

## LETTER

# Floral resource diversification promotes solitary bee reproduction and may offset insecticide effects – evidence from a semi-field experiment

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### Abstract

Pollinator declines in agricultural landscapes are driven by multiple stressors, but potential interactions of these remain poorly studied. Using a highly replicated semi-field study with 56 mesocosms of varying wild plant diversity (2–16 species) and oilseed rape treated with a neonicotinoid, we tested the interacting effects of resource diversity and insecticides on reproduction of a solitary wild bee. Compared to mesocosms with oilseed rape monocultures, availability of resources from wild plants complementing oilseed rape doubled brood cell production. In addition, bee reproduction increased due to plant diversity and identity effects. Exposure to neonicotinoid-treated oilseed rape reduced bee larval to adult development by 69%, but only in mesocosms with oilseed rape monocultures. Availability of complementary flower resources can thus offset negative effects of neonicotinoid-treated oilseed rape on wild bee reproduction. Policy should encourage the implementation of diverse floral resources mitigating negative effects of crop monocultures and insecticides, thereby sustaining solitary bee populations in agricultural landscapes.

### Keywords

Complementary resources, neonicotinoids, *Osmia bicornis*, plant diversity and identity, pollinator declines.

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## INTRODUCTION

Pollinators are important for the reproduction of 88% of flowering wild plants and increase yields of 75% of the world's economically most important crops (Klein *et al.* 2007; Ollerton *et al.* 2011; Potts *et al.* 2016). Bees are the most important group of insect pollinators, with solitary bees making up over 95% of all bee species globally (Corbet *et al.* 1991; Goulson *et al.* 2015). However, wild bee populations are declining in intensified agricultural landscapes, due to multiple and potentially interacting stressors, threatening pollination service provision (Goulson *et al.* 2015; Potts *et al.* 2016; Powney *et al.* 2019). Notable stressors driving bee declines are habitat loss, lack of floral resources and pesticide use (Goulson *et al.* 2015; Potts *et al.* 2016; Woodcock *et al.* 2016).

With habitat loss and fragmentation, floral resource availability in agricultural landscapes is decreasing, which can be a limiting factor for pollinator populations (Biesmeijer *et al.* 2006; Carvell *et al.* 2006; Potts *et al.* 2010; Scheper *et al.* 2014; Goulson *et al.* 2015; Samuelson *et al.* 2018). Non-crop flowering resources are often seen as important for the persistence of pollinators in the agricultural landscape, and different methods of increasing floral resources have been discussed and implemented (Dicks *et al.* 2015). Flower strips, for example, are part of agri-environmental schemes to aid pollinators

(Blaauw & Isaacs 2014; Jönsson *et al.* 2015). There is still ongoing research, however, on whether mass-flowering crops may make other flower resources redundant, which plants in flower strips are best for pollinators, and whether diversity, identity or quantity of floral resources is more important (Westphal *et al.* 2009; Blüthgen & Klein 2011; Fründ *et al.* 2013; Woodcock *et al.* 2016). In addition to resource diversity contributing to a more balanced diet promoting bee reproduction ('complementarity effect'), bee reproduction could also be affected by the presence of certain plant species in diverse plant communities, playing an exceptional role by offering large quantities and nutritional traits of nectar or pollen over an extended flowering period ('selection effect') (Loreau & Hector 2001; Blüthgen & Klein 2011; Filipiak 2019; Lawson *et al.* 2020).

Another factor potentially negatively affecting solitary bee densities and reproductive success are neonicotinoid insecticides (Rundlöf *et al.* 2015; Woodcock *et al.* 2017). Neonicotinoids are systemic insecticides used against sucking and chewing pest insects and are commonly applied as seed treatment. They are taken up by the plant after germination and all plant parts end up containing the insecticide to some extent, including nectar, pollen and guttation fluid (Bonmatin *et al.* 2015). Depending on the levels of uptake by visiting pollinators, these may be negatively affected through lethal or

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sublethal effects (e.g. impaired navigation, nest behaviour, social networks and thermoregulation) (Gill *et al.* 2012; Whitehorn *et al.* 2012; Fischer *et al.* 2014; Jin *et al.* 2015; Crall *et al.* 2018). In contrast to honeybees and bumblebees (Whitehorn *et al.* 2012; Arce *et al.* 2017; Tsvetkov *et al.* 2017), field and semi-field studies investigating these threats of exposure for solitary bees, are still rare and inconclusive (Rundlöf *et al.* 2015; Peters *et al.* 2016; Woodcock *et al.* 2017; Ruddle *et al.* 2018). In particular, effects of insecticides on pollinator reproduction, including multiple development stages from egg to adult, are poorly understood.

In this study, we focus on the combination of the potentially interacting stressors of low floral resource availability and exposure to a neonicotinoid insecticide affecting solitary bee reproduction. There is limited research available suggesting that high amount of natural habitat, potentially because of food or nesting resources, can buffer negative effects of pesticides on wild bees (Park *et al.* 2015; Centrella *et al.* 2020). However, experimental evidence of the relative roles of flower resource abundance, diversity, plant identity and exposure to neonicotinoids for the performance of solitary bees is missing. Complementary resources could reduce the number of interactions with treated crop plants and thereby the amount of uptake of neonicotinoid by adult bees and their offspring. In addition, diverse flower resources may add more and different nutrients to bees' diets, which may counteract potential negative effects of the insecticide (Filipiak 2019; Lawson *et al.* 2020). To fill this research gap, we study the reproductive success of the solitary bee species *Osmia bicornis* in a highly replicated semi-field study with 56 mesocosms of neonicotinoid-treated vs. untreated oilseed rape, with and without additional flower strips of varying flowering plant diversity (2–16 species seeded) and identity. Oilseed rape is a mass-flowering crop plant, that relies on insect pollination for better yields (Stanley *et al.* 2013; Woodcock *et al.* 2019). Systemic insecticides have been commonly used in the past in Europe to treat oilseed rape against sucking insect pests, until their outdoor use has been prohibited for crops attractive to bees in the European Union in 2013 (Elbert *et al.* 2008; European Commission 2013). In other parts of the world, however, neonicotinoid insecticides continue to be heavily used in flowering crops, potentially threatening pollinating insects.

We focus on comparing the importance of effects of systemic insecticide exposure and complementary floral resource availability on *Osmia* offspring production. We study the influence of insecticide treatment, as well as floral resource availability regarding quantity, plant species identity, number of flowering plant species, and their Shannon diversity. We address the following hypotheses:

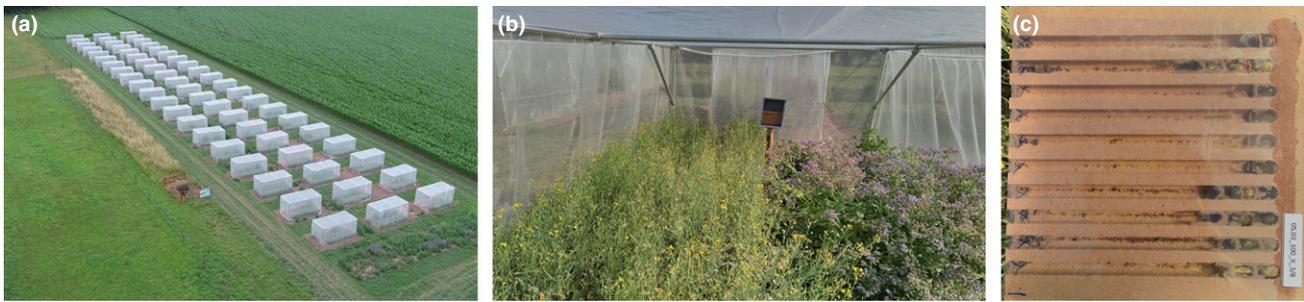
- (1) Negative effects of neonicotinoid treatment of oilseed rape are mitigated by the availability of complementary floral resources.
- (2) Diversity of floral resources is a better predictor of reproductive success compared to resource quantity alone.
- (3) In addition to diversity effects (resource complementarity), identity effects of specific plant species (selection effects) promote wild bee reproduction.

## MATERIALS AND METHODS

The study took place at Göttingen University's experimental farm Reinshof in central Germany (coordinates: 51°29'46.1" N 9°55'53.1" E). Fifty-six mesocosms of 2 m × 4 m × 2 m were set up after seeding of plants in April 2018. Mesocosms either contained 50% summer oilseed rape (variety 'Trapper', male fertile hybrid) and 50% flower strip (48 mesocosms, Figure 1b), or 100% oilseed rape (8). The flower strips in the 48 mesocosms containing both oilseed rape and flower strip were of different diversity levels (2, 4, 8, 12 or 16 seeded plant species; see Table S8 for details). There was a pair of two mesocosms for each plant identity/diversity combination, for which the configuration of the flower strips was the same. Half of these mesocosms had summer oilseed rape with a neonicotinoid treatment. Here, commercial summer oilseed rape seeds treated with Bayer's MODESTO were used. The seed treatment contained clothianidin, a neonicotinoid insecticide, as well as thiram, a fungicide, and beta-cyfluthrin, a pyrethroid insecticide. To focus on the effect of the neonicotinoid, oilseed rape seeds in the other half of the mesocosms were treated only with thiram and beta-cyfluthrin as a control. Standard application rates were used (23.15 µg of clothianidin per oilseed rape seed; see Table S6 for details). Because of high densities of pollen beetles, all oilseed rape plants (with and without neonicotinoid treatment) were treated with Karate Zeon (Syngenta) (standard application rate) containing lambda-cyhalothrin, a non-systemic pyrethroid insecticide, before mesocosms were closed on 12 June 2018, seven weeks after seeding and three weeks before the introduction of bees. Flower strips were not treated with any insecticides or fungicides.

Annual plant species naturally occurring in the agricultural landscape or being used in commercial flower strip mixes were chosen for the flower strips. Plant species were required to be flowering at the same time as the summer oilseed rape (June/July) and to be attractive to *O. bicornis*, with bee-flower-interactions on record. A set of 16 species from six different plant families, Asteraceae, Boraginaceae, Brassicaceae, Fabaceae, Papaveraceae and Resedaceae, was used in the experiment. For lower diversity levels, we made sure that plant species in each mesocosm would be from different plant families, if possible. For a detailed overview of combinations of plant identity and diversity levels, as well as instances where flowering weed species occurred in mesocosms, which were included in the analysis, see Table S2. Mesocosms were set-up in 20 rows consisting of three mesocosms each (Figure 1a). For logistical reasons, one row always either had treated or untreated oilseed rape. Besides that, the set-up was randomised.

*Osmia bicornis*, a cavity-nesting, polylectic solitary bee species, was used as the study organism. It is a common species, representing solitary bees in trials testing new insecticides (EFSA 2013). Cocoons were bought from a commercial breeder in Northern Germany (BIENENHOTEL.DE) and taken from 4 °C storage conditions to room temperature before the start of the experiment. After emerging, bees were sorted by gender and 12 males and 12 females were introduced into each mesocosm on 3 July 2018, which marked the start of the experiment. After mating, females readily started nesting. Bee



**Figure 1** (a) Overview of mesocosm setup. (b) A mesocosm with 50% oilseed rape, 50% flower strip and *Osmia* nesting boards. (c) A nesting board with ten linear *O. bicornis* nests.

nests consisting of ten wooden nesting boards with ten nesting cavities each (8 mm diameter) were set up in the mesocosms (Figure 1c). Furthermore, a hole of 20 cm depth and diameter was dug in the soil and kept wet throughout the experiment to provide mud for building nests, and plastic bowls with water and expanded clay were set up to ensure water supply (see Figure S3 for more photographs).

Starting on day 10 (13 July 2018) after the start of the experiment, all mesocosms were sampled every 3–4 days, six times in total (Table S1). *Osmia* bees are relatively short-lived and the experiment was ended on day 27 (30 July 2018), when brood cell construction had ceased (Szentgyörgyi & Woyciechowski 2013). In every sampling round, the number of flowers of all flowering plant species in each mesocosm were estimated, to be able to quantify resources available to nesting bees. For flower estimations, small patches of each flowering species were counted, and the total numbers for each mesocosm were then estimated based on these counts. For Asteraceae, we defined one flower as one flower head. Furthermore, the nesting progress was marked on the nesting boards, to later be able to tell when each brood cell was constructed.

Open flowers from treated and untreated oilseed rape were sampled to quantify levels of neonicotinoid residues. Samples were stored at  $-18^{\circ}\text{C}$  and analysed using a validated multi-residue method following Böhme *et al.* (2017) and validated by spiking samples with the target substance clothianidin.

At the end of the experiment, nesting boards were carefully removed from the mesocosms and stored at ambient temperatures, protected from rain under a roof and from birds/insects using fine mesh. Nesting boards were examined again in December 2018 and the numbers of brood cells were counted for each mesocosm and sampling round. Cocoons were then removed from nesting boards, transferred to glass vials and stored in a refrigerating unit at  $4^{\circ}\text{C}$ . In April 2019, after overwintering, vials were taken out of the refrigerator and kept at room temperature. Emerging males and females were counted. After one month, remaining cocoons were opened to see whether there was a larva or adult inside. In total, we discriminated for each mesocosm and sampling round the numbers of *O. bicornis* offspring in the following live stages: brood cells (all cells containing at least an egg), cocoons (with alive/dead larvae), fully developed adults inside cocoons (alive/dead) and emerged adults (alive).

### Statistical analysis

First, the number of offspring in each development stage was compared between mesocosms with treated vs untreated oilseed rape to test the effects of the neonicotinoid insecticide treatment on bee reproductive success. The unbalanced design prompted us to separately analyse the mesocosms containing 50% oilseed rape and 50% flower strip ( $n = 48$ ) and the mesocosms containing 100% oilseed rape ( $n = 8$ ). Additionally, to test whether *O. bicornis* reproduction was enhanced when complementary floral resources were available, numbers of individuals in the different life stages in 50% oilseed rape/50% flower strip mesocosms were compared to those in 100% oilseed rape mesocosms. Generalised linear mixed effects models with Poisson distribution and mesocosm ID and sampling round as random effects were included to account for non-independence of repeated measures per mesocosm.

Second, we investigated whether floral resource abundance, flowering plant species richness, or Shannon diversity (based on flower estimates) are better predictors of bee reproductive success. The number of emerged offspring in 50% flower strip/50% oilseed rape mesocosms ( $n = 48$ ) was used as the response variable. Pairwise correlations of predictor variables were first checked for covariances using Pearson correlation tests. Species richness and Shannon diversity showed a strong correlation (correlation coefficient  $r = 0.77$ ,  $P < 0.001$ ). Floral resource abundance was neither strongly correlated with flowering plant species richness ( $r = 0.18$ ,  $P = 0.002$ ), nor with Shannon diversity ( $r = 0.04$ ,  $P = 0.501$ ). As a result, our models included floral abundance and either species richness or Shannon diversity, but not both. Generalised linear mixed effects models with Poisson distribution and mesocosm ID and sampling round as random effects were used. The respective explanatory variables were scaled to zero mean and unit variance to allow for comparisons of model estimates.

Third, we aggregated flower estimates over all sampling rounds for each 50% flower strip/50% oilseed rape mesocosm to evaluate the importance of single plant species for *O. bicornis* reproductive success. Aggregated data were analysed using the random forest approach (with default parameters of 500 trees and 7 variables tried at each split) with number of emerged offspring as the response variable. This method is able to determine important factors predicting a response variable from a large set of different factors by calculating

importance scores for each one (Breiman 2001). Mean Decrease Accuracy and Mean Decrease Gini were obtained for each flowering plant species. The more the accuracy of the random forest decreases when excluding a variable, the more important the variable. Higher values therefore indicate a higher importance for data classification. Negative values indicate poorer model performance than under random permutations.

All statistical analyses were performed in *R* version 3.6.2 ('R Development Core Team 2019') using dedicated packages *lme4* (version 1.1-21, Bates et al. 2019), *MuMIn* (version 1.43.15, Bartoń 2019), *randomForest* (version 4.6-14, Liaw et al. 2018) and *ggplot2* (version 3.2.1, Wickham et al. 2019). Models were created based on our hypotheses without any subsequent model simplification. Significances of predictor variables were tested using z-tests. All models met assumptions of normality of residuals and homoscedasticity.

## RESULTS

In mesocosms with 50% oilseed rape and 50% flower strip, the neonicotinoid treatment did not have an effect on any stage of *Osmia* development (brood cells, cocoons, developed and emerged individuals) (Figure 2a, Table S3). In 100% oilseed rape mesocosms, there were no differences in numbers of brood cells, cocoons and emerged individuals, but there was a 69% reduction in the numbers of larvae that developed into adults inside cocoons in mesocosms with treated oilseed rape ( $P = 0.004$ , Figure 2b, Table S3). The number of offspring was significantly higher in 50% oilseed rape/50% flower strip mesocosms for all stages of development compared to 100% oilseed rape mesocosms, where flowering ended earlier (Table S4; Table S11; Figure S4). Residue analyses confirmed that the seed treatment was successful with treated oilseed

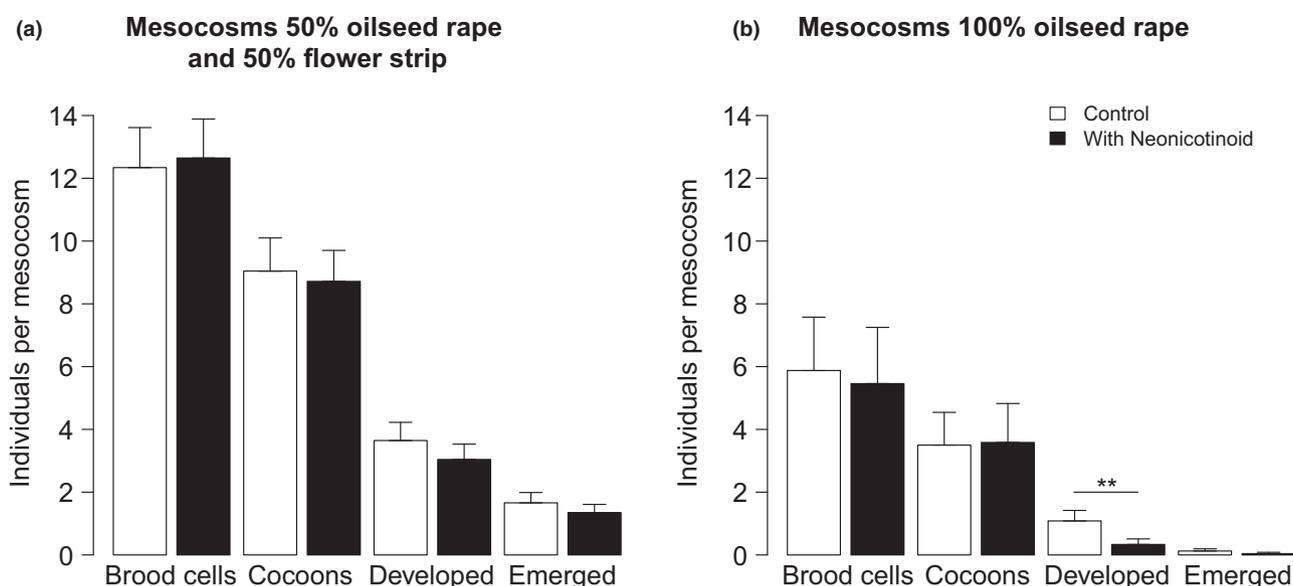
rape flowers containing  $3.00 \pm 0.15 \text{ ng g}^{-1}$  of clothianidin (mean  $\pm$  standard error of the mean) (see Table S7 for details).

The number of emerged offspring increased with abundance, species richness, and Shannon Diversity of flowering plants (Table S5). Comparing model estimates, Shannon Diversity and species richness predicted the number of emerged individuals better than flower abundance (Table S5). Offspring numbers were significantly positively correlated with Shannon Diversity of flowering plants for all stages of development (Figure 3b; Table S10). Flower numbers were not significantly correlated with Shannon Diversity of flowering plants (Figure 3a; Table S9).

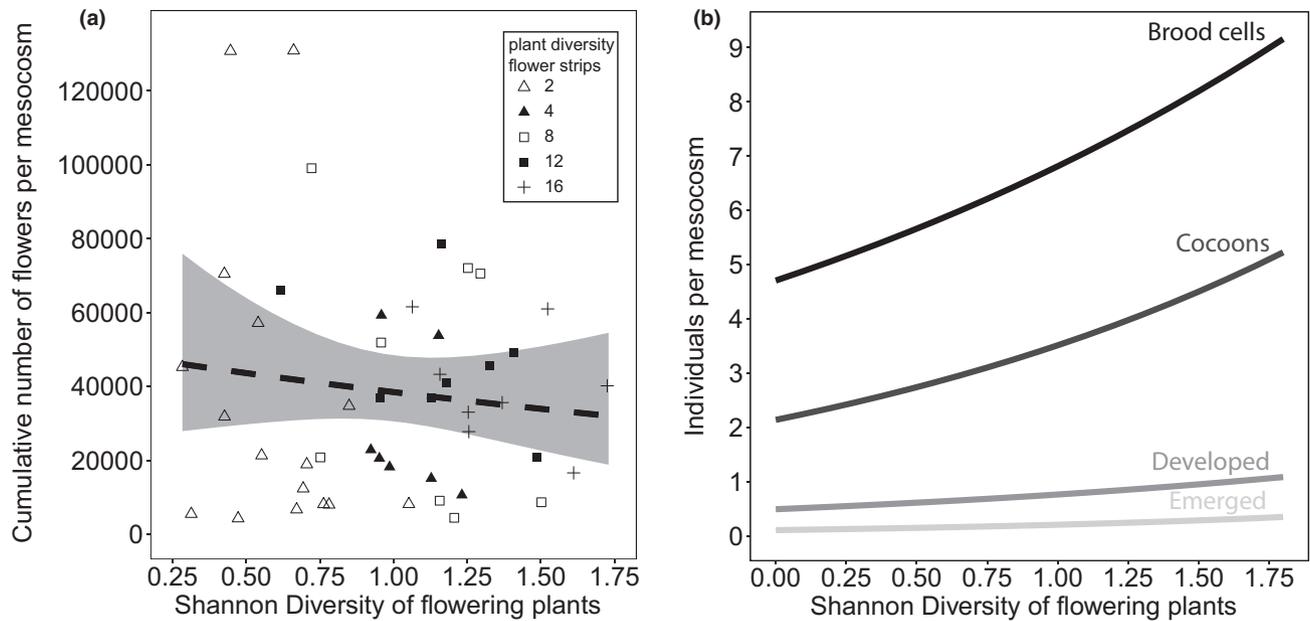
Results of the random forest analysis (with 37.47% variance explained) emphasised the importance of two plant species, *Phacelia tanacetifolia* and *Raphanus sativus*, predicting the number of emerged *Osmia* offspring (Figure 4; Figure S2). Flower estimates over all sampling rounds and mesocosms were relatively high for *Phacelia* (median: 10925.5 flowers) and lower for *Raphanus* (median: 1813.5; Figure S1).

## DISCUSSION

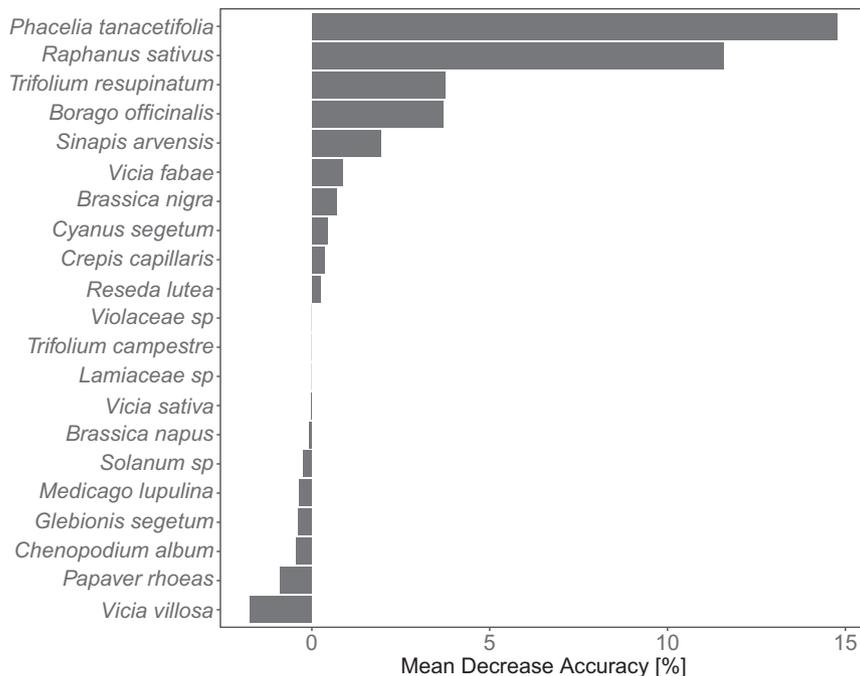
Here, we showed with a semi-field experiment that the neonicotinoid treatment of oilseed rape did not affect the offspring production of *O. bicornis* bees when complementary resources were offered. Bee development (from the larval to the adult stage) was negatively affected only when oilseed rape was the sole resource available, that is, in resource-poor environments of oilseed rape monocultures. This highlights the importance of complementary floral resources apart from mass flowering crops for bee reproductive success in the agricultural landscape, which may even mitigate negative effects of neonicotinoid insecticides. Our results highlight the importance of



**Figure 2** Number of individuals per mesocosm and sampling round for different *O. bicornis* life stages comparing mesocosms with treated and untreated oilseed rape. Panel (a) shows mesocosms with 50% oilseed rape and 50% flower strip. Panel (b) shows mesocosms with 100% oilseed rape. Shown are arithmetic means + 1 standard error of the mean. Asterisks denote significant pairwise difference within developmental stage ( $P = 0.004$ ).



**Figure 3** Cumulative number of flowers per mesocosm (a) and number of individuals per mesocosm (b) in relation to Shannon Diversity of flowering plants. Shapes/colours of raw data points in (a) are corresponding to seeded plant diversity in respective mesocosms. Dashed line represents non-significant relationship ( $P = 0.429$ ) and envelope shows 95% confidence interval. Solid lines for all offspring life stages in (b) represent significant relationships ( $P < 0.05$ ).



**Figure 4** Mean Decrease Accuracy of flowering plant species in mesocosms for random forest models predicting the numbers of emerged *Osmia* offspring. Higher values indicate a higher importance.

flower resource diversity, compared to just resource abundance. This is in line with other studies showing the importance of pollen diversity for pollinators, for example, for enhancing bumblebee colony growth (Hass *et al.* 2019). In addition to diversity effects, we found single plant species contributing extraordinarily to the number of emerging offspring. The two species of highest importance were *Phacelia tanacetifolia* and *Raphanus sativus*. *Phacelia* was offering large

quantities of floral resources and is known to be beneficial for different pollinator species (Williams & Christian 1991). *Raphanus* may be a beneficial resource because of the high lipid content of its pollen (Singh *et al.* 1999).

Seminal studies on biodiversity-ecosystem functioning have partitioned diversity effects into complementarity and selection effects (e.g. Hector *et al.* 1999; Loreau & Hector 2001 for biodiversity-productivity relationships in grassland). Lacking

monocultures of plant species from flower strips because of limited mesocosm numbers, we cannot adopt the same statistical approaches to directly compare these effects here. However, using indirect analyses, our results suggest, that both selection (plant species identity) and complementarity effects (plant diversity) are at play influencing bee reproduction (Loreau & Hector 2001). These findings have important implications for management of bee populations in human-dominated landscapes, as they indicate that not only diverse flower resources are key for bee maintenance (e.g. through landscape diversification or targeted flower strips; Tscharrnke *et al.* 2005; Albrecht *et al.* 2020; Batáry *et al.* 2020), but that also key plant species with specific traits (e.g. high nutritional value; Filipiak 2019; Lawson *et al.* 2020) are needed to maintain bee populations.

#### Neonicotinoid effects on reproductive success contingent on resource availability

*Osmia* reproductive success was not affected by oilseed rape neonicotinoid treatment when complementary flower resources were present. In resource-poor environments, however, where oilseed rape was the only resource offered, the number of developed offspring was 69% lower, when seeds were treated with the systemic insecticide. Because of limited mesocosm availability and the need for replicates for each diversity level and insecticide treatment, the sample size for the 100% oilseed rape mesocosms was relatively small. For future studies, larger sample sizes are recommended.

To the best of our knowledge, neonicotinoid effects on *Osmia* bees in field/semi-field studies have so far only been shown to affect the number of brood cells (Sandrock *et al.* 2014; Rundlöf *et al.* 2015; Woodcock *et al.* 2017), whereas studies showing effects on subsequent life stages are missing. In a laboratory study, effects on larval development have been studied by Nicholls *et al.* (2017), where *Osmia* larvae were feeding on provisions from orchard sites laced with neonicotinoids with no effects reported. In our semi-field experiment, pollinators were able to forage in near-natural conditions. Larvae managed to spin cocoons, but their development to adults inside the cocoons seemed to have been impaired, when exposed to neonicotinoid insecticides from oilseed rape and without pollen provisioning from complementary flowering plants. Previous studies focusing on *Osmia* brood cells may thus have underestimated negative effects of neonicotinoids on wild bee reproduction that intensify in subsequent developmental stages.

In environments with complementary resources, potential negative effects of neonicotinoid treatment were mitigated, as suggested by Park *et al.* (2015). This result contributes significantly to the limited knowledge on interactive effects of floral resource abundance and exposure to insecticides. It highlights the importance of floral resource availability apart from mass-flowering, and potentially insecticide-treated, crops in agricultural landscapes. Complementary resources, for example from flower strips, can increase resource diversity and abundance throughout the season and limit exposure to pollen and nectar containing neonicotinoids or having other unfavourable traits (Eckhardt *et al.* 2014). While clothianidin has been banned from fields in the European Union, it is still widely used in

other parts of the world, where introducing complementary, untreated floral resources, for example by landscape diversification, could help to mitigate potential negative effects of the insecticide on pollinators (Simon-Delso *et al.* 2015; European Commission 2018).

#### Floral resource abundance and diversity effects on reproductive success

Higher offspring numbers in mesocosms with flower strips emphasised the importance of complementary floral resources in agricultural landscapes offering nectar and pollen throughout the season. Mass-flowering crops can offer abundant resources (Westphal *et al.* 2003), but resource availability in the agricultural landscape decreases drastically when they stop flowering and there are no complementary floral resources (e.g. flower strips) available (Blüthgen & Klein 2011). Our study further showed that *Osmia* reproductive success is not simply determined by resource abundance but can be better predicted using the diversity and species richness of flowering plants. This suggests that a diverse pool of resources offers benefits additional to their combined floral resources. Focusing on the plant species contributing most to *Osmia* emerging success, it can be assumed that in addition to resource abundance (provided by, e.g., *Phacelia tanacetifolia*), a diverse mix of pollen, increasing the availability of different nutrients, is important for larval development. Filipiak *et al.* (2019), for example, highlight the need of a balanced diet for *Osmia* larvae. *Raphanus sativus* has been shown by Singh *et al.* (1999) to have a high lipid content, which may explain its importance for *Osmia* offspring production. Wildflower plantings should therefore suit the needs of target species, with different bee species relying on different key flowering plant species (see also Mallinger *et al.* 2019; Nichols *et al.* 2019). Future studies should focus on species-specific and combined effects of flowering plants on bee reproduction to identify additive and potentially interactive effect of nectar and pollen nutrient composition (e.g. Baude *et al.* 2016).

#### CONCLUSIONS

Our study demonstrates that complementary floral resources are of major importance for *O. bicornis* reproductive success. Providing a diversity of flowering plant species can help to sustain big pollinator populations in the agricultural landscape and may even mitigate potential negative effects of systemic insecticides. In addition to the diversity of flowering plants, the abundance of key plant species benefiting the bee target species most is important. Future research should analyse in more detail to what extent resource-rich environments may mitigate insecticide applications and determine the amount and composition of complementary resources needed. Diverse floral resources are needed to sustain solitary bee populations, and to mitigate crop monocultures and potential negative effects of insecticide applications in agricultural landscapes. Policy should therefore encourage land managers to increase and diversify floral resources, particularly in simplified agricultural landscapes that are dominated by mass-flowering crops treated with insecticides potentially detrimental to bee reproductive success.

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## DECLARATIONS OF INTEREST

None.

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## AUTHORSHIP

FK, TT and IG designed the study; FK performed the research; GB provided chemical analyses; FK wrote the first manuscript draft, all authors contributed to the final manuscript version.

## DATA AVAILABILITY STATEMENT

We confirm, that, should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository and the data DOI will be included at the end of the article (<https://doi.org/10.5061/dryad.wdbrv15n7>).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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