



# Effects of three flower field types on bumblebees and their pollen diets

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## Abstract

Agri-environment schemes, like flower fields, have been implemented in the EU to counteract the dramatic decline of farmland biodiversity. Farmers in Lower Saxony, Germany, may receive payments for three flower field types: annual, perennial (five years old), and mixed flower fields composed of yearly alternating annual and biannual parts. We assessed the effectiveness of these flower field types in providing bumblebee foraging habitat compared to control cereal fields. We sampled bumblebees with transect walks and assessed the richness of exploited pollen plants using DNA meta-barcoding and direct observations.

All flower field types enhanced bumblebee abundance and species richness compared to control fields but attracted mostly three generalist species. Although we expected highest benefits from the more heterogeneous mixed flower fields, abundance was highest in annual, only intermediate in mixed, and lowest in perennial flower fields. Bumblebee species richness did not differ between flower field types.

Overall, the proportion of sown plants in pollen loads was surprisingly low (< 50%). *Bombus pascuorum*, but not *B. terrestris* agg., exploited 10% of the sown plant species in perennial, 36% in annual and 45% in mixed flower fields, respectively. Compared to direct observations, pollen samples revealed 4.5 times more visited plant species and thus assessed floral resource use more reliably. Plant species richness in pollen loads decreased with local flowering plant species richness and increased with proportion of annual crops in the landscape, potentially due to the exploitation of more diverse and scattered resources, including flowering crops, in homogenized landscapes to fulfil dietary requirements.

Our results indicate that under the current management, both annual and mixed flower fields provide the most attractive food resources, while perennial flower fields offered the poorest foraging habitats. Conclusively, flower fields seem important but resources from the surrounding landscape are still needed to sustain bumblebees in agricultural landscapes.

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## Introduction

Flower-visiting insects are crucial for the sexual reproduction of wild plants (Biesmeijer, 2006) and most crop species (Garibaldi et al., 2013; Klein et al., 2007). However, while the dependence of global production on animal-mediated pollination is increasing, populations of both managed and wild pollinators are shrinking (Potts et al., 2016). These declines have been linked to the expansion of cropped areas and agricultural intensification during the 20<sup>th</sup> century (Foley, 2005). One important pollinator group that has suffered from agricultural intensification are bumblebees (*Bombus* spp.) (Goulson et al., 2008). Bumblebees depend on a high quantity and diversity of floral resources available continuously from early spring to October that have become rare during the last decades (Goulson et al., 2008). Within the second pillar of the EU's Common Agricultural Policy, agri-environment schemes have been introduced to mitigate the negative effects of intensive farming and to enhance biodiversity and the delivery of associated ecosystem services like pollination (Batáry et al., 2015; Carvell et al., 2006; Geppert et al., 2020). Flower fields are an agri-environment scheme designed to mitigate the food scarcity for pollinators in agricultural landscapes. They can be implemented as annual fields that are ploughed and (re-) sown every year or as perennial flower fields that persist without ploughing for several years. Annual flower fields often provide high flower cover (Williams et al., 2015), and therefore may support higher pollinator abundances than continuous perennial flower fields (Krimmer et al., 2019). In contrast, perennial flower fields might provide important food resources for pollinators offered by biannual or perennial plant species which are not present in annual flower fields and therefore promote more different bumblebee species. Moreover, in Lower Saxony, Germany, mixed flower strips or fields are implemented as a unique third flower field type (FFT). These mixed flower fields are separated in two parts that are ploughed and re-sown alternatingly each year, resulting in the simultaneous presence of an annual and a biannual part. Therefore, this scheme might combine the advantages of annual and perennial flower fields and might support both, relatively high bumblebee species richness and abundance, but studies of mixed flower fields are missing so far. In addition to the local flower field management, landscape complexity, i.e. the amount of intensively managed annual crops or semi-natural habitats, is an important factor for bumblebees potentially impacting the landscape-wide floral resource availability and moderating the effects of flower fields (Krimmer et al., 2019; Scheper et al., 2015). However, comparisons between flower strips or fields with different

disturbance and sowing interval regimes and their effect in different landscapes have rarely been studied.

Bumblebees collect pollen and nectar as floral resources. Pollen has a high protein content and is therefore essential for reproduction (Roulston & Cane, 2000). Bumblebees mix pollen of different plant species to optimize nutritional value (Kriesell et al., 2017; Vaudo et al., 2015), and a high pollen diversity can have a positive impact on colony growth (Hass et al., 2019). High bumblebee visitation rates in the flower fields might suggest that they are used as the main pollen resource (Wood et al., 2015), but this could be misleading because, firstly, visiting bumblebees might collect nectar instead of pollen (Konzmann & Lunau, 2014), and, secondly, a large proportion of the bumblebee pollen diet may consist of sources collected outside the flower fields in the agricultural landscape, especially woody plant species (Bertrand et al., 2019; Kämper et al., 2016). Whether bumblebees foraging in flower fields carry a high proportion of pollen from non-flower-field plant resources is rarely assessed, but a study from the U.K. suggests that bumblebees show a high degree of constancy to plant species sown in flower fields (Carvell et al., 2006). Likewise, bumblebees in urban flower strips mainly collect pollen from plants present on the strips (Potter et al., 2019). However, observational and pollen transfer networks can differ significantly, because pollinators may forage outside the observation plots (Potter et al., 2019), indicating that pollen samples are needed to give insight into the resource use of pollinators and to evaluate flower fields as foraging habitats.

In this study, we investigated the effectiveness of the three FFTs that were implemented in Lower Saxony as part of agri-environment schemes (annual, perennial and mixed flower fields) in promoting local bumblebee abundance and species richness compared to conventionally managed cereal fields. We also collected pollen samples of two bumblebee species commonly found in all FFTs (*Bombus terrestris* agg. and *B. pascuorum*) to analyse the pollen richness collected, the proportion of collected plant species that were sown in the flower fields, and to compare visitation and pollen networks. We hypothesized,

- (1) Bumblebee abundance will be lowest in cereal fields and highest in annual fields due to the highest flower cover. Mixed flower fields will take an intermediate position between annual and perennial flower fields due to the combination of both characteristics. Landscape simplification will impact bumblebee abundance negatively.
- (2) Bumblebee species richness will be lowest in cereal fields and highest in perennial fields due to the highest plant species richness. Mixed flower fields will take an intermediate position and landscape simplification will have a negative impact.
- (3) Plant species sown in the flower fields will be the main pollen resource. The proportion of sown plant species in the bumblebee pollen loads will be highest in annual flower fields due to their attractively high

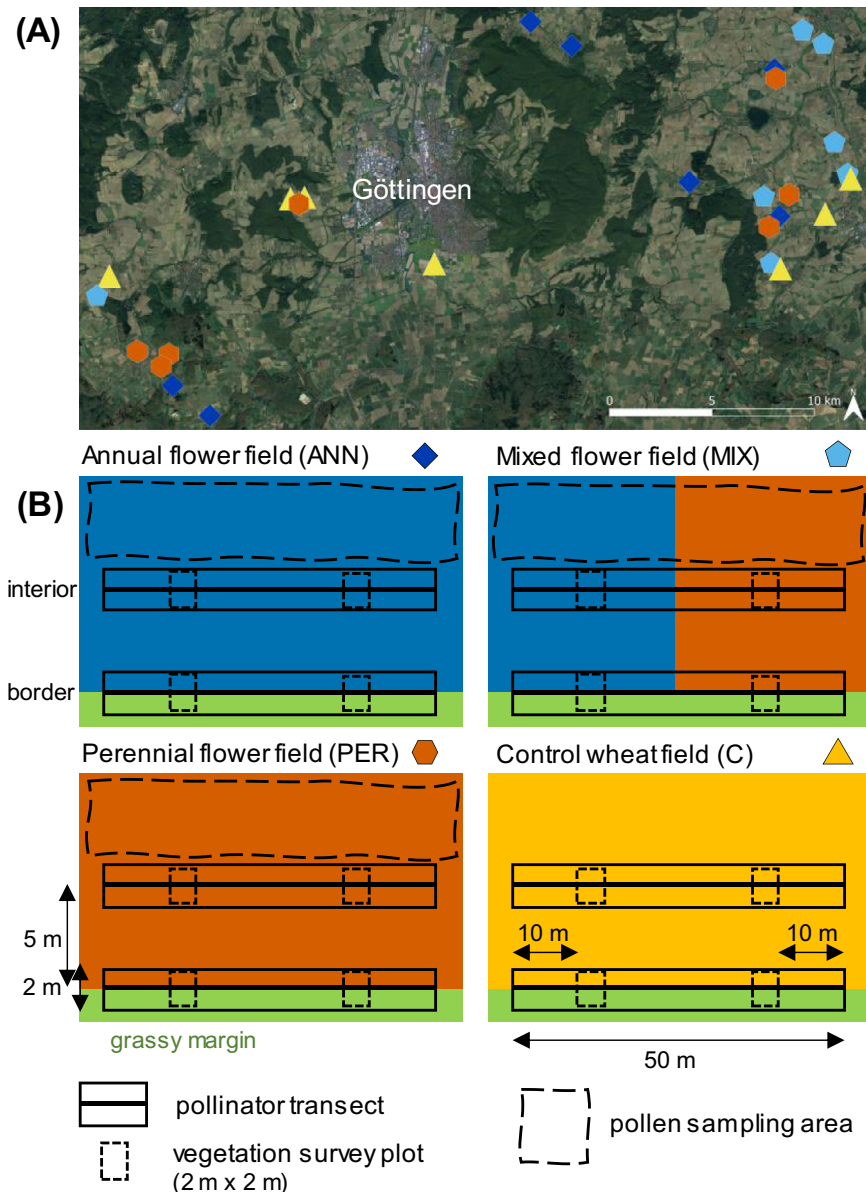
flower cover and lowest in perennial flower fields. Moreover, we expect that the proportion of sown plants will increase with landscape simplification due to a lack of alternative food resources.

- (4) The richness of collected plant taxa in the pollen loads will increase with plant species richness in the respective flower fields and will therefore be highest in perennial flower fields. However, richness of collected plant taxa will decrease with landscape simplification. Flower fields will be the main foraging sites and therefore the number of plant taxa the bumblebee is interacting with will be similar in pollen load networks and in flower visit observation networks. Plants with the highest visitation frequency will also constitute the highest share in pollen samples.

## Materials and methods

### Study sites and landscape analysis

We selected 28 sites around Göttingen in southern Lower Saxony, Germany (Fig. 1A). The region is characterized by intensively managed agricultural land and forests, with the most common crops being cereals, oilseed rape, maize and sugar beet (Hass et al., 2018). Each site represented either an annual, mixed, or perennial flower field or a



**Fig. 1.** (A) Map of study sites around the city of Göttingen in the federal state of Lower Saxony, Germany; satellite image from Google Satellite, 2019. (B) Schematic overview of the study design. We selected 7 fields per management type and sampled bumblebees and their visited plant species for 10 min on two transects per field ( $N = 56$  transects), three times between June and August 2018. All fields were bordered by grassy margins adjacent to field roads. During the second sampling round we also recorded flowering plant species in two 2 m x 2 m plots per transect and collected pollen samples from bumblebees.

conventionally managed winter wheat field as control (seven replicates per field type) (Appendix A: Table S1). Sample field size ranged from 0.28–1.50 ha for 26 fields, but two control fields were larger (3.9 ha and 4.5 ha). The three different flower field types (FFT) did not differ in their field sizes, as indicated by pairwise comparisons with Wilcoxon rank sum test ( $p > 0.05$ ). Details on management and seed mixture of all flower field types are presented in Appendix A: Table S2. All fields were bordered by grassy margins. All mixed and perennial flower fields were at least in their second year of management.

To include the effect of landscape composition within foraging distance, we used QGIS 3.4.10. to set buffer zones of 1,000 m around the centre of each field (Greenleaf et al., 2007). We used information of agricultural fields in land-use maps (SLA, 2018) and digitized all other land cover types based on satellite images (Map data ©2019 Google based on GoogleSatellite plugin). For each buffer zone, we assessed the percentage of annual crops as a proxy for agricultural intensity and landscape simplification. Average percent cover of forests, permanent grassland, annual crops, woody habitats, and seminatural habitats did not differ between field types (Appendix A: Table S3). Overall, mean cover of annual crops was  $56.5 \pm 3\%$  (mean  $\pm$  SE), and mean cover of seminatural habitats was  $4.3 \pm 0.47\%$ . We decided to only consider percentage of open habitats, i.e., cover of annual crops, seminatural habitats, and permanent grassland as landscape level covariates. However, testing with Spearman's rank correlation showed that percentage cover of annual crops was significantly negatively correlated with both covariates (Appendix A: Table S4). Therefore, we only included cover of annual crops in the models.

## Bumblebee sampling

We sampled bumblebees along two transects (border and interior), because their communities might differ. The border transect was located directly at the border of the flower field (or control wheat field) and the grassy margin, and the interior transect was placed 5 m further inside the field (Fig. 1B). We chose this distance to avoid disturbance of potentially nesting birds in the field centre. The transects were 50 m long and 2 m wide. In mixed flower fields, transects covered the annual and biannual part equally (25 m in each part). In three cases (wet corner at field edge hindering germination; nutrient-rich field corner dominated by ruderal plants (nettle); heavy vehicle damage), transect position was slightly shifted to obtain a representative sample of the vegetation. We walked along the transect for 10 minutes (excluding handling time of insects) and recorded all bumblebees and their interactions with plant species, i.e. when the bumblebee was in contact with the inflorescence. Individuals sitting on other plant parts were included in the abundance and species richness analysis, but not visitation rates. In case reliable identification under field conditions was impossible, the specimens were caught with a sweep net, transferred to a

jar with ethyl-acetate and identified in the laboratory with stereomicroscopes. The *Bombus terrestris/lucorum* species complex was referred to as “*Bombus terrestris* agg.”.

Bumblebees were surveyed from 09:00–18:00 in dry weather with little or no clouds, low wind (below Beaufort scale 4) and temperatures above 15°C. To account for the diurnal variability of pollinator activity, we sampled individual fields at different times of the day across the three sampling rounds (observer ALH: June 20<sup>th</sup>–July 7<sup>th</sup>; observers JP and CG: July 16<sup>th</sup>–24<sup>th</sup>; observers JP and CG/VC: July 27<sup>th</sup>–August 6<sup>th</sup>, 2018; as far as possible, we made sure there was no systematic bias of observers in the assignment to transects and/or treatments.). To assess floral resource availability on surveyed transects, we divided each transect into sections of 10 m. During each round of the pollinator survey, we estimated the percentage area covered by floral units in each 10  $\times$  2 m section.

During the second round, we recorded all flowering plant species and separately those that had recently flowered in four 2 m  $\times$  2 m plots per field. Two vegetation plots were located on the border transect, and two on the interior transect, 10 m from the respective transect end (Fig. 1B).

## Pollen collection

During the second sampling round, we collected pollen samples from bumblebees foraging in the flower fields. On each flower field, we aimed to capture five individuals each of two abundant bumblebee species of this survey with contrasting traits, *Bombus terrestris* agg. (short tongue and large colony) and *B. pascuorum* (long tongue and small colony). Individuals were caught outside the transects (Fig. 1B) with a clean vial and were transferred to a second vial with ca. 2 ml of water. The glass was gently shaken to wash the pollen from the bumblebee, then the bee was released and the water with pollen was transferred to an Eppendorf reaction tube. Total sampling time was limited to 20 minutes, excluding handling time. Samples were stored at 10°C in a cooling room until preparation in October 2018.

## Pollen sample preparation

From the 168 collected pollen samples (less than ten foraging individuals in the set time frame), we selected three samples per field for each *B. terrestris* agg. and *B. pascuorum* for further analysis as this number resulted in the most well-balanced distribution of sample sizes across species, sites, and field types (overall 110 samples). Samples with a high amount of visible pollen were preferred. If no difference was visible, and more than three samples were available, we chose them randomly. After centrifugation (10,000 rpm, 20°C, 10 min + additional 10 min for single samples where pollen was not set after first centrifugation step), the pollen pellets were dried in the open reaction tubes.



## Metabarcoding and bioinformatic processing

Next generation sequencing is a feasible approach to identify pollen in mixed samples to genus and species level even without expert palynological knowledge (Keller et al., 2015). In general, samples were processed strictly according to the protocol of Sickel et al. (2015) for the ribosomal internal transcribed spacer 2 (ITS2) (see Appendix A: section 1.4 for details).

Raw sequencing reads were joined and quality filtered (maxEE=1.0, >150 bp, no ambiguous base-pairs) using VSEARCH (v2.8.1, Rognes et al., 2016). With the same software, reads were dereplicated, denoised and chimeras removed to generate unique amplicon sequence variants (ASVs). All filtered reads were mapped against these ASVs to receive read counts per sample using VSEARCH and global alignments with 0.99% sequence identity threshold. ASVs were further taxonomically classified against multiple reference databases created with BCdatabaser (Keller et al., 2020) by species lists (see below) with global alignments using VSEARCH and top hit selection but a minimum threshold of 97%. Priority of reference databases was (1) created by a list of all plant species identified during our bumblebee and vegetation survey (2) then all unclassified ASVs by the Red List of plants for Lower Saxony (Garve, 2004). (3) Lastly, remaining unidentified ASVs were classified to the highest taxonomic level possible (threshold 0.8) with VSEARCH and the SINTAX algorithm. In R 3.6.1 (R Core Team, 2019) we used the ‘phyloseq’ package (McMurdie & Holmes, 2013) to manage the data, filter out samples with less than 100 reads, agglomerate ASVs on species level, transform to relative abundances (percent contribution of each species in a sample to the sample’s total number of reads) and discard species per sample that contributed less than 1% to the sample’s read count.

## Statistical analyses

Data exploration (Zuur et al., 2010) and all statistical analyses were performed in R v3.6.3 (R Core Team, 2019). We compiled separate data sets for testing our hypotheses because we had to aggregate the observations from different sampling rounds and at different levels to address each hypothesis (resulting in different sample sizes).

### Hypotheses (1) and (2): Bumblebee abundance and species richness

To compare the effectiveness of the FFTs over the whole survey period, we pooled abundance and species richness at the field level, summing observations over transects and sampling rounds to increase representativeness of each sample point (different number and identity of observers between sampling rounds). We calculated the sum instead of the mean because we wanted to use GLMs with Poisson or negative binomial error

distribution and these require integers. Flower cover data were pooled by calculating the mean per transect, and then calculating the overall mean flower cover per field over all three rounds. We used the mean of means because for one mixed and one perennial flower field, flower cover data for the interior transect in the second round was missing. In this case we calculated the mean cover for the interior transect of those two fields over 2 rounds. Flowering plant taxa were only recorded during the second sampling round, so we merged the observations from the four vegetation survey plots per field to obtain total species richness of flowering plants. Mean flower cover was significantly positively correlated with the number of flower taxa on the field (Spearman’s  $\rho = 0.77$ ,  $p < 0.0001$ ), so we decided to use only one of them in the model. We used GLMs to analyse the effects of field type, flower cover and percentage cover of annual crops in the landscape on total bumblebee abundance and bumblebee species richness. In the variable “mean flower cover”, there was an outlier (perennial flower field BS2.4). We decided to keep the site in the data set as an example of a field with high flower cover but low bee abundance, but we repeated all models excluding the outlier. Those models and results are presented in Appendix B: Tables S1–S15.

### Hypothesis (3): Proportion of sown plants in pollen loads

To quantify the extent to which bumblebees use the floral resources offered by the different FFTs, we categorized plant species based on their presumed origin. We assumed that a plant species in the pollen load of a bumblebee caught on a given flower field was ‘sown’ if it was listed on the seed mixture of the respective field type’s management regulations (Lower Saxonian Ministry for Food, Agriculture and Consumer Protection, 2017a, 2017b, 2017c). If not listed, plant origin was classified as ‘other’. For each pollen load, we calculated the proportion of sown plants as the ratio of the sum of sown plants’ percent of reads (numerator) and the sum of sown and other plants’ percent of reads (denominator). Then we averaged pollen samples from one site (field) for each bee species (which was necessary to improve model fit. We used a GLMM with field ID as a random factor to test whether the proportion of sown plants (cumulative relative abundance) in pollen loads pooled per field depended on FFT, bumblebee species, interaction of both factors, the species richness of flowering plants in the field (correlated with flower cover:  $\rho = 0.55$ ,  $p = 0.0003262$ ), and the percentage cover of annual crops in the landscape.

### Hypothesis (4): Plant species richness in pollen loads and comparison of the richness of plant taxa interactions in pollen and field observation networks

We calculated plant species richness per pollen sample collected from one bumblebee individual. We did not pool

samples per site because we were interested in the variation between pollen loads. Then we fitted a GLMM with field ID as random factor to test whether plant species richness depended on FFT (annual, mixed, perennial), bumblebee species, the interaction of FFT and bumblebee species, the species richness of flowering plants in the field (correlated with flower cover:  $\rho = 0.51$ ,  $p < 0.0001$ ), and the percentage cover of annual crops in the landscape.

For visual comparison, we constructed pollen and visitation networks with the ‘bipartite’ package v2.15 (Dormann & Fruend, 2008). We used visits of *B. terrestris* agg. and *B. pascuorum* from the second sampling round, which was conducted at the same time as the pollen sampling. To compare the information from both survey methods, we first transformed the number of observed visits into relative abundance (i.e., percentage of visits to a plant species at a site divided by the total number of visits to all plant species at that site) for each bumblebee species. However, since data from metabarcoding is semi-quantitative, a direct comparison of relative abundance from the two methods is inappropriate. Therefore, instead of relative abundance, we calculated the richness of visited plant species for each bee species per field (for pollen data: per sample). Because we did not pool pollen samples from the same bumblebee species and field, this resulted in unequal numbers of observations between method but reflects variability between pollen samples/bee individuals. Ultimately, we tested the effects of FFT, survey method, percentage cover of annual crops, and flowering plant species richness on the richness of visited plant taxa with a GLMM.

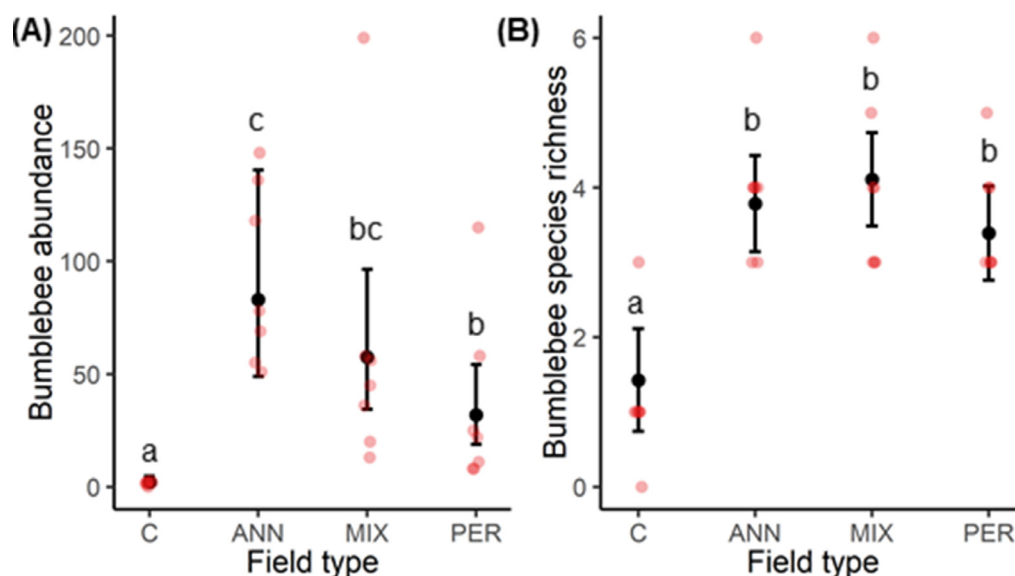
For all models with count data we first fitted models with Poisson error distribution and then we used the ‘DHARMA’ package (Hartig, 2020) to assess diagnostic plots of the

residuals. In case of overdispersion, we used a negative binomial distribution. However, in some cases a normal distribution resulted in the best model fit. The functions used for modelling were ‘glm’, ‘glm.nb’ in the ‘MASS’ package (Venables & Ripley, 2002) and the ‘glmmTMB’ function of the ‘glmmTMB’ package (Brooks et al., 2017). An overview of all models and assumed error distributions is presented in Appendix A: Table S5. We checked for spatial autocorrelation of model residuals with Moran’s I in ‘DHARMA’, based on sample field centre coordinates (Appendix A: Table S6). Because all variables are relevant for our hypotheses, we decided against further model simplification. For pairwise comparison of elements, we applied Tukey’s post hoc test on the respective final model using pairwise comparisons between estimated marginal means computed with the ‘emmeans’ package v1.4.7 (Lenth, 2020), using the ‘relevel’ function to change reference level and compute all relevant comparisons.

## Results

### Bumblebee abundance and species richness

In total, we observed 1,342 bumblebees from 10 species (Appendix A: Table S7). The most abundant taxa were *Bombus terrestris* agg. (578 individuals), *B. lapidarius* (376 individuals), and *B. pascuorum* (302 individuals). Pairwise comparison of estimated marginal means (EMM) from our GLM revealed that bumblebee abundance was significantly enhanced in all FFTs compared to the control cereal fields (Fig. 2A, Appendix A: Table S10–S11), namely by factors of  $39.1 \pm 19.8$  observed visits in annual flower fields,  $27.1 \pm 13.1$  in mixed flower fields, and  $15 \pm 7.6$  in perennial flower



**Fig. 2.** Effects of flower field type (C = control wheat field, ANN = annual, MIX = mixed, PER = perennial flower field) on (A) bumblebee abundance and (B) bumblebee species richness. Data from 7 replicates per treatment level were pooled across transects and sampling rounds. Predictions of GLM with 95% confidence intervals are shown.

fields. Within FFTs, bumblebee abundance was significantly higher in annual (EMM $\pm$ SE = 83 $\pm$ 22 observed visits) than in perennial flower fields (EMM $\pm$ SE = 32 $\pm$ 9 observed visits) (EMM ratio $\pm$ SE = 2.6 $\pm$ 0.95,  $p$  = 0.0440), with mixed flower fields taking an intermediate position (EMM $\pm$ SE = 57 $\pm$ 15 observed visits) (Fig. 2A, Appendix A: Table S10–S11). Species richness of bumblebees was also significantly enhanced in all FFTs compared to the control cereal fields, namely by factors of 2.36 $\pm$ 0.51 in annual flower fields ( $p$  = 0.0008), 2.68 $\pm$ 0.47 in mixed flower fields ( $p$  = 0.0001), and 1.97 $\pm$ 0.497 in perennial flower fields ( $p$  = 0.0038) but did not differ between the FFTs (Fig. 2B, Appendix A: Table S10–S11).

In the models based on all 28 sites, neither percentage cover of annual crops in the landscape nor local mean flower cover affected bumblebee abundance and species richness (Appendix A: Table S10). Moreover, flower cover and number of flowering plant taxa were higher in all FFTs compared to control fields but did not differ between FFTs (Appendix A: Fig. S1A,B, Table S8–S9). However, when a perennial flower field (BS2.4) with high flower cover was excluded, the positive effect of mean flower cover on bumblebee abundance became significant whereas the difference between annual and perennial flower fields became non-significant, and for bumblebee species richness the difference between annual flower fields and control fields became non-significant (Appendix B: Table S6–S7).

### Proportion of sown plant species

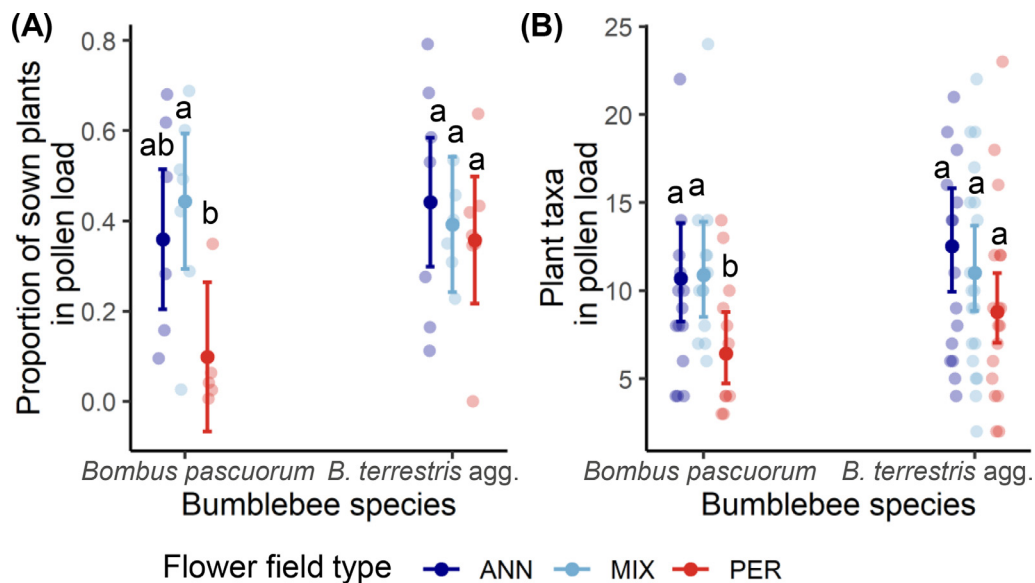
Metabarcoding yielded in total 378,076 classified reads after merging and quality filtering. This corresponded to a

mean of 4,019 reads per sample (minimum 148, maximum 14,736). After quality filtering, 91 samples with a total of 125 identified plant taxa remained.

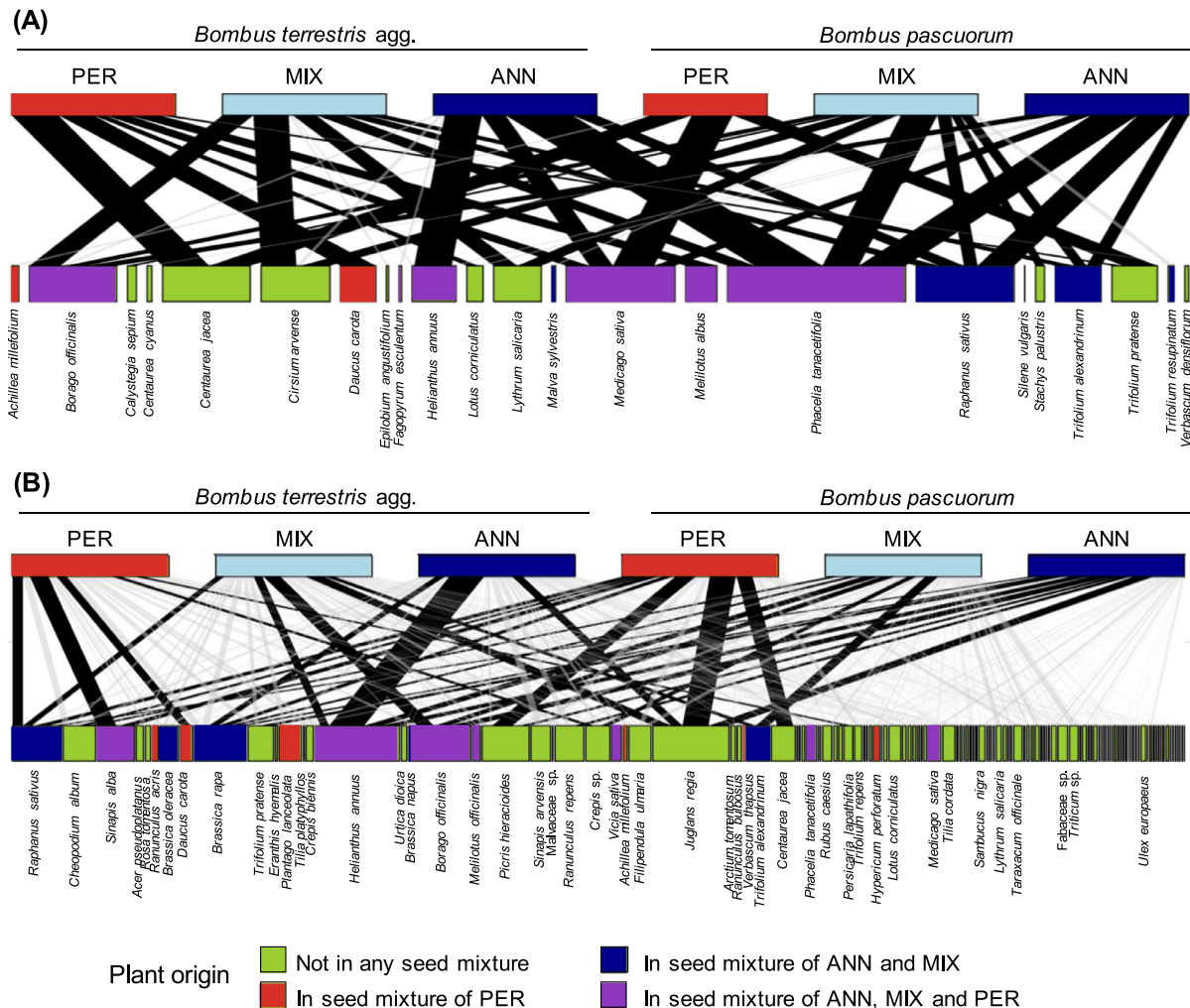
The predicted proportion of sown plants in the pollen loads of *B. pascuorum* was significantly lower in perennial (9.73% $\pm$ 8.12%, EMM $\pm$ SE) than in mixed (44.52% $\pm$ 7.19%) and annual flower fields (36.07% $\pm$ 7.63%), but not in the pollen loads of *B. terrestris* agg. (Fig. 3A, Appendix A: Table S15). In the model, neither annual crop cover nor plant species richness in the field affected the proportion of sown plants in the pollen loads (Appendix A: Table S14).

### Plant species richness in pollen loads and comparison of the richness of visited plant taxa in pollen and field observation networks

The richness of visited plant taxa in the bumblebees' pollen loads was lower in perennial (6.26 $\pm$ 1.01) than in mixed flower fields (10.58 $\pm$ 1.31), but only for *B. pascuorum* (Fig. 3B, Table S12–S13). Plant species richness in the pollen loads of both bumblebee species increased with percentage cover of annual crops in the surrounding landscape and decreased with higher flowering plant species richness on the flower field (Appendix A: Table S12). During three sampling rounds, we observed 1,302 interactions between bumblebees and inflorescences of 41 plant species. *Phacelia tanacetifolia* (519 visits), *Helianthus annuus* (128 visits), and *Borago officinalis* (121 visits) received most visits (Appendix A: Fig. S3). Comparing the field observation (Fig. 4A) and pollen networks (Fig. 4B) based on data from the second sampling



**Fig. 3.** Effects on the plant taxa in the pollen loads of *Bombus terrestris* agg. and *B. pascuorum*. (A) Interactive effects between flower field type (ANN = annual, MIX = mixed, PER = perennial flower field) and bumblebee species on the proportion of pollen (contribution to read count per sample) collected from plant species sown in the flower fields (pollen samples were averaged on field level). (B) Interactive effects of flower field type and bumblebee species on the number of plant taxa in the pollen loads. Predictions of GLMMs with 95% confidence intervals are shown. Lower case letters denote different ( $p < 0.05$ ) estimated marginal means (Tukey's test).



**Fig. 4.** Interaction networks of *Bombus terrestris* agg. and *B. pascuorum* and their respective forage plants in the different flower field types (ANN = annual, MIX = mixed, PER = perennial flower field) based on (A) observed visits during transect walks and (B) DNA meta-barcoding of pollen washed from whole body. Both surveys were conducted simultaneously in each sampled field during July 16–24, 2018. Interaction width indicates interaction strength. Interactions with > 5% of reads or visits are highlighted in black, < 5% in grey. Fill of lower boxes indicates assumed origin of plant, based on agri-environment scheme seed mixture regulations. Note that pollen loads were collected outside the transect area.

round, we found for *B. terrestris* agg. and *B. pascuorum* broadly the same main forage plants in the three FFTs (Fig. 4A,B), but metabarcoding detected additional exploited plant taxa, resulting in  $4.52 \pm 0.595$  (EMM ratio  $\pm$  SE) times more interactions than the visitation survey (Tukey's post hoc test on GLMM,  $df=121$ ,  $t$  ratio = 11.498,  $p < 0.0001$ ) in all FFT (Fig. 5A, Table S17). Both survey methods showed a positive effect of increasing cover of annual crops and a negative effect of increasing local flowering plant species richness on the interaction richness (Fig. 5B,C). Moreover, in the pollen network we found a high proportion of weak interactions ("tails") (Appendix A: Fig. S5,S6).

## Discussion

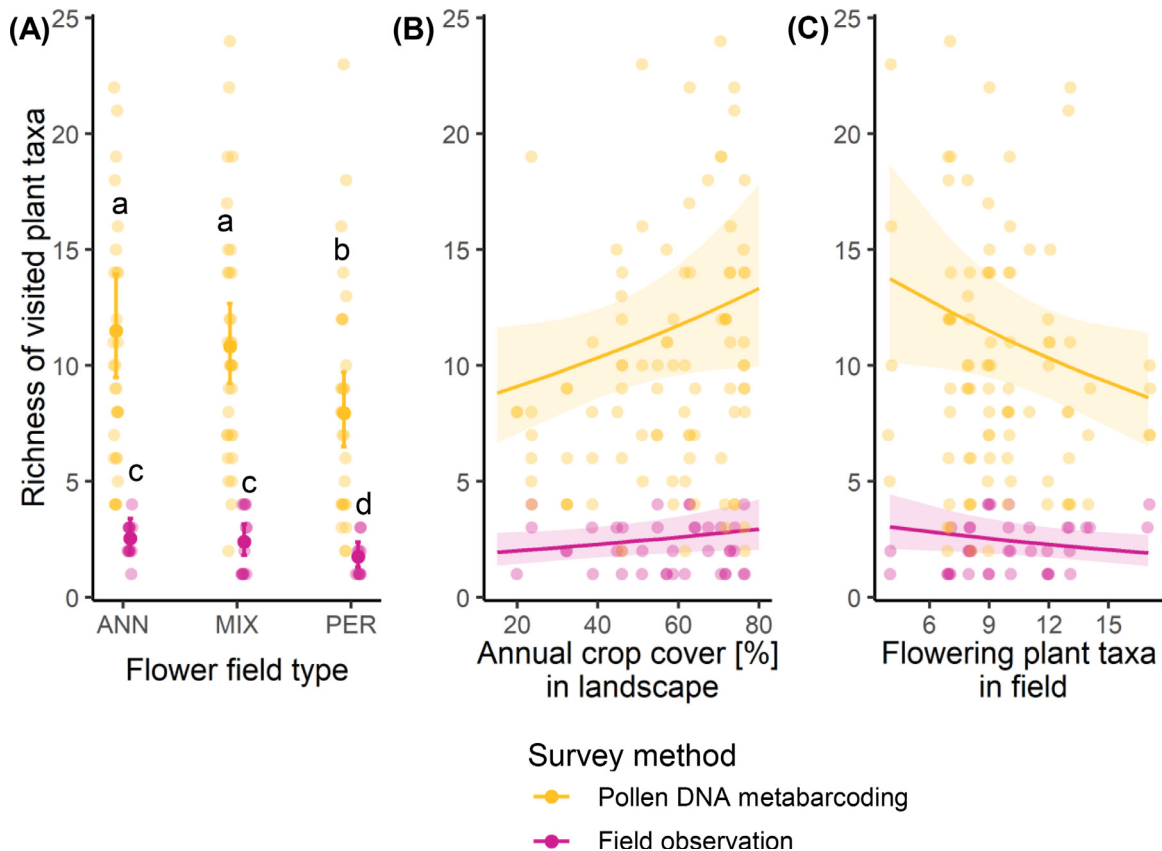
Our study showed that all flower field types (FFT) enhanced bumblebee abundance and species richness

compared to cereal fields. However, annual flower fields attracted the highest abundance of bumblebees whereas species richness was similar in all FFTs. A high proportion of the bumblebees' pollen diet originated from plants not sown in the flower fields, especially for *B. pascuorum* in perennial flower fields. Similarly, the pollen analysis revealed a high number of additional bumblebee-plant interactions compared to the observed visits in the flower fields. Surprisingly, the number of interactions increased with a higher proportion of annual crops in the landscape.

## Bumblebee abundance and species richness

The enhanced bumblebee abundance and species richness in all three FFTs compared to control cereal fields confirm the effectiveness of these agri-environment schemes as shown in previous studies (Scheper et al., 2013). However,





**Fig. 5.** Effects on the richness of visited plant taxa of *Bombus terrestris* agg. and *B. pascuorum*: (A) Interactive effect between flower field type (ANN = annual, MIX = mixed, PER = perennial flower field) and method (pollen DNA meta-barcoding or observed visits); (B) Effect of annual crop cover (%) within 1000 m; (C) Effect of plant species richness in the field. Predictions of GLMMs with 95% confidence intervals are shown. Different lower-case letters = significant ( $p < 0.05$ ) differences between estimated marginal means (Tukey's test). Note that each data point in the pollen data represents one pollen load/sample, but each data point from field observation is the total number of observed visits from a field during the second sampling round.

the FFTs differed in their effectiveness to promote bumblebee abundance. For bumblebee abundance we could confirm our hypothesis that annual flower fields have the highest and perennial flower fields the lowest bumblebee abundance, with intermediate levels in mixed flower fields. Therefore, we support the results of previous studies showing that newly established flower fields have the highest attractiveness (Krimmer et al., 2019; Williams et al., 2015). When the outlier was excluded, we found a weak effect of flower cover on bumblebee abundance. In our study, plant species with high nectar and/or pollen rewards like *Phacelia tanacetifolia*, *Helianthus annuus* and *Borago officinalis* were most often visited in annual and mixed flower fields and represented key flower resources for wild bees (Warzecha et al., 2018). However, these positive effects of non-native species like *P. tanacetifolia* on bumblebee abundance are not necessarily transferable to other pollinator species, e.g. more specialized solitary bees, because of species-specific flower preferences (Potter et al., 2019). Biannual and perennial vegetation seemed less attractive for bumblebees, lowering the overall bumblebee abundance on mixed and perennial flower fields. The low attractiveness of perennial flower

fields might be due to the increasing dominance of non-insect pollinated plants like grasses after several years without ploughing. Additionally, the fixed seed mixture composition in the perennial scheme might not be optimal for many locations with site specific characteristics leading to a low flower cover of focal species; especially during hot summers like 2018.

We found mainly common and widespread bumblebee species in the flower fields and species richness of bumblebees was similarly enhanced compared to control fields in all FFTs and was on the same level as reported from flower strips near our study region in 2017 (Geppert et al., 2020). That annual and mixed flower fields could only attract more bumblebee individuals, but not species, might be due to the low number of required plant species in the seed mixture (min. five) as it is well established that species richness increases with plant species richness in flower fields (Scheper et al., 2013; but see Warzecha et al., 2018). However, given the high observed variability for bumblebee abundance for all FFTs, local management or site-specific characteristics of individual fields seems to play a major role. Alternatively, an increased bumblebee species richness

in these FFTs might have been impeded by a poor species diversity in the wider landscape.

### Proportion of sown plant species in the pollen loads

We found surprisingly low proportions of sown plant species in pollen samples of bumblebees foraging in the flower fields (model estimates below 50% for all FFTs). In contrast to previous studies (Carvell et al., 2006; Potter et al., 2019) this result highlights the importance of floral resources outside the flower fields and bumblebees probably complement their pollen diet from flower fields to optimize the nutritional values for colony growth (Hass et al., 2019; Kriesell et al., 2017; Vaudo et al., 2015). Hence, flower fields can only partly compensate for monotonous, simplified landscapes lacking flower resources. We expected that the proportion of sown plants in the pollen samples would be highest in mixed and annual flower fields due to the high attractiveness of single annual plant species and high overall flower cover. Our results only partly support this hypothesis, as only the pollen loads from *B. pascuorum* contained significantly less pollen from sown plants in perennial flower fields than in mixed flower fields. This indicates that flowering plants in perennial flower fields are visited only infrequently and that the main foraging sites are situated in other habitat types.

### Plant species richness in pollen loads and comparison of the richness of visited plant taxa in pollen and field observation networks

The lower quality of perennial flower fields as a foraging habitat was also confirmed by a lower richness of collected plant taxa in pollen loads of *B. pascuorum*. This indicates that perennial flower fields are the least suitable foraging habitat as already indicated by the low bumblebee abundance and the low proportion of sown plants in pollen samples of this FFT (Warzecha et al., 2018). Interestingly, and contrary to our expectations, the number of plant species in the pollen loads was negatively affected by the number of presently flowering plant species on the field level, which could indicate niche partitioning due to adaptive foraging (Valdovinos et al., 2016; Westphal, Steffan-Dewenter & Tschamtkke, 2006). Especially, short-tongued and most common bumblebee species tend to optimize their foraging behaviour and prefer mass-abundant resources (Westphal, Steffan-Dewenter et al., 2006). Hence, single plant species dominating the floral aspect in the fields could drive the observed patterns of declining richness in pollen loads with increasing plant species richness. In contrast, richness of visited plants was positively influenced by the cover of annual crops in the surrounding landscape. The reason for the positive effect of annual crops on the pollen richness collected could be that in simple landscapes

bumblebees need to fly further to fulfil their foraging requirements (Redhead et al., 2016; Westphal et al., 2006) and therefore cover a larger area with patchily distributed flowering resources, thereby increasing the chance of collecting pollen from different plant species. Moreover, they might also exploit late flowering crops, such as sunflowers, weeds in arable fields and other (annual) flowering fields in the surrounding landscape.

When comparing bumblebee-plant interactions from field observation and pollen loads, we found that both *B. terrestris* agg. and *B. pascuorum* visited a wide variety of plant species. We expected that plants with the highest visitation frequency would also compose the highest share in pollen samples, which was only partially supported by our results. For example, being a nectar plant for bumblebees (Williams & Christian, 1991), *Phacelia tanacetifolia* was much more dominant in the visitation network than in the pollen network. In contrast, *Juglans regia* (walnut) is a wind-pollinated tree species and only appeared in the pollen network. However, walnut is an important forage plant for *Osmia* and *Apis* bees (Bertrand et al., 2019), and might have been collected deliberately here, given the importance of woody plants for the pollen diet of bumblebees (Bertrand et al., 2019; Kämper et al., 2016).

We found a 4.5 times higher number of interactions in pollen networks compared to visitation networks, emphasizing the limited information provided by local visitation networks alone. Similarly, Pornon et al. (2016) detected 2.5 times more plant species involved in plant-pollinator interactions via metabarcoding than by the observation of visits alone. A major reason for the difference may be that each observation in our transect walks corresponds to one interaction at one point in time, while each pollen sample comprises the result of several interactions within the bees' foraging ranges up to several kilometres (Greenleaf et al., 2007; Westphal et al., 2006b) and over a longer time period while the bee was foraging. Most importantly, pollen samples also reveal which plants the bumblebees had been foraging outside the flower fields (Pornon et al., 2016), e.g. seminatural habitats like woody or grassy field margins or suburban garden areas (Samuelson et al., 2018; Steffan-Dewenter et al., 2002). Additionally, the pollen network revealed few plant species that had been flowering earlier (e.g., *Eranthis hyemalis*, spring). An explanation could be that such pollen is still present in the storage of the nests and might in small amounts be passively taken out again by individuals and detected by the sensitivity of metabarcoding. Due to methodological reasons, weak links were much more frequent in the pollen network compared to the visitation network where fewer plants were observed for a limited time, thus interaction strength should not be compared directly between the two networks. Also, it has to be noted that metabarcoding data has some limitations, e.g. only semi-quantitative abundances, potential amplification biases that can alter relative amounts between each species and its classification depends on quality and completeness of the

reference database (Bell et al., 2016, 2019). We reduced risks of the latter two points by performing PCRs in triplicate (Darling & Mahon, 2011) and by using dedicated custom reference databases (Keller et al., 2020). Given the strength and limitation of both approaches, the combination of both is a great opportunity to acquire a more holistic picture of resource utilisation. Therefore, our findings emphasize the benefits of combining observational and pollen metabarcoding data to assess the uptake of conservation measures by targeted pollinators.

## Conclusions

We conclude that flower field agri-environment schemes (AES) in Lower Saxony effectively enhance bumblebee abundance compared to conventionally managed cereal fields, however mainly common and widespread species. All flower field AES could be optimised to better support bumblebees. For example, in annual and mixed flower fields the seed mixture could be diversified and enhanced with key plant species to attract more different bumblebee species. In perennial flower fields attractive plant species seemed to have a low flower cover resulting in only low bumblebee abundances and low proportions of sown species in the pollen diets of *B. pascuorum*. A promising scheme optimization could be a higher flexibility that allows for adaptations of seed mixtures to local conditions (Schmidt et al., 2020) and of management practices (e.g. Schmidt et al., 2020), e.g. scarification by breaking up the grass sward to allow germination of a wide range of seeds from the seed bank (Blake, Woodcock, Westbury, Sutton, & Potts, 2011). Additionally, better counselling for farmers regarding flower field maintenance (e.g., re-seeding, mowing) might improve the effectiveness of perennial flower fields to promote bumblebees. Besides, the implications of different management options should be considered also with respect to other flower pollinators, such as solitary wild bees or hoverflies.

Pollen sampling through DNA meta-barcoding revealed more bumblebee-plant interactions than found in the visitation network indicating that both methods complement each other and that a landscape perspective is needed to fully understand resource use of bumblebees within their large foraging ranges. Furthermore, high proportions of non-sown plants in the bumblebees' pollen diet highlight the importance of other habitat types as foraging habitats. We conclude that optimised flower field AES in combination with other semi-natural habitat types have the potential to support and sustainably promote bumblebees as an important pollinator group in agricultural landscapes.

## Authors' contributions

JP, PB and ALH developed the conceptual foundations for this manuscript. JP, CG and ALH performed the field

surveys and JP and ALH identified species. AK performed pollen DNA extraction, sequencing, and bioinformatics. JP with help of ALH performed statistical analysis and JP and ALH wrote the first draft of the manuscript. All authors contributed substantially to revisions and gave final approval for submission.

## Data accessibility

Cleaned bumblebee and plant data and processed tables are available in the Göttingen Research Online database (Piko et al., 2021), and sequencing data are available via the NCBI Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra/PRJNA702032>, BioProject accession number PRJNA702032).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.baae.2021.02.005](https://doi.org/10.1016/j.baae.2021.02.005).

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