

Bee diversity effects on pollination depend on functional complementarity and niche shifts

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Abstract. Biodiversity is important for many ecosystem processes. Global declines in pollinator diversity and abundance have been recognized, raising concerns about a pollination crisis of crops and wild plants. However, experimental evidence for effects of pollinator species diversity on plant reproduction is extremely scarce. We established communities with 1–5 bee species to test how seed production of a plant community is determined by bee diversity. Higher bee diversity resulted in higher seed production, but the strongest difference was observed for one compared to more than one bee species. Functional complementarity among bee species had a far higher explanatory power than bee diversity, suggesting that additional bee species only benefit pollination when they increase coverage of functional niches. In our experiment, complementarity was driven by differences in flower and temperature preferences. Interspecific interactions among bee species contributed to realized functional complementarity, as bees reduced interspecific overlap by shifting to alternative flowers in the presence of other species. This increased the number of plant species visited by a bee community and demonstrates a new mechanism for a biodiversity–function relationship (“interactive complementarity”). In conclusion, our results highlight both the importance of bee functional diversity for the reproduction of plant communities and the need to identify complementarity traits for accurately predicting pollination services by different bee communities.

Key words: biodiversity–ecosystem functioning; complementarity effect; functional diversity; interspecific competition; niche plasticity; plant–animal interactions; plant pollinator network; plant reproduction; specialization.

INTRODUCTION

Positive effects of biodiversity on functioning of terrestrial ecosystems have mainly been shown by experimental manipulations of plant diversity (Loreau et al. 2001, Cardinale et al. 2006, Scherber et al. 2010, Isbell et al. 2011), whereas experiments manipulating diversity of higher trophic levels are more sparse (Duffy et al. 2007, Finke and Snyder 2008). Biotic pollination is important for wild plant reproduction (Ollerton et al. 2011) and crop pollination as an ecosystem service (Klein et al. 2003). Recent large-scale declines in pollinator diversity (Biesmeijer et al. 2006, Potts et al. 2010) raise concerns about the maintenance of pollination services, but biodiversity experiments focusing on pollinators are surprisingly limited. Previous studies on pollinator diversity effects have employed natural gradients of diversity, leaving species richness confounded with other variables such as environment, abundance,

or community composition (Kremen et al. 2002, Klein et al. 2003, Potts et al. 2006, Gómez et al. 2007, Hoehn et al. 2008). Most of these studies suggest a positive effect of bee species diversity on pollination, but this has still not been confirmed experimentally. There is only a single published experiment on diversity effects on pollination (Fontaine et al. 2006), which did not separate effects of diversity from number of pollinator individuals, considered only two levels of functional group diversity without controlling species richness, and did not include nesting sites for bees. To understand consequences of pollinator declines and to bridge the gap between the previous studies, experiments assessing the effects of pollinator diversity on plant reproduction independently of important confounding factors and in more realistic settings are highly needed.

Mechanistic explanations of biodiversity effects commonly involve functional niche complementarity (Loreau and Hector 2001), which has also been suggested for pollinator diversity (Fontaine et al. 2006, Hoehn et al. 2008, Tylianakis et al. 2008). Bee species could have complementary pollination niches because they differ in the plant species they visit and in the place and time of visitation (Hoehn et al. 2008, Blüthgen and Klein 2011), but this need not be the case because most bee species

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are considered to be trophic generalists (Williams et al. 2010). An “interactive complementarity effect” can arise when interspecific interactions such as facilitation, interference, intraguild predation, or competition change the outcome expected from fundamental niches or a species’ behavior in isolation (Cardinale et al. 2002, Casula et al. 2006, Ashton et al. 2010). For example, interactions with wild bees have been shown to increase honey bees’ pollination effectiveness on hybrid sunflowers (Greenleaf and Kremen 2006). Interspecific competition should force species to reduce niche overlap with coexisting species (interspecific repulsion), which may be realized by niche contraction or niche shifts (Morse 1974). Thus pollinators may respond to interspecific competition by increasing specialization (Inouye 1978) and shifting to less preferred plant species (Walther-Hellwig et al. 2006). Both responses might have positive effects on pollination function: pollinator specialization may increase conspecific pollen deposition (Wissel 1977), while niche shifts to alternative plants may cause more complete coverage of the whole plant community.

In this study, we performed a biodiversity experiment manipulating species richness of bees as the most important group of pollinators (Potts et al. 2010) and tested the effects of bee diversity and functional complementarity on seed production of a sown plant community including nine pollinator-dependent plant species. We established caged bee communities of one to five species in different combinations, standardizing the total number of bees per cage to identify bee diversity effects independent of total bee abundance and to vary the degree of intraspecific and interspecific competition. Niche complementarity in flower visitation was quantified for two niche dimensions: temporal niche partitioning driven by differences in temperature dependence of activity (Willmer and Corbet 1981, Willmer 1983) and niche partitioning regarding the plant species visited (Fründ et al. 2010, Blüthgen and Klein 2011). We test whether the presence of other species leads to larger realized differences in flower visitation, and whether the relationship between bee diversity and plant community pollination can be explained by functional niche coverage as the outcome of fundamental and interactive complementarity.

METHODS

Experimental design

We established 55 caged “mesocosms” (each $4 \times 2 \times 2$ m = 16 m³) with one to five wild bee species (Fig. 1a, b) in different combinations, following a substitutive design aimed at analyzing bee diversity effects independent of total bee abundance (39 cages with bees; eight control cages without bees, “syrphids only”; four control cages without any pollinators, “no pollinators”; and four open cages; Table 1; see the Appendix for details). Plants were allowed to be colonized by other insects (mainly hoverflies) before cages were closed. All insects were regularly removed from the no pollinators

controls. The total number of bees per cage was 20.5 ± 2.7 (mean \pm SD). Treatments were randomly assigned to cages (see Fig. A1 in the Appendix). The experiment was performed on the experimental field of the Agroecology group in Göttingen, Germany, in 2008. Seeds of the same plant community in each cage were sown in April 2008. Some plants started flowering in early June. On 12–16 June, early blooming flowers were cut. By the end of June most species were in flower. The main experimental period started with the introduction of target bee species on 3–5 July and lasted five weeks until 8 August 2008. Fruits were harvested from 30 July to 27 August 2008.

In this study, we used a substitutive design (see Appendix for further details), focusing on the effect of diversity independent of total abundance. Each bee species was present in cages across the diversity gradient. As is often the case in biodiversity experiments, it was impossible to realize all possible species combinations (Bell et al. 2009); in our case this was due to limited availability of some bee species.

Plant community and reproductive success

Seeds were purchased from commercial suppliers of wild plants (Appendix). In each cage, a community of 16 plant species was sown in a regular pattern with two patches per plant species (Appendix: Fig. A2), with intraspecific aggregation reducing interspecific competition among plants (Wassmuth et al. 2009). Aiming for a standardized target plant density, the number of seeds sown per species was based on prior germination tests. Seeds were sown into standardized commercial soil substrate (Fruhstorfer Erde T25, Hawita, Lauterbach, Germany; 600 L per cage) that was evenly spread on the tilled soil. After sowing, plants were first allowed to grow without isolation netting and watered as needed. Unsown plants were controlled by weeding and grass-specific herbicide (Fusilade; Syngenta, Basel, Switzerland) before the cages were closed on 2 June 2008 with netting of 0.8-mm mesh size (Rantai Typ S48; Schachtrupp, Hamburg, Germany).

The number of flowering units per plant species and cage was counted twice during the main phase (counting dates 9–11 July and 28–30 July; see Appendix: Table A1 for plant species details). Eleven of the sown plant species were in full bloom during the main experimental phase (*Centaurea cyanus*, *Chrysanthemum segetum*, *Consolida regalis*, *Crepis capillaris*, *Legousia speculum-veneris*, *Lotus corniculatus*, *Medicago × varia*, *Orlaya grandiflora*, *Scandix pecten-veneris*, *Sinapis arvensis*, and *Vicia villosa*).

For these 11 plant species (others were considered for flower visitation, but not for seed production), fruiting units were harvested when a species had mostly ripened, three to four weeks after its peak flowering time. For a given plant species, all cages were harvested on the same day. The number of seeds per cage and plant species were assessed by counting the number of fruiting units in

the cage and then counting the number of seeds in a harvested subset (eight fruiting units per patch). A “fruiting unit” was defined as a flower head in Asteraceae, *Lotus* and *Medicago*, as an umbel in Apiaceae, as a pedicle for *Consolida*, *Sinapis*, and *Vicia*, and as a single fruit in *Legousia*. Harvested fruiting units were dried and dissected in the laboratory. In general, all clearly developed seeds in these samples were considered viable and counted.

It was not possible to reliably distinguish fruits containing a seed from empty fruits in Asteraceae, whose fruits form an inseparable unit with the single seed. Therefore, viability of seeds was assessed by germination tests for *Centaurea*, *Chrysanthemum*, and *Crepis*; the number of viable seeds was calculated by multiplying the number of fruits by percentage germination (20 fruits of each patch, i.e., 40 per cage, from separately harvested fully ripe fruiting units were placed in petri dishes with wet filter paper for four weeks in dark climate chambers with 14°C and 96% relative humidity; visibly empty fruits were excluded). To estimate the number of seeds ($N_{i,j}$) per cage i for each plant species j , the number of fruiting units in the cage was multiplied by the number of seeds per fruiting unit in the harvested subset. In *Crepis*, seeds in unripe flower heads could not be counted and were therefore excluded from the calculation.

Nine plant species (all except *Orlaya* and *Scandix*) produced significantly lower numbers of viable seeds in control cages than in cages with bees. These species were considered as pollinator-dependent plants, hereafter “the plant community.” Seed production of the plant community (SP_i , the main response variable) was calculated per cage i as the mean of standardized seed numbers (i.e., observed number of seeds divided by the maximum of the respective plant species) across plant species:

$$SP_i = \frac{\sum_{j=1}^9 \frac{N_{i,j}}{\max_{1-55,j}}}{9}.$$

Pollinator communities

We used five bee species in the experiment: bumble bees (*Bombus terrestris*) inhabiting nest boxes and four solitary bee species inhabiting reed internodes (*Heriades truncorum*, *Hylaeus communis*, *Megachile centuncularis* group, *Osmia bicornis*). These five bee species were selected to represent a relatively realistic distribution of traits within the constraint of sufficient availability. Typical for bee communities in Central Europe (Westrich 1989), one in five species was primitively eusocial (*Bombus*), one in five species was oligolectic (specialized on one plant family, Asteraceae: *Heriades*), body length ranged from 5 mm (*Hylaeus*) to larger than 15 mm (*Bombus*), and the different species collect pollen on different parts of their body.

Small bumble bee colonies were purchased (STB Control, Aarbergen, Germany) and set to the target number of workers (12, 6, 4, 3, and 3 in 1, 2, 3, 4, and 5 species treatments, respectively). Colonies also included a queen and brood, favoring natural behavior. To keep abundance constant despite newly hatching bees, colonies were reduced to the target number of workers every week (removing excess workers and brood cells).

Overwintering solitary bees were collected from trap nests that had been exposed in the field in 2007 around Göttingen, Germany (reed internodes in plastic tubes), and close to Leipzig, Germany (bundles of bamboo internodes). Trap nests were brought into the laboratory in autumn 2007 and stored at 4°C until spring 2008, while opening stems throughout the winter to inspect the type and number of bee brood cells. Due to shortage of material, we used two closely related and functionally similar species of *Megachile* in a constant ratio (2:1 *M. centuncularis* [L.] and *M. versicolor* Smith) and treated them as a functional unit comparable to a species (*M. centuncularis* group) for the experiment (see Westrich 1989 for data on their similar floral niches). After hibernation, bee nests were incubated at room temperature (incubation time per bee species based on prior experience). Bee species combinations (using a 1:1 sex ratio) were prepared in cardboard boxes with paper tube exits. Bees hatching before the start of the experiment were stored in dark climate chambers (12°C). Once all boxes were prepared, they were placed in the cages to allow for near-natural emergence behavior. At this time, bumble bee nest boxes and reed internodes as nesting substrate for solitary bees had already been placed in the cages. See the Appendix for further pollinator details.

Throughout the main phase, flower visitation was observed for all cages. In each of six rounds, all cages were observed in randomized order. In each observation session, one observer slowly walked around the cage and noted the number of flower visitors of each pollinator species per plant patch (average observation time of 7 minutes per session). All observations were performed between 08:00 and 19:00 hours during different weather conditions but not during heavy rain. The total number of visits per cage did not differ significantly among diversity treatments (ANOVA, $F_{4,34} = 0.87$, $P = 0.49$; linear regression, $P = 0.19$, $N = 39$).

Statistical analysis

We compared different models with seed production as the response, and analyzed how three variables based on flower visitation relate to bee diversity. All analyses were performed with the software R 2.11.1 (R Development Core Team 2010). If not stated otherwise, a replicate is considered to be a cage ($N = 39$ cages excluding controls).

Predictors of seed production.—Different functions have been proposed to describe the form of the biodiversity–functioning relationship (Cardinale et al. 2006). These previously proposed functions (Michaelis–

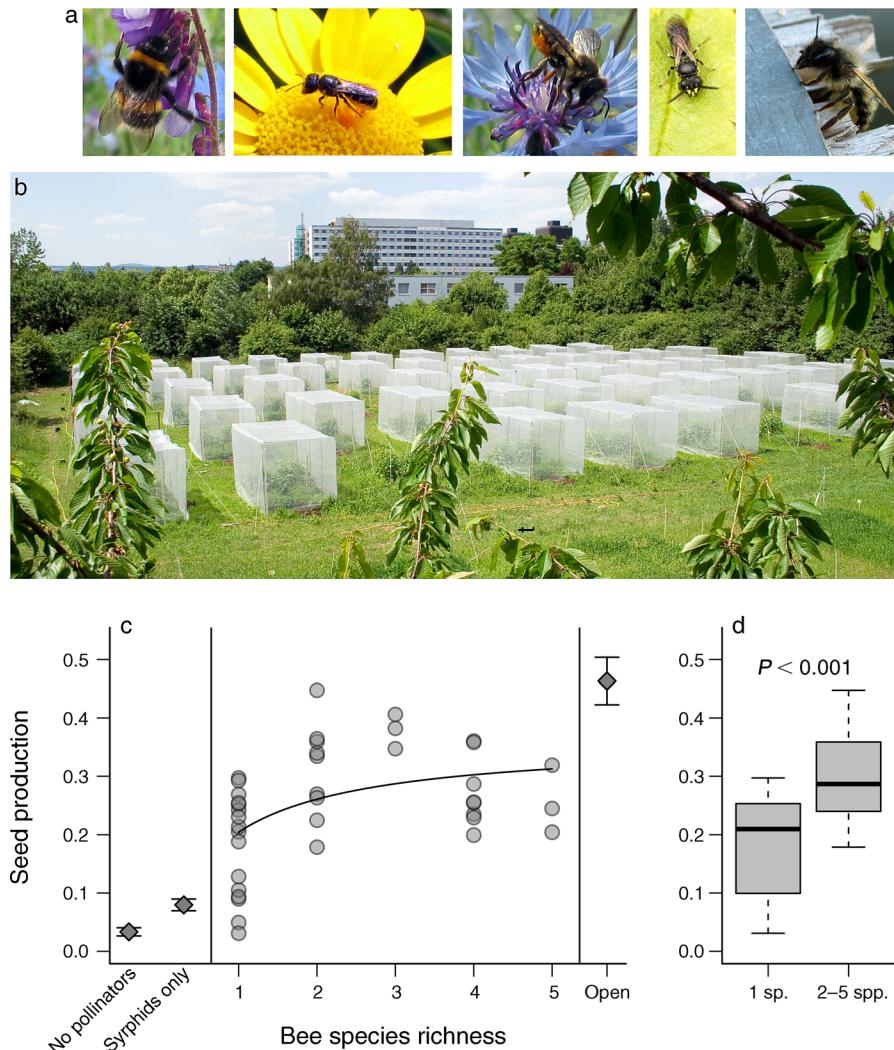


FIG. 1. Experimental test of wild bee species diversity effects on pollination of plant communities. (a) The five bee species inhabiting the cages in different combinations, from left to right: *Bombus terrestris*, *Heriades truncorum*, *Megachile centuncularis* group, *Hylaeus communis*, *Osmia bicornis* (photos by J. F. Fründ, except *H. communis* by J. K. Lindsey). (b) View of the bee diversity experiment (photo by M. von Fragstein). (c, d) Seed production of the plant community (number of seeds per cage, standardized relative to maximum and averaged across nine plant species) in relation to the number of bee species present in a cage (circles represent one data point per cage, $N = 39$). Control treatments: no pollinators, closed cages and regular insect removal, syrphids only, closed cages with syrphid recruitment allowed; open cages, access for natural pollinator community. Diamonds show mean \pm SE for the three types of controls ($N = 4, 8,$ and 4 cages). The solid line shows the best-fitting curve (Michaelis-Menten saturation). For the boxplots, the line in the box is the median; box endpoints indicate the interquartile range; whiskers show the data range.

Menten saturation, power law, log-linear, linear, and an intercept-only null model) were fitted to the relationship between seed production and bee diversity by nonlinear least squares regression and compared with AIC_c (Akaike's information criterion with sample size correction). Models with smallest AIC_c values are best supported by the data, with a difference in $AIC_c > 2$ indicating that the alternative model is clearly worse (Burnham and Anderson 2001). Seed production was related to mechanistic variables (functional niche coverage or bee specialization) by multiple regression, in which explanatory variables were only weakly

correlated ($r \leq 0.34$). Partial residual plots (component plus residuals plots) were used to show the influence of one variable while holding the values of other variables in the model constant (on their mean value). Positive diversity effects on seed production or functional coverage could not be explained by variation in species composition among diversity levels (Appendix: Fig. A3).

Temperature coverage.—Temperature coverage was defined as the integral of bee community activity in relation to temperature. To calculate this, the relationship between temperature and bee foraging activity was estimated for each species by fitting a Gaussian response

TABLE 1. Overview of the design (treatments) of the wild bee species diversity experiment.

Bee species richness	Bee identity/treatment	N replicates	Number of bee individuals†
1	A (<i>Bombus</i>)	4	24 A
1	B (<i>Heriades</i>)	3	24 B
1	C (<i>Hylaeus</i>)	1	24 C
1	D (<i>Megachile</i>)	4	24 D
1	E (<i>Osmia</i>)	4	24 E
2	AB	2	12 A + 12 B
2	AC	1	12 A + 12 C
2	AD	2	12 A + 12 D
2	AE	2	12 A + 12 E
2	DE	2	12 D + 12 E
3	ADE	3	8 A + 8 B + 8 C
4	ABCE	2	6 A + 6 B + 6 C + 6 E
4	ABDE	2	6 A + 6 B + 6 D + 6 E
4	ACDE	2	6 A + 6 C + 6 D + 6 E
4	BCDE	2	6 B + 6 C + 6 D + 6 E
5	ABCDE	3	6 A + 4 B + 4 C + 4 D + 6 E
	Open‡	4	unknown
	Syrphids only§	8	0
	No pollinators¶	4	0

† Letters indicate bee species (see second column); realized number may be slightly lower (21 individuals per cage on average); every week, the number of bumble bee workers was adjusted to half the number given for A (because new workers hatched continuously).

‡ Northward side of cage open during main experimental phase, visits by pollinators from surroundings.

§ Control cages without bees, but without active exclusion of insects during main experimental phase.

¶ Control cages without pollinators, active exclusion of all flying insects and visible pupae every 2–4 days.

curve, using function “nls” in R with a formula adapted from Jongman et al. (1987):

$$\sqrt{\text{activity}_i} = c \cdot \exp\left(\frac{-0.5(\text{temp} - u)^2}{t^2}\right)$$

where activity_{*i*} is the number of visits by bee species *i* observed during an observation session (see last paragraph of *Pollinator communities* for sampling of flower visitation), temp is temperature measured during the observation session (i.e., reflecting variation within and between days), and the fitted parameters are *c* (square root of maximum activity), *u* (optimum temperature), and *t* (tolerance or niche width).

From these species-specific response curves, temperature coverage was calculated in three steps. First, activity_{*i*} was rescaled to a maximum of 1 for each species. Rescaling was used to have temperature-related effects independent of total activity, but temperature coverage without rescaling yielded similar results (Appendix). Second, rescaled response curves were averaged across all bee species present in a community, yielding bee community activity (activity_{com}). Third, the integral of this composite function was calculated numerically:

$$\text{temperature coverage} = \int_{15^\circ\text{C}}^{44^\circ\text{C}} \text{activity}_{\text{com}}(\text{temp})d\text{temp}.$$

Plant species coverage.—Plant species coverage was defined as the number of visited plant species, which may be influenced by low numbers of observations and

variation in flower abundance. To correct for these types of possible sampling artifacts, the number of plant species was divided by the mean of a null model that closely resembled null model “IR” of Ulrich and Gotelli (2010), but also incorporated independently measured data about all available flower resources. The null model simulated completely nonselective flower choice by randomly redistributing interactions among plants within bee species and cage, i.e., it generated data with the same number of visits per bee species and cage. The probability of each plant species to be visited was proportional to its flower abundance in the cage. Results were similar when plant species coverage was not corrected by the null model (Appendix).

Aiming to separate effects of fundamental and realized preferences on plant species coverage, we simulated visitation network data using the following approach: the number of visits per bee species and cage was randomly redistributed among plant species, using interaction probabilities based on flower availability and flower preferences. Flower preferences (forage ratio sensu Krebs 1998, termed “fundamental preferences” here) for each bee species were calculated comparing visits pooled across all cages with one bee species to overall flower availability. These simulations were similar to the null model used to correct plant species coverage, but the probability for a plant species to be visited by a bee species also depended on the estimated flower preferences of this bee species. Plant species coverage calculated from these simulated visitation data

is referred to as “prediction without shifts.” An extended presentation of this simulation can be found in the Appendix. Quantitative niche overlap (O) between a bee species pair was calculated as

$$O = 1 - \frac{\sum_j |p_{1,j} - p_{2,j}|}{2}$$

where $p_{1,j}$ and $p_{2,j}$ are proportional visitation to plant species j by bee species 1 and 2, respectively.

Bee specialization.—Bee specialization was calculated as the index d' (Blüthgen et al. 2006), which describes how strongly resource use (visitation) differs from resource availability. Flower availability per cage was calculated from the counted number of flower units, weighted for each plant species by the total visits received per flower unit across all cages. Bee specialization was compared between cages with one and cages with more than one bee species with a linear mixed-effects model including random effects of cage identity and fixed effects of bee species. Bee community-wide specialization per cage was quantified by the weighted mean of d' (weights equal number of visits of a bee species in that cage). Results for specialization were robust to the choice of alternative specialization metrics (see Appendix). The related index H_2' (Blüthgen et al. 2006) was used for testing for complementarity in networks pooled across cages, but could not be used for individual cages because it is not defined for monospecific communities. Significance of preference differences among bee species in the pooled network was assessed by comparing H_2' to a null model (Blüthgen et al. 2006). Specialization indices were calculated with the R-package “bipartite” 1.15 (Dormann et al. 2009).

RESULTS

Treatments and seed production

First, we looked at the effect of bee species richness on pollination. Overall, a positive nonlinear effect of bee diversity on seed production by the plant community was supported by the data (Fig. 1c). Among the a priori selected functions, a saturating function (Michaelis-Menten) best described the relationship between bee diversity and seed production, followed by log and power functions, while a linear model and the null model clearly performed worse (Table 2). Some uncertainty remains about the exact form of this relationship because species composition was not fully balanced among diversity treatments in our design (Appendix). Plants in cages with two to five bee species produced significantly more seeds than plants in cages with a single bee species (Welch t test, $P < 0.001$, $N = 23$ vs. 16; Fig. 1d; see Appendix: Table A3 for detailed statistics), and this increase could not be explained by additive effects (Appendix: Fig. A4). Seed production in controls was significantly lower than in bee cages of all diversity levels (syrphids only vs. bees, Welch t test, $P < 0.001$, $N = 8$ vs. 39; Fig. 1c), but active exclusion of hoverflies

further lowered seed production (syrphids only vs. no pollinators, Welch t test, $P = 0.002$, $N = 8$ vs. 4; Fig. 1c). Plants in open cages, which were visited by diverse pollinators from the surrounding area, produced more seeds than plants visited by experimental bee communities (Welch t test, $P = 0.004$, $N = 4$ vs. 39; Fig. 1c).

Temperature complementarity

We considered complementarity as the potential underlying driver of bee diversity effects (Figs. 2–4). Complementarity might arise from interspecific variation in temperature responses (Fig. 2a). To quantify temperature responses, a Gaussian curve was fitted to the relationship between foraging activity and current temperature for each bee species (Fig. 2b; $N = 66$ –144 observation sessions per species). These curves were used to estimate temperature niche coverage, i.e., the completeness of flower visitation across variable weather conditions. Temperature niche coverage did not increase monotonically with bee diversity (Fig. 2c; $P > 0.20$ in both linear and log-linear regression, $N = 39$). It tended to be higher when different bee species were combined (Welch t test, $P = 0.021$, $N = 23$ vs. 16; Fig. 2d), but it did not increase beyond two bee species (Fig. 2c), as all bees except *Bombus* had the highest activity during warm and sunny days (Fig. 2b).

Plant species complementarity and niche shifts

Functional complementarity among bee species was also related to differences in the plant species they visited (Fig. 3). These differences were larger when more than one bee species were present, i.e., interspecific overlap was reduced among co-occurring species (a “niche shift”; Fig. 3a; Appendix: Fig. A5). Floral preferences differed significantly among bee species both in a network pooled across single-bee-species treatments and in a network pooled across multiple-bee-species treatments (Fig. 3b, c; specialization index, $H_2' = 0.19$ and 0.22, respectively; excluding the known specialist *Heriades*, $H_2' = 0.15$ and 0.20, respectively; all four $P < 0.001$ in comparison to 1000 null model replicates). Half of all plant species were observed to be visited only in cages with more than one bee species (Fig. 3c; Appendix: Fig. A6).

To link flower preferences to bee diversity and seed production, plant species (niche) coverage was quantified as the number of plant species visited by all bees in a cage, corrected for possible sampling bias. Plant species coverage tended to increase with bee diversity ($P = 0.11$ in linear regression and $P = 0.04$ in log-linear regression, $N = 39$), but the highest values were not observed in cages with the highest number of bee species (Fig. 4a). Plant species coverage was higher in cages with two to five bee species than in cages with one bee species (Welch t test, $P = 0.007$, $N = 23$ vs. 16; Fig. 4b). Importantly, the increase could not be predicted by flower preferences of bees in cages without other species; when preferences were estimated from monospecific

TABLE 2. Comparison of different models explaining plant community seed production.

Model and explanatory variables	AIC _c	ΔAIC _c	R ²
Null model	-67.9	26.9	NA
Bee species richness: linear	-70.8	24.0	0.13
Bee species richness: power	-73.5	21.3	NA
Bee species richness: log-linear	-74.5	20.3	0.21
Bee species richness: saturating (Michaelis-Menten)	-76.2	18.6	NA
Bee species richness: 1 sp. vs. 2–5 spp. (two-level factor in linear model)	-82.1	12.7	0.35
Plant spp. coverage + temperature coverage: linear	-94.8	0	0.56
Plant spp. coverage + temperature coverage + specialization <i>d'</i> : linear	-92.2	2.6	0.56

Note: $N = 39$ cages in all models. NA means not available.

cages and used to simulate visitation in multispecific cages, plant species coverage was underestimated (Fig. 4b; Appendix: Fig. A5). In addition to visits to alternative plant species and higher plant species

coverage by the bee community, the niche shift in the presence of other bee species also involved a significant increase in bee specialization *d'* (linear mixed-effects model, including bee species and random effect of cage,

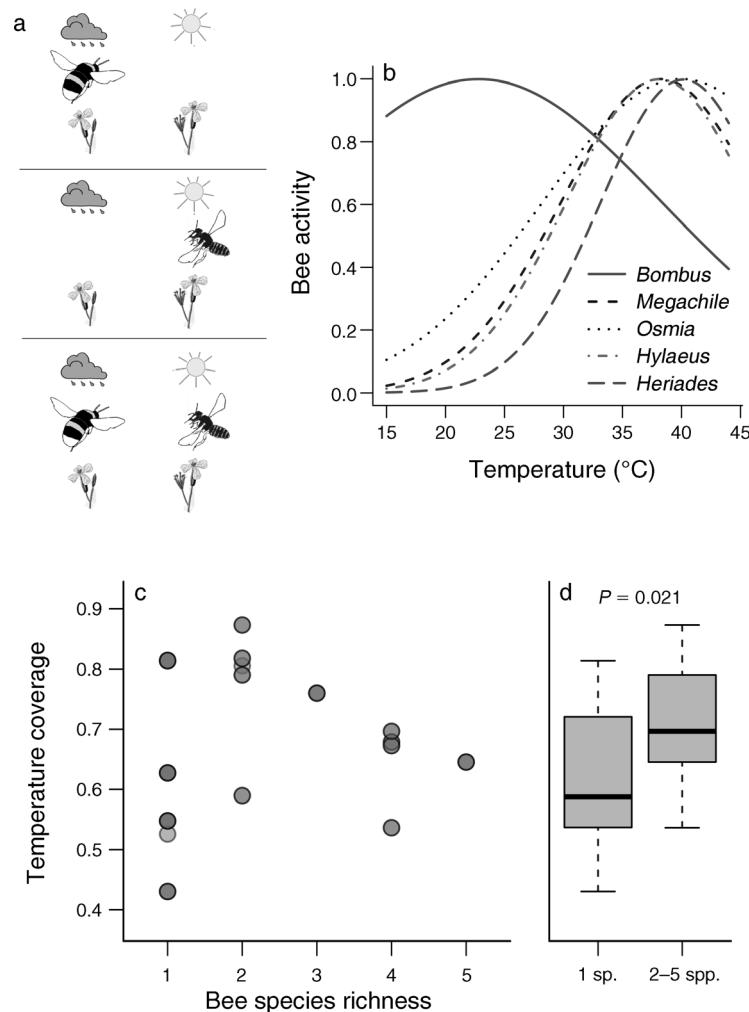


FIG. 2. Functional complementarity among bee species related to weather conditions. (a) Bee species active under different weather conditions provide more complete pollination (visitation) when they occur together. (b) Relationship between temperature (of partly shaded thermometers, integrating air temperature and solar radiation) and flower visitation activity of the five bee species used in the experiment (fitted Gaussian niche curves). (c, d) Functional temperature niche coverage (how evenly a bee community covers the whole temperature range, a measure of complementarity) in relation to the number of bee species present in a cage. Boxplot components are as in Fig. 1d.

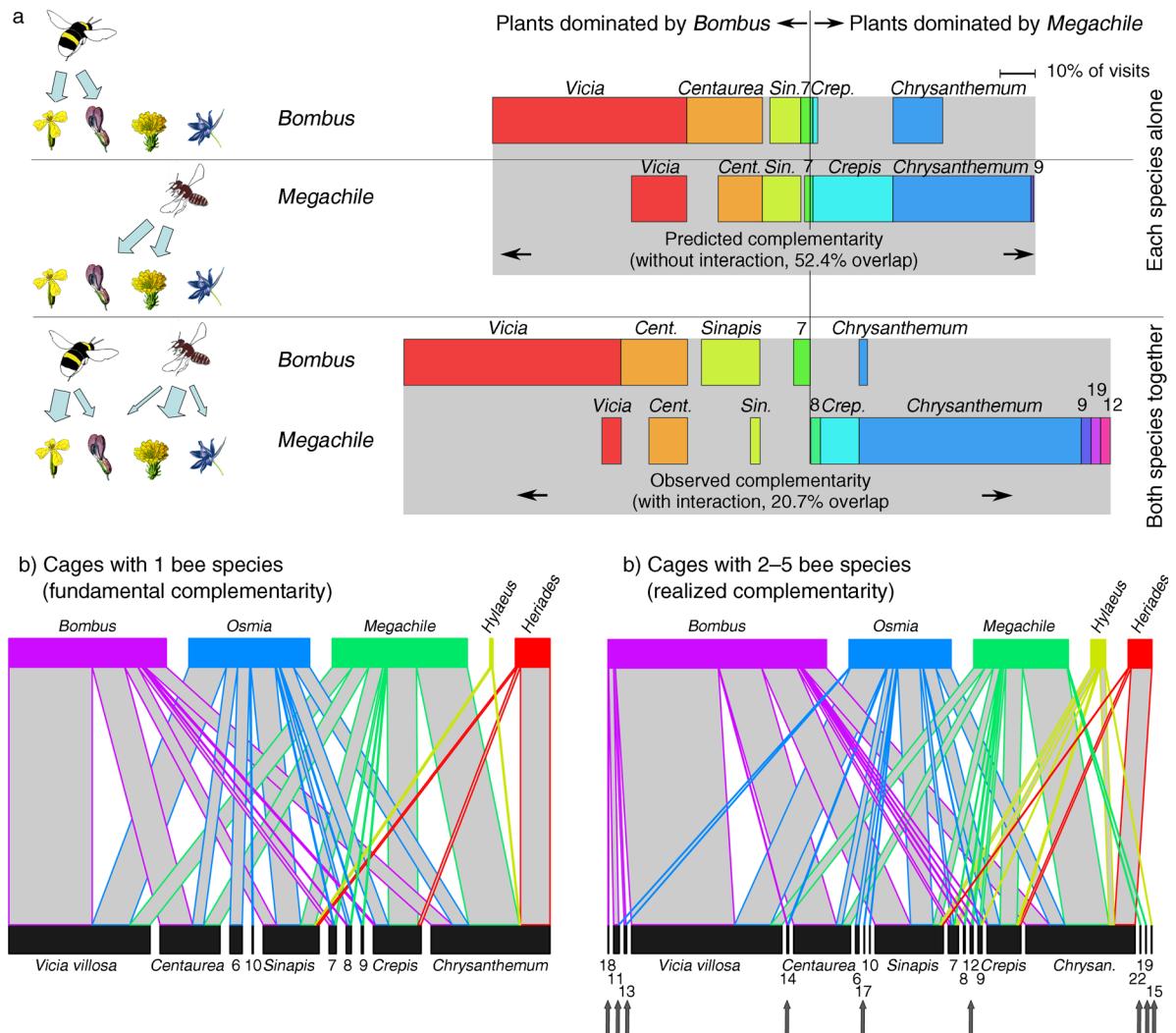


Fig. 3. Functional complementarity among bees in plant species visited. Bee species combinations visit more plant species both due to fundamental differences in flower preferences and due to interspecific interactions. (a) On the left a conceptual illustration of the complementarity mechanism; on the right a corresponding example from our experiment, showing flower visitation patterns of two bee species in communities with one species (top) and with both species (bottom). The proportion of different plant species (color-coded) in all visits is shown for each bee species and community context. Co-occurring bee species reduce niche overlap, which amplifies complementarity. A null model shows that the decrease in overlap is significant (Appendix: Fig. A5). (b, c) Pooled quantitative interaction networks; arrows indicate plant species only visited in cages with 2–5 spp., see also Appendix: Fig. A6. Plant species are referred to by number where there is no room to give the genus; see Appendix (Table A1) for full scientific names.

single- vs. multiple-bee treatments, $P < 0.001$, $df = 37$; Fig. 4c).

Complementarity and seed production

Plant community seed production was explained far better by a multiple regression model combining both variables of functional niche coverage (temperature coverage, $P = 0.01$, and plant species coverage, $P < 0.001$, $N = 39$) than it was explained by bee species richness (Table 2, Fig. 5a–c). In contrast, pollinator specialization (d' , weighted mean per cage) did not

explain plant community seed production ($P = 0.76$, when added to the multiple regression; Table 2).

DISCUSSION

Our experiment showed that higher bee diversity leads to higher seed production, but this required that an increase in richness caused functional niches to be covered more completely (i.e., flower visitation to more plant species and under more environmental conditions). Functional niche coverage, which reflects the mechanism of complementarity effects, predicted seed production far better than species richness per se did.

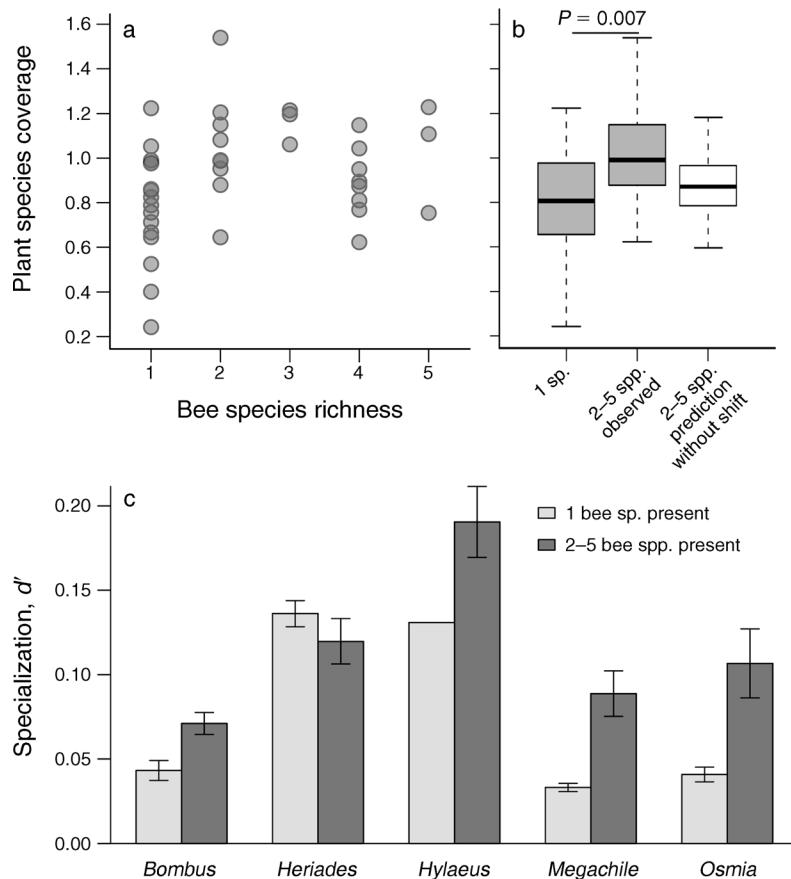


FIG. 4. Bees' floral niche shifts in response to community context. (a) Plant species coverage (i.e., the number of plant species visited per cage, corrected for sampling effects) in relation to the number of bee species present. (b) Plant species coverage per cage in cages with one vs. more than one bee species (P value based on Welch t test). The white box on the right shows the expectation if there were no changes in flower preferences (simulation based on visitation in cages with one bee species; see Appendix (Fig. A5) for an extended version including an appropriate test showing that observed coverage is significantly higher than expected). (c) Specialization per bee species and cage (mean \pm SE). Specialization increased under interspecific competition, but less so for *Bombus* (strong competitor) and not for *Heriades* (specialist). Boxplot components are as in Fig. 1d.

The relationship between seed production and bee diversity was relatively weak and tended to saturate because not all combinations of bee species showed high complementarity. Functional complementarity was related to interspecific differences in temperature dependence and in flower preferences. Flower preferences appeared to be flexible and depended on community context; interspecific overlap was reduced in multispecies treatments, resulting in both higher specialization and higher functional coverage (i.e., more plant species being visited) than expected from single-species treatments. We will first discuss the relationship between bee diversity and pollination, second bee functional complementarity and its relation to bee diversity, third the novel finding that interspecific interactions modify functional complementarity by niche shifts, and, fourth, wrap up by linking bee complementarity to seed production of plant communities.

Relationship between bee diversity and seed production

We found that pollination of a diverse plant community tended to be higher when more bee species were present. This confirms that bee diversity is important for pollination as suggested by field studies (Kremen et al. 2002, Klein et al. 2003, Potts et al. 2006, Hoehn et al. 2008), and shows that this diversity effect is independent of bee abundance or environmental effects. In contrast to the results of Fontaine et al. (2006) showing a benefit of combining bees and flies, we also show that diversity within bees matters for pollination. Moreover, by using all species in different combinations across the diversity gradient, we showed that the relationship is not merely driven by effective bee species being present mostly in more diverse communities (a sampling or selection effect), which has been considered as an explanation for field data (Klein et al. 2003, Larsen et al. 2005). In contrast, the bee diversity effect shown

here reflects a complementarity effect rather than a sampling or selection effect (Loreau and Hector 2001), as the most effective species had the highest abundance in single-species cages, but maximum seed production was not observed in these cages. That our results were not simply driven by differences in pollination effectiveness was further supported by seed production in cages with two bee species being higher than expected for additive single-species effects (known as “over-yielding” in productivity studies). However, bee species diversity per se was not a very good predictor of reproductive output of the plant community, and the strongest difference was found comparing communities with one bee species to communities with several bee species.

The diversity effect tended to saturate with higher bee diversity. Such an effect could arise when few bee species already provide optimal pollination (i.e., no pollen limitation). However, this cannot explain the pattern found here; pollination did not appear to be optimal in any of the bee diversity treatments because seed production was higher in open cages. The saturating model for the bee diversity–pollination relationship must still be treated with care and does not mean that two bee species are sufficient for pollination of plant communities. First, the non-saturating logarithmic model was not much worse. Second, the exact form of the relationship may depend on the spatiotemporal scale (Bengtsson et al. 2002), the selected plant and bee species combinations, and environmental conditions. Third, as species abundances in real communities are often highly skewed, effective diversity levels used in our experiment are probably comparable to natural communities with much higher species richness. Fourth, in many real-world scenarios of bee diversity declines, total bee abundance declines in parallel because density compensation by the remaining species is incomplete or lacking (Winfree and Kremen 2009), resulting in steeper declines of function (Larsen et al. 2005).

Biodiversity–function relationships in the real world may also be determined by extinction order (Larsen et al. 2005, Srivastava and Vellend 2005). For example, hoverflies are likely to be still present in real landscapes after declines in bee diversity (Jauker et al. 2009) and might buffer pollination. In our experiment, hoverflies contributed only little to pollination, despite similar number of flower visits and higher number of individuals compared to bees. Low pollination effectiveness of hoverflies compared to bees is consistent with previous studies (e.g., Jauker et al. 2012), questioning how far they can offset loss of pollination by bees.

Functional complementarity and bee diversity

Bee species differed in their response to temperature or weather conditions. While bumble bees showed highest flower visiting activity during colder periods, other bees were mostly active under warm and sunny conditions (similar to natural communities; Fründ et al. 2010). This means that they perform complementary

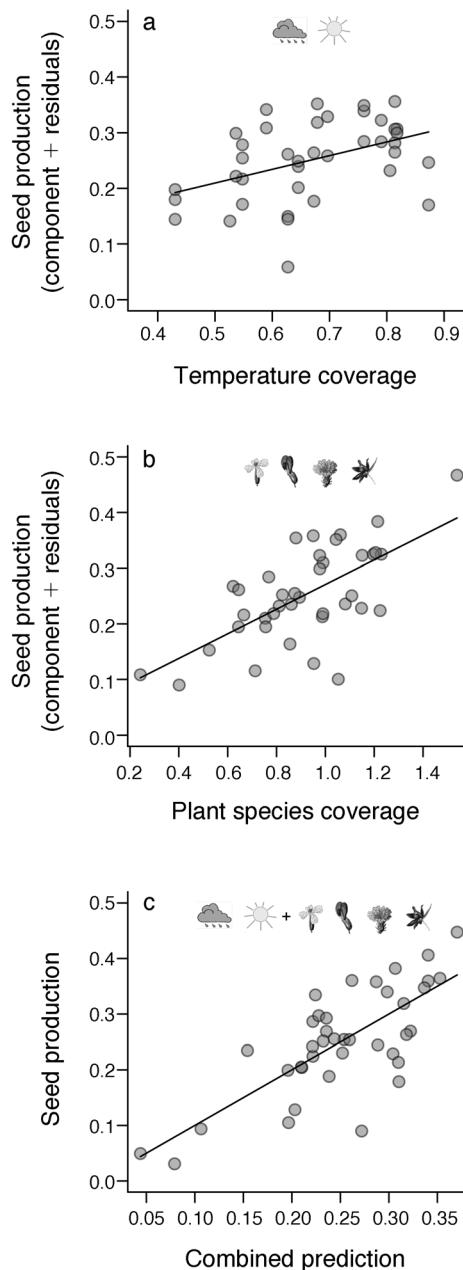


FIG. 5. Relationship between seed production of the plant community and two variables of functional niche complementarity. (a, b) Partial residual plots (component + residuals) of a multiple regression including both variables (temperature coverage, $P = 0.01$, and plant species coverage, $P < 0.001$, respectively). (c) Seed production in relation to the model prediction of that multiple regression. See Figs. 2 and 4 for explanation of variables. $N = 39$ cages in all three panels.

functions. A balanced combination of cold-tolerant bees (such as bumble bees) and other bees covers different environmental conditions particularly well.

In our study, more plant species were visited in cages with two or more bee species than in cages with one bee species. Bee species differed in their floral preferences

and thus performed complementary functions for the plant community. This confirms that there is complementary specialization among polylectic (“generalist”) bee species (Fründ et al. 2010). This is an important contribution to overall specialization of bee communities because highly specialized oligolectic bees are only a minority in most bee communities (Fründ et al. 2010). For the optimal pollination of a plant community, it is essential that all plant species are visited, which will be determined by the interplay between generalization and complementarity.

Functional coverage in both dimensions did not increase linearly with bee diversity and was not highest in cages with the highest number of bee species. This can be explained by a variable degree of functional redundancy among species pairs, and consequentially higher overlaps in some combinations than in others. Functional complementarity of a bee community will be determined by differences among species’ realized niches and the evenness of niche coverage. Thus, a bee community providing high coverage of all relevant niche dimensions can have better pollination function than a randomly composed community with more species.

In addition to the functional niche dimensions important in our study (weather and plant species), bee species can also be complementary in their behavior in individual flowers (Blüthgen and Klein 2011), in preferred resource density (Tylianakis et al. 2008), in preferred flower height (Hoehn et al. 2008), and in time of pollinating activity (Hoehn et al. 2008). Temporal niche complementarity, both within a day (Willmer and Corbet 1981) and among days, may be related to complementarity in response to temperature, which we focused on here. Future studies should try to design diversity gradients of bee communities with realistic trait distributions that help to disentangle the relative importance of the different dimensions of functional complementarity.

Niche shifts

Most importantly, the floral niche partitioning among polylectic bee species was not simply due to fundamental preferences, as we observed shifts in bees’ floral niches when other bee species were present. These shifts were probably a result of interspecific competition. In cages with multiple bee species, bees visited plant species that were not visited in any of the cages with one bee species. Fundamental flower preferences (i.e., without competing species) were not sufficient to explain the high plant species coverage in cages with multiple bee species. Note that our quantitative concept of fundamental niche puts more emphasis on the relative use of different resources than a possible alternative concept that views the fundamental niche as a list of all resources that a species can potentially use. This finding highlights that competition for floral resources influences the structure of interaction networks and may explain why flower preferences in the field are variable (Fründ et al. 2010).

As predicted by competition theory (Inouye 1978), niche shifts not only involved use of novel resources, but also increased specialization on previously preferred plant species (niche contraction). Both effects reduce interspecific overlap and are not mutually exclusive; community niche coverage can increase at the same time as niche breadth of individual bee species decreases. These shifts in floral niches enhanced functional complementarity among bee species. An understanding of the underlying mechanisms and how they shape biodiversity–functioning relationships needs further attention.

We showed that fundamental flower preferences and competition among bee species both shape the realized pattern of plant pollinator interactions. This finding of dynamic plant pollinator linkages adds to recently shown temporal dynamics of plant pollinator networks (Alarcón et al. 2008, Olesen et al. 2008, Fründ et al. 2011), cautioning against assumptions of homogeneity in plant pollinator associations (e.g., Memmott et al. 2004). Pollinator generalization increases with intraspecific competition (Fontaine et al. 2008) and thus should occur in scenarios of pollinator species loss with density compensation. This effect occurred also in our study; bees were more generalized in single-species cages where intraspecific competition was highest. However, more plant species were visited in multispecies cages, which highlights that responses to intraspecific competition cannot compensate for the decrease in total niche coverage resulting from reduced interspecific competition and loss of complementary species. Thus, predictions based on single-species studies can underestimate functional niche coverage in multispecies communities.

Determinants of seed production

Seed production was highest when bee communities displayed a broad range of temperature dependence and floral preferences. In contrast, plant community seed production was not related to pollinator specialization, so we could not confirm the long-standing hypothesis in pollination ecology that plants benefit from pollinator specialization due to increased conspecific pollen delivery (Wissel 1977). This might be because our specialization index focuses on the consumer’s overall resource specialization (i.e., selectiveness, or the diversity of plant species used by a bee species), while the benefit of pollinator specialization for pollination effectiveness might be better estimated by considering the sequence of visits, which drives the purity of deposited pollen. It is thus possible that despite higher bee specialization in treatments with highest bee diversity, heterospecific pollen deposition may have been higher and contributed to low seed production. The frequency of consecutive visits to the same plant species can also be high in generalist pollinator species that show floral constancy (Wilson and Stine 1996). A positive effect of specialization on function was found for aphid parasitoids when functional niche coverage was standardized by the

experimental design (Finke and Snyder 2008). In our case, functional niche coverage was the best predictor of reproductive success of the plant community. The likely underlying mechanism is that a more even distribution of flower visitation ensures pollination where it is most limiting; additional visits bring the highest benefit when they avoid failure of pollination and relatively low benefit when a plant is already well pollinated.

Conclusion

We demonstrated that bee diversity tends to improve pollination success in plant communities, but this relationship depends on the species-specific traits that drive functional complementarity. This corroborates recent studies reporting functional complementarity due to niche partitioning as the main driver of biodiversity effects (Loreau and Hector 2001, Finke and Snyder 2008, Cardinale 2011). Furthermore, interspecific interactions are important in determining the functional value of bee communities, showing that compilation of traits from single-species studies can be misleading. Our study suggests that “interactive complementarity effects” are important for biodiversity–functioning relationships. Our results provide experimental evidence that functionally diverse bee communities are needed to maintain plant communities that rely on pollination for high reproductive success.

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SUPPLEMENTAL MATERIAL

Appendix

Supporting methods, including detailed descriptions of experimental methods and (supporting) statistical analyses, as well as three tables and six figures ([Ecological Archives E094-186-A1](#)).

Supplement

Simulation source code (R-script text file) underlying Fig. A5 in the Appendix, and example data set (Rdata file) to run with the code. The example data set is based on bee preferences calculated from different sets of cages, illustrating a “niche shift” ([Ecological Archives E094-186-S1](#)).