Limitation of complementary resources affects colony growth, foraging behavior, and reproduction in bumble bees

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Abstract. Resource availability in agricultural landscapes has been disturbed for many organisms, including pollinator species. Abundance and diversity in flower availability benefit bee populations; however, little is known about which of protein or carbohydrate resources may limit their growth and reproductive performance. Here, we test the hypothesis of complementary resource limitation using a supplemental feeding approach. We applied this assumption with bumble bees (*Bombus terrestris*), assuming that colony growth and reproductive performance should depend on the continuous supply of carbohydrates and proteins, through the foraging for nectar and pollen, respectively. We placed wild-caught bumble bee colonies along a landscape gradient of seminatural habitats, and monitored the colonies' weight, foraging activity, and reproductive performance during the whole colony cycle. We performed supplemental feeding as an indicator of landscape resource limitation, using a factorial design consisting of the addition of sugar water (carbohydrate, supplemented or not) crossed by pollen (protein, supplemented or not). Bumble bee colony dynamics showed a clear seasonal pattern with a period of growth followed by a period of stagnation. Higher abundance of seminatural habitats resulted in reducing the proportion of pollen foragers relative to all foragers in both periods, and in improving the reproductive performance of bumble bees. Interestingly, the supplemental feeding of sugar water positively affected the colony weight during the stagnation period, and the supplemental feeding of pollen mitigated the landscape effect on pollen collection investment. Single and combined supplementation of sugar water and pollen increased the positive effect of seminatural habitats on reproductive performance. This study reveals a potential colimitation in pollen and nectar resources affecting foraging behavior and reproductive performance in bumble bees, and indicates that even in mixed agricultural landscapes with higher proportions of seminatural habitats, bumble bee populations face resource limitations. We conclude that the seasonal management of floral resources must be considered in conservation to support bumble bee populations and pollination services in farmlands.

Key words: Bombus terrestris; feeding experiment; landscape structure; pollen and nectar resources; reproductive performance; seasonal phenology.

INTRODUCTION

The fitness of organisms is related to the availability and intake of multiple nutrients. As well established in plant ecology, this ecological process can follow the multiple limitation hypothesis predicting that an organism's growth and reproductive performance can be limited by more than one nutrient simultaneously (Gleeson and Tilman 1992, Rubio et al. 2003, Sperfeld et al. 2012). The multiple limitation hypothesis was also tested with herbivorous consumers, with evidence that colimitation in the availability of nutrients nitrogen (N), phosphorus (P), and carbohydrates affects the foraging activity and

reproductive performance of terrestrial (e.g., Joern and Behmer 1997, Raubenheimer and Simpson 2004, Elser et al. 2007) and aquatic insects (e.g., Elser et al. 2007, Sperfeld et al. 2012, Richard and De Roos 2018).

Over the past century, intensified human activity has caused widespread environmental changes and disturbances of resource availability for many organisms (Tilman et al. 2001). Particularly, the decline of seminatural habitats and the disturbance in flower resource availability has led to the decline of pollinator populations in farmlands (Biesmeijer et al. 2006, Krauss et al. 2010, Potts et al. 2010). This directly concerns human well-being, as pollination is critical for the reproduction of many wild plant species and crop yields (Klein et al. 2007, Ollerton et al. 2011, Potts et al. 2016).

Bees are central-place foragers and exploit flower resources available in the surrounding landscape. They

collect nectar and pollen of plants as resources of carbohydrate and protein, respectively (Donkersley et al. 2017). Carbohydrates are required to fulfill energy-intensive flights and thermoregulatory functions in the nest of social bees (Haydak 1970), and proteins are essential for growth, egg production, and larval rearing (Haydak 1970, Harder 1990, Goulson et al. 2010). Many bee species are generalists and collect pollen from diverse plant species, which can greatly differ in the composition, quantity, and quality of proteins, carbohydrates, and other nutrients (e.g., minerals; Tasei and Aupinel 2008, Requier et al. 2015, Wright et al. 2018).

Low flower resource availability negatively affects bee foraging activity, growth, and reproductive performance (e.g., Westphal et al. 2006, Crone and Williams 2016, Spiesman et al. 2017). Particularly, temporal scarcity in flower availability and peak supply by mass-flowering crops impact the diversity and abundance of pollen intake and the demographic dynamics of bee populations (Riedinger et al. 2015, Requier et al. 2017, Hass et al. 2018). Conversely, seminatural habitats (e.g., grasslands, woodlands, hedgerows) can positively affect the growth and reproductive performance of wild bees through the continuous availability of diverse flower resources (e.g., Banaszak 1992, Fussell and Corbet 1992, Dramstad and Fry 1995), and further pollinator diversity and abundance in mixed landscapes (Steffan-Dewenter and Tscharntke 2001, Carvell et al. 2006, Biesmeijer et al. 2006, Krauss et al. 2010). Despite these established positive effects of (semi-)natural habitats on flower resource availability for bees, little is known about which resource (e.g., pollen or nectar) limits growth and reproductive performance of bee populations in differently managed landscapes.

In this study, we assessed the response of bumble bees (*Bombus terrestris*) to possible colimitation of different food resources along a landscape gradient. Bumble bees belong to the most important wild pollinators of a wide range of crops and wild plants (Williams et al. 2012, Kennedy et al. 2013, Goulson et al. 2015). Bumble bee populations are declining in farmlands (Goulson et al. 2015), as a consequence of the loss of seminatural habitats and the loss of continuous resource supply in agricultural landscapes (Carvell et al. 2008, Williams et al. 2012). We performed a landscape-scale experiment with 32 wild-caught bumble bee colonies placed along a landscape gradient in the amount of seminatural habitats. We then monitored the colonies' weight, foraging activity, and reproductive performance along the colony cycle (from June to mid-August). We expected that these colony traits should be positively affected by the amount of seminatural habitats surrounding the colonies, as they offer diverse and continuous pollen and nectar resources (Carvell et al. 2008, Williams et al. 2012).

We also experimentally manipulated resource availability using supplemental feeding (Fig. 1), for which we attributed colonies to a factorial design consisting in the constant addition of sugar water (carbohydrate, yes/no)

crossed by pollen (protein, yes/no). This supplemental feeding approach aims to depict the nature of landscape limitations for bumble bees (e.g., carbohydrate or protein or both resources), whenever the food supplementation interacts with landscape gradient effects on the bumble bee traits (e.g., colony weight, foraging activity, and reproductive performance; Fig. 1). Thus, we tested whether the supplemental feeding reveals the limitation of resources, as an indicator of the landscape limitation in the availability of carbohydrates and/or proteins in simplified landscapes. Finally, given evidences in temporal variations in both flower availability in agricultural landscapes (Riedinger et al. 2015, Requier et al. 2017, Hass et al. 2018) and bumble bee colony life cycle (e.g., Duchateau and Velthuis 1988, Pereboom et al. 2003, Alaux et al. 2006, Hovestadt et al. 2018), we tested whether the magnitude of the interaction between seminatural habitats and supplemental feeding differed over time, or occurred during a restricted critical period of bumble bee colony development.
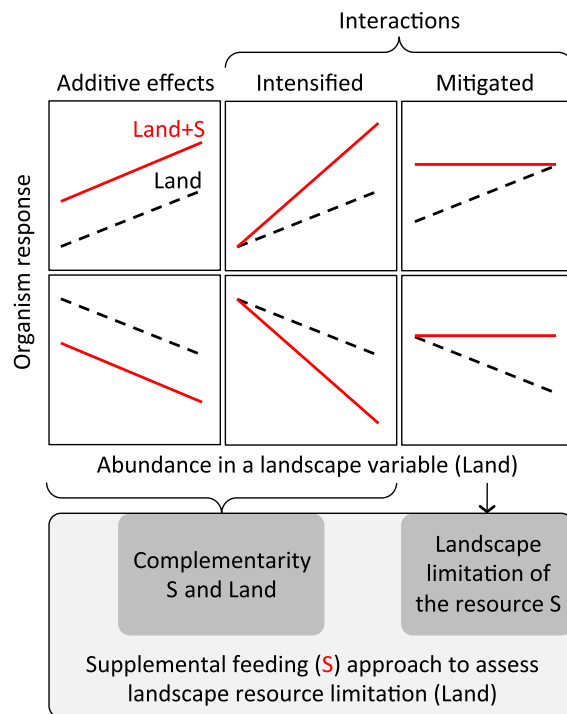

FIG. 1. Conceptual framework of the supplemental feeding approach to assess landscape resource limitation on organisms. In a context of positive (top row) or negative (bottom row) effect of the landscape on organism response, a constant supplemental feeding (S, in red) can have an additive effect, or interact with the landscape. Interactions can intensify or mitigate the effect of the landscape. Additive effects and intensified interactions can be interpreted as functional complementarity between the supplemented resource S and the landscape. Mitigated interactions indicate limitation of the supplemented resource S in the landscape.

MATERIAL AND METHODS

Study area and experimental design

The study was carried out in central Germany (49°47′ N, 9°57′ E, Fig. 2a), in a 20 × 20 km area in Lower Franconia during 2014. The region is characterized by mixed agricultural landscape, mainly composed of arable land, seminatural habitats, and settlement areas (see Redlich et al. 2018). We selected eight sites to cover a gradient in the amount of seminatural habitats in the surrounding landscape. Seminatural habitats include set-aside land, forest, orchards, and meadows that are extensively managed, and linear structures such as hedgerows and roadsides. Such habitats host a high diversity of plants that offer continuous flowering resources over the season (Requier et al. 2017, Hass et al. 2018) and high pollen quality (Donkersley et al. 2014, Requier et al. 2015, Vaudo et al. 2015). The gradient of seminatural habitats ranged from 0.7 to 35% over the eight sites (Fig. 2b, c). The proportion of seminatural areas was calculated within a 2-km radius, in ArcMap v. 10 (ESRI, Redlands, CA), using official digital topological maps ATKIS DTK 25 (Bayerische Vermessungsverwaltung, 2015). The choice of the radius

distance is based on the foraging ecology of *Bombus terrestris* that shows 95% of the foraging activity within a radius of 1,750 m around the nest (Walther-Hellwig and Frankl 2000, Osborne et al. 2008).

Establishment of colonies

Four colonies of *B. terrestris* were placed at each site (Fig. 2a), totaling a monitoring of 32 colonies (eight sites × four colonies). All colonies came from the same population in the neighborhood of the Hubland campus at the University of Würzburg (49°47′ N, 9°58′ E, Fig. 2b). In spring (April), foraging queens were caught from the wild, placed in individual rearing boxes, and kept in climate chambers. Temperature, light, and humidity were controlled to provide conditions of darkness, 25°C, and more than 60% humidity, respectively. Queens were fed with carbohydrates and proteins, consisting of sugar water (ApiInvert®) and freeze-dried pollen, respectively. The sugar-water treatment consisted of a 50% sucrose solution. We used polyfloral pollen samples collected from honey bee colonies from the same study area (i.e., the University campus) over the summer season (June–July) of the year before the experiment.
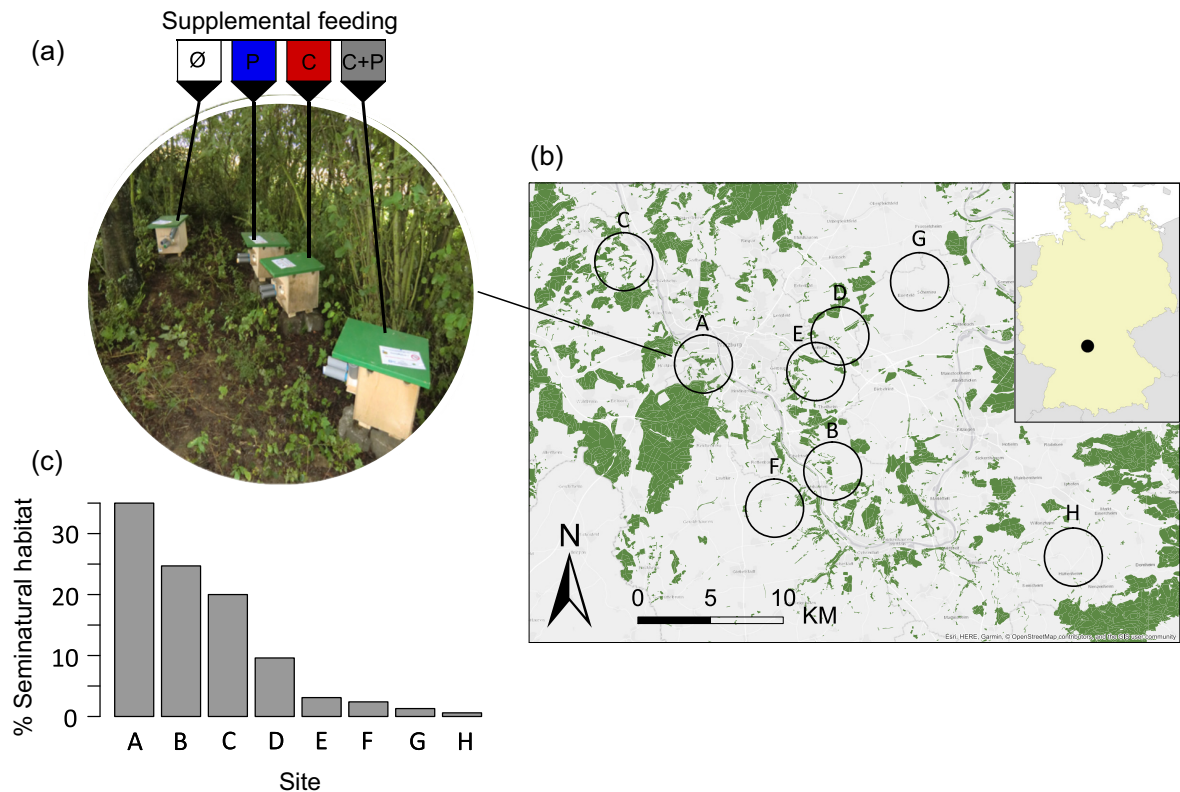

FIG. 2. Landscape-scale monitoring of wild-caught bumble bee colonies with a supplemental feeding experiment. (a) Supplemental food resources were given to assess the hypothesis of resource limitation to bumble bee colonies in mixed landscapes. The supplemental feeding consists of the addition of sugar water (carbohydrate, C), pollen (protein, P), both (C + P), or none (control, ∅). (b) Thirty-two wild-caught colonies of *Bombus terrestris* were monitored in central Germany, (c) along a gradient of seminatural habitats. Seminatural habitats are shown in green in (b), and circles represent a 2-km radius around the sites.

The pollen samples were dried deep-frozen until the experiment started. Unfortunately, we did not analyze the botanical origin of the pollen samples used in this study, but a related study on pollen resource uses by honey bee colonies from the same study region showed that the dominant plant species foraged upon during summer include the following six genera: *Brassica*, *Centaurea*, *Picris*, *Plantago*, *Potentilla*, and *Trifolium* (Danner et al. 2017). After 4–5 weeks of queen rearing (from the first date of egg laying), the number of brood cells (including larvae and pupae) and adult workers were counted and summed to measure the initial colony size (as number of individuals). The average colony structure was composed of 51.3 ± 24.2 larvae (mean $\pm$ SD) and 43.1 ± 20.9 adults. The colonies were also weighed to the nearest 0.1 g (Mettler Toledo PM 3000, Mettler-Toledo, Columbus, OH). Each colony was then transferred in a cardboard box (with addition of kapok and moss for isolation and nesting facility) inside a wooden nesting box ($30 \times 30 \times 30$ cm, Fig. 2a and Appendix S1: Fig. S1). The occupied wooden nesting boxes were placed in the selected field sites at beginning of June 2014 (between June 9 and 15), with a random attribution of colonies to sites.

Supplemental feeding

At each site ($n = 8$), we randomly assigned a feeding treatment to each colony. Thus, the four colonies were distinguished at each site by supplemental feeding of (1) sugar water (carbohydrate), or (2) pollen (protein), or (3) both sugar water and pollen (see above for details), or (4) none as a control (Fig. 2a). Bumble bees had access to the supplemental resources through a tube connecting the in-nest space to two types of external tanks (Appendix S1: Fig. S2). Every week, tanks were refilled with sugar water (or nothing) in the first, and pollen (or nothing) in the second tank, according to the treatment assigned. We provided resources in excess so that tanks were never empty.

Monitoring colony weight, foraging activity, and reproductive performance

The 32 colonies were monitored from June 9 to August 15 (Requier et al. 2019). The end date of the monitoring was considered by observing young queens flying out of the colonies before the end of the experiment, meaning the colonies had produced new queens; that is, the last stage of the colony cycle was reached. Once a week the foraging activity of each colony was observed and colony weight was taken. We first observed the in-and-out flight activity of the four colonies during a standardized observation time of 30 min per colony (each colony was observed independently). All the observations were randomly performed between 8 a.m. and 9 p.m. for each colony and landscape to cover potential daily variation in foraging activity and prevent biased observational data. A single observer did the observations at about 2–3-m distance from the nest entrance so as not to disturb flight traffic and bumble bee behavior. The number of individuals flying back to the nest was recorded to estimate the number of returning foragers. We also recorded the presence (or absence) of pollen loads on the returning foragers as an indicator of the colonies' investments to pollen collection. After each observation the weight of each colony was taken (subtracting the box's weight previously measured before the experiment). The colony weight (expressed in kilograms) was considered as an indicator of colony size, given the high correlations between number of individuals and weight at the start of the experiment ($n = 32$, $t = 9.21$, $P < 0.001$, $R^2 = 0.86$) and at the end ($n = 32$, $t = 7.97$, $P < 0.001$, $R^2 = 0.82$). At the end of the experiment (August 16), the 32 colonies were collected to quantify reproductive performance. First, in the field, the entrance of the colony was closed after sunset to ensure that no bumble bees were outside the nest. Then, colonies were brought back to the laboratory, weighed, and frozen in their cardboard box at $-30°C$. The number of males and young queens produced (brood cells and newly emerged queens) were counted to estimate the reproductive performance of the colony. Males were discarded from the analysis, given the high correlation with queens' production ($n = 32$, $t = 2.90$, $P = 0.007$, $R^2 = 0.47$).

Data analysis

All statistical analyses were performed using the R Project for Statistical Computing version 3.3.3 (R Development Core Team 2018).

Exploring seasonal patterns.—The seasonal pattern of the colony weight ($n = 248$, with 8 ± 1 repeated observations per colony per site) was modeled as a function of the day of year, using generalized additive mixed models (GAMMs) and a Gaussian error structure (*gamm* function in the *mgcv* R-package). The same procedure was applied with the seasonal dynamic of foraging activity ($n = 216$, restricted sample size, as no foraging activity could be observed at the initial date of the experiment) with a Poisson error structure. GAMMs are modeling techniques that allow temporal spline fitting while taking account of repeated measurements on statistical units in a nested design. Herein, the identities of the site and colony were specified as a suit of nested random grouping variables. Time was rescaled on Julian dates from the first of January. Model residuals were extracted and inspected against fitted values (residuals vs. fitted plot and normal Q-Q plot) to ensure residual normality and homoscedasticity assumptions were fulfilled. Finally, the seasonal distinction between colony growth and stagnation periods (see Results) was a posteriori determined using the *breakpoints* function of the *strucchange* R-package (Zeileis et al. 2002). We then

detrended the data (Requier et al. 2017) to control for the variations arising from seasonal effects and to isolate the effects of the landscape gradient in seminatural habitats and supplemental feeding treatments. Detrended data were obtained by subtracting the best-fit line of the GAMM.

Identifying causal links underlying seasonal colony activity and performance, and testing landscape resource limitation.—We used path analyses (Shipley 2009) to disentangle direct and indirect effects of seminatural habitats, supplemental feeding, and season on colony dynamics. Path analysis helps to disentangle the most plausible direct and indirect links in multivariate data sets by assessing conditional independence among indirectly linked variables. We applied the path analysis using the *PiecewiseSEM* R-package (Lefcheck 2016). We first averaged colony traits within growth and stagnation periods at the colony level ($n = 32$ colonies for each of the two time periods) so that we could compute all explanatory and response variables at the same level (the colony). We then built a basic path model that reproduced the hypothesized mechanistic structure underlying bumble bee colony dynamics, linking initial colony size, colony weight (i.e., the detrended data of the colony weight by subtracting the best-fit line of the GAMM), foraging activity, foraging behavior (proportion of foragers collecting pollen), and reproductive performance throughout the colony life cycle. In particular, we expected that (1) reproductive performance would increase with colony weight (both at growth and stagnation periods), and also with initial colony size and that (2) each step of this causal chain would potentially influence foraging activity and behavior. We also tested the possible effect of landscape resource limitation. For that, we analyzed the effects of the proportion of seminatural habitat in the surrounding 2-km radius, the supplemental feeding treatment, and the interaction between supplemental feeding and seminatural habitat in each causal link of the path model. This procedure allowed us to integrate and manage the potential links between seminatural habitats, supplemental feeding, and season on colony dynamics statistically. See Appendix S1: Table S1 for the complete list of paths tested. Each causal link in the path model was depicted as a linear model (LM) or a generalized linear model (GLM), using *lm* and *glm* function in the *base* R-package, respectively, depending on the nature of the involved variables. We used GLMs with logit-link function for the proportion of foragers collecting pollen, meaning that the number of pollen foragers was considered relative to the number of other foragers (nonpollen foragers). We used a GLM with a Poisson error structure for the reproductive performance, and LMs with Gaussian error structure for other variables. Once computed, the basic path model was refined by dropping nonsignificant links and by sequentially adding any link that was initially ignored until the path model was judged statistically supported by the data.

New links were added by order of increasing P value, and deviation from expected conditional independence assessed using Shipley's d-separation test (Shipley 2013). The final path model was consistent with data according to the Fisher's chi-square distribution C-statistic comparing observed correlations across independence claims to random variation (Fisher's $C = 12.74$, $P = 0.338$; Shipley 2009). All quantitative explanatory variables were then standardized using Z scores, so that coefficient estimates could be readily compared to determine the most influential explanatory variables in the candidate path models (see Appendix S1: Table S1).

RESULTS

Seasonal patterns in bumble bee colony dynamics

Bumble bee colony weight (in kilograms) was highly seasonal and nonlinear (temporal variations tested using GAMM, $F_{6,241} = 146.6$, $P < 0.001$), with a clear-cut distinction between a period of colony growth followed by a period of stagnation (Fig. 3a). The foraging activity (measured as number of returning foragers) also showed a seasonal pattern with significant temporal variation ($F_{6,209} = 23.57$, $P < 0.001$; Fig. 3b). No temporal trend was detected on the foraging behavior (i.e., the proportion of foragers collecting pollen). The seasonal changes of colony weight and foraging activity were synchronized, with a specific seasonal breakpoint at day 182 (July 1) for colony weight, and at day 189 (July 8) for foraging activity. The growth period showed an increase of colony weight with an average of 5.65 ± 0.15 kg and a peak of foraging activity with an average maximum weight of 20 ± 21 returning foragers. The stagnation period included a decrease in colony weight growth with an average of 5.99 ± 0.22 kg and a strong decrease in foraging activity with an average of 13 ± 13 returning foragers.

Internal links underlying seasonal colony activity and performance

The path analysis depicted consistent direct links between initial colony size, colony weight, foraging activity, foraging behavior, and reproductive performance, with indirect links that did not significantly deviate from conditional independency requirements (Fisher's $C = 12.74$, $P = 0.338$; Fig. 4). The main links are fitted in Appendix S1: Fig. S3. Coefficients and detailed P values underlying the path analysis are presented in Appendix S1: Table S1. Among the causal links underlying colony dynamics, the links among growth in colony size and reproductive performance were the most notable effects (Appendix S1). Following the significant links, the colony weight during the stagnation period was positively affected by the colony weight during the growth period, and the latter by the initial colony size. The reproductive performance (i.e., the number of young
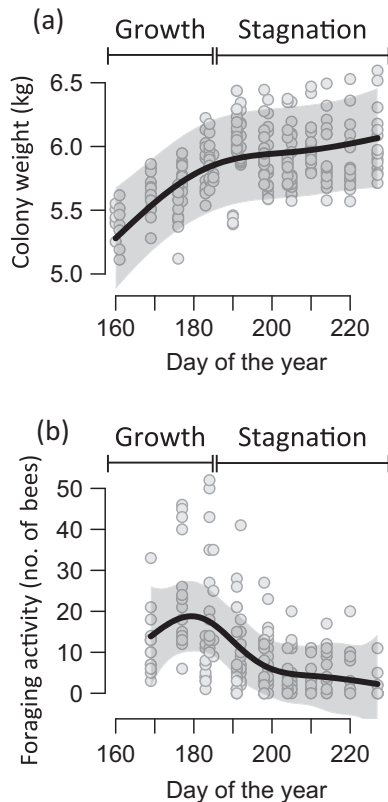
Fig. 3. Seasonal patterns of bumble bee colony dynamics in mixed landscapes showing (a) colony weight (in kilograms, $n = 248$) and (b) foraging activity (number of returning foragers, $n = 216$). Each dot represents a measurement of colony dynamics; thick lines show the generalized additive mixed models (GAMM) predictions, and shaded areas show the 95% confidence interval envelope. The horizontal lines delineate the seasonal extent of growth and stagnation periods (distinguished at day 185.5, see Results).

queens produced) was mainly explained by the colony weight during the growth period, suggesting a critical stage of the growth period for colony fitness. The foraging activity (i.e., the number of individuals flying back to the nest) was positively affected by the colony weight and the initial colony size, showing that larger colonies foraged more. On the other hand, the colony weight during the growth period changed foraging behavior, indicating that larger colonies allocated a smaller proportion of foragers to pollen collection than smaller colonies. This relationship was reversed during the stagnation period. Moreover, delayed effects occurred between these two traits (colony weight and foraging behavior), suggesting that the foraging behavior could be a critical colony trait to adjust colony dynamics.

Landscape resource limitation

The supplemental feeding had a marginal, positive effect on the detrended data of colony weight during the stagnation period of the bumble bee life cycle ($P = 0.053$, Fig. 5C and Appendix S1: Table S1). In turn, the landscape gradient (% seminatural habitat) significantly affected the foraging behavior (Appendix S1: Table S1). Higher abundance of seminatural habitats resulted in reduced forager's investment to pollen collection, independently of the supplemental feeding, during the period of colony growth (Fig. 5a), and—in the absence of supplemental feeding—also during the stagnation period (Fig. 5b). The supplementation of pollen mitigated the landscape effect on foraging behavior, and combined supplementation of pollen and sugar water interacted positively with seminatural habitats and increased forager's investment to pollen collection. The supplementary feeding of carbohydrates or carbohydrates + proteins and the proportion of seminatural habitats significantly increased the reproductive performance of bumble bee colonies (Fig. 5d). The strongest effects of supplementary feeding of carbohydrates on reproductive performance were observed in landscapes with high proportion of seminatural habitats. Interestingly, even in these assumedly high-quality landscapes, reproductive performance was very low without supplementary feeding (Fig. 5d and Appendix S1: Table S1). The supplementation treatment with pollen only had no clear effect on reproductive performance, indicating a strong limitation by carbohydrates or a limited attractivity of the freeze-dried pollen resource compared to flowers (Fig. 5d).

Discussion

Our study underpins the importance of seminatural habitats to conserve pollinator populations in agricultural landscapes and suggests that even landscapes with a higher proportion of seminatural habitats were characterized by limited resource availability. By using a novel experimental approach of supplemental feeding in differently structured landscapes, we can demonstrate the critical role of seminatural habitats as a foraging resource of pollen and nectar, affecting the reproductive performance and behavior of *Bombus terrestris* colonies. Moreover, the results suggest colimitation in these two resources (pollen and nectar) in mixed landscapes and pinpoint the critical growth period for colony fitness, providing new perspectives on improving pollinator-friendly practices for the conservation of pollinators in farmlands.

Bumble bees are critical crop pollinators (Williams et al. 2012, Kennedy et al. 2013, Goulson et al. 2015); however, little is known about their colony dynamics in farmlands. In this study, we demonstrate that *B. terrestris* colonies show typical seasonal patterns in colony growth and foraging activity. Particularly, we established a clear-cut period of change between colony growth—defined as an increase in colony weight and high foraging activity—and stagnation—described as a stable phase in colony weight with a low foraging activity (see also Bowers 1986, Duchateau and Velthuis 1988). This
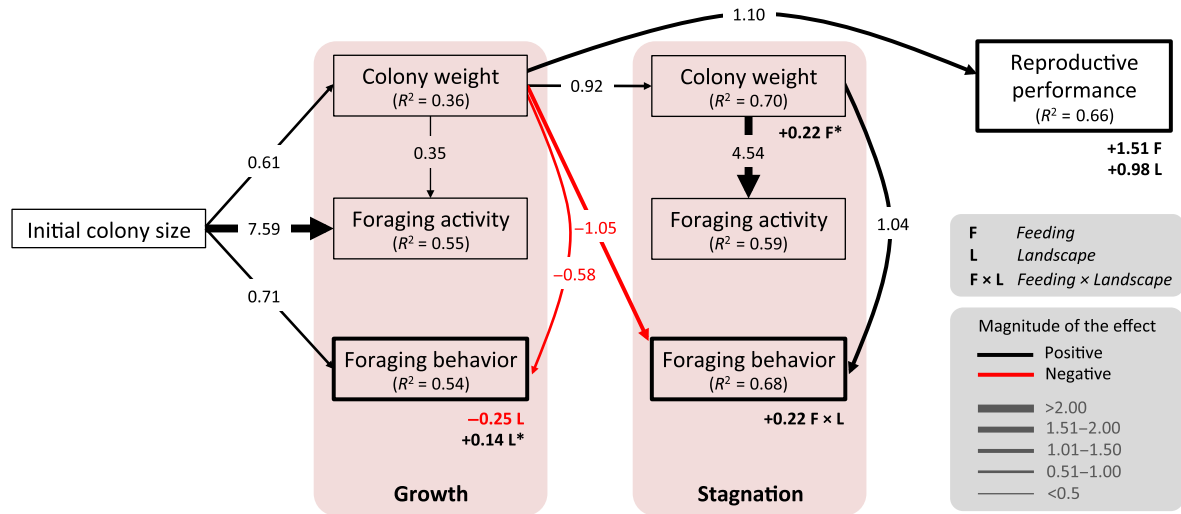
FIG. 4. Path analysis revealing the mechanistic structure underlying bumble bee colony dynamic, helping to disentangle direct and indirect effects of colony dynamics, seminatural habitats (L, in bold), and supplemental feeding (F, in bold). Only significant links are shown (* considered as marginally significant with $P < 0.06$). Landscape (L) and feeding (F) effects are only shown in letters for simplicity of illustration. See Appendix S1: Table S1 for detailed statistical properties of the path model and links, and Appendix S1: Fig. S3 for the graphical representation of the main links. Total explained variance (R^2) is indicated in the box for each response variable. The thickness of an arrow represents the magnitude of the (standardized) effect and the color shows the correlation sign (positive or negative).

evidence of seasonal phenology of colony dynamics demonstrates the importance of analyzing potential different responses of colonies to feeding and landscape effects across the season. Moreover, the internal links underlying seasonal colony activity and performance showed that the reproductive performance of *B. terrestris* depends on the colony growth in spring. This interesting result argues for carry-over effects; that is, the colony growth in spring is the most important driver of colony reproductive performance later in the season, and pinpoints a critical stage of the growth period for the reproductive performance of bumble bee colonies. This result confirms general understanding that larger colonies early in the season have higher reproductive performance later in the season (Müller and Schmid-Hempel 1992, Westphal et al. 2009), and could explain the difficulties finding direct timing links between these two colony traits (e.g., Williams et al. 2012). Integrating colony dynamic processes with potential complex mechanisms of feedback and carry-over effects are needed to predict the effect of environmental disturbances on pollinator ecology better.

Interestingly, the abundance of seminatural habitats did not affect weight and foraging activity of bumble bee colonies when considering this typical seasonal pattern (i.e., with detrended data; see also Westphal et al. 2003, Hass et al. 2018). There is no consensus in the literature on the effect (e.g., Hass et al. 2018) or lack of effect (e.g., Goulson et al. 2002, Westphal et al. 2003, Kämper et al. 2016) of seminatural habitat on colony growth and foraging of bumble bees. Nevertheless, the abundance of seminatural habitats affects the foraging behavior of

bumble bees in mixed landscapes. Although some studies already established this effect with forage resource preferences (e.g., Dramstad and Fry 1995, Kells et al. 2001), here a decrease in forager's investment to pollen collection related to the availability of seminatural habitats could be demonstrated. This suggests that seminatural habitat allows bumble bee colonies to allocate fewer foragers to pollen collection, implying that for the same amount of pollen intake, pollen from seminatural habitats would be higher quality than pollen from other habitats (e.g., croplands). This hypothesis converges with works of Donkersley et al. (2014) showing that the quality of pollen collection increases with the abundance of seminatural habitats in the surrounding landscape. Indeed, seminatural habitats can improve the nutritional value of pollen collected through the availability of a wide diversity of flowering plants and subsequently through a critical availability in nutrient diversity for bee development (Tasei and Aupinel 2008, Requier et al. 2015, Vaudo et al. 2015, Danner et al. 2016, Donkersley et al. 2017). Moreover, the diversity of plant species in seminatural habitats increases the temporal spread of food availability for bees, by phenological succession, and can buffer the resource gap after mass-flowering crops (Danner et al. 2016, Requier et al. 2017, Hass et al. 2018).

Agricultural landscapes can lack pollen and nectar resources for bumble bee populations (Carvell et al. 2008, Potts et al. 2010, Williams et al. 2012, Goulson et al. 2015). Here, we found evidence that the increase of the reproductive performance of bumble bees is related to the abundance of seminatural habitats. Moreover, the
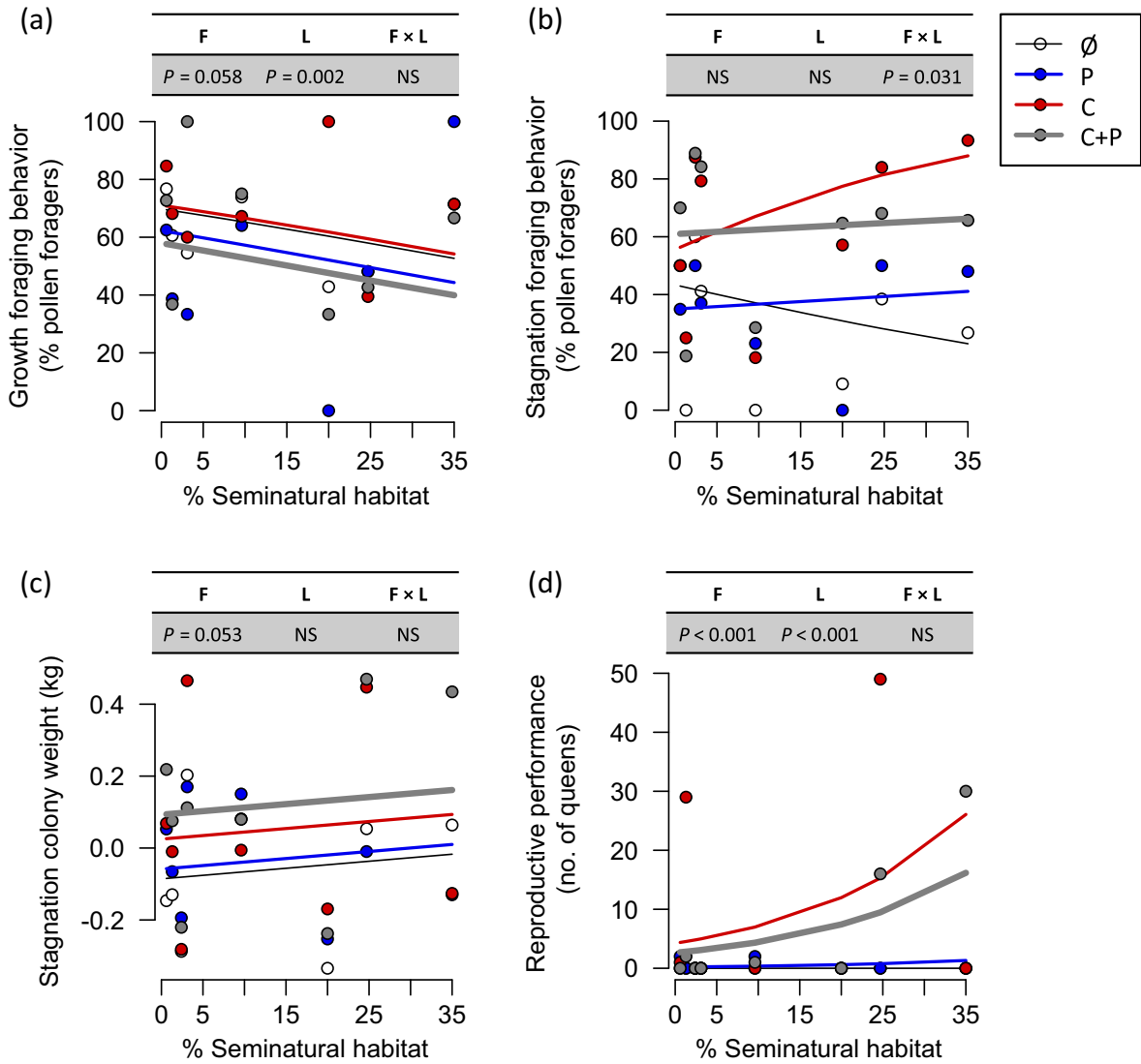
Fig. 5. Effects of seminatural habitat and supplemental feeding on (a) the foraging behavior during the period of colony growth, (b) the foraging behavior during the stagnation period, (c) the colony weight during the period of colony stagnation, and (d) the reproductive performance of bumble bees. Each dot represents a measurement of colony dynamics. Colors depict the different supplemental feeding treatments (pollen in green, sugar water in red, both pollen and sugar water in gray, and the control in black). Lines show the model predictions with respective treatment colors and significance of the landscape (L) and feeding treatments (F) are shown for each fit. See Appendix S1: Table S1 for more details on statistical properties.

results suggest that within the range of seminatural habitats studied in our landscape gradient (i.e., from 0.7% to 35%), the availability of food resources for *B. terrestris* populations is limited, as indicated by the beneficial effect of supplemental feeding in all landscapes. In particular, the supplementary feeding of carbohydrates (i.e., nectar) increased the reproductive performance of colonies placed in landscapes with a high abundance of seminatural habitats, but not in simple landscapes. This result demonstrates that there is significant resource shortage of nectar supplies even in landscapes with abundant seminatural habitat. Moreover, this result suggests that seminatural habitats are complementary to

carbohydrates, likely because of the benefits of diverse pollen resource availability. An assumption could be that the addition of carbohydrate resources allows forager bees to exploit the more diverse pollen resources in these complex landscapes and produce sexual offspring. Indeed, sexual offspring, especially new gynes, need more resources (proteins and carbohydrates) for breeding than nonsexual individuals, because they need increased larval feeding (Ribeiro et al. 1999) and have a longer larval developmental time (Duchateau and Velthuis 1988).

In simple landscapes, even if workers got supplemental carbohydrates to forage, the absence of

seminatural habitats limited sexual offspring production. Seminatural habitats are sources of high plant diversity (Dramstad and Fry 1995) and provide high pollen quality (Donkersley et al. 2014, Requier et al. 2015, Vaudo et al. 2015). Surprisingly, we found no clear effect of our pollen supplementation on reproductive performance even in landscapes with low abundance of seminatural habitats. This could be related to the rather low amount of freeze-dried pollen consumed by the bumble bees during our experiment (K. K. Jowanowitsch and K. Kallnik, *personal observation*). Possibly, the attractivity of the used freeze-dried pollen resource was reduced because of a potential change of the digestibility and nutritive value of our 1-yr-old freeze-dried pollen compared to fresh pollen available on flowers (Vásquez and Olofsson 2009). Nevertheless, our result suggests that food resources should be limited in mixed landscapes for bumble bee fitness, in particular in simplified landscapes with a low proportion of seminatural habitats. We are aware that, because of the complexity of ecological systems and several possible explanatory parameters, a larger sample size would be advantageous, as in most ecological studies. However, several parameters must be taken into account. Although wild-caught bumble bee colonies guarantee a great realism in contrast to nonlocal bought colonies, rearing viable colonies from wild-caught queens is very challenging. This requires a huge experimental effort —capturing a large number of queens, keeping them in controlled conditions (e.g., climate chambers), and feeding them until the establishment of the colony. On average, it is necessary to capture and breed three times more queens than the final number of viable colonies expected for the experiment (see also Samuelson et al. 2018). Further, the sample size of this study is placed in the same range than many other ecological studies working with bumble bee colonies (e.g., ranging from 24 to 43 colonies in Westphal et al. 2009, Kämper et al. 2016, Hass et al. 2018, Samuelson et al. 2018, Vaudo et al. 2018). Based on these experimental parameters, we recommend further investigation and we believe that the supplemental feeding in differently structured landscapes and thus resource environments provides a promising novel approach for experimentally testing multiple resource limitations in ecology.

In the context of existing conservation measures to counteract the decline in pollinators and pollination service in agricultural landscapes (Biesmeijer et al. 2006, Krauss et al. 2010, Potts et al. 2010, Carvell et al. 2015, Potts et al. 2016), this study supports the benefit of conserving seminatural habitats for bumble bee populations. Moreover, the results pinpoint the critical stage of spring colony growth as a potential early warning indicator of reproductive performance. We recommend integrating processes of colony dynamics in future studies of environmental risk assessment on bee ecology. Exploring how seasonality of resource limitation could affect pollinator performance would also help calibrate suitable management measures for the conservation of biodiversity-driven services in farmlands.

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Data Availability

The data presented in this manuscript are available through the Dryad Digital Repository: https://doi.org/10.5061/dryad.fxpnvx0n2

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