Oecologia (2019) 189:149–158
https://doi.org/10.1007/s00442-018-4296-y

PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Establishment of wildflower fields in poor quality landscapes enhances micro-parasite prevalence in wild bumble bees

Niels Piot[1] · Ivan Meeus[1] · David Kleijn[2,3] · Jeroen Scheper[2] · Theo Linders[2,4,5] · Guy Smagghe[1]

Received: 6 March 2018 / Accepted: 29 October 2018 / Published online: 7 November 2018

Abstract

The current worldwide pollinator decline is caused by the interplay of different drivers. Several strategies have been undertaken to counteract or halt this decline, one of which is the implementation of wildflower fields. These supplementary flowers provide extra food resources and have proven their success in increasing pollinator biodiversity and abundance. Yet such landscape alterations could also alter the host–pathogen dynamics of pollinators, which could affect the populations. In this study, we investigated the influence of sown wildflower fields on the prevalence of micro-parasites and viruses in the wild bumble bee *Bombus pascuorum*, one of the most abundant bumble bee species in Europe and the Netherlands. We found that the effect of sown wildflower fields on micro-parasite prevalence is affected by the composition of the surrounding landscape and the size of the flower field. The prevalence of micro-parasites increases with increasing size of sown wildflower fields in landscapes with few semi-natural landscape elements. This effect was not observed in landscapes with a high amount of semi-natural landscape elements. We elaborate on two mechanisms which can support these findings: (1) "transmission hot spots" within the altered flower-networks, which could negatively impact hosts experiencing an increased exposure; (2) improved tolerance of the hosts, withstanding higher parasite populations.

Keywords Host–pathogen · Bumble bee · Conservation · Parasites · Flower mixes

Introduction

Parasites are essential components of well-functioning ecosystems, in which the dynamic equilibrium between host and parasite is key in shaping populations (Henson et al. 2009). A host population size is determined by the environmental carrying capacity controlled by both bottom-up (e.g., food availability) and top-down forces. Herein parasites, as the highest trophic level, act as a top-down force on the host population. Landscape alterations will greatly affect bottom-up forces on the host population, as they may alter food or nest availability. They will also affect the higher trophic levels (e.g., parasites); indirectly by influencing the hosts (e.g., affecting the host's overall physiological status due to altered food sources, thereby also affecting its immune competence) or directly by altering potential transmission routes between hosts. The net effect of landscape alterations on host population is, therefore, an interplay between its direct effect on bottom-up forces and its influence on top-down forces (Lafferty 2012; Cable et al. 2017).

Current conservation efforts to counteract or halt the decline of bee pollinators often include landscape

Communicated by Candace Galen.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00442-018-4296-y) contains supplementary material, which is available to authorized users.

✉ Guy Smagghe
guy.smagghe@ugent.be

[1] Laboratory of Agrozoology, Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, Ghent, Belgium

[2] Plant Ecology and Nature Conservation Group, Wageningen University, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands

[3] Resource Ecology Group, Wageningen University, PO Box 47, 6700 AA Wageningen, The Netherlands

[4] CABI, Rue des Grillons 1, 2800 Delémont, Switzerland

[5] Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

alterations. Understanding the effects of landscape alterations on bee parasites is of great importance, since parasites are regarded as an important driver of their current decline (Goulson et al. 2015). Both wild and domesticated bees, providing essential ecosystem services (Gallai et al. 2009), are affected by a wide range of different pathogens and parasites. Over 20 different viruses are reported for honey bees (Flenniken and Andino 2013; McMenamin and Genersch 2015) and most of these viruses have also been detected in other non-domesticated bee species (Gisder and Genersch 2017). Aside from viruses, there is also a wide array of protozoan and microsporidian pathogens described in honey bees and bumble bees (Goulson and Hughes 2015; Meeus et al. 2011). From hereon we will refer to these pathogens as micro-parasites, i.e., protozoan and microsporidian pathogens excluding viruses.

Until now the alterations of local host–parasite dynamics in pollinators have mainly been studied in relation to spillover events of domesticated honey bees or bumble bees towards wild bees. The main focus in these studies has been how the presence of domesticated pollinators affects the host–parasite dynamics in wild bees (Graystock et al. 2016; Arbetman et al. 2012; Colla et al. 2006; Fürst et al. 2014). Studies assessing the relation of landscape alterations and pathogen prevalence in bees remain scarce (Henson et al. 2009).

In Europe, agri-environmental measures have been implemented to enhance biodiversity on farmland and counteract the loss of pollination services. The influence of such measures on pollinator species richness and abundance has been investigated in several studies (reviewed in Scheper et al. 2013). However, implementing measures to enhance pollinators, such as sowing of wildflower fields, does not only alter the quality of the landscape for pollinators but can also change the plant-pollinator interactions (Geslin et al. 2017). Both could lead to changes in the host–pathogen dynamics (Holdenrieder et al. 2004).

Flowers are considered as hotspots for horizontal transmission of pathogens between pollinators (Graystock et al. 2015; Durrer and Schmid-Hempel 1994). Flowers visited by a larger number of pollinator species will presumably serve as a greater hotspot for pathogen transmission compared to less attractive flowers. One could expect that addition of a large amount of high quality attractive flowers (i.e., wildflower fields) decreases pathogen prevalence as the likelihood of pathogen transmission via flowers decreases because of a flower dilution effect. This has been shown for *T. grandiflorum* where there is a density dependent pollinator visitation, here high plant density decreases pollination visits per plant (Steven et al. 2003). Nevertheless, it is unknown whether implementation of flower fields result in an enhanced or reduced prevalence of pathogens in wild pollinators, as these flower fields can also attract pollinators. Here the amount of natural available floral resources is an important factor to be taken into consideration. Previous studies showed that the amount of semi-natural landscape has a substantial influence on the effect size of wildflower field implementation on pollinator diversity and density (Scheper 2013; Carvell et al. 2011).

The landscape context-dependent effect mainly seems to operate through direct positive effects of floral resource availability (Scheper et al. 2015). In areas with a low amount of semi-natural habitat, i.e., resource poor landscapes, pollinators could be attracted towards the sown flower fields. In these resource poor landscapes the contrast of adding a large amount of additional flowers is much larger than in areas with many semi-natural landscape elements, i.e., resource rich landscapes. Incorporating the surrounding habitat is, therefore, of importance when assessing the effect of wildflower field implementation.

In this study we assessed the effect of pollinator enhancing landscape alterations in the form of sown wildflower fields on the prevalence of micro-parasites and viruses in the common carder bee *Bombus pascuorum* in the Netherlands. We set up a paired sampling design, with locations in the middle and southern parts of the Netherlands, to answer the following questions: (i) does implementation of wildflower fields alter the prevalence of micro-parasites and viruses? (ii) do all pathogens display a similar response? and (iii) to what degree does the surrounding landscape composition influence the prevalence of micro-parasites and viruses in the implemented wildflower fields?

Materials and methods

Experimental design

To measure the influence of sown wildflower fields on the prevalence of micro-parasites and viruses, we used 16 rectangular 50 ha study areas in agricultural landscapes, mainly dominated by grasslands, winter wheat and maize, located across the middle and southern parts of the Netherlands (See Figure S1 in Supporting Information and Table S1). The size of the study area is essentially based on the average size of a Dutch farm (42 ha; CBS 2015) and so it represents the management unit in which measures to mitigate pollinator loss can be independently implemented in the landscape. In autumn 2012 or spring 2013, wildflower fields or strips ranging in size from 0.4 to 4.9 ha were sown in the center of eight study areas. Each experimental area with sown wildflower fields was paired with a control area that had a similar soil type and landscape context but did not have sown wildflower fields. Each flower field was sown with two different wildflower seed mixtures sown separately on half of the field. One mixture targeted long-tonged bee species (Mixture 1),

while the other mixture targeted short-tonged bees, hover flies and parasitoid wasps (Mixture 2) (for seed composition see supplementary material Table S2). Differences in bee species composition between mixtures were outside the scope of this paper and the pooled area sown with the two mixtures was therefore considered as the sown wildflower field in this study. The control sites were located at least 2 km from the sown flower fields.

Landscape characterization

In order to incorporate possible influences of surrounding landscape, we determined the landscape composition in each of the 50 ha study areas. We used ArcMap 10 (ESRI, Redlands, CA) to calculate the relative coverage of the different land-use types in each 50 ha study landscape and quantified the landscape complexity as the proportion of semi-natural habitat (mainly forests, heathlands, extensive grasslands, roadside verges and ditch banks) in the landscape.

Bumble bee collection

Bombus pascuorum is one of the most widespread and abundant species in the Netherlands and Europe. It is mostly a surface-nesting species, which is active from the beginning of April to the end of October. *B. pascuorum* makes medium-sized nests of up to 150 workers and has a foraging range of around 450 m from the nest, depending on the nest density (Knigtht et al. 2005). In this study we used this species as focal species, as this is one of the most widespread and abundant species of bumble bees in the Netherlands (together with: *B. lapidarius* and *B. terrestris*), for which adequate numbers could be sampled (i.e., a minimum of 10 individuals) at each site. From mid-August 2014 to early September 2014, workers of *B. pascuorum* ($N = 10–24$, median: 13), were caught in the center of each study area. The sampling was done on sunny, calm days. At each site the sampling was completed within a single day, and the paired sites were sampled on the same day. Bees were caught in a 2 m wide transect walk along the center of the sown wildflower fields, i.e., in the sown flower fields sampling across both flower mixes and along the center of the control study areas, i.e., field margins and road ditches. The transect walk was repeated until a minimum of 10 *B. pascuorum* bees were caught. After the 10th *B. pascuorum* was caught, sampling continued until the end of the transect walk. Bees were caught on flowers and individually stored in plastic containers (Ø: 7.5 cm, height: 7 cm). Bees were kept alive until they arrived at the lab, where they were processed. At least 10 individuals were caught at each site (for details see supplementary material Fig.S1 and accompanying Table S1).

Pollinator counts

Most bee pathogens are multi-host pathogens and can infect both honey bees and bumble bee species. The presence and abundance of other potential host populations may have an impact on the prevalence of micro-parasites and viruses in the focal species of this study, i.e., *B. pascuorum*. Hence transects were surveyed to estimate the abundance of these pollinators to have an idea of the exposure of *B. pascuorum* to the different pollinators.

Five transects of one by twenty meters were surveyed at each study site (Westphal et al. 2008). Transects were performed between 9 am and 5.30 pm on dry sunny days from early July to mid-August. Each transect had a net observation time of 10 min. Transects were randomly allocated in the center of each field, either the center of the flower fields or the center of the control fields. Species were identified in the field when possible, while difficult to recognize species were identified in the lab.

Bumble bees were identified to species level, to enable discrimination between *B. pascuorum* and other bumble bee species (Supplementary material Table S3 and Table S4). *Bombus terrestris* and *Bombus lucorum* were grouped together and identified as "*Bombus terrestris* group" as they are cryptic species.

Nucleic acid extraction

The abdomen of each collected individual of *B. pascuorum* was cut off and ground with mortar and pestle in 1.5 ml of RLT buffer (Qiagen, Venlo, the Netherlands) supplemented with 15 µl of β–mercapto-ethanol. Mortar and pestle were sterilized in between crushing (2 times rinsing with 90% ethanol and 5% bleach followed by rinsing with ultrapure water). The homogenate was centrifuged for 2 min at 2000*g*. The supernatant was transferred to a fresh tube and stored at − 80 °C until further use.

For DNA extraction, 200 µl of the homogenate was used and 400 µl of Lysis buffer G was added along with 40 µl of proteinase K and vortexed thoroughly. This was followed with a 1 h incubation at 52 °C and shaking (400 rpm). Further steps proceeded according to the manufacturer's protocol (Invisorb Spin Tissue Mini kit, Stratec Biomedical, Birkenfeld, Germany).

For RNA extraction, 200 µl of the homogenate was mixed with 200 µl of 70% ethanol. Further steps proceeded according the manufacturer's protocol (RNeasy Mini Kit, Qiagen). RNA was stored at − 80 °C until further use.

Micro-parasite detection

DNA was used to screen for protozoan and microsporidian pathogens. The PCR-based detection of *Crithidia* sp. and

Apicystis bombi was performed as in Meeus et al. (2010) and the detection of *Nosema* sp. as in Menail et al. (2016). PCR products from the micro-parasite screening were visualized on a 1.5% agarose gel. The pathogen identity was determined for several positive samples of each pathogen using direct Sanger sequencing (LGC, Middlesex, UK).

Virus detection

RT-PCR was performed with random hexamers primers, according to the manufacturer's protocol, using 500 ng of total RNA (SuperScript II Reverse Transcriptase, Life Technologies, Carlsbad, CA).

A multiplex PCR was used to screen for six viruses, i.e., Israeli acute paralysis virus (IAPV), Deformed wing virus (DWV), Sacbrood bee virus (SBV), Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV) and Chronic bee paralysis virus (CBPV) as described by Sguazza et al. (2013). Positive samples were sent for sequencing to confirm their identity. BQCV was not found in our screening.

Statistical analysis

Effect of the implementation of wildflower fields on prevalence of micro-parasites and viruses

All statistical analyses were performed using the computing environment R (R Core Team 2015). To test the effect of sowing wildflower fields on the prevalence of micro-parasites and viruses we used Generalized Linear mixed models (GLMM), this was done with the lme4 Package (Bates et al. 2015). This effect was tested for all micro-parasites, i.e., *Apicystis bombi*, *Crithidia bombi* and *Nosema bombi*. The influence of sown wildflower fields on the infection prevalence of pollinator RNA viruses was analyzed in a similar manner as the micro-parasites. However, it was not possible to analyze all viruses separately as the incidence of some of the viruses was too low, and so we opted to analyze all viruses together. The prevalence of the micro-parasites and viruses was a binomial response variable, for which the link function log of the odds ratio (logit) was used. The area of sown wildflower field (ha), area semi-natural habitat (ha) and their interaction served as fixed factors. As we used a paired setup, pair served as random factor.

Presence of potential host species

Since all of the investigated micro-parasites and viruses are multi-host pathogens the presence of other pollinators and the exposure of *B. pascuorum* to these potential host species could be a possible explanatory factor for the pathogen prevalence in *B. pascuorum*. Therefore we looked at the presence of different potential hosts (i.e., *B. pascuorum*, other *Bombus* sp. and *Apis mellifera*) in the center of each site. The ln (x + 1) transformed bee counts served as response variable in a Linear mixed model. Inspection of the residuals confirmed the absence of over-dispersion. Fixed factors were the size of the implemented wildflower fields (ha), area of semi-natural habitat (ha) and their interaction. Pair (i.e., paired experiment and control plot) and location (i.e., each study site) served as random factors. Location was nested within pair to account for multiple measures at each study site. Comparison of the abundance of the different potential hosts (Fig. 2) between control sites and flower field sites was done as described above where the fixed factor was Treatment (i.e., implementation of a flower field vs control fields).

To look at the effect of the abundance of other potential hosts on virus and micro-parasite prevalence in *B. pascuorum*, we used GLMMs where the fixed factors were the abundance of other bumble bees, the abundance of honey bees and their interaction. Pair served as a random factor. The prevalence of the micro-parasites and viruses was a binomial response variable, for which the link function log of the odds ratio (logit) was used.

Simulation study and power analysis

To inspect the sensitivity of our results we performed a simulation study. For each micro-parasite (i.e., *A. bombi*, *C. bombi* and *N. bombi*) a separate simulation was performed. The amount of infected individuals at each site (both flower field sites and control sites) was randomly fluctuated. Either the amount of infected individuals was increased by 1, remained unchanged or was decreased by 1 (Supplementary Table S8). For each micro-parasite 1000 simulations were run using the computing environment R (R Core Team 2015). The GLMMs used in the simulation study were the same as described above.

To investigate the power of our sampling we performed a power analysis (Supplementary Figure S6). For this analysis we used the power curve function from the SIMR package as described by Green and MacLeod (2016) and used the effect size of the interaction effect as reported for each micro-parasite (Table 3).

Results

Prevalence of micro-parasites and viruses in the wild bumble bee, *Bombus pascuorum*

In total 217 workers of *B. pascuorum* were caught and analyzed for the presence of micro-parasites (i.e., *A. bombi*, *C. bombi* and *N. bombi*) and viruses. Across all sites 86.2% of the screened bumble bees were infected with at least one of the investigated pathogens. The overall prevalence of

micro-parasites and viruses for each pathogen is displayed in Table 1. From all screened pathogens, *A. bombi* infections were the most abundant with a prevalence of 48.8% across all sites. The overall viral infections were somewhat lower than the micro-parasite infections: 55.8% of the bumble bees were infected with at least one of the six screened viruses, while this was 67.7% for the infection with at least one micro-parasite.

Effect of sown of wildflower fields on the prevalence of micro-parasites and viruses

Across all locations, 73.3% of the screened bees in wildflower fields were infected with at least one of the investigated micro-parasites: for the control sites this was 62.9%. The implemented flower fields (i.e., presence or absence of the flower fields, not taking into account the size of the fields) increased the micro-parasite prevalence (Supplementary Table S5), yet this was not significant. When the size of the flower fields and the composition of the surrounding landscape were taken into account we found that their interaction (wildflower field size × landscape composition) significantly affected the micro-parasite prevalence. This interaction was present for all three investigated micro-parasites (*A. bombi*: $\chi^2 = 11.445$, $p = 0.001$; *C. bombi*: $\chi^2 = 8.013$, $p = 0.005$; *N. bombi*: $\chi^2 = 9.386$, $p = 0.002$). In areas with a low amount of semi-natural habitat the implementation of a flower field increased the prevalence of micro-parasites. In these areas the prevalence of micro-parasites increased with the size of the implemented flower fields [see Supplementary Figure S3, for the confidence intervals in the area's which are extrapolated by the model, i.e., locations with a large flower field and low amount of semi-natural habitat (< 9 ha)]. In areas with a large amount of semi-natural habitat, the implementation and size of the flower field did not appear to affect the prevalence of the three micro-parasites compared to the control. For all three micro-parasites the effect of wildflower field implementation was highest in the areas with a low amount of semi-natural habitat. (Figure 1a–c, Table 2 and Supplementary Figure S3).

Our simulation study for the micro-parasites confirmed the significant effect of the interaction (wildflower field size × landscape composition) on the micro-parasite prevalence (Supplementary Figure S4, Table S7 and Table S8). After 1000 simulations, fluctuating the infection prevalence for each micro-parasite at each site, the interaction effect (wildflower field size × landscape composition) remained significant in 100% of the simulations of *A. bombi* infection prevalence. For *C. bombi* and *N. bombi* infection prevalence the interaction effect remained significant in 95.3% and 92.4% of the simulations, respectively. The directionality of the effect remained unchanged in all simulations for each micro-parasite (Supplementary Figure S5).

Analysis of the prevalence of pollinator RNA viruses showed no significant trends. The wildflower implementation and size as well as the surrounding landscape had no significant effect on the prevalence of the investigated RNA viruses ($p > 0.2$) (Fig. 1d, Table 2 and Supplementary Table S5).

Presence of potential host species

The overall abundance of *B. pascuorum*, that is the species used for pathogen analysis, was not different between the control sites and the sites where flower fields were implemented ($F_{1,7} = 0.002$, $p = 0.964$) (Fig. 2a). On the other hand, the overall abundance of the other potential hosts, i.e., other *Bombus* species ($F_{1,7} = 1.378$, $p = 0.26$) (Fig. 2b) and honey bees ($F_{1,7} = 2.964$, $p = 0.13$) (Fig. 2c) was higher in the sites where flower fields were implemented compared to the center of the

Table 1 Prevalence of different pathogens across both flower-sites and control sites

Pathogen	Type	Prevalence		
		Overall[a] (%)	Flower-sites (%)	Control sites (%)
Micro-parasite infection		67.7	73.3	62.9
Apicystis bombi	Neogregarinorida	48.8	55.4	43.1
Crithidia bombi	Trypanosomatidae	33.6	37.6	30.2
Nosema bombi	Nosematidae	15.2	15.8	14.7
Virus infection		55.8	55.4	56.0
ABPV	Dicistrovirus	0.9	0.0	1.7
BQCV	Dicistrovirus	0.0	0.0	0.0
CBPV	Unclassified RNA virus	39.2	34.7	43.1
DWV	Iflavirldae	3.2	2.0	4.3
IAPV	Dicistrovirus	16.1	9.9	21.6
SBV	lflauiriri3P	18.9	20.8	17.2

[a]Flower-sites and control sites taken together

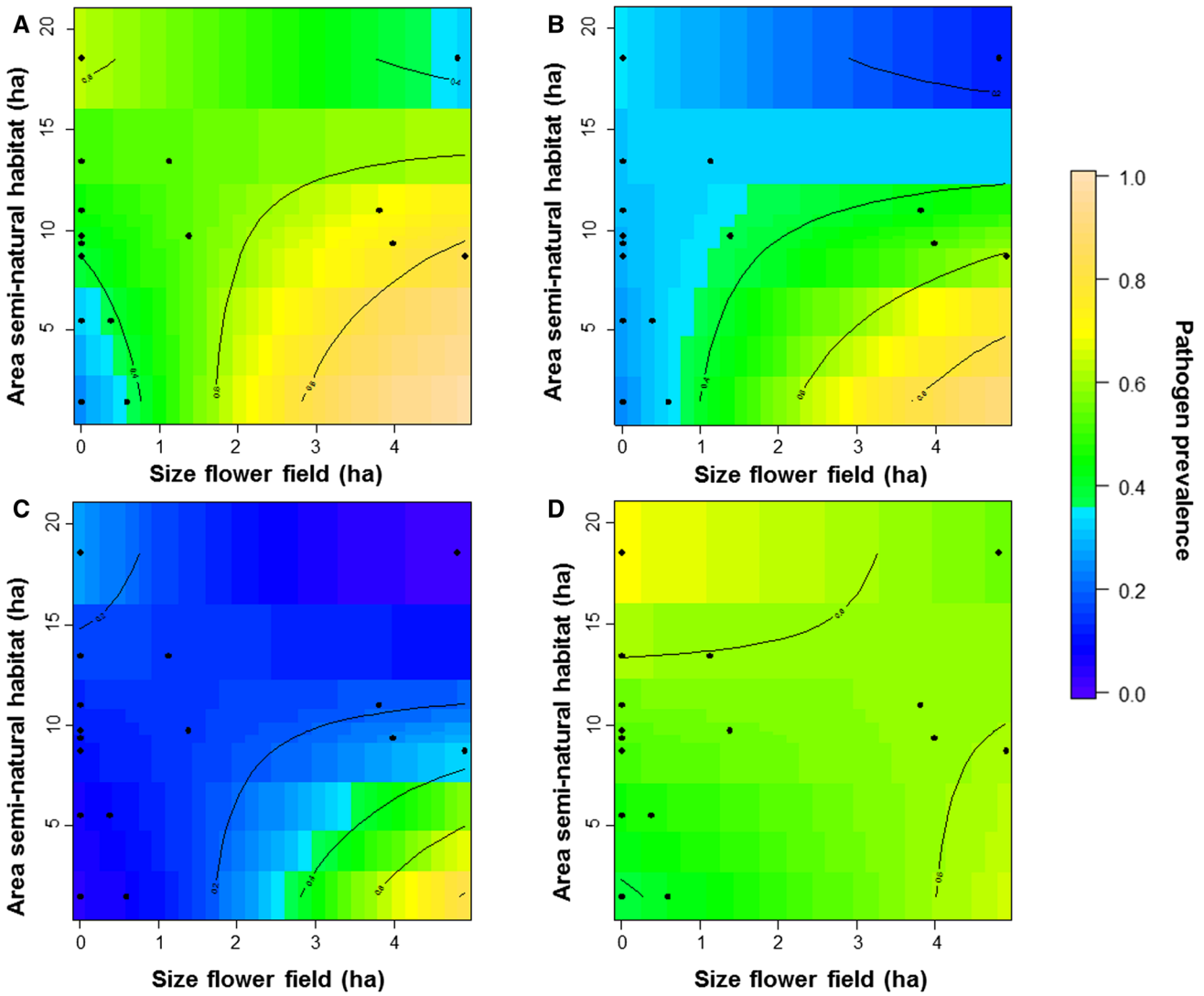

Fig. 1 Contour plots representing the interacting effect of wildflower field size (*x*-axis) and the area of semi-natural habitat in the surrounding landscape (*y*-axis) on **a** prevalence of *Apicystis bombi,* **b** *Crithidia bombi,* **c** *Nosema bombi* and **d** viruses. Black dots represent each site

control fields albeit not significant. Yet when looking at the total amount of other potential host species (i.e., other bumble bee species and honey bees together) they show a significantly higher abundance in the center of sites where flower fields were implemented compared to the center of the control fields ($F_{1,7}=5.695$, $p=0.048$) (Fig. 2d). The potential exposure of *B. pascuorum* to other pollinators (i.e., other bumble bee species and honey bees) was, therefore, higher in the flower fields as compared to the control fields. When we looked at the effect of the size of the implemented flower field and the surrounding landscape we found a significant effect of their interaction on the abundance of honey bees ($\chi^2=4.627$, $p=0.032$). Here the abundance of honey bees increased with the size of the flower field, and this increase was most pronounced in areas with a low amount of semi-natural habitat. The abundance of *B. pascuorum* and the other bumble bee species was not significantly influenced by either the size of the flower field or the surrounding landscape (Table 3 and Supplementary Figure S2).

When looking at the prevalence of the micro-parasites in *B. pascuorum* we find that the abundance of both honey bees and other bumble bees together had a positive effect on all investigated micro-parasites, i.e., a higher abundance of both honey bees and bumble bees increased the prevalence of all the investigated micro-parasites (Supplementary Table S6).

Table 2 Results of the GLMM with the effect of sown wildflower field size and the amount of semi-natural habitat on pathogen prevalence

	Parameter	β	χ^2	*p* value
Apicystis bombi				
	Flower-site	0.972	1.943	0.163
	Area SN	0.094	0.364	0.546
	Flower-site:Area SN	−0.066	11.445	**0.001**
Critbidia bombi				
	Flower-Site	0.742	1.226	0.268
	Area SN	0.026	0.250	0.617
	Flower-site:Area SN	−0.054	8.013	**0.005**
Nosema bombi				
	Flower-site	1.012	0.067	0.795
	Area SN	0.112	0.916	0.339
	Flower-site:Area SN	−0.084	9.386	**0.002**
Viruses				
	Flower-site	0.247	$3e^{-0.4}$	0.987
	Area SN	0.074	1.011	0.315
	Flower-site:Area SN	−0.020	1.079	0.299

Significant factors are indicated in bold

F*lower-site* Sown wildflower field size (ha), *Area SN* area of semi-natural landscape elements (ha), *Flower-Site:Area SN* interaction term

Discussion

Does flower field implementation alter pathogen prevalence?

To date several hypotheses exist concerning the influence of landscape alterations on population dynamics and biodiversity (Tscharntke et al. 2012). However, none of these hypotheses take into account the impact of landscape alterations on the top-down force, such as parasites and diseases. With this study we wanted to investigate the effect of landscape alterations, in the form of wildflower field implementation, on pathogen prevalence in the common carder bee *B. pascuorum*. We used a paired setup to sample bumble bees in similar locations where most factors interfering with parasite dynamics are mainly fixed and randomized over multiple paired locations. With this setup we wanted to assess if wildflower field implementation altered the parasite presence in *B. pascuorum*.

We found that the implementation of wildflower fields significantly changed the micro-parasite prevalence compared to the paired control sites and that this change was dependent on the size of the flower field and the surrounding habitat. In areas with a low amount of semi-natural habitat the prevalence of micro-parasites in the wild bumble bee, *B. pascuorum,* increased as the size of the flower field increased. This effect was observed for all three investigated

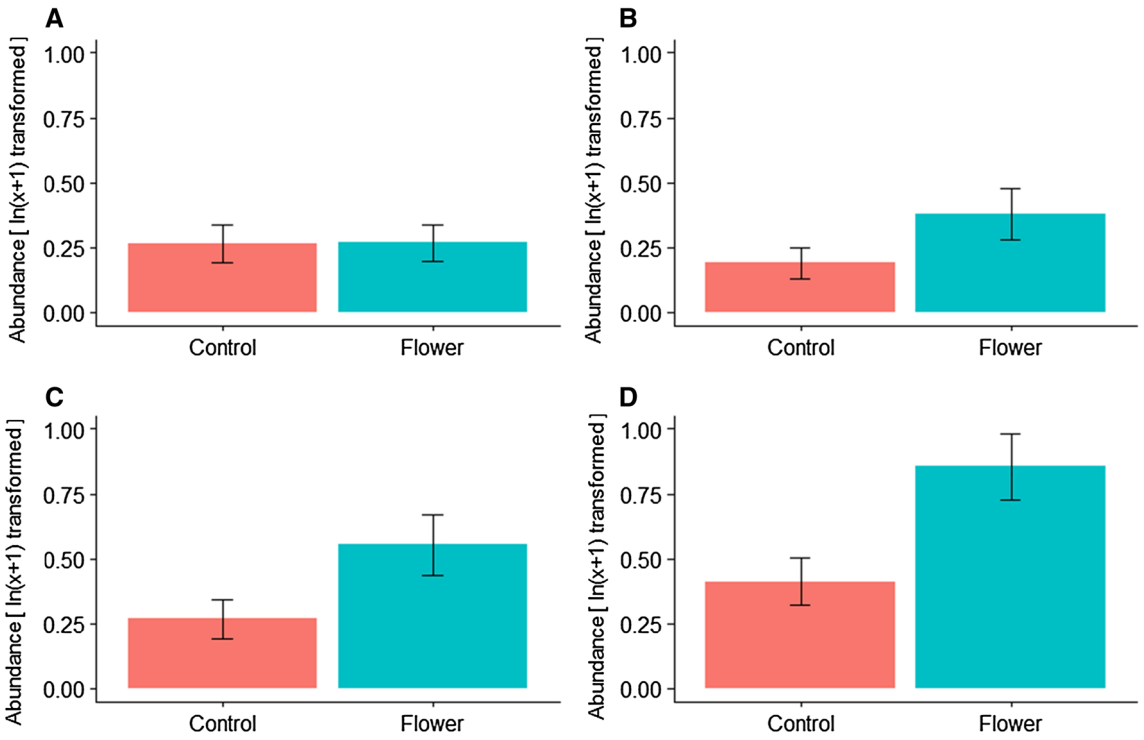

Fig. 2 The effect of flower field implementation on the abundance (mean ln($x+1$); $n=40$) of **a** *Bombus pascuorum,* **b** other bumblebee species, **c** *Apis mellifera* and **d** other bumble bee species and *Apis mellifera* together. Error bars show the standard error

Table 3 Results of the linear mixed models looking at the effect of sown wildflower field size and the amount of semi-natural habitat on the pollinator abundance [ln $(x+1)$ transformed] in the center of each site

	Parameter	β	χ^2	p value
B. pascuorum				
	Flower-site	0.109	0.234	0.629
	Area SN	0.018	0.199	0.656
	Flower-site:Area SN	−0.011	2.592	0.107
A. mellifera				
	Flower-site	0.333	3.775	0.052
	Area SN	−0.018	2.986	0.084
	Flower-site:Area SN	−0.019	4.627	0.032
Other *Bombus* sp.				
	Flower-site	0.065	0.158	0.691
	Area SN	−0.007	0.302	0.583
	Flower-site:Area SN	−0.004	0.178	0.673
Other *Bombus* sp. and *A. mellifera*				
	Flower-site	0.311	3.653	0.056
	Area SN	−0.022	2.026	0.156
	Flower-site:Area SN	−0.018	3.117	0.078

Flower-site: Sown wildflower field size (ha), *Area SN*: area of semi-natural landscape elements (ha), *Flower-Site: Area SN* interaction term

micro-parasites. Although we found a clear effect for the micro-parasites, we did not find this significant trend for the grouped viruses. The prevalence of the viruses was not significantly influenced by the implementation of the flower fields and their size, the amount of semi-natural habitat or their interaction. We expected that the prevalence of the RNA viruses would be influenced by the presence of honey bees as the investigated viruses are typically honey bee-associated (Goulson and Hughes 2015), and the abundance of honey bees has been reported as a good predictor for the prevalence of pollinator viruses in wild pollinators such as bumble bees (Fürst et al. 2014). Even though the abundance of honey bees was significantly affected by the size of the flower field and the surrounding environment (Table 3), the virus prevalence was not affected by these factors (Table 2). Furthermore, virus prevalence was not affected by honey bee presence (see supplementary Table S6).

What is the influence of the surrounding landscape composition on parasite prevalence?

Our results showed that the role of the surrounding environment on parasite prevalence is of importance. In areas with a low amount of semi-natural environment we noticed an increase of micro-parasites with an increasing size of the implemented flower field as compared to the control sites. This relation provides evidence that a landscape alteration is an important interaction factor for parasite prevalence in wild bee populations. Either the shift in parasite prevalence is caused by an indirect effect of the landscape changes on the host, or the landscape changes directly affect the parasite through an altered transmission dynamic. Here we explored these two plausible mechanisms which could explain the observed results.

(i) Wildflower field implementation could directly affect pathogen prevalence through the addition of flowers, which function as a transmission spot for pathogens. Implementing flower field could then alter the transmission potential of pathogens via flowers.

The addition of wildflower fields in areas with a low amount of semi-natural landscape (i.e., poor in available flower resources), could create an attraction effect towards the flower-rich sown wildflower fields. This phenomenon has previously been suggested by Kleijn and van Langevelde (2006) and was demonstrated for bumble bees by Heard et al. (2007) and Carvell et al. (2011) who showed this effect was dependent on the surrounding environment. This was recently also confirmed by Kleijn et al. (2018), using the same locations as this study, where they measured bee abundance in the flower field and in the adjacent area of the flower fields in 2 consecutive years (i.e., 2013-2014). In the first year they saw that the implementation of a flower field results in a clear attraction effect. In the second year they saw this effect was still present yet less pronounced, as the implementation of the flower fields had a landscape wide effect on the abundance of bumblebees.

It is, however, unknown if increased pollinator abundance results in an increased flower visitation frequency. If this were the case then flowers in these highly attractive patches would function as hotspots, driving pathogen transmission and increasing pathogen prevalence (Graystock et al. 2015; Durrer and Schmid-Hempel 1994; Cisarovsky and Schmid-Hempel 2014). To explore the transmission hypothesis we looked at the exposure of our focal species, *B pascuorum*, to other bumble bee species and honey bees. In our analysis we saw that *B. pascuorum,* did not really display this attraction effect, as the abundance of *B. pascuorum* in the flower field sites was not different from the control sites. When we look at the abundance of other bumble bee species and honey bees (Fig. 2b, c), the difference between the flower fields and the control fields was not significant. A more detailed analysis revealed that the size of the implemented flower field and the surrounding environment significantly influenced the abundance of honey bees, no significant effect was found for the other bumble bee species (Table 3). The presence of honey bees therefore appears to be an important contributing factor to the observed increase in micro-parasite prevalence. However, we did not observe the previously described association between honey bee abundance and virus prevalence

in bumble bees (Fürst et al. 2014), nor did we find any significant effect of honey bee abundance on the micro-parasite prevalence (Table S6).

From current knowledge we can infer that both honey bees and bumble bees can contribute to the parasite prevalence within *B. pascuorum*. Both honey bees and bumble bees can vector the investigated pathogens (Graystock et al. 2015), yet their role in the transmission network will differ. For example, honey bees could be less suitable hosts for the certain micro-parasites, as shown for *Crithidia bombi* (Ruiz-Gonzalez and Brown 2006), but could still play an important role in the vectoring between flowers (Graystock et al. 2015). While in bumble bee species this parasite can multiply within the gut, thereby this hosts will play an important role in increasing the micro-parasite inoculum in the network. We find that the interaction of honey bee abundance and bumble bee abundance had a positive effect on the prevalence of *A. bombi*. Here an increased abundance of both honey bees and bumble bees significantly increases the prevalence of *A. bombi* (Supplementary Table S6).

Overall the exposure of *B. pascuorum* to the other potential hosts (i.e., honey bees and other bumble bees) was higher in the flower fields and appeared to coincide with an increased prevalence of micro-parasites. As we used bee abundance to explore this transmission hypothesis we are aware that this is only an approximation of flower visitation. To test if the observed increase in pathogen prevalence in areas with a low amount of semi-natural habitat is caused by an increased transmission potential, i.e., an increased visiting frequency per flower by different competent host species, one should measure the flower visitation frequency at flowers.

(ii) Sowing wildflower fields can indirectly affect the pathogen prevalence through the host. Adding extra floral resources increases the availability of high quality food and this in turn can increase the tolerance of the bee-host towards parasite infections.

In our study we saw that the implementation of a wildflower field in areas with a low amount of semi-natural habitat increased the micro-parasite prevalence. Yet due to the low amount of natural resources in these areas pollinators benefit most from the addition of food resources in such locations (Scheper 2013). The availability of high quality and diversity of pollen and nectar resources have been shown to influence honey bee health and pathogen tolerance (Di Pasquale et al. 2013). Similar results have been found for bumble bees, where starved bees showed a higher mortality after infection (Brown et al. 2000). Moreover, the nutritional status of bumble bees has been shown to affect the population dynamics and development of micro-parasites. Logan et al. (2005) showed that pollen-starved bees supported significantly smaller populations of the micro-parasite *C. bombi* and that malnutrition disrupts the parasite's developmental processes. Addition of high quality resources, for instance by sowing wildflower fields, could therefore increase the parasite populations within individual hosts. The effect of additional high quality food resources, such as sown wildflower fields, can compensate the shortage of semi-natural habitat. The effect of wildflower field implementation on the nutritional status of the bees in areas with a low amount of semi-natural habitat is, therefore, expected to be larger than in locations with a larger amount of semi-natural habitat. This could explain the observed increase in micro-parasite prevalence with the increase in size of the flower field in the areas with a low amount of semi-natural habitat. Similarly, we saw that in areas with a high amount of semi-natural habitat the implementation of a flower field did not have a large impact on the micro-parasite prevalence irrespective of the size of the field.

Conclusion

In our study we could show that landscape alterations, intended to boost diversity and populations of pollinators (i.e., sown wildflower fields), can alter the local micro-parasite prevalence. Here the impact of the implemented flower field is dependent on both the surrounding environment and the size of the field. Currently there is a focus on the effect of bottom-up forces when assessing and evaluating the impact of landscape alterations on bee population. However, it can be expected that the population size will be determined by an interplay between these bottom-up forces and the top-down forces. Here we have shown that landscape alterations can affect parasite prevalence and potentially top-down forces. Our result shows that especially in semi-natural poor regions the addition of flower fields can affect parasite prevalence. This observation opens new areas of research, as it complicates the relations between (bee) population dynamics and landscape alterations as proposed by Tscharntke et al. (2012). If higher pathogen prevalence is induced through the implementation of "attractive hot spots", then such measures could prove to be counterproductive. We encourage more research into the importance of flower mix compositions to support pollinators. Each flower has its importance in terms of forage provisioning (bottom-up force), yet it also determines the contact network between bees, an important factor for multi-host parasites to encounter new hosts (top-down force).

Acknowledgements This project was supported in part by the Ghent University and the Research Foundation-Flanders (FWO-Vlaanderen) for the Belgian team, and the team in the Netherlands by the Netherlands Organization for Scientific Research (NWO-ALW; Biodiversity Works Program, 841.11.001) and Syngenta. D.K. was additionally supported by EC FP7 project LIBERATION (311781; www.fp7liberat

ion.eu) and EC COST Action SUPER-B (FA1307; http://www.superb-project.eu). We also thank Dr. Tibor Bukovinszky for his assistance.

Author contribution statement DK, GS and IM conceived the ideas and designed methodology; NP and TL collected the data; NP, IM and JS analyzed the data; NP and IM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Data accessibility The data will be made publicly available at Dryad.

References

Arbetman MP et al (2012) Alien parasite hitchhikes to Patagonia on invasive bumblebee. Biol Invasions 15(3):489–494

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67(1):1–48

Brown MJF, Loosli R, Schmid-Hempel P (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees. Oikos 91:421–427

Cable J et al (2017) Global change, parasite transmission and disease control: lessons from ecology. Philos Trans R Soc B Biol Sci 372(1719):20160088

Carvell C et al (2011) Bumble bee species' responses to a targeted conservation measure depend on landscape context and habitat quality. Ecol Appl 21(5):1760–1771

Cisarovsky G, Schmid-Hempel P (2014) Combining laboratory and field approaches to investigate the importance of flower nectar in the horizontal transmission of a bumblebee parasite. Entomol Exp Appl 152(3):209–215

Colla SR et al (2006) Plight of the bumble bee: pathogen spillover from commercial to wild populations. Biol Cons 129(4):461–467

Di Pasquale G et al (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? Zeil J (ed) PLoS ONE 8(8):p.e72016

Durrer S, Schmid-Hempel P (1994) Shared use of flowers leads to horizontal pathogen transmission. Proc R Soc B Biol Sci 258(1353):299–302

Flenniken ML, Andino R (2013) Non-specific dsRNA-mediated antiviral response in the honey bee. G. Smagghe (ed) PLoS ONE 8(10): p.e77263

Fürst MA et al (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506(7488):364–366

Gallai N et al (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68(3):810–821

Geslin B et al (2017) Massively introduced managed species and their consequences for plant–pollinator interactions. Adv Ecol Res 57:147–199

Gisder S, Genersch E (2017) Viruses of commercialized insect pollinators. J Invertebr Pathol 147(July):51–59

Goulson D, Hughes WOH (2015) Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. Biol Cons 191:10–19

Goulson D et al (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347(6229):1255957

Graystock P, Goulson D, Hughes WOH (2015) Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proc R Soc B Biol Sci 282(1813):20151371

Graystock P et al (2016) Do managed bees drive parasite spread and emergence in wild bees? Int J Parasitol Parasites Wildl 5(1):64–75

Green P, MacLeod CJ (2016) SIMR: an R package for power analysis of generalized linear mixed models by simulation. Nakagawa S (ed) Methods Ecol Evol 7(4):493–498

Heard MS et al (2007) Landscape context not patch size determines bumble-bee density on flower mixtures sown for agri-environment schemes. Biol Let 3(6):638–641

Henson KSE, Craze PG, Memmott J (2009) The restoration of parasites, parasitoids, and pathogens to heathland communities. Ecology 90(7):1840–1851

Holdenrieder O et al (2004) Tree diseases and landscape processes: the challenge of landscape pathology. Trends Ecol Evol 19(8):446–452

Kleijn D et al (2018) Scaling up effects of measures mitigating pollinator loss from local- to landscape-level population responses. Carvalheiro L (ed) Methods Ecol Evol:1–12

Kleijn D, van Langevelde F (2006) Interacting effects of landscape context and habitat quality on flower visiting insects in agricultural landscapes. Basic Appl Ecol 7(3):201–214

Knigtht ME et al (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Mol Ecol 14(6):1811–1820

Lafferty KD (2012) Biodiversity loss decreases parasite diversity: theory and patterns. Philos Trans R Soc B Biol Sci 367(1604):2814–2827

Logan A et al (2005) The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. Parasitology 130(6):637–642

McMenamin AJ, Genersch E (2015) Honey bee colony losses and associated viruses. Curr Opin Insect Sci 8:121–129

Meeus I et al (2010) Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers. J Appl Microbiol 109(1):107–115

Meeus I et al (2011) Effects of invasive parasites on bumble bee declines. Conserv Biol 25(4):662–671

Menail AH et al (2016) Large pathogen screening reveals first report of *Megaselia scalaris* (Diptera: Phoridae) parasitizing *Apis mellifera intermissa* (Hymenoptera: Apidae). J Invertebr Pathol 137:33–37

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ruiz-Gonzalez MX, Brown MJF (2006) Honey bee and bumblebee trypanosomatids: specificity and potential for transmission. Ecol Entomol 31(6):616–622

Scheper, J. et al., 2013. Environmental factors driving the effectiveness of European agri-environmental measures in mitigating pollinator loss - a meta-analysis J. Gomez, ed. *Ecology Letters*, 16(7), pp.912–920

Scheper J et al (2015) Local and landscape-level floral resources explain effects of wildflower strips on wild bees across four European countries. Diamond S (ed). J Appl Ecol 52(5):1165–1175

Sguazza GH et al (2013) Simultaneous detection of bee viruses by multiplex PCR. J Virol Methods 194(1–2):102–106

Steven JC et al (2003) Density-dependent pollinator visitation and self-incompatibility in upper great lakes populations of *Trillium grandiflorum*. J Torrey Bot Soc 130(1):23

Tscharntke T et al (2012) Landscape moderation of biodiversity patterns and processes—eight hypotheses. Biol Rev 87(3):661–685

Westphal C et al (2008) Measuring bee diversity in different European habitats and biogeographical regions. Ecol Monogr 78(4):653–671