

Local and landscape threats to bee and wasp populations in agricultural landscapes

Dissertation

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Felix Klaus

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Thesis committee

Prof. Dr. Teja Tscharntke

Agroecology, Department of Crop Sciences, Georg-August-Universität Göttingen

Prof. Dr. Johannes Isselstein

Institute of Grassland Science, Department of Crop Sciences, Georg-August-Universität Göttingen

Prof. Dr. Kerstin Wiegand

Department of Ecosystem Modelling, Buisgen-Institute, Georg-August-Universität Göttingen

Prof. Dr. Thomas Kneib

Chairs of Statistics and Econometrics, Faculty of Business and Economics, Georg-August-Universität Göttingen

Prof. Dr. Ingo Grass

Department of Ecology of Tropical Agricultural Systems, Institute of Agricultural Sciences in the Tropics,
University of Hohenheim

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Summary

The transformation and intensified use of the agricultural landscape in central Germany in the past decades has led to the fragmentation and loss of semi-natural habitats, threatening biodiversity including insects. Bees and wasps are highly diverse groups of insects providing ecosystem services such as pollination and pest control. Different species experience the landscape at different scales, have different requirements regarding food and nesting habitats, and show different responses to the exposure to stressors such as insecticides. Calcareous grasslands are biodiversity hotspots in the agricultural landscape around Göttingen, but their numbers and sizes have vastly decreased in the past. Their importance as habitat for bees and wasps, also compared to effects of the surrounding landscape, and how they contribute to the export of pollinators and their services to the surrounding landscape have been studied and are reported in this thesis. The thesis consists of four chapters:

- 1) A framework chapter giving an overview of the entirety of the thesis and providing context for the following chapters
- 2) Floral resource diversification promotes solitary bee reproduction and may offset insecticide effects – evidence from a semi-field experiment
- 3) Calcareous grassland fragments as sources of bee pollinators for the surrounding agricultural landscape
- 4) Trophic level and specialization moderate effects of habitat loss and landscape diversity on cavity-nesting bees, wasps and their parasitoids

In the second chapter, the importance of the availability and diversity of food resources and of the exposure to insecticides for orchard bee populations were studied in a highly replicated semi-field experiment. Bees were kept in mesocosms with flowering oilseed rape, which was either treated with a neonicotinoid insecticide or untreated, and flower strips differing regarding plant identity and diversity. We found that the availability of flower strips per se, flower strip diversity, and the availability of specific flowering plant species all positively affected brood cell production of bees. Exposure to insecticide-treated oilseed-rape reduced bee larval to adult development only in mesocosms with oilseed-rape monocultures, which suggests complementary flower resources offsetting these insecticide effects. Our findings emphasize the importance of alternative and diverse food resources for bee populations in the agricultural landscapes, which can be provided, for example, by plants in flower strips, hedgerows, or field margins.

In the third chapter, the focus is on the export of pollinators and pollination services from calcareous grasslands into the surrounding landscape. Calcareous grasslands are biodiversity hotspots in the agricultural landscape, and provide food and nesting resources for pollinators, which also forage in the close surroundings, thereby pollinating flowering crop and wild plants. This spillover was studied for solitary bees, social bees and hoverflies by establishing potted flowering plants at different distances (0-400m) from the focal grasslands and observing pollinator visits, as well as measuring pollination success by counting seeds developed by the exposed plants. The number of visits decreased with distance only for solitary bees, suggesting

the importance of calcareous grasslands as their main habitat. The size of the grasslands was correlated with solitary bee numbers, with larger grasslands supporting more than twice as many bees individuals. Seed production of the focal plant did not decrease with distance, suggesting that other pollinators, such as bumble bees and hoverflies, which seemed to be less dependent on the grasslands, or to have greater foraging ranges, were compensating for the loss of solitary bees. These results show the significance of calcareous grasslands as major bee habitats, and calls for their conservation and restoration to support bee populations, which pollinate plants in the surrounding landscape.

The fourth and final chapter is about the effects of habitat loss and landscape diversity on cavity-nesting insects of different trophic levels and grades of food and habitat specialization. Using trap nests on calcareous grasslands differing regarding size and diversity of the surrounding landscape, the following patterns were revealed: Species from higher trophic levels were positively affected by semi-natural habitat at larger spatial scales, compared to those from lower trophic levels, but only, when they were generalists. Specialist species responded to habitat loss at the same scales as their hosts, suggesting bottom-up effects as the driving factor. While bees, being habitat specialists, were mainly driven by local habitat loss, wasps as habitat generalists were mostly affected by landscape diversity. These findings highlight the need to consider different special scales contingent on trophic level and specialization of target species for conservation, maintaining or restoring both local habitats and high landscape diversity.

In summary, this thesis shows the importance of a unique habitat type (calcareous grasslands) especially for bees, and how their pollination services spillover into the surrounding landscape, the different effects of local and landscape factors on species with different trophic levels, and the scales, at which they experience the landscape. Furthermore, the significance of the availability and diversity of food resources in supporting bee populations and potentially offsetting negative effects of other stressors was shown. The findings can help to evaluate the relative importance of different stressors affecting bees in the agricultural landscape and they underline the need to conserve and restore semi-natural habitats in the landscape, and to foster diverse landscapes, to support populations of various beneficial insects.

Chapters 2, 3 and 4 have been published in scientific journals and are presented in their published form. All photographs by Felix Klaus, unless otherwise stated.

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Chapter 1

Introduction

Agricultural intensification during the past decades has been associated with several threats to ecosystems, such as habitat loss and fragmentation and the use of pesticides (Foley et al., 2005; Robinson & Sutherland, 2002; Tscharntke et al., 2005).

Extensively managed grasslands have been turned into intensively managed grasslands, or crop fields (Poschlod & WallisDeVries, 2002). Extensively used grasslands contain and support a highly biodiverse community of plants and animals, which lose habitat, when land-use is intensified (Poschlod & WallisDeVries, 2002). Habitat loss is often accompanied by habitat fragmentation, when patches of grassland are not completely transformed, but broken up (fragmented) into smaller patches (Krauss et al., 2010). Depending on the distance between fragments and the permeability of the surrounding matrix, grassland patches can be more or less isolated, depending also on the mobility of the species of interest (Hagen et al., 2012). Consequently, reduced gene flow and re-colonization of habitat patches can lead to local extinctions of species (Krauss et al., 2010). Overall, habitat loss and fragmentation thus lead to less biodiverse ecosystems.

Another threat to biodiversity in intensified agricultural landscapes is the use of chemical pesticides (Woodcock et al. 2016). Non-target organisms can be affected by pesticides both directly and indirectly. Pollinating insects, for example, can be directly affected, when they are exposed to pesticides, for example via contaminated nectar, pollen, or guttation water, when flowering plants are treated with systemic insecticides (Bonmatin et al., 2015), or indirectly affected, for example by herbicide applications, causing reduced availability of flowering weeds in crop fields, used by pollinators as food sources (Hardman et al., 2016). Similarly, predatory insects, for example, can be affected by insecticide applications either directly through exposure, or indirectly, when prey numbers are reduced (Sánchez-Bayo, 2021).

Consequently, biodiversity has declined in the agricultural landscape, with smaller plant and animal populations of reduced fitness being less capable to reproduce and disperse (Dudley & Alexander, 2017; Hallmann et al., 2021). The loss of species acting as pollinators or biological control agents negatively affects the provision of ecosystem services, upon which agriculture is dependent (Letourneau et al., 2009; Potts et al., 2016). The economic value of these ecosystem services is immense. Regarding pollination, for example, 35% of the world food production has been shown to be dependent on pollinators (Klein et al., 2007).

While the main drivers of biodiversity decline have been identified, knowledge about potential interactive effects of multiple factors is still scarce (Dicks et al., 2021; Goulson et al., 2015; Potts et al., 2016; Powney et al., 2019). For example, bees being stressed by a scarcity of floral resources may be more susceptible to exposure to insecticides (Goulson et al., 2015). Furthermore, the importance of local and landscape factors and the scale at which the landscape is experienced is highly variable (Steffan-Dewenter et al., 2002; Tscharntke et al.,

2012). Traits of species that influence their responses to these factors need to be further examined.

The restoration and maintenance of agricultural ecosystems is crucial to support biodiversity and associated ecosystem services (Samways et al., 2020). This thesis aims to further knowledge about how populations of beneficial arthropods (bees, wasps, and their natural enemies) are affected by a combination of stressors, determining the scales at which they experience the landscape, and how ecosystem service provisioning is affected by remnants of semi-natural habitat in the agricultural landscape. Understanding how beneficial insects respond to interacting stressors, and at what scales they use the landscape, is critical for the design and implementation of effective and efficient conservation measures.

Study region

The study region was the agricultural landscape in the surroundings of the city of Göttingen in central Germany. Agriculture has been intensified since the end of World War II, but average field sizes are relatively small (around 3.5 hectares) (Batáry et al., 2017). The landscape consists primarily of crop fields (mostly corn, wheat, barley, sugar beets and oilseed rape) and forests, and a smaller percentage is made up of semi-natural habitats such as field margins, hedgerows, or calcareous grasslands. The studies took place on agricultural fields and calcareous grassland fragments embedded in the landscape.

Study system

Calcareous grasslands are biodiversity hotspots in the agricultural landscape in Central Europe (WallisDeVries et al., 2002) (Fig. 1a). They are a result of human management over the past centuries, with extensive grazing or mowing retaining their open grassland character (Poschlod & WallisDeVries, 2002). To retain their open and nutrient-poor character, grazing is best done only for part of the season, and using low stocking densities (Jefferson, 2005). Alternatively, or additionally, they can be mown, ideally late in the, and only once or twice per season, gradually mowing sections of larger grassland patches over several weeks (Kühne et al., 2015). Their shallow and nutrient-poor soil allows a diverse community of plant species to coexist, because of competitive abilities being restricted by the limited availability of nutrients (Römermann et al., 2008; Stevens et al., 2004). The high plant diversity in turn allows for and facilitates high insect diversity.

In the past decades, most calcareous grasslands have been lost or fragmented due to agricultural intensification or abandonment (Grass et al., 2018; Krauss et al., 2010; Poschlod & WallisDeVries, 2002). To keep the grasslands profitable, land managers increased fertilizer input, stocking density of grazing animals, or the number of mowing events, causing a decline of diversity of plants and associated organisms (Gossner et al., 2016). In cases, where profitable management was not possible, grazing and mowing were often abandoned (Isselstein et al., 2005). Consequently, woody plant encroachment led to the loss of the open grassland character and its associated biodiversity (Tokarczyk, 2017).

The remaining fragments provide food and nesting habitat for many insect species, including bees and wasps, and act as source habitats and stepping stones for the re-colonization of smaller habitat patches in the surrounding landscape. Thus, these fragments also facilitate the provision of ecosystem services, such as pollination and pest control in the surrounding crop fields (Jauker et al., 2009; Öckinger & Smith, 2007; Ricketts et al., 2008).

Our study region around the city of Göttingen in central Germany, is dominated by intensive agricultural land use. There are 285 extensively managed calcareous grasslands, making up 0.26% of the total area (Krauss et al., 2003). The grasslands we used as study sites were privately owned, but owners were obliged to extensive management (guidelines regarding grazing densities/number and time in the year of mowing events) due to contracts with the local conservation agency.

In this thesis, the focal species were cavity-nesting insects, which can be studied using trap nests (Fig. 1b). Trap nests are artificial nesting devices. They are highly attractive to cavity-nesting bees and wasps as they offer additional nesting cavities in landscapes where the availability of nesting resources is often scarce (Staab et al., 2018; Tschardt et al., 1998). The populations of cavity-nesting species and their interactions with food and natural enemies, can be easily studied using trap nests (Fig. 1c, d). Nests can be moved to the laboratory after completion to study their contents and monitor emergence. Species associated with trap nests belong to different trophic levels (from primary producers to quaternary consumers) and exhibit different grades of food (generalist bee to specialist parasitoid species) and habitat (grassland-associated bee to generalist wasp species) specialization.



Figure 1: (a) Calcareous grassland fragment embedded in agricultural landscape. (b) Trap nest consisting of plastic tubes filled with reed. (c) Bees' nest in reed showing pollen/nectar provision (yellow), bee larvae (white) and cell partitions (green). (d) Wasps' nest in reed showing cocoons (light brown), caterpillar larvae as food provision (red/green), and cell partitions (grey).

Objectives

The main objective of this thesis was to study the importance and potential interactions of local and landscape threats to bee and wasp populations in agricultural landscapes.

At the local scale, the combined impact of limited food resources and exposure to insecticides were studied in a semi-field experiment. Different scenarios of food resource availability and insecticide exposure were simulated, to determine the importance of these factors for bee fitness, as well as potential interactive effects. Knowledge on how bees are impacted by interactions of different threats is important to be able to better focus conservation efforts to protect and enhance pollinator populations in the agricultural landscape (chapter 2).

At the landscape scale, the correlation of pollination service provision to the distance to nearby calcareous grassland fragments was studied to determine the dependence of different groups of pollinating insects on the grasslands. This is relevant for the assessment of the availability of pollination potential in the landscape, to identify areas with a need for creating or restoring additional habitats, or for implementing measures supporting pollinators not associated with calcareous grasslands (chapter 3).

Furthermore, considering both local and landscape factors, the abundance of cavity-nesting insects from different trophic levels was studied with relation to spatial scales and different grades of food and habitat specialization in order to determine traits of species influencing their responses to these factors. These results can be used to individually evaluate agricultural landscapes regarding the conservation potential and needs for species from different trophic levels and exhibiting different grades of specialization (chapter 4).

The thesis should fill knowledge gaps regarding how species with different traits respond to stressors differently and at different scales, which can help to make conservation efforts more targeted and effective.

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Chapter 2

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

Felix Klaus^{1*}, Teja Tschardt¹, Gabriela Bischoff² and Ingo Grass^{1,3}



¹University of Göttingen, Agroecology, Göttingen, Germany

²Julius Kühn-Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Berlin, Germany

³University of Hohenheim, Ecology of Tropical Agricultural Systems, Stuttgart, Germany

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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: Pollinator declines; neonicotinoids; *Osmia bicornis*; plant diversity and identity; complementary resources

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 sublethal effects (e.g. impaired navigation, nest behavior, 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 x 4 m x 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 A. 8 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 A. 6 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 June 12th 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 A. 2. 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 randomized.

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 July 3rd 2018, which marked the start of the experiment. After mating, females readily started nesting. Bee 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 20cm 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 A. 3 for more photographs).



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.

Starting on day 10 (July 13th 2018) after the start of the experiment, all mesocosms were sampled every 3-4 days, six times in total (Table A. 1). *Osmia* bees are relatively short-lived and the experiment was ended on day 27 (July 30th 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°C and analyzed 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°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 analyze 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. Generalized 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. Generalized 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 analyzed 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.

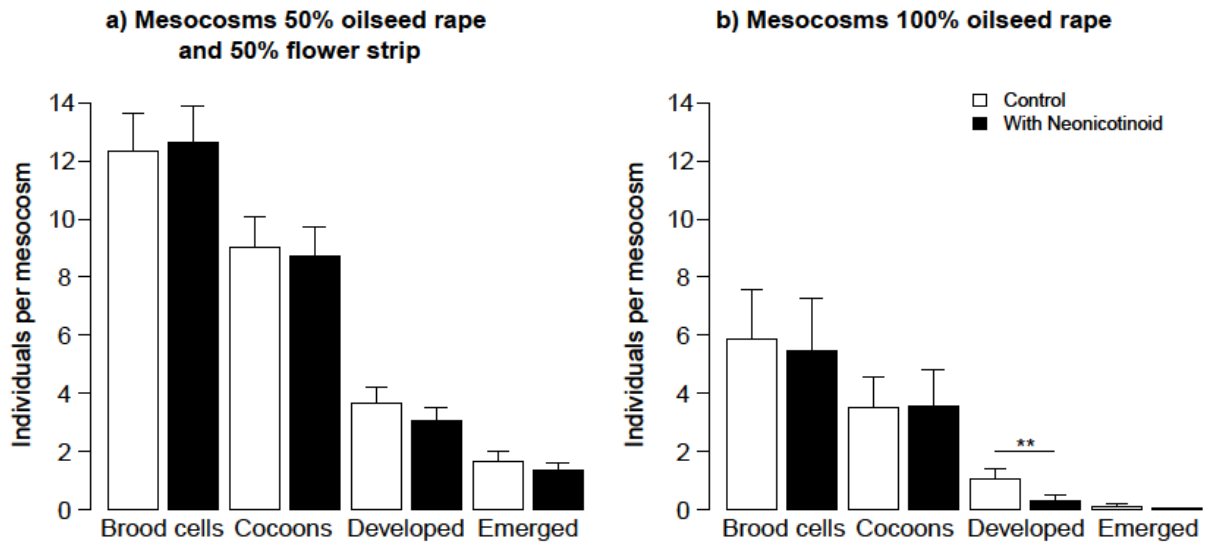


Figure 2: Number of individuals per mesocosm and sampling round for different *O. bicornis* life stages comparing mesocosms with treated and untreated oilseed rape. Panel (a) shows mesocosms with 50% oilseed rape and 50% flower strip. Panel (b) shows mesocosms with 100% oilseed rape. Shown are arithmetic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

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 A. 3). 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 A. 3). 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 A. 4; Table A. 11; Figure A. 4). 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 A. 7 for details).

The number of emerged offspring increased with abundance, species richness, and Shannon Diversity of flowering plants (Table A. 5). Comparing model estimates, Shannon Diversity and species richness predicted the number of emerged individuals better than flower abundance (Table A. 5). Offspring numbers were significantly positively correlated with Shannon Diversity of flowering plants for all stages of development (Figure 4b; Table A. 10). Flower numbers were not significantly correlated with Shannon Diversity of flowering plants (Figure 4a; Table A. 9).

Results of the random forest analysis (with 37.47% variance explained) emphasized the importance of two plant species, *Phacelia tanacetifolia* and *Raphanus sativus*, predicting the

number of emerged *Osmia* offspring (Figure 3; Figure A. 2). 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 A. 1).

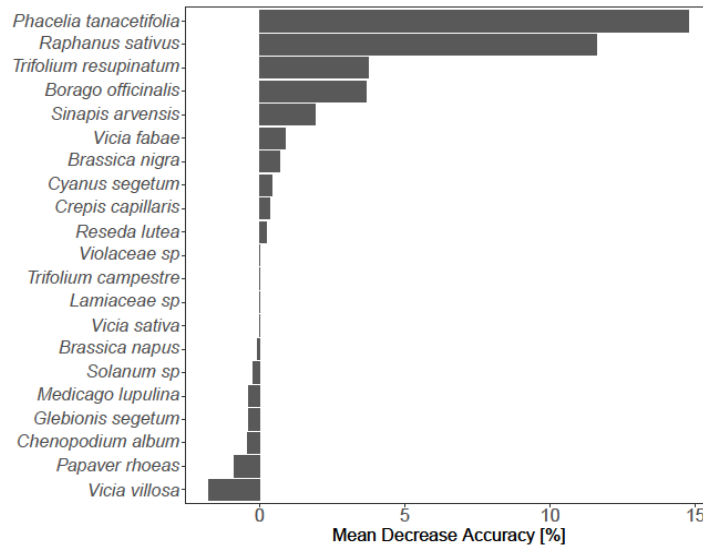


Figure 3: Mean Decrease Accuracy of flowering plant species in mesocosms for random forest models predicting the numbers of emerged *Osmia* offspring. Higher values indicate a higher importance.

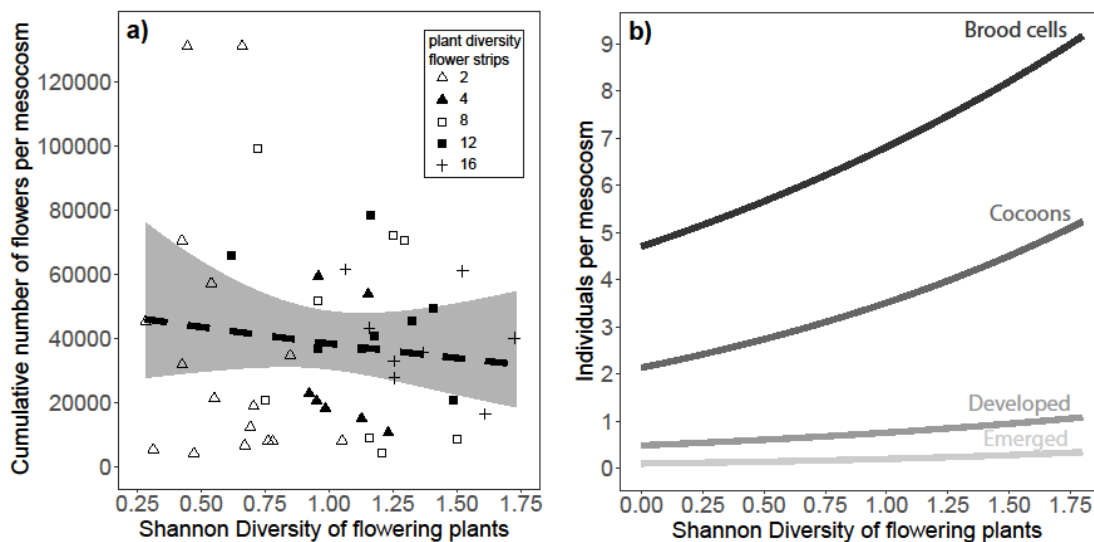


Figure 4: Cumulative number of flowers per mesocosm (a) and number of individuals per mesocosm (b) in relation to Shannon Diversity of flowering plants. Shapes/colors of raw data points in (a) are corresponding to seeded plant diversity in respective mesocosms. Dashed line represents non-significant relationship ($p=0.429$) and envelope shows 95% confidence interval. Solid lines for all offspring life stages in (b) represent significant relationships ($p<0.05$).

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, i.e. 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 flower resource diversity, compared to just resource abundance. This is in line with other studies showing the importance of pollen diversity for pollinators, e.g. 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; Tscharrntke *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 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 unfavorable 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 emphasized 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 analyze 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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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>).

Appendix

Table A. 1: Overview of sampling dates and corresponding number of days after start of experiment. Numbers of flowers for all flowering plant species were estimated and nesting progress was observed for each mesocosm and sampling round.

Sampling Round	Sampling date	Days after start of experiment
1	13.07.2018	10
2	17.07.2018	14
3	20.07.2018	17
4	23.07.2018	20
5	26.07.2018	23
6	30.07.2018	27

Table A. 2: Overview of plant species seeded and flowering weed species occurring in each mesocosm.

Mesocosm ID	Seeded Species										Flowering Weed Species									
	<i>Brassica napus</i> (Brassicaceae)	<i>Glebionis segetum</i> (Asteraceae)	<i>Crepis capillaris</i> (Asteraceae)	<i>Cyanus segetum</i> (Asteraceae)	<i>Borago officinalis</i> (Boraginaceae)	<i>Phacelia tanacetifolia</i> (Boraginaceae)	<i>Raphanus sativus</i> (Brassicaceae)	<i>Sinapis arvensis</i> (Brassicaceae)	<i>Brassica nigra</i> (Brassicaceae)	<i>Trifolium resupinatum</i> (Fabaceae)	<i>Trifolium campestre</i> (Fabaceae)	<i>Medicago lupulina</i> (Fabaceae)	<i>Vicia faba</i> (Fabaceae)	<i>Vicia sativa</i> (Fabaceae)	<i>Vicia villosa</i> (Fabaceae)	<i>Papaver rhoeas</i> (Papaveraceae)	<i>Reseda lutea</i> (Resedaceae)	<i>Chenopodium album</i> (Amaranthaceae)	<i>Lamiaceae sp</i> (Lamiaceae)	<i>Solanum sp</i> (Solanaceae)
01	treated 100%																x			
100%_I_1/16	untreated 100%																			
02	untreated 100%																			
100%_I_2/1	treated 100%																			
03	untreated 100%																			
100%_II_3/8	treated 100%																			
04	untreated 100%																			
100%_II_2/11	treated 100%																			
05	untreated 100%																			
100%_III_1/18	treated 100%																			
06	untreated 100%																			
100%_III_3/3	treated 100%																			
07	untreated 100%																			
100%_IV_2/18	treated 100%																			
08	untreated 100%																			
100%_IV_1/3																				
09_2_I_1/10	treated 50%			x									x				x			
10_2_I_1/13	untreated 50%				x								x				x			x
11_2_II_3/20	treated 50%	x																x		x
12_2_II_2/17	untreated 50%		x															x		x
13_2_III_2/16	treated 50%							x							x					
14_2_III_2/13	untreated 50%								x							x				
15_2_IV_2/2	treated 50%												x							
16_2_IV_2/3	untreated 50%													x						
17_2_V_1/2	treated 50%		x															x		

46_12_III_3/17	untreated 50%	x		x	x			x	x	x	x	x	x	x			x	x
47_12_IV_1/4	treated 50%	x	x		x	x	x	x	x	x		x		x		x	x	x
48_12_IV_1/7	untreated 50%	x	x		x	x	x	x	x		x		x		x	x	x	x
49_16_I_3/14	treated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
50_16_I_1/15	untreated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
51_16_II_2/6	treated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
52_16_II_3/7	untreated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
53_16_III_3/10	treated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
54_16_III_2/19	untreated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
55_16_IV_3/6	treated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
56_16_IV_1/17	untreated 50%	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table A. 3: Model results from generalized linear mixed models with random effects mesocosm and sampling round with family poisson. (a) Mesocosms with 50% oilseed rape and 50% flower strip (n=48). (b) Mesocosms with 100% oilseed rape (n=8). The effects of treatment of oilseed rape with neonicotinoid on the number of individuals of bee offspring in different life stages (brood cells, cocoons, developed, emerged) are shown. Estimates, standard errors, Z values and p values are reported. Significant results (p<0.05) are shown in bold.

	Brood cells				Cocoons				Developed				Emerged			
	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p
<i>(a) Response: # Individuals per mesocosm and sampling round for 50% oilseed rape/50% flower strip mesocosms</i>																
(Intercept)	1.827	0.503	3.632	0.001	1.087	0.660	1.647	0.100	-0.441	0.797	-0.553	0.580	-1.814	0.947	-1.916	0.055
Treatment: untreated	-0.031	0.155	-0.202	0.840	0.048	0.178	0.270	0.787	0.054	0.323	0.168	0.867	-0.046	0.449	-0.102	0.919
<i>(b) Response: # Individuals per mesocosm and sampling round for 100% oilseed rape mesocosms</i>																
(Intercept)	-0.999	1.508	-0.663	0.508	-1.213	1.383	-0.877	0.380	-3.274	1.585	-2.065	0.039	-3.931	1.523	-2.581	0.010
Treatment: untreated	0.074	0.135	0.549	0.583	-0.019	0.229	-0.083	0.934	1.179	0.404	2.916	0.004	1.099	1.154	0.952	0.341

Table A. 4: Model results from generalized linear mixed models with random effects mesocosm and sampling round with family poisson. Mesocosms with 100% oilseed rape (n=8) are compared to those with 50% oilseed rape and 50% flower strip (n=48) regarding the number of individuals of bee offspring in different life stages (brood cells, cocoons, developed, emerged). Estimates, standard errors, Z values and p values are reported. Significant results ($p < 0.05$) are shown in bold.

Response: # Individuals per mesocosm and sampling round																
	Brood cells				Cocoons				Developed				Emerged			
	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p
(Intercept)	1.072	0.537	1.995	0.046	0.254	0.693	0.366	0.714	-1.740	0.870	-1.999	0.046	-4.508	1.176	-3.833	0.001
Mesocosms 50% flower strip/50% oilseed rape	0.703	0.194	3.625	0.001	0.823	0.228	3.616	0.001	1.313	0.434	3.026	0.002	2.683	0.780	3.439	0.001

Table A. 5: Model results from generalized linear mixed models with random effects mesocosm and sampling round with family poisson. The effects of (a) flower resource abundance (flower estimates) and species richness of flowering plants, and (b) flower resource abundance and Shannon Diversity of flowering plants on the number of emerged individuals per mesocosm and sampling round for 50% oilseed rape/50% flower strip mesocosms (n=48) were tested in two models. Explanatory variables were scaled to zero mean and unit variance. Estimates, standard errors, Z values and p values are reported. Significant results (p<0.05) are shown in bold.

Response: # Emerged individuals per mesocosm and sampling round for 50% oilseed rape/50% flower strip mesocosms				
	Estimate	Std. Error	Z	p
(a)				
(Intercept)	-1.821	0.850	-2.141	0.032
Species richness of flowering plants	0.428	0.144	2.965	0.003
Flower estimates	0.257	0.086	3.003	0.003
(b)				
(Intercept)	-1.769	0.791	-2.237	0.0253
Shannon Diversity of flowering plants	0.522	0.118	4.428	<0.001
Flower estimates	0.390	0.091	4.279	<0.001

Table A. 6: Application rates of oilseed rape seeds.

Treatment	Clothianidin content		Thiram content [g/kg oilseed rape seeds]	Beta-Cyfluthrin content [g/kg oilseed rape seeds]
	[g/kg oilseed rape seeds]	[µg/oilseed rape seed]		
With neonicotinoid	5	23.15	4	1
Control without neonicotinoid	-	-	4	1

Table A. 7: Clothianidin (neonicotinoid) residues in oilseed rape flower samples. The LOD (limit of detection) was 0.3 ng g⁻¹ and the LOQ (limit of quantification) was 0.7 ng g⁻¹, based on the average weight (4.0 g) of all samples.

Treatment	Number of samples	Number of samples >LOD	Mean concentration of clothianidin	Standard error of the mean	Median concentration of clothianidin	Maximum concentration of clothianidin
Treated oilseed rape	10	10	3.00	0.15	3.00	3.75 ng g ⁻¹
Untreated oilseed rape	10	1	<LOQ		<LOQ	<LOQ

Table A. 8: Number of replicates for different levels of flowering plant diversity. There were 56 mesocosms in total, 48 of which containing a flower strip.

Flowering plant species seeded	Oilseed rape cover	Replicates treated oilseed rape	Replicates untreated oilseed rape
-	100%	4	4
2	50%	8	8
4	50%	4	4
8	50%	4	4
12	50%	4	4
16	50%	4	4

Table A. 9: Model results from a generalized linear mixed model with family negative binomial. The effects of Shannon Diversity of flowering plants (calculated using cumulative number of flowers) on the cumulative number of flowers per mesocosm for 50% oilseed rape/50% flower strip mesocosms are shown. Estimates, standard errors, Z values and p values are reported.

Response: Cumulative number of flowers per 50% oilseed rape/50% flower strip mesocosm				
	Estimate	Std. Error	Z	p
(Intercept)	10.808	0.331	32.69	<0.001
Shannon Diversity of flowering plants	-0.250	0.317	-0.79	0.429

Table A. 10: Model results from generalized linear mixed models with random effects mesocosm and sampling round with family poisson. The effects of Shannon Diversity of flowering plants on the number of individuals of bee offspring in different life stages (brood cells, cocoons, developed, emerged) are shown. Estimates, standard errors, Z values and p values are reported. Significant results ($p < 0.05$) are shown in bold.

Response: # Individuals per mesocosm and sampling round																
	Brood cells				Cocoons				Developed				Emerged			
	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p
(Intercept)	1.549	0.465	3.331	<0.001	0.759	0.610	1.246	0.213	-0.714	0.745	-0.958	0.338	-2.254	0.865	-2.605	0.009
Shannon Diversity of flowering plants	0.370	0.079	4.681	<0.001	0.497	0.096	5.155	<0.001	0.441	0.160	2.751	0.006	0.663	0.239	2.775	0.006

Table A. 11: Model results from generalized linear models with family negative binomial. The effects of mesocosm type (50% oilseed rape/50% flower strip vs. 100% oilseed rape) on the number of estimated flower numbers per mesocosm were tested individually for all sampling rounds ((a) – (f)). Estimates, standard errors, Z values and p values are reported. Significant results ($p < 0.05$) are shown in bold. Model results for sampling round 6 are not presented as no converging model could be fitted. Patterns are resembling those of sampling rounds 1-5 however (see Fig. A.4).

Response: # Estimated flower numbers per mesocosm and sampling round				
	Estimate	Std. Error	Z	p
(a) Sampling Round 1				
(Intercept)	8.669	0.193	44.811	<0.001
Mesocosms 50% flower strip/50% oilseed rape	0.501	0.209	2.393	0.017
(b) Sampling Round 2				
(Intercept)	7.636	0.266	28.745	<0.001
Mesocosms 50% flower strip/50% oilseed rape	1.114	0.287	3.875	<0.001
(c) Sampling Round 3				
(Intercept)	5.508	0.345	15.956	<0.001
Mesocosms 50% flower strip/50% oilseed rape	3.240	0.373	8.679	<0.001
(d) Sampling Round 4				
(Intercept)	3.597	0.384	9.375	<0.001
Mesocosms 50% flower strip/50% oilseed rape	4.858	0.414	11.723	<0.001
(e) Sampling Round 5				
(Intercept)	0.223	0.580	0.384	0.701
Mesocosms 50% flower strip/50% oilseed rape	8.337	0.614	13.575	<0.001

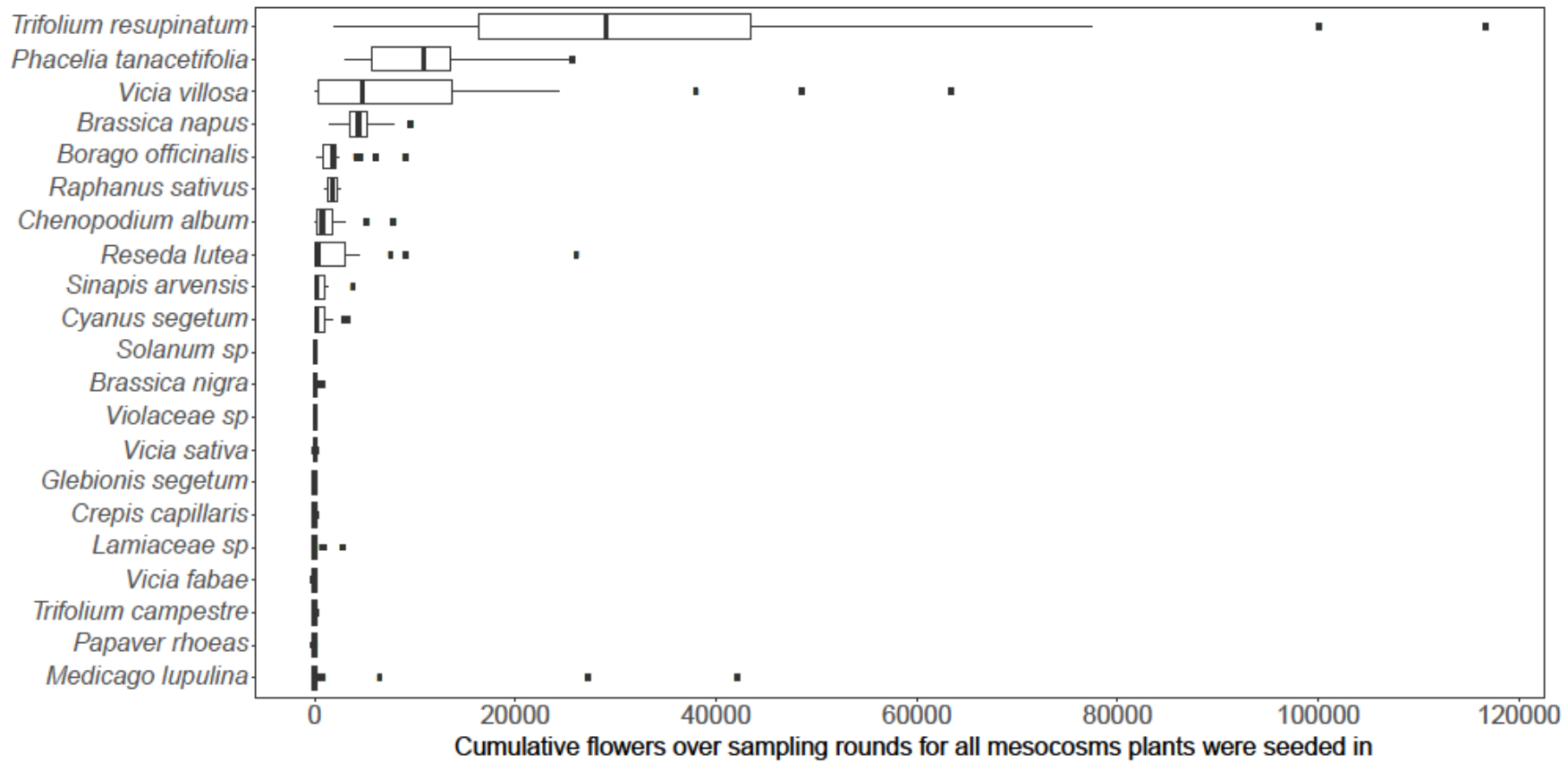


Figure A. 1: Boxplots of cumulated flowers over all sampling rounds per mesocosm for each plant species. Displayed are minimum, first quartile, median, third quartile, maximum and outliers. For each plant species, only those mesocosms were considered, where the species of concern was seeded (or occurred as flowering weed species). Plant species are sorted by median in decreasing order.

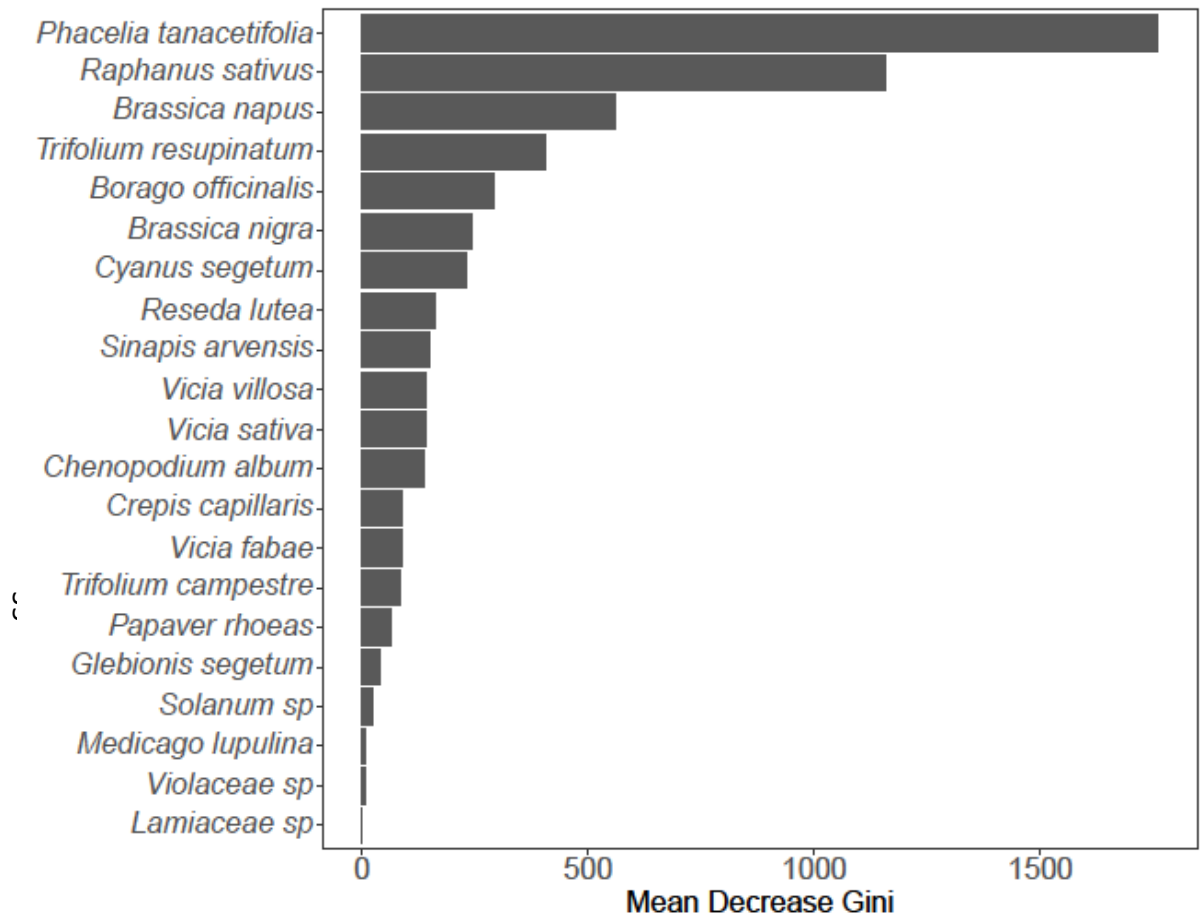


Figure A. 2: Mean Decrease Gini of flowering plant species in mesocosms for random forest models predicting the numbers of emerged *Osmia* offspring. Higher values indicate higher variable importance.

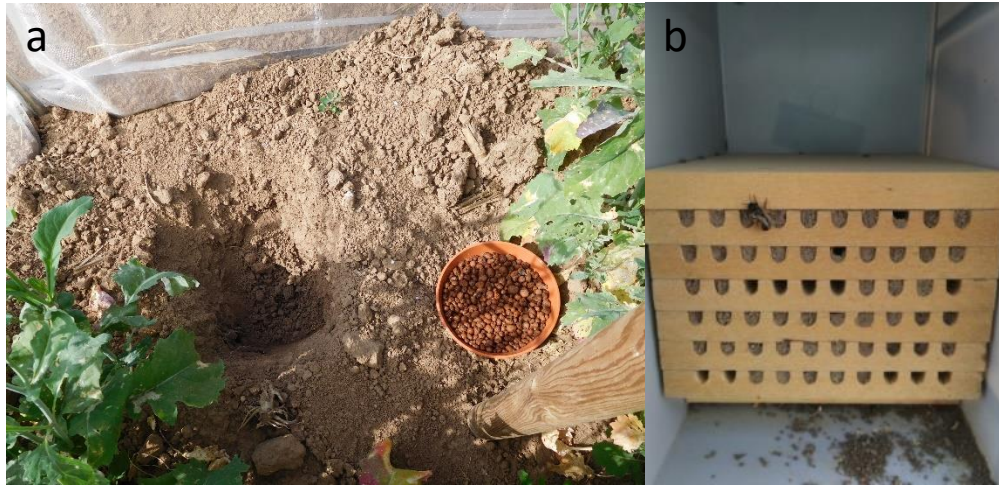


Figure A. 3: (a) Plastic bowl with expanded clay and mud hole were kept wet throughout the experiment to ensure water and mud supply for bees to drink and build nest walls and plugs. (b) Nesting block with plugged nests and a female *O. bicornis*.

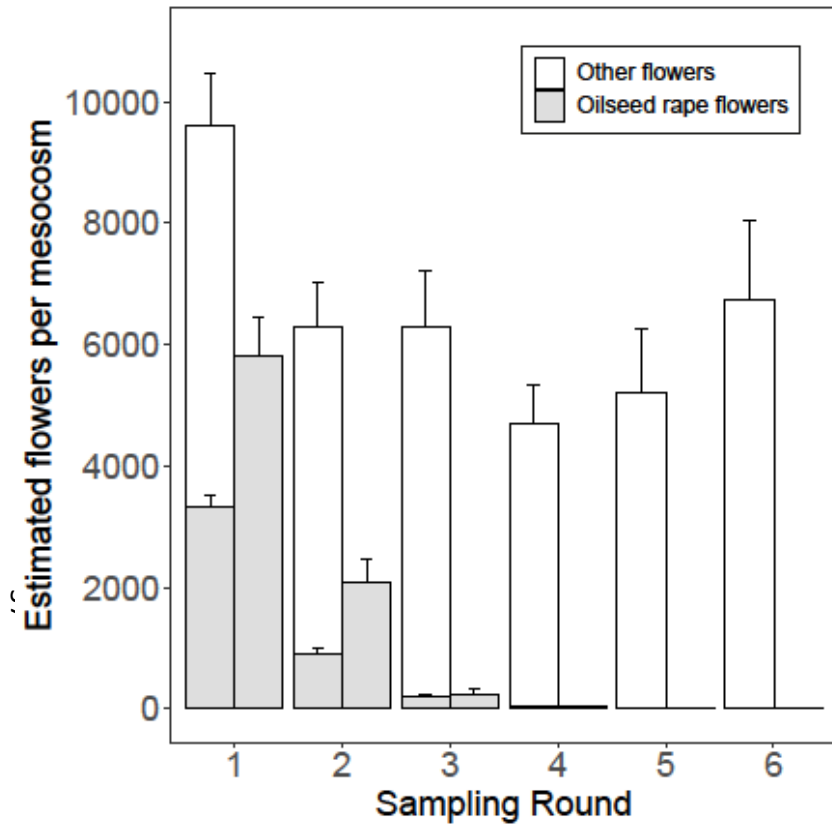


Figure A. 4: Mean (bar) and standard error of the mean (error bar) of flower estimates per mesocosm for each sampling round. The left bars represent 50% oilseed rape/50% flower strip mesocosms (in white) and their share of oilseed rape flowers (grey part of white bars). The right bars (in grey) represent flowers in 100% oilseed rape mesocosms.

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Chapter 3

Calcareous grassland fragments as sources of bee pollinators for the surrounding agricultural landscape

Felix Klaus^{a,*}, Teja Tscharntke^a, Johannes Uhler^{a,b} and Ingo Grass^c



^aUniversity of Göttingen, Agroecology, Göttingen, Germany

^bUniversity of Würzburg, Department of Animal Ecology and Tropical Biology, Würzburg, Germany

^cUniversity of Hohenheim, Ecology of Tropical Agricultural Systems, Stuttgart, Germany

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Abstract

In Central Europe, agricultural intensification has led to increasing fragmentation and loss of semi-natural habitats. In turn, ecosystem services such as pollination are being lost when insect pollinators depend on semi-natural habitats. Calcareous grasslands are a highly threatened, biodiverse type of semi-natural habitat that may substantially support wild pollinators and pollination services to surrounding habitats. Here, we studied spillover of pollinators and pollination services from calcareous grassland fragments of different sizes into the surrounding landscape for solitary and social bees, as well as hoverflies. We selected eight grassland fragments of varying sizes (0.05-9.41 ha) surrounded by simplified agricultural landscapes near Göttingen, Germany. To quantify pollination spillover, we established *Phacelia tanacetifolia* (Boraginaceae) plots at distances ranging from 0-400 meters from grassland edges alongside field roads. Our experiment revealed the importance of calcareous grassland fragments as sources of solitary bees, of which visitation to *Phacelia* plants decreased with increasing distance from fragments. Larger grassland fragments supported more than twice as many solitary bees as smaller ones. The limited foraging range of solitary bees appeared to be compensated by other groups, such as bumblebees and hoverflies, which were less affected by distance, suggesting a greater forage radius and/or independence from the grassland fragments as habitat. Seed production of *Phacelia* plants increased with overall pollinator visitation and solitary bee visits specifically, but did not decrease with distance. In conclusion, calcareous grasslands need to be conserved or restored as major bee habitats, which support spillover of pollination services into the adjacent agricultural landscape.

Keywords: Spillover; fragmentation; semi-natural habitat; pollination; wild bee; hoverfly

Highlights

- Calcareous grasslands support spillover of solitary bees into agricultural landscapes
- Large fragments (at least 8.4 ha) support spillover of more than twice as many solitary bees than small ones (up to 3.0 ha)
- *Phacelia* seed set increased with pollinator visitation, particularly by solitary bees
- Pollination at larger distances may be compensated by bumblebees and hoverflies
- Conservation and restoration of calcareous grasslands are key to sustain solitary bees

Introduction

In the agricultural landscape, insect pollination is an ecosystem service that plays an important role for yields of many crop plants and the reproduction of most non-crop plants (Klein et al., 2007). Important groups of pollinating insects in the central European agricultural landscape include social and solitary bees and hoverflies (Rader et al., 2016). Flowering plant species benefit from a diverse set of pollinators ensuring successful pollination in different landscapes of varying complexity (Brittain et al., 2013; Ellis et al., 2017; Holzschuh et al., 2012). The abundance as well as diversity of pollinating insect species is therefore important for successful pollination of many crop and wild plant species in the agricultural landscape.

Pollinating insects are negatively affected by the intensification and expansion of agricultural land and the associated loss of habitat providing nesting and food resources (Kremen et al., 2002; Ricketts et al., 2008). Due to unspecific or widely available food and nesting requirements, some groups of pollinators, such as bumblebees and hoverflies, are better suited for conditions in intensified agricultural landscapes (Jauker et al., 2009; Steffan-Dewenter et al., 2002; Steffan-Dewenter and Tscharntke, 1999; Westphal et al., 2006). Solitary bees, on the other hand, are affected more, because small potential habitats such as field margins or hedgerows can often not sustain viable populations, which rely on larger habitats such as grassland fragments (Krewenka et al., 2011; Öckinger and Smith, 2007). Pollination service provision is therefore threatened by a decreasing number and diversity of solitary bees in the agricultural landscape (Kremen et al., 2002; Ponisio et al., 2019).

The intensification of the agricultural landscape and abandonment of formerly extensively managed grasslands make remaining managed grassland fragments valuable sources of biodiversity (Grass et al., 2018; Poschlod and WallisDeVries, 2002). They play an important role in exporting individuals to other habitats to sustain smaller populations, as well as to provide pollination services in the landscape surrounding the main habitat (Albrecht et al., 2007; Grass et al., 2019; Kremen et al., 2002; Öckinger and Smith, 2007). The export of pollinators and their pollination services from calcareous grasslands into the surrounding agricultural landscape have not yet been studied however.

Calcareous grasslands are biodiversity hotspots in the Central European agricultural landscape and are important semi-natural habitats harboring a diversity of plant and pollinator species (WallisDeVries et al., 2002). They are highly threatened by fragmentation and nutrient inputs from surrounding intensified agriculture (Krauss et al., 2010). Habitat loss and reduced grassland size may negatively affect the abundance and spillover of species relying on them as primary habitat. Pollinating insects on calcareous grasslands will forage in the surrounding landscape especially when food resources on the grassland itself are scarce, or resources in the surroundings are abundant or highly attractive (Holzschuh et al., 2011). Bigger populations at larger grassland fragments may also be more likely to disperse further into the surroundings in search of alternative nesting or foraging habitats. Regarding daily foraging movements into the surrounding landscape, smaller and solitary species, such as solitary bees, are more restricted in their movement and will be bound to forage only in the close surroundings of

their nest (Gathmann and Tschardtke, 2002; Kremen et al., 2002; Zurbuchen et al., 2010). Common and large bumblebee species, on the other hand, are also abundant in intensively managed agricultural landscapes and are thus less dependent on larger semi-natural habitats (Steffan-Dewenter et al., 2002; Steffan-Dewenter and Tschardtke, 1999; Westphal et al., 2006). Knowledge about pollination services of these different pollinator groups to wildflowers in the agricultural landscape surrounding calcareous grassland fragments is still missing, however. Knowing about the importance of nearby grasslands for the abundance of different pollinators could help decision makers to achieve more targeted conservation measures in the future.

In this study, we address the role of different pollinator groups for providing pollination services in the surroundings of calcareous grassland fragments using *Phacelia tanacetifolia* (Boraginaceae) as a phytometer plant. *Phacelia* is a highly attractive, nectar and pollen rich plant species with generalist flowers visited by a variety of pollinating insect groups (Carreck and Williams, 2002). It is commercially grown and used as honeybee forage and green manure crop (Stevenson, 1991). Its seed set is pollinator-dependent and hence bees or other pollinating insects are required for maximum seed set (Stevenson, 1991; Saatzucht Steinach, personal communication, 11/2019). We focus on the export of pollinating insects from calcareous grasslands, their foraging in the agricultural surroundings, and their impact on pollination success of *Phacelia*. We study the influence of distance to grassland fragments as well as fragment size on the abundance of different groups of pollinating insects. The following hypotheses are addressed:

- (1) Spillover of solitary bees is restricted to close surroundings of calcareous grasslands by their limited foraging radius, whereas other pollinator groups, such as the common and large bumblebees as well as hoverflies, are not restricted to these habitats.
- (2) Floral visitation of grassland-dependent pollinators is higher in the surroundings of larger than smaller grassland fragments.
- (3) The seed set of *Phacelia tanacetifolia* is enhanced by pollinator visits. *Phacelia* seed set will decrease at larger distances from calcareous grasslands, due to the loss of habitat specialists, which are restricted to the close surroundings of the grassland fragments.
- (4) Pollinators from larger grassland fragments disperse further into the surroundings compared to those from smaller fragments.

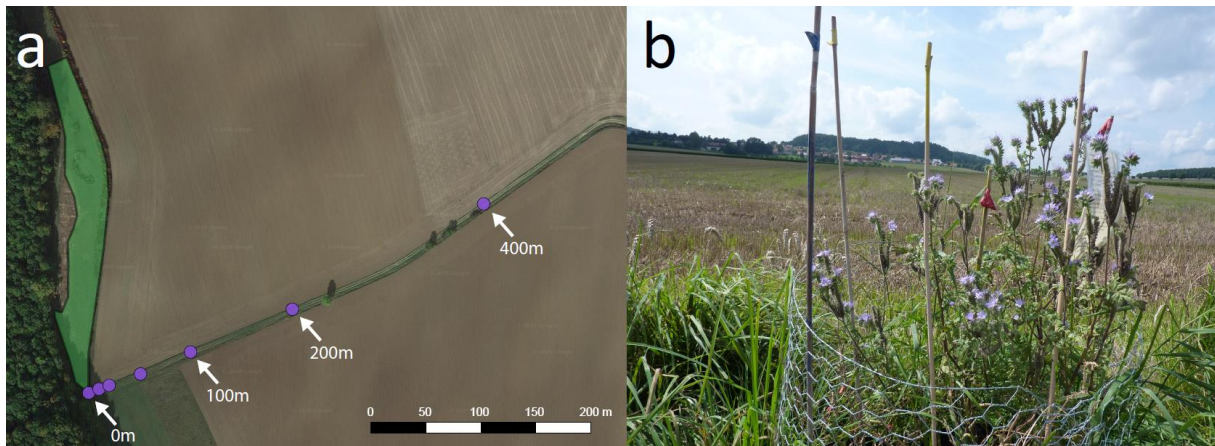


Figure 1: (a) Example grassland fragment (green shade, site # 08) with plots consisting of two *Phacelia* plants at different distances (purple dots) (Google Maps, 2020). (b) *Phacelia* plants protected by chicken wire.

Materials and Methods

The study took place in central Germany in the surroundings of the city of Göttingen (lat: 51.532717, long: 9.935154, 20 km radius around the city). The region is dominated by intensive agricultural land use. There are 285 extensively managed calcareous grasslands in the study region making up 0.26% of the total area (Krauss et al., 2003). Eight of these grassland fragments were used as study sites. The intensity of the management of the sites (grazing or mowing) was required to not differ substantially to avoid differences in habitat quality. One requirement was the availability of a field road perpendicular to the grassland edge (at least 200m in length) accompanied by similar grassy field margin strips and cereal fields and without other semi-natural habitat elements potentially interfering in its surrounding. Eight fragments, ranging in size from 0.05 to 9.41 hectares (see Table A.1 for all fragment sizes), with suitable roads were used to study pollination services in the surroundings of grasslands of different sizes. All sites were more than 1000 m apart from each other to avoid spatial autocorrelation. Adjacent forest fragments or hedgerows offered nesting habitat for cavity-nesting bees at all sites.

Phacelia tanacetifolia (Boraginaceae) was chosen as phytometer plant because of its attractiveness to pollinators and its dependence on pollinating insects to increase seed production (Stevenson, 1991). Plants were sown in early May 2017 and planted in small pots on May 22nd. They were transferred into larger 8l pots and standard NPK fertilizer as well as water retaining granulate ("Broadleaf P4 - Polyacrylamid", Broadleaf Industries Inc., Costa Mesa, USA) were added on May 31st. Because of plants starting to flower early, possibly due to a lack of nutrients before being replanted into larger pots, flowering branches were cut to induce further plant growth. The plants were transferred to the study sites just before they started flowering again on June 20th and watered depending on weather conditions, usually every third day.

Plots consisting of two potted plants were set up at distances of 0, 10, 20, 50, 100, 200 and 400 meters from the edge of each grassland alongside a road (Fig. 1a). It was made sure, that there were no semi-natural habitats such as hedgerows, forest margins, or nutrient-poor grasslands (except for the calcareous grassland) within 200m of all plots. Chicken wire fences were used to protect plants against wind and herbivores (Fig. 1b).

Observations of pollinating insects interacting with flowers were carried out between July 3rd and August 8th every 10 to 15 days, depending on weather conditions. Sampling was restricted to days with suitable environmental conditions for pollinators. The weather was required to be sunny, with no rain, no strong winds, and temperatures of at least 15°C. The time of season was chosen to avoid potentially confounding effects of mass-flowering oilseed rape. Plants at a plot were observed for three intervals of five minutes each, recording all flower-pollinator interactions. One to four observation rounds were carried out for each plot, depending on flower availability (Table A.2). Some plants were destroyed by farming machinery and could therefore not be observed anymore (Table A.2). In cases where sampling was not possible, no data points were generated, which could have been used in the statistical analyses.

After plants had stopped flowering, the seed buds of each plant were counted. Seeds were then cleaned using a sample-cleaning device (“MLN”, Pfeuffer, Kitzingen, Germany) and counted using a seed-counting machine (Contador-e, Pfeuffer, Kitzingen, Germany).

Generalized linear mixed models with site and sampling round as random effects were used to evaluate effects of distance from grasslands and grassland size, as well as their interaction, on the number of pollinators from different groups visiting flowers. To model the count data, family poisson was used for all models at first, but was replaced by negative binomial for the bumblebee and all pollinator models to correct for overdispersion or deviation. The interaction of distance from grassland and grassland size was only kept in the bumblebee model, where it was significant (Table 1, Table A.3). Generalized linear mixed models with site as random effect and family negative binomial were used to evaluate effects of (raw data-) mean numbers of pollinators from different groups visiting flowers and distance from grasslands on the number of seeds per *Phacelia* plant. The explanatory variable mean number of visits was square root transformed in the solitary bee model, and distance from grassland was scaled to zero mean and unit variance in all models to improve model fit and achieve normality of residuals and homogeneity of variance. Observation numbers of 5 minute intervals were extrapolated to hourly visits.

All statistical analyses were performed in R version 3.6.1 (“R Development Core Team 2019”) using dedicated packages *lme4* (version 1.1-21, Bates et al. 2019), *DHARMA* (version 0.3.0, Hartig & Lohse 2020), *ggplot2* (version 3.2.1, Wickham et al. 2019), and *MuMIn* (version 1.43.17, Barton 2020). Models were created based on our hypotheses and were subsequently simplified, when interaction terms did not come out as significant. Significances of predictor variables were tested using z-tests. All models met assumptions of normality of residuals and homoscedasticity. Model fits and 95% confidence intervals were calculated for all models.

Results

One to four observation rounds were conducted at each plot (see Table A.2 for details). Overall, 361 pollinators were observed interacting with flowers, including 160 bumblebees, 54 hoverflies and 121 solitary bees (see Table A.4 for details). *Bombus terrestris* made up the majority of the observed bumblebees with 98 flower visits, followed by *B. pratorum* (31 visits) and *B. pascuorum* (19). Solitary bees were mainly furrow bees, dominated by the genus *Lasioglossum* (96). Observed hoverflies were mostly of the genera *Episyrphus* (30) and *Sphaerophoria* (13). The three main pollinator groups (bumblebees, hoverflies, and solitary bees) dominated the observed community and were analyzed separately. Other groups that visited flowers more rarely and were therefore not looked at individually were honeybees (*Apis mellifera*, 11 visits), butterflies (Lepidoptera, 12), and non-syrphid flies (*Empis* spp., 3).

The number of pollinator visits per observation interval significantly decreased with increasing distance from the grasslands for solitary bees ($P = 0.011$; Fig. 2b, Table 1). This translates to about a 61% decrease at distances from 0 to 400 meters. Hoverflies ($P = 0.459$) and all pollinators combined ($P = 0.076$) were not significantly correlated with distance (Fig. 2a, Table 1). For bumblebees, in contrast to the additive effects ($P = 0.058$ for grassland size and $P = 0.116$ for distance from grassland), the interaction of distance from grassland and grassland size was significant ($P = 0.034$; Fig. 6b, Table 1), with numbers of bumblebee visits increasing more strongly with grassland size at large distances, compared to smaller distances. The interaction term was excluded from the other models, where it did not have a significant effect, but the output from the original model of all pollinators combined including the interaction term was plotted as comparison (Fig. 6a, Table A.3). Grassland size was significant for solitary bees ($P = 0.026$; Fig. 3b, Table 1), with more than twice as many visits to *Phacelia* plants near large fragments (predicted model fits for fragments of at least 8.4 hectares) compared to small ones (up to 3.0 ha of size), as well as for all pollinators combined ($P = 0.003$; Fig. 3a, Table 1), but not for hoverflies ($P = 0.732$).

The number of seeds per *Phacelia* plant significantly increased with an increasing mean number of pollinator visits per observation interval ($P = 0.027$; Fig. 4a, Table 1). Looking at the three main groups individually, a significant correlation could only be shown for mean number of solitary bee visits ($P = 0.032$; Fig. 4b, Table 1). Bumblebees ($P = 0.206$) and hoverfly ($P = 0.407$) visits were not significantly correlated to seed numbers (Fig. 4c+d, Table 1).

The number of seeds per plant was not significantly correlated to distance from grassland (Fig. 5, Table 2).

The variance was mostly explained by the random factors site and sampling round for all models (Table A.5).

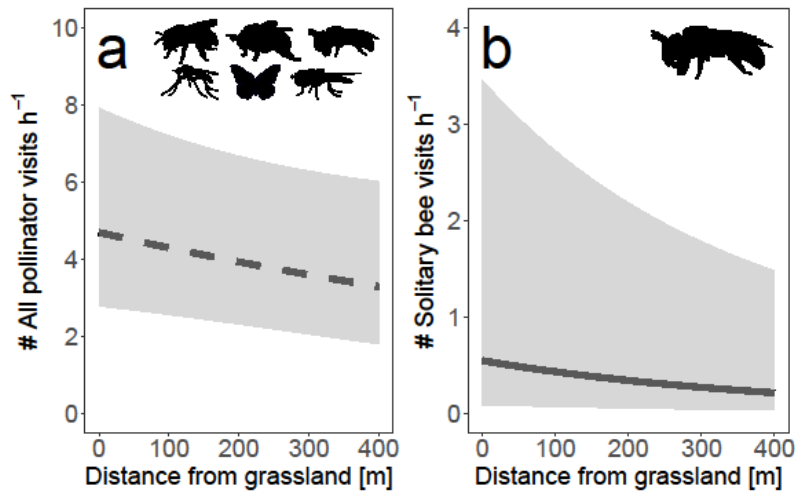


Figure 2: Number of visits of all pollinators combined and solitary bees and hoverflies individually at different distances from grassland fragments. Solid lines represent significant relationships ($p < 0.05$). Dashed lines represent marginally significant relationships ($p \geq 0.05$ and < 0.10). Envelopes show 95% confidence intervals. Observation numbers of 5 minute intervals were extrapolated to hourly visits.

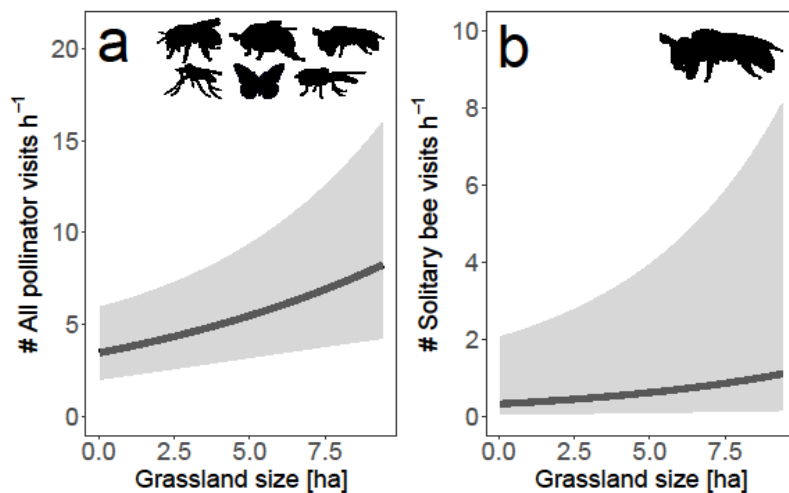


Figure 3: Number of visits of all pollinators combined and solitary bees and hoverflies individually at *Phacelia* plants in the surroundings of grassland fragments of different size. Solid lines represent significant relationships ($p < 0.05$). Envelopes show 95% confidence intervals. Observation numbers of 5 minute intervals were extrapolated to hourly visits.

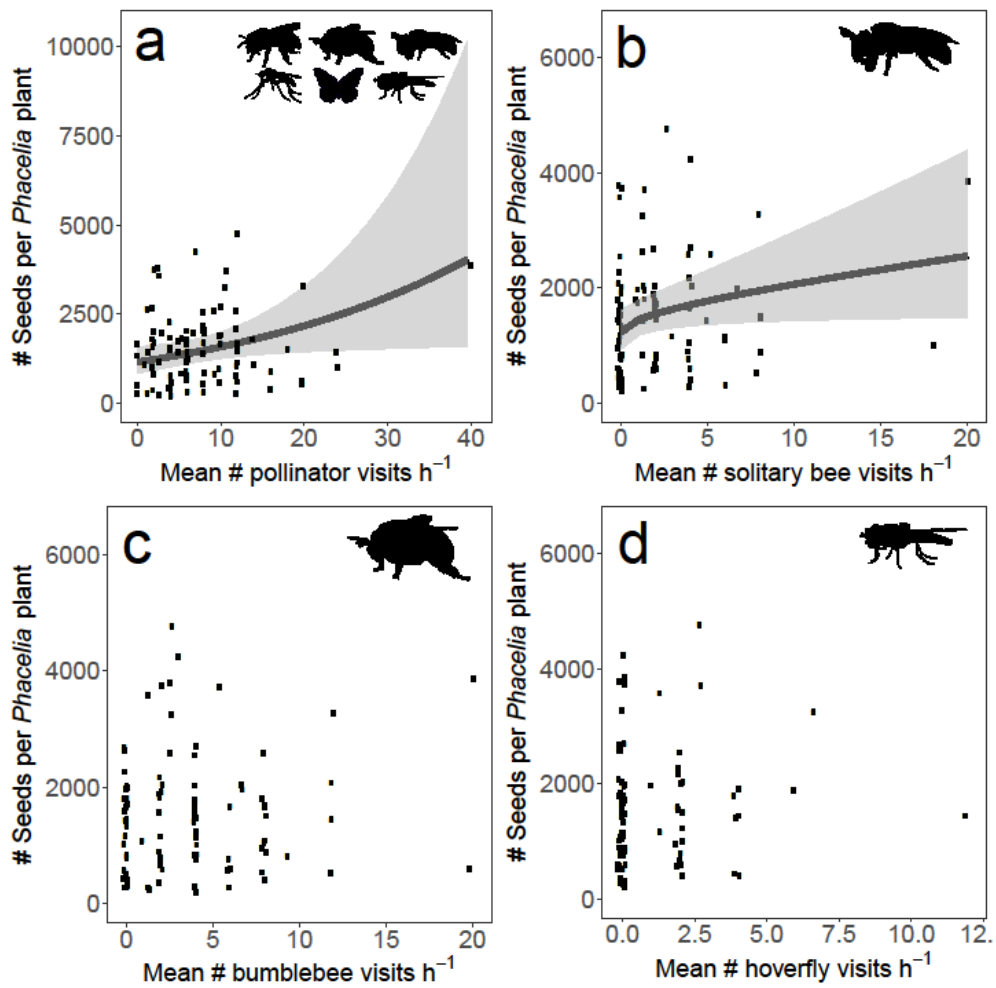


Figure 4: Number of seeds per *Phacelia* plant and mean number of pollinator visits (all pollinators combined, and three main groups individually). Solid lines represent significant relationships ($p < 0.05$). Envelopes show 95% confidence intervals. Raw, untransformed data points are represented by black dots. Observation numbers of 5 minute intervals were extrapolated to hourly visits.

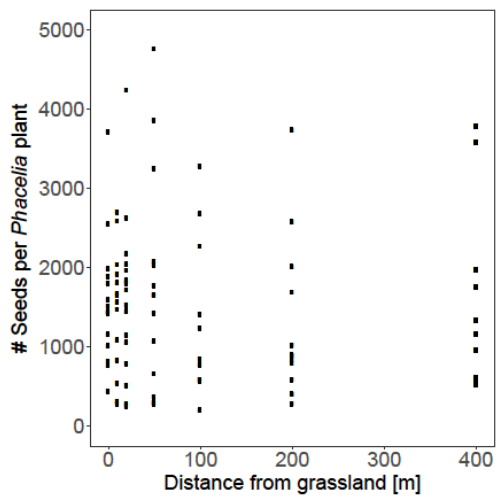


Figure 5: Number of seeds per *Phacelia* plant in relation to distance from grassland fragments. Raw, untransformed data points are represented by black dots.

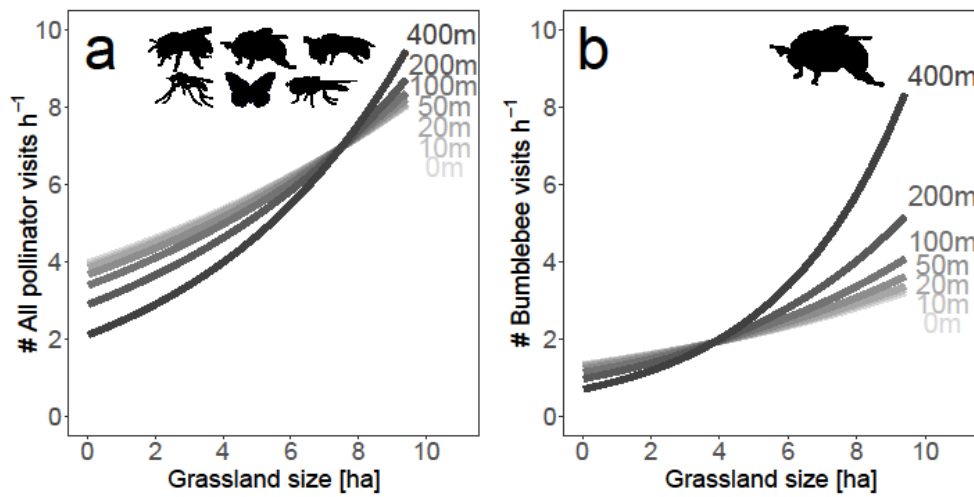


Figure 6: Interactive effects of grassland size and distance from grasslands on visitation of a) all pollinators and b) bumblebees to *Phacelia* plants. Observation numbers of 5 minute intervals were extrapolated to hourly visits.

Table 1: (a) Model outputs from generalized linear mixed models with random effects site and sampling round (family poisson for solitary bees and hoverflies; family negative binomial for all pollinators and bumblebees). The effects of distance and size of grassland fragments on the number of visits of different pollinator groups (all pollinators, solitary bees, bumblebees, and hoverflies) are shown. For bumblebees, the interaction term was kept in the model, because it was significant. (b) Model outputs from generalized linear mixed models with random effect site (family negative binomial for all models). The effects of number of visits of the pollinator groups on the number of seeds per *Phacelia* plant are shown. Distance from grassland was scaled to zero mean and unit variance. Estimates, Z values and P values rounded to three digits after the comma are reported. Significant predictors ($P < 0.05$) are shown in bold.

	All pollinators (n=361)			Solitary bees (n=121)			Bumblebees (n=160)			Hoverflies (n=54)		
	Estimate	Z	P	Estimate	Z	P	Estimate	Z	P	Estimate	Z	P
<i>(a) Response: # Visits h⁻¹</i>												
(Intercept)	-1.266	-4.589	<0.001	-3.689	-3.855	<0.001	-2.395	-5.086	<0.001	-2.659	-7.179	<0.001
scale(Distance from Grassland [m])	-0.123	-1.776	0.076	-0.327	-2.551	0.011	0.144	1.898	0.058	-0.121	-0.740	0.459
Grassland size [ha]	0.093	2.996	0.003	0.133	2.226	0.026	-0.228	-1.570	0.116	-0.033	-0.343	0.732
Grassland size [ha] * scale(Distance from Grassland [m])	-	-	-	-	-	-	0.059	2.120	0.034	-	-	-
<i>(b) Response: # Seeds per Phacelia plant</i>												
(Intercept)	7.054	44.244	<0.001	7.118	51.995	<0.001	7.197	52.683	<0.001	7.293	84.960	<0.001
Mean # visits h ⁻¹	0.379	2.213	0.027	-	-	-	0.345	1.265	0.206	0.404	0.830	0.407
sqrt(Mean # visits h ⁻¹)	-	-	-	0.565	2.145	0.032	-	-	-	-	-	-

Table 2: Model output from generalized linear mixed model with random effect site (family negative binomial). The effects of distance of grassland fragments on the number of seeds per *Phacelia* plant are shown. Distance from grassland was scaled to zero mean and unit variance. Estimates, Z values and P values rounded to three digits after the comma are reported.

	Estimate	Z	P
<i>Response: # Seeds per Phacelia plant</i>			
(Intercept)	7.317	86.487	<0.001
scale(Distance from Grassland [m])	-0.032	-0.404	0.686

Discussion

Here, we studied the spillover of pollinators and pollination services from calcareous grassland fragments into the surrounding agricultural landscape. While visitation of solitary bees to phytometer plants decreased with distance, hoverfly numbers were not affected and, depending on fragment size, bumblebee visits even increased. This highlights the importance of calcareous grasslands for solitary bees. Their reduced visitation at more distant plots appeared to be compensated by the more mobile and less grassland-reliant hoverflies and bumblebees, leading to a seed set of *Phacelia* that was independent from distance.

Pollinator visits at different distances from grasslands

Solitary bee floral visits decreased with increasing distance from calcareous grassland fragments by about 61% from 0 to 400 meters. A loss of about 0.5 bees per hour may not sound like much, but considering the whole flowering period of approximately four weeks and approximately ten hours of pollinator-friendly conditions every day, this would result in 280 hours, so 140 more bees, which is quite substantial. This result therefore highlights the importance of extensively managed grasslands as habitat for these bee species and suggests that even small spatial distances can lead to steep declines in pollinator activity adjacent to grassland fragments (Öckinger and Smith, 2007). Since the 1960s, the calcareous grassland fragments in the study region have experienced an average loss of approximately 50% in area (Krauss et al., 2010). With this ongoing loss and fragmentation, our results indicate that solitary bee visitation rates will decrease in large parts of the agricultural landscape. Hoverflies did not show a decrease in number of visits and, depending on fragment size, bumblebees even increased at larger distances from the grasslands, suggesting greater foraging distance to isolated patches and/or independence from the grassland fragments as habitat. Bumblebees have been shown to be highly mobile covering longer distances and less reliant on semi-natural grassland habitats (Walther-Hellwig and Frankl, 2000; Westphal et al., 2006). Also, in our study, all bumblebees observed were from generalist species and are therefore expected not to be limited to or dependent on calcareous grassland habitats locally, but more to be affected by landscape factors (Hopfenmüller et al., 2014). Likewise, hoverflies are often

common even in intensified agricultural landscapes, in which flower strips and field margins can provide sufficient suitable habitat for them (Haenke et al., 2009; Jauker et al., 2009). This is further supported by our finding that bumblebee visitation could even increase at larger distances when calcareous grassland size was large. Hence, a combination of large semi-natural habitats and additional habitats and resources in the agricultural matrix may support populations of generalist bumblebee species.

Pollinator visits in the surroundings of grasslands of different sizes

Our results also show that solitary bee floral visits in the adjacent agricultural landscape increases with the size of calcareous grassland fragments. They seem to be driving the trend of all pollinator interactions, which are also positively correlated with fragment size. This suggests that larger grasslands support larger populations of solitary bees, which rely on them as primary habitat (Steffan-Dewenter et al., 2006). With solitary bee populations declining because of nesting sites and food resources being lost in simplified European agricultural landscapes, semi-natural habitats like calcareous grasslands are key to support remaining populations and re-establish areas they have disappeared from (Grass et al., 2018; Kremen et al., 2002; Ricketts et al., 2008). Conservation measures implemented in such extensively managed grasslands, with lower mowing or grazing intensities and lower fertilizer input, have also been shown to be more (cost-) effective compared to more intensified sites (Kleijn et al., 2009).

Pollinator visit effects on *Phacelia* seed production

Phacelia plants showed an increase in seed production with increasing pollinator visits, emphasizing their important role for plant fitness. The increase seemed to be driven by but not reliant on solitary bees, which were the only group that also showed a relationship to seed set when looked at individually. After a strong initial increase, seed set seems to taper off, suggesting that a successful and complete pollination of all flowers of a plant is achieved. In another study, solitary bees have been shown to pollinate oilseed rape more efficiently than hoverflies (Jauker et al., 2012).

***Phacelia* seed production at different distances from grasslands**

The positive correlation of solitary bee visits on seed set suggests higher pollination efficacy compared to other pollinator groups, such as the studied bumblebees and hoverflies. However, the export of pollination services of solitary bees into the surrounding agricultural landscape is limited by their restricted foraging radius (Gathmann and Tschardt, 2002; Kremen et al., 2002). In line with their relatively small foraging distances, we found decreasing visitation of the isolated *Phacelia* plants by solitary bees with increasing distance from the grasslands, however, this did not affect *Phacelia* seed set. These findings suggest that other pollinators potentially compensated for the loss of solitary bees at larger distances. Therefore, despite the likely higher pollination efficiency of solitary bees, pollinator abundance may be more important than pollinator identity for reproduction of *Phacelia tanacetifolia*. This has been found often, but not always, to be the case (e.g. Hoehn et al., 2008). Holzschuh et al.

(2012) for example found that honeybees were the most abundant flower visitors in cherry orchards, but wild bees drove the differences in fruit set. In our case, the generalist flowers of *Phacelia tanacetifolia* have been shown to attract a variety of different pollinator groups (Carreck and Williams, 2002). Other pollinating insects such as bumblebees and hoverflies which have a greater foraging radius or do not rely on calcareous grasslands can therefore make up for the reduced visitation of solitary bees at larger distances from grassland fragments (Steffan-Dewenter and Tschardt, 1999; Walther-Hellwig and Frankl, 2000; Westphal et al., 2006).

In contrast, we hypothesize that more specialized plants, relying solely on solitary bee species for pollination would be threatened in simplified landscapes (large-scale monoculture cropping systems lacking semi-natural habitat). This idea is supported by studies emphasizing parallel declines in pollinators and the plants relying on them (Biesmeijer et al., 2006).

Conclusions

Our study suggests that calcareous grasslands can provide spillover of pollinators and pollination services in otherwise simple agricultural landscapes. Solitary bees in particular appeared to rely on calcareous grasslands and make important contributions to pollination services in surrounding agriculture. However, visitation rates by solitary bees strongly declined with distance from grassland, whereas bumblebees and hoverflies were less or not affected. These findings emphasize the great importance of calcareous grasslands as nesting and foraging habitat for solitary bees, with many species being threatened, and suggest that generalist and more mobile species may compensate for habitat loss with larger foraging ranges and usage of alternative habitats and resources in the agricultural matrix (Greenleaf et al., 2007; Jauker et al., 2013; Theunert, 2002).

Specialized plant species (e.g. with more specialized floral traits or short flowering phenology), which are more reliant on solitary bee pollination may therefore be particularly threatened by insufficient pollination in intensified agricultural landscapes lacking semi-natural habitats such as grasslands (Bond, 1994; Kleijn et al., 2015). Further studies should look into more specialized pollination systems to give recommendations for conserving existing and establishing new semi-natural habitat fragments of sufficient size and suitable spatial arrangement to ensure the abundance of solitary bees as valuable pollinators throughout the landscape.

Declarations of interest: none

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

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Appendix

Table A.1: Grassland locations and sizes.

Site #	Size [ha]	Latitude	Longitude
01	4.93	51.59724725	9.84320102
02	9.41	51.54028185	9.763150289
03	0.81	51.52063249	9.802044149
04	3.11	51.45014072	9.793686699
05	0.30	51.44866555	9.776368287
06	0.05	51.43281082	9.770519397
07	2.30	51.50721448	10.01621637
08	0.83	51.55398265	10.04631486

Table A.2: Percentage of plots sampled for all grassland sites and sampling rounds. A plot is a set of two potted plants set up at a certain distance from a grassland. Plots were not sampled when plants were not flowering anymore or when they were destroyed. These cases did not generate any data points and were therefore not included in statistical analyses. “Total” indicates the total percentage of plots sampled at each site over all sampling rounds. Sampling dates for each round are shown in brackets.

Site #	% plots sampled				Total
	Sampling round 1 (03. - 11.07.2017)	Sampling round 2 (18. - 21.07.2017)	Sampling round 3 (28.07. - 06.08.2017)	Sampling round 4 (07.08.2017)	
01	100	100	33	0	58
02	100	29	0	0	32
03	100	17	0	0	29
04	100	86	29	0	54
05	100	100	14	0	54
06	100	100	67	17	71
07	100	100	57	57	79
08	100	100	43	0	61

Table A.3: Model outputs from generalized linear mixed models with random effects site and sampling round (family poisson for solitary bees and hoverflies; family negative binomial for all pollinators). The effects of distance, size of grassland fragments, and their interaction, on the number of visits of different pollinator groups (all pollinators, solitary bees, and hoverflies) are shown. Because of a lack of significance, the interaction term was later removed in the model selection process. Outputs of the final models are presented in Table 1. Distance from grassland was scaled to zero mean and unit variance. Estimates, Z values and P values rounded to three digits after the comma are reported. Significant predictors ($P < 0.05$) are shown in bold.

	<i>All pollinators</i>			<i>Solitary bees</i>			<i>Hoverflies</i>		
	Estimate	Z	P	Estimate	Z	P	Estimate	Z	P
<i>Response: # Visits h⁻¹</i>									
(Intercept)	-1.295	-4.650	<0.001	-3.672	-3.834	<0.001	-2.665	-7.123	<0.001
scale(Distance from Grassland [m])	-0.221	-2.150	0.032	-0.285	-1.481	0.139	-0.138	-0.640	0.522
grassland size [ha]	0.100	3.183	0.001	0.129	2.084	0.037	-0.030	-0.310	0.757
Grassland size [ha] * scale(Distance from Grassland [m])	0.029	1.331	0.183	-0.011	-0.285	0.776	0.008	0.125	0.900

Table A.4: Overview of number of visits of individuals from different pollinator groups, and genera and species within these groups to *Phacelia* plants.

Pollinator Group	Species	Number of visits
Bumblebees		160
	<i>Bombus terrestris</i>	98
	<i>Bombus pratorum</i>	31
	<i>Bombus pascuorum</i>	19
	<i>Bombus lapidaries</i>	8
	<i>Psithyrus sp.</i>	3
	<i>Bombus hortorum</i>	1
Solitary bees		121
	<i>Lasioglossum sp.</i>	96
	<i>Halictus sp.</i>	18
	<i>Hylaeus sp.</i>	5
	<i>Andrena sp.</i>	2
Honeybees	<i>Apis mellifera</i>	11
Hoverflies		54
	<i>Episyrphus sp.</i>	30
	<i>Sphaerophoria sp.</i>	13
	<i>Scaeva sp.</i>	3
	<i>Melanostoma sp.</i>	3
	<i>Paragus sp.</i>	2
	<i>Xanthogramma sp.</i>	1
	<i>Parasyrphus sp.</i>	1
	<i>Syrphus sp.</i>	1
Butterflies		12
	<i>Pieris sp.</i>	5
	<i>Lycaenidae</i>	5
	<i>Melanargia sp.</i>	1
	<i>Inachis sp.</i>	1
Flies	<i>Empis sp.</i>	3
Total visits		361

Table A.5: Marginal and conditional R^2 values (delta; rounded to three digits after the comma) for all models.

Model	Random terms	Marginal R^2 (delta)	Conditional R^2 (delta)
All pollinators ~ scale(Distance) + Size	Site + sampling round	0.021	0.097
Solitary bees ~ scale(Distance) + Size	Site + sampling round	0.024	0.351
Bumblebees ~ scale(Distance) * Size	Site + sampling round	0.020	0.131
Hoverflies ~ scale(Distance) + Size	Site + sampling round	0.002	0.034
Seeds per plant ~ mean visits all pollinators	Site	0.083	0.215
Seeds per plant ~ sqrt(mean visits solitary bees)	Site	0.064	0.170
Seeds per plant ~ mean visits bumblebees	Site	0.030	0.118
Seeds per plant ~ mean visits hoverflies	Site	0.009	0.025
Seeds per plant ~ scale(Distance)	Site	0.002	0.034

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Chapter 4

Trophic level and specialization moderate effects of habitat loss and landscape diversity on cavity-nesting bees, wasps and their parasitoids

Felix Klaus^{a,b,*}, Teja Tschardt^a, Ingo Grass^c



^aUniversity of Göttingen, Agroecology, Göttingen, Germany

^bJulius Kühn-Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Bee Protection, Braunschweig, Germany

^cUniversity of Hohenheim, Ecology of Tropical Agricultural Systems, Stuttgart, Germany

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Abstract

Habitat loss is a primary driver of biodiversity decline, but differences in species responses to habitat loss from local to landscape scales are poorly understood. Trophic level, food and habitat specialization have been suggested to be important predictors of species' responses to habitat loss, landscape diversity, and landscape scale. Using cavity-nesting communities of bees, wasps and their parasitoids on calcareous grasslands as a model system allowed us to compare responses of species differing regarding their trophic level, and degree of specialization on habitat and food. We found that species from higher trophic levels experienced semi-natural habitat at larger spatial scales than those of lower trophic levels, but only, when they were generalists (abundance of bees, 150 m radius, vs. wasps feeding on herbivores, 450 m radius), not specialists (bees, 150 m, vs. bee parasitoids, 150 m). Parasitoids, which are typically more specialized regarding their food resources (hosts), compared to predators such as predatory wasps, responded to habitat loss at the same spatial scales as their hosts, suggesting strong bottom-up effects of resource availability, i.e., host availability driving parasitoid abundance. Bees were mostly habitat specialists of calcareous grasslands and mainly driven by local habitat loss, whereas wasps as habitat generalists were mostly affected by landscape diversity. Our study highlights the need to consider the different spatial scales contingent on trophic level and specialization of target species groups, maintaining or restoring both local habitat and landscape diversity, as this is needed for their successful conservation.

Keywords: Bee; wasp; trap nest; parasitoid; calcareous grassland; spatial scale

Introduction

Habitat loss is a primary driver of local and global biodiversity decline (Dobson et al. 2006; Brondizio et al. 2019). However, differences in species responses to habitat loss, such as the strength of their population decline, are difficult to predict. One reason species respond differently to habitat loss is their trophic position in food webs and the spatial scale at which they experience the surrounding landscape, which drives their response to habitat loss at local and landscape scales (van Nouhuys 2005; Cagnolo et al. 2009; Steckel et al. 2014; Mayr et al. 2020). A reduction in local spatial extent of habitat (within a few hundred meters) should mostly affect species at lower trophic levels, such as bees as primary consumers, which often have lower dispersal abilities and thus are dependent on plants as locally available resources (Raffaelli 2004; Holt 2009). By contrast, species at higher trophic levels, such as predators or parasitoids, ought to be more mobile to follow their prey and to switch between prey populations (Holt 1996). Consequently, species at higher trophic levels should often perceive the landscape at larger spatial scales. Their occurrence is therefore not only dependent on local habitat quantity but also on the availability of habitat patches and habitat diversity at the landscape scale (Tscharntke et al. 2005; Grass et al. 2018).

However, the notion that the spatial scale at which species respond to habitat loss increases with their trophic position is not always true and does not appear to apply to all food webs (Thies et al. 2003). A potential reason is that the scale at which the landscape is perceived by predators also depends on their level of food and habitat specialization. Regarding food, parasitoids, for example, which are often specialized on one or few host species, are strongly affected by host availability, and hence may experience the landscape at similar scales as their hosts (Thies et al. 2003). By contrast, more generalist predators may be more mobile to switch between prey populations (Holt 1996; Rand & Tscharntke 2007; Green 2009; Grass et al. 2018; Fornoff et al. 2021). Regarding habitat specialization, habitat area has been shown to be most important for habitat specialist species, while generalists are mainly driven by habitat diversity and connectivity (Steffan-Dewenter 2003; Holzschuh et al. 2010).

Trap nests for bees, wasps and their parasitoids, which are of high ecological importance providing ecosystem services such as pollination and pest control (Tscharntke et al. 1998; Klein et al. 2007; Staab et al. 2018), provide the opportunity to study and compare a small and well-defined community of species from different trophic levels from primary to quaternary consumers and with different grades of specialization in a standardized manner (Tscharntke et al. 1998; Steckel et al. 2014; Staab et al. 2018; Fornoff et al. 2021) (Fig. 1a). These artificial nesting resources for cavity-nesting insects are often made from common reed and placed at study sites to attract females to build nests, which can then be studied (MacIvor 2017). Trap nests enable us to compare responses of both specialized (parasitoids), as well as generalist (hosts) species (Krombein 1967). In addition, the community of trap nesting insects can be related to the spatial scale at which species from different trophic levels experience local and landscape-level habitat amount (Holzschuh et al. 2010).

Calcareous grasslands are hotspots of plant and insect diversity in central Europe (Steffan-Dewenter & Tscharrntke 2002; WallisDeVries et al. 2002). Most of the calcareous grasslands have greatly decreased in area and distribution during agricultural intensification in the 20th century, so that today mostly small and isolated fragments can be found in the agricultural landscape (Poschlod & WallisDeVries 2002; Krauss et al. 2010; Grass et al. 2018). In this study, we use trap nests on calcareous grasslands to study the effects of habitat loss and habitat diversity at local and landscape scales on species responses at different trophic levels.

Differences between trophic levels regarding their responses to habitat loss, diversity and spatial scales may be expected because their food resources are either directly (bees as herbivores) or more indirectly (wasps as carnivores) driven by the habitat types (Kruess & Tscharrntke 2000; Raffaelli 2004). More specialized consumers with a more narrow diet breadth such as parasitoids can be expected to be more closely linked to the availability and distribution of their food resources (hosts), and to be more affected by landscape change (Kruess & Tscharrntke 2000), compared to more generalist predators.

Regarding responses to habitat loss and habitat diversity, primary consumers such as bees have been shown to be affected mainly by habitat availability, while secondary and tertiary consumers (wasps) respond positively to higher landscape heterogeneity (Holzschuh et al. 2010). The responses of species from the same trophic level with different grades of specialization (e.g. bee parasitoids and wasps feeding on herbivorous prey, both being secondary consumers) to habitat loss and diversity can also be expected to differ. Compared to the responses of primary consumers (here: bees), bee parasitoids can be expected to respond at similar scales due to the strong tie to their hosts, while the generalist wasps may respond at larger scales.

The following hypotheses were addressed:

- (1) Generalist species of higher trophic levels, i.e. wasps, perceive the landscape at larger scales compared to species at lower trophic levels (bees), while specialist species at higher trophic levels, i.e., parasitoids, are affected at similar scales as their hosts.
- (2) Bee abundance in trap nests is more strongly driven by calcareous grassland area than additional semi-natural habitats in the surrounding landscape, making bees habitat specialists.
- (3) Bees, which are mainly habitat specialists on calcareous grasslands, are mainly driven by local habitat loss, while habitat generalists (predatory wasps) are mostly affected by habitat diversity at the landscape scale.

Materials and Methods

The study took place in the agricultural landscapes of the surroundings of the city of Göttingen, central Germany (lat: 51.532717, long: 9.935154, 20 km radius around the city). The region is dominated by intensive agricultural land use. There are 285 extensively managed calcareous grasslands in the study region making up 0.26% of the total area (Krauss et al., 2003). Twenty-three of these grassland fragments were used as study sites (see Figure 1b for an example; see Figure 2 for a map of the study area and distribution of study sites in the landscape). The sites were selected along independent gradients (\triangleq treatments) of grassland area (minimum: 82 m², maximum: 50673 m², mean 6902 m², median 3465 m²), amount of other semi-natural habitat in the surroundings, and landscape diversity (based on Shannon Index of habitat types) (Table A.2). The intensity of the management of the sites (grazing or mowing) was required to not differ substantially to avoid differences in habitat quality. All sites were more than 300 m apart from each other (2406 m \pm 444 m; mean \pm 1 standard error), and spatial independence was ensured by calculating spatial autocorrelation for all relevant variables and for residuals of all models (Moran's I with $p > 0.05$ in all cases). Adjacent forest fragments or hedgerows offered nesting habitat for cavity-nesting bees and wasps at all sites.

Six trap nests were set up at each site in mid-April 2017. They were evenly spread across each site, and placed in spots that were not shaded most of the day and close to vegetation to resemble preferred natural nesting sites. Each trap nest consisted of two plastic tubes with a diameter of 10.5 cm, which were filled with common reed (*Phragmites australis* (Cav.) Trin. ex Steud.; approximately 200 reeds per tube) with diameters between 2 and 10 mm, cut to the length of the tube (20 cm), and attached to a wooden post 1.3 m above the ground (Figure 1c) (Tschardt et al. 1998; Staab et al. 2018). Sites were sampled every three weeks, starting end of May 2017 (when we noted the first nests had been plugged, meaning closed by a bee or wasp using natural materials such as mud or resin, indicating a nest was built and completed) and until mid-October 2017 (when nesting had stopped; total of seven sampling rounds). Plugged nests were collected and replaced with reeds of a similar diameter, to ensure the constant availability of nesting sites and to not miss the nests of the first generation of those species that have two generations per year. Plugged nests were brought to the lab and dissected, to determine the identity of host species, number of brood cells, parasitoid species, and number of parasitized brood cells. Nest inhabitants (hosts and parasitoids) were identified to species level, if possible, using a stereomicroscope (for literature used for identification, see Table A.3). For later analyses, nests were categorized into species groups based on the type of food provided to the larvae. The six groups were *bees* (providing larvae with pollen and nectar), *wasps hunting herbivorous prey* (such as aphids), *wasps hunting carnivorous prey* (spiders), and their respective parasitoids (*parasitoids of bees*, *of wasps hunting herbivorous prey*, and *of wasps hunting carnivorous prey*). For the analyses, all nests from a site were pooled across sampling rounds and trap nests to obtain total abundance (number of brood cells \triangleq sampling unit) for each of the species groups.

The landscape within a 500 m radius around each study site was mapped by ground-truthing. Habitats were categorized in sixteen categories: *oilseed rape field, grain field, maize field, other crop field, open canopy forest, closed canopy forest, field margin, hedgerow, pasture, nutrient poor grassland, orchard, settlement, water body, street, field road, and quarry*. The landscape data was digitized and analyzed using the software QGIS, version 2.14.3 (QGIS Development Team 2016) and R (R Core Team 2020). The variables *open canopy forest, field margin, hedgerow, nutrient poor grassland, orchard, field road* and *quarry* were combined to *semi-natural habitats*. The proportion of semi-natural habitat (excluding the focal grassland), and the diversity of landscape types were calculated for different scales (from 100 m to 500 m, 50 m steps) using the R package ‘landscapemetrics’ (Hesselbarth et al. 2019). The maximum scale of 500 m was chosen to avoid spatial autocorrelation between sites, and has been shown to be suitable resembling the maximum foraging distance for most bee species of trap nests (Gathmann & Tschardt 2002; Zurbuchen et al. 2010).

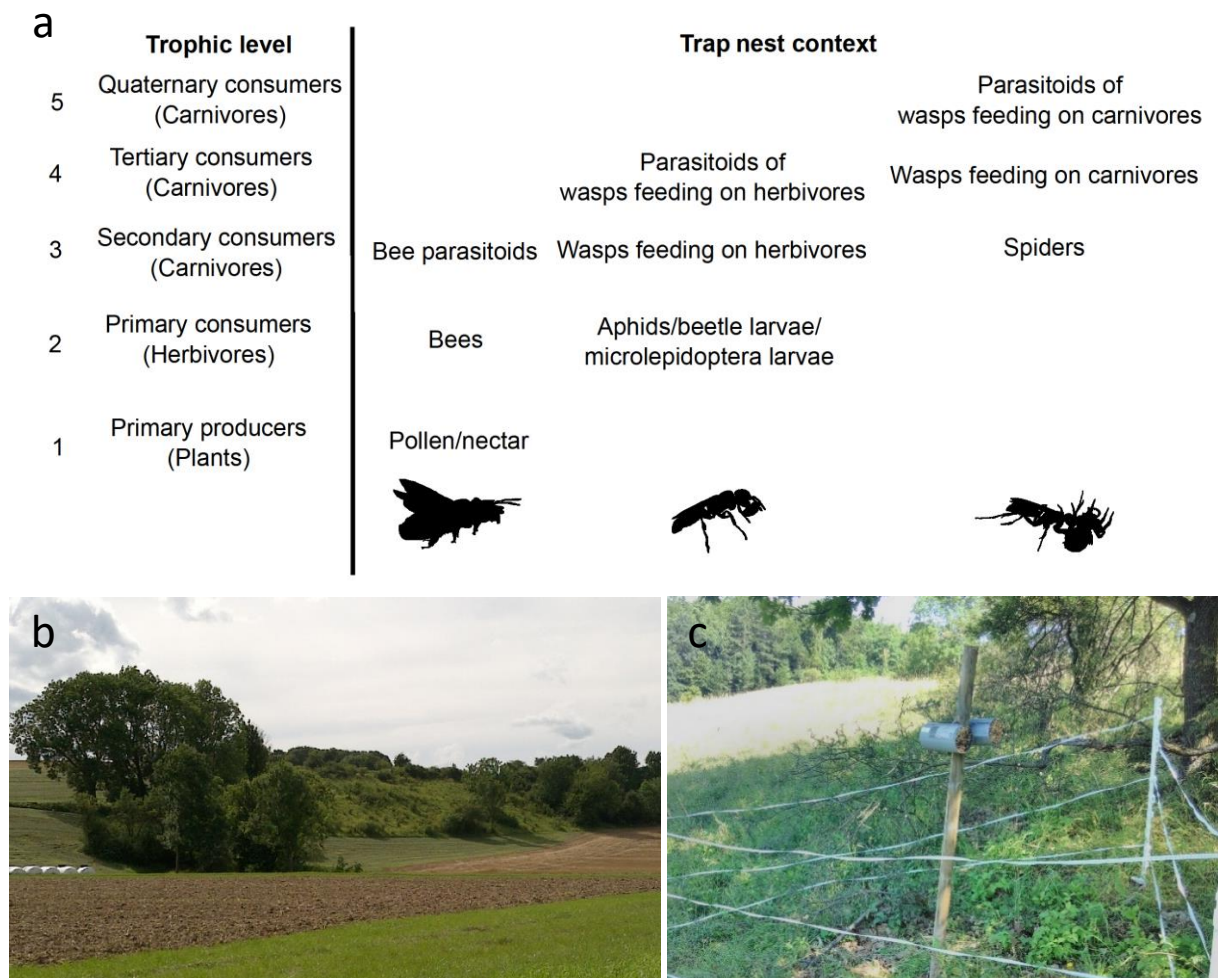


Figure 1. (a) Overview of the trophic levels in the food chain and their representatives in the trap nest system. (b) A calcareous grassland fragment (center) embedded in the agricultural landscape. (c) A trap nest consisting of two plastic tubes filled with reeds attached to a wooden post, and protected from grazing animals by a fence.

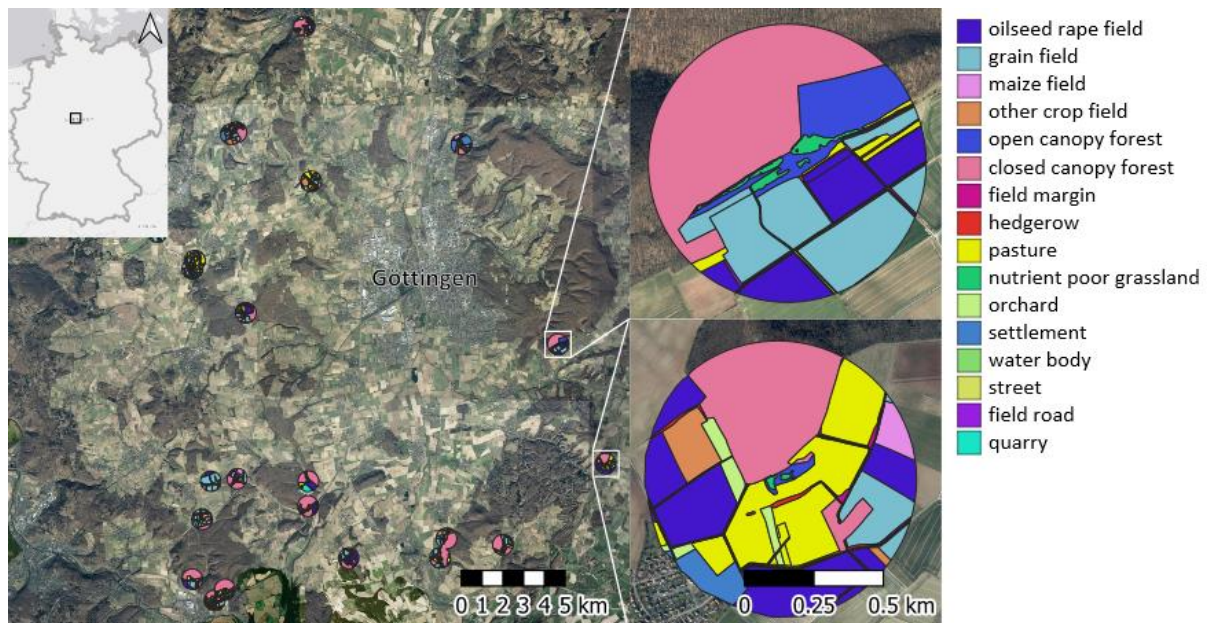


Figure 2. Map of the location of the study region within Germany (top left); locations of the calcareous grasslands studied in the surroundings of the city of Göttingen, with 500 m buffers showing the landscape types (center), and detailed maps of contrasting landscapes around two sample sites (right). Basemap © ESRI.

Statistical Analysis

First, to determine the spatial scales at which the species groups at the different trophic levels were affected by the landscape composition, the correlation coefficients (using the Spearman method) of abundance (using brood cell numbers in trap nests) and the proportion of semi-natural habitat (including extensively managed grasslands) and the landscape diversity within different radii around the center of each grassland were calculated (Figure 3a and b). The most appropriate scales (highest correlation coefficients, but choosing the same scale for parasitoids and hosts of one trophic level) were used as variables for further analyses. The correlation coefficients of the hosts and respective parasitoids for the chosen scale were for semi-natural habitats: 0.50 and 0.57 for bees (150 m scale), 0.32 and 0.35 for wasps feeding their larvae with herbivorous prey (450 m scale), and -0.20 and 0.08 for wasps feeding their larvae with carnivorous prey (450 m scale), and for landscape diversity: 0.37 and 0.36 for bees (200 m scale), 0.38 and 0.45 for wasps feeding their larvae with herbivorous prey (200 m scale), and 0.23 and 0.13 for wasps feeding their larvae with carnivorous prey (100 m scale) (see Fig. 3).

Trap nest inhabitants were split into three groups based on their trophic levels: bees, wasps feeding their larvae with herbivorous prey, and wasps feeding their larvae with carnivorous prey. All three groups were attacked by parasitoids. Effects of *grassland area*, proportion of *semi-natural habitat* (excluding extensively managed grasslands) in the surrounding landscape and *landscape diversity* on species abundance were analyzed separately for each group.

Generalized linear models (GLMs) with negative binomial distribution were used. All models included the three explanatory variables *grassland area*, *semi-natural habitat* and *landscape diversity*. All predictors were scaled to zero mean and unit variance to be able to compare effect sizes, and *grassland area* was additionally log-transformed. Model assumptions of generalized linear models were met and we tested for potential collinearity of predictor variables for all models. We refer to results as statistically significant when $p < 0.05$ and marginally statistically significant when $0.05 \leq p < 0.10$.

All statistical analyses were done in R version 4.0.3 (R Core Team 2020) using dedicated packages lme4 (for the implementation of mixed effect models) (version 1.1.26; Bates et al. 2015), DHARMA (for residual model diagnostics) (version 0.3.3.0; Hartig 2020), multcomp (for univariate testing via z- tests of estimated model coefficients) (version 1.4.15; Hothorn et al. 2008), effects (for extracting model outputs) (version 4.2.0; Fox, John 2003, 2019), ggplot2 (for plotting) (version 3.3.6; Wickham 2016), raster (for reading shapefiles) (version 3.4.5; Hijmans 2020), sf (for calculating distances between points) (version 1.0.7; Pebesma 2018), and corrplot (for extracting correlation coefficients) (version 0.84; Wei & Simko 2017).

Results

From the 138 trap nests (23 sites x 6 trap nests each), 3,124 nests were collected throughout the study period, containing 10,736 brood cells. Of these, 6,470 brood cells belonged to bees, 438 of which to their parasitoids, 1,874 to wasps hunting herbivorous prey, 544 of which to their parasitoids, and 2,392 to wasp hunting carnivorous prey, 733 of which to their parasitoids.

Sixteen species of bees were collected. *Osmia bicornis* (Linnaeus, 1758) (Megachilidae) was by far the most abundant bee species (73% of all bee brood cells), followed by *Osmia truncorum* (Linnaeus, 1758) (Megachilidae) and *Hylaeus communis* (Nylander, 1852) (Colletidae). There were 26 species of wasps hunting herbivorous prey, with *Ancistrocerus nigricornis* (Curtis, 1826) (Vespidae) being the most abundant (43% of all brood cells belonging to this species), followed by *Ancistrocerus gazella* (Panzer, 1798) (Vespidae) and *Ancistrocerus antilope* (Panzer, 1789) (Vespidae). The wasps hunting carnivorous prey (six species) were dominated by *Trypoxylon clavicerum* (Lepeletier de Saint Fargeau & Audinet-Serville, 1828) (Crabronidae; 72%) followed by *Deuteragenia subintermedia* (Magretti, 1886) (Pompilidae). The most abundant of the 27 parasitoid species were *Melittobia acasta* (Walker, 1839) (Eulophidae; in 6.4% of all brood cells), *Chrysis terminata* (Dahlbom, 1854) (Chrysididae; 2.2%) and *Cacoxenus indagator* (Loew, 1858) (Drosophilidae; 1.5%). As a side note, to our knowledge, this is the first record of the wasp species *Microdynerus timidus* (Saussure, 1856) (3 brood cells) and *Passaloecus vandeli* (Ribaut, 1952) (20 brood cells) in the German state of Lower Saxony. They have previously been recorded in the state of Hessen, to the south of Lower Saxony (Jacobs 2007; Tischendorf et al. 2015), and seem to be moving north, presumably due to climate change. See Table A.1 for a full list of species and their abundances. Because of trap nests

being a system relatively poor in species numbers, especially, when the community is split into sub-groups, we focused on abundances, and did not consider species richness here.

Abundances of bees (primary consumers) and their parasitoids were most strongly positively correlated with semi-natural habitat (including extensively managed grasslands) at small scales (150 m; Fig. 3a). Wasps feeding on herbivores (secondary consumers) and their parasitoids were similarly positively affected by semi-natural habitats, but at larger scales (450 m; Fig. 3a) and landscape diversity at small to medium scales (200m; Fig. 3b). When splitting up this group into sub-groups based on prey type, it became apparent, that this pattern was driven by the most numerous group of wasps feeding on Microlepidoptera larvae, and not by those feeding on aphids or Chrysomelidae larvae (Fig. A.1). Abundances of wasps feeding on carnivores (tertiary consumers) and their parasitoids were not well predicted by semi-natural habitat and landscape diversity (correlation coefficient < 0.3) (Fig. 3a, b).

Regarding local and landscape effects, bee and bee parasitoid abundances were significantly positively correlated with the local area of the focal grassland fragments ($p < 0.001$; Fig. 4a, d; Table 1). The host species from higher trophic levels and their parasitoids were not significantly correlated with local grassland area (Fig. 4 b, c, e, f; Table 1). Regarding landscape diversity, no significant correlations were found for bees and their parasitoids (Fig. 5a, d; Table 1). The parasitoids of wasps feeding on herbivores were marginally significantly correlated with landscape diversity ($p = 0.06$; Fig. 5e; Table 1), while their hosts were not (Fig. 5b; Table 1). Wasps feeding on carnivores and their parasitoids were positively affected by landscape diversity (marginally significant for parasitoids; $p = 0.01$; $p = 0.07$; Fig. 5c, f; Table 1). None of the groups were significantly correlated with semi-natural habitat other than the focal grassland (Table 1).

In general, the patterns of hosts and their parasitoids were similar, while the host species from different trophic levels showed different patterns regarding responses to scale, grassland area and landscape diversity (Fig. 3; Fig. 4; Fig. 5; Table 1).

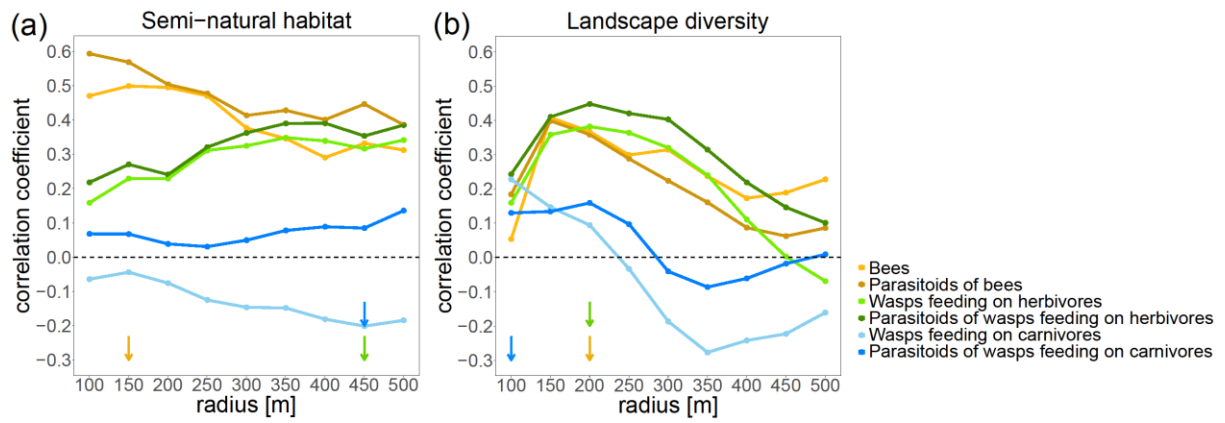


Figure 3. Correlation coefficient of abundance of the different groups (brood cell numbers in trap nests per site; $n = 23$ sites) and (a) semi-natural habitat (including focal grasslands) and (b) landscape diversity respectively, at different scales (radii around center of focal grasslands). Scales most correlated (using the same scale for each pair of host and parasitoid) and used for further analyses for the different trophic levels are pointed out by arrows.

Table 1. Model results of generalized linear models (GLMs) for abundance across species groups. The effects of *grassland area*, *semi-natural habitat* excluding extensive grasslands in the surrounding landscape and *landscape diversity* on the abundances (brood cell numbers) are shown. All three predictor variables were scaled to zero mean and unit variance and additionally, *grassland area* was log-transformed. Estimates, standard errors, Z values and p values rounded to three digits after the comma are reported. Significant and marginally significant predictors ($p < 0.10$) are shown in bold.

	Bees				Wasps feeding on herbivores				Wasps feeding on carnivores			
	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p
<i>Response: Abundance (number of brood cells) per site</i>												
(Intercept)	5.481	0.135	40.500	<0.001	4.363	0.097	45.075	<0.001	4.610	0.119	38.684	<0.001
scale(log(Grassland area))	0.555	0.153	3.615	<0.001	-0.015	0.118	-0.124	0.902	0.006	0.145	0.044	0.965
scale(Semi natural habitat)	-0.066	0.205	-0.322	0.748	0.150	0.129	1.165	0.244	-0.204	0.147	-1.394	0.163
scale(Landscape diversity)	0.139	0.198	0.701	0.484	0.167	0.118	1.417	0.157	0.337	0.130	2.604	0.009

	Parasitoids of bees				Parasitoids of wasps feeding on herbivores				Parasitoids of wasps feeding on carnivores			
	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p	Estimate	Std. Error	Z	p
<i>Response: Abundance (number of brood cells) per site</i>												
(Intercept)	2.668	0.184	14.539	<0.001	3.119	0.095	32.812	<0.001	3.431	0.152	22.557	<0.001
scale(log(Grassland area))	0.830	0.208	3.995	<0.001	-0.098	0.119	-0.822	0.411	0.123	0.185	0.664	0.507
scale(Semi natural habitat)	-0.293	0.275	-1.066	0.286	0.188	0.126	1.488	0.137	-0.098	0.187	-0.525	0.600
scale(Landscape diversity)	0.301	0.269	1.117	0.264	0.219	0.118	1.854	0.064	0.298	0.166	1.789	0.074

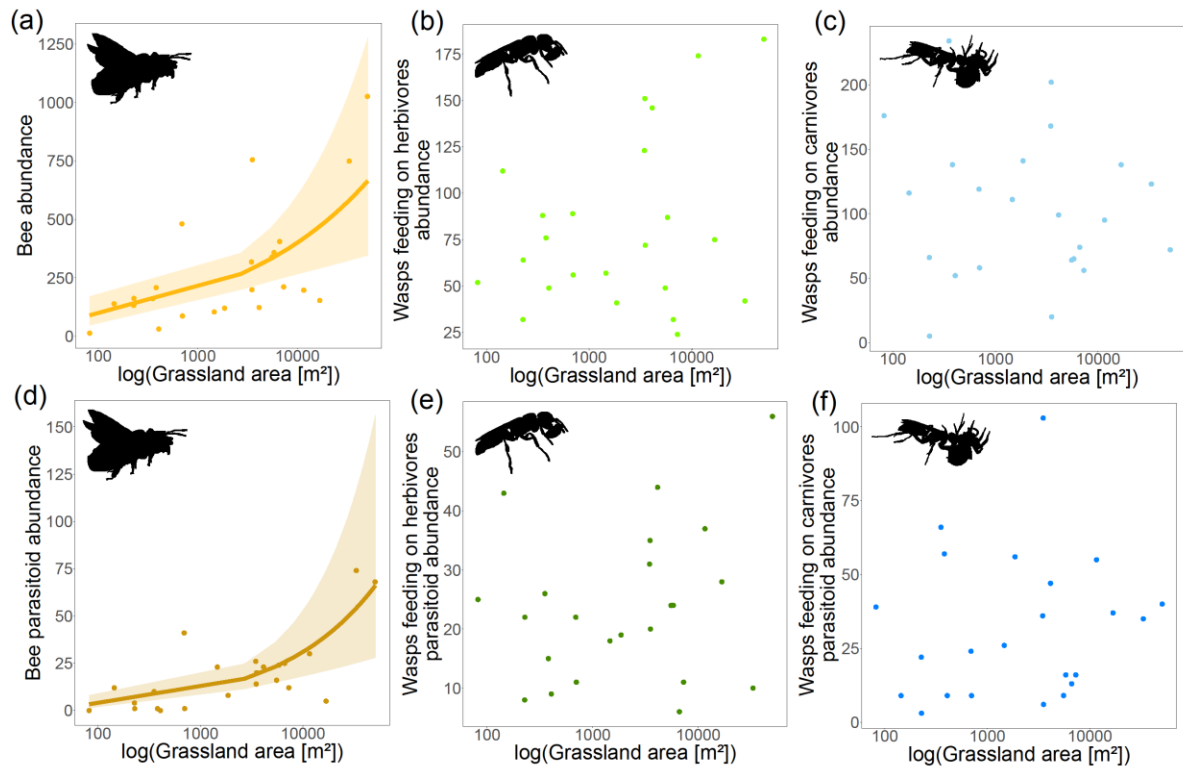


Figure 4. Abundance (number of brood cells per site) of different trophic levels in relation to grassland area (log-transformed). Solid lines represent significant relationships ($p < 0.05$). Envelopes show 95% confidence intervals.

Discussion

We found in this study that species from different trophic levels and with different food and habitat specialization are differently affected by habitat area and landscape diversity. Using cavity-nesting bees, wasps, and their parasitoids on calcareous grasslands as a model systems, we found that species at different trophic levels perceive habitat loss and landscape diversity of the agricultural landscape at different spatial scales. Depending on the target species/community, maintaining and restoring local habitat islands may not be sufficient for conservation, but the landscape must be considered as well, especially for species of higher trophic levels and habitat generalists.

Bee and wasp abundance was influenced by the availability of semi-natural habitats, with species of higher trophic levels (wasps) perceiving the landscape at larger spatial scales than those of lower trophic levels (bees). This is in line with the concept by Holt (1996), stating, that higher trophic levels perceive the landscape at larger spatial scales, which is assumed to be caused by the higher mobility of the predators' prey and the predators' need to switch between prey populations, compared to herbivores that feed on non-mobile food, i.e., sessile plants. Comparing trophic levels of parasitoids and their hosts, no differences regarding the landscape scale best suited to explain their abundances were found. This has been shown before and is likely due to the typically high food specialization of parasitoids on their hosts,

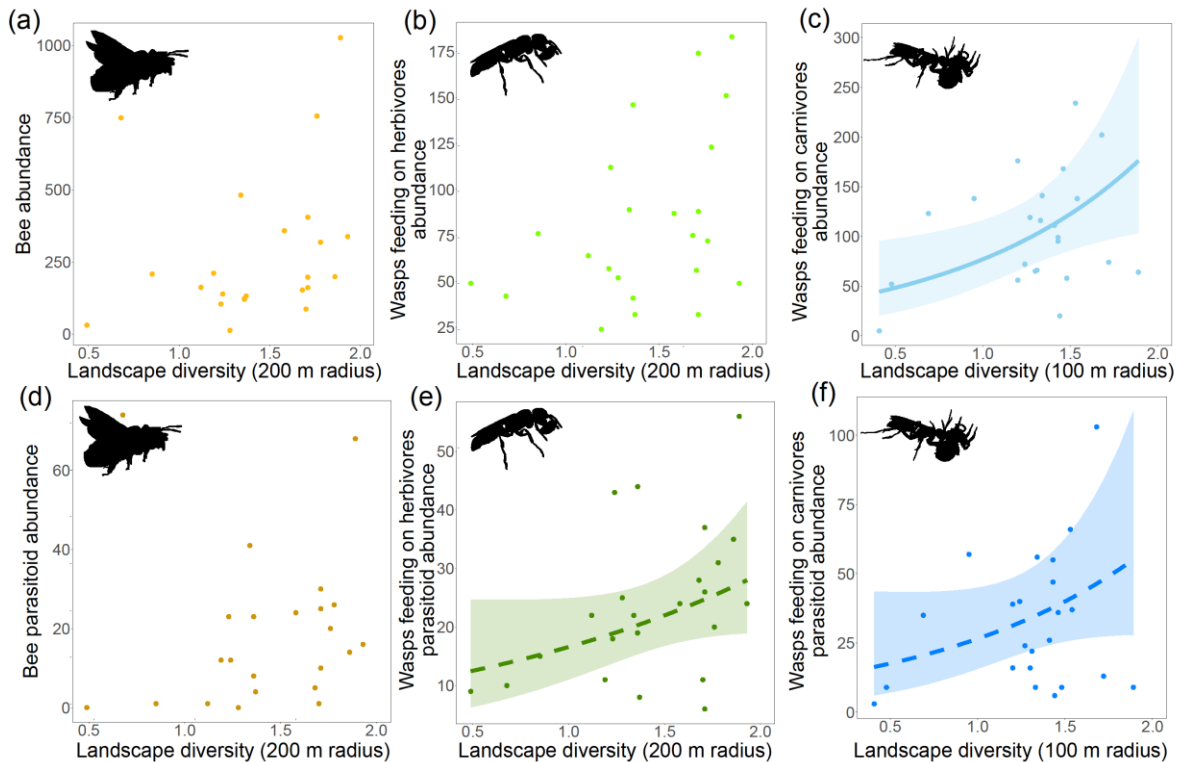


Figure 5. Abundance (number of brood cells per site) of different trophic levels in relation to the diversity of the surrounding landscape (different radii). Solid lines represent significant relationships ($p < 0.05$). Dashed lines represent marginally significant relationships ($p \geq 0.05$ and < 0.10). Envelopes show 95% confidence intervals.

causing them to be tied more closely to the spatial scale at which their hosts respond to local- and landscape-level habitat availability than generalists (Steffan-Dewenter & Tscharrntke 2000; Thies et al. 2003; Rand & Tscharrntke 2007; Holt 2009; Grass et al. 2018). These results suggest that in addition to the trophic level of consumers, the degree of food specialization mediates how species experience the landscape.

We found that the scales at which abundances of the same trophic level relate to different landscape variables can be quite different. This could be shown by their responses to the amount of *semi-natural habitat* and *landscape diversity*. This contrast was most distinct for wasps feeding on herbivores, representing the trophic level of secondary consumers, being affected at large scales by *semi-natural habitat*, and at smaller scales by *landscape diversity*. This may be caused by flexible foraging strategies. The availability of large amounts of semi-natural habitat at larger scales seem to be sufficient for providing herbivorous prey to the wasps. When large amounts of semi-natural habitat are not available, a high diversity of habitats at smaller scales may be needed, with edge habitats providing both food sources and ensuring connectivity and permeability of the landscape (Krewenka et al. 2011; Mallinger et al. 2016). When further dividing the group of wasps feeding on herbivores by prey type, different responses are revealed, with wasps feeding on Microlepidoptera larvae responding strongly to semi-natural habitat, which may be because of the association of their prey with

(fruit) trees and shrubs, which are abundant at semi-natural habitats (MacKay 1962; Hoffmann et al. 2018). Wasps feeding on aphids and Chrysomelidae larvae on the other hand are not associated with semi-natural habitat, which can be explained by their prey not being associated with semi-natural habitats, but with annual crop plants (e.g. aphids as pest species in wheat fields) (Dedryver et al. 2010; Jolivet et al. 2012).

Bee abundance was strongly correlated to grassland area, but not to the amount of additional semi-natural habitats in the surrounding landscape, suggesting that the studied cavity-nesting bees are habitat specialists of the calcareous grasslands. As we provided artificial nesting sites at all sites, food requirements can be expected as the limiting factor for bee occurrence. Bees rely on flowering plants offering nectar and pollen, which were widely available at the focal grasslands (Steffan-Dewenter & Tscharrntke 2002; WallisDeVries et al. 2002). Solitary bees, depending on their body size, can have maximum foraging ranges of up to 1100 m, however, realized foraging distances may be much lower, when resources are available in close proximity to the nest, as was the case at the calcareous grasslands (Gathmann & Tscharrntke 2002; Zurbuchen et al. 2010).

By contrast, abundances of wasp species were not positively correlated to grassland area, which may be due to their higher trophic level and hence higher mobility weakening species-area relationships, as suggested by Holt (2009). It has also been shown and is supported by our results, that habitat specialists (in our case bees) are mainly driven by local habitat loss, while habitat generalists (wasps) are mostly affected by landscape diversity and connectivity (Steffan-Dewenter 2003; Holzschuh et al. 2010). Furthermore, the prey of most cavity-nesting wasps, such as aphids, Chrysomelidae larvae, and spiders, is mostly not associated with extensively managed grasslands, but annual crop fields, suggesting the wasps to use the grasslands for nesting and feeding, but not as much for hunting (Dedryver et al. 2010; Jolivet et al. 2012; Hoffmann et al. 2018).

Conclusions

In conclusion, we showed that trophic level and specialization moderate species' responses to local habitat loss and landscape diversity and that they perceive the landscape at different scales. These findings highlight the need for conservation or restoration projects to foster habitat heterogeneity, providing resources essential either in close proximity for less mobile species from lower trophic levels and their specialist antagonists or within the wider landscape, while also ensuring high landscape diversity and permeability, for more mobile species from higher trophic levels.

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Declarations of interest: none

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Appendix

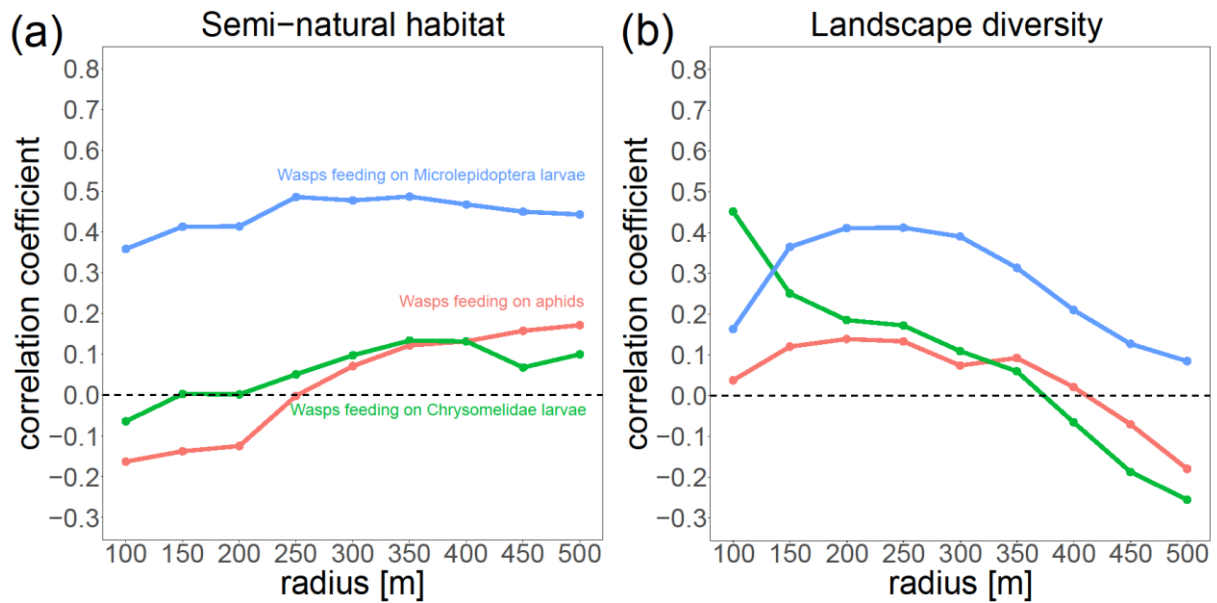


Figure A.1. Correlation coefficient of abundance of different sub-groups of wasps feeding on herbivorous prey (brood cell numbers in trap nests per site; $n = 23$ sites) and (a) semi-natural habitat (including focal grasslands) and (b) landscape diversity respectively, at different scales (radii around center of focal grasslands). The sub-groups are based on the type of prey: wasps feeding on Microlepidoptera larvae (8 out of 25 species, 1,330 out of 1,874 brood cells), wasps feeding on aphids (7 spp, 264 brood cells), and wasps feeding on Chrysomelidae larvae (6 spp, 240 brood cells). Additional prey types (Curculionidae – 2 spp, Thysanoptera, – 1 sp, Diptera – 1 sp, 40 brood cells in total) are not shown.

Table A.1. List of species recorded at different trophic levels in trap nests and their total brood cell numbers.

Species	# brood cells
Bees	6470
<i>Hylaeus communis</i>	283
<i>Hylaeus confusus</i>	26
<i>Hylaeus difformis</i>	135
<i>Hylaeus leptocephalus</i>	9
<i>Hylaeus sp</i>	22
<i>Megachile alpicola</i>	18
<i>Megachile centuncularis</i>	58
<i>Megachile ligniseca</i>	41
<i>Megachile sp</i>	11
<i>Megachile versicolor</i>	175
<i>Osmia bicornis</i>	4711
<i>Osmia brevicornis</i>	77
<i>Osmia caeruleascens</i>	54
<i>Osmia campanularum/cantabrica/florisomnis/rapunculi</i>	2
<i>Osmia florisomnis</i>	248
<i>Osmia florisomnis/rapunculi</i>	4
<i>Osmia leiana</i>	59
<i>Osmia rapunculi</i>	79
<i>Osmia truncorum</i>	328
<i>Osmia truncorum/crenulatus</i>	109
<i>Osmia uncinata</i>	21
Bee parasitoids	438
Acari	20
<i>Cacoxenus indagator</i>	165
<i>Chrysis cyanea</i>	3
<i>Chrysis fulgida</i>	3
<i>Chrysis terminata</i>	6
<i>Chrysura hirsuta</i>	5
<i>Coelioxys inermis</i>	4
<i>Coelioxys mandibularis</i>	6
<i>Ephialtes manifestator</i>	3
<i>Gasteruption assectator</i>	12
<i>Gasteruption erythrostomum</i>	1
<i>Gasteruption jaculator</i>	2
<i>Megatoma undata</i>	18
<i>Melittobia acasta</i>	140
<i>Sapyga clavicornis</i>	24
<i>Sapygina decemguttata</i>	15
<i>Stelis breviscula</i>	9
<i>Stelis minuta</i>	2
Wasps hunting herbivorous prey	1874
<i>Ancistrocerus antilope</i>	121
<i>Ancistrocerus claripennis</i>	42
<i>Ancistrocerus gazella</i>	201
<i>Ancistrocerus nigricornis</i>	813
<i>Ancistrocerus parietinus</i>	74
<i>Ancistrocerus parietum</i>	3
<i>Ancistrocerus trifasciatus</i>	24
<i>Crossocerus cetratus</i>	28
<i>Discoelius zonalis</i>	52
<i>Gymnomerus laevipes</i>	5

<i>Microdynerus timidus</i>	3
<i>Nitela/Pemphredon/Psenulus sp</i>	38
<i>Passaloecus brevilabris</i>	13
<i>Passaloecus corniger</i>	103
<i>Passaloecus eremita</i>	29
<i>Passaloecus insignis</i>	39
<i>Passaloecus singularis</i>	8
<i>Passaloecus sp</i>	14
<i>Passaloecus vandeli</i>	20
<i>Spilomena beata/troglydytes</i>	4
<i>Symmorphus bifasciatus</i>	45
<i>Symmorphus connexus</i>	16
<i>Symmorphus crassicornis</i>	13
<i>Symmorphus debilitatus</i>	7
<i>Symmorphus gracilis</i>	93
<i>Symmorphus murarius</i>	35
<i>Symmorphus sp</i>	31
Parasitoids of wasps hunting herbivorous prey	544
Acari	6
<i>Chrysis angustula</i>	2
<i>Chrysis corusca</i>	39
<i>Chrysis cyanea</i>	18
<i>Chrysis fulgida</i>	2
<i>Chrysis solida</i>	44
<i>Chrysis terminata</i>	228
<i>Ephialtes manifestator</i>	6
<i>Ephialtes sp</i>	9
<i>Megatoma undata</i>	13
<i>Melittobia acasta</i>	136
<i>Nematopodius debilis</i>	12
<i>Omalus aeneus</i>	1
<i>Poemenia collaris</i>	7
<i>Poemenia hectica</i>	8
<i>Poemenia notata</i>	3
<i>Poemenia sp</i>	1
<i>Pseudomalus auratus</i>	9
Wasps hunting carnivorous prey	2392
<i>Agenioideus cinctellus</i>	8
<i>Auplopus carbonarius</i>	2
<i>Deuteragenia subintermedia</i>	306
<i>Trypoxylon clavicerum</i>	1727
<i>Trypoxylon figulus</i>	137
<i>Trypoxylon figulus/minus</i>	16
<i>Trypoxylon minus</i>	195
<i>Trypoxylon sp</i>	1
Parasitoids of wasps hunting carnivorous prey	733
<i>Chrysis corusca</i>	17
<i>Chrysis cyanea</i>	138
<i>Chrysis terminata</i>	3
<i>Ephialtes manifestator</i>	5
<i>Gasteruption assectator</i>	1
<i>Ichneumonidae sp</i>	4
<i>Megatoma undata</i>	15
<i>Melittobia acasta</i>	414
<i>Nematopodius debilis</i>	136

Table A.2. Correlation matrices showing Pearson correlation coefficients comparing explanatory variables used in the same models for a) bees, b) wasps feeding on herbivores, and c) wasps feeding on carnivores.

a) Bee models

	Grassland area	Semi-natural habitat (150 m scale)	Landscape diversity (200 m scale)
Grassland area			
Semi-natural habitat (150 m scale)	0.257		
Landscape diversity (200 m scale)	0.123	0.712	

b) Wasps feeding on herbivore models

	Grassland area	Semi-natural habitat (450 m scale)	Landscape diversity (200 m scale)
Grassland area			
Semi-natural habitat (450 m scale)	0.339		
Landscape diversity (200 m scale)	0.123	0.530	

c) Wasps feeding on carnivore models

	Grassland area	Semi-natural habitat (450 m scale)	Landscape diversity (100 m scale)
Grassland area			
Semi-natural habitat (450 m scale)	0.339		
Landscape diversity (100 m scale)	-0.089	0.305	

Table A.3. Literature used for the identification of nest inhabitants.

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Synthesis

Agricultural intensification has been associated with several threats to ecosystems during the past decades and is still ongoing (Foley et al., 2005; Robinson & Sutherland, 2002; Tschardt et al., 2005). This thesis aims to contribute to the understanding of potential interactive effects of multiple stressors in biodiversity decline. In three experimental field and semi-field studies, threats to bee and wasp populations in the agricultural landscape at the local and landscape scale were studied. Effects on trap nesting insect communities were studied on a local scale, using mesocosms simulating different scenarios of interacting stressors acting on a solitary bee species, and on a landscape scale, using a gradient of semi-natural habitat and diversity in the surrounding landscape. It was hypothesized, that bees are more associated with calcareous grasslands, exporting pollination services only on a small scale, while species from higher trophic levels, such as predatory wasps, experience the landscape at larger scales, and that a higher abundance and diversity of floral resources can mitigate negative effects of insecticide exposure in bees.

Firstly, the interactive effect of floral resource availability and diversity and exposure to a neonicotinoid insecticide were examined using trap-nesting bees in a large-scale semi-field study. The availability of additional floral resources, their diversity, and flower species identity were important for bee reproductive success. We found indications of interactive effects of flower resource availability offsetting insecticide effects, with insecticide exposure reducing bee development only in mesocosms without additional floral resources. These results emphasize the importance of non-crop floral resource abundance and diversity for bees in the agricultural landscape, which can be provided by plants in flower strips, hedgerows, or field margins. With various stressors acting on insect populations in the agricultural landscape, other combinations, such as the use of different pesticides applied as mixtures, or at similar times, should be tested in experimental studies in the future, to identify further interactive effects.

Secondly, the export of pollination services into the surrounding landscape were quantified utilizing phytometer plants at different distances from calcareous grassland fragments as potential source habitats. Solitary bee numbers decreased with increasing distance, suggesting the importance of calcareous grasslands as their main habitat. Pollinators either less bound to the source habitats, or with larger foraging radius, such as bumble bees and hoverflies, were able to compensate for the loss of solitary bee pollination services at larger distances. Further research in more simplified landscapes could replicate this study with larger distances, to identify, if and at which distance pollination services start to decrease, in order to identify possible ecosystem service gaps in the landscape.

Thirdly, trap nesting insect communities were used to study how habitat loss and landscape diversity affect abundances of species depending on their trophic level and grade of food and habitat specialization. It was found that species from higher trophic levels are positively affected by semi-natural habitat at larger spatial scales, compared to species from lower trophic levels, but only when they are generalists. Parasitoids, typically being more

specialized, were affected at similar scales as their hosts, demonstrating the need to consider different spatial scales for the conservation of species from different trophic levels and with different grades of specialization. We also found evidence for species within trophic levels being affected differently, depending on their type of prey. Establishing species-specific responses would require even larger datasets with appropriate numbers of observations for single species, and could be the focus of future research.

Intensification has transformed agricultural landscapes and is still doing so today (Robinson & Sutherland, 2002). Associated threats to biodiversity and ecosystem services need to be explored and addressed, to be able to sustain multifunctional agricultural ecosystems (Foley et al., 2005; Tscharntke et al., 2005). In summary, this thesis shows how threats associated with agricultural intensification, namely habitat loss and fragmentation, landscape simplification, and exposure to insecticides, can have different and interactive effects at different scales on beneficial insects and their associated ecosystem services, depending on their trophic level and grade of food and habitat specialization. Understanding how species with certain traits are affected differently is critical for the design and implementation of effective and efficient conservation measures, and the results from this thesis hopefully contributed to filling some of the knowledge gaps. The importance of both the size of fragments of semi-natural habitats, as well as of landscape diversity have been shown, and are to be considered for a landscape-wide approach of species conservation. Furthermore, the potential of food resource availability and diversity in offsetting negative effects of other stressors such as insecticide exposure should be further explored and considered in future conservation programs and agri-environmental schemes.

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Curriculum vitae

Felix Klaus
felix.klaus.sc@gmail.com

EDUCATION

- 2016-2023 **GEORG-AUGUST-UNIVERSITY GÖTTINGEN** Göttingen, GERMANY
PhD Candidate, to be submitted
- Agroecology Group
 - Funded by Research Training Group 1644 “Scaling Problems in Statistics”
- 2014-2016 **GEORG-AUGUST-UNIVERSITY GÖTTINGEN** Göttingen, GERMANY
Master of Science in “Biological Diversity, Ecology & Evolution”, March 2016
- Master’s thesis in Agricultural Ecology analyzing pollen from trapnesting wild bees
- 2013-2014 **UNIVERSITY OF CALIFORNIA, DAVIS** Davis, USA
Fulbright Scholarship
- Research in Neal Williams Lab on wild bee establishment for crop pollination
- 2009-2013 **GEORG-AUGUST-UNIVERSITY GÖTTINGEN** Göttingen, GERMANY
Bachelor of Science in “Biological Diversity and Ecology”, March 2013
- Bachelor’s thesis in Agricultural Ecology
- 2011 **LINCOLN UNIVERSITY** Christchurch, NEW ZEALAND
Abroad studies
- Studying Southern Hemisphere- and island-ecology
- 1999-2008 **HEINRICH VON KLEIST-GYMNASIUM** Bochum, GERMANY
German Abitur
- Majoring in Biology and Geography

EXPERIENCE

- 2020 - 2023 **INSTITUTE FOR BEE PROTECTION** Braunschweig, GERMANY
Julius Kühn-Institut
- Research fellow in BeesUp project
 - Wild bee monitoring
 - Development and evaluation of bee conservation measures
 - Work on planning tool for wild bee friendly enhancement of urban areas
- 2016 - 2023 **AGROECOLOGY GROUP** Göttingen, GERMANY
University of Göttingen
- Project studying combination of stressors (insecticides and limited floral resources) affecting solitary wild bees
Klaus, F., Tscharnke, T., Bischoff, G., & Grass, I. (2021). Floral resource diversification promotes solitary bee reproduction and may offset insecticide effects—evidence from a semi-field experiment. Ecology Letters, 24(4), 668-675.
 - Project studying effects of habitat loss and landscape diversity on cavity-nesting insects of different trophic levels and grades of specialization
Klaus, F., Tscharnke, T. & Grass, I. (2023) Trophic level and specialization moderate effects of habitat loss and landscape diversity on cavity-nesting bees, wasps and their parasitoids. Insect Conservation and Diversity.

- Project studying pollinator spillover from calcareous grasslands into surrounding agricultural landscape
Klaus, F., Tschardtke, T., Uhler, J., & Grass, I. (2021). Calcareous grassland fragments as sources of bee pollinators for the surrounding agricultural landscape. Global Ecology and Conservation, 26, e01474.
 - Presenting at GfÖ conference 2019 in Münster
 - Presenting at esa (entomological) conference 2018 in Vancouver, CA
- 2016 **INSTITUTE FOR BEE PROTECTION** Braunschweig, GERMANY
Julius Kühn-Institut
- Field assistant
 - Effects of pesticides on non-target organisms
 - Field, semi-field and laboratory studies of honeybees, bumblebees and solitary wild bees
 - Poster presentation at yearly meeting of German bee research institutes
- 2012 - 2016 **AGROECOLOGY GROUP** Göttingen, GERMANY
University of Göttingen
- Master's thesis "Benefits of Hedgerows for Cavity-Nesting Insect Communities in the Agricultural Landscape"
 - Project on hedgerows and pollinator/pollen movement
Klaus, F., Bass, J., Marholt, L., et al. (2015). Hedgerows Have A Barrier Effect And Channel Pollinator Movement In The Agricultural Landscape. Journal of Landscape Ecology, 0(0), pp. -. Retrieved 16 Jul. 2015, from doi:10.1515/jlecol-2015-0001
 - Bachelor's thesis on post-harvest quality of strawberries linked to pollination success
Klatt, B., F. Klaus, C. Westphal, and T. Tschardtke. 2014. Enhancing crop shelf life with pollination. Agriculture & Food Security 3:14.
 - Research assistant 10/2012 – 07/2013 and 11/2014 – 03/2016
- 2014 **NEAL WILLIAMS LAB** Davis, USA
University of California, Davis
- 10 weeks position as junior specialist
 - Fulbright postacademic training
 - Research on cavity-nesting pollinators in the agricultural landscape
- 2013 **LAHMANN'S BIOLAND** Voigtholz, GERMANY
Organic Farm
- 5 weeks internship on organic farming practices (WWOOF)
- 2011 **LANDCARE RESEARCH** Christchurch, NEW ZEALAND
Environmental Research Organization
- 8 weeks internship on Classical Biological Control
 - Learning about experimental design, field and lab methods, data collection and entry, statistics, and time- and project-management

ADDITIONAL

Languages: Fluent in English and German
Basic knowledge of French and Swedish

Skills include: Project Management & literature research
Managing & surveying trapnesting wild bees, wasps & honey bees
Pollen analysis (morphological/molecular)
Identification of insects (esp. Hymenoptera, Diptera, Coleoptera)
Statistical analysis in R, Excel, OpenBUGS, & JAGS
GIS in ArcGIS & R
Programming in Visual Basic
Driving license (German class B)

Interests include: Bee-keeping, Wildlife gardening, Running, Soccer

