Managing strawberry pollination with wild bees and honey bees:

Facilitation or competition by mass-flowering resources?

Dissertation

zur Erlangung des Doktorgrades

der Fakultät Agrarwissenschaften

der Georg-August-Universität Göttingen, Germany

vorgelegt von

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geboren in Northeim, Germany

Göttingen, Januar 2019

D7

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Tag der mündlichen Prüfung: 05 Februar 2019

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General Introduction

Introduction

Pollination is an important ecosystem service for the fruit yield and quality of 70 % of the world's most important crops (Klein *et al.* 2003). Fruits and vegetables, which have a high economic and nutritional value, particularly benefit from pollination (Gallai *et al.* 2009; Potts *et al.* 2010; Eilers *et al.* 2011). The economic value of pollination services for agricultural production is estimated to 153–577 billion US$ (Gallai *et al.* 2009; Lautenbach *et al.* 2012). As global pollinator-dependent fruit and vegetable production intensifies, the demand for pollination is increasing (Aizen & Harder 2009). The honey bee (*Apis mellifera* L.) is the most dominant pollinator globally, but the demand for pollination is increasing faster than the number of managed colonies (Aizen & Harder 2009). Although most pollination services to crops are mainly delivered by a few generalist bee species (Kleijn *et al.* 2015), a greater diversity of bees and functional traits will sustain resilient pollination services over space and time (Hoehn *et al.* 2008; Albrecht *et al.* 2012). However, pollinators are currently threatened and in decline, due to multiple stressors associated with agricultural intensification, such as fragmentation of flower-rich semi-natural habitats (SNH), homogenous cropping systems and reduced landscape heterogeneity (Potts *et al.* 2010, 2016; Kovács-Hostyánszki *et al.* 2017). Local changes in habitat quality often lead to reduction in flowering plant diversity and loss of suitable bee habitats for nesting. When native and domesticated pollinators are rare or absent, farmers growing entomophilous crops are exposed to high economic risks due to reduced pollination rates (Potts *et al.* 2016).

To face the demand for pollination services in crop production and increase pollinator efficiency, it is important to understand the relative importance of wild and managed bees in crop pollination, with respect to bee functional traits, and in response to spatial and temporal changes in landscape-wide floral resource availability. Different bee species show various responses to landscape-wide floral resource availability, as they differ in their foraging range and food plant preferences (Steffan-Dewenter *et al.* 2002; Rollin *et al.* 2013). Honey bees and bumble bees build large colonies, and have long foraging radii (approximately 1–3 km), enabling them to explore a relatively large area surrounding their colonies (Steffan-Dewenter & Kuhn 2003; Westphal *et al.* 2006; Osborne *et al.* 2008). Most wild bees, which are mainly solitary bees, have only a limited foraging range; often less than a few hundred meters around their nests (Gathmann & Tscharntke 2002; Zurbuchen *et al.* 2010). They forage on more scattered resources, but are also found within mass-flowering crops (Rollin *et al.* 2013; Stanley & Stout 2014). Common mass-flowering resources such as oilseed rape (OSR, *Brassica napus* L.; Westphal *et al.* 2003; Holzschuh *et al.* 2016) and apple (*Pyrus malus* L.; Grab *et al.* 2017) can provide ample nectar and pollen. These resources positively influence the colony development and reproductive success of bees (Pelletier & McNeil 2003; Westphal & Tscharntke 2009; Crone & Williams 2016). As honey bees and bumble bees are known to prefer mass-flowering resources (Rollin *et al.* 2013), it is likely that they will use those abundant resources over minor flowering resources. In particular, honey bees are most vulnerable to distraction, as they are able to communicate resources using the waggle dance (Couvillon 2012). It has also been shown that bumble bees can adjust their pollen foraging to the colonies' needs (Leonhardt & Blüthgen 2012).

When native pollinators are absent, farmers may decide to boost pollinators in target crop fields by establishing managed bee colonies adjacent to the fields. Managed bees (e.g. *A. mellifera* and *Bombus terrestris* L.) are easy to handle and can provide many individuals as they have a social nesting behaviour. Large colonies of *A. mellifera* consist of up to 80,000 individuals and *B. terrestris* colonies of up to 600 individuals (Felix & Krebs 2012). However, a high number of individuals will not necessarily translate into visits in the target crop field, as other co-flowering resources may be visited instead (Bobiwash *et al.* 2017). High mass-flowering resource availability can reduce the number of bees in target crop fields and pollination of co-flowering wild plants (Holzschuh *et al.* 2011; Grab *et al.* 2017). Selection of different bee species or colony sizes could enhance pollinator abundance in target crop fields. Small honey bee colonies have been found to have shorter foraging radii (Beekman *et al.* 2004), and foragers from small colonies visit adjacent resources more often than foragers from large colonies (Boecking & Kreipe 2013). Colony size effects in bumble bees are so far unknown.

Bee pollinators collect both pollen for the broods' nutrition, and nectar to meet their energy costs (e.g. foraging, temperature regulation; Haydak 1970). The identification of pollen resources can reveal part of their food plant preferences and requirements. To identify the pollen foraging preferences, next-generation sequencing (NGS) can be used which can give qualitative data of visited plant species (Keller *et al.* 2015; Sickel *et al.* 2015; Bell *et al.* 2017). In addition, microscopic pollen analysis allows for the pollen grain count of certain plants, and to estimate pollination efficiency for crop pollination services (Gyan & Woodell 1987; Balfour & Ratnieks 2017; Marzinzig *et al.* 2018).

The foraging of honey bees can be investigated in more detail by using the waggle dance decoding (Von Frisch 1967; Couvillon 2012). Investigating the honey bees' dance language is a unique way of understanding the movement of bees in the landscape, and can give information about distance and direction of exploited nectar and pollen resources (Couvillon 2012). Waggle dance decoding has previously been used to study the foraging behaviour of honey bees in agricultural landscapes (Danner *et al.* 2017) and to evaluate the effectiveness of honey bees for pollination management in crop production (Balfour & Ratnieks 2017).

The interactive effects of facilitation and competition among crops for pollinators are largely unknown. This knowledge may be particularly important for agriculture when a minor flowering crop such as strawberry is co-flowering with mass-flowering resources (e.g. OSR). OSR and strawberry (*Fragaria x ananassa* Duch.) are both economically important. Strawberries are a potential cash crop, with increasing cultivation worldwide (FAOSTATS 2018). On average, 0.16 million ha of strawberry are cropped in Europe, compared to 8.62 million ha of oilseed rape (five-year average). Insufficient pollination can cause malformations of strawberry fruits, decreasing their commercial value and shelf life (Klatt *et al.* 2013; Wietzke *et al.* 2018). To meet the demand for future crop pollination services, it is important to understand the foraging ecology of wild bees and managed bees with respect to spatial and temporal changes in agricultural landscapes.

Hypotheses and research questions

In the following chapters of my PhD thesis, I study the foraging behaviour of wild and managed bees in agricultural landscapes (Figure 1a–c), for potential crop pollination services in strawberry fields, along a landscape-gradient of OSR availability (Figure 1d).

Figure 1 Pollinators on strawberry flowers: a) honey bee (*Apis mellifera* L.), b) bumble bee (*Bombus terrestris* L.), c) *Andrena* sp. and d) a flowering strawberry field (front) adjacent to a simultaneously flowering OSR field (back).

In the *first chapter*, the relative importance of social and solitary bee abundance in strawberry fields in response to OSR availability (product of OSR land cover and OSR flower cover in the nearest field) and the consequences on strawberry fruit weight was investigated. Generally, we expected that social bee abundance in strawberry fields will decrease with high OSR availability, while solitary bee abundance may be facilitated by decreasing resource competition with social bees (Figure 2a). Further, potential losses in strawberry fruit weight are expected to be counterbalanced by complementary resource use of social and solitary bees in strawberry fields. Moreover, we hypothesize that insect pollination will in general benefit strawberry fruit weight and quality.

In the *second chapter*, the utility of small and large honey bee and bumble bee colonies for strawberry pollination, and potential distraction by OSR availability is studied. The pollen resource use was investigated by using pollen DNA metabarcoding and microscopic pollen analysis. We aimed to identify the main pollen resources during early spring, and explore how the pollen richness and foraging for strawberry pollen is affected by mass-flowering OSR availability. Generally, we expected that high OSR availability would decrease pollen richness, and the amount of collected strawberry pollen, in large colonies more than in small colonies, due to possible landscape exploitation (Figure 2b).

The *third chapter* focus on the foraging ecology of honey bees using waggle dance analysis. We aimed to reveal the preferred foraging habitats, and to study the foraging distance and the preference for strawberry fields, in response to OSR availability and flower-providing semi-natural habitats (SNH). Our general expectation is that OSR availability decreases the foraging distance, and the preference for strawberry fields, as well as the pollen collection from strawberry (Figure 2c). Moreover, we aim to identify the most abundant pollinators in strawberry fields and co-flowering foraging habitats.

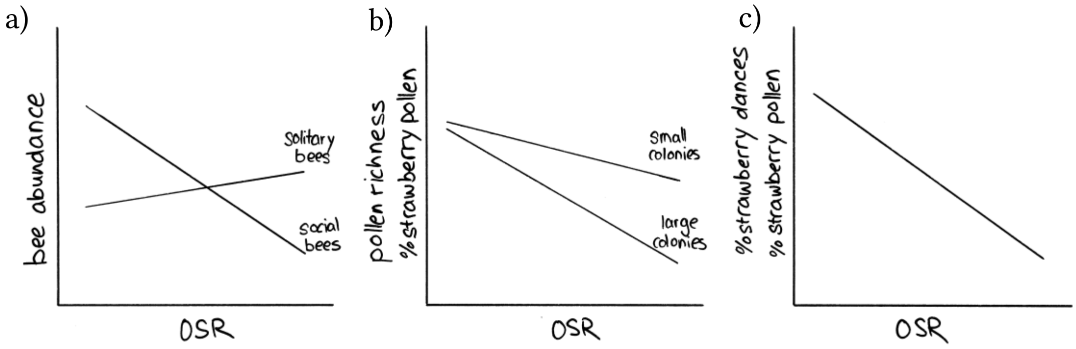

Figure 2 Hypotheses graphs. a) Increasing mass-flowering OSR availability (i.e. OSR flower cover multiplied by OSR land cover) decreases social bee abundance but enhances solitary bee abundance. b) Pollen richness (number of plant genera) and the proportion of strawberry pollen will decline more in large than small colonies with high OSR availability. c) High OSR availability will decrease the preference of honey bees for strawberry foraging (e.g. % dances for strawberry and pollen collection from strawberry plants).

Study regions and landscapes

The studies were conducted in agricultural landscapes in Lower Saxony and Northern Hesse, Germany, in three consecutive years (2015–2017, Figure 3a,b). Depending on the study year, we established our experiments adjacent to 8–11 strawberry fields with field sizes of on average 2.24 ha (±1.02 SE; range 0.92–3.6 ha). Fields were surrounded by a landscape mosaic of arable crop fields, fragments of semi-natural habitats (SNH), forests and settlements.

To map the location of used habitats indicated by the waggle dance, we produced digital maps of land cover types surrounding our study fields using a geographic information system (Figure 3c, ESRI ArcGIS, Version 10.3.1). We classified the land cover types cropland, settlement, forest, SNH, OSR and strawberry within a 2000 m radius, but used different scales for the three chapters. The agricultural data (InVeKoS: database of agricultural cropping; https://www.zi-daten.de) were provided by the agricultural departments of the German states Lower Saxony (Landwirtschaftskammer Niedersachsen) and Hesse (Landesbetrieb Landwirtschaft Hessen). Other land cover types were mapped using satellite images (Bing Maps) and validated with observations in the field. In the first chapter, we decided to use landscape surveys within a 1000 m radius around the study fields to cover the main foraging range of social bees (Steffan-Dewenter & Kuhn 2003; Westphal *et al.* 2006) but also, and more importantly, the foraging range of solitary bees (Gathmann & Tscharntke 2002). In the second chapter, a radius of 2000 m was selected, as only honey bees (*A. mellifera*) and bumble bees (*B. terrestris*) have been investigated, and they are known to have long foraging ranges (Beekman & Ratnieks 2000; Steffan-Dewenter & Kuhn 2003; Westphal *et al.* 2006; Osborne *et al.* 2008). In the third chapter, landscape surveys were performed within a 750 m radius, as the mean foraging distance of foragers from the small honey bee observation hives was small (mean 653 m ±16.25 SE).

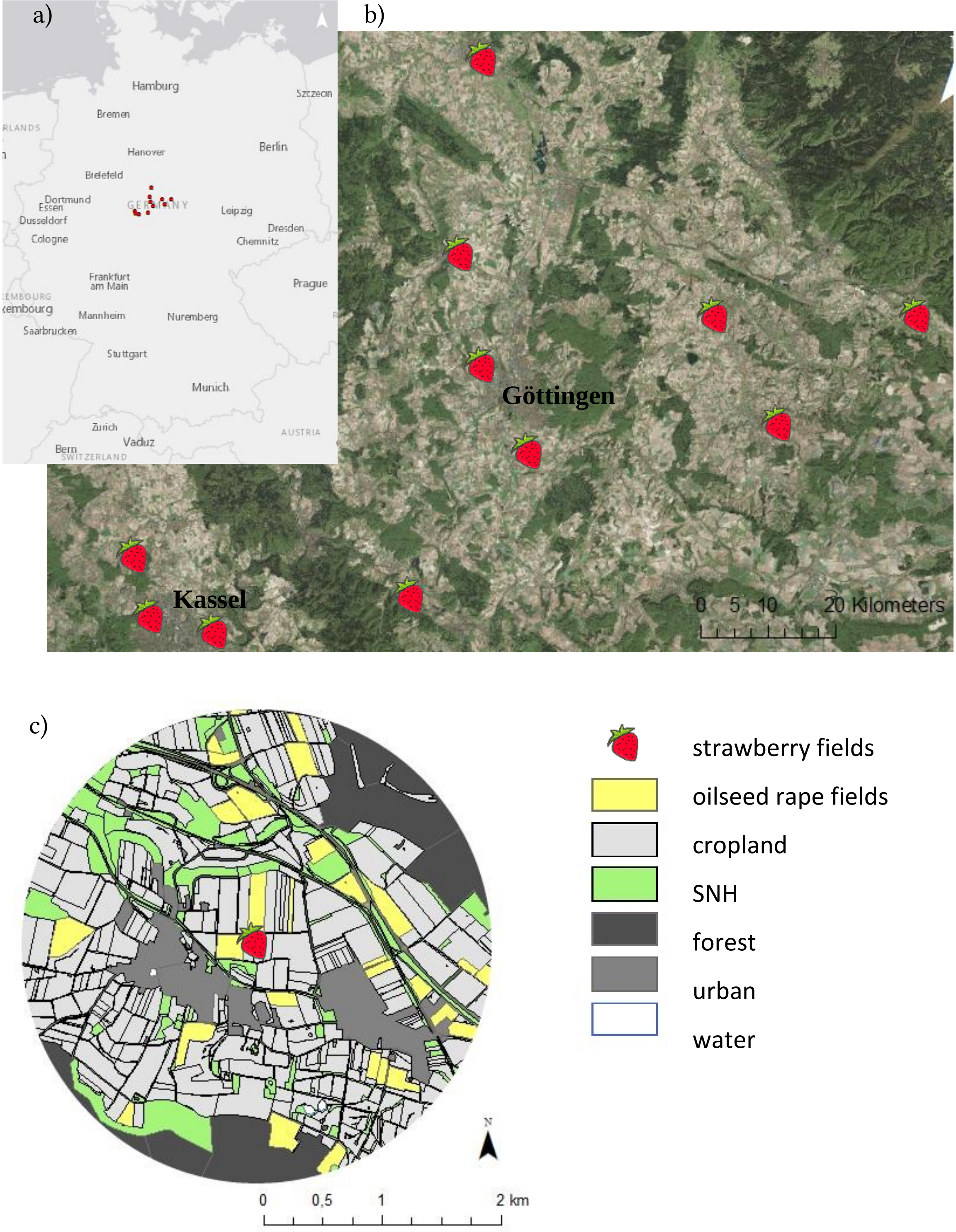

Figure 3 GIS map (ArcGIS, version 10.3.1., ESRI) of our study locations in central Germany (a) in the regions surrounding Göttingen and Kassel (b, scale 1:700,000). b) The land cover types (e.g. oilseed rape fields, cropland, semi-natural habitats (SNH), forest, urban area and water) were mapped within a radius of 2000 m around our study fields (strawberry); scale 1:60,000. Basemap source: Esri basemap (Bing).

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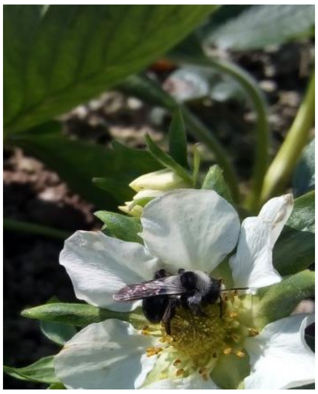
Chapter 1

Crop pollination services: complementary resource use by social vs solitary bees facing crops with contrasting flower supply



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The content of the chapter is published in Journal of Applied Ecology, 2020

doi: 10.1111/1365-2664.13777

Abstract

Many farmers are facing high economic risks if pollinator declines continue or temporal and spatial variation in wild bee communities cause reduced pollination services. Co-flowering crops might compete for pollinators, while they also might facilitate the delivery of pollination services. This rarely studied topic is of particular interest with respect to the foraging decisions of bees from different functional groups and when more sparsely and mass-flowering crops are in bloom at the same time.

The abundance of honey bees, bumble bees and solitary bees in strawberry fields was quantified with transect walks along a gradient of oilseed rape (OSR) availability (product of OSR land cover and temporally changing OSR flower cover). We established a pollination experiment with pollination treatments (open-, wind- and self-pollination) to study the effects of insect pollination on strawberry fruit weight and quality.

Changes in OSR availability exhibited contrasting effects on social versus solitary bees in strawberry fields. Bumble bees and honey bees were less abundant in strawberry fields when OSR availability was high, whereas solitary bees were facilitated. With more strawberry flowers we found more bees in general.

When flowers were open-pollinated they resulted in heavier fruits with better commercial grades compared to wind-and self-pollinated flowers. A higher bee abundance enhanced the strawberry fruit weight and quality but depended on flower order and variety.

Synthesis and applications. Sparsely flowering crops may compete with mass-flowering crops for social bee pollinators while solitary pollinators in the field might be even facilitated. To ensure best fruit weight and quality it can be beneficial to support bee abundance in the field. While some social and solitary bee species can be managed for pollination services, wild bees, in particular solitary species, should be conserved and promoted for stable crop pollination services in dynamic agricultural landscapes.

Introduction

The increasing global production of pollinator-dependent crops is causing a great demand for pollination services (Aizen and Harder 2009). Many farmers are prone to high economic risks if pollinator numbers decline (Potts *et al.* 2016) or temporal and spatial variation in pollinator communities result in reduced pollination services and consequent yield losses (Garibaldi *et al.* 2011).

The effectiveness of a pollinator community is determined by multiple environmental factors, including competition or facilitation for pollinators among co-flowering plants (Willcox *et al.* 2017). The temporal and spatial co-occurrence of flowering plants affect the bees' foraging behaviour, their visitation rates and finally crop yields, especially if more sparsely and inconspicuously flowering plants compete with mass-flowering resources (Danner *et al.* 2017, Grab *et al.* 2017). To date, the effects of spatio-temporal variation in floral resources on the foraging behaviour of bees from different functional groups, and consequences for crop pollination services have rarely been studied (Danner *et al.* 2017, Grab *et al.* 2017).

Highly rewarding mass-flowering resources in the landscape can draw pollinators away from co-flowering plants leading to interactions between those plant species (Magrach *et al.* 2017). Important mass-flowering crops are, for instance, oilseed rape (OSR) (Westphal *et al.* 2003, Holzschuh *et al.* 2016) or apple (Grab *et al.* 2017). Recently, Grab *et al.* (2017) demonstrated that the overall pollinator abundance in strawberry fields was reduced particularly in landscapes with a high land cover of mass-flowering apple orchards. Similar, large proportions of mass-flowering OSR fields can result in increased competition for pollinators with negative effects on the pollination of wild plants (Stanley and Stout 2014, Holzschuh *et al.* 2016). In contrast, facilitative interactions may occur if pollinators are attracted by high densities of mass-flowering resources and they visit adjacent flower resources in the same location as well (Hegland 2014).

Social bees, in particular honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.), prefer mass-flowering resources to a great extent (Westphal *et al.* 2006, Rollin *et al.* 2013). Because of their large foraging distances up to several kilometres they are able to exploit most rewarding resource patches at larger spatial scales than solitary bees (Bänsch *et al.* 2020a, Westphal *et al.* 2003), which mostly forage within a few hundred meters around their nests (Gathmann and Tscharntke 2002, Zurbuchen *et al.* 2010). Within their relatively small foraging ranges, solitary bees exploit both scattered floral resources (Rollin *et al.* 2013) and mass-flowering crops (Holzschuh *et al.* 2013).

After mass-flowering, honey bees and bumble bee species spillover (or return) to semi-natural habitats or to more sparsely flowering crops (Blitzer *et al.* 2012) where high densities of honey bees may alter the solitary bee communities and their abundance (Lindstrom *et al.* 2016, Magrach *et al.* 2017) by spatial displacement of solitary bees (Goulson 2003, Hudewenz and Klein 2015). Thus, the phenological sequence of crop flowering can play a major role in explaining indirect competition between different functional groups of pollinators (Kovacs-Hostyanszki *et al.* 2013, Magrach *et al.* 2017). However, during peak bloom of mass-flowering crops, solitary bees might benefit from reduced indirect competition for floral resources when social bees are rare in minor rewarding resource patches, e.g. by competitive release (González-Varo and Vilà 2017, Magrach *et al.* 2017).

Oilseed rape (Brassica napus L.) and strawberry (Fragaria x ananassa Duch.) are both economically important crops that can benefit from insect pollination and are flowering at the same time in our study regions (central Germany). Insect-pollinated strawberry fruits are usually heavier, have a better commercial grade and a longer shelf life than self- or wind-pollinated fruits (Klatt *et al.* 2014, Castle *et al.* 2019). In OSR, seed yield, seed weight and oil content increase when the flowers are pollinated by insects (Bommarco, Marini and Vaissière 2012; Stanley, Gunning and Stout 2013).

The aim of our study was to investigate competitive and facilitative interactions among mass-flowering OSR and more sparsely flowering strawberries (see Appendix Fig. S1a in Supporting Information) on the foraging behaviour of social versus solitary bees in strawberry fields. Various social and solitary bees are using strawberries and OSR as foraging resources (Wietzke *et al.* 2018, Appendix Figs S1b-d). Changes in bee foraging behaviour of different functional groups may have significant consequences for the provisioning of pollination services and the strawberry fruit weight and quality. To our knowledge, these indirect interactions and their implications for fruit production have not been studied so far. We focused on OSR availability which takes the phenological and spatial occurrence of OSR into account (product of OSR flower cover and OSR land cover). To develop effective pollinator management schemes for wild but also manageable pollinators, a better understanding of crop pollination is necessary. Especially with regard to the temporal shifts in the foraging behaviour of bees from different functional groups and in co-flowering crops. For this reason, we tested the following hypotheses:

OSR availability affects bees in the strawberry fields differently, as social bees will be withdrawn away from strawberry fields, whereas solitary bees on strawberries increase due to decreasing resource competition and their local orientation in foraging behaviour.

Pollination and bee abundances in strawberry fields benefit the strawberry fruit yield and quality.

Methods

Study region and study sites

The study was conducted on strawberry fields from May to July in 2015. Study sites were chosen in central Germany around the cities Göttingen in Southern Lower Saxony and Kassel in Northern Hesse (map and coordinates in Appendix Figs S2 a-c). Eight experimental fields were located in the centre of circular landscapes (radius 1000 m) along a gradient of OSR land cover and separated by at least 6 km. Experimental strawberry field size was on average 2.5 ha (± 0.4 SE [=standard error]) while the size of oilseed rape fields was on average 2.1 ha (± 0.2 SE). We chose only strawberry fields on which, among other varieties, Sonata or Honeoye was grown, because these varieties flower simultaneously with OSR and are commonly grown by our cooperating farmers. Although it is known that semi-natural habitats affect bee densities in agricultural landscapes (Ricketts *et al.* 2008), we did not include them in our analyses since the land cover gradient within 1000 m was only small (mean 2.1 % ± 1.4 SE, range from 0.6 % – 5.1 %).

OSR availability is the product of OSR land cover at landscape scale and OSR flower cover within the next field to our study fields. We used digital land cover maps (InVeKoS: database of agricultural cropping; https://www.zi-daten.de/) that were provided by the agricultural departments of the German states Lower Saxony (Landwirtschaftskammer Niedersachsen, 2015) and Hesse (Landesbetrieb Landwirtschaft Hessen, 2015) to calculate the OSR land cover; i.e. the percentage of OSR fields that covered the defined area of a landscape circle within a 1000 m radius around our experimental fields. We decided upon 1000 m as it covers the foraging distances of most relevant bee species (Bänsch *et al.* 2020a, Gathman and Tscharntke 2002, Zurbuchen *et al.* 2010). Although honey bees and bumble bees can fly larger distances (Bänsch *et al.* 2020a, Osborne *et al.* 2008), distances are often below < 1000 m, which has been shown in particular for honey bees in spring (Bänsch *et al.* 2020a). Additionally, we mapped our landscapes to validate the InVeKoS data using ESRI ArcGIS 10.3.1. OSR land cover in our study landscapes ranged from 4.1 % to 17.6 % (mean 9.8 % ± 0.8 SE).

OSR flower cover within the nearest field was visually estimated at each survey as percentage of ground that was covered by flowers within a 2 m^2 square at a scale ranging from 0 to 100 % using increments of 5 % for different levels of flower cover and 1 % for flower cover below 5 %. Flower cover of OSR ranged from 1 to 70 % (mean 28.8 % ± 3.0 SE). The flower cover in the square was representative for the field. Due to similar management regimes of the farmers in our regions, OSR flowering was largely synchronized within the landscapes, i.e. main bloom was at the same time. Data collection started with the beginning of strawberry bloom, which can differ between the landscapes mainly due to their elevation and microclimatic differences (details on temporal variation in the flower phenology of OSR and strawberry are given in Appendix Fig. S3).

Pollinator abundance

We used standardised transect walks to sample flower-visiting bees in our study fields. Other pollinators such as syrphid flied were observed only in a few isolated cases which is in line with Klatt *et al.* (1.6 % non-bee pollinator in strawberry fields; 2014). Therefore, we focused on bee pollinators and we had four observation periods during the strawberry flowering period in May and June. Flowering of both crops started around the end of April and co-flowering continued for four to five weeks depending on the location and weather conditions. Two transects (each 50 m x 4 strawberry rows) were established within the strawberry fields: one at the edge and one inside the field (15 m from edge) to account for edge effects. Each transect lasted 15 min and was conducted between 9 am and 6 pm while we visited each field in morning and afternoon hours. Hence, eight transects have been conducted in each strawberry field during the study period. For the observations we chose good weather conditions, i.e. days with a minimum temperature of 12°C, no rain, low wind speed and low cloud cover (Westphal *et al.* 2008). Flower-visiting bees within the transect area were counted and identified to species level in the field or caught with an insect net for later identification in the laboratory. Solitary bees were identified by SB and Frank Creutzburg (JenInsect, Jena, Germany; see species list in Appendix Table S1). Bee individuals that escaped from the insect net were not identified to species level, but their abundance was counted if they could be assigned to one functional group. Bee abundance is quantified as number of individuals per transect.

We assigned the bees to functional groups according to their sociality and level of domestication (i.e. honey bees, bumble bees and solitary bees, Appendix Table S1). However, we found one individual of Halictus tumulorum which is thought to be primitively eusocial. Due to its morphological similarity with other solitary bees, we included it in the group of solitary bees for our analyses.

We quantified strawberry flower cover by counting the number of open flowers along two meters of a strawberry row within each transect area (edge and inside of the field separately).

Pollination experiment

Fruit weight and commercial grades

To investigate the importance of insect pollination for strawberry fruit quantity and quality, we established a pollination experiment with three treatments (open-, wind- and self-pollination). Open-pollinated flowers were left open to allow access for all flower-visitors as well as for airborne pollen. To exclude only insects, but allow airborne pollen flow, we bagged individual flowers in bags with mesh sizes of 1 mm in the wind-pollination treatment. We used Osmolux bags (Pantek, France), which are permeable for water vapour (http://www.pantek-france.fr/agriculture.html), for the self-pollination treatment (exclude insects and airborne pollen; Klatt *et al.* 2014). All bags were removed after blooming to standardise the fruit ripening.

In total, we had 40 plants per field, 20 plants at the edge and 20 plants in the inside of the strawberry field according to transect location. Five plants per transect location were assigned to the self- and wind-pollinated treatment and ten plants to the open-pollination treatment. Strawberry flowers can be assigned to different orders according to their temporal occurrence and position on the stalk. One primary flower (first order) usually occur first on the main stalk, followed by two secondary flowers which branch off the main first stalk, and followed by up to four third order flowers which branch off the second stalks. Generally, higher flower orders produce berries with a reduced size and weight (Free 1993). For this reason, we included only fruits from 1st to 2nd flower orders. Every strawberry fruit was weighted directly after harvest and categorized into commercial grades with respect to size, shape and colour according to the European Commission (2011). Commercial grades were categorized as G1/E, where we pooled Grade Extra and one, G2 as Grade two and NM for non-marketable fruits.

To investigate the direct effects of bee abundance, pollination treatment, variety and order on strawberry fruit weights and commercial grades, we marked the strawberry flowers that were open during the transect walks which correspond to our measurements of bee abundance at that point in time (see Appendix Table S2). While we have four observation periods for bees in the strawberry fields, we used a subset of three observation periods since the combined data of bee abundance and subsequent fruit yield and quality is only available for three points in time.

Data analysis

Pollinator abundance

Statistical analyses were done with the software R version 3.3.2 (R Development Core Team 2016). We analysed the effects of OSR availability on bee abundance with generalized linear mixed effect models using the glmmTMB package (Brooks *et al.* 2017). Bee abundance (i.e. number of individuals) was included as response variable. Bee functional group (i.e. honey bee, bumble bee, solitary bee), OSR availability, strawberry flower cover and interactions between bee functional group and OSR availability and bee functional group and strawberry flower cover were the explanatory variables. Observation period nested within location of the transect (edge/inside) nested within the study landscape was included as random effect. The effects of transect location on the number of bee individuals per transect of functional bee groups in the strawberry field was tested beforehand and we found no relevant differences in bee abundances between edge and inside. Continuous explanatory variables (i.e. strawberry flower cover and OSR availability) were scaled to a mean of zero and a standard deviation of 1 to improve convergence of the models. We found no significant correlations between fixed effects (all p > 0.1, rPearson < 0.1). We fitted the global model with negative binomial distribution because of overdispersion in the Poisson model. Further, we compared the fit of global models with OSR availability at different spatial scales (i.e. 500 m and 1000 m radius). These global models showed the same patterns and did not differ in their fit (delta AICc <2). We conducted subsequent analyses at the 1000 m scale as this scale comprises most likely the foraging ranges of both solitary and social bees in our study (Bänsch *et al.* 2020a, Gathman and Tscharntke 2002, Zurbuchen *et al.* 2010). We selected the best fitting models based on the multimodel inference approach (Burnham and Anderson 2002) by fitting candidate models that contained all possible combinations of explanatory variables from the global model (function 'dredge', package 'MuMIn' ; Barton 2018). Additionally, appropriateness of model assumptions was assessed by plotting residuals vs. fitted values and vs. explanatory variables, respectively. We ranked the models by the AICc and used the Akaike weight (wi) to estimate the probability of the individual models to have the best fit across models (Burnham and Anderson 2002). All models within delta AICc (dAICc) < 2 in comparison to the best fitting model were considered to have substantial empirical support and are reported together with the null and the global model. We considered only the best fitting model (dAICc=0) for our pollinator abundance analysis since the dAICc of all models was above 2 in comparison to the best fitting model. The best fitting model was refitted with restricted maximum likelihood for model interpretation. The relative importance of each explanatory variable was assessed using the sum of Akaike weights (Σwi) over all candidate models that included the respective variables (function 'importance', package 'MuMIn'; Barton 2018). We report effects of variables with Σwi > 0.2.

Pollination experiment: fruit weight and commercial grades

Fruit weight

The effects of bee abundance and pollination treatment on strawberry fruit weight were analysed using linear mixed effect models (function 'glmmTMB', package 'glmmTMB'; (Brooks *et al.* 2017) . We log-transformed strawberry fruit weight to homogenize variances after inspecting residual versus fitted values. The global model was fitted with the fixed effects bee abundance (i.e. number of bee individuals), pollination treatment (open, wind, self), variety (Sonata, Honeoye) and strawberry flower order (1st, 2nd) and following interactions: bee abundance and order, bee abundance and variety, and bee abundance and pollination treatment. The random effects included plant ID nested in observation period nested in location of the transect nested within landscape. We used multimodel inference to select best fitting candidate models. Models were fitted with maximum likelihood for comparison using AICc. The best models (dAICc < 2 in comparison to the best fitting model) were then refitted with restricted maximum likelihood for model interpretation. Post-hoc test and 95 % confidence intervals were obtained for interpretation (function 'emmeans', package 'emmeans'; Lenth 2017).

Commercial grades

We tested the effects of bee abundance, pollination treatment, variety and strawberry flower order, and interactions between bee abundance and order, bee abundance and variety and bee abundance and pollination treatment on fruit quality using cumulative linked mixed models (function 'clmm', package 'ordinal'; Christensen 2018). Commercial grade was used as response variable and the random term was included as described above in the fruit weight section. Again, we used the multimodel inference to select best fitting models.

Results

In total, we recorded 527 bees visiting strawberry flowers in our experimental field sites. From these, 471 were identified to species level or at least on genus level (except 11 individuals which were only recorded as solitary bees). *Apis mellifera* represented 46.5 % of the bee community, followed by bumble bees with 29.9 % and solitary bees with 23.6 %. Bumble bees were dominated by *Bombus terrestris* (83.7 %) and solitary bee community consisted mainly of *Andrena* species (11 species, 81.1 %) with only a few individuals of *Osmia bicornis* and only one individual of *Lasioglossum parvulum* and *Halictus tumulorum*, respectively (see Appendix Table S1).

Effects of OSR on pollinator abundance in strawberry fields

The multimodel inference approach resulted in one best fitting model (Appendix Table S3a, see Table S4a for CIs and model estimates). The interaction between OSR availability and bee functional group was the most important predictor variable as indicated by the high Σwi of 0.95 (for all values see Appendix Table S5). The abundance of honey bees and bumble bees in strawberry fields decreased with increasing OSR availability, while solitary bee abundance increased (Fig. 1). The decrease in social bees was 3.7 times higher in bumble bee compared to honey bees. Bumble bees dominated the bee community in strawberry fields at low OSR availability while solitary bees were most prevalent at high OSR availability. Increasing strawberry flower cover resulted in higher bee abundances of all functional groups (Σwi=0.79, Appendix Fig. S4).

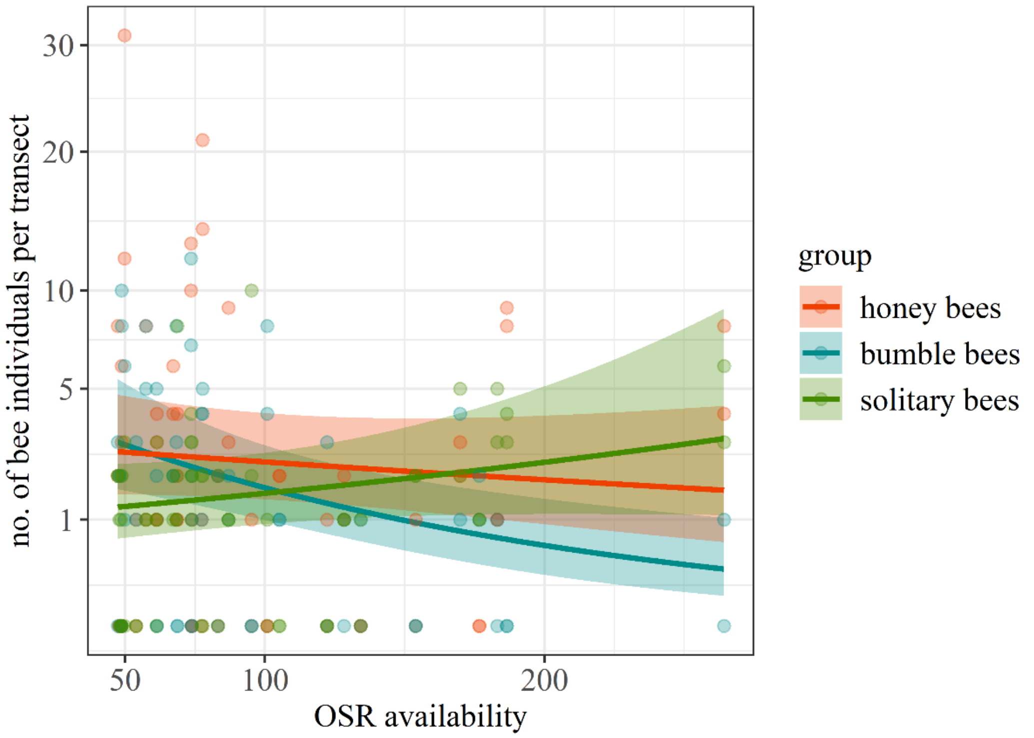


Figure 1 Effects of OSR availability (product of OSR flower cover and OSR land cover) on bee abundance (no. of individuals per transect, shown on a square root scale) in strawberry fields for each functional group. Honey bee and bumble bee abundances decrease with increasing OSR availability while solitary bee abundance increases. We show regression lines and 95 % confidence intervals obtained from mixed model estimates.

Pollination experiment: fruit weight and commercial grades

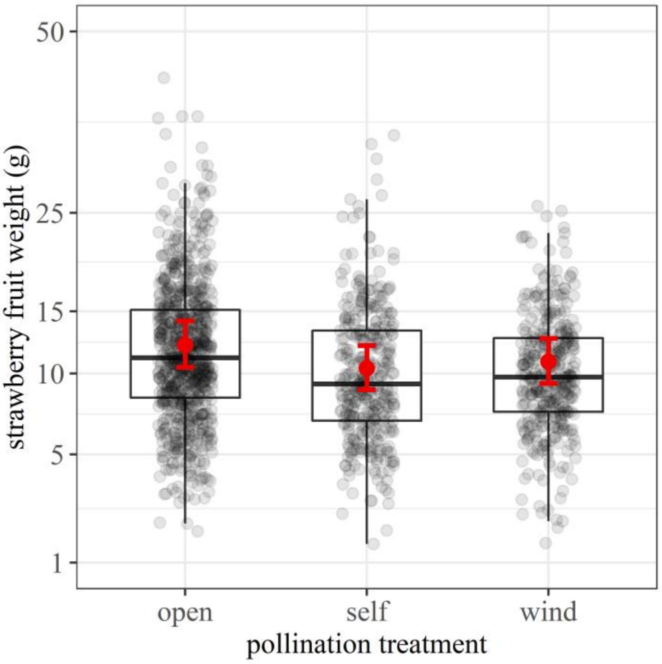
Fruit weight

Two models within a range of dAICc < 2 explained the strawberry fruit weight (Appendix Table S3b, CIs and model estimates can be found in Table S4b). Greatest Akaike weights were found for the main effects of pollination treatment and order (Σwi = 1 respectively), followed by bee abundance (Σwi = 0.94) and last variety (Σwi = 0.64), however the direct comparability of the sum of Akaike weights is limited due to the slightly different number of models in which the variable occur (Table 1a). We found high sums of Akaike weight for the two-way interactions between bee abundance and order (Σwi = 0.87) and between bee abundance and variety (Σwi = 0.51). Open-pollinated flowers had 6.9 % higher fruit weight than wind-pollinated fruits and 4,8 % higher fruit weight than fruits from self-pollinated flowers (Fig. 2a). Increasing bee abundance showed a positive effect on the fruit weight of the variety Honeoye, while this was not observed for the variety Sonata (Fig. 2b). A higher bee abundance did particularly benefit the fruit weight of fruits from first order flowers while second flower orders were less affected (Fig. 2c). In general fruits from second order flowers had a 10.9 % lower fruit weight than fruits from first order flowers.

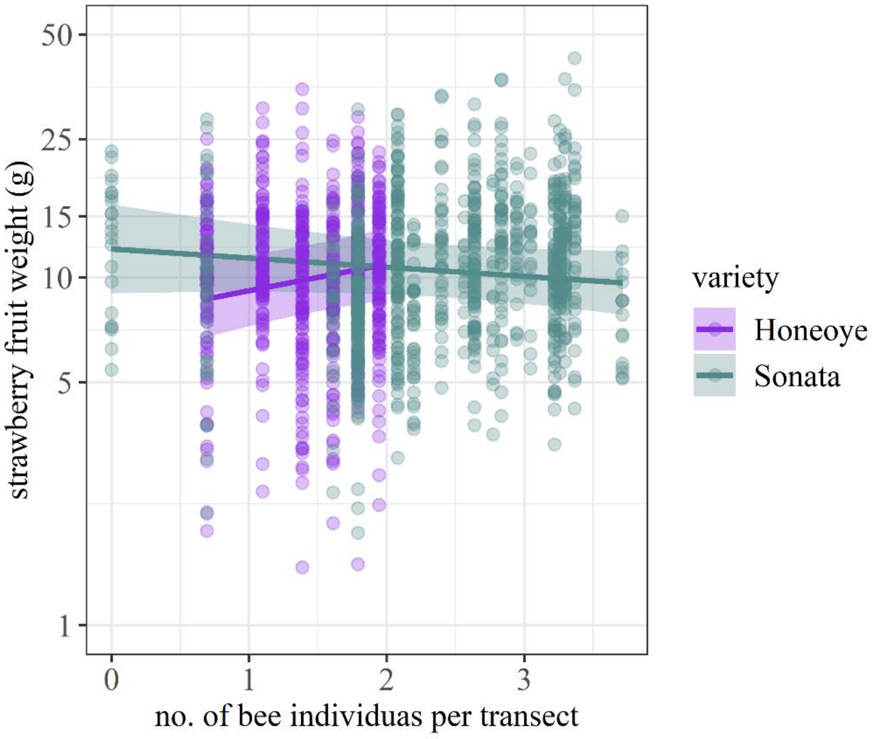
Table 1 The relative importance of explanatory variables expressed by the sum of Akaike weights (Σwi) for models to explain the effects of the number of bee individuals per transect (bees), pollination treatment (PT), flower order and variety on strawberry fruit weight (a) and commercial grades (b). Number of models in which the variable occur is shown in brackets.

Response Variable	bees	PT	Variety	Order	Bees: variety	Bees: order	Bees: PT
(a) Fruit weight (g)	0.95 (27)	1.00 (22)	0.64 (22)	1.00 (22)	0.51 (9)	0.88 (9)	0.22 (9)
(b) Commercial grade	0.68 (27)	1.00 (22)	0.80 (22)	1.00 (22)	0.38 (9)	0.25 (9)	0.25 (9)

a)


strawberry fruit weight (g)
50
25
15
10
5
1
open
self
wind
pollination treatment

b)


strawberry fruit weight (g)
50
25
15
10
5
1
0
1
2
3
no. of bee individuas per transect
variety
Honeoye
Sonata

c)

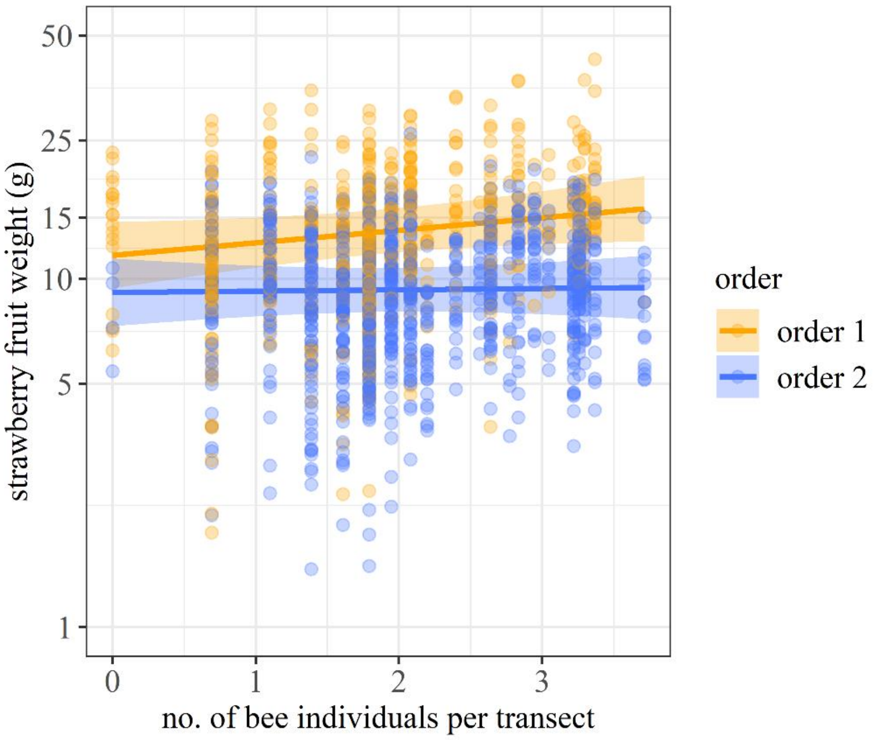


Figure 2 a) Effect of pollination treatment (a) and the number of bee individuals per transect (b, c) on strawberry fruit weight (g) (shown on log scale in all figures). Open pollinated flowers resulted in heavier fruits than self- and wind-pollinated flowers (a). Increasing bee abundance increased strawberry fruit weight from the variety Honeoye but not from Sonata (b). Higher bee abundance promotes strawberry fruit weight from first order flowers while second order flowers are less affected (c). We show regression lines and 95 % confidence intervals obtained from mixed model estimates. Datapoints are jittered in (a).

Commercial grades

We found five models with substantial empirical support (Appendix Table S3b). The best fitting model (dAICc = 0) included the main effects variety, order and pollination treatment with Σwi ranging from 0.68 to 1 (see Table 1b for all Σwi). The interactions between bee abundance and variety (Σwi = 0.38), between bee abundance and order (Σwi = 0.25), and bee abundance and pollination treatment (Σwi = 0.25) were included in candidate models within the range of dAICc < 2 (Appendix Table S3b). Fruits of open-pollinated flowers had a high probability of being placed in the best grade (G1/E), while self-pollinated and wind-pollinated flowers were less likely to reach the best grade. The probability of a fruit being assigned to the second grade (G2) was similar for all treatments, while the probability of fruits being assigned to the non-marketable grade (NM) was highest for self-pollinated flowers and lowest for open-pollinated flowers in general (Appendix Fig. S5a). Further, we found that bee abundance increased the probability of achieving the best commercial grade in the open-pollinated treatment. Similar patterns were observed for fruits from the self- and wind-pollinated treatments but the effect was lower (Fig. S6c). Fruits of the variety Sonata had a higher probability of occurrence of G1/E compared to fruits of the variety Honeoye (Appendix Fig. S5b). However, higher bee abundances increased the probability that fruits from the variety Honeoye, but not from Sonata, were assigned to the best commercial grade. As a result, fruits were less likely to be classified in the third commercial grade when bee abundance was high for the variety Honeoye (Appendix Fig. S6a). In general, second order fruits were more likely to receive G1/E compared to first order flowers (Appendix Fig. S5c). The interaction of variety and order with bee abundance showed that a high bee abundance was particularly beneficial for the quality of first order fruits and the variety Honeoye (Appendix Fig. S6b).

Discussion

We could demonstrate that bees of different functional groups exhibit trait-based responses to temporal changes in local and landscape-wide resource availability. In contrast to other studies that analysed only landscape effects in terms of OSR land cover (Westphal *et al.* 2003, Holzschuh *et al.* 2013), we additionally identified temporal shifts in the flower cover within OSR fields. By multiplying OSR flower and land cover we calculated an index which represents the temporal OSR flower availability in the surrounding landscape. We demonstrate that social bees are attracted by mass-flowering resources while solitary wild bees do not react to increased mass-flowering resource availability and keep foraging in more sparsely flowering crops where they provide essential pollination services. Particularly bumble bees showed a strong decrease in abundance in sparsely flowering strawberry fields when OSR was in full bloom. The bumble bee community consisted mainly of B. terrestris (> 80 %) which is a generalist species and known to prefer mass-flowering resources such as OSR (Walther-Hellwig and Frankl, 2003). Honey bees showed a less steep decrease but their overall density in the landscape depend on beekeeping activities. While large and old forest areas may contain wild honey bee colonies (Requier *et al.* 2019), only small, disturbed and fragmented forest patches occur in our agricultural landscapes. Thus, honey bees in our study belong to managed colonies. Beekeepers may favour landscapes with many and large OSR fields to increase the honey harvest. Therefore, the density of managed honey bees is likely to be linked to OSR availability (in which measurement OSR land cover is included).

In contrast to honey bees and bumble bees, solitary bees were facilitated in strawberry fields by increasing mass-flowering OSR availability. Lower densities of honey bees and bumble bees may reduce resource competition for solitary bees thereby promoting their abundances in the fields (Lindstrom *et al.* 2016). Solitary bees might have spilled over from mass-flowering OSR to sparsely flowering strawberry fields. Similar spillover processes have been observed from mango (Simba *et al.* 2018) or OSR (Kovacs-Hostyanszki *et al.* 2013) to natural vegetation. Both crops, OSR and strawberry, can provide pollen and nectar as a reward (Bänsch *et al.* 2020a, Leidenfrost *et al.* 2020, Knopper *et al.* 2016). It has been shown that the attractiveness of OSR to honey bees and some solitary bee species is higher than the attractiveness of strawberry while the attractiveness of both crops to bumble bees is similar (Knopper *et al.* 2016). Nevertheless, the amount of pollen and nectar resources provided by OSR at landscape scale is much higher than strawberry (based on the cover).

In general, pollinator facilitation and competition have been rarely studied with respect to interactions between crops including their flowering phenology. Phenological shifts in bees foraging have been shown by Grab *et al.* (2017) who found that mass-flowering apple blossom in their early and main flowering can decrease bee abundance in strawberry, while bee abundance can be facilitated after peak flowering.

Honey bees made up to almost half of the bee community in our study but relying completely on social bees could cause pollination shortage in crops or other plants which are in bloom at the same time but offer smaller flower resources than mass-flowering crops. Obtaining or promoting greater species diversity, in particular of solitary bees, is likely to ensure (Wietzke *et al.* 2018) and increase pollination (Hoehn *et al.* 2008). In other regions and crop types, not only bees but also other pollinators should be considered as they can play a major role in crop pollination as well (Rader *et al.* 2016). In correspondence with Klatt *et al.* (2014), who found just 1.6 % non-bee pollinators in strawberry field, we rarely observed non-bee flower-visitors in our study field.

Strawberry flowering showed, in general, positive effects on the bee abundance in strawberry fields. Through targeted variety selection, farmers could provide high flower coverages over a longer period of time to promote bee abundance in the fields. In addition, the energy supply by nectar can be variety-dependend and influences the choice of flowers by bees (Abrol 1992).

In line with Klatt *et al.* (2014) we found that open-pollination can benefit strawberry fruit weight and quality. As we observed only few non-bee pollinators in our study fields (like Klatt *et al.* (2014): 1.6 % non-bee pollinator), bees are most likely the most important pollinators for strawberries in our regions. As also shown by other studies, higher pollinator abundance can enhance the fruit weight of strawberries (Castle *et al.* 2019) and many other crops (Garibaldi *et al.* 2013, Grab *et al.* 2017). Additionally, we demonstrate that increasing numbers of bees in the field enhanced the probability of fruits being classified in the highest quality grade in the open pollination treatment. Unexpectedly, we found a similar pattern for self- and wind-pollinated fruits that could be related to random confounding factors we could not control for in our field experiment. However, the effect size was low and hence from minor importance. The advantages of open-pollination can be variety-dependent as shown by our results and also by Klatt *et al.* (2014). In particular, the quality of fruits from the variety Honeoye benefited from a high bee abundance compared to Sonata, which showed weaker responses. Generally, the share of non-marketable fruits was higher in the self- than in the open-pollination treatment. As social versus solitary bees showed contrasting foraging behaviour in strawberry fields due to OSR availability, strawberry flower-visiting bees were abundant during the whole strawberry flowering season. Together, social and solitary bees can provide potential pollination services to strawberry flower throughout the season. Complementarity among pollinators has been mainly described for seasonal changes (Pisanty *et al.* 2014, Ellis *et al.* 2016), but not for changes in response to flower phenology of co-flowering crops.

Flower order effects can be explained by the flower biology. Flowers of low order (e.g. first flower order) are larger and have a greater amount of achenes which have to be pollinated to induce fruit growth (Roussos *et al.* 2009). Hence, fruits from low flower orders have a greater potential to gain higher weights and benefits from pollination.

Conclusions

Pollination services for crops with rather inconspicuous flowering are strongly influenced by the temporal changes in availability of simultaneously mass-flowering crops., Solitary bees in strawberry are promoted with increasing OSR availability while honey bees and bumble bees are pulled away. Continuous pollination services during the flowering of crops with inconspicuous flowering are likely provided by complementary abundance of social and solitary bees. It has been shown that not only bee pollination in general but also an increasing number of bees in the strawberry fields benefits strawberry fruit weight and quality. While farmers could manage different bee species for pollination services (e.g. *Apis mellifera*, *Bombus* species, and *Osmia* species; Garibaldi *et al.* 2017), focus should be the promotion of pollinator-friendly landscapes. This can increase the natural occurrence and diversity of pollinators of different functional groups.

Acknowledgements

We are extremely grateful to the cooperating strawberry farmers. We thank Prof. Dr. E. Pawelzik and her working group (University of Göttingen) for providing the laboratory facilities for the post-harvest analysis of strawberry fruits. Furthermore, we thank Jascha Conrad, Susanne Jahn, Brigitte Jünemann and Martin Greve for their valuable help during the field season, Nicole Beyer for bee trait data and Ingo Grass for statistical advice. Further, we thank Fabrice Requier and three anonymous reviewers for their helpful comments on the initial manuscript. CW is grateful for funding by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - project number 405945293. SB acknowledges her funding by the Deutsche Bundesstiftung Umwelt DBU (German Federal Environmental Foundation) through a PhD scholarship.

Data accessibility

Data available from the Dryad Digital Repository: doi:10.5061/dryad.q2bvq83h7 (Bänsch *et al.*, 2020b).

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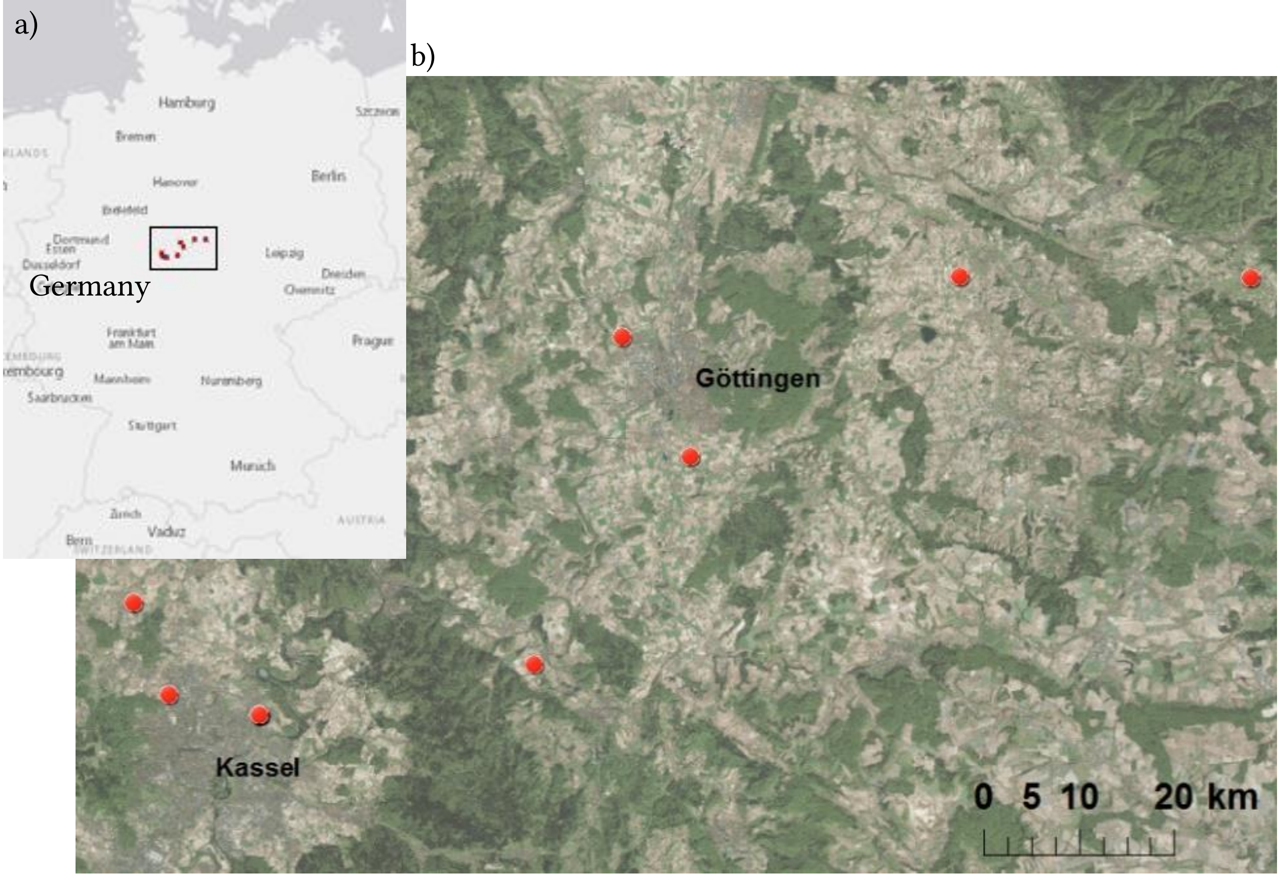
Supplementary material - Chapter 1

Figures

Figure S1 (a) Flowering strawberry field (front) and oilseed rape field (back) during main flowering periods. Strawberry flower visitors from different functional groups are shown with a honey bee (*Apis mellifera*; b), a bumble bee (*Bombus terrestris*; c) and a solitary bee (*Andrena* family; d).



c) Coordinates of the study sites

Study landscape	Longitude	Latitude
R1	10.1949849	51.5979663
R2	9.93980671	51.4918522
R3	10.4684523	51.5971525
R4	9.87591442	51.5622997
R5	9.79322019	51.3700203
R6	9.41459669	51.4060073
R7	9.53336284	51.3402018
R8	9.44850076	51.3521854

Figure S2 Location of study sites in central Germany (a). The fields are located in the region around Göttingen and Kassel (b; scale 1:500.000). Coordinates of the study sites can be seen in the table below (c; format: Datum D_WGS_1984, Prime Meridian Greenwich, unit: degree). Maps were created with ArcGIS and basemaps were provided by BingMaps.

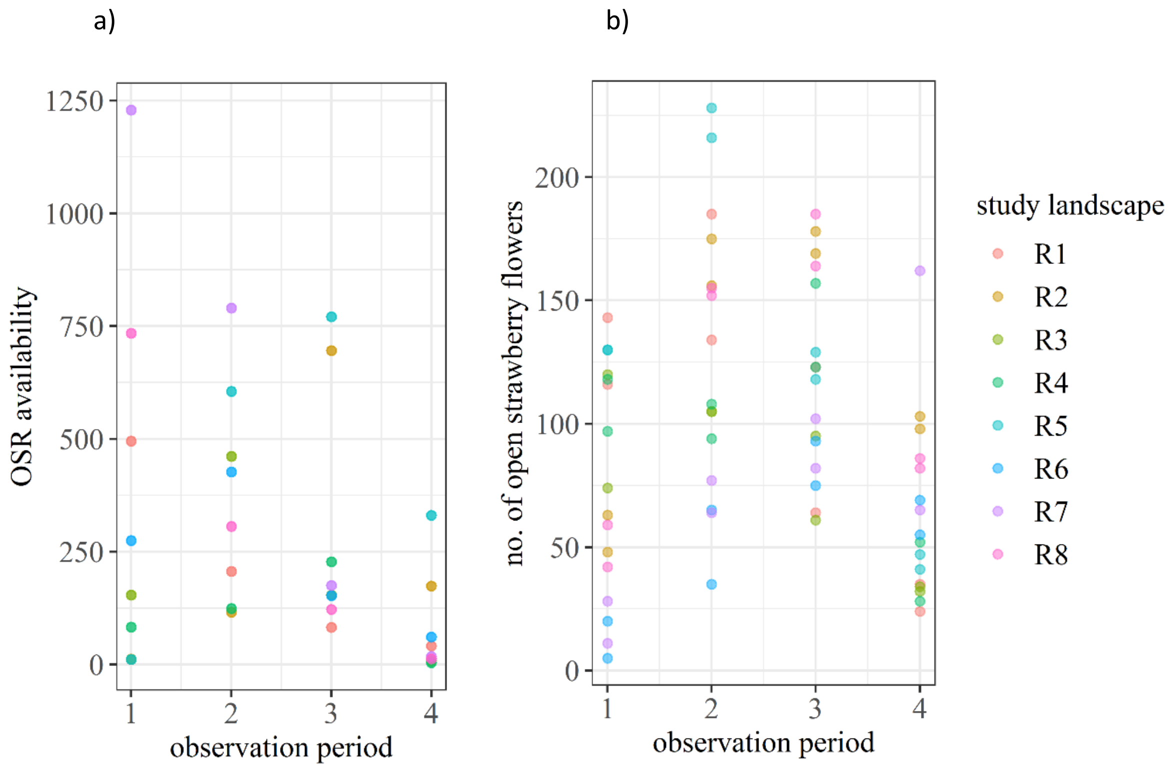


Figure S3 a) OSR availability (product of OSR flower cover and OSR land cover) and b) the number of open strawberry flowers (along 2 x 2 m^2) over the study period. Measurements were taken during four observation rounds (chronological order from 1 to 4). Each point represents a study landscape in figure (a), while we have two observations per study landscape in figure (b) for each transect per observation round.

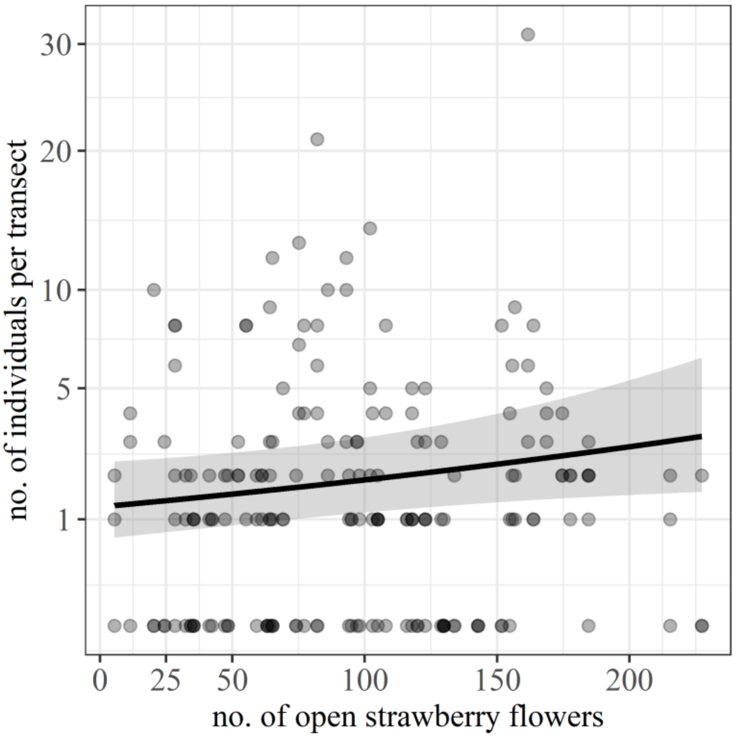


Figure S4 The number of bee individuals per transect in strawberry fields increases when the number of open strawberry flowers (along 2 x 2 m² within the strawberry rows) increases. The regression line and 95 % confidence interval are obtained from mixed effect model estimates. Please note that bee abundance is shown on a square root scale.

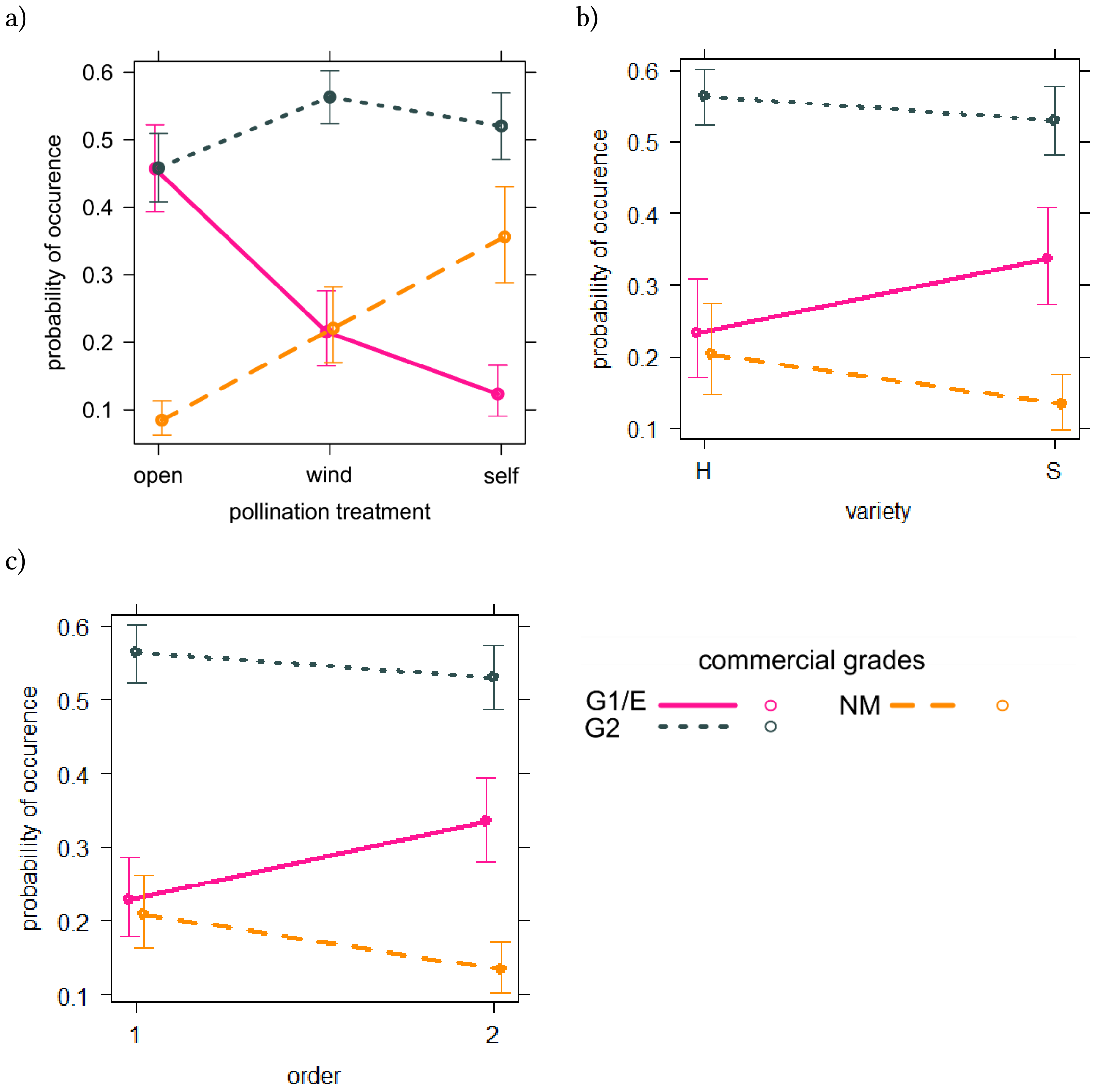


Figure S5 The predicted probability of occurrence (and 95 % confidence intervals) of the three commercial grades in relation to a) pollination treatment, b) strawberry variety and c) flower order. The probability of fruits achieving the best commercial grade (G1/E) are greatest in the open pollinated treatment while fruits from self-pollinated fruits will most likely result in non-marketable fruits (NM). No clear pattern was found for fruits which achieved the intermediate commercial grade (G2). Strawberry fruits from the variety Sonata (S) have a greater probability to achieve the best commercial grade compared to Honeoye (H). Similar pattern has been found for flower order, where fruits from the second flower order (order 2) have a greater probability to achieve the best commercial grade in comparison to first order flowers (order 1).

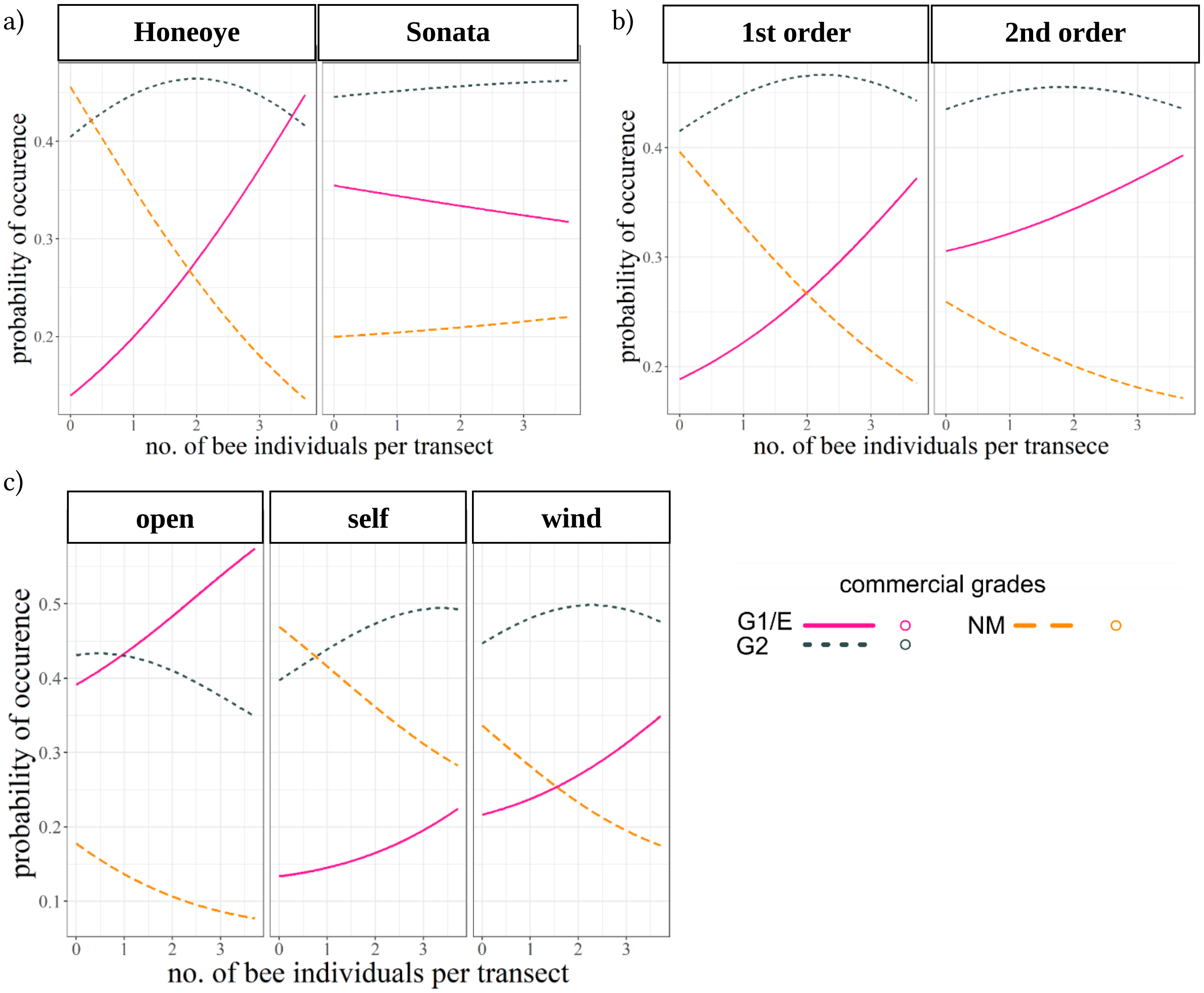


Figure S6. The probability of occurrence of the three commercial grades in relation to a) the number of bee individuals per transect for the varieties Honeoye and Sonata, and b) the number of bee individuals per transect for the flower orders 1 and 2, and c) the number of bee individuals per transect for the pollination treatments open-, self- and wind-pollination. The probability of fruits achieving the best commercial grade (G1/E) increases with a greater number of bee individuals per transect for the variety Honeoye but not for Sonata (a). A greater number of bee individuals per transect increases the probability of fruits achieving the best commercial grade in the first order while the second order was less affected (b). Fruits in the open-pollinated treatments had a higher probability to achieve the best commercial grade G1/E when the number of bee individuals per transect increased while the patterns are less clear for the self- and wind-pollinated treatments (c).

Tables

Table S1 Species list of observed and caught species, body size and sociality. The foraging range is mostly correlated with the body size (i.e. body length) of bees: the larger the bee, the longer the foraging range (Greenleaf *et al.* 2007). Some solitary bee species have been observed but were not caught and/or identified.

Species	No. of individuals	Body size (mm)	Sociality
Andrena chrysosceles	7	9-10(1)	solitary
Andrena cineraria	4	14(1)	solitary
Andrena fucata	1	12-13(1)	solitary
Andrena gravida	18	13(1)	solitary
Andrena haemorrhoa	10	11(1)	solitary
Andrena helvola	9	11-12(2)	solitary
Andrena lapponica	1	9-11(1)	solitary
Andrena nigroaenea	19	14(1)	solitary
Andrena nitida	7	15(1)	solitary
Andrena ssp.	3	NA	solitary
Andrena scotica	9	13(1)	communal
Andrena subopaca	2	7(1)	solitary
Apis mellifera	219	10-20(3)	social
Bombus hypnorum	2	13(4)	social
Bombus lapidarius	18	14(4)	social
Bombus ssp.	1	NA	social
Bombus pomorum	2	18-20(2)	social
Bombus terrestris	118	14(4)	social
*Halictus tumulorum**	1	7(1)	social
Lasioglossum parvulum	1	6-7(5)	solitary
Osmia bicornis	8	10-11(1)	solitary
Not identified solitary bees	11	NA	solitary
Not caught	56	NA	NA

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* *Halictus tumulorum* is thought to be primitively eusocial but we decided to include this one individual in the group of solitary bees for our analysis since most species of the family Halictidae are solitary and *H. tumulorum* is morphologically similar to the majority of observed solitary wild bees (Allen 2012).

Allen, G. 2012, *Halictus tumulorum* (Linnaeus,1758) | BWARS.
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Table S2 Mean values (± SE) of OSR availability (product of OSR flower cover and OSR land cover), no. of open strawberry flowers (along 2 x 2 m^2 within the strawberry rows) and no. of bee individuals per transect (subdivided into functional groups: honey bees, bumble bees, solitary bees) at different flowering periods. T1 = early, T2 = intermediate, T3 = late.

Mean of	T1 (n = 603)	T2 (n = 540)	T3 (n = 340)
OSR availability	237.62 ± 10.52	235.81 ± 7.79	243.29 ± 15.41
no. of open strawberry flowers	105.62 ± 2.33	125.24 ± 2.15	105.64 ±2.33
no. of bee individuals (all functional groups)	8.12 ± 0.34	10.76 ± 0.37	10.00 ± 0.48
no. of honey bee individuals	3.18 ± 0.17	5.81 ± 0.30	5.06 ± 0.42
no. of bumble bee individuals	1.99 ± 0.15	2.07 ± 0.11	2.81 ± 0.13
no. of solitary bee individuals	1.98 ± 0.05	1.68 ± 0.08	2.10 ± 0.12

Table S3 Summary of best fitting candidate models (dAICc < 2), null- and global models (X0, X_global) for a) the effects of OSR availability (product of OSR land cover and OSR flower cover) on the number of bee individuals per transect and b) the pollination experiments: strawberry fruit weight (g) and commercial grades of strawberry fruits.
Explanatory variables are strawb_fc = no. of open strawberry flowers (along 2 x 2 m^2 within the strawberry rows), OSR = OSR availability, order = flower order, PT = pollination treatment, variety = strawberry variety, bees = no. of bee individuals per transect.

Response variable	Model	DF	AICc	dAICc	Akaike weight (wi)	Explanatory variables
a) Effects of OSR availability on pollinator abundance						
No. of bee individuals per transect	Bee1	11	766.3	0	0.641	Bees+ OSR + strawb_fc + group + OSR:group
	Bee0	5	775.4	9.16	0.007	1 (intercept)
	Bee_global	13	284.37	770.5	4.2	Bees+ OSR + strawb_fc + group + straw_fc:group + OSR:group
b) Pollination experiments						
Strawberry fruit weight (g) (n=1438)	FW1	10	1547.4	0	0.473	Bees + PT + order + variety + bees:order + variety:order
	FW2	8	1548.4	0.97	0.292	Bees + PT + order + bees:order
	FW0	6	1877.2	329.77	0	1 (intercept)
	FW_global	15	1549.9	2.43	0.109	Bees + PT + order + variety + bees:order + bees:variety + bees:PT
Commercial grade (n=1483)	Q1	10	2891.50	0	0.226	PT + order + variety
	Q2	12	2892.30	0.86	0.147	Bees + PT + order + variety + bees:variety
	Q3	13	2893.20	1.75	0.094	Bees + PT + order + variety + bees:order + bees:variety
	Q4	9	2893.30	1.87	0.089	PT + order
	Q5	14	2893.40	1.9	0.087	Bees + PT + order + variety + bees:order + bees:PT
	Q0	6	3063.8	172.32	0	1 (Intercept)
	Q_global	15	2894.5	3.05	0.049	Bees + PT + order + variety + bees:order + bees:variety + bees:PT

Table S4 Estimates and 95% upper and lower confidence intervals (CI) of the best fitting models (dAICc < 2 in comparison to the best fitting model with dAICc = 0) for a) the number of bee individuals per transect and b) the pollination experiments (strawberry fruit weight & commercial grades).

Abbreviations: OSR = oilseed rape availability (product of OSR flower cover and OSR land cover), Strawb fc = no. of open strawberry flowers (along 2 x 2 m² within the strawberry rows), BB = no. of bumble bees per transect, SB = no. of solitary bees per transect, order 2 = flower order (2nd), PT = pollination treatment, wind = wind-pollination, self = self-pollination, Variety S = strawberry variety Sonata, bees = no. of bee individuals per transect.

Model	Explanatory variable	95% CI lower limit	95% CI upper limit	Estimate
a) Effects of OSR availability on pollinator abundance (response: no. of bee individuals per transect)				
Bee1	(Intercept)	0.314	1.421	0.867
	Strawb fc	0.019	0.410	0.215
	BB	-0.717	0.109	-0.304
	SB	-0.842	-0.022	-0.432
	OSR	-0.373	0.134	-0.120
	BB: OSR	-0.862	-0.022	-0.442
	SB: OSR	-0.036	0.713	0.339
b) Pollination experiments				
Response: strawberry fruit weight (g)				
FW1	(Intercept)	1.964	2.555	2.260
	Bees	0.074	0.372	0.222
	Order2	-0.362	-0.132	-0.246
	PT wind	-0.22	-0.1	-0.160
	PT self	-0.173	-0.054	-0.113
	Variety S	0.026	0.833	0.452
	Bees:order2	-0.127	-0.02	-0.074
	Bees:Variety S	-0.405	-0.051	-0.237
FW2	(Intercept)	2.289	2.72	2.504
	Bees	-0.024	0.158	0.067
	PT wind	-0.221	-0.101	-0.161
	PT self	-0.173	-0.054	-0.114
	Order 2	-0.365	-0.133	-0.249
	Bees:order2	-0.126	-0.019	-0.073

Response: commercial grades				
Q1	Order 2	-0.774	-0.295	-0.535
	PT wind	0.829	1.411	1.120
	PT self	1.483	2.094	1.788
	Variety S	-0.991	-0.038	-0.514
	1|2	-1.232	-0.381	-0.807
	2|3	1.307	2.183	1.745
Q2	Bees	-1.211	0.107	-0.552
	Order 2	-0.775	-0.292	-0.533
	PT wind	0.827	1.409	1.118
	PT self	1.479	2.09	1.785
	Variety S	-3.005	-0.328	-1.666
	Bees:varietyS	-0.046	1.416	0.685
	1|2	-2.687	-0.562	-1.624
	2|3	-0.128	1.984	0.928
Q3	Bees	-1.314	0.02	-0.647
	PT wind	0.824	1.406	1.115
	PT self	1.477	2.088	1.783
	Order 2	-1.502	-0.215	-0.858
	Variety S	-3.071	-0.418	-1.745
	Bees:varietyS	-0.01	1.424	0.707
	Bees:order2	-0.134	0.458	0.162
	1|2	-2.924	-0.718	-1.821
	2|3	-0.366	1.826	0.730
Q4	PT wind	0.830	1.412	-1.121
	PT self	1.481	2.092	1.787
	Order 2	-0.771	-0.290	-0.531
	1|2	-0.826	-0.154	-0.490
	2|3	1.696	2.428	2.062
Q5	Bees	-1.284	0.091	-0.579
	PT wind	0.601	2.123	1.362
	PT self	0.569	2.079	1.324
	Order 2	-0.771	-0.289	-0.530
	Variety S	-3.002	-0.338	-1.670

Bees:PT self	-0.117	0.563	0.223
Bees:PT wind	-0.468	0.226	-0.121
Bees:varietyS	-0.042	1.412	0.685
1|2	-2.772	-0.585	-1.679
2|3	-0.214	1.960	0.873

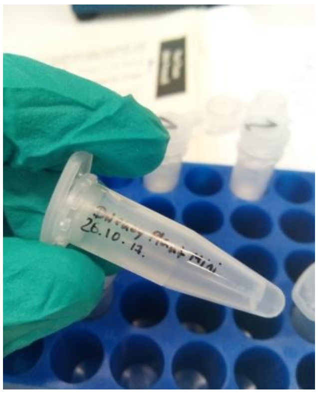
Table S5 The relative importance of explanatory variables expressed by the Σwi for models to explain the effects of functional bee group (= group), no. of open strawberry flowers along 2 x 2m^2 within the strawberry row (= Strawb fc) and OSR availability (= OSR, i.e. product of OSR flower cover and OSR land cover) on the number of bee individuals per transect in strawberry fields. Number of models in which the variable occur is shown in brackets.

Response variable	OSR	group	OSR:group	Strawb fc	Strawb fc: group
No. of bee individuals per transect	0.95 (8)	0.95 (9)	0.91 (3)	0.79 (8)	0.08 (3)

***

Chapter 2

Pollen foraging in honey bees and bumble bees is dependent on species-specific responses to varying mass-flowering crop availability



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The content of the chapter is published in Molecular Ecology, 2020

doi: 10.1111/mec.15675

Abstract

Worldwide pollinator declines lead to pollination deficits in crops and wild plants, and managed bees are frequently used to meet the increasing demand for pollination. However, their foraging can be affected by flower availability and colony size.

We investigated how mass-flowering oilseed rape (OSR) can influence the pollen resource use of small and large honey bee (*Apis mellifera* L.) and bumble bee (*Bombus terrestris* L.) colonies. Colonies were placed adjacent to strawberry fields along a gradient of OSR availability in the landscapes. We used ITS2 metabarcoding to identify the pollen richness based on ITS2 amplicon sequencing and microscopy for quantification of target pollen.

Bumble bees collected pollen from more different plant genera than honey bees. In both species, strawberry pollen collection decreased with high OSR availability but was facilitated by increasing strawberry flower cover. Colony size had no effect. The relationship between NGS-generated ITS2 amplicon reads and microscopic pollen counts was positive but pollen type-specific.

Bumble bees and, to a lesser degree, honey bees collected pollen from a wide variety of plants. Therefore, in order to support pollinators and associated pollination services, future conservation schemes should sustain and promote pollen plant richness in agricultural landscapes. Both bee species responded to the availability of flower resources in the landscape. Although honey bees collected slightly more strawberry pollen than bumble bees, both can be considered as crop pollinators. Metabarcoding could provide similar quantitative information as microscopy, taking into account the pollen types, however there remains still high potential to improve the methodological weaknesses.

Introduction

Pollinators not only contribute to the yield and quality of many crops, but also deliver pollination services to many wild plants, enhancing seed set (Klein *et al.* 2007; Klatt *et al.* 2014; Wietzke *et al.* 2018). However, pollinators worldwide are at risk due to multiple stressors, such as agricultural intensification, habitat loss, and accompanying reduction in the diversity and abundance of host plant species (Potts *et al.* 2010; Vaudo *et al.* 2015). At the same time, global pollinator-dependent crop production is intensifying and the demand for pollination services is increasing (Aizen & Harder 2009). When native and domesticated pollinators are rare or absent, farmers are exposed to high economic risks due to reduced pollination rates (Potts *et al.* 2016).

The use of managed bees, such as honey bees (*Apis mellifera* L.) and bumble bees (i.e. *Bombus terrestris* L.), can contribute to meeting the pollination demand in crop production. Both species are generalist pollinators that visit a great variety of plant species and are suitable for the pollination of many crops (Kleijn *et al.* 2015). Crop pollination can be promoted through the use of honey bee pollination services offered by beekeepers or by placing commercially available bumble bee colonies in or next to crops. The use of bumble bees in particular has become a widely used practice for pollination in greenhouses, but is also frequently used in fields (Velthuis & van Doorn 2006; Gosterit & Baskar 2016), while keeping honey bees is an established practice in many field crops (Klein *et al.* 2007; Potts *et al.* 2010).

Bees collect pollen because it provides proteins, vitamins, and minerals, for larval development (Thorp 2000). Bees often forage on a large number of different plant species to meet their dietary requirements (Di Pasquale *et al.* 2013; Alaux *et al.* 2017, Leidenfrost *et al.* 2020) and they can balance nutrient deficits by collecting either greater amounts or diversity of pollen (Hendriksma & Shafir 2016; Danner *et al.* 2017). Colony development can be enhanced through increasing intake of higher amounts and/or better pollen quality in terms of pollen diversity or species composition (Brodschneider & Crailsheim 2010; Vaudo *et al.* 2018; Kämper *et al.* 2016). To counteract ongoing bee declines and to sustain vital populations of managed and wild pollinators in agricultural landscapes, it is important to understand the temporal and spatial dynamics of pollen resource exploitation (Kämper *et al.* 2016; Bertrand *et al.* 2019), the effects of pollen richness on reproductive success (Requier *et al.* 2017; Vaudo *et al.* 2018; Hass *et al.* 2019), and plant-pollinator interactions and networks (Bell *et al.* 2017; Arceo-Gomez *et al.* 2020). High flower and pollen constancy of bees may imply high carry-over of the respective pollen on the stigma, and hence could be used as an indicator of pollination efficiency (Gyan & Woodell 1987; Montgomery 2009, Marzinzig *et al.* 2018).

Pollen richness can be investigated using ITS2 (internal transcribed spacer) metabarcoding. In comparison to traditional methods (e.g. microscopy), metabarcoding can provide a higher resolution of taxon richness and has a higher throughput with a predictable cost- and timeframe (Keller *et al.* 2015; Smart *et al.* 2017; Bell *et al.* 2019). However, metabarcoding is restricted in its applicability in pollen quantification. Several factors may affect the quantification results obtained by NGS-based amplicon sequencing, such as DNA extraction, PCR amplification, or barcode copy number (Peel *et al.* 2019), which can lead to over- or underestimation of actual pollen counts (Richardson *et al.* 2015; Pornon *et al.* 2016; Baksay *et al.* 2020). The number of metabarcoding reads may be more likely positively related to microscopy-based counts for commonly occurring taxa than for rare pollen taxa in the samples (Smart *et al.* 2017). Hence, the abundance of metabarcoding amplicons could be used as estimate of relative abundance but should be applied with respect to the investigated taxa and research questions (Danner *et al.* 2017; Smart *et al.* 2017; Nürnberger *et al.* 2019). Complementary to metabarcoding, microscopic pollen analysis can be used for pollen quantification as less processing steps are needed that could bias the pollen counts. However, great expertise is needed to identify all plant taxa present in pollen pellets using microscopy (Keller *et al.* 2015), but target pollen can be counted by non-experts, especially when pollen grains have a characteristic surface structure (Beug 2015). However, rare pollen taxa may not be detected with microscopy, which can also depend on the number of counted pollen per sample (Smart *et al.* 2017; Lau, Bryant & Rangel 2018). Up to now, only few studies compared quantitative results from metabarcoding and traditional microscopy and indicate that outcomes are not necessarily correlated and can depend on plant species and species composition (Bell *et al.* 2019; Richardson *et al.* 2015).

Bumble bees build colonies with up to 600 individuals (e.g. *B. terrestris*) while honey bee colonies (*A. mellifera*) can achieve colony sizes up to 80,000 individuals (Felix & Krebs 2012). Thus, both bee species can provide many foraging and pollinating individuals. Yet, using large colonies for pollination services does not guarantee a high number of individuals in target crop fields since both honey bees and bumble bees are able to explore landscapes extensively due to their large foraging radii (Steffan-Dewenter & Kuhn 2003; Westphal *et al.* 2006). However, bumble bees tend to exploit more diverse resources in the close surroundings of their colonies (Wolf & Moritz 2008) since they are not able to communicate as effectively as honey bees do by waggle dance (Dornhaus & Chittka 2001; Couvillon 2012).

Especially honey bees but also some bumble bee species, like *B. terrestris*, preferably exploit highly rewarding mass-flowering crops, such as oilseed rape (OSR, *Brassica napus* L.; Rollin *et al.* 2013). The availability of mass-flowering resources within the foraging ranges can lead to lower bee densities in minor flowering crops, such as strawberries (*Fragaria x ananassa* Duch.) (Grab *et al.* 2017; Bänsch *et al.* 2020a) and hence, potentially affect pollination services. Many entomophilous crops (e.g. strawberry) produce high value fruits (e.g. greater fruit weight and quality) when insect-pollinated (Bommarco *et al.* 2012; Klatt *et al.* 2014; Wietzke *et al.* 2018). In order to apply adapted pollination management it is important to study bees foraging preferences, for instance, with pollen analyses (Marzinzig *et al.* 2018).

Simply increasing the number of managed bee colonies in crop fields is presumably not the optimal solution for pollination management since this would result in higher costs for farmers and high managed bee densities may negatively affect wild bees (Herbertsson *et al.* 2016; Mallinger *et al.* 2017). As an alternative, the selection of pollinator species and adaptation of colony size may be more efficient in directing foraging bee pollinators to crops in agricultural landscapes. To our knowledge, research on this possible adaption of colony size in pollinator management is still very rare but can have severe implications for pollination management. Few studies suggest that foraging can vary between colonies of different sizes (e.g. shorter foraging distance of smaller colonies; Beekman *et al.* 2004; Westphal *et al.* 2006; Böcking and Kreipe 2013).

In general, we focus on the following questions in the present study: Which pollen resources do managed pollinators use in agricultural landscapes and to what extent? Is the pollen foraging behaviour bee species-specific and influenced by (mass-)flowering resource availability and colony size? Do quantitative analyses of pollen samples based on ITS2 metabarcoding and microscopy yield comparable results?

More specifically, we hypothesized that honey bees and bumble bees will use flowering crops (i.e. strawberry and OSR) as major pollen resources in agricultural landscapes because flowering crops provide ample pollen and can cover large areas. Further, we expected that bumble bees collect pollen from a greater number of plant genera than honey bees and that they collect pollen from resources close to the colonies (i.e. strawberry) in greater proportions and more frequently due to less effective communication. Considering the bees' preference for mass-flowering crops, we hypothesized that pollen richness and the proportion of strawberry pollen, as minor flowering target crop, will decrease in the pollen loads of both bee species with increasing OSR availability. Larger colonies were expected to collect greater pollen richness while smaller colonies should collect greater proportions of crop pollen close to the hive. Due to several factors that influence outcomes of pollen quantification based on metabarcoding (Peel *et al.* 2019; Bell *et al.* 2019), we hypothesized a positive and pollen type-specific relationship between the number of metabarcoding reads and microscopic pollen counts for the two target crop species, i.e. strawberry and OSR.

We investigated the pollen foraging preferences of two managed bee species, *A. mellifera* and *B. terrestris*, during the co-flowering of two economically important crops (strawberry and OSR) by placing large and small honey bee and bumble bee colonies next to strawberry fields in agricultural landscapes that represented a gradient of mass-flowering OSR availability. Making use of the advantages offered by ITS2 metabarcoding, we identified the plant genera that were present in mixed pollen samples collected by the honey bee and bumble bee colonies. Traditional microscopy was applied to quantify the proportions of strawberry and OSR pollen in the samples. Finally, we analysed the pollen type-specific relationships between the number of metabarcoding reads and microscopic pollen counts for the two crop species as only few studies have compared pollen quantification by metabarcoding and microscopy so far, which did not achieve clear results (Bell *et al.* 2019; Richardson *et al.* 2015).

Material and Methods

Study location

We studied the pollen foraging behaviour of bee colonies adjacent to nine commercial strawberry fields in central Germany in the surrounding regions of the cities Göttingen (southern Lower Saxony) and Kassel (northern Hesse) in 2016 (Supporting Information Figure S1). Most study fields were managed for public pick-your-own harvesting and usually had three different strawberry varieties to extend the harvesting season. Study field sizes were on average 2.23 ha (±0.92 SD, range 1.01–3.61 ha). The strawberry study fields were surrounded by an agricultural landscape matrix which we mapped using a geographic information system (ESRI ArcGIS, Version 10.3.1). We classified the land cover types into strawberry fields, OSR fields, other cropland (mainly non-entomophilous crop fields with adjacent field margins, single trees and country lanes), semi-natural habitats (e.g. hedges, fallow land, and meadow orchards), forests, and urban areas. The landscapes were mapped within a typical honey bee and bumble bee foraging distance of about 2,000 m radius around our study fields (Westphal *et al.* 2006; Härtel & Steffan-Dewenter 2014). Availability of OSR was calculated as the product of OSR land cover (ha) and OSR flower cover (%). OSR flower cover was estimated at each observation date within the nearest OSR field to the strawberry field along a transect of 50 m x 4 m. All OSR fields had relatively uniform germination and flowering within our study landscapes. To validate the OSR gradient, we assessed the OSR land cover in autumn 2015 (winter OSR plants can already be identified in autumn). Strawberry flower cover within the adjacent field was determined by counting the open flowers along 2 m of the row with greatest flower abundance, as this area was most likely the most attractive for bees.

Experimental set-up

We established an experiment with small and large honey bee (*A. mellifera carnica* Pollmann 1879) and bumble bee (*B. terrestris* Linnaeus, 1758*)* colonies adjacent to strawberry fields in order to study pollen foraging in relation to OSR availability and strawberry flowering. One small and one large colony of each species was placed at the edge of each of the nine study fields (in a distance of ≤ 5 m to the strawberry cultivation). Hence, we studied 36 colonies. Large honey bee colonies had around 20,000 workers at the beginning of the season and one queen. Small honey bee colonies were built as nuclei with around 4,000 workers from additional large colonies. Honey bee colonies sizes were estimated following the ‘Liebefeld Method’ by visually estimating the number of adults on the combs surface (Imdorf *et al.* 1987; Dainat *et al.* 2020). All small colonies successfully raised their own queen, which emerged a few days after experimental set up in the field. Even immature queens produce queen substance pheromone and stimulate pollen collection in foragers (Free, Ferguson and Simpkins 1984; Boch 1979). Hence we do not expect large differences in foraging behaviour due to the queens’ age.

At the end of the experiment, large honey bee colonies comprised approximately 30,000 to 35,000 workers and small colonies about 6,000 to 8,000 workers. Small and large bumble bee colonies (*B. terrestris*) were purchased from a German bumble bee breeder (STB Control, Aarbergen, Germany). The colonies consisted of one queen bee and 40 and 80 workers, respectively. In order to monitor the development of the bumble bee colonies, we weighed them as the number of individuals is difficult to quantify (e.g. they may hide in the complex structure of their nests; Lefebvre & Pierre 2006). We monitored the colony weight during the first observation round (small colonies: mean 1,045.33 g ± 45.68 SE and large colonies: mean 1,155.11 g ± 50.59 SE) and third observation round (small colonies: mean 1533.50 g ± 69.85 SE and large colonies: mean 1754.75 g ± 114.32 SE). Data collection began on 6th of May 2016 with the beginning of the strawberry blossom.

Pollen sampling and preparation

We collected pollen loads of honey bees and bumble bees in front of their colonies during the strawberry flowering period on, if possible, five observation days per study landscape. Since we could not analyse pollen from each colony at each sampling date (e.g. due to low colony activity and hence, pollen material below 0.05 g), the number of samples per colony type can differ (small honey bee colonies n = 34, large honey bee colonies n = 40, small bumble bee colonies n = 38, large bumble bee colonies n = 37). We set a threshold of 0.05 g to have enough pollen material for the metabarcoding process (0.015 g), repetitions in case something went wrong (e.g. contamination) and microscopy. The study period lasted from 6th May to 6th June 2016, depending on the microclimatic conditions within the study landscapes. Pollen loads from honey bees were collected using commercial pollen traps installed in front of the colony. The traps guide the bees through a 5 mm grid that removes pollen loads from the hind tibia. Traps were installed in front of each colony for 30 minutes on each observation day. Pollen loads from bumble bees were collected by capturing, if possible, five individual bumble bees in front of their colonies with an insect net and put them into marking cages, respectively. Pollen was removed from the hind tibia with tweezers and stored in 1.5 mL reaction tubes. Bumble bees were released after this procedure. To account for foraging preferences of bees for either pollen or nectar resources at certain times of the day we varied sampling times across landscapes systematically at each visit (i.e. visiting each field equally in morning hours and afternoon hours between 9 am and 5 pm, respectively). Pollen sampling was only conducted on days with low wind speed, no rain and a minimum temperature of 14°C.

We pooled the pollen loads of each observation date by colony and homogenised them in 70 % ethanol (ratio 1 : 4 pollen : 70 % ethanol). From this mixture, we prepared 1 mL aliquots in 1.5 mL reaction tubes for microscopic and molecular pollen analysis. The tubes were centrifuged for 10 min at 15,400 x *g*. We then removed and discarded the supernatant ethanol. Afterwards, samples were dried with open lids in a fume cupboard with an air throughput of 1000 m^3/h for 72 h.

To study the pollen collection constancy (homogeneity in pollen samples) of in-field foragers in strawberry fields, we collected honey bee (n=37) and bumble bee individuals (n=36) visiting strawberry flowers. Standardized transects walks were conducted at two locations within strawberry fields (2 x 50 m in 30 minutes at the edge and in the centre of the field) for the two most common strawberry varieties (Sonata and Honeoye) on five observation dates in seven out of the nine landscapes in 2015. Transect times were varied systematically between 9 am and 5 pm (i.e. visiting each field equally in morning hours and afternoon hours, respectively). The collected bees were washed with 70 % ethanol individually in 1.5 mL reaction tubes in order to remove the pollen from the bees' bodies. Subsequently, the bees were removed, and the pollen-ethanol mixture was centrifuged for 10 min at 15,400 x *g* to remove and discard the residual ethanol and dried as described in the paragraph above.

Pollen analysis

We analysed the richness and amount of target pollen grains in pollen loads using two methodologies: ITS2 metabarcoding and microscopy. To assess the relationship and variability between the quantitative outcomes for different pollen types, we compared the number of ITS2 amplicon reads and pollen grain counts for *Fragaria* and *Brassica* pollen types in the samples. Both methods have the same taxonomic resolution in our study. We assume that ITS2 amplicon reads from *Fragaria* and *Brassica* indicate strawberry and OSR, as they were the most common flowering plant species belonging to these genera in our study landscapes during the study period. *Fragaria vesca* is flowering at the same time like *Fragaria x ananassa* but flowers in much lower abundancies in our study landscapes, and mainly in woody habitat structures. Other congeneric species of *Brassica* type flower most likely only later in the year (e.g. in flower strips).

We used metabarcoding of ITS2-region PCR-amplicons (polymerase chain reaction) to quantify the richness of pollen collected by small and large honey bee and bumble bee colonies. In the present study, we used the advantages of metabarcoding techniques (e.g. high efficiency and resolution; Bell *et al.* 2019; Baksay *et al.* 2020) for qualitative high-throughput identification of PCR-amplified ITS2 sequences from plant genera present in pollen loads collected from honey bees and bumble bees to study pollen richness.

DNA was extracted from aliquots of ca. 0.015 g pollen using the DNeasy Plant Mini Extraction Kit from Qiagen according to the manufacturer's instructions. Cell lysis and homogenisation of the samples were modified as follows: 150 g ceramic beads (1.4 mm), one tungsten carbide bead (3 mm) and 200 µL buffer AP1 were added to each dried sample. Samples were homogenised twice with a FastPrep Instrument (FastPrep®FP120, ThermoSavant) for 45 seconds at 6.5 m/s with a cooling step with ice in-between. Another 200 µL buffer AP1 were added. Finally, the standard protocol was followed until the DNA was eluted with 50 µL of elution buffer. DNA concentration and quality were measured using a Nanodrop (Thermo Scientific, Massachusetts, USA).

For each sample, we performed three PCR reactions in separate 10 µL reactions to reduce PCR bias (Sickel *et al.* 2015) using the primers ITS2F (Chen *et al.* 2010) and ITS4R (White *et al.* 1990). Each reaction contained 0.3 µL FastStartTaq Polymerase (5 U/µL, Roche), 0.5 dNTPs (0.5 µL), 0.75 µL of each forward and reverse primer (10 pmol/µl), 2.5 µL 10x PCR Puffer with $MgCl_2$ at a concentration of 20 mM (Roche), 19.2 µL PCR grade water, and 1 µL DNA template. The PCR conditions were optimised to the following conditions: initial denaturation at 95°C for 10 minutes, 37 cycles of denaturation at 95°C for 40 s, annealing at 49°C for 40 s and elongation at 72°C for 40 s. Final extension was performed at 72°C for 5 min.

All reactions were checked for successful amplifications and contaminations by gel electrophoresis (1.5 % agarose gels stained with ethidium bromide, 120 V for 30 min). Triplicate PCR products were pooled per sample and purified using the QIAquick PCR purification Kit. 500 ng of each PCR product were used for library preparation according to the manufacturer's protocol NEBNext Ultra II DNA Library Prep Kit for Illumina (New

England Biolabs, Munich, Germany). Paired-end sequencing (2x150 bp) was performed on a NextSeq500 platform using a Mid-output flowcell (150 cycles).

In order to increase the accuracy of assignment of amplicon sequencing reads to plant-specific ITS2 sequences, we extracted all ITS2 sequences from a global eukaryota database (Förster 2015) that have previously been described for plants occurring in Lower Saxony, Germany (Garve 2004, 2007). The resulting subset was made non-redundant by clustering identical entries with VSEARCH (version 2.9.1; Rognes *et al.* 2016) and then used to create a magicBLAST database (version 1.4; Boratyn *et al.* 2019). After blasting the ITS2 amplicon reads against this database, all paired reads that both aligned to a database entry (plant ITS2 sequence) with at least 50 bp each and a similarity greater than 98 % were kept.

For each matching read, we calculated an alignment score by multiplying the alignment length with the alignment identity; the scores for the forward and reverse read were summed to get the final score for each read-pair. Read-pairs that matched several entries were ordered by this score. Only the top scoring match (plant species) per read was counted. As some plant species have very similar ITS2 sequences and we, therefore, cannot unambiguously distinguish them on a species level, we decided to use amplicon read-based identification at the genus level only. If there were multiple scoring matches with an identical score, we decided on the match with higher reliability based either on personal observations of flowering plants in the field or otherwise a distribution atlas of plants in Lower Saxony (Garve 2007). Only in the case of *Hirschfeldia incana* L., we decided to reject the first match since several other matches for this read indicated the genus *Brassica* (e.g. *Brassica napus* L., *Brassica rapa* L.). In comparison to other studies (e.g. Danner *et al.* 2017; Nürnberger *et al.* 2020) who calculated the relative abundance of plant taxa based on metabarcoding sequences, we based our analysis on the presence/absence of ITS2 sequences of certain plant genera only, as the quantitative output (e.g. number of reads) can be biased (Sickel *et al.* 2015). Pollen richness represents the number of plant genera occurring in one pollen sample.

Strawberry and OSR pollen grains were quantified by microscopy for colony samples and the in-field foragers. One aliquot per sample was diluted with distilled water (ratio 1 : 4 pollen : 70 % ethanol). One drop of the pollen-water mixture was applied to a microscopic slide together with one drop of Kayser's gelatine stained with fuchsine and fixed with a cover slide. We counted 200 pollen grains with 400 x magnification on each slide (Lau, Bryant & Rangel 2018). For this purpose, we randomly selected one or when necessary more rows on the slides until we reach a number of 200 pollen grains and categorized the pollen into strawberry pollen, OSR pollen and others according to a self-made reference collection and a determination key (Beug 2015).

Data analysis

All statistical analyses were performed with the software R version 3.6.2 (R Core Team 2016). Continuous explanatory variables (i.e. OSR availability and strawberry flower cover) were scaled to a mean of zero and a standard deviation of one to improve convergence of the models using the scale function (R Core Team 2016). We found only weak if any correlation between fixed effects ($-0.3 > r < 0.3$; Hinkle *et al.* 2003). Data were visualised using the package *ggplot2* (Wickham 2017) and mixed model fit was visualised using the package *effects* (function *allEffects*, Fox and Weisberg 2019).

Pollen richness

Given that the number of collected pollen samples differed between small and large honey bee and bumble bee colonies (i.e. 34, 40, 38 and 37 samples, respectively), we created species accumulation curves and present both, the total number of plant genera (using all samples) but also the rarefied number of plant genera based on 34 samples using the function *specaccum* from the *vegan* package (Oksanen *et al.* 2019).

Effects of bee species, colony size, and mass-flowering resource availability on pollen richness

To analyse the effects of bee species, colony size and flowering resource availability on pollen richness we fitted generalised linear mixed effects models using the *glmmTMB* package (Brooks *et al.* 2017). In a first step, we fitted a global model with pollen richness as response variable and bee species (honey bee/bumble bee), colony size (small/large), OSR availability, and strawberry flower cover and all two-way interactions as explanatory variables. Colony ID nested in study landscape was included as random effect. Two global models containing all explanatory variables were fitted with Poisson and negative binomial error distributions, respectively, and compared using the second order Akaike Information Criterion (AICc) (Burnham & Anderson 2004). The smaller the

AICc, the better is the fit of the model. We decided for the negative binomial model since the AICc was lowest and over-dispersion was detected in the Poisson model. We applied the multimodel inference approach on our global model using the function *dredge* (package *MuMIn*; Burnham & Anderson 2002; Barton 2018), which creates a list of candidate models with all possible model combinations. To avoid overfitting, we limited the number of parameters in each candidate model to three (Crawley 2007). Appropriateness of model assumptions was assessed by plotting residuals vs. fitted values. We ranked the models by AICc. All models within delta AICc (dAICc) < 2 from the best fitting model were considered to have substantial empirical support and are reported together with the null model (Table 1). The relative importance of each explanatory variable was assessed using the sum of Akaike weights (Σw_i) over all candidate models which included the respective variables (function *importance*, package *MuMIn*; Barton 2018). We considered all explanatory variables in the best fitting models (dAICc < 2) if $\Sigma w_i > 0.2$ to explain the effects on our response variables. We applied post hoc comparisons using the function *emmeans* to test for differences between bee species and colony sizes with alpha of 0.05 (Lenth 2017). To analyse how pollen composition in pollen loads of small and large honey bee and bumble bee colonies differ, we performed presence/absence-based non-metric multidimensional scaling (NMDS, function *nmds*, package *vegan*; Oksanen *et al.* 2019) using Bray Curtis dissimilarity (Clarke *et al.* 2006). Differences between colony types were tested with an analysis of variance using distances matrices including study landscape as a strata variable (function *adonis2*, package *vegan*; Oksanen 2019).

Pollen collection

In order to describe the pollen resource utilisation of honey bee and bumble bee colonies, we created heat maps displaying the number of ITS2 amplicon reads obtained from metabarcoding. Using only the presence of amplicon reads for certain plant genera, we extracted the five most common plant genera in pollen samples of small and large colonies summarized over the whole study period (i.e. frequency). These data are merely described and not statistically analyzed.

Table 1 Summary of best fitting candidate models (dAICc < 2) and null models for the response variables a) pollen richness and b) strawberry pollen collection. Candidate models are subset from the global models with a maximum of three parameters. Global models for both, pollen richness and strawberry pollen collection, include OSR availability, bee species, colony size, strawberry flower cover and all two-way interactions as explanatory variables. Response variable for pollen richness is the number of plant genera in pollen samples based on metabarcoding and response variable of strawberry pollen collection is the proportion of strawberry pollen found in colony pollen samples counted with the microscope.

Model estimates for models indicated with † are shown in Supplementary Information Table S2. Explanatory variables: OSR = OSR availability, species = bee species (honey bee/bumble bee), size = colony size (small/large), straw_fc = strawberry flower cover.

Model	df	logLik	AICc	dAICc	Akaike weight (w_i)	Explantory variables
a) Pollen richness (n=152)						
R1 †	6	-384.40	781.37	0.00	0.17	OSR + species
R2	5	-385.48	781.38	0.00[1]	0.17	species
R3 †	7	-383.42	781.61	0.24	0.15	OSR + species + size
R4	6	-384.58	781.74	0.37	0.14	species + size
R5	7	-383.69	782.17	0.79	0.11	species + size + species:size
R6 †	7	-384.17	783.12	1.75	0.07	OSR + species + straw_fc
Null model	4	-438.91	886.09	104.72	0.00	1
b) Strawberry pollen collection (n=157)						
P1 †	7	-580.76	1176.28	0.00	0.50	OSR + species + straw_fc
P2	6	-582.66	1177.89	1.61	0.23	OSR + straw_fc
Null Model	4	-592.68	1193.63	17.35	0.00	1

[1] dAICc=0.004

Collection of strawberry pollen

Colony level

Microscopic pollen counts were used to determine the proportion of strawberry and OSR pollen. The effects of bee species, colony size, OSR availability, and strawberry flower cover on the proportions of collected strawberry pollen were analysed with generalised linear mixed effects models (*glmmTMB*, Brooks *et al.* 2017). We fitted global models with the proportion of strawberry as the response using the *cbind* function. Explanatory variables were bee species, colony size, OSR availability, strawberry flower cover, and all two-way interactions. Since a binomial model resulted in over-dispersion, the global model was fit using a betabinomial error distribution. We included colony ID nested in study landscape as random effects to account for our nested study design. We then followed the multi-model inference approach as described in the previous paragraph, and again, allowed only three parameters in candidate models.

In-field foragers

To analyse the proportion of strawberry pollen in the pollen loads of honey bees and bumble bees (*B. terrestris*) in the field we fitted generalised linear mixed effects models with a beta-binomial error distribution (*glmmTMB,* Brooks *et al.* 2017). The proportion of strawberry pollen was used as the response variable using the function *cbind* and bee species as explanatory variable. Location of bee collection in the field (edge/center) was nested in the study landscape as random effects. We applied post hoc comparisons to test for differences between bee species with alpha of 0.05 (function *emmeans*, Lenth 2017).

Relationship between quantitative outcomes of metabarcoding and microscopy

To test for pollen type-specific associations between quantitative outcomes of ITS2 metabarcoding and microscopy, we fitted linear regression models for log ITS2 amplicon reads +1 reads versus log microscopic pollen grain counts +1 for each crop type (i.e. strawberry and OSR) separately.

Results

Pollen richness

Using ITS2 metabarcoding we identified a total of 112 ITS2 sequences in the pollen samples. We found that bumble bee colonies (*B. terrestris*) collected in total about four to five times more plant genera than honey bee colonies (Table 2). We identified 22 plant genera in small (n = 34) and 27 in large honey bee colonies (n = 40, rarefied for n = 34: 25.67 ± SD 1.23 genera, Supplementary Information Figure S2), whereas higher plant genera richness was identified in small bumble bee colonies (95 plant genera, n = 38, rarefied for n = 34: 92.61 ± SD 2.15 genera) and large bumble bee colonies (94 plant genera, n = 37, rarefied for n = 34: 92.34 ± SD 1.73, Supplementary Information Figure S2).

Table 2 Mean number (±SE) and range of identified plant genera is shown. The total number of plant genera represents identified plant genera across all observation dates and landscapes.

Bee species	Colony size	Plant genera	Range	Total number of plant genera
Honey bee	small	2.97 ±0.19	1–5	22
	large	3.81 ±0.24	1–9	27
Bumble bee	small	16.66 ±1.12	3–39	94
	large	17.32 ±0.94	7–37	95

Effects of bee species, colony size, and mass-flowering resource availability on pollen richness

The effects of bee species, colony size, OSR availability, and strawberry flower cover on the richness of pollen, collected by honey bee and bumble bee colonies, were explained by several models with empirical support (dAICc < 2) (Table 1a). Based on the relative importance of each explanatory variable, assessed using the sum of Akaike weights (Σw_i), we found that greatest importance was assigned to the effect of bee species, indicated by a high Σw_i of 1, followed by the effect of size and OSR availability ($\Sigma w_i = 0.45$ for both, Supplementary Information Table S1). Bee species and OSR availability were included in the best fitting model (dAICc = 0, Table 1a). Pollen richness revealed by metabarcoding was 4.9 times higher in bumble bee colony samples compared to honey bees (Figure 1a) and increased with increasing OSR availability (Figure 1b, Supplementary Information

Table S2a). Colony size also affected pollen richness, though to a smaller extent. In general, large colonies collected about 20 % more different pollen genera than small colonies (Supplementary Information Figure S3a). Strawberry flower cover ($\Sigma w_i = 0.2$) correlated negatively with pollen richness, independently of bee species and colony size. However, this effect is only of minor importance as indicated by the low sum of Akaike weight and low effect size (see model estimates in Supplementary Information Table S2a, Figure S3b). The interaction between species and size was included within the best fitting models, but the sum of Akaike weight was quite low (= 0.11) and hence not considered to have a substantial effect on our response variable. We calculated model-averaged coefficients which support our results (Supplementary Material Figure S4a).

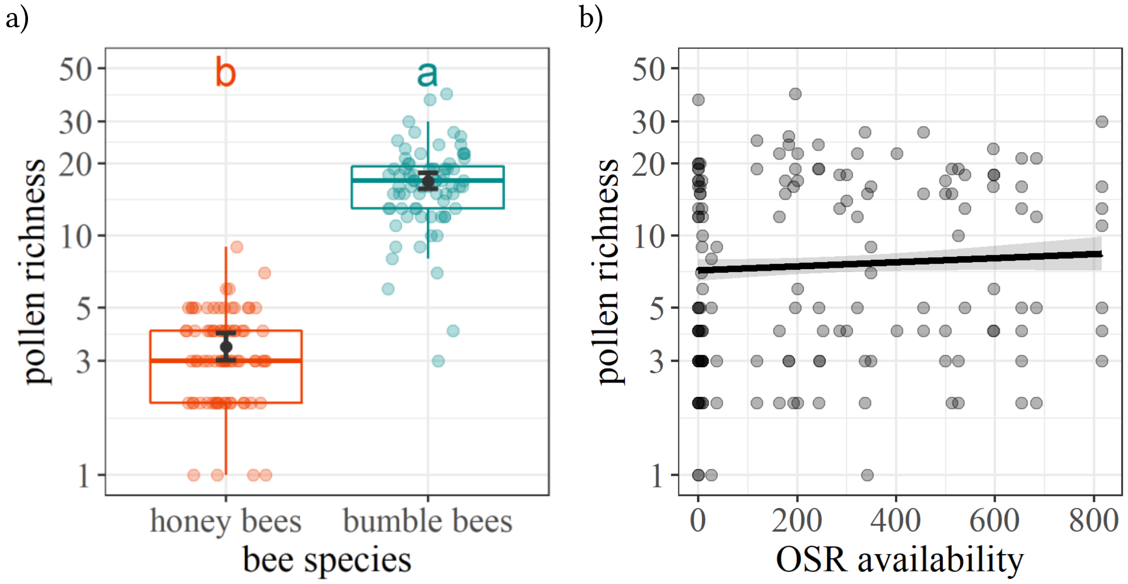

Figure 1 Effect of bee species (a, Σwi = 1) and oilseed rape (OSR) availability (b, Σwi = 0.45) on pollen richness (i.e. number plant genera). Pollen richness in pollen loads is higher in colony samples collected from bumble bees than from honey bees (a). Different letters indicate significant differences obtained from post hoc Tukey test (significance level of 0.05). Predicted values and 95% confidence interval (in black) from mixed effect models are shown. Further, pollen richness increases with high OSR availability (b). The regression line is obtained from mixed model estimates (model R1, see Table S2a) and 95 % confidence region is shown (in grey). Note that pollen richness is shown on a log scale in both plots.

Pollen community composition

The taxonomic composition of the pollen samples originating from small and large colonies was very similar within bee species but differed significantly between bee species ($R^2 = 0.46$, $p = 0.005$, stress value = 0.18, Supplementary Information Figure S5).

Pollen collection

ITS2 sequences of *Fragaria* and *Brassica* were consistently identified in pollen loads of returning honey bees continuously during the study period (*Fragaria* sequences were found in 57 samples and *Brassica* sequences in 60 samples from 77 pollen samples in total, Table 3). Other pollen resources were typically restricted to a shorter time period (e.g. *Salix*) or differed between study landscapes. Sequences of *Brassica* were also identified on many observation dates in bumble bee pollen loads (59 out of 75 pollen samples), while a reduced frequency was observed for the collection of *Fragaria* pollen (43 out of 75 pollen samples). In comparison to honey bees, bumble bee colonies collected pollen from more diverse resources (Supplementary Information Figure S6a,b). A great number of amplicon reads were mapped to ITS2 sequences of plant genera other than the flowering crops strawberry or OSR, such as woody and herbaceous plant genera (e.g. *Salix*, *Prunus* and *Acer*). Without the application of metabarcoding these would have escaped out attention.

Table 3 Five most common pollen resources of small and large honey bee and bumble bee colonies across all landscapes and observations dates. The study period lasted from 6th May to 5th June. The frequency shows the number of samples in which the ITS2 sequence was detected and is based on presence /absence data of plant genera in pollen samples.

Bee species	Colony size	Genus	Frequency
Honey bees n = 77	large n = 42	*Brassica*	35
		Fragaria	31
		Salix	12
		Rosa	11
		Prunus	8
	small n = 35	*Fragaria*	26
		Brassica	25
		Rosa	8
		Crataegus	4
		Ranunculus	4
		Salix	4
Bumble bees n = 75	large n = 38	*Brassica*	29
		Acer	28
		Monotropa	28
		Sedum	25
		Betonica	22
		Fagus	22
		Malus	22
		Spergula	22
		Viola	22
	small n = 37	*Brassica*	30
		Acer	29
		Monotropa	29
		Fragaria	26
		Cornus	24

Collection of strawberry pollen

Colony level

The ITS2-sequences of *Fragaria* were among the five most frequent genera in the pollen samples of all small colonies and of the large honey bee colonies. However, the ITS2 sequences of *Fragaria* were not recorded within the most frequent genera collected by the large bumble bee colonies (Table 3).

Based on microscopic quantification, strawberry pollen grains amounted on average to 26.30 % of the pollen collected by honey bee colonies and 18.58 % of the pollen collected by bumble bee colonies, while the collection of OSR pollen was below 8 % for both bee species and both colony sizes (Table 4). We found two models with empirical support explaining the effects of bee species, colony size, OSR availability, and strawberry flower cover on the proportion of collected strawberry pollen (Table 1b). In the best fitting model (dAICc = 0), strawberry flower cover, bee species and OSR availability were included. Based on the assessment of the relative importance of each explanatory variable, strawberry flower cover was identified as the most important predictor variable ($\Sigma w_i = 1$), followed by OSR availability ($\Sigma w_i = 0.94$) and bee species ($\Sigma w_i = 0.56$, Supplementary Information Table S3). Strawberry pollen collection increased with increasing strawberry flower cover (Figure 2a). Increasing OSR availability decreased the proportion of collected strawberry pollen independently of bee species or colony size (Figure 2b). We found a higher proportion of strawberry pollen in honey bee samples by 69.61 % compared to bumble bee samples (Figure 2c). Colony size and interactions were not included in the best fitting model (dAICc < 2). The results are support by the model-averaged coefficients (Supplementary Material Figure S4b).

Table 4 Mean percentages (±SE) of strawberry and oilseed rape pollen in pollen loads of small and large honey bee and bumble bee colonies.

Species	Colony size	Strawberry pollen	Oilseed rape pollen
Honey bee	small (n=36)	26.30 ±4.63	3.76 ±2.36
	large (n=39)	15.74 ±3.30	6.29 ±2.30
Bumble bee	small (n=40)	18.58 ±3.78	7.56 ±2.25
	large (n=42)	14.98 ±3.60	6.62 ±2.31

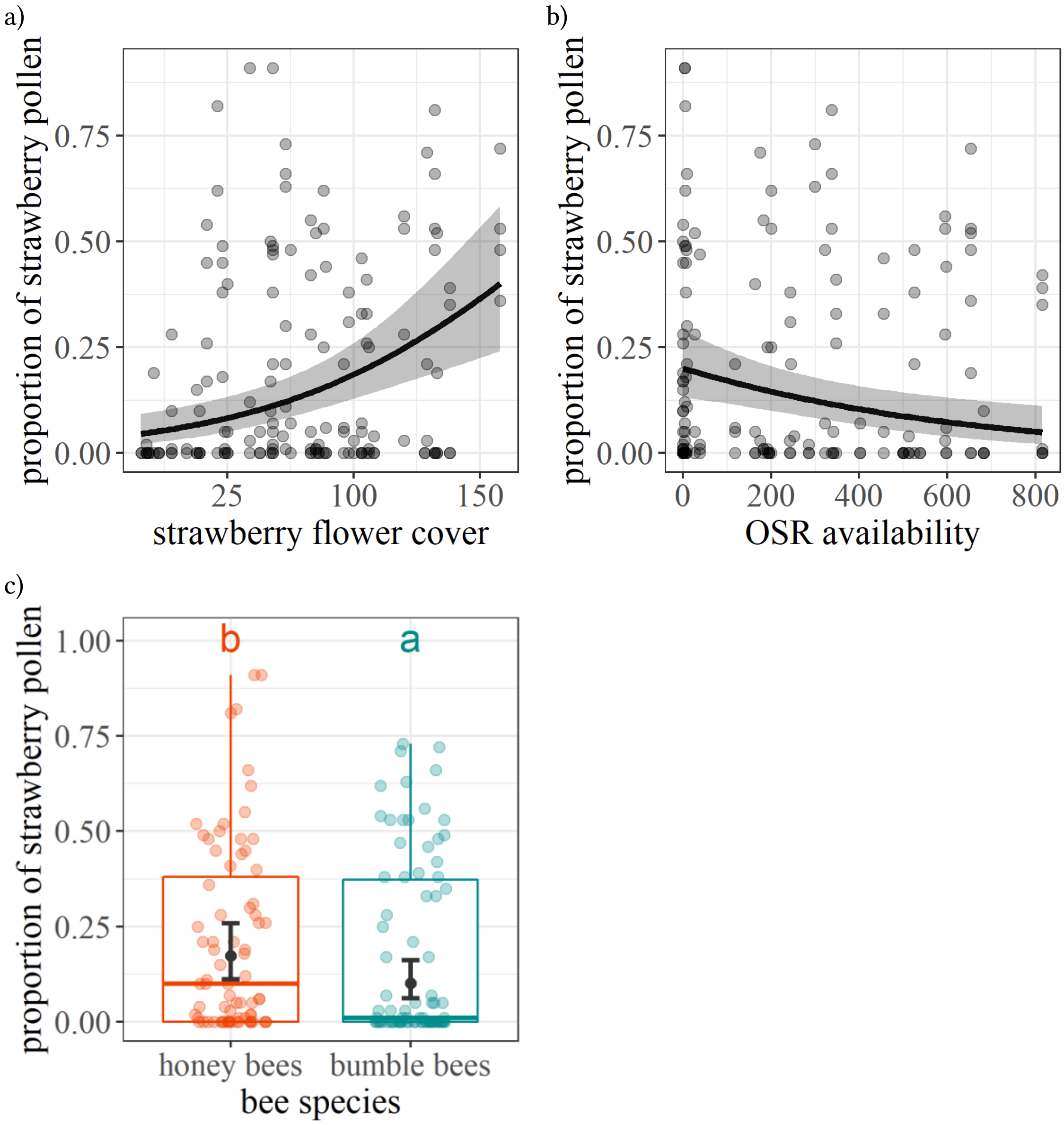

Figure 2 Effects of (a) strawberry flower cover (Σwi = 1), (b) OSR availability (Σwi = 0.94) and (c) bee species (Σwi = 0.56) on the proportion of strawberry in pollen loads (n = 157). High strawberry flower cover increased the proportion of strawberry pollen independently of bee species and colony size (a). High OSR availability decreased the proportion of strawberry pollen loads of both species and colony sizes (b). The regression lines are obtained from mixed model estimates (model P1, see Table 2b) and 95 % confidence region is shown (in grey). Honey bees collected greater proportions of strawberry pollen compared to bumble bees (c). Different letters indicate significant differences obtained from post hoc Tukey test (significance level of 0.05). Predicted values and 95% confidence interval from the mixed effect model P1 are shown (in black).

In-field foragers

Honey bee foragers in strawberry fields collected a 1.27 times greater proportion of strawberry pollen compared to *B. terrestris* foragers ($p < 0.001$, Figure 3).

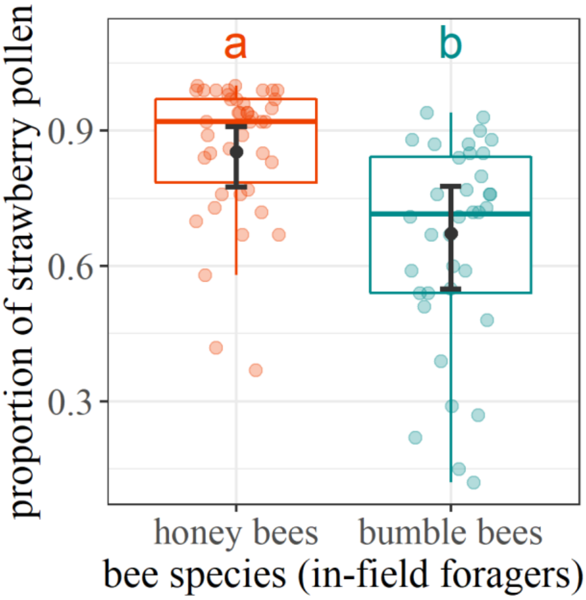

Figure 3 Effect of bee species on the proportion of strawberry pollen in pollen loads from the hind tibia of in-field foragers. Strawberry pollen had a greater share in pollen loads of honey bees (n=37) than in bumble bees (n=36). Different letters indicate significant differences obtained from post hoc Tukey test with a significance level of 0.05. Predicted values and 95% confidence interval from mixed effect models are shown (in black).

Relationship between quantitative outcomes of metabarcoding and microscopy

We found positive relationships between the number of ITS2 amplicon reads and microscopic pollen counts which differed for the two pollen types (see Figure 4 for details on intercept and slope) being stronger for strawberry pollen ($R^2 = 0.69$) than for OSR ($R^2 = 0.15$). The positive intercepts for both plant genera (i.e. when microscopic pollen counts = zero), indicate that ITS2 metabarcoding was able to detect pollen when microscopy failed. When no pollen grains were detected by microscopy, the average number of ITS2 reads of strawberry was 3.2 (95% CI [= Confidence Interval] 1.5-5.9) and for OSR 113.4 (95% CI 62.4-203.4).

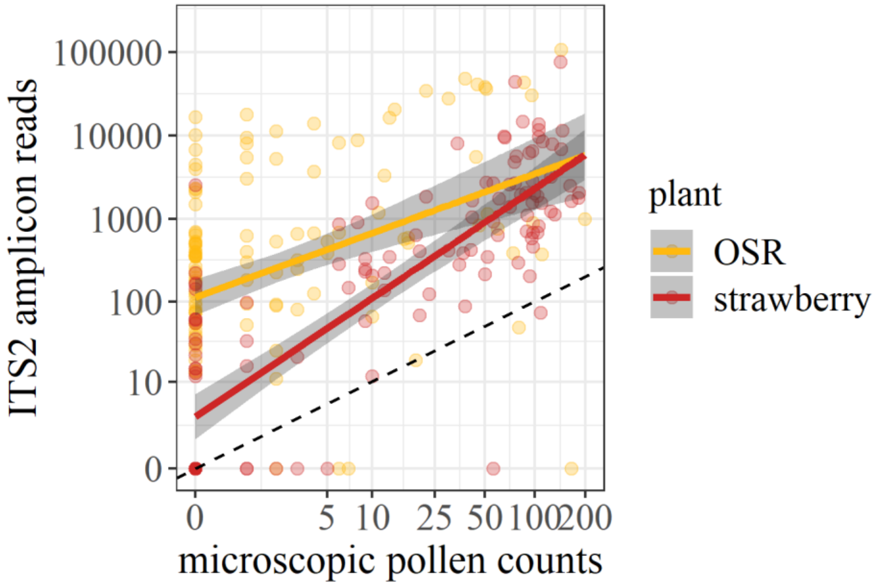

Figure 4 Relationships between quantitative outcomes of ITS2 amplicon reads and microscopic pollen counts for strawberry pollen (red; intercept = 1.43 [95% CI 0.94-1.93], slope = 1.37 [95% CI 1.21-1.53], $R^2 = 0.69$, n = 132) and OSR pollen (yellow; intercept = 4.74 [95% CI 4.15-5.32], slope = 0.74 [95% CI 0.43-1.05] $R^2 = 0.15$, n = 131). The regression was calculated for strawberry and OSR pollen separately as follows: lm(log(no. of reads + 1) ~ log(microscopic counts + 1)). Solid lines show the regression lines, and dotted line the angle bisector with intercept = 0 and slope = 1.

Discussion

Pollen richness

Honey bee pollen demand was met continuously by crop plants (i.e. OSR and strawberry). However, the majority of analysed pollen in this study (> 70 % in many samples) was collected from non-crop plants. Bumble bees (*B. terrestris*) exploited a greater richness of plant genera in agricultural landscapes compared to honey bees and hence can contribute to the pollination of a greater variety of plant species. Although both bee species are generalists and able to collect pollen rewards and nectar from many plant species (Felix & Krebs 2012), honey bees are known to focus on only a few species (de Vere *et al.* 2017). This is likely due to their ability to communicate the most profitable resources in the landscape using the waggle dance (Couvillon 2012), and the fact that individual foragers alternate pollen and nectar resources only to a limited extent during foraging trips (Keller *et al.* 2005). Additionally, pollen composition differed greatly between bee species. Great amounts of pollen came from woody and herbaceous plant genera which can be found in e.g. hedgerows and field groves and have been highlighted by other studies as important source for bee nutrition and colony growth (Requier *et al.* 2015; Kämper *et al.* 2016; de Vere *et al.* 2017).

Interestingly, we found evidence that high landscape-wide OSR availability increased the pollen richness collected by both bee species, supporting the findings of Requier *et al.* (2015). Bees may have focussed on a greater diversity of pollen rather than on quantity (Leonhardt & Blüthgen 2012). In contrast, increasing local strawberry flower availability appears to reduce the collected pollen richness, presumably because bees focused on the resources next to their colonies.

Collection of strawberry pollen

Both bee species collected a fairly high amount of strawberry pollen. But with high increasing OSR availability, they collected less strawberry pollen. As honey bees can communicate the location of most profitable resources using the waggle dance (Couvillon 2012), a shift to mass-flowering or other high-reward flower patches is likely. However, we found only limited pollen foraging on OSR, which is in accordance with Garbuzov *et al.* (2015), but in contrast to Danner *et al.* (2017). Those contrasting results may be highly dependent on the flower availability in the surrounding landscape. Both honey bees and bumble bees have a known preference for mass-flowering resources to satisfy their high resource demand (Rollin *et al.* 2013). But at the same time, they are known to favour foraging close to their colonies to reduce energy costs (Seeley 1995, Lihoreau 2010). At the time of high OSR flowering, we identified ITS2 sequences of several other genera, e.g. *Salix*, *Prunus* and *Taraxacum* in the pollen loads using amplicon metabarcoding. Hence, bees have collected pollen on co-flowering plants that may be more attractive pollen resources than OSR. However, they may have used OSR as nectar source. While low pollen diversity is likely to increase the pollination potential of target crops (Marzinzig *et al.* 2018), higher pollen diversity likely benefits bee health (Brodschneider & Crailsheim 2010; Alaux *et al.* 2017).

In addition, local strawberry flower availability in the adjacent strawberry field benefitted the strawberry pollen foraging of both bee species. Thus, a high strawberry flower cover will facilitate the pollen collection of the respective crop but may result in pollinator dilution in the field.

In contrast to Boecking & Kreipe (2013), we did not find that small colonies collected greater proportions of strawberry pollen in pollen samples. However, based on the frequency of pollen in the samples, strawberry was collected by small colonies more frequently than by large colonies. Based on these descriptive data, we found first indications that the foraging behaviour of small and large bumble bee colonies can differ. In addition, honey bee colonies collected strawberry pollen more frequently than bumble bee colonies. Additional data from in-field honey bee foragers showed a higher flower constancy for honey bee individuals than bumble bee individuals (Rollin *et al.* 2013). A high flower and pollen constancy is likely to be linked to pollination success and even higher seed set (Gyan & Woodell 1987; Montgomery 2009). Due to a large number of individuals in honey bee colonies, they may have a slight advantage in pollinating adjacent crops, e.g. strawberries, in comparison to bumble bees. Professional beekeepers are rare in the investigated region while many hobby beekeepers create nuclei early in the year to prevent swarming and to increase their number of colonies. Hence, farmers could take advantage of individual beekeepers and invite them to place their nuclei next to their strawberry fields. This would create a win-win situation: farmers benefit from the nuclei through the provided pollination services while beekeepers could place new nuclei at a distance from their colonies to prevent workers from returning to their former hive. Although our study examines some advantages of honey bees in terms of pollen collection from adjacent crops, the value of recommending one bee species over another for pollination services is limited. The bee species-specific foraging on the flower (e.g. pollen deposition) for example is not considered in our study nor did we measure the direct pollination success (Ne'eman *et al.* 2010). While pollination success of plants can depend on functional bee traits (Marzinzig *et al.* 2018) it may depend even more on the functional diversity of traits (Hoehn *et al.* 2008). The proportion of fertile achenes per fruit, which can be linked to higher fruit weight (Klatt *et al.* 2014), will be higher if several bee species, and not only the honey bee, visit the flower (*Chagnon et al.* 1993).

Relationship between quantitative outcomes of metabarcoding and microscopy

In general, we observed pollen type-specific, positive relationships (i.e. slopes and different proportions of explained variation) between microscopic pollen counts and ITS2 metabarcoding reads for frequently occurring taxa in our samples, i.e. *Fragaria* and *Brassica.* This finding is in accordance with Smart *et al.* (2017), who also found positive relationships between commonly occurring pollen taxa in mixed samples. Due to the high amount of unexplained variation, especially for OSR pollen, further research is needed that takes potentially confounding factors into account, for instance pollen type identities, standardized amounts of pollen and defined compositions of mixed samples to confirm a general positive relationship between the quantitative outcomes of both methods (Richardson *et al.* 2015; Pornon *et al.* 2016; Smart *et al.* 2017; Baksay *et al.* 2020). Previous research has shown that the number of ITS2 reads does not reflect the actual number of pollen grains (Pornon *et al.* 2016; Baksay *et al.* 2020). Quantitative outcomes derived from ITS2 metabarcoding can be affected by contamination of the samples as well as DNA extraction and amplification biases. Pollen species, pollen counting methodology and the chosen marker may affect quantification as well (Pornon *et al.* 2016; Baksay *et al.* 2020). The number of pollen grains we used for metabarcoding is unknown and does exceed the number of pollen grains used for microscopy. The probability to detect rare pollen taxa increases with the number of analysed pollen grains. To identify the species composition in mixed pollen samples standards of 100-300 pollen grains are considered to be sufficient (Marzinzig *et al.* 2018; Bertrand *et al.* 2019; Lau *et al.* 2019) while a more specific assessment of rare pollen species would likely need a 500 pollen grain count (Lau, Bryant & Rangel 2018). As our correlation analysis is conducted with strawberry and OSR pollen, which are major pollen resources in our study landscapes, we are confident that both pollen species are well represented in our samples and that our data sets provide a sound basis for the analysis. In comparison to microscopy, ITS2 metabarcoding is more advantageous in that it achieves a high taxon richness, allows for a higher throughput with a predictable cost- and timeframe, and does not need specific expert knowledge in palynology (Keller *et al.* 2015; Bell *et al.* 2019).

New developments in microscopic pollen detection using deep learning techniques (Gallardo-Caballero *et al.* 2019) or in full-length amplicon or genome sequencing with.

e.g. nanopore sequencing techniques (Lang *et al.* 2019; Peel *et al.* 2019; Leidenfrost *et al.* 2020) could improve the weaknesses of both approaches (e.g. time expenditure in microscopy or quantification accuracy in molecular methods). However, studies are needed to compare and evaluate the accuracy of those new developments.

Conclusions

We demonstrate that honey bee and bumble bee (*B. terrestris*) colonies differ substantially in their pollen resource use in agricultural landscapes. Bumble bees collected pollen from a much larger variety of plant genera compared to honey bees. Thus, conservation schemes should consider bees foraging preferences by taking diverse plant communities into account to promote pollinators and associated pollination services for wild and crop plants. Annual flowering crops and in particular floral resources in permanent landscape elements, such as hedges, are important in fulfilling the foraging requirements of bees. Both honey bee and bumble bee foragers adapted their foraging behaviour to the availability of mass-flowering resources, which could affect the provisioning of pollination services to minor flowering crops. Honey bees carried slightly more strawberry pollen and less diverse pollen loads than bumble bees but consequences for pollination services need to be studied in more detail. If bee densities are low, farmers can use managed bee colonies for crop pollination. However, we would rather recommend designing pollinator-friendly agricultural landscapes that provide species-rich flower resources for wild and managed pollinators, which in turn can provide pollination services to crops and wild plant species.

IT2 metabarcoding is a suitable method to study the richness of bee-specific pollen diet using mixed pollen samples of unknown plant communities. However, associations between quantitative outcomes of microscopic pollen grain counts and ITS2 amplicon reads were pollen type-specific, weak and large proportions of variation were not explained. Our results can contribute to ongoing discussions that apply and test different methods to quantify pollen grain counts (Pornon *et al.* 2016; Baksay *et al.* 2020). Considering the growing interest in both microscopic (Gallardo-Caballero *et al.* 2019) and molecular (Baksay *et al.* 2020; Leidenfrost *et al.* 2020) pollen analyses for pollen identification and quantification, our study highlights that the methods should be chosen carefully and in a targeted manner.

Acknowledgements

We would like to thank the contributing strawberry farmers for allowing us to keep our honey bees and bumble bees on their fields. Further, we would like to thank Susanne Jahn and Brigitte Jünemann for their valuable help with beekeeping activities, and Sabrina Pach and Delia Niehaus for their help in the molecular biology lab of livestock Göttingen. Many thanks to Hermann Behling and Siria Biagioni who supported our microscopic analysis in the Palynology & Climate Dynamics lab in Göttingen. SB acknowledges her PhD Scholarship funding by the Deutsche Bundesstiftung Umwelt (DBU). CW is grateful for funding by the Deutsche Forschungsgemeinschaft (DFG) (Project number 405945293). RW is grateful for support from the Federal Ministry of Education and Research (BMBF) and the Saxon Ministry of the Arts and Sciences (SMWK) funded Saxony^5 Initiative.

Data accessibility

Data and R Script: https://doi.org/10.6084/m9.figshare.12777401.v3 (Bänsch *et al.* 2020b)

DNA sequences: NCBI BioProject ID PRJNA662345

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Supplementary material – Chapter 2

Tables

Table S1 The relative importance of explanatory variables expressed by the Σw_i for models explaining the effects of bee species (species), colony size (size), oilseed rape availability (OSR), and strawberry flower cover (straw_fc) on pollen richness. All interactions, which are not shown in the table, have $\Sigma w_i < 0.2$ or are not included within the best-fitting models (dAICc > 2).

Response variable	species	size	OSR	straw fc	size:species
Pollen richness	1	0.45	0.45	0.2	0.11

Table S2 Model summaries of chosen models (see Table 2) explaining the effects of bee species (species), colony size (size), OSR availability (OSR), and strawberry flower cover (straw_fc) on a) pollen richness (i.e. no. of plant genera within the pollen samples) and b) strawberry pollen collection (i.e. proportion of strawberry pollen in the pollen samples). Estimates and standard errors are shown.

Model	Explanatory variables	Estimate	Std. Error
a) Pollen richness (n=152)			
R1	(Intercept)	2.8276	0.03901
	OSR	0.05014	0.03390
	species HB	-1.59319	0.07761
R3	(Intercept)	2.87475	0.05145
	OSR	0.05185	0.03385
	size small	-0.09434	0.06744
	species HB	-1.59919	0.07767
R6	(Intercept)	2.82762	0.03895
	OSR	0.06283	0.03876
	species HB	-1.59369	0.07757
	straw_fc	-0.02606	0.03872
b) Strawberry pollen collection (n=157)			
P1	(Intercept)	-2.1739	0.2698
	OSR	-0.4930	0.1643
	species HB	0.6100	0.3066
	straw_fc	0.6751	0.1608

Table S3 The relative importance of explanatory variables expressed by the Σw_i for models explaining the effects of strawberry flower cover (straw_fc), bee species (species) and OSR availability (OSR) on the proportion of strawberry pollen in pollen loads. All interactions, which are not shown in the table, have an $\Sigma wi < 0.2$ or are not included within the best-fitting models ($dAICc > 2$)

Response variable	straw fc	OSR	species
Strawberry pollen	1	0.94	0.56

Figures

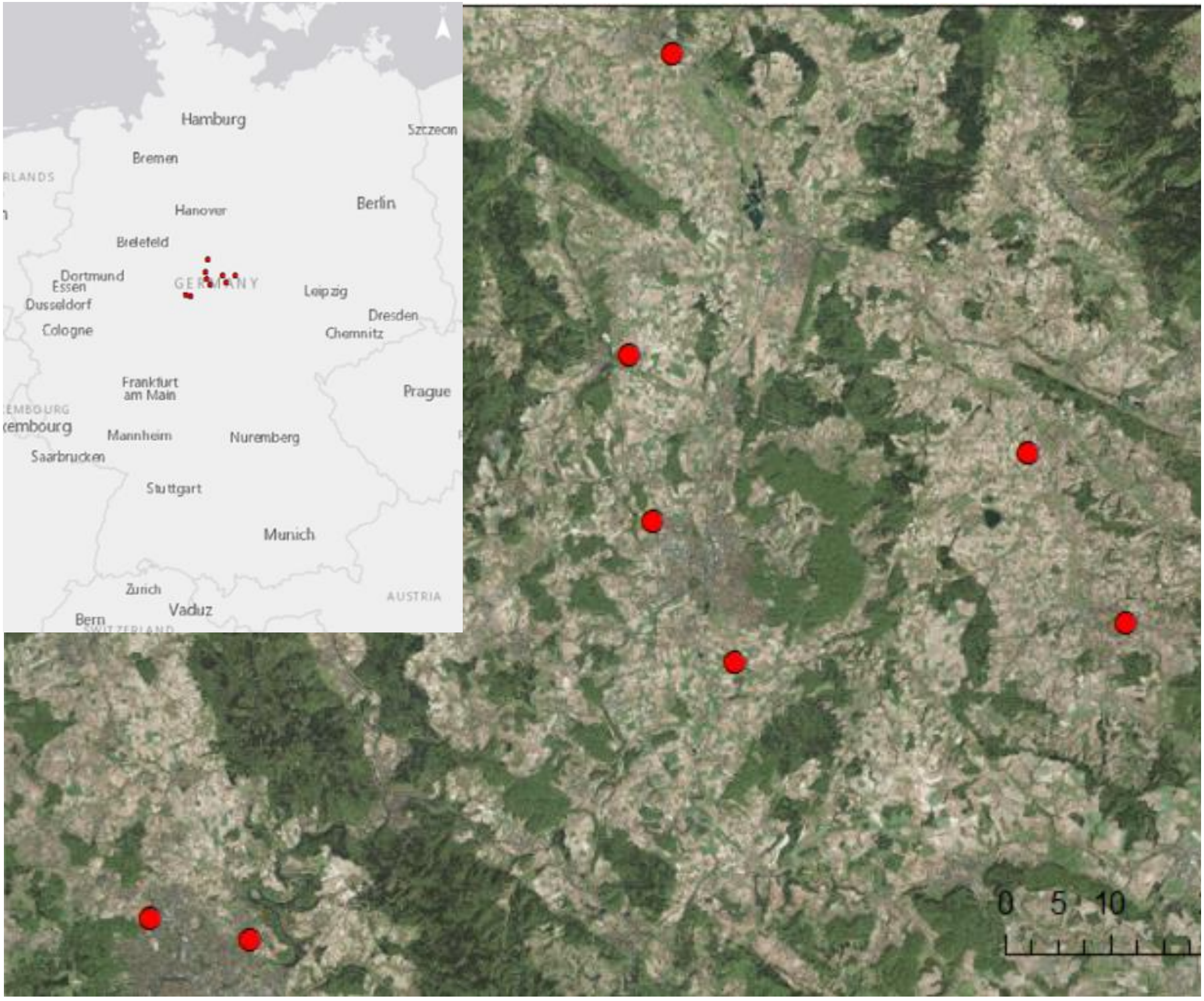

Figure S1 Study locations (red circles) in central Germany (a) in the regions surrounding Göttingen and Kassel (b). Scale 1:700,000; Basemap source: ESRI basemap (Bing).

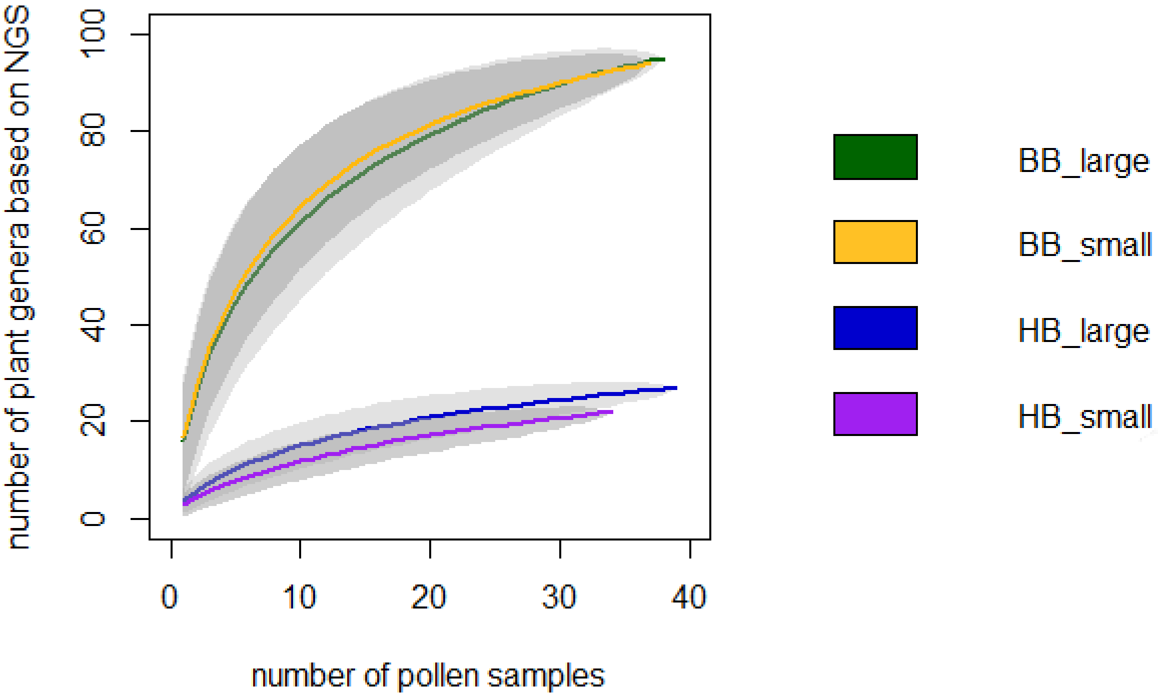

Figure S2 Rarefaction curves (Mao Tau method) and 95 % confidence intervals for pollen samples collected by small (n=34) and large (n=40) honey bee colonies (HB) and small (n=38) and large (n=37) bumble bee colonies (BB). Pollen richness (number of plant genera) is shown on the y axis.

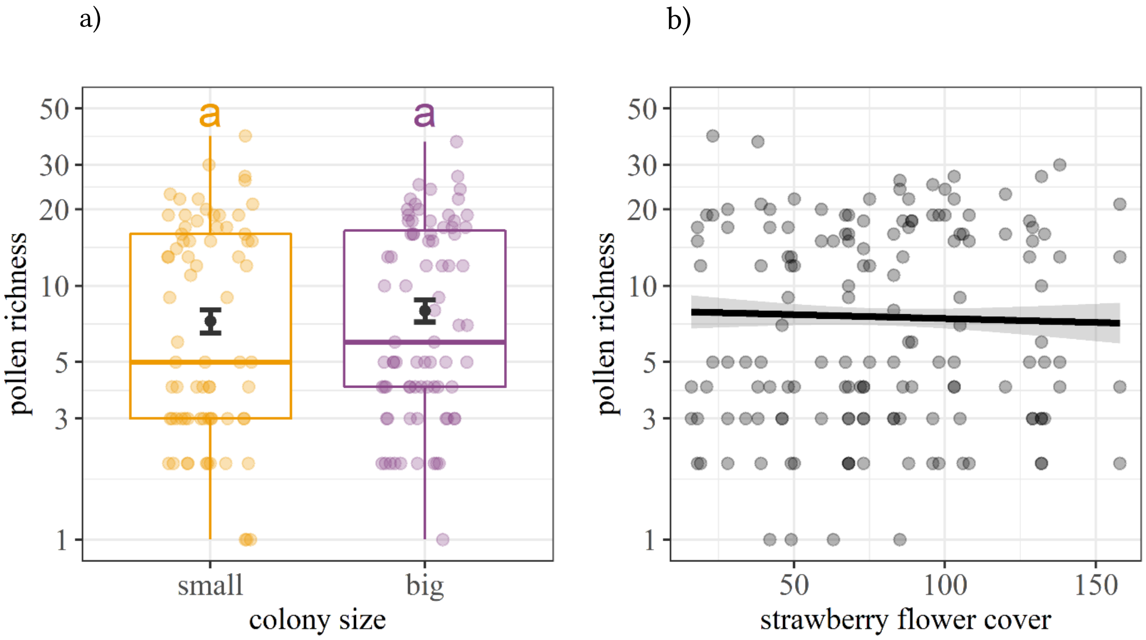

Figure S3 Effects of a) colony size on pollen richness (no. of plant genera in pollen samples and b) strawberry flower cover (no. of open strawberry flowers). According to the estimates, large colonies tend to collect a greater number of plant genera compared to small colonies, however, this effect is rather small. Different letters indicate a significance level of 0.05 obtained from post hoc Tukey test. Predicted values from mixed effect models are shown (in black). As indicated by the low Akaike weight (=0.2) and low effect size (0.026), the effect of strawberry flower cover on pollen richness is quite low. The regression line is obtained from mixed model estimates (model R1, see Table 3) and 95 % confidence region is shown. Please note, that pollen richness is shown on a log scale in both plots.

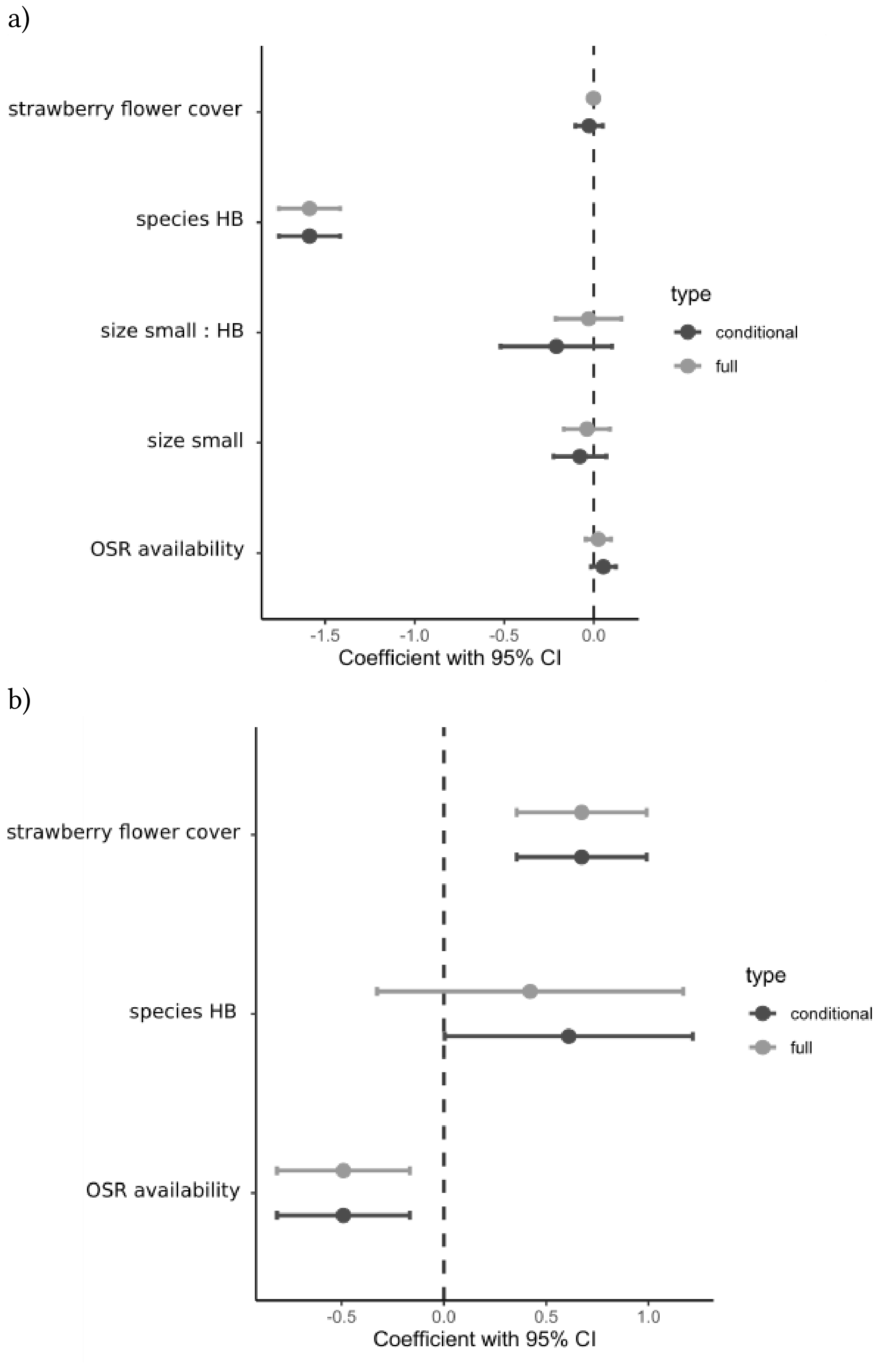

Figure S4 Full and conditional model averaged coefficients for pollen richness (a) and the proportion of strawberry pollen (b). Coefficients were averaged across candidate models within dAICc < 2. Note: Intercept is not displayed.

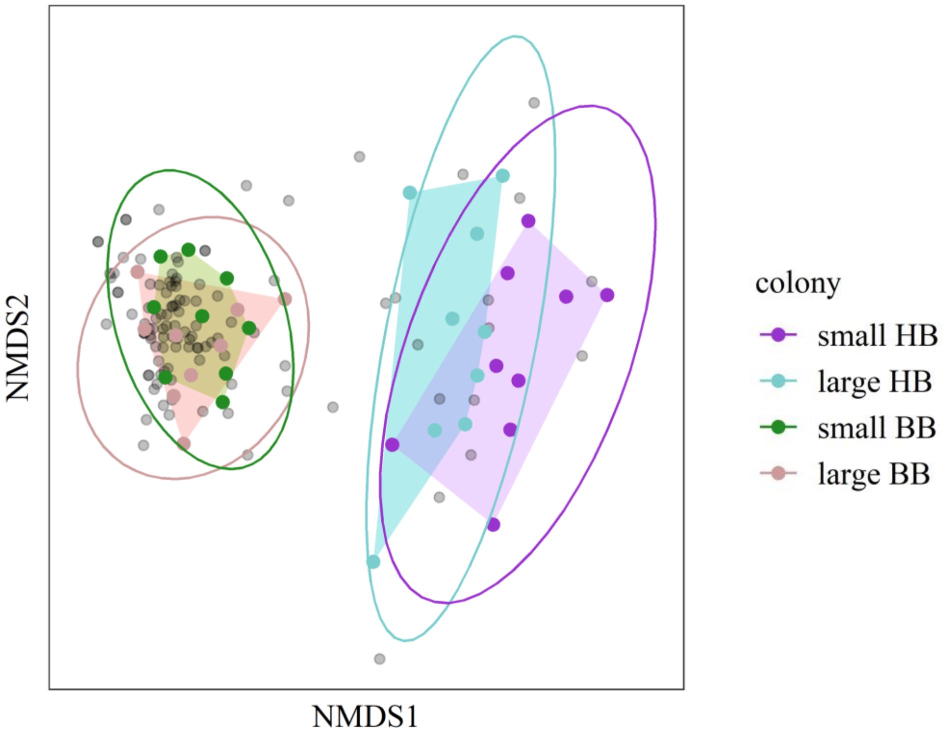

Figure S5 Pollen community composition differs greatly between honey bees (HB) and bumble bees (BB) ($R^2 = 0.46$, $p = 0.005$, stress value = 0.18). However, plant communities used for pollen collection greatly overlap between small and large colonies for honey bees and bumble bees.

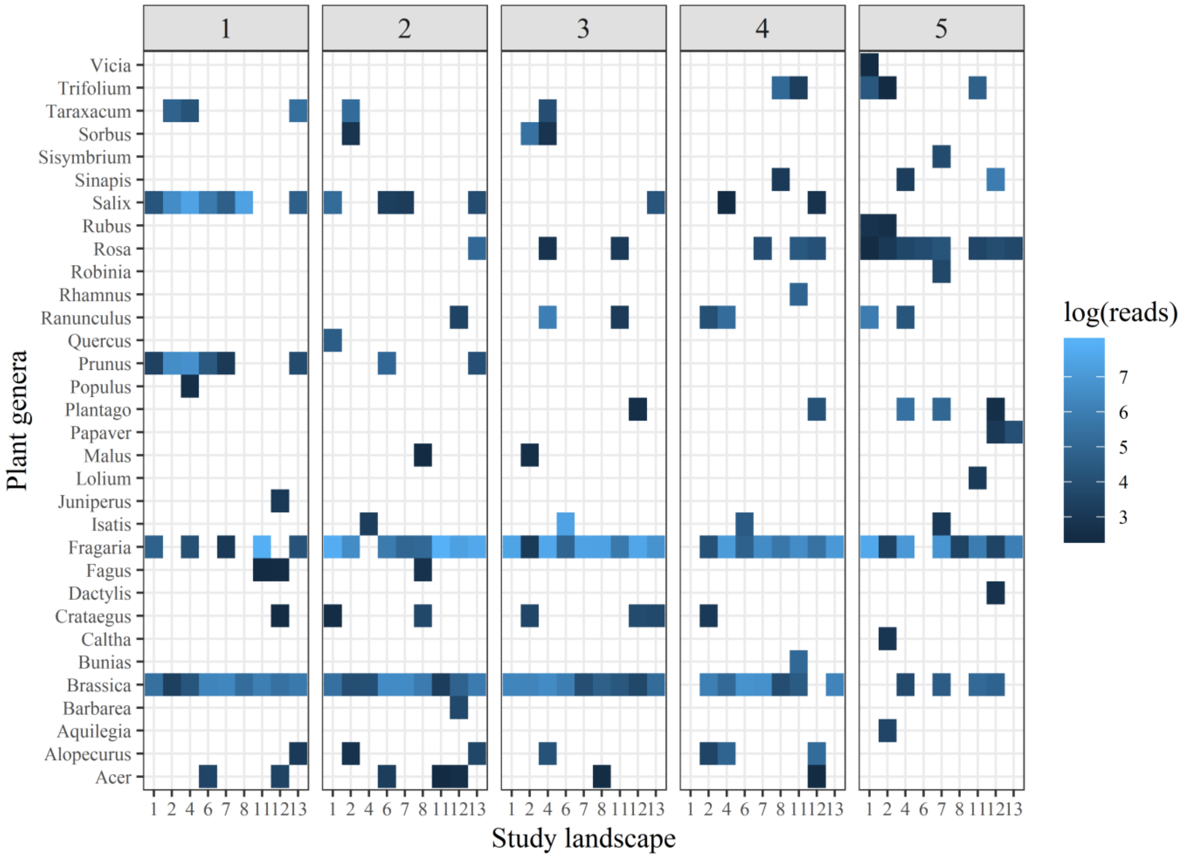

Figure S6a Present ITS2 sequences in honey bee pollen samples for each study landscape and observation round (1–5). The shading represents the number of reads and is log-transformed for better visualisation.

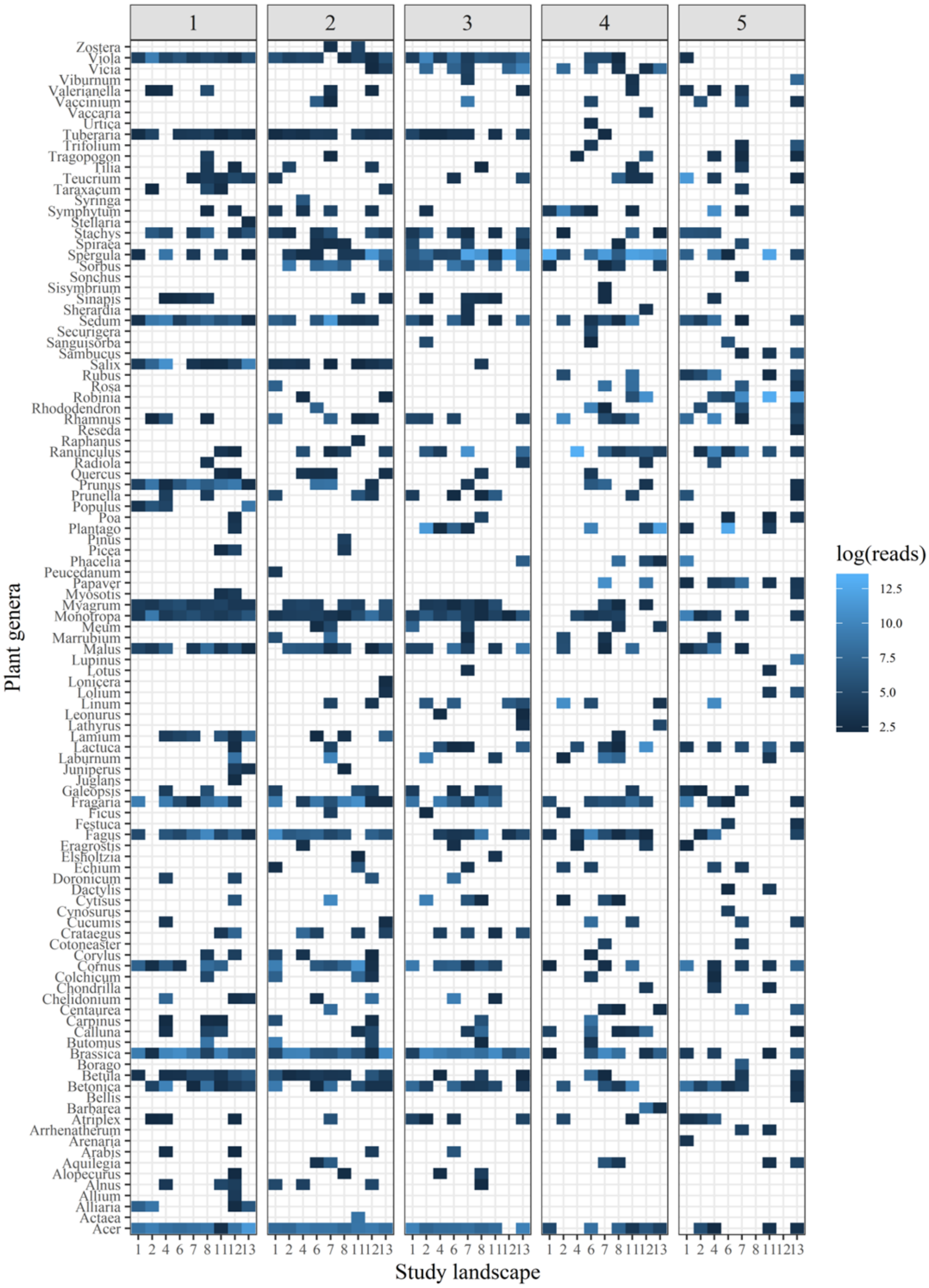

Figure S6b Present ITS2 sequences in bumble bee pollen samples for each study landscape and observation round (1–5). The shading represents the number of reads and is log-transformed for better visualisation.

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Chapter 3

Foraging of honey bees in agricultural landscapes with changing patterns of flower resources

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The content of the chapter is published in Agriculture, Ecosystems and Environment, 2020

doi: 10.1016/j.agee.2019.106792

Abstract

The demand for crop pollination is increasing and honey bees are frequently used, in particular as wild pollinators are in decline. Temporal and spatial variation of flower resources affects foraging decisions of wild and honey bees. To optimise crop pollination management a better understanding of potential competition for pollinators in mass- and minor-flowering crops is needed.

We combined waggle dance decoding, pollen load analysis and field surveys to identify the habitat preferences and pollen use of honey bees in response to spatio-temporal changes in resource availability. Observation hives were placed on the edge of eleven fields of blooming strawberries (mean 2.24 ha) located in landscapes with different amounts of oilseed rape (OSR), semi-natural habitats (SNH) and apple trees in Germany. In addition, we surveyed honey bees and wild bees in strawberry fields.

Honey bee dances more often indicated strawberry, OSR fields and SNH than expected given their landscape-wide areas. Honey bees collected on average 7.9% strawberry, 49.0% OSR, 30.2% Pyrus type (e.g. apple) and 12.9% other pollen types. The mean honey bee foraging distance was 740 m, and decreased with OSR availability. In the observation hives, dances for strawberry fields were not directly affected by OSR availability or SNH land cover. But large amounts of OSR reduced overall honey bee and bumble bee abundance in strawberry fields, but solitary bees were unaffected. Bumble bees were most abundant in strawberry fields (62.6%) and together with solitary bees (7%) they represented about 70 % of the observed bees.

Minor-flowering strawberry fields represent a preferred resource for honey bees, especially for small colonies as indicated by decoding of waggle dances. However, the availability of more attractive OSR and local strawberry flower cover moderates the abundance of social bees (honey bees and bumble bees) in strawberry fields while other wild bees were less affected. Hence, we conclude that wild bee conservation plays a major role for strawberry pollination. If pollination services by solitary bees are limited, small honey bee hives can be used scrupulously to supplement pollination services in strawberries.

Introduction

Insect pollination can increase the yield and quality of many crops and wild plants worldwide (Klein *et al.* 2007; Klatt *et al.* 2013). The economic value of pollination services in agricultural production is estimated to be 153–577 billion US$ (Gallai *et al.* 2009; Lautenbach *et al.* 2012). As global fruit production intensifies, the demand for pollination is increasing (Aizen & Harder 2009). However, wild pollinators are currently threatened and in decline, due to multiple stressors associated with agricultural intensification, such as fragmentation of flower-rich semi-natural habitats (SNH) and homogenous cropping systems (Potts *et al.* 2010, 2016; Kovács-Hostyánszki *et al.* 2017). The resource availability of entomophilous crops and flowers in SNH habitats can be limited to certain time periods. A temporal shortage of foraging resources is likely to decrease population dynamics of wild and honey bees in agricultural landscapes (Schellhorn *et al.* 2015, Wintermantel *et al.* 2019).

A better understanding of the foraging ecology of honey bees can contribute to maintaining crop pollination services. The honey bee, *Apis mellifera L.*, is the most important pollinator in crop production and is relevant globally (Aizen & Harder 2009; Kleijn *et al.* 2015). Worker honey bee collect pollen and nectar from a great variety of plant species (polylectic) and are known to collect pollen with high flower constancy, which can be linked to improved pollination efficiency (Montgomery 2009). However, the spatial ecology of honey bee foraging in relation to spatio-temporal shifts in resource availability at the landscape scale is not well understood. The waggle dance honey bees use to communicate rewarding resources is a unique way of investigating foraging at a landscape level, and can give information about the distance and direction of the most profitable nectar and pollen resources being visited (Von Frisch 1967; Couvillon *et al.* 2012a). Decoding the dances can help to understand the foraging of honey bees with respect to seasonal changes in resource availability and in target crops (Danner *et al.* 2016, Balfour & Ratnieks 2017, Garbuzov *et al.* 2015). Pollen foraging can also be determined by identifying the abundance of collected plant species in pollen loads (Danner *et al.* 2016; Balfour & Ratnieks 2017; Marzinzig *et al.* 2018 Garbuzov *et al.* 2015). Recently, it has been shown that honey bees with an intact dance communication were able to collect a greater pollen diversity compared to colonies with experimentally disturbed communication (Nürnberger *et al.* 2019).

Honey bees prefer to forage in agricultural landscapes on flowers in SNH, on flowering woody structures, weeds and (mass-) flowering crops such as oilseed rape (OSR, Brassica napus L.) (Rollin *et al.* 2013; Requier *et al.* 2015; Danner *et al.* 2016; Sponsler *et al.* 2017). Routinely, they forage in distances of 1.5 km, but can also forage on resources in distances up to 12-14 km (Visscher & Seeley 1982; Beekman & Ratnieks 2000; Steffan-Dewenter & Kuhn 2003). As honey bees tend to optimise their foraging (Seeley 1995) the availability of resources and landscape structures can affect their foraging behaviour (Steffan-Dewenter *et al.* 2002; Garbuzov *et al.* 2015; Danner *et al.* 2016). The availability of mass resources can be linked to shorter foraging distances (e.g., Danner *et al.* 2016). Foraging distance in spring, when mass resources such as OSR and flowers in SNH are in bloom, is likely less than 1 km (Beekman & Ratnieks 2010, Couvillon *et al.* 2014, Danner *et al.* 2016; Balfour & Ratnieks 2017, Danner *et al.* 2017)

These previous studies show that improved understanding of the spatial foraging pattern of honey bees in response to temporal changes of resource availability at the landscape scale is relevant to the use of honey bees for crop pollination. In this study we investigated the pollination of strawberry, a fruit crop which often flowers at the same time as mass-flowering OSR, by honey bees and wild bees. Previous studies found that honey bee abundance in strawberry fields with minor rewards can be negatively affected by co-flowering mass resources such as OSR or apple (Bänsch *et al.* 2020; Grab *et al.* 2017). Our study combined three methodologies: 1) waggle dance decoding from small hives at the edge of strawberry fields; 2) analysis of pollen loads of honey bees collected in pollen traps at the hives; 3) surveys of bees foraging in strawberry fields. The data collected were used to address three questions: 1) What land use types with pollen-providing plant species are most used by honey bees in the agricultural landscape? 2) Do alternative resources (e.g. OSR and SNH) affect honey bee foraging distance? 3) Does the availability of these alternative resources affect the proportion of waggle dances for strawberry fields, the proportion of strawberry pollen collected, and bee abundance in strawberry fields?

Material and Methods

Study fields and landscapes

We studied eleven conventionally managed strawberry fields in central Germany in the regions surrounding the cities of Kassel and Göttingen in 2017 (Fig. 1). Most strawberry fields were managed for harvesting via public self-pick harvesting and usually about three varieties of different peak flowering periods were grown to extend the harvesting season. Mean field size was 2.24 ha (±1.02 SE; range 0.92–3.6 ha). The study fields were surrounded by a landscape mosaic consisting of arable crop fields, fragments of SNH, forests and urban area.

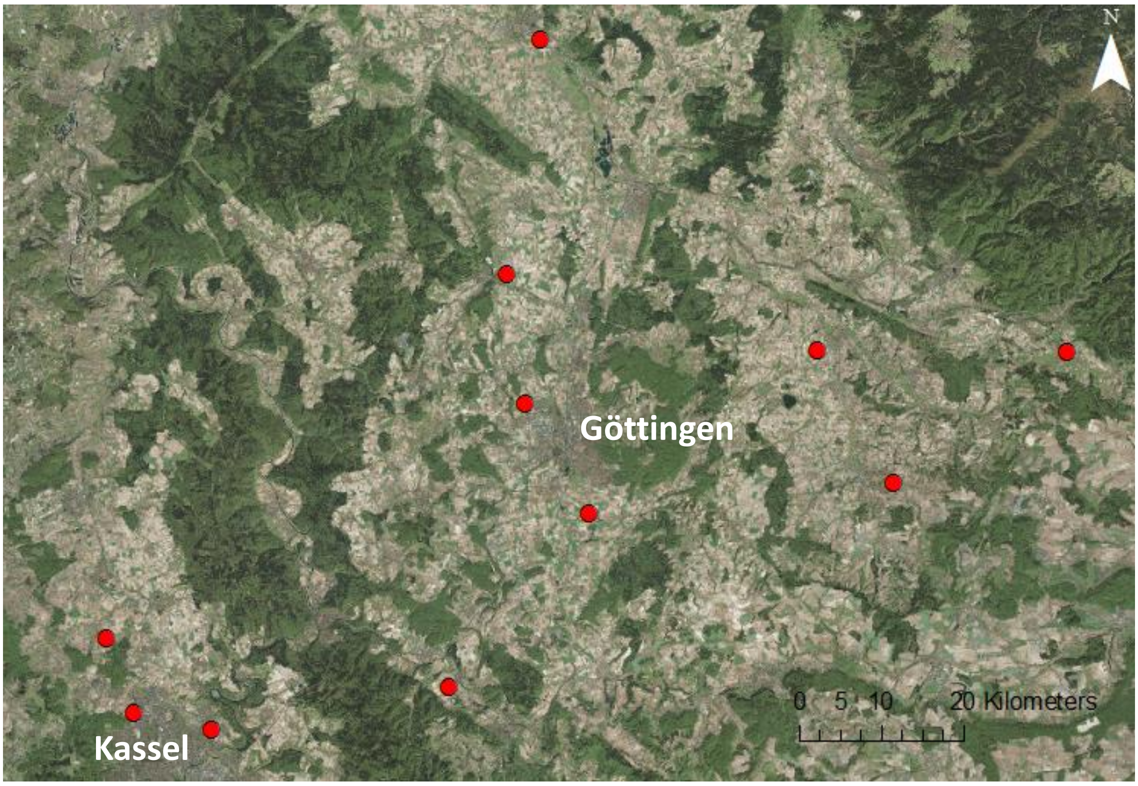

Figure 1 Location of study fields (red circles) in the surroundings of Göttingen and Kassel, central Germany (a); 1:700,000. Basemap source: ESRI basemap (Bing).

To identify the preferred land use types of honey bees, we created digital maps of the land cover types of the study landscapes surrounding our study fields in 750 m radii using a geographic information system (ESRI ArcGIS, Version 10.3.1). We focused on 750 m since the observed mean foraging distances of honey bees were rather short during the study period (740 m ± 26 SE). We calculated the proportion of land cover (%) within study landscapes of following land use types: strawberry fields (only one study field per study landscape; 1.3% ±0.2), OSR fields (8.6% ±2.4), SNH (6.7% ±0.9), cropland (66.4% ±4.0), forest (0.9% ±0.4) and urban area (16.2% ±5.0) (Table S1). Agricultural field data (e.g. cropping type) are based on InVeKos data (database of agricultural cropping; https://www.zi-daten.de/) provided by the German states Lower Saxony (Landwirtschaftskammer Niedersachsen, 2017) and Hesse (Landesbetrieb Landwirtschaft Hessen, 2017). The land use category 'cropland' comprises the entire agricultural landscape matrix, i.e. crop fields (mainly non-flowering annual crops, such as cereals or sugar beets), field margins and country lanes. Scattered trees (including fruit trees, such as apple) along roads and country lanes have not been mapped separately. Flowering crops (i.e. strawberry and OSR fields) have been treated as separate category. SNH include non-cultivated landscape elements, such as hedges, meadows, shrub lands and meadow orchards with fruit trees, like apple, that provide potential foraging resources for honey bees and nesting for wild bees. Cropland and urban area can provide various and attractive resource patches (e.g. flowering weeds and homegarden plants) but we were unable to map them in detail due to an insufficient resolution of maps and the large amount and diversity of resource types.

As both the area of OSR fields in the landscape and the flower cover within OSR fields can determine the abundance of OSR nectar and pollen resources, we calculated an index for OSR availability. OSR availability is the product of OSR land cover (ha) within a radius of 750 m and the OSR flower cover estimated in the nearest OSR field to the hive along a transect of 50 x 4 m. The distance to the nearest OSR field was correlated with the OSR land cover within a radius of 750 m (r= -0.8, p=0.0032). The more OSR land cover within the landscape surveys the closer was the nearest OSR field. All OSR fields had a relatively uniform germination and performance in the study landscapes. The average distance to SNH was 71.5 ± 20.3 m (range 10 – 193 m) but land cover and distance was not correlated.

Observation hives

To study the waggle dance, we used two-frame observation hives (Supplementary Information Fig. S1), each with approximately 4,000 worker bees and a queen bee of same age (*A. mellifera carnica* Pollmann, provided by W. Seip Biozentrum GmbH & Co. KG, Germany). The observation hives were modified so that returning bees were led to one site of the comb first, where we did video recordings following Danner *et al.* 2016. One hive per study field was positioned at the edge of the strawberry field on the 27th or 28th April 2017. Additional food (sugar solution) was provided if needed.

Waggle dance decoding

To observe the waggle dances we made video recordings on four recording days during the strawberry and OSR flowering period from the 30th April until 5th June 2017, using a camcorder (Sony HDR CX240E). For the recording dates we chose only days with low wind speed, no rain and a minimum temperature of 14°C. The recording times were equally distributed to morning and afternoon hours (9 am – 5 pm) to account for pollen and nectar availability of different plant species throughout the day.

Each video recording lasted 90 minutes and we decoded, if possible, 20 dances per record (mean 18.07 ±0.37 SE). To differentiate between potential nectar and pollen foragers, we noted whether dancing bees carried visible pollen or not. Bees without pollen loads were presumably nectar forager. After two rounds of video recordings we rotated the hives once between the study fields to reduce potential effects of intrinsic differences among hives. In addition, we accounted for potential hive effects within our statistical analysis.

We decoded waggle dances following a protocol from Couvillon *et al.* (2012b). For each waggle dance we measured the angle relative to vertical, which then can be converted into the direction by adding the azimuth of the sun at the time of the dance (macro by W. Towne, Kutztown University, Pennsylvania, USA). The time of the waggle run can be translated into the foraging distance, but short dance durations can result in low or even negative values, based on the calibration curve, especially for values that would result in distances < 100 m (Schürch *et al.* 2013). Those dances were set to 100 m, as honey bees (A. mellifera carnica P.) typically communicate resources in distances below 100 m by round dances and not waggle dances (Von Frisch 1967). As round dances point to a resource close to the hive, we counted them as visits in strawberry fields that were the most rewarding resource in the close surroundings of the hives (< 100 m). In three out of the eleven landscapes single fruit trees or bushes may have been also flowering close to the hives, so we cannot entirely preclude that bees have performed the round dance for these resources.

To identify the visited land use types indicated by the waggle dance we combined the spatial information (distance and direction) with our digitalised land cover maps. We then counted the number of dances, which were assigned to certain mapped land cover types (i.e. strawberry field, OSR fields, SNH, cropland, forest and urban area, Supplementary Information Fig. S2). Dances that were assigned to streets (25 dances) were excluded from the data set.

Pollen analysis

At each observation date, we collected pollen loads from homecoming bees for ten minutes (or at least from 10 bees) directly after the waggle dance recordings to analyse exploited pollen resources. Pollen samples were pooled per hive and date, and stirred with 500-1000 µl water, depending on the sample size, to homogenise them. A drop of each sample was fixed on microscopic slides with glycerine gelatine. Following the guidelines of pollen identification in honey samples (Dustmann 2006), 500 pollen grains per sample were identified and counted by a pollen identification expert (S. Böhrs, Mellisopal Pollenanalytik, Germany). Pollen belonging to the Pyrus type can be mainly assigned to pome fruits, such as apples (Malus sp.) and pears (Pyrus sp.). In our study region most common fruit trees are apple trees that flower until beginning of May which overlaps with the OSR bloom for a short period of time.

Surveys of bees in strawberry fields

We conducted transect walks in strawberry fields during the video recordings and chose rows with the highest strawberry flower cover for transects (50 m x 4 m). Flower cover was quantified by counting open flowers along two meters of a representative strawberry row within the transect walk. All flower-visiting bees were caught with an insect net and either identified in the field (particularly honey bees, common bumble bees and characteristic solitary bees) or killed with ethyl-acetate and identified by F. Creutzburg (Jeninsect, Jena). We assigned the bees to functional groups, namely honey bees, bumble bees and solitary bees, according to their sociality, level of domestication and foraging behaviour (e.g. foraging ranges, see also Rollin *et al.* 2013).

Data analysis

Identification of preferred foraging habitats

We assume that in a homogenously attractive landscape the proportion of land cover of different land use types (strawberry, OSR, SNH, cropland, forest and urban) should equal the frequency of observed dances pointing to these. Hence, we calculated the frequency of expected dances as the product of the land cover (%) of each land use type and the sum of observed dance frequencies. We identified preferred foraging habitats by comparing the frequency of observed dances versus the proportion of expected dances for certain land uses using Chi^2 Test. Habitats with higher frequencies of observed dances than expected were classified as preferred foraging habitats. Standard residuals were extracted to explain the strength of difference between observed and expected values.

Effects of landscape-wide resource availability on foraging distances

We tested how OSR availability and SNH land cover affected the foraging distances using linear mixed effect models (function 'lmer', package 'lme4', (Bates, Machler, Bolker, & Walker, 2014)). Round dances were excluded from this analysis since they do not indicate a quantifiable foraging distance. The foraging distance was square root transformed to achieve a random distribution of the residuals and homogeneity of variances. We included the observation round nested in landscape and hive as crossed random terms in the models.

We first fitted a global model including the following explanatory variables: OSR availability, SNH land cover, pollen collection (pollen present/absent), and their two-way interactions. All explanatory variables were checked for potential correlations. We found only little if any correlation ($-0.3 < r > 0.3$, (Hinkle *et al.* 2003)). Continuous explanatory variables were scaled to a mean of zero and a standard deviation of 1.

We employed the multimodel inference approach (Burnham & Anderson 2002) to select the best fitting models. Models were ranked by the second order Akaike Information Criterion (AICc) and we used the Akaike weight (wi) to estimate the probability of the individual models to have the best fit across all models (Burnham & Anderson 2004). All models within delta AICc (dAICc) < 2 from the best fitting model were considered to have substantial empirical support and are reported together with the null model (model which is not taking any explanatory variables into account) and the global model (Supplementary Information Table S2). Appropriateness of model assumptions was assessed by plotting residuals vs. fitted values. The relative importance of each explanatory variable was assessed using the sum of Akaike weights (Σwi) over all candidate models that included the respective variables (function 'importance', package 'MuMIn', (Barton 2018)). We considered Σwi > 0.2 to explain effects on our response variables.

Exploitation of strawberry fields

To analyse whether OSR availability and the proportion of SNH land cover alter the proportion of dances pointing to the strawberry field and the proportion of collected strawberry pollen, we used generalised linear mixed effects models with binomial distribution (function 'glmmTMB', package 'glmmTMB', (Brooks *et al.* 2017; Bolker 2018)) for both response variables. OSR availability, SNH land cover and the interaction between both were included as explanatory variables and region and hive as crossed random terms. We accounted for overdispersion by observation level random effects in both models (Harrison 2015). Again, we used the multimodel inference approach to identify the best fitting models and the relative importance of each explanatory variable as described above.

Bee abundance in strawberry fields

To investigate the effects of OSR availability, SNH land cover and local strawberry flower cover on bee abundances we grouped the bees into honey bees (HB), bumble bees (BB) and other mostly solitary bees (SB). Models were fitted for each functional group with generalised linear mixed effect models using the 'glmmTMB' package (function 'glmmTMB', (Brooks *et al.* 2017)). The abundance of honey bees followed a Poisson distribution and we accounted for overdispersion in bumble bee and solitary bee abundance with negative binomial distribution. Again, we used the multimodel inference approach to choose the best fitting models and to identify the relative importance of explanatory variables as described above.

All statistical analyses were performed with the software R version 3.5.0 (R Core Team 2016).

Results

In total we observed 759 dances (743 waggle dances and 16 round dances). Almost half of dancing bees (45.85 %) carried visible pollen loads.

Preferred foraging habitats

We found that 42 of 784 dances (5.4% of all dances observed) pointed to the neighbouring strawberry fields, including both waggle dances and round dances. About half of the dancing bees (20 out of 42) carried visible pollen. Another 95 dances pointed at OSR fields (12.1%) and only 28 bees carried visible pollen loads. We found 59 dances (7.5%) pointing to SNH and most bees (37 bees) carried visible pollen loads. The majority of dances pointed to unspecified cropland (60.0% of all dances observed), 11.0% dances pointed to urban area and 4.1 % at forest.

The observed frequencies of dances were higher than expected (based on the land cover of respective land use types) for the land use types strawberry, OSR, SNH and forest while it was lower for urban area and cropland (Chi^2=248.82, df=5, p<0.001, Fig. 2).

Our pollen analysis showed that strawberry pollen amounted to 7.9% ±2.7 SE on average in the pollen samples (up to 85.0% in individual samples). OSR was the most dominant pollen type that reached proportions of up to 100.0% (mean 49.0% ±5.7). Another major pollen resource was identified as Pyrus L. type (mean 30.2% ±5.9). Pyrus includes all pome types, but we expect apple pollen to represent the largest amount because apple bloomed at a similar time and is cropped frequently in our landscapes in orchards, old meadows, along roadsides, and in gardens. The remaining pollen was collected to a small extent from plants such as Salix L., Rubus L. and Taraxacum. In general, pollen diversity was low with only one to five different pollen types per sample.

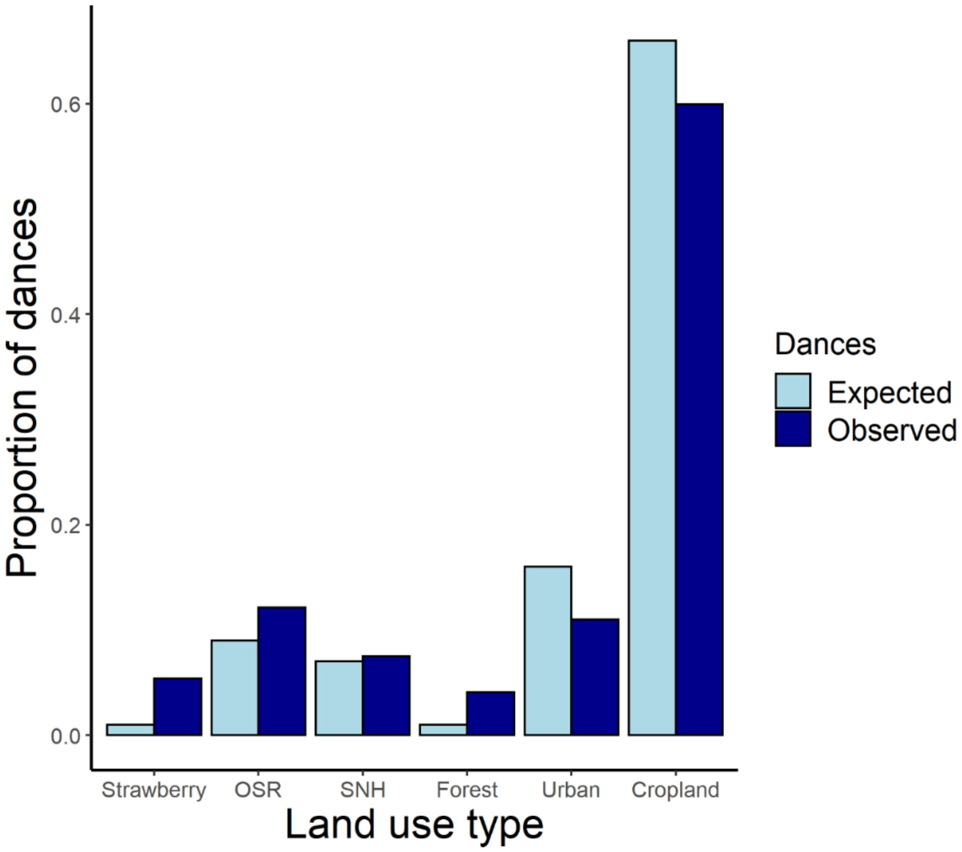

Figure 2 Expected proportions of dances (light blue) versus the observed proportions of dances (dark blue) for the dominant land use types (n=784). The observed proportion of dances is higher for strawberry, oilseed rape (OSR), semi-natural habitats (SNH) and forest compared to the expected proportion. While the proportion of expected dances is higher for urban areas and cropland than observed. Residuals according to Pearsons Chi Square test are for strawberry 12.26, for OSR 3.05, for SNH 0.58, for forest 8.67, for urban area -3.84 and for cropland -3.58.

Effects of landscape-wide resource availability on foraging distances

The foraging distance estimated by the duration of waggle dances of honey bees was on average 740 m ± 26 SE, range 100 – 7783 m. The foraging distance of honey bees with pollen was on average 697 m (± 40 SE) and differed only slightly from the foraging distance of bees without visible pollen 775 m (± 35 SE). The effects of OSR availability, SNH land cover, and pollen collection on the foraging distance were explained by several models with empirical support (dAICc< 2, Supplementary Information Table S2a). In the best fitting model all main effects and an interaction between OSR availability and SNH land cover were included (Supplementary Information Table S2a). Pollen resource use was the most important explanatory variables indicated by the high Σwi= 0.94, followed by OSR (Σwi= 0.93), followed by SNH land cover (Σwi =0.74) and an interaction between OSR availability and SNH land cover (Σwi= 0.47, Supplementary Information Table S3). The foraging distance of honey bees decreased when OSR availability increased and SNH land cover was high (Fig. 3).

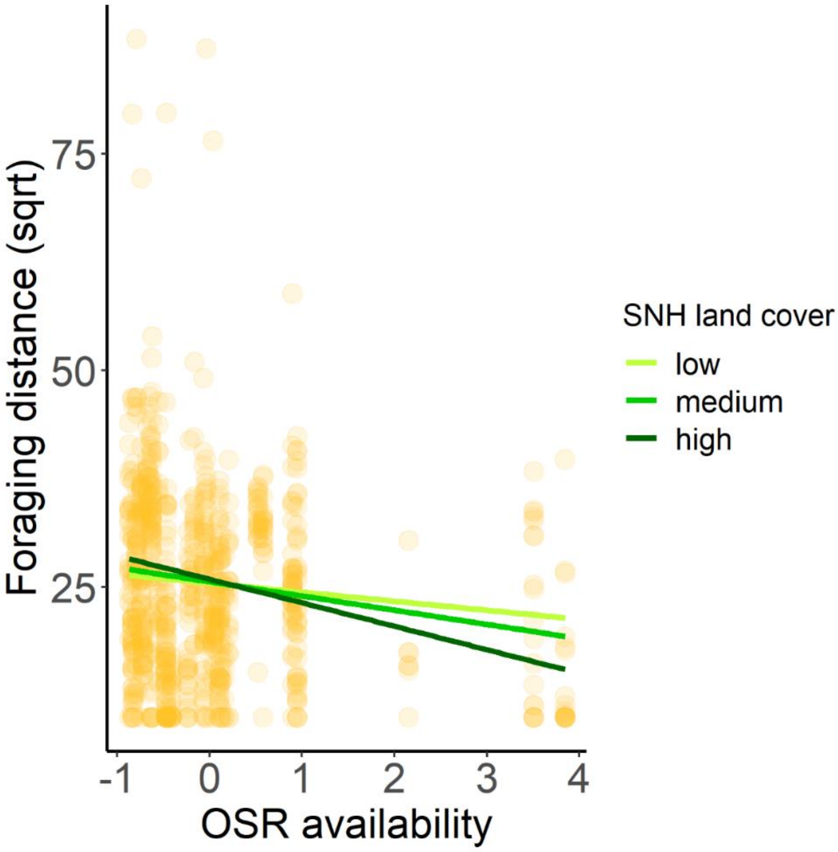

Figure 3 Interactive effects of OSR availability and SNH land cover on the foraging distance of honey bees (n=743). The foraging distance of honey bees decrease with increasing OSR availability, particularly when SNH land cover is high. Foraging distance is shown on square root scale in response to scaled OSR availability (scaled to a mean of zero and standard deviation of 1) as fitted in the model and data points are jittered. Regression lines are obtained from mixed model estimates and are predicted for low, medium and high SNH land cover (i.e. 10, 50, 90- percentile of observed data).

While the main effect of OSR availability was negatively correlated with foraging distances (e.g. short foraging distances), the main effect of SNH land cover was positively correlated with foraging distances (e.g. increasing foraging distance, estimates can be found in Supplementary Information Table S4a). Further, marginal interactive effects of OSR availability and pollen use (Σwi =0.42) and of SNH land cover and pollen use (Σwi =0.35) were found in the second and third best fitting models. The interactive effects showed that foragers without visible pollen loads showed stronger responses to OSR availability and SNH land cover (Supplementary Information Fig. S3a-b). Forager without visible pollen loads indicated longer foraging distances when SNH land cover increased and shorter foraging distances when OSR availability increased compared to forager with visible pollen loads.

Exploitation of strawberry fields

Neither was the frequency of dances pointing to strawberry fields nor the proportion of strawberry pollen in the pollen samples related to OSR availability or SNH land cover. The best fitting models were either the null model or differed not substantially from it (dAICc< 2) (Supplementary Information Table S2b-c).

Bee abundance in strawberry fields

We recorded in total 719 pollinator-flower interactions in strawberry fields, 218 bees belong to honey bees, 450 bees to the genus *Bombus* (mainly *B. terrestris* L.), 164 individuals were assigned to solitary bees and 51 bees were excluded as they were neither caught or identified (species list can be found in Supplementary Information Table S5). OSR availability and local strawberry flower cover determined the honey bee and bumble bee abundances in the strawberry fields (Supplementary Information Table S2d). Strawberry flower cover was the only predictor variable in the best fitting model and the most important predictor variable for both functional groups (Σwi for HB: 0.82, for BB: 0.81, Supplementary Information Table S6). Increasing strawberry flower cover correlated positively with social bee abundances in the field (Fig. 4). In contrast, increasing OSR availability, which was included in the second best fitting model, correlated negatively with social bee abundances, however the effect was only of minor importance (Σwi for HB: 0.37, BB: 0.35, Supplementary Information Table S4b and Fig. S4). SNH land cover was not included within the best fitting models for social bees. We found no effect of OSR availability, SNH land cover and strawberry flower cover on solitary bee abundance in the strawberry field (Supplementary Information Table S2d).

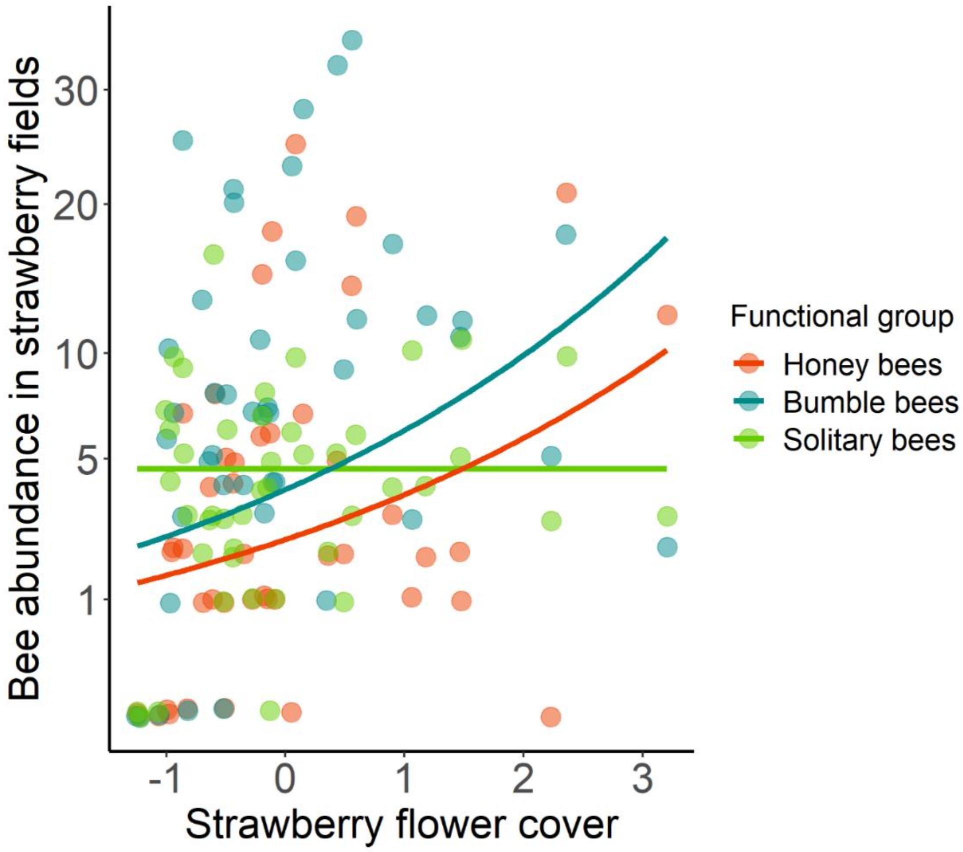

Figure 4 Effects of strawberry flower cover (%) on the number of bees per transect in strawberry fields (bee abundance) for each functional group. The number of honey bees and bumble bees per transect in the strawberry fields is enhanced with increasing strawberry flower cover while solitary bee abundance was not affected. Strawberry flower cover is scaled (to a mean of zero and standard deviation of 1) as fitted in the model and data points are jittered. Regression lines are obtained from mixed effect model estimates.

Discussion

Our study showed that OSR fields, SNH and strawberry fields were the preferred foraging land use types of honey bees, while cropland and urban area were less visited. Increasing both OSR availability and the proportion of SNH land cover shortened the foraging distance and therefore should have benefited the energy balance of honey bees. However, we found no effects of OSR availability and SNH land cover on the strawberry foraging behaviour of honey bees. Bumble bees, together with solitary bees, represented the majority of bees in the strawberry fields.

Preferred foraging habitats

OSR and SNH were similarly preferred by honey bees, while Danner *et al.* (2016) found even greater preference for SNH. This may depend largely on the quantity and quality of flowering resources in SNH (Kleijn & van Langevelde 2006). A great number of dances were performed for cropland in general, as this kind of habitat can provide flowering weed patches, woody and herbaceous structures (Requier *et al.* 2015). A great frequency of dances has been observed for forest as well but many dances were performed in one landscape at one time suggesting a mass-flowering effect that might be caused by flowering maples (de Vere *et al.* 2017). Although only a small frequency of dances pointed to the neighbouring strawberry field it was still more preferred than OSR and SNH when taking the land cover into account. The strawberry field is only a very small fraction within our study landscapes, but bees prefer to forage close to their hives (Seeley 1995). Hence, the distance to the hive can also affect the attractiveness of a resource and strawberry pollination may benefit from the close distance to the hives. However, important flower resources such as OSR fields and SNH were easily accessible to our honey bee hives as well. The average distance to the next resource patches were rather small (for OSR 323 m and for SNH 72 m). Moreover, with increasing availability of OSR the distance to the next OSR field declined. Besides the distance to potential foraging resources, the attractiveness of a habitat can also depend on flower availability since honey bees are attracted by mass-flowering resources (Rollin *et al.* 2013) but they also rely on diverse flower resources to fulfil their foraging requirements (Requier *et al.* 2015, Hendriksma & Shafir 2016).

Analyses of pollen loads revealed that bees have used OSR as their main pollen resource, supporting previous studies (Rader *et al.* 2009; Danner *et al.* 2017), but contrasting with studies that found only limited pollen foraging on OSR (Garbuzov *et al.* 2015). The second most abundant pollen was apple. Apple trees are common in agricultural landscapes in our region as well as in urban areas and have been shown to be highly attractive for honey bees (Grab *et al.* 2017). The importance of small flowering patches and single flowering trees in urban area and cropland might be underestimated with waggle dance analysis as it was not possible to map all minor patches due to dance inaccuracy. In addition, honey bees forage rather for the most profitable resources (Donaldson-Matasci & Dornhaus, 2014, Nürnberger *et al.* 2019).

Effects of landscape-wide resource availability on foraging distances

OSR availability and SNH land cover moderated the foraging distance of honey bees. Our results suggest that foraging distances can be shortened due to high resource availability, in particular in OSR fields, which might increase colony fitness (Requier *et al.* 2015; Danner *et al.* 2016). The interactive effect of OSR availability and SNH suggested by our results may be of minor significance, as the effect size is rather small and the variability increased with high SNH land cover.

In support of the results of previous studies, we found evidence that the foraging distance is shorter when pollen is collected (Danner *et al.* 2016; Balfour & Ratnieks 2017). Honey bees carrying pollen may require more energy since foraging flights take longer when pollen is collected (Winston 1991). Thus, they might aim to shorten their flight distance. In contrast to Danner *et al.* (2016), who found that pollen foraging distance decreased when SNH were close to the study hives, we found that a high SNH land cover tends to increase the foraging distance, particularly of nectar foragers. The importance of SNH for foraging bees can be highly variable due to different plant communities in the SNH and due to temporal shifts in flowering phenology. Semi-natural habitats in our landscapes were, for example, hedges with spring-flowering bushes, such as hawthorn (Crataegus sp.) but also extensively managed meadows with different plant species communities. The probability of dancing can be further affected by pollen quality (Waddington *et al.* 1998), which we did not account for in our study. Nevertheless, SNH was especially important as pollen source habitat in our study since about 75.0% of bees indicating visitation of SNH carried visible pollen loads.

Exploitation of strawberry fields and implications for strawberry pollination management

Surprisingly, we did not find direct effects of OSR availability and SNH land cover on the frequency of dances pointing at strawberry fields or on the proportion of collected strawberry pollen in pollen loads but on the abundance of honey bees in the strawberry fields. Since only a small frequency of dances pointed at the adjacent strawberry field and strawberry pollen collection was minor, the dataset might not be sufficient to make profound statements on the effects of co-flowering resources on target crop foraging. A larger dataset would presumably help to disentangle those effects.

In terms of pollination management strategies, what does it mean that only 5.4% of active forager (about 30.0%) from a small hive (around 4,000 bees in our study) indicate the strawberry field as valuable resource? Many flower can still be visited, as 5.4% of the foragers means about 200 honey bees and one honey bee visits about 7.45 flowers per minute in strawberry crops (Albano *et al.* 2009). However, those calculations likely underestimate the actual number of foragers, since the majority of foragers is not recruited by waggle dances but rather visits known resources within the close surrounding of the hives. In addition, the probability of recruitment is lower, the closer the flower resource is. In particular, small colonies, that we had in our study, tend to visit adjacent resources frequently (Boecking & Kreipe 2013). Our results are more in favour of nuclei hives (i.e. small colonies built by beekeepers for breeding and to prevent swarming) than for large commercial bee hives. Hence, one small honey bee hive placed at the edge of a field may significantly contribute to the pollination of strawberries.

Bee abundance in strawberry fields

Like Connelly *et al.* (2015), we found that *Bombus* species were the most dominant group in strawberry fields (62.6%). Together with solitary bees (7.0 %), they represent about 70% of the bee community in strawberry fields. Honey bees made up only 30.0% which is in line with previous studies in strawberry fields in the same regions (28.0–33.9%) (Klatt *et al.* 2013; Wietzke *et al.* 2018), but contrasts Grab *et al.* (2017) (1.3%). Since almost all honey bees in Germany are managed by beekeepers, their abundance depends strongly on beekeeping activities in the surrounding landscapes. Moreover, honey bee but also bumble bee abundance in strawberry fields can be negatively impacted by mass-flowering events such as OSR flowering, supporting previous findings of competition between minor and mass-flowering crops (Bänsch *et al.* 2020; Grab *et al.* 2017). Contrarily the abundance of solitary bees was not affected by mass-flowering resources and even facilitated in previous studies (Bänsch *et al.* 2020). Hence, to assure pollination services in minor-flowering crops it is vital to sustain and promote wild pollinators which are often even more efficient pollinators than honey bees, while managed pollinator may still add to crop pollination (Winfree *et al.* 2008; Garibaldi *et al.* 2013).

Conclusions

Our study shows that landscape-wide mass-flowering resources can influence the foraging distance of honey bees. They have shorter foraging distances with high OSR availability and thus, visited highly rewarding OSR resources nearby more frequently. Interestingly, neither strawberry pollen collection nor the waggle dances towards strawberry fields were negatively affected by high OSR availability indicating a constant exploitation of the fields by the observation hives. Nevertheless, the overall abundance of honey bees in strawberry fields decreased with high OSR availability and low local flower cover whereas solitary bees were less affected. Hence, the conservation of wild bees and their pollination services plays a major role for strawberry pollination. As solitary wild bees are not strongly influenced by the local flower cover in the fields and mass-flowering crop availability at landscape scale, priority should be given to restore and sustain their pollination services for strawberries and other minor-flowering crops. Managed pollinators can be used scrupulously if natural pollination services are limited. In this case, particularly small honey bee hives with small population sizes appear to be a promising tool for minor crop pollination services since their foraging was rather unaffected by mass-flowering OSR. Otherwise minor-flowering crops may compete with mass-flowering resources such as OSR in the surrounding landscapes.

Acknowledgement

We are grateful to the cooperating strawberry farmers who allowed us to keep our honey bees on their strawberry fields. Further, we would like to thank Susanne Jahn and Brigitte Jünemann for their invaluable help with beekeeping activities and Julia Wellinghoff and Klara Küpers for their assistance during field work and waggle dance decoding. CW is funded by the Deutsche Forschungsgemeinschaft (DFG) (Project number 405945293).

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Supplementary material – Chapter 3

Tables

Table S1 Mean coverage and range of habitat types (% within a 750 m radius).

Habitat type	Land cover (%)	Range
Strawberry	1.34 ±0.18	0.57–2.42
OSR	8.58 ±2.42	1.71–29.95
SNH	6.66 ±0.86	2.74–11.96
Forest	0.88 ±0.38	0–3.61
Urban	16.20 ±5.03	0.96–55.09
Cropland	66.36 ±4.02	37.57–82.97

Table S2 Summary of best fitting models within dAICc < 2, the global model, the Null model and the corresponding explanatory variables are shown. Model estimates for models indicated with * are shown in the Supplementary Information Table S2. Abbreviations of explanatory variables: OSR= oilseed rape availability, SNH= SNH land cover, pollen= pollen present/absent, straw_fc= strawberry flower cover

Models	df	logLik	AICc	dAICc	Akaike weight (*wi*)	Explanatory variable
a) Foraging distance (n=743)						
F1*	9	-2828.423	5675.1	0	0.122	OSR + SNH + pollen + OSR:SNH
F2*	10	-2827.474	5675.2	0.15	0.113	OSR + SNH + pollen + OSR:pollen + OSR:SNH
F3*	10	-2827.475	5675.2	0.16	0.112	OSR + SNH + pollen + OSR:SNH + SNH:pollen
Global model	11	-2826.584	5675.5	0.43	0.098	OSR + SNH + pollen + OSR:pollen + OSR:SNH + SNH:pollen
F5	7	-2830.792	5675.7	0.65	0.088	OSR + pollen
F6	8	-2829.859	5675.9	0.82	0.081	OSR + pollen + OSR:pollen
F7	8	-2829.97	5676.1	1.05	0.072	OSR + SNH + pollen
F8	9	-2829.023	5676.3	1.2	0.067	OSR + SNH + pollen + OSR:pollen
F9	9	-2829.025	5676.3	1.2	0.067	OSR + SNH + pollen + SNH:pollen
F10	10	-2828.135	5676.6	1.48	0.058	OSR + SNH + pollen + OSR:pollen + SNH:pollen
Null model	5	-2835.973	5682	6.94	0.004	1
b) Dances for strawberry fields (n=759)						
Null model	3	-48.738	104.1	0.31	0.299	1
Global model	6	-46.416	107.2	3.38	0.064	(OSR + SNH) ^2
c) Pollen use of strawberry (n=42)						
Null model	4	-122.615	254.3	0	0.579	1
Global model	7	-121.973	261.2	6.93	0.018	(OSR + SNH) ^2
d) Bee abundances (n=375)						
Honey bees						
HB1*	4	-113.326	235.677	0	0.574	straw_fc
HB2*	5	-112.859	237.297	1.62	0.255	OSR + straw_fc
Null model	3	-115.771	238.143	2.466	0.167	1
Global model	9	-111.152	245.599	9.922	0.004	(OSR + SNH + straw_fc)^2
Bumble bees						
BB1*	5	-113.096	237.77	0	0.573	straw_fc
BB2*	6	-112.561	239.392	1.622	0.255	OSR + straw_fc
Null model	4	-115.591	240.208	2.438	0.169	1
Global model	10	-110.988	248.643	10.873	0.002	(OSR + SNH + straw_fc)^2
Solitary bees						
Null model	4	-111.379	231.783	0	0.992	1
Global model	10	-107.44	241.546	9.762	0.008	(OSR + SNH + straw_fc)^2

Table S3 The relative importance of explanatory variables expressed by the Σwi for models to explain the effects of oilseed rape availability (OSR; product of OSR land cover and OSR flower cover), pollen and the land cover of semi-natural habitats (SNH) on the foraging distance of honey bees. All interactions, which are not shown in the table, have an Σwi < 0.2.

Response variable	pollen	OSR	SNH	OSR:SNH	OSR:pollen	SNH:pollen
Foraging distance	0.94	0.93	0.79	0.47	0.42	0.35

Table S4 Model summaries of chosen models to explain a) the effects of oilseed rape availability (OSR), semi-natural land cover (SNH) and pollen resource use (pollen loads present/absent) on the foraging distance of honey bees (F1, F3, F4) and to explain b) the effects of OSR availability and SNH land cover on honey bee (HB1+HB2) and bumble bee (BB1+BB2) abundance. Explanatory variables, estimates and standard error are shown.

	Estimate	Std. Error
a) Foraging distance		
F1		
(Intercept)	25.6894	1.1992
OSR	-1.8372	1.1041
pollen	-1.5768	0.7852
SNH	0.1491	1.0091
OSR:SNH	-0.552	1.813
F2		
Intercept	25.6843	1.205
OSR	-2.1107	1.165
pollen	-1.5696	0.785
SNH	0.1801	1.016
pollen:OSR	n1 0.5917	0.766
OSR:SNH	-0.5204	1.824
F3		
(Intercept)	25.6649	1.1829
OSR	-1.8751	1.093
pollen	-1.5481	0.7863
SNH	0.4355	1.064
OSR:SNH	-0.6263	1.7954
pollen:SNH	5 -0.5956	0.7633
b) Bee abundances		
HB1		
(Intercept)	0.8439	0.221
straw_fc	0.4606	0.2049
HB2		
(Intercept)	0.8385	0.2209
OSR	-0.2129	0.229
straw_fc	0.5345	0.2187
BB1		
(Intercept)	1.3497	0.3069
straw_fc	0.4707	0.2177
BB2		
(Intercept)	1.3243	0.2756
OSR	-0.2272	0.2162
straw_fc	0.5622	0.2293

Table S5 Number of bee species in strawberry fields, oilseed rape fields (OSR) and semi-natural habitats (SNH) across all landscapes and observation dates.

Genus	Species	No. of indiviuals
Andrena	*bicolor*	1
	chrysosceles	2
	cineraria	7
	fulva	3
	gravida	18
	haemorrhoa	26
	helvola	16
	minutula	1
	nigroaenea	11
	nitida	3
	scotica	9
	ssp	10
Apis	*mellifera*	218
Bombus	*hortorum*	1
	hypnorum	1
	lapidarius	38
	pascuorum	5
	pratorum	4
	ssp	1
	terrestris	400
Halictus	*tumulorum*	1
Lasioglossum	*calceatum*	3
	laticeps	1
	parvulum	3
	ssp	1
Megachile	*ssp*	3
Nomada	*lathburiana*	1
Osmia	*bicornis*	16
	cornuta	1
	ssp	1
Unknown wild bees (no bumble bees)		26
Not caught		51

Table S6 The relative importance of explanatory variables expressed by the Σ*wi* for models to explain the effects of oilseed rape availability (OSR; product of OSR land cover and OSR flower cover), the land cover of semi-natural habitats (SNH) and strawberry flower cover (straw_fc) on the abundance of honey bees (HB) and bumble bees (BB) in strawberry fields. All interactions, which are not shown in the table, have an Σ*wi* < 0.2.

Response variable	straw_fc	OSR	SNH
HB	0.82	0.37	0.3
BB	0.81	0.35	0.28

Figures

Figure S1 Observation hive next to a strawberry field (left) and view into the hive (right)

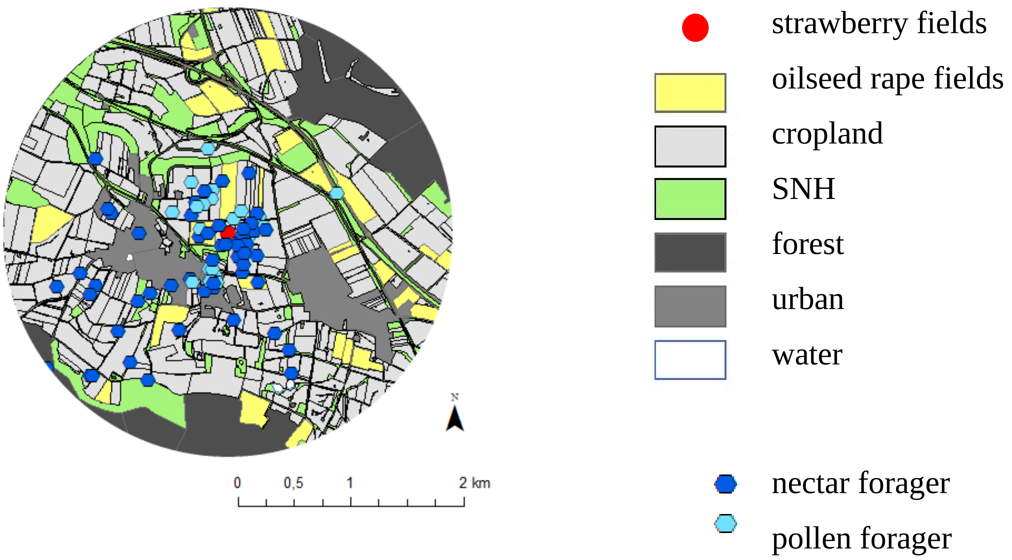

Figure S2 Example of one study landscape (radius 2000m) with mapped dances of nectar and pollen forager (pollen loads absent/present). Scale 1:60,000; basemap source: ESRI basemap (Bing).

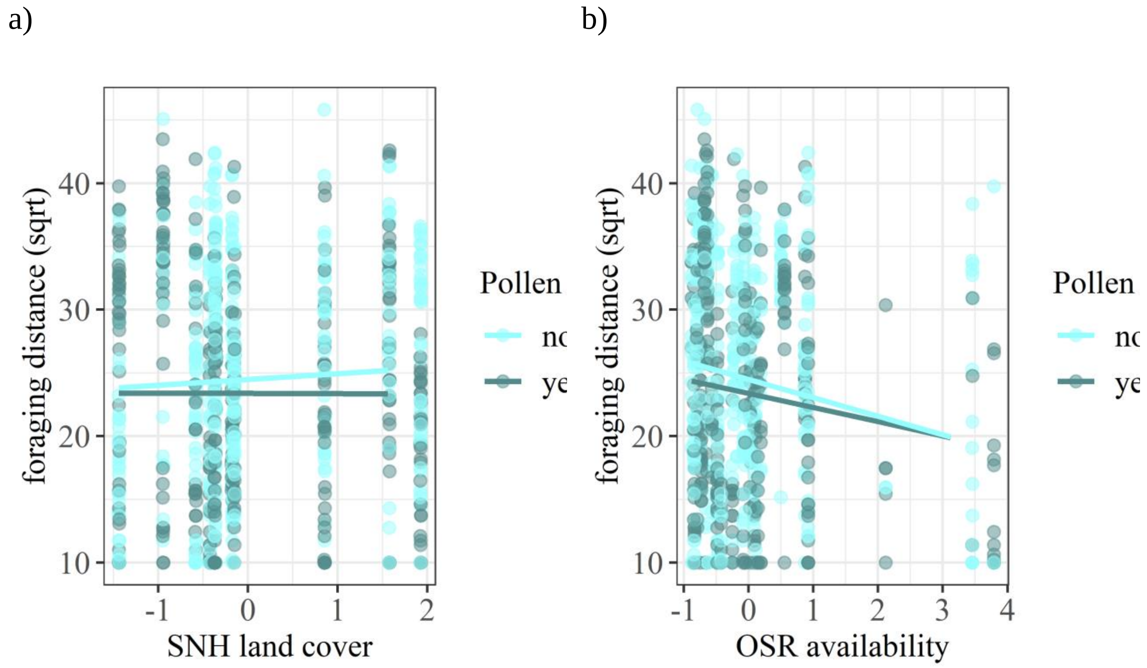

Figure S3 a-b Interactive effects of SNH land cover and pollen resource use (a) and OSR availability and pollen resource use (b) on the foraging distance of honey bees are shown (n=743). The foraging distance is shown on square root scale in response to the scaled explanatory variables (to a mean of zero and standard deviation of 1) as fitted in the model. Please note that data points are jittered due to better representation. Regression lines are obtained from mixed effect model estimates.

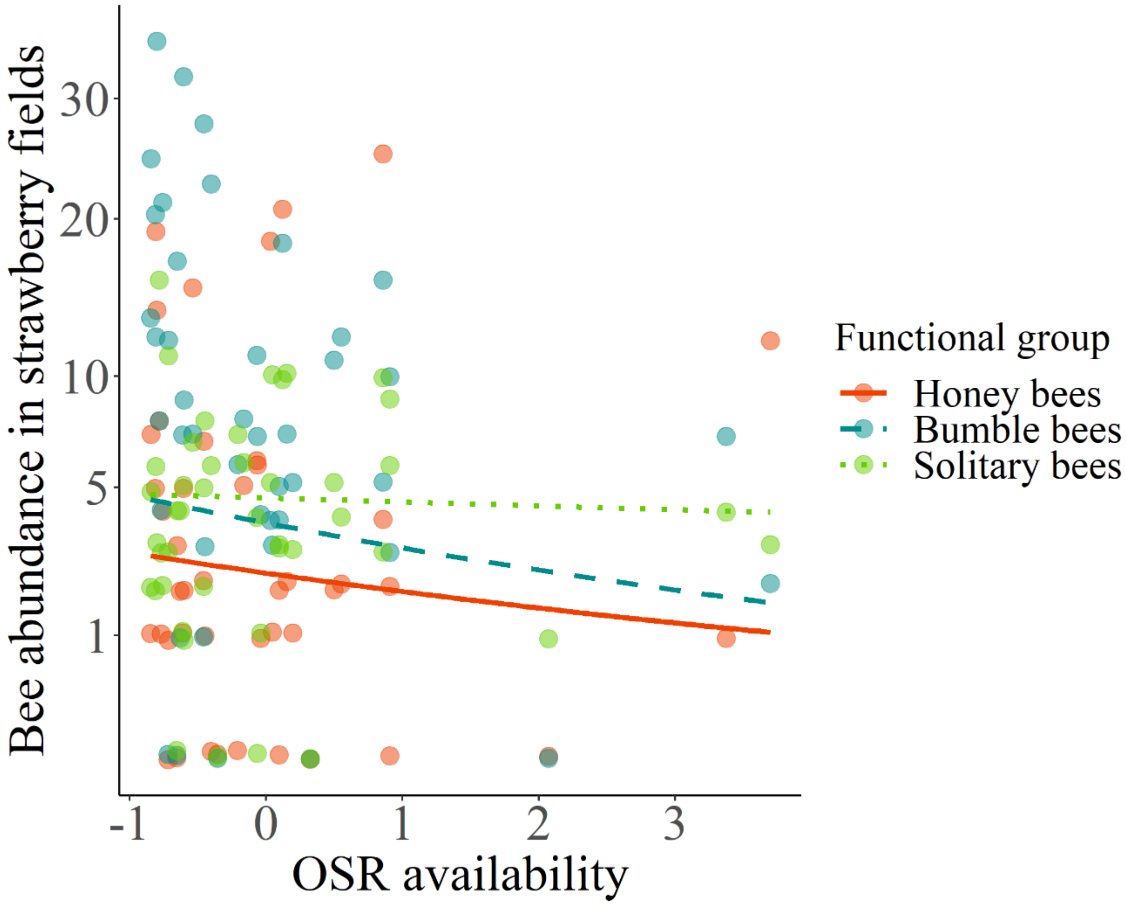

Figure S4 Effects of OSR availability (product of OSR land cover and flower cover) on the number of bees per transect in strawberry fields (bee abundance) for each functional group. The number of social bees (honey bees and bumble bees) per transect in the strawberry fields is decreasing with high OSR availability while solitary bee abundance was not affected. OSR availability is scaled (to a mean of zero and standard deviation of 1) as fitted in the model and data points are jittered for better representation. Regression lines are obtained from mixed effect model estimates.

***

Summary and general conclusion

Pollination can benefit the yield and quality of the majority of the world's most important crops and has a great economic value. But today, pollinator abundance and species richness are in decline. One factor driving these declines is agricultural intensification and accompanying fragmentation and loss of important nesting and foraging habitats for bees, which are the most important pollinators. As global pollinator-dependent fruit and vegetable production intensifies, the demand for pollination is increasing, and farmers who grow those crops may be vulnerable to high economic risks if pollinators are rare.

To meet the demand for future crop pollination services, it is important to understand the relative importance of wild bees and managed bees in crop pollination with respect to bee functional traits and responses to spatial and temporal floral resource availability in the landscapes. Facilitation or competition among plants for pollinators is likely when they flower simultaneously, particularly when mass-flowering is involved. In my PhD thesis, I study the foraging of bees in a minor-flowering crop (strawberry, *Fragaria x Ananassa* Duch.) in relation to the spatial and temporal availability of mass-flowering oilseed rape (OSR; *Brassica napus* L.). Moreover, I considered different functional traits of bee pollinators, such as sociality, foraging ranges and food plant preferences.

In the *first chapter*, the relative importance of social and solitary bees for strawberry pollination along a landscape-wide gradient of OSR availability was assessed with transect walks in eight strawberry fields. OSR availability (product of OSR land cover within 1000 m around the study field and OSR flower cover within the nearest field to the study fields) exhibited contrasting effects on social versus solitary bee abundance. Social bees were drawn away from strawberry fields when OSR availability was high, while solitary bee abundance was facilitated. Strawberry fruits from open-pollinated flowers had the highest fruit weight and best quality compared to wind- and self-pollination. Further, fruit weight increased with the number of bees in the strawberry fields. Hence, the results can have great implications for crop profitability.

The *second chapter* investigates the pollen resource utilisation of managed small and large honey bee (*Apis mellifera* L.) and bumble bee (*Bombus terrestris* L.) colonies and their responses to OSR availability (OSR land cover within 2000 m around our study fields multiplied by OSR flower cover). One large and one small hive of each species was placed adjacent to nine strawberry fields. To study the pollen resource utilisation, DNA metabarcoding (Next-Generation Sequencing; NGS) was applied to identify the pollen richness based on the presence of ITS2 sequences, and microscopy was used to identify the proportion of target pollen (i.e. strawberry and oilseed rape) in pollen loads of returning foragers. Bumble bees collected pollen from more different plant genera than honey bees. In both species, strawberry pollen collection decreased with high OSR availability but was facilitated by increasing strawberry flower cover. Colony size had no effect. Metabarcoding reads and microscopic pollen counts correlated positively but the strength of the correlation differed between pollen types.

The *third chapter* focused on the habitat preferences and pollen resource utilisation of honey bees by combining waggle dance decoding and microscopic pollen analysis. Further, the bee abundances in strawberry fields were assessed by transect walks. Honey bee observation hives were placed adjacent to eleven strawberry fields with differing amounts of OSR availability and semi-natural habitats (SNH) in the surroundings. As the mean foraging range of honey bees was relatively small (653 m), the 750 m radius land cover surrounding our study fields was taken into account. Honey bees danced more often for the strawberry field but also for OSR fields and SNH than the land cover of respective habitats would suggest. The foraging distances were shorter when the resource availability, in particular OSR availability, increased. Surprisingly, foraging in strawberry (e.g. dance and pollen proportion) was not affected by OSR availability or SNH land cover. Bumble bees were most abundant in strawberry fields and, together with other wild bees, formed 75 % of the bee community.

In conclusion, the results of my PhD thesis show that the availability of mass-flowering resources can influence the species-specific foraging behaviour and pollen resource use of bees. Mass-flowering resources can decrease the abundance of social bees in simultaneously flowering minor flowering crops, while solitary bees could even be facilitated. Although social bees collected pollen from minor flowering target crops quite frequently, they collected less pollen as the availability of mass-flowering resources increased. Managed social bees (i.e. honey bees and bumble bees) could be considered as additional pollinators when wild bees are rare, but farmers should not rely on the option of replacing declining bee populations with managed bees. Rather, they should take action to maintain and restore wild bee communities, as they appear to play a major role for pollination in minor flowering crops such as strawberries. To determine the pollen richness in mixed pollen samples, *ITS2 metabarcoding* is a promising method, but it is restricted in its quantitative results. The pollen richness collected from bumble bee colonies was much higher in comparison to honey bee colonies. Although both species have a social behaviour and are closely related, their foraging preferences were very different. Therefore, the specific foraging preferences, not only of the managed bee species, but of the bee communities within the landscapes in general, should be considered in conservation management.

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Zusammenfassung und Fazit

Viele der weltweit angebauten Nutzpflanzen können von Bestäubung profitieren. Der Ertrag und die Qualität kann oftmals verbessert werden und die Bestäubungsleistung trägt damit zum Wirtschaftswert der Agrarproduktion bei. Allerdings gibt es einen Rückgang in der Anzahl als auch der Diversität der Bestäuber. Ein Faktor, der zu einem großen Teil zu diesem Rückgang beiträgt, ist die landwirtschaftliche Intensivierung und die damit einhergehende Fragmentierung und der Verlust wichtiger Lebensräume und Nahrungsressourcen von Bestäubern. Die wichtigsten Bestäuber sind Bienen.

Landwirte, die Bestäuber-abhängige Nutzpflanzen anbauen, können hohen wirtschaftlichen Risiken ausgesetzt sein, wenn Bestäuber fehlen. Um in Zukunft die Bestäubung von Nutzpflanzen gewährleisten zu können, ist es wichtig die Bedeutung von Wildbienen und domestizierten Bienen (wie Honigbienen und Hummeln) für die Nutzpflanzenbestäubung zu verstehen, vor allem im Hinblick auf ihre funktionellen Merkmale und ihr Sammelverhalten in den Agrarlandschaften. Da Bienen Blüten besuchen, um Pollen und Nektar zu sammeln, kann ihr Sammelverhalten von den verfügbaren floralen Ressourcen in der Landschaft beeinflusst werden und Pflanzen, die gleichzeitig blühen, können um Bestäuber konkurrieren. In meiner Dissertation untersuche ich das Sammelverhalten von Bienen in Bezug auf die Bestäubungsleistung in Erdbeerfeldern (*Fragaria x Ananassa* Duch.) unter Berücksichtigung der funktionellen Merkmale von Bienen (z. B. Lebensweise, Sammeldistanzen, Präferenzen für Nahrungsressourcen) und der räumlichen und zeitlichen Verfügbarkeit von Massentrachten wie Raps (*Brassica napus* L.).

Im ersten Kapitel wird die relative Bedeutung von sozial und solitär lebenden Bienen für die Erdbeerbestäubung entlang eines Gradienten der Rapsverfügbarkeit in der Landschaft anhand von Transekten im Erdbeerfeld untersucht. Die Rapsverfügbarkeit (Produkt aus Raps-Landschaftsdeckung und Rapsblütendeckung) zeigte gegensätzliche Effekte auf die Abundanz von sozial und solitär lebenden Bienen. Die Anzahl der sozialen Bienen (Honigbienen und Hummeln) im Erdbeerfeld nahm mit zunehmender Rapsverfügbarkeit ab, die Anzahl der Solitärbienen nahm dagegen zu. Generell konnten Früchte von größerem Gewicht und höherer Qualität geerntet werden, wenn die Blüten offen bestäubt wurden (d.h. Zugang von allen Bestäubern hatten), im Gegensatz zu

Wind- und Selbstbestäubung. Zusätzlich nahm das Fruchtgewicht mit steigender Anzahl von Bienen im Erdbeerfeld zu.

Im zweiten Kapitel wird die Nutzung von Pollenressourcen von kleinen und großen Honigbienen- und Hummelvölkern (*Apis mellifera* L. und *Bombus terrestris* L.) in Bezug auf die landschaftsweite Rapsverfügbarkeit untersucht. Jeweils ein großes und ein kleines Volk beider Arten wurde neben insgesamt neun Erdbeerfeldern platziert und die Pollenhöschen von heimkehrenden Bienen wurden gesammelt. Die Nutzung der Pollenressourcen wurde durch die Kombination zweier Methoden untersucht: mittels DNA *Metabarcoding* (*Next-Generation-Sequencing*) wurde die Pollenvielfalt (Anzahl der Pflanzengattungen) bestimmt und die mikroskopischen Pollenanalyse wurde genutzt, um den Anteil von Zielpollen (Erdbeere und Raps) zu quantifizieren. Hummelvölker haben im Vergleich zu Honigbienenvölkern Pollen von mehr unterschiedlichen Pflanzenarten gesammelt. Bei beiden Arten nahm der Anteil von Erdbeerpollen mit zunehmender Rapsverfügbarkeit ab, aber wurde durch eine steigende Erdbeerblütendeckung gefördert. Die Größe der Völker zeigte keinen Effekt. Die Anzahl der Sequenzen, die anhand der *Metabarcoding* Methode ermittelt wurden, stehen im positiven Zusammenhang zu der Anzahl mikroskopisch gezählter Pollen (für die Pollentypen Erdbeere und Raps). Die Stärke von dem Zusammenhang ist jedoch abhängig von den unterschiedlichen Pollentypen.

Im dritten Kapitel liegt der Fokus auf der Landschafts- und Pollenressourcennutzung von Honigbienen in Abhängigkeit der landschaftsweiten Verfügbarkeit von Raps. Die Landschaftsnutzung wurde durch die Beobachtung der Bienentänze untersucht und die Pollenressourcen mikroskopisch bestimmt. Zudem wurde die Bienengemeinschaften im Erdbeerfeld und anderen Blühhabitaten anhand von Transekten erfasst. Zur Beobachtung des Bienentanzes und zur Sammlung der Pollenhöschen wurden Beobachtungskästen (kleine Honigbienenvölker) an Erdbeerfeldern in elf Landschaften aufgestellt, die sich in ihrem Anteil von Raps und naturnahen Habitaten (NNH) unterschieden. Da die Honigbienen zu dem Zeitpunkt eine kurze Flugdistanz aufwiesen (im Mittel 653 m), wurde die Landschaftsdeckung von Raps und NNH in einem Radius von 750 m berücksichtigt. Die Tänze der Honigbienen zeigten, dass das Erdbeerfeld, Raps und NNH häufiger mittels des Bienentanzes kommuniziert wurden als die Landschaftsdeckung der jeweiligen Habitate vermuten lässt. Insbesondere bei hoher

Rapsverfügbarkeit in der Landschaft, konnten kurze Flugdistanzen beobachtet werden. Die Nutzung der Erdbeerfelder (Anteil von Tänzen bzw. Pollen) wurde jedoch nicht direkt von der Rapsverfügbarkeit oder der Landschaftsdeckung von NNH beeinflusst. Hummeln waren die am häufigsten vertretene Gruppe in den Erdbeerfeldern und bildeten zusammen mit den anderen Wildbienen 75 Prozent der Bienengemeinschaft.

Die Ergebnisse, die im Rahmen meiner Dissertation gewonnen wurden, zeigen, dass in der Agrarlandschaft blühende Massentrachten das artspezifische Sammelverhalten und die Ressourcennutzung von Bienen beeinflussen können. Eine hohe Verfügbarkeit von Raps in der Landschaft kann die Abundanz von Solitärbienen in kleinflächig blühenden Kulturen wie Erdbeere fördern, die Abundanz sozialer Bienen jedoch verringern. Honigbienen und Hummeln (soziale Bienen) sammelten zwar relativ häufig Pollen von der Zielkultur, jedoch weniger sobald die Verfügbarkeit von Massentrachten in der Umgebung zunahm. Ein kombiniertes Management mit Honigbienen- und Hummelvölkern könnte daher eine kontinuierliche Bestäuberaktivität im Erdbeerfeld gewährleisten. Jedoch sollten sich Landwirte nicht auf die Möglichkeit verlassen, den Rückgang an Bestäubern mit domestizierten Bienen ausgleichen zu können. Vielmehr sollten der Erhalt und die Förderung von Wildbienen unterstützt werden, da diese einen wichtigen Beitrag für die Bestäubung von Nutzpflanzen, wie der Erdbeere, in unseren Agrarlandschaften leisten können. Zur Bestimmung der Pollenvielfalt in gemischten Pollenproben ist *ITS2-Metabarcodierung* eine vielversprechende Methode, die jedoch in ihrer quantitativen Aussagekraft eingeschränkt ist. Hummelvölker sammelten von wesentlichen mehr unterschiedlichen Pflanzenarten Pollen als Honigbienen. Obwohl beide Arten ein soziales Verhalten haben und eng miteinander verwandt sind, kann ihr Pollen-Sammelverhalten sehr unterschiedlich sein. Daher sollte das artspezifische Sammelverhalten, nicht nur von diesen beiden Arten, sondern von der gesamten Bienengemeinschaft in den Landschaften, für eine nachhaltige Landschaftsplanung berücksichtigt werden.

***

Acknowledgements

Firstly, I would like to thank both my supervisors Catrin Westphal and Teja Tscharntke for their excellent supervision. Thank you for giving me the opportunity to be part of the Functional Agrobiodiversity/Agroecoloy group! I really appreciated all the advice and support you gave me throughout the previous years. Many thanks to Prof. Dr. Holger Kreft who kindly provided the second opinion.

I would like to thank my lovely colleagues and friends from the world's best working groups! Thank you for the great working atmosphere, your support, your feedback, your friendship and the unforgettable time we spent together. I am particularly grateful to my current and former office friends Anoush Ficiciyan, Julia Rosa, Manuel Toledo, Ingo Grass and Maraja Riechers. Further, I am thankful to Laura Sutcliffe, Emmeline Topp, Christine Venjakob and Kevin Li for their corrections and Hannah Reininghaus for her great support and motivation.

I would like to acknowledge the Deutsche Bundesstiftung Umwelt (DBU) who awarded me a PhD scholarship. I thank all members of the scholarship program for the friendly mentoring, particularly Dr. Hedda Schlegel-Starmann. Thank you Maria L., Maria S., Alex and Marcus for the great time.

I greatly appreciate the help and support from Susanne Jahn, Brigitte Jünemann and Heike Francoli. Thank you for your invaluable help. Furthermore, I am grateful to my student helpers Martin Greve, Jascha Conrad, Julia Wellinghoff and Klara Küpers for their great help – I could not have finished all that work without you. Thanks to Leonie Netter, my Bachelor student, who did a great job on pollen identification. Many thanks to Hermann Behling and Siria Biagioni who supported our microscopic analysis in the Palynology & Climate Dynamics lab.

I am thankful to all the strawberry farmers who allowed me to work on their strawberry fields and many thanks for the delicious strawberries; I guess the bees did a good job on your fields. I especially would like to thank Prof. Dr. Elke Pawelzik who allowed me to work in her facilities for post-harvest analysis. Further, I appreciate the statistical advice from Doreen Gabriel.

I would like to express further appreciation to Prof. Dr. Bertram Brenig who offered me the opportunity to conduct the NGS analysis. Additionally, I would like to thank Sabrina

Pach and Delia Niehaus for their support and advice on laboratory work. I am grateful to Röbbe Wünschiers who conducted the bioinformatics with me. Thank you for your enthusiasm and fruitful discussions.

I am very grateful for the cooperation with Francis Ratnieks and Stephan Härtel. Thank you for your discussions and support on the waggle dance analysis.

Last but not least, I would like to thank my family and friends. Thank you my lovely friends (from Einbeck and elsewhere) for your moral support. Thank you Verena, I would have not gotten here had we not met in Hevensen. Thank you Natalie for your long-lasting friendship and your optimism. Thank you Rebecka for your invaluable help, discussions and motivation. Thank you Jana and Kerry for your hospitality and your great help. And finally, thank you Mama, Papa and Benjamin, and thank you Christoph for your love, your endless support and always believing in me! I am and always will be grateful.

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Curriculum Vitae

The CV is available in the printed version only.

Publications

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Bänsch S., Tscharntke T., Gabriel D. & Westphal C. (2020) Crop pollination services: complementary resource use by social vs solitary bees facing crops with contrasting flower supply. *Journal of Applied Ecology,* https://doi.org/10.1111/1365-2664.13777

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Talks and conference contributions

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Invited talk, beekeepers‘ association meeting, Moringen

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Declarations

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

   Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

   Göttingen, den ....

   ....

   (Unterschrift)

2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

   Göttingen, den ....

   ....

   (Unterschrift)