ranunculus ficaria* (ranunculaceae) biomass and reproduction. American Midland Naturalist 173:30–37.

Myers, J. A., and K. E. Harms. 2009. Seed arrival, ecological filters, and plant species richness: a meta-analysis. Ecology Letters 12:1250–1260.

Obeso, J. R. 2002. The costs of reproduction in plants. New Phytologist 155:321–348.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Reinhart, K. O., G. W. T. Wilson, and M. J. Rinella. 2012. Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. Ecology Letters 15:689–695.

Sargent, R. D., and D. D. Ackerly. 2008. Plant–pollinator interactions and the assembly of plant communities. Trends in Ecology and Evolution 23:123–130.

Stampfli, A., and M. Zeiter. 2004. Plant regeneration directs changes in grassland composition after extreme drought: a 13-year study in southern Switzerland. Journal of Ecology 92:568–576.

Traveset, A., C. Tur, K. Trøjelsgaard, R. Heleno, R. Castro-Urgal, and J. M. Olesen. 2016. Global patterns of mainland and insular pollination networks. Global Ecology and Biogeography 25: 880–890.

van der Heijden, M. G. A., and T. R. Horton. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology 97:1139–1150.

West, H. M., A. H. Fitter, and A. R. Watkinson. 1993. The influence of 3 biocides on the fungal associates of the roots of *Vulpia ciliata* ssp *ambigua* under natural conditions. Journal of Ecology 81:345–350.

Wolfe, B. E., B. C. Husband, and J. N. Klironomos. 2005. Effects of a belowground mutualism on an aboveground mutualism. Ecology Letters 8:218–223.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2357/suppinfo