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A two-part modelling approach reveals a positive effect of pollinator biodiversity in boosting the pollination of apple flowers

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ABSTRACT

Pollinator biodiversity may benefit crop pollination. Yet benefits in agro-ecosystems may be context-dependent and offset by agronomic or other limiting orchard-specific or tree-specific factors that obscure biodiversity-ecosystem service relationships. To test if crop pollination benefitted from pollinator biodiversity, we sampled local wild bee communities in five organic and five Integrated Pest Management (IPM) apple orchards in Germany and experimentally measured the pollination success of apple flowers, quantified as the number of pollen tubes reaching the base of styles. Using standard statistical modelling approaches for over-dispersed count data, we found little or no effect of wild bee biodiversity on pollination success, irrespectively of farm management (organic versus IPM). There was, however, a positive relationship between the number of pollen tubes in insect-pollinated and pollen-supplemented flowers across trees and orchards, suggesting confounding effects of local tree- or orchard-specific factors limiting pollination success. Using a statistical two-part hurdle model which allowed us to separate (i) the probability of pollination and (ii) the quantity of pollination, we were able to demonstrate that the collective effects of local tree/orchard factors acted as a primary limiting threshold for pollination success. Once the threshold was crossed, the hurdle model demonstrated that increasing wild bee abundance enhanced pollination success of apple trees. We advocate the use of statistical two-part models as a more powerful approach to identifying limiting factors of, and the role of biodiversity in, crop pollination and potentially other ecosystem services in agro-ecosystems.

1. Introduction

Biodiversity is considered to underpin ecosystem functions and services to society (Hooper et al., 2012; Lister and Garcia, 2018) but it is vanishing at alarming rates, with potential major ecological and economic consequences (The Economics of Ecosystems and Biodiversity (TEEB), 2010; Cardinale et al., 2012). For instance, a recent study by Hallmann et al. (2017) revealed >75 % decline across 27 years in total flying insect biomass around the city of Krefeld, Germany, a loss recorded more broadly across Germany (Seibold et al., 2019) and the world (van Klink et al., 2020v). As flying insects pollinate many wild and crop plants (Klein et al., 2007; Ollerton et al., 2011), it is of great importance to understand the relationships between pollinator diversity and the ecosystem service of pollination they deliver (Winfree, 2013), especially as world agriculture becomes more pollinator-dependent without a concomitant increase in crop diversity (Aizen et al., 2019).

Various components of pollinator (particularly bee) biodiversity can benefit crop pollination. Both species richness (Klein et al., 2003; Garibaldi et al., 2013; Winfree et al., 2018) and abundance (Garibaldi et al., 2013; Winfree et al., 2015; Castle et al., 2019; Perrot et al., 2018) of wild bees as well as functional (trait) diversity (Hoehn et al., 2008; Martins et al., 2015; Woodcock et al., 2019), trait matching (Garibaldi et al., 2015) and phylogenetic diversity (Grab et al., 2019) have been shown to be positively associated with improved pollination services (that is, the transfer of viable, compatible pollen from anthers to stigmas) and, subsequently, crop production (fruit or seed set of crops) (reviewed in Dainese et al., 2019).

Agricultural practices that are thought to be environment- and biodiversity-friendly, e.g. organic farming, support pollinator communities in intensively managed areas (Kennedy et al., 2013; Tuck et al.,

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2015; Carrié et al., 2018). They can ensure stable production for some crops (Andersson et al., 2012), although reduction in fruit yield of up to 48 % in organic compared to IPM apple orchards was recently reported (Samnegård et al., 2019). In summary, the overall effects of organic land management on pollinator diversity and, especially, crop pollination are highly variable and sometimes insignificant (Kremen et al., 2002; Bengtsson et al., 2005; Brittain et al., 2010; Porcel et al., 2018). Generalizing the impact of organic agriculture therefore still requires additional tests of the relationships between organic land management, pollinator biodiversity and ensuing pollination service provision across crop species and cropping systems.

The benefits of pollinator biodiversity for pollination and, subsequently, for the production of pollinator-dependent crops may be context dependent (Bos et al., 2007; Boreaux et al., 2013; Bartomeus et al., 2015; Garibaldi et al., 2018), a topic that has been largely neglected. In agricultural systems, pollination success may be limited not only by pollinator shortfall but also by other local biotic and abiotic conditions and resources, which we term here *local factors*. These factors may be provided to the plant by biodiversity (e.g. nutrient cycling), or by farmers as an external input (e.g. water, fertilizers, pesticides), or as a management intervention (e.g. removal of weeds, tillage, provision of appropriate pollinizer cultivar, spatial arrangement of pollinizer and cropping variety) (Klein et al., 2015; Marini et al., 2015; Tamburini et al., 2017, 2019). If these local orchard factors collectively limit pollination success, they represent a primary threshold for a successful pollination event. Pollination of pollinator-dependent crops is mainly studied from the perspective of pollinator limitation, or factors negatively affecting pollinator species richness and abundance, assuming *a priori* no limitation due to suboptimal supply of local orchard factors (cf. Martins et al., 2015). Existing evidence, however, suggests that local farming practices in real-world agricultural systems may be suboptimal with respect to a range of local factors (e.g. fertilizer or pesticide regime), which themselves impact pollination success (Boreaux et al., 2013).

Fruit, nut and seed production of pollinator-dependent crops may be limited by local factors operating before, during or after pollination *per se*, defined as the movement of viable, cross-compatible pollen from anther to receptive stigma (Willmer, 2011). The bottom-up effects of one local factor, limited resources to a plant, are known to modify or even cancel the benefits of enhanced pollen transfer in fruit or seed set during the post-pollination period (Bos et al., 2007; Klein et al., 2015; Tamburini et al., 2017). But local factors may also impact the probability of a successful pollination event, either before or as pollinators come into play. For instance, the longevity of ovules and the development of floral features associated with pollinator attraction, such as flower number, size, production of floral volatiles or the quality and amount of nectar, are affected by water and nutrient availability to a plant (Williams, 1965; Muñoz et al., 2005; Cardoza et al., 2012; Ceulemans et al., 2017). For crops with complex cross-cultivar incompatibility systems, like many rosaceous fruit trees, choice and spatial placement of compatible pollen sources (pollinizers) may additionally modify the chances of successful pollen transfer. This may be especially the case if a pollinizer differs from the main cultivar in attractiveness to pollinators (Klatt et al., 2013) or is planted in a spatial configuration that ignores typical pollinator foraging behaviour (Free, 1993). Thus, in sub-optimally managed agro-ecosystems, a complex interplay of diverse local factors other than pollinators may represent a primary site-specific or tree-specific (and consequently variable) limiting threshold to successful pollination. Benefits for pollination from naturally or artificially enhanced pollinator biodiversity can be achieved only upon surmounting this threshold. Pollinator supplementation, either by buying or renting managed pollinators or devoting part of the agricultural land to support wild pollinator species, increases farmer expenses. Thus, it is of applied relevance to identify the operation of such local factors *versus* lack of pollinator biodiversity in affecting pollination success.

However, it may be extremely challenging under field conditions to detect a threshold and to identify individual local factors acting upon a crop plant as a prerequisite for successful pollination. Accounting for their interactive effects over short- and long-time scales is all the more challenging. For instance, Muñoz et al. (2005) found a more than two-fold increase in floral display and flower visitation rates to experimental shrubs only after two years of fertilization with a nitrogen-containing fertilizer. A standard technique in pollination ecology, namely manual pollen supplementation with fresh pollen from the closest local compatible pollen source (which is most likely to be used by insect pollinators), can be employed instead to discern local factors acting as a threshold in pollination success. Manual pollination can potentially summarize into one variable the effects on pollination of all on-site interacting factors other than pollinator biodiversity. Thus, manual pollination of a crop variety is expected to be independent of pollinator supply and to give comparable results across sites of the same crop. If local factors besides pollinators considerably limit pollination, the success of manual pollination may correlate with the success of insect pollination (Martins et al., 2015) and vary substantially across sites, potentially obscuring any relationship between pollinator biodiversity and pollination. Finding a correlation between manual pollination and the success of insect pollination may therefore be one means of detecting thresholds to pollination success caused by local factors. But it has so far rarely been measured in agro-ecological studies.

Here, we implemented pollen supplementation experiments with apple (*Malus domestica* Borkh.), an economically important and highly pollinator-dependent crop (Free, 1993; Garratt et al., 2014; Pardo and Borges, 2020). We aimed to i) explore the relationship between pollinator biodiversity and pollination success, measured as the number of pollen tubes at the base of a style, and ii) test the benefits of organic land management for pollinators and pollination. Because we found effects of site- or tree-specific local factors on pollination, we formally tested for their threshold effect in limiting pollination services to apple using several statistical modelling approaches, including a two-part hurdle model (Zuur et al., 2009). Finally, we advocate the use of hurdle models in the detection of threshold effects in pollination and potentially other biodiversity-ecosystem service relationships.

2. Material and methods

2.1. Study sites

Fieldwork was carried out in spring 2014 at ten apple orchards across the federal state of Saxony-Anhalt, Germany (Fig. 1, Table 1) to compare the effects of certified organic management *versus* Integrated Pest Management (IPM) on local pollinator diversity and provision of pollination services. As landscape-scale factors may influence pollinator community richness and abundance, a paired experimental design was used (Fig. 1). To ensure spatial independence of our field sites, we selected orchards located >3 km away from each other within an organic/IPM pair and >38 km between pairs (Fig.1).

All orchards under study were diversified (other fruit crops such as cherries, pears, plums and strawberries as well as apple were grown) and varied in size from 0.3–18 ha (7.48 ha ± 6.59 ha; mean ± SD), with the apple-planted area ranging from 0.24 ha to 12 ha (5.02 ha ± 4.36 ha; mean ± SD). Trees of the same cultivar, grafted on semi-dwarf MM106 rootstock, were grown in blocks of 3–4 rows. The distance between rows was 2−3 m, with ~1.5 m between trees in the same row. Understory vegetation was mown before apple flowering in all but two sites. A cross-compatible pollinizer cultivar had been planted either as a neighbouring block or as single trees at the beginning and end of each row (Table A.1). Only two orchards had pollinizer trees interspersed within the line of the main cultivar (Table A.1). Pollinizer cultivars varied across sites (Table 1).

Information on additional, local orchard-specific factors was gathered from each site, namely: total area of the orchard, area planted with apples, tree age, total number of apple cultivars per orchard, spatial

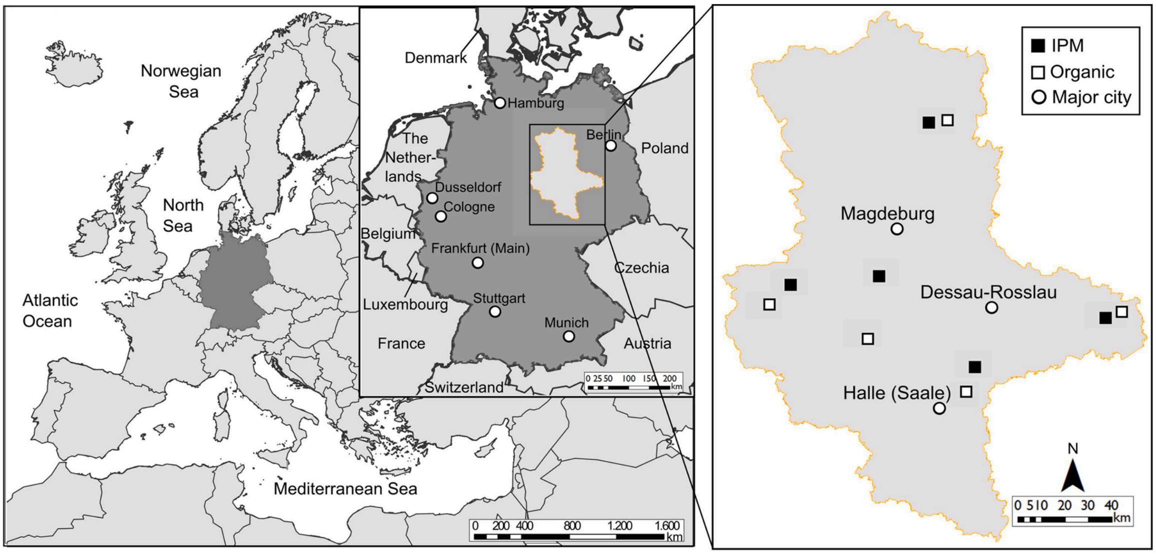

Fig. 1. Location of the ten study orchards (square boxes) in the federal state of Saxony-Anhalt, Germany; IPM – Integrated Pest Management.

Table 1
Description of the study sites.

Orchard	Management	Orchard pair	Latitude	Longitude	Experimental cultivar	Pollinizer	PropIP	Proportion of urban land use at 1000 m	Shannon landscape diversity at 1000 m	Mean No. of pollen tubes per style in pollen-supplemented flowers (± SE)
Altenweddingen	IPM	b	52° 2′2.34″N	11°32′1.38″E	Elstar	Gloster	0.60	1.00	0.00	2.8 (±0.3)
Dingelstedt	IPM	d	51°58′22.40″N	10°59′59.61″E	Belle de Boskoop	James Grieve	0.70	0.02	0.72	4.9 (±0.5)
Schweinitz	IPM	c	51°47′42.69″N	13° 0′15.95″E	Elstar	Idared	0.72	0.24	1.20	8.7 (±0.6)
Stendal	IPM	e	52°37′35.36″N	11°50′32.45″E	Elstar	Gloster	0.44	0.24	1.31	3.4 (±0.3)
Zörbig	IPM	a	51°36′16.95″N	12° 6′40.42″E	Elstar	Gala	0.74	0.96	0.21	13.4 (±0.6)
Aschersleben	Organic	b	51°44′42.89″N	11°27′7.40″E	Elstar	Gloster	0.38	0.30	0.64	3.1 (±0.3)
Heudeber	Organic	d	51°54′58.07″N	10°51′22.60″E	White transparent	Alkmene	0.56	0.02	1.03	2.2 (±0.2)
Lindwerder	Organic	c	51°49′41.31″N	13° 1′45.30″E	Elstar	Idared	0.62	0.28	1.18	3.4 (±0.3)
Plößnitz	Organic	a	51°32′21.21″N	12° 3′35.16″E	Elstar	crab apple	0.78	0.95	0.26	NA (failed)
Storkau	Organic	e	52°37′36.56″N	11°56′45.32″E	James Grieve	Braeburn	0.48	0.18	0.64	4.1 (±0.3)

IPM – Integrated Pest Management, PropIP – proportion of insect-pollinated styles with at least one pollen tube growing, Proportion of urban land use - proportion of urban land use (parks, allotments, cemetery, residential, industrial, retail, quarry) in the surrounding landscape at 1000 m radius.

arrangement of pollinizer in relation to the cropping variety (blocks of rows *versus* interspersed pollinizer trees), irrigation and tillage (Table A.1).

2.2. *Landscape variables*

Land-use simplification due to agricultural intensification is considered to reduce local bee diversity and abundance, and can lead to reduced crop pollination (Kennedy et al., 2013; Martin et al., 2019). In contrast, semi-natural habitats in the surrounding landscape provide alternative foraging and nesting resources for pollinators and have been shown to have a positive impact on their diversity and abundance (Kennedy et al., 2013). Urban land use has been reported to decrease (Bates et al., 2011) as well as to boost (Theodorou et al., 2017, 2020) pollinator diversity and pollination. To account for variation in surrounding land use, we quantified the proportion of arable land (annual tilled crops), orchards, forest, semi-natural habitats (meadow/pasture and scrub) and urban areas in the landscape surrounding each orchard at 250, 500, 750, 1000, 1500 and 2000 m radii using land cover maps (Geofabrik GmbH, Germany) in Quantum GIS (http://www.qgis.org/).

To identify the scale at which the surrounding land use had the most power to explain variance in the abundance of flower-visitors, we correlated wild bee abundance (derived from pan trap material) with landscape diversity (Shannon diversity (Shannon and Weaver, 1949) of proportions of land use types) at each of our study sites at all six radii (Table A.2). Correlation coefficients peaked at the 1000 m scale, which was then chosen as the spatial scale for statistical modelling. This scale is within the foraging range of many bee species (e.g. Greenleaf et al., 2007). At this scale, the Shannon landscape diversity index did not differ significantly between organic and IPM orchards (GLMM, $P > 0.05$, Fig. A.4). Also, the proportion of each land use class surrounding sites (Tables 1 and A.1) did not differ significantly between organic and IPM orchards (GLMM, $P > 0.05$).

2.3. *Sampling of bees and ground vegetation*

During the peak of apple flowering at each orchard, we quantified the species richness and abundance of local flying insect communities using six pan traps per site (two yellow, two white and two blue, each of diameter 24 cm and height 3 cm), mounted on a stick at the height of the apple blossom and two-thirds filled with odourless soapy water. Pan traps were exposed along the row of experimental trees (see 2.5 Pollination experiments) at each site for 48 h of sunny, warm (21.5 ± 2.1 °C, mean ± SD), moderately humid (45.4 ± 7%, mean ± SD) calm weather during apple bloom. Temperature, wind speed and humidity were recorded at each site. Collected bees were preserved in 70 % ethanol and identified in the lab under a stereo-microscope. Identification to species level of morphologically cryptic specimens was confirmed by DNA barcoding of the COI gene (Supplementary methods B). Prior to statistical analysis, bee species richness was rarefied to a common sample size to account for different sample sizes across study sites.

While pan-trapping bees, we also randomly placed ten 1 m^2 quadrats in each orchard to measure the richness of flowering plant morphospecies in the understory vegetation and the percentage of bare soil at each site as proxies for alternative foraging and nesting resources, respectively, for ground nesting bees like *Andrena* spp., which are frequent temperate-zone apple flower visitors (Mallinger and Gratton, 2015; Park et al., 2016). We defined a flowering plant morphospecies on site as a group of plants with visually identical morphological flower traits (e.g., flower shape, size, colour of petals). A list of recorded plant genera in apple orchards is provided in Appendix C.

2.4. Surveys of flower visitors

During pan trapping, we also performed a transect walk of 500 m over 30 min at each site between 11:00 and 15:00, when we recorded all observed apple flower visitors which made contact with reproductive parts of a flower. Transects were placed along rows of the cultivar used for pollination experiments, including experimental trees. Flower visitors were assigned to five insect morphogroups: honey bees (*Apis mellifera* L.), bumble bees (*Bombus* spp.), *Andrena* spp., other bees and Diptera.

2.5. Pollination experiments

We studied one of the most common modern apple cultivars in Saxony-Anhalt, “Elstar”, which is self-sterile (S-alleles: S_3S_5) and requires transfer of cross-cultivar compatible pollen for successful seed and fruit development (Matsumoto, 2013). At three sites where “Elstar” was absent, we chose other self-sterile cultivars: “White Transparent” (S_1S_5), “James Grieve” (S_5S_8) and the triploid “Belle de Boskoop” ($S_2S_3S_5$) (Table 1).

We performed pollination experiments at each site to quantify the relative success of self-/wind and insect pollination. Counting pollen grains deposited on stigmas is the most straightforward approach to measure pollination directly and to avoid potential underestimation due to post-pollination effects on initial or final fruit/seed set (Kremen et al., 2002). However, it is not applicable to apple because cross-compatible pollen cannot be readily differentiated based on pollen sculpture alone. Furthermore, genetic incompatibility occurs not on the stigmatic surface but during pollen tube growth through the style, where S-RNases are expressed (Losada and Herrero, 2014). Therefore, we used the number of pollen tubes at the base of the style as a measure of pollination, which has been shown to be informative in other fruit crops (Brittain et al., 2013).

At each site, we selected 10 trees of the same cultivar in the same row, with 3–5 trees separating each experimental tree, and each at least 10 m away from the orchard edge. Three experimental treatments were applied to each tree, using one “king bud” flower per treatment per tree: 1) pollination exclusion (representing self-/wind pollination); 2) insect pollination (representing pollination provided by the local pollinator community); and 3) manually applied pollen supplementation.

As not all flowers start to flower at the same time, two relatively small branches with 4–5 inflorescences were covered with a PVC mesh bag (mesh size = 1 mm) shortly before blooming to prevent insect visitation. One of them was assigned to the pollination exclusion treatment and the other to future pollen supplementation. At the same time, we chose an additional branch at the same height and side of the tree and marked it with tape as the insect pollination treatment.

We returned to each site during peak apple bloom and evaluated the receptivity of flowers on experimental branches based on criteria in the Supplementary methods 2. The insect-pollinated flowers were collected once the receptive period of all five stigmas of a flower was finished (the surface of a stigma is not sticky anymore and turns brownish, petals fade and get loose). Hence, the insect-pollinated flowers were open for insect visitation for a maximum 7 days. For manual pollination experiments, we used the first receptive “king bud” flower on each branch that fulfilled these criteria. Stigmas of each receptive flower assigned to the pollen supplementation treatment were manually pollinated until they were fully covered by pollen grains. For each flower, we used fresh pollen from at least three trees belonging to the closest compatible pollinizer present in the orchard. Manually pollinated, insect-pollinated and pollinator-excluded flowers were then collected into open 1.5 mL Eppendorf tubes with 300 μL of tap water, ensuring that peduncles reached the water and stigmas did not touch the surface of the tube. Petals and anthers were removed before flowers were placed into tubes. Flowers were kept out of direct sunlight, returned immediately to the laboratory at ambient temperature and held at room temperature (22 °C) for 72 h to ensure full pollen tube growth. Then we fixed flowers in FAA (1:1:18, formalin, acetic acid, 70 % ethanol) and stored them at 4 °C until we counted pollen tubes at the base of the styles.

2.6. Counting of pollen tubes

Collected flowers were processed and stained following Losada and Herrero (2014). After staining with aniline blue, softened hypanthium tissue was removed, styles carefully separated from each other and squashed on a microscope slide. Freshly prepared slides were immediately examined by fluorescence microscopy (Zeiss Axioplan 2, Carl Zeiss, Jena, Germany). We scored the numbers of pollen tubes reaching the base of a style; we assume that these pollen tubes would have penetrated and fertilized the flower’s ovules.

The number of pollen tubes directly measures the transfer of viable, compatible pollen (i.e. pollination) in apple. To test how the number of pollen tubes reaching the base of a style relates to final fruit and seed set, we simultaneously repeated the pollination experiment as described above on the same trees at one pair of orchards (IPM orchard Zörbig and paired organic orchard Plößnitz, Table 1). Instead of collecting the flowers, we now marked them after the treatment application and rebagged them. Bags were removed after petal fall and experimental flowers were monitored over the season till fruit maturity. We collected experimental fruits before harvesting and counted their seeds.

2.7. Statistical analyses

To test the effects of the 1) proportion of arable land, 2) orchards, 3) urban land cover 4) forest, and 5) semi-natural (meadow/pasture and scrubs) habitats at the 1000 m radius, as well as 6) the Shannon landscape diversity at 1000 m, 7) management (IPM vs. organic), 8) the richness of local flowering plant morphospecies and 9) the percentage of bare soil on wild bee abundance (derived from pan trap material), we constructed Generalized linear mixed-effects models (GLMMs) with a negative binomial error distribution. Linear mixed-effects models (LMMs) were used to evaluate the effects of the same predictors listed above on rarefied bee species richness. Orchard pair was included as a random factor in both GLMMs and LMMs.

We tested for the effects of pollination treatment (pollination exclusion vs. insect pollination vs. pollen supplementation) on the number of pollen tubes at the base of a style using a GLMM with negative binomial error distribution. Styles nested within flowers, trees within an orchard, and orchards within an orchard pair were included as random factors to account for the hierarchical structure of our experimental design. As pollen supplementation completely failed in one organic orchard possibly due to inviable pollen of the crab apple pollinizer (Plößnitz, Table 1), we excluded this site and its paired IPM orchard (Zörbig, Table 1) from statistical modelling that incorporated this treatment. A Spearman rank correlation was used to test for an association between insect pollination and pollen supplementation treatments.

GLMMs with automated model selection were then applied to assess the relationship between pollination success (the number of pollen tubes at the base of a style in insect-pollinated flowers) and the following independent explanatory variables: 1) the collective effect of local factors (measured as the number of pollen tubes at the base of a style in pollen-supplemented flowers), 2) individual local factors (orchard size, tree age, total number of cultivars, spatial arrangement of pollinizer, presence of irrigation), 3) local microclimate (temperature, wind speed, humidity), 4) farming type (IPM vs. organic), 5) the proportion of arable land, orchards, forest, semi-natural and urban land cover in the surrounding landscape, 6) the Shannon landscape diversity at 1000 m radius, 7) rarefied wild bee species richness (derived from pan trap material), 8) the abundance of wild bees (derived from pan trap material) and 9) the abundances of each morphogroup of flower visitor (honey bees, bumble bees, *Andrena* spp., and other bees, derived from transect observations). The proportion of forest in the surrounding landscape was significantly correlated with the Shannon landscape diversity (Spearman's rho = 0.84, P = 0.001). Therefore Shannon landscape diversity only was included in the modelling. Pollination success was modelled at the style level. We accounted for our hierarchical sampling design and the resulting pseudoreplication in numbers of pollen tubes at the base of styles by nesting styles within a tree, trees within an orchard, and orchards within an orchard pair. Therefore, the effects revealed by the model are at the tree or orchard level. The abundance of Diptera was not included because it accounted only for a marginal number of visits to apple flowers (2%). Initially we included apple variety as a covariate to control for potential variation in pollination success due to physiological differences between experimental cultivars. But it was consistently insignificant and therefore we removed it from final analyses.

Due to over-dispersion in our response variable 'number of pollen tubes at the base of a style', we applied a negative binomial error distribution, a standard approach to deal with over-dispersed count data, in our analyses. However, our dataset had a high frequency of zero observations (41.5 %). To account for zero-inflation in our response variable 'number of pollen tubes at the base of a style', we additionally modelled the relationship between pollination success and the same nine predictor variables listed above using two zero-inflated and one zero-altered GLMM for count data and compared them using the Akaike information criterion (AIC) (Zuur et al., 2009). These models were as follows: i) zero-inflated Poisson; ii) zero-inflated negative binomial; and iii) zero-altered two-part hurdle model (Mullahy, 1986; Zuur et al., 2009; Zuur and Ieno, 2016). We used the Akaike information criterion to evaluate the relative goodness of fit to our data of the candidate models listed above and the negative binomial GLMM, a standard means of analyzing skewed data.

Standard count-based zero-inflated models are limited by the assumption that zeros (absence of pollen tubes) and non-zeros (presence, but also a count of the number of pollen tubes) result from the same ecological process. In contrast, the underlying idea of a two-part hurdle model does not constrain these two processes to be the same. Thus, one ecological process or factor could affect the presence/absence of pollen tubes, whereas another ecological process or factor could influence the number of pollen tubes. In the first part of the hurdle model, a Bernoulli probability model was used to model the presence/absence of pollen tubes. The hurdle was crossed only if the realization was positive (pollen tube growth was initiated), and then the non-zero count data (number of pollen tubes) were modelled with a (zero-truncated) count distribution (Zuur et al., 2009). In the count part of our hurdle model, we applied a zero-truncated negative binomial error distribution due to over-dispersion present in our dataset. The R code used to set up the two-part hurdle model is provided in Appendix E.

When models included several factors, we used an all-subset (i.e. all combinations of predictors of interest), automated model selection approach based on the Akaike Information Criterion, corrected for small sample size (AICc). We only allowed up to three predictors in each candidate model to avoid model over-fitting. After model selection, the final models predicting pollination success included just the collective effect of local factors and wild bee abundance as explanatory variables. Model selection was conducted using the *dredge* function in the R package *MuMIn* (Bartoń, 2013).

All variables included in the models described above were standardized to a mean of zero and standard deviation of one to derive comparable estimates. We used variance inflation factors with a cut-off value of 3 to check for collinearity among the explanatory variables (Zuur et al., 2009); variables included in models were not collinear. All mixed-effect models were performed using the R package *glmmTMB* (Brooks et al., 2017). R 3.2.4 software (R Core Team, 2013) was used to perform statistical analyses.

3. Results

3.1. Bee communities in apple orchards

Across the ten orchards surveyed, we sampled 329 bee individuals belonging to 38 species in pan traps (Table A.3.). Pan trapped bee communities had 10.3 ± 3.4 species per site (mean ± SD, range 5–15) and were numerically dominated by wild bees (98 %), especially ground-nesting species belonging to the genera *Andrena* (Andrenidae) (51 %, 15 species) and *Lasioglossum* (Halictidae) (34 %, 12 species).

Although honey bees were vastly underrepresented in sampling with pan traps (pan traps are known to under sample large bees, see Westphal et al., 2008), they were the dominant visitor of apple flowers observed during transect walks. Honey bees accounted for 73 % of 1024 recorded flower visits, followed by bumble bees (11 %), *Andrena* spp. (9%), other bees (5%, mainly *Anthophora* spp.) and Diptera (2%). Though the abundance of honey bees observed on transect walks increased with the abundance of wild bees measured by pan-trapping, the relationship was not statistically significant (Spearman's rho = 0.515, P = 0.133, Fig. A.1).

3.2. Local and landscape-scale effects on bee biodiversity

Bee abundance, as measured from pan trapped material, was not statistically related to any local-scale factor (GLMM, $P > 0.05$). In relation to landscape-scale factors at the 1000 m scale, wild bee abundance dropped with increasing Shannon landscape diversity (GLMM, z = -4.72, $P < 0.001$, $R^2 = 0.71$, Fig. 2a) and increased with the proportion of urban land use in the surrounding landscape (GLMM, z = 3.32, $P < 0.001$, $R^2 = 0.53$, Fig. 2b).

Rarefied bee species richness, as also measured from pan trapped material, increased with flowering plant morphospecies diversity in apple orchards (LMM, $t_8 = 2.6$, $P = 0.03$, $R^2 = 0.39$, Fig. 3). We did not find differences in either wild bee species richness (LMM, $P > 0.05$) or abundance (GLMM, $P > 0.05$) between organic orchards and their paired counterparts under IPM (Fig. 4a, b). This lack of difference between IPM and organic management may be because the richness of flowering plant morphospecies in the understory did not differ markedly between management types (LMM, $P > 0.05$, Fig. 4c).

Honey bee abundance, as measured by transect walks, was not influenced by local or landscape-scale factors (GLMM, $P > 0.05$). Neither did it vary between organic orchards and their paired counterparts under IPM (GLMM, $P > 0.05$) (Fig. 4d).

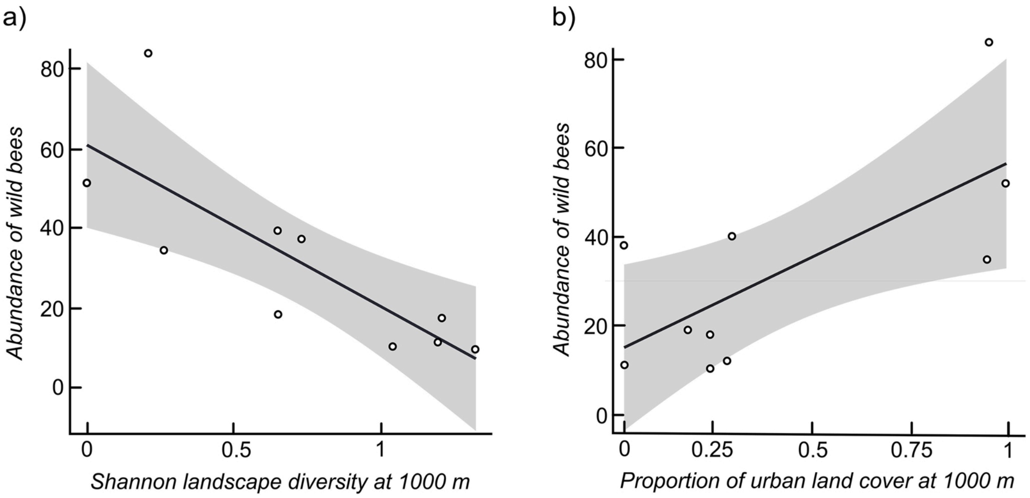

Fig. 2. Association between wild bee abundance in pan traps and a) Shannon landscape diversity or b) proportion of urban land use, both at 1000 m radius of an orchard (GLMM, $P < 0.001$); dots represent actual data points ($n = 10$ orchards) whereas the plotted line and the shaded areas (95 % confidence intervals) are the predicted relationships from statistical modelling by GLMM.

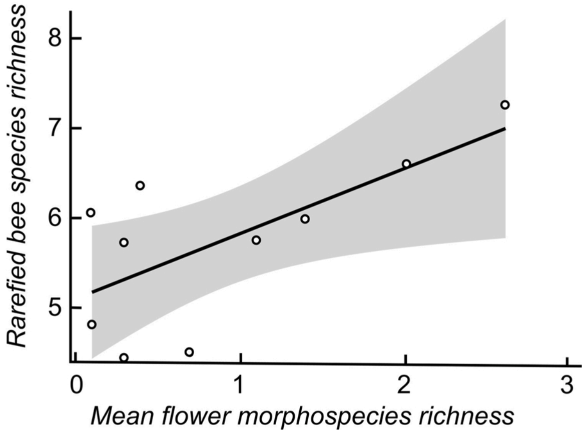

Fig. 3. Positive association between flower morphospecies richness in understory vegetation (averaged per 10×1 m^2 quadrats within each orchard) and rarefied wild bee species richness (LMM, $P < 0.05$); dots represent actual data points ($n = 10$ orchards) whereas the plotted line and the shaded areas (95 % confidence intervals) are the predicted relationships from statistical modelling by LMM.

3.3. Pollen limitation

The number of pollen tubes at the base of a style differed significantly between experimental pollination treatments (GLMM, $z = -13.11$, $P < 0.001$). It was higher in insect-pollinated (1.12 ± 0.06, mean ± SE) and pollen-supplemented flowers (5.08 ± 0.21, mean ± SE) than in the insect-excluded treatment (0.03 ± 0.01, mean ± SE), and highest in the pollen-supplemented treatment (Tukey's HSD, $P < 0.001$). Insect-excluded flowers had almost no pollen tubes, confirming self-incompatibility of apple cultivars under study and the irrelevance of wind pollination for this crop. A significantly higher number of pollen tubes in pollen-supplemented compared to insect-pollinated flowers suggests that orchards under study were pollen-limited.

The number of pollen tubes at the base of a style in insect- and hand-pollinated flowers was significantly correlated with seed set of the corresponding treatment of flowers on the same tree (Spearman's rho = 0.846, $P < 0.001$; Fig. A.2). This indicates that a large number of pollen tubes at the base of a style equates with high fruit set and crop yield for a given tree in a given orchard.

Both insect and manual pollination, measured as pollen tubes at the base of a style, varied considerably across orchards (Fig. A.3).We surprisingly found a significant correlation in counts of pollen tubes between insect-pollinated and experimental pollen-supplemented flowers across orchards (Spearman's rho = 0.800, $P = 0.01$; Fig. 5). This suggests that orchards are predisposed to either high or low pollination success, which represents evidence for local, site-specific factors (hurdles) limiting pollination.

3.4. Local orchard management and pollen tube number

To test for local site- or tree-specific factors (hurdles) limiting pollination, we statistically modelled pollination service provision to apple in relation to orchard management, landscape structure, bee biodiversity and other local (tree or orchard-specific) factors (see Table 1 and Table A.1). The two-part hurdle model (AICc = 1105.4, log-likelihood= -542.70) clearly outperformed the zero-inflated Poisson GLMM (AICc = 1232.4, log-likelihood=-609.18), the zero-inflated negative binomial GLMM (AICc = 1234.4, log-likelihood=-609.19) and the negative binomial GLMM (AICc = 1235.5, log-likelihood=-610.75). Thus, the two-part hurdle model was used to interpret associations between tested predictors and pollination.

The hurdle model showed significant effects of both local tree or orchard-specific factors and wild pollinator abundance on pollination success (Table 2). Only one of these two explanatory variables was identified by other candidate models. Our hurdle model revealed that the occurrence of a successful pollination event (the presence of pollen tubes at the base of a style, irrespective of their number, and representing the first or binary part of the hurdle model) was significantly associated with the collective effect of local factors, which we term '*local tree/orchard effects*' ($z = 3.43$, $P < 0.001$), but not with bee diversity and not with management type (organic versus IPM) (Table 2). The number of pollen tubes reaching the base of a style (representing the second or quantitative part of the hurdle model), in contrast, was positively associated with the abundance of wild bees in the pan traps ($z = 2.05$, $P = 0.04$, Fig. 6), but not with local tree/orchard effects and not with orchard management type (organic versus IPM) or honey bee abundance on transect walks.

Individual local factors that entered our initial model selection, namely orchard management (organic vs. IPM), orchard size, tree age, total number of cultivars, spatial arrangement of pollinizer and presence of irrigation (Table 1, Table A.1), were consistently non-significant in model selection. Other local factors we recorded, namely tillage and pollinizer variety (Table A.1), were not included in statistical modelling

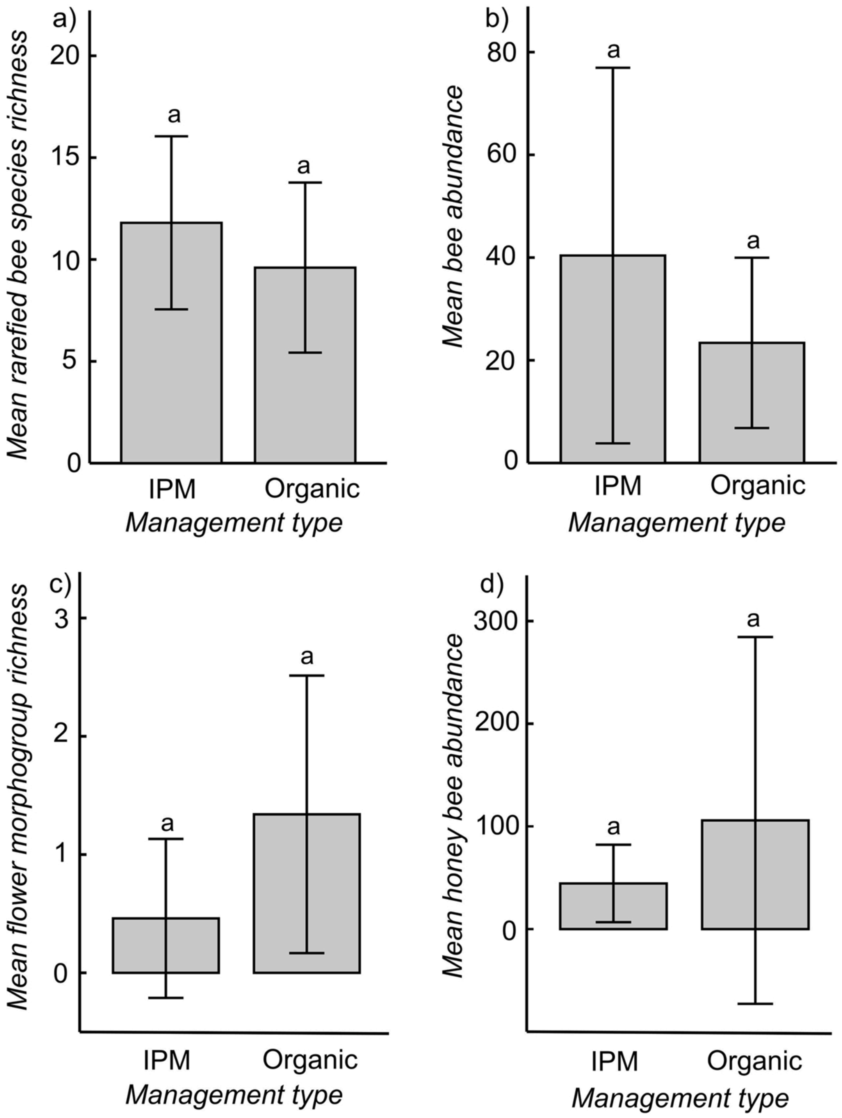

Fig. 4. Effects of orchard management (five Integrated Pest Management (IPM) *vs.* five organic orchards) on a) mean rarefied wild bee species richness, b) mean wild bee abundance, and c) mean understory flower morphospecies richness, and d) mean abundance of honey bees observed during the transect walk (Tukey's HSD for all comparisons, $P > 0.05$); bars indicate 95 % confidence intervals.

due to lack of variation across orchards or lack of replication. We therefore could not identify the specific cause or causes of collective local tree/orchard effects.

4. Discussion

We found that orchard management (IPM *versus* organic) did not directly impact pollinators or provision of the ecosystem service of pollination to commercial apple trees. Pollinator biodiversity was, though, related to the diversity of flowering wild plant species in orchards. For some trees and orchards, pollination *per se* was foremost hindered by local factors other than pollinators. For trees and orchards above the threshold imposed by local factors, the provision of pollination increased with wild bee abundance but not with that of managed honey bees.

4.1. Two-part hurdle model

Our results highlight the strength of a two-part modelling approach in identifying factors limiting pollination success and in unraveling the masked effects of biodiversity on ecosystem service provision. Through the partitioning of our statistical model into a binary distribution sub-model and a truncated count distribution sub-model, we were better able to provide a natural interpretation of the context-dependency of crop pollination in real-world agroecosystems. For instance, one may assume that a proportion of flowers have zero probability to be pollinated due to lack of nutrients necessary for an apple tree to produce flower volatiles and high-sucrose nectar to attract pollinators. These local tree/orchard effects determine the probability for stigmas in a flower to be pollinated at all. A second factor or process, namely wild bee abundance, then determines how many pollen grains will be deposited on the stigmas of the flowers that produce sufficient volatiles and nectar to attract flower visitors. In line with previous studies that have shown context-dependent post-pollination effects of pollinator biodiversity on crop yield (Bos et al., 2007; Boreaux et al., 2013; Klein et al., 2015), our findings suggest that similar interactive effects between pollinator abundance and local factors also apply at the pollination *per se* stage of plant reproduction.

Additionally, two-part models are suitable for over-dispersed and heavily zero-inflated data, as is typical of measurements of pollination success, i.e. fruit and seed set, and potentially in other areas of agricultural ecology. Despite their obvious advantages, two-part models are

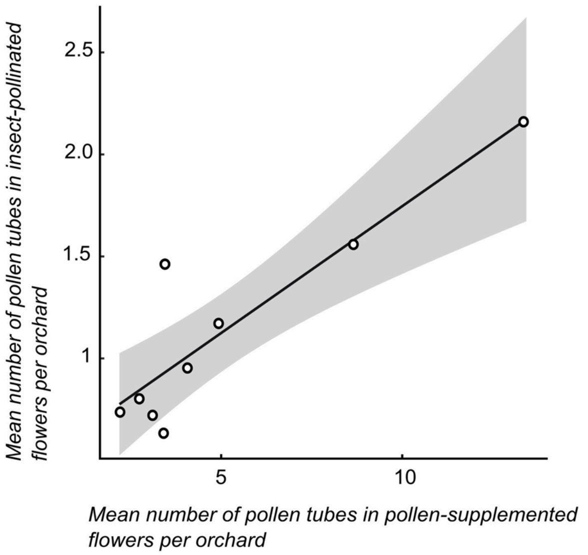

Fig. 5. Scatterplot of pollen tube number at the base of a style in insect and manually pollinated flowers across orchards (n = 9 orchards, Spearman's rho = 0.800, $P = 0.01$). One orchard was excluded due to failed pollen supplementation. Dots indicate actual data points, the black solid line represents predicted relationship and the shaded area represents 95 % confidence intervals (using linear regression function).

Table 2
Model coefficients of local tree/orchard effects and wild bee abundance on pollination in the negative binomial GLMM (a), zero-inflated models (b, c) and the two-part hurdle model (d); all estimates are based on standardized values.

Response (Number of pollen tubes in insect-pollinated flowers)	Estimate	Std. Error	z value	*P*-value
(a) Negative binomial				
(Intercept)	−0.16	0.13	−1.24	0.21
Local orchard effect	0.03	0.01	2.41	0.01*
Abundance of wild bees	0.14	0.10	1.44	0.14
(b) Zero-inflated Poisson				
(Intercept)	0.09	0.16	0.58	0.56
Local orchard effect	0.02	0.01	1.51	0.13
Abundance of wild bees	0.20	0.10	1.94	0.05*
(c) Zero-inflated negative binomial				
(Intercept)	0.10	0.17	0.61	0.54
Local orchard effect	0.02	0.01	1.44	0.14
Abundance of wild bees	0.21	0.10	1.94	0.05*
(d, part i) Hurdle model, binary component				
(Intercept)	−0.14	0.20	−0.69	0.48
Local orchard effect	0.10	0.03	3.43	<0.001 ***
(d, part ii) Hurdle model, count component				
(Intercept)	0.31	0.13	2.42	0.01
Abundance of wild bees	0.20	0.102	2.05	0.04*

* $P < 0.05$.
*** $P < 0.001$.

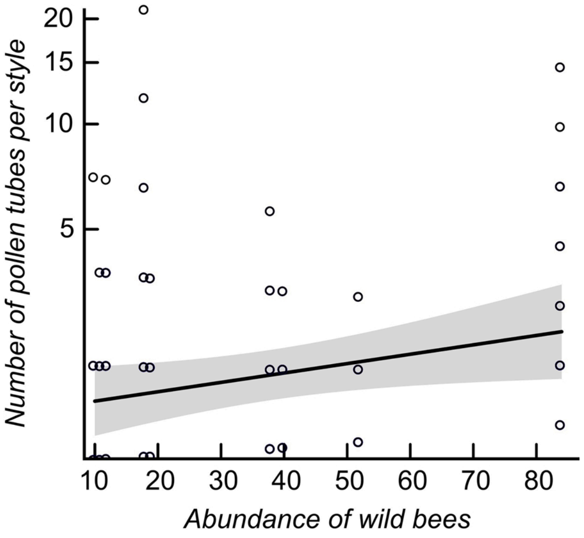

Fig. 6. Positive effect of overall bee abundance on number of pollen tubes at the base of styles in insect-pollinated apple flowers at 8 orchards, estimated using the hurdle model (count part) ($P < 0.05$); plotted line shows predicted relationship and the shaded areas indicate 95 % confidence intervals (count part of hurdle model).

barely applied in crop pollination research (cf. Gillespie et al., 2015), although they are a well-established statistical framework in other fields of ecology e.g. in species-abundance (Potts and Elith, 2006) or epidemiological studies (Chipeta et al., 2014). We advocate their wider employment for exploration of bi-partite relationships, as seen in our analysis of pollinator biodiversity and the ecosystem service of pollination.

4.2. Local factors

Using a two-part statistical model, we found that local factors collectively (our local tree/orchard effects) had a major impact on pollination success. Orchards below a threshold, namely the collective hurdle of local factors, experienced low pollination success, regardless of wild bee abundance.

Our study did not find a direct effect on pollination success of any individual local factor tested, such as irrigation or spatial arrangement of pollinizer trees. This is possibly because no one local factor on its own was closely related to the first or binary part of our hurdle model in predicting whether a pollination event would occur at all. Alternatively, interactions between two or more local factors might have driven the association between local orchard effects in the first part of our hurdle model but our sample size was not sufficient to test it.

Although we were not able to identify what factor exactly caused a threshold for pollination, we hypothesize that one local tree/orchard effect that we could not formally test due to lack of replication, namely the lack of viable, cross-cultivar compatible pollen, could have played a role. Our apple orchards as well as those reported by others (e.g. Garratt et al., 2014; Blitzer et al., 2016) were pollen-limited. Pollen limitation is usually attributed to lack of effective pollinators for pollen transfer. But, for self-incompatible plants like apple, incompatible pollen, even if deposited by pollinators on a stigmatic surface in large amounts, will not result in pollen tube growth and subsequent fertilization of ovules (Free, 1993). Lack of appropriate pollinizer may therefore lead to low realized pollination in an orchard, independent of pollen vectors. Our lack of success in hand pollination at the Plößnitz orchard points to this local factor as a potential cause of pollination shortfall.

For the ca. 7 500 apple varieties grown worldwide for direct consumption or for cider and other edible products, the suitability of diploid cultivars as pollinizers of cropping varieties is mainly determined by S-alleles and overlapping flowering time (Matsumoto, 2013). However, a variety's relative attractiveness to pollinators, a trait crucial for successful pollination, is rarely considered either in horticultural recommendations for choice of pollinizer when planning an orchard or in selection programmes for development of new varieties (Klatt et al., 2013). Additionally, the spatial configuration of pollinizer trees in an orchard could reduce the benefits of even a highly compatible pollen source on crop pollination if it does not correspond to the behavioural foraging preferences of dominant pollinators in the area (Brittain et al., 2013).

4.3. Effects of biodiversity on pollination

Relative effects of species richness *versus* abundance on ecosystem service provision have been extensively debated, in large part because understanding the functional consequences of biodiversity loss has become a central issue in ecology (Cardinale et al., 2006, Winfree, 2013; Dainese et al., 2019). The count part of our hurdle model showed the importance of wild bee abundance for pollination, but it was not affected by bee species richness and honey bee abundance (Table 2). In our study system, both organic and IPM orchards had diverse bee communities, even though highly mobile species like honey bees or bumble bees were underrepresented in pan trap material due to sampling bias typical to this sampling method (Westphal et al., 2008). During the relatively short pan-trapping time period of 48 h, we recorded on average 10 species per site (ranging from 5 to 15), in line with the results of long-term monitoring of orchard bee diversity (on average 36.5 species per site across the whole apple flowering season) in the United States of America by Russo et al. (2015). Field-based correlational and experimental research suggests that species richness is more important for high pollination provision in extremely species-poor systems, in study systems with gradients in species diversity that include very species-poor sites (Albrecht et al., 2012; Connelly et al., 2015; Blitzer et al., 2016) or at high spatial (e.g., regional) scales due to species turnover (Winfree et al., 2018). Controlled cage experiments by Fründ et al. (2013) showed the highest increase in pollination service provision when augmenting species richness from one to more than one species. In relatively species-rich communities, like in our study system, the species richness-function relationship is most likely saturated due to niche complementarity and selection effects (Loreau and Hector, 2001). They may explain why we did not find a relationship between bee species richness and pollination.

The overall abundance of wild bees in our study orchards was, however, associated with increased pollination service. Although the abundance of flower visitors is thought to translate into increased visitation rates (Morris et al., 2010), bee species dominating the pan-trap communities (mainly bees of the genera *Andrena* and *Lasioglossum*) were not among the most common flower visitors observed during our transect walks. They nevertheless may have contributed to enhance pollination indirectly via facilitation effects (Greenleaf and Kremen, 2006; Brittain et al., 2013) rather than directly via pollination.

Following the mass ratio hypothesis (Vázquez et al., 2005), the numerically dominant pollinator should have the highest functional effects, even if it is less effective per visit than less abundant species. The honey bee was the most common flower visitor in our orchards and performed almost three quarters of all observed insect-flower interactions. But we found that the abundance of honey bees was not associated with pollination success. Similar findings have been reported both for apple (Mallinger and Gratton, 2015; Blitzer et al., 2016, reviewed in Pardo and Borges, 2020) and a diversity of crops worldwide (Garibaldi et al., 2013). It could reflect the poor pollination efficiency of honey bees, possibly attributable to poor colony health (Geslin et al., 2017), or the side-working behaviour of nectar-collecting honey bee workers visiting apple flowers (Russo et al., 2017). It could also reflect the tendency of honey bee to move from one tree to another of the same cultivar along a row rather than to switch between rows of different cultivars (Free, 1993), or reflect the saturation of visits by honey bees to individual flowers or trees (Garibaldi et al., 2020). However, this still has to be explicitly addressed by future studies.

Limitations to apple pollination we observed could have potentially arisen also due to interactions among pollination and other ecosystem services supporting crop yield, e.g. biological pest control (Garibaldi et al., 2018; Tamburini et al., 2019). For instance, decreased pest regulation in an orchard may entail outbreaks of florivorous pests like the apple blossom weevil, *Anthonomous pomorum* (Gratwick, 1992). Damage it causes to apple blossoms modifies the final floral display and floral attractiveness, which may result in lower visitation rates by pollinators and less pollen deposited on stigmas. Conversely, high levels of pest control reduce pest pressure and thus can interact complementarily (Bartomeus et al., 2015) or synergistically with pollination (Sutter and Albrecht, 2016).

4.4. Effects of local habitat and organic orchard management on bee diversity

Alternative foraging and nesting resources for wild bees, provided either by local management within a field site or in the surrounding landscape, are thought to promote wild bee biodiversity in agro-ecosystems (Kennedy et al., 2013, Campbell et al., 2017, Samnegård et al., 2019). In line with these findings, we found the richness of flowering plant morphospecies in the ground vegetation of our orchards to be positively related to bee species richness, though not with bee abundance or pollination. Bee abundance was, however, affected by the surrounding landscape. An increasing proportion of urban land around orchards supported higher numbers of bee pollinators, potentially due to diverse green infrastructure and abundant foraging and nesting resources for bees in residential areas, allotment gardens etc. (Theodorou et al., 2020). The negative effect of landscape heterogeneity on wild bee abundance was most likely driven by the proportion of forest (the two landscape variables were significantly correlated) because the majority of wild bee species recorded in apple orchards require open soil for nesting.

Although the positive impact of pesticide-free practices like organic management on pollinators has been frequently documented (Holzschuh et al., 2008; Kennedy et al., 2013; Schneider et al., 2014; Lichtenberg et al., 2017; Samnegård et al., 2019), we did not detect a significant difference in bee diversity and abundance between organic and IPM orchards. We have two explanations for this result. Firstly, benefits of organic management for highly mobile organisms like bees were shown by previous studies to be dependent on landscape context, and greater in simple compared to complex landscapes (Batáry et al., 2011; Concepción et al., 2012). But they diminished or were even undetectable when controlling for landscape heterogeneity (Chateil and Porcher, 2015). The landscape heterogeneity surrounding organic and IPM orchards in our study did not differ significantly (Fig. A.4), suggesting that organic orchardists need to consider management of the wider landscape to enhance pollinators. Secondly, IPM, if strictly following IPM guidelines (e.g., avoid spraying pesticides during the flowering peak) and maintaining high on-farm habitat heterogeneity and diverse crops, potentially could support the same level of biodiversity as organic agriculture (Kennedy et al., 2013; Porcel et al., 2018). Indeed, in our study, the richness of flowering plant morphospecies in the understory did not differ between management types, suggesting similar availability of alternative foraging resources for pollinators in both IPM and organic orchards. However, the effect of IPM on pollinator biodiversity has to be explicitly tested by future studies.

4.5. Conclusions

Two-part hurdle models may be more powerful in revealing relationships between biodiversity and ecosystem services than commonly applied statistical approaches, especially for data structured with two components: binary and quantitative. Application of a two-part hurdle model enabled us to reveal the context-dependence of pollination in a pollinator-dependent crop, and found that crop plants were negatively impacted by local tree/orchard effects, which collectively constituted a primary limiting threshold to pollination in both IPM and organic orchards. Above the threshold, pollination benefitted from an abundance of wild bees, the numbers of which did not differ between IPM and organic orchards. We advocate the experimental and statistical approaches employed here to identify the threshold(s) imposed by local factors on pollination and other ecosystem services in agro-ecosystems. We conclude that pollination would likely be enhanced at our study orchards first and foremost by management focussed on the elimination of local agronomic factors that present thresholds to any successful pollination.

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 A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.107197.

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