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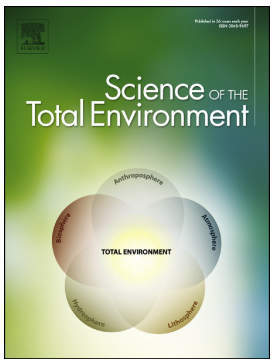

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Landscape structure affects temporal dynamics in the bumble bee virome: landscape heterogeneity supports colony resilience

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ABSTRACT

Virus spillovers from managed honey bees, *Apis mellifera*, are thought to contribute to the decline of wild pollinators, including bumble bees. However, data on the impact of such viruses on wild pollinators remain scarce, and the influence of landscape structure on virus dynamics is poorly understood. In this study, we

deployed bumble bee colonies in an agricultural landscape and studied changes in the bumble bee virome during field placement under varying habitat composition and configuration using a multiscale analytical framework. We estimated prevalence of viruses and viral loads (i.e. number of viral genomic equivalent copies) in bumble bees before and after placing them in the field using next generation sequencing and quantitative PCR. The results show that viral loads and number of different viruses present increased during placement in the field and that the virus composition of the colonies shifted from an initial dominance of honey bee associated viruses to a higher number (in both viral loads and number of viruses present) of bumble bee associated viruses. Especially DWV-B, typical for honey bees, drastically decreased after the time in the field. Viral loads prior to placing colonies in the field showed no effect on colony development, suggesting low impacts of these viruses in field settings. Notably, we further demonstrate that increased habitat diversity results in a lower number of different viruses present in *Bombus* colonies, while colonies in areas with well-connected farmland patches decreased in their total viral load after field placement. Our results emphasize the importance of landscape heterogeneity and connectivity for wild pollinator health and that these influences predominate at fine spatial scales.

KEYWORDS: Cross-species virus transmission, host shifts, farmland, habitat fragmentation, habitat heterogeneity, pollinators

INTRODUCTION

Interspecies disease transmission can have profound impacts on human and animal health (Daszak et al. 2000). Emerging infectious diseases have been linked with rapid declines of global entomofauna (Potts et al. 2010; Sánchez-Bayo & Wyckhuys 2019), where disease transmissions between domesticated animals and wildlife populations are of particular concern (Daszak et al. 2000; Blitzer et al. 2012). Managed western honey bees (Apis mellifera) typically host a range of pathogens (Ratnieks & Carreck 2010). Therefore, they may act as an important source of pathogens for wild arthropods (Blitzer et al. 2012). Overlapping species ranges, niches and

behaviors promote cross-species disease transmission (Woolhouse et al. 2001; Manley et al. 2015). Thus, commercial pollinators have been mentioned as key drivers of disease emergence in other insects and pathogen spillover from honey bees is a possible cause for the decline of wild pollinators, including bumble bees (*Bombus* spp., Blitzer et al. 2012; Fürst et al. 2014; Manley et al. 2015).

When investigating potential impacts of viruses on wild bee populations, it is important to consider additional stressors affecting their fitness and resilience. Among the most important drivers of current insect declines are habitat loss and fragmentation (Sánchez-Bayo & Wyckhuys 2019). Both processes can lead to homogenized landscapes poor in floral resources and, in consequence, lowered pollinator abundance and species diversity (Potts et al. 2010; Kennedy et al. 2013) as well as altered pathogen prevalence through changes in plant-pollinator networks (Figueroa et al. 2020). Fragmented agroecological systems pose significant challenges to biodiversity, not only regarding their landscape composition and configuration, but also in terms of pollution through agrochemicals (Goulson et al. 2015). Consequently, pollinators inhabiting such landscapes inevitably face multiple intertwined stressors, which may amplify adverse effects (Tilman et al. 2001). Both nutritional stress and exposure to pesticides have been shown to impact honey bee immune pathways (Di Prisco et al. 2013; Dolezal & Toth 2018), potentially leading to synergistic effects where immunosuppression increases pathogen susceptibility (Di Prisco et al. 2013; Annoscia et al. 2020). Furthermore, habitat composition and configuration in fragmented landscapes influence transmission dynamics of infectious diseases via shared floral resources (Figueroa et al. 2020), which act as viral hotspots and are the most likely route for cross-species virus transmission among pollinators (Alger et al. 2019; Yañez et al. 2020). The probability of susceptible hosts being exposed to pathogens is likely increased in fragmented landscapes with limited and spatially clumped floral resources (Woolhouse et al. 2005; Piot et al. 2019). Availability of essential floral resources throughout the flowering season can increase the tolerance of an organism to withstand pathogens (Dolezal & Toth 2018; Piot et al. 2019; Zhang et al. 2020) as well as insecticides (Crone & Grozinger 2021), since the availability of high quality and diverse pollen and nectar resources positively influences bee health (Brown et al. 2000; Dolezal et al. 2019).

Hence, host responses to landscape structure or increased floral resources, whether through altered foraging patterns (Maurer et al. 2020), colony fitness (Maurer et al. 2020), or diet breadth (Gómez-Martínez et al. 2022), may shape pathogen prevalence and disease outbreaks in bee communities (Figueroa et al. 2020; Graystock et al. 2020; Manley et al. 2023). Modeling suggests that benefits from floral resources, corridors and other connections may outweigh the possible risks of increased pathogen transmission (McCallum & Dobson 2002; Burnham et al. 2021). In addition, empirical studies have found that decreased landscape heterogeneity reduces pathogen prevalence through an increased diet breadth of the dominant bee species, thus leading to a dilution effect (Figueroa et al. 2020). Spatial landscape heterogeneity therefore influences pathogen transmission dynamics with evidence for both increased (Figueroa et al. 2020) as well as decreased transmission or persistence rates (Rees et al. 2013; White et al. 2018). Such contrasting evidence likely stems from context-dependent factors such as species behavior (e.g. movement), species densities, or transmission dynamics (White et al. 2018). Yet, empirical studies linking landscape structure and disease transmission, and their impact on pollinator fitness, are rare because controlled experimental manipulations of landscapes, target hosts and pathogens, are often infeasible (Tracey et al. 2014).

RNA viruses have a high potential to cross species barriers due to their large population sizes and rapid adaptive changes (Moya et al. 2004; Woolhouse et al. 2005). Many viruses that were first described in honey bees have subsequently been detected across the wider arthropod community (e.g. Levitt et al., 2013, Radzevičiūtė et al. 2017; Martin & Brettell 2019). Notably, even though most of these viruses were described first in honey bees, it generally remains unclear which species is the original host and whether the observed interspecific transmission events constitute true host shifts, i.e. viruses invading and establishing in a new host species (Longdon et al. 2014), or whether the viruses are simply generalist arthropod viruses. However, for some viruses such as the Deformed Wing Virus (DWV) and Black Queen Cell Virus (BQCV) evidence indicates that honey bees are the primary host (e.g. Levitt et al., 2013; Radzevičiūtė et al, 2017), henceforth referred to as honey bee associated viruses. Other viruses like *Bombus cryptarum* densovirus (BcDV), Castleton burn virus (CBV) and Mayfield virus -1 (MV 1) are more tightly linked to the *Bombus* genus (Schoonvaere et al. 2018;

Pascall et al. 2021); henceforth called bumble bee associated viruses. Based on the co-occurrence of viruses and shared viral strains within sites, frequent interspecific virus transmission is suspected, especially between honey bees and bumble bees (Fürst et al. 2014; Wilfert et al. 2016; Manley et al. 2019). In contrast to burgeoning evidence for viruses first associated with honey bees also being detected in other arthropods, knowledge on their impacts in alternative hosts is limited (Yañez et al. 2020). The few studies that investigated pathogenicity outside of honey bees report potential clinical symptoms in *Bombus* spp. (Tehel et al. 2016), wasps (*Vespa crabro*, Forzan et al. 2017), ants (*Lasius niger*, Schläppi et al. 2020), and spiders (*Agelena labyrinthica*, Schläppi et al. 2023). However, except for DWV associated wing deformities in field observations (Genersch et al. 2006), reports on pathogenicity in non-honey bee hosts are rare and primarily based on laboratory assays (e.g. Fürst et al. 2014; Tehel et al. 2020). Thus, it remains unclear if pollinator populations in the field are affected by frequent exposure to viruses from managed honey bees.

Here, we take advantage of a highly contrasted landscape mosaic dominated by intensively managed vineyards, in which herbicide spraying produces extensive bare ground, intermixed with vineyards that are managed to allow ground vegetation to grow and thus potentially providing floral resources to pollinators (Maurer et al. 2020). By considering the wider surroundings around the vineyards (henceforth landscape structure) at multiple spatial scales, we investigated whether landscape structure affects viral load dynamics in bumble bee field colonies, and how this is linked to colony development by experimentally deploying bumble bee colonies in vineyard fields of varying management intensities and surrounding landscape structures. More specifically, we addressed the following four major research questions: i) Does the composition of viruses in commercially purchased bumble bee colonies change following placement in the field; ii) does the initial viral load (before placement in the field) negatively affect colony development; iii) does the landscape structure as well as local vineyard field condition affect the viral load, number of different viruses present, and turnover in bumble bee colonies, and iv) does the landscape structure influence bumble bee colony development? Regarding the landscape structure, we focused on habitat area and connectivity metrics of four main land cover types in the

study area as well as overall landscape heterogeneity. For the vineyard field conditions we considered the ground vegetation cover, floral resources, and size of vineyard fields where the bumble bee colonies were placed.

MATERIAL AND METHODS

2.1 Study area and design

The study was conducted in Central Valais, Switzerland from 08-May to 23-June-2017. The study landscape is dominated by vineyards interspersed with small patches of dry oak stands and steppe on steep terrain, and orchards in the plains. Vineyards are the most abundant land cover type in the study area and are characterized by contrasting management practices. These include fields with bare ground due to herbicide spraying or tillage, and fields with vegetated ground as a result of a more extensive and nature-friendly management (i.e. with no or less pesticide and herbicide applications; e.g. Bosco et al. 2019a, b, Bosco et al. 2023). A bout 70%–80% of the vineyards are intensively managed and support virtually no ground vegetation cover, whereas the remaining 20%–30% are allowed to grow ground vegetation (henceforth vegetated vineyards; Arlettaz et al., 2011; Bosco et al. 2019a). Vegetated vineyards have been shown to be important for bumblebee colony development and foraging behavior as they improve connectivity and amount of quality floral resources (Maurer et al. 2020). Buff tailed bumble bee (*Bombus terrestris*) colonies were purchased from Andermatt Biocontrol (Biobest, Belgium) at the "two-week old" stage which included at least 20 workers along with the starting queen (average colony weight: 653±27 mg). They were allocated to vineyard fields with varying ground-vegetation coverage and surrounded by different landscape composition and configuration (Fig. 1; see SI Table S2). The semi-experimental set-up controlling for high vs low habitat area and fragmentation of vegetated vineyards stemmed from a study on habitat area and fragmentation effects on bumble bee colony development and foraging behavior. Here, habitat area refers to the area covered by vegetated vineyards within 250m radius while fragmentation refers to the number of separated, vegetated vineyard patches within 250m radius (details in Maurer et al. 2020, Bosco et al. 2019b). In summary, the colonies were placed in vineyards fields in a stratified random manner dependent on low vs high habitat area and fragmentation of vegetated vineyards (see SI Table S1) and to account for variability in slope steepness and surrounding landscape (Maurer et al. 2020). Colony

vineyard fields were on average 19km apart, with a minimum of 366m (Fig. 1). Workers (N = 5) were collected from 35 colonies before placing them in the field (day 0) and after collecting them from the field (day 45). Both times, the specimens were stored at -20°C for three days before being transferred to the laboratory for storage at -80°C before subsequent virus analyses (see below). Weight gain of colonies, number of workers, queens, and total number of cells (as a sum of healthy, dead and hatched pupal cells, pollen and nectar pots), as well as infections by parasitic moths, *Aphomia sociella*, were assessed after collecting the colonies from the field (see SI Table S3; details in Maurer et al. 2020).

2.2 Environmental predictors at multiple spatial scales

To assess the role of the landscape structure around the experimental fields, we used the Swiss land use and land cover (LULC) data SwissTLM3D (copyright@swisstopo 2020, resolution 1-3m), complemented by CORINE LULC data (© European Union, Copernicus Land Monitoring Service 2020, European Environment Agency EEA). We aggregated the LULC to eight main classes (Fig. 1, see SI section 1.2) and calculated the relative area covered by the four most abundant LULC: farmland (arable land, orchards, grasslands), forests (mixed, coniferous, deciduous and forest groves), residential areas (residential, public, industrial, and agricultural buildings), and vineyards (irrespective of their ground vegetation coverage) within 9 spatial scales ranging from 100m up to 1,500m radii in 100m increments (100–500m) and 250m increments (500–1,500m), respectively (see SI Fig. S1) using the metric percentage of landscape (PLAND) in the R package landscapemetrics (Hesselbarth et al. 2019). For those four classes we also calculated the mean distance between patches of the same LULC class as a measure of configuration and connectivity (mean of Euclidean nearest-neighbor distance ENN_MN). Lastly, we obtained an estimate of landscape heterogeneity across the same 9 spatial scales by calculating a Shannon diversity index based on all eight land-cover classes, taking both the number of land-cover classes and the abundance of each class into account (metric Shannon diversity SHD). Given the importance of vegetated vineyards for arthropods (Bosco et al. 2019a, b; Maurer et al. 2020; Bosco et al. 2022; Bosco et al. 2023), we additionally calculated area (PLAND) and fragmentation (patch density, PD) of vegetated vineyard fields across the 9 spatial scales.

To investigate field-scale effects of vineyards on virus susceptibility of bumble bees, we measured field size of the experimental field (Table 1) as well as ground vegetation density and flower resources in a random subset of 6 vegetated vineyard fields surrounding the experimental field within a buffer zone of 250m radius. The flower resources were measured in two ways according to Maurer et al. (2020): blooming flower species richness and coverage of blooming flowers per vineyard field. The species richness of blooming plants was assessed along two transects (1m width, 20m length) per vineyard field, while the coverage of blooming flowers was estimated in one square meter per field, placed 10m from the field margin (see details in Maurer et al. 2020). To address the influence of proximity of known honey bee apiaries on potential transmissions to bumble bee colonies, we included the nearest-neighbor distance to the next apiary and the number of apiaries within the 9 spatial scales (see SI Table S2 for details). In Switzerland, apiaries are recorded, and GPS data was provided by the cantonal apiary inspector. Influence of feral honey bee colonies was not considered, as self-sustaining feral honey bee populations have not been demonstrated (Kohl et al. 2022) and nearly all honey bees in Switzerland are managed and dependent on apicultural activities. All metrics were calculated in R (R Core Team 2022).

2.3 Virus analyses

Following standard protocols for RNA extraction we performed next generation sequencing (NGS) to identify bee viruses present in the colonies before and after placement in the field. Subsequently, reverse transcription and quantitative PCR (RT-qPCR) were conducted to quantify the viruses at colony level. From the NGS, the viruses with the highest number of reads, which represented 99.9% of reads related to bee viruses, were then selected for RT-qPCR quantification. All data analysis referring to viral loads is based on these RT-qPCR results.

RNA extraction – RNA was extracted from bumble bees (N = 5 per colony per sampling session) using a NucleoSpin® RNA II kit (Macherey-Nagel, Oensingen, Switzerland). Individual bees were crushed in PBS Buffer (0.5 mg tissue/µL) with a 5-mm metal bead in 2-mL Eppendorf® (Basel, Switzerland) tubes using a Retsch® (Haan, Germany) MM 300 mixer mill for 1 min at 25 Hz (de Miranda et al. 2013). Fifty µL of the homogenate was used for the extraction and RNA was stored in 60µL elution buffer at -80 °C (de Miranda et al. 2013).

Next generation sequencing – RNA from every colony was used for the identification of bee viruses present in the bumble bee colonies. Briefly, after QC evaluation, RNA from each individual (5 µl) was first pooled per colony. Then, 2µl from each colony pool were mixed for each of the two sampling sessions (before and after placement in the field). Libraries comprising each sampling session were prepared using the Corall total RNA-Seq Library kit (Lexogen, Vienna, Austria). NGS were performed using an Illumina SP flow cell (100 Mio reads/pool, 300 cycles) in paired-end mode (2 × 150 bp).

Bioinformatics analysis – Reads were quality-trimmed with fastp (Chen et al. 2018, Version 0.12.5) and mapped to the *Bombus terrestris* genome (iyBomTerr1.2, ncbi bioproject PRJEB45694) using STAR (Dobin et al. 2013, Version 2.7.3a). Quality-trimmed and unmapped reads were assembled via SPAdes (Prjibelski et al. 2020, Version 3.14.0). Resulting scaffolds were then aligned to virus nucleotide and protein and sequence databases (Genbank and RefSeq viral nucleotide sequences downloaded on 21-01-2021, UniProt viral amino acid sequences downloaded on 21-01-2021) using BLASTn (Camacho et al. 2009, Version 2.0.0+, default settings) and DIAMOND (Buchfink et al. 2015), Version 0.9.18, default settings). To exclude false positives, the scaffolds with a virus hit were aligned to an in-house non-viral database consisting of archaeal, bacterial, fungal, mammal, and protozoal sequences. Scaffolds were considered false positive if they had a longer hit on a sequence of the in-house database compared to the virus databases or if they had a nucleotide hit of more than 10% of their own length to any sequence of the non-viral database.

Reverse transcription and qPCR – cDNA synthesis was performed using a M-MLV RT Kit (Promega, Dübendorf, Switzerland) for two pooled RNA samples (each pooled from 5 individuals) per colony, with one sample for each sampling session (N=35*2). A thermocycler (Biometra, Analytik Jena, Jena, Germany) was used to incubate 0.75 µL of a random hexamer oligonucleotide (100 µM) and H2O for 5 Minutes at 70 °C with 0.5 µg of template RNA. Then, 5 µL of 5x buffer, 1.125 µL dNTPs (10 mM) and 1 µL reverse transcriptase (M-MLV) were added followed by incubation at 37 °C for 60 min. For the absolute virus quantification, the group of bee viruses with the highest number of reads from the above-described libraries were selected: DWV-A, DWV-B, Black queen cell virus (BCQV), Lake Sinai Virus (LSV), CBV, MV 1, Duke bunyavirus (DuBV), BcDV,

Apis/Bee rhabdovirus-1 (ARV-1; SI Table S4). qPCR reactions were prepared using 6 µL of 2X reaction buffer (SensiFAST™ SYBR® No-ROX Kit, Meridian Bioscience, London, UK), 0.24 µL forward and reverse primer (SI Table S5), 2.52 µL water and 3 µL of ten-fold diluted cDNA. The qPCR reactions were performed in a CFX96TM Real-Time PCR Detection Systems (BioRadⓒ, CA, USA): 3 min for 95°C, 40 cycles of 3 sec at 95°C and 30 sec at 57°C. Following, melting curve analysis reading the fluorescence at 0.5 °C increments from 55 to 95 °C was used to verify product specificity. Samples were run in duplicate for all target viruses and the Rps5 reference gene which was used to assess the quality of the process (RNA extraction, cDNA synthesis and qPCR). Furthermore, each plate was run with 5 ten-fold serial dilution (10-2 to 10-6 ng) of purified PCR products serving as standard curves and positive controls and for viral quantification plus two no-template negative controls (Bustin et al. 2009). Based on the qPCR output, the plotting using the standard curves and the experimental dilution factors during the RNA extraction and reverse transcription processes, the estimated viral loads were derived. Samples were considered negative if no peak or a shifted peak was observed in the melting curve analysis.

2.4 Statistical Analyses

For each colony and sampling session we calculated (i) viral load defined as log10 genomic equivalent (GE) copies per colony and virus, (ii) total viral load defined as the sum of the combined viral loads of all nine viruses; and (iii) the number of viruses present per colony. To analyze how viral loads and number of viruses changed from before to after field placement (research question i), we ran paired t-tests with total viral load, number of viruses present, and viral loads of the six most abundant viruses (DWV-B, BCQV, CBV, MV 1, DuBV, BcDV) as response variables against the sampling session (i.e. before and after placement in the field).

To test if initial viral loads affect colony development parameters (research question ii) we used viral loads of the six most abundant viruses, total viral load, and number of viruses present at day 0 (before placement in the field) as predictors in single-predictor linear models. The following colony development parameters were used as response variables: number of workers (log-transformed), number of queens (log-transformed), number

of total cells, number of hatched pupal cells, weight gain, moth infestation index, and number of parasitic larvae or pupae (log-transformed, see Table 1 and SI Table S4). We fitted linear models and log-transformed the response variables if necessary to follow a normal error distribution.

To assess the influence of landscape structure on virome change from before to after field placement (research question iii) we calculated the change in total virus load per colony as $log(viral\ load)_{day\ 45} - log\ (viral\ load)_{day\ 0}$) across all nine viruses. Similarly, for the change in number of viruses present, we calculated the difference in the number of viruses present at day 45 and at day 0. Further, we calculated the turnover and appearance of viruses during the field placement (package codyn, Hallett et al. 2016). For each of those response variables we performed scale-optimization and multi-scale species distribution modeling (after e.g. Bosco et al. 2019b, McGarigal et al. 2016), shown to be the most robust and parsimonious method for this purpose (Cushman et al. 2023, personal communication): i) First, scale optimization was conducted for landscape predictors measured at multiple spatial scales (see Table 1). To do so we ran single-predictor models for all scales to identify the best scale per predictor, based on lowest AICc (Burnham & Anderson 2002). Thus, variables could enter the full model at different scales – following a multi-scale analytical framework (McGarigal et al. 2016). ii) Second, we ran single-predictor models for all remaining predictors measured at a single scale. iii) We tested for collinearity among the selected (scale-optimized) variables with Pearson correlation. For pairs of intercorrelated variables with $|r| > 0.6$ the variable with the lower AICc in the univariate model was retained in the modeling. In addition, to avoid overfitting of the full model relative to the sample size, we reduced the numbers of covariates by selecting only those with $P < 0.1$ in the single-predictor models to enter the full model. For all response variables we used linear mixed-effects models with a Gaussian distribution since residuals of all response variables followed a normal distribution. From the full models, we did a stepwise backwards selection until only (near-) significant variables were left in the model (i.e. $p<0.1$). Among those candidate models from the stepwise backwards selection, we chose the best model based on a set of different indices (R package 'performance', Lüdecke et al. 2021). No interaction effects were considered.

To model the influence of field- and landscape-scale variables on colony development we ran the same statistical protocol including scale optimization and variable preselection as for virome changes using the following response variables: number of workers (negative binomial model, function glm.nb, package MASS, Venables & Ripley 2002), number of queens (negative binomial), number of cells (normal linear model), and number of parasitic larvae and pupae (negative binomial).

All predictors were standardized prior to modeling. For all models, assumptions such as normality of residuals, homoscedasticity, and outliers were checked (package 'performance'; see Appendix section 3). Based on a composite outlier score (R package 'performance') obtained via the joint application of multiple outlier detection algorithms (median absolute deviation (MAD)-based robust z scores, Leys et al. 2013; and Cook's distance, Cook 1977) we checked for influential outliers in all models; with none detected (Fig. S6-S13). Spatial autocorrelation of model residuals was tested for each model (package DHARMa, Hartig 2021) with none found. All data and code are available from zenodo online repository (Bosco et al. 2024).

RESULTS

3.1 Change in virus composition before and after placement in the field

The most prevalent viruses in the bumble bee colonies were DWV-A, DWV-B, BQCV, LSV, ARV-1. In addition, several viruses that are associated with bumble bees were abundant: BcDV, CBV and MV 1 (Table S4; Schoonvaere et al. 2018; Pascall et al. 2021). Total viral load across all viruses (total viral GE copies per colony on log scale) increased on average by 1.34±2.21 GE copies (mean±SD) from the beginning to the end of the field placement (day 0 = 3.67±1.47; day 45 = 5.00±1.61; t = -3.580, df = 34, p= 0.001; Fig. 2a, d), while the number of viruses present on average increased by a factor of 1.46±1.75 from day 0 to day 45 (day 0 = 3.14±1.03; day 45 = 4.60±1.31; t = -4.913, df = 34, p<0.001; Fig. 2a, c, d). Among the 9 screened viruses, only 1 to 5 were present at day 0, whereas at day 45 between 2 and 7 viruses were present among the colonies (Fig. 2b). The viral load range for each virus type or species is presented in Fig. 2c.

The increase in the number of viruses present was driven by the appearance of new and mostly bumble bee associated viruses, such as CBV ($t = -6.258$, $df = 34$, $p<0.001$; Fig. 2c, d) or MV 1 ($t = -5.596$, $df = 34$, $p<0.001$; Fig. 2c, d) as well as DuBV ($t = -9.743$, $df = 34$, $p<0.001$; Fig. 2c, d). Although DuBV has been isolated from bees, it is known that bunyaviruses infect trypanosomatids (e.g. Grybchuk et al. 2018); thus whether the bee or its parasites are infected is unclear. At day 0 colonies had either viral loads dominated by BcDV (significant increase; $t = -2.135$, $df = 34$, $p = 0.040$; Fig. 2c, d) or a combination of two honey bee associated viruses: DWV-B (significant decrease; $t = 2.619$, $df = 34$, p-value = 0.013; Fig. 2c, d) and BQCV (significant increase; $t = -3.466$, $df = 34$, $p = 0.001$; Fig. 2c, d).

3.2 Influences of initial colony viral loads on colony development

Among all measured colony development parameters, only the moth infestation index was related to the initial viral loads of viruses, with increasing loads of DWV-B colonies being related to higher moth infestation rates at the end of the field placement (estimate±SE=0.210±0.081, p=0.012), while increasing BcDV loads were associated with lower moth infestations (-0.218±0.079, p=0.008; SI Table S6; Fig. S3).

3.3 Change in viral patterns in relation to landscape structure

From the scale-optimization process, we found that most predictors entering the full model had their optimal scale at local or meso-scale (here, 100m up to 500m) with only one predictor at 1250m. The spatial scale of each predictor is hereafter indicated in brackets.

Total Viral Load Change – The mean distance among forest patches (1250m radius) had a marginally significant and positive relationship with the total viral load change in bumble bee colonies. Mean distance among farmland patches showed a large positive and significant relationship (Table 2A, Fig. 3a), meaning that bumble bee colonies located in areas with more isolated (or less connected) farmland patches had a higher increase in viral loads after field placement (SI Fig. S4a).

Change in number of viruses present – Habitat diversity (300m), a measure of landscape heterogeneity, was significantly negatively related to the change in number of viruses present, showing that bumble bee colonies

placed in areas with fewer land-cover types (lower heterogeneity) had a higher number of different viruses after placement in the field (Table 2B, Fig. 3b). On the other hand, forest area (100m) was significantly positively related to the change of number of viruses present; with increasing forest cover in the close surroundings of a colony, the number of detected viruses increased (Table 2B; SI Fig. S4b, c).

Virus Turnover – The area covered by forests (100m) had the strongest effect on viral turnover. Colonies in areas with more forest showed a higher change in their virus composition (Table 2C). However, with increasing distances (decreasing connectivity) among forest patches (400m), virus turnover also increased significantly. Also, fragmentation of vegetated vineyards (400 m) significantly influenced the turnover, such that the viral composition changed less for colonies in areas with higher patch density of vegetated vineyards (Table 2C; SI Fig. S5a-c).

Virus Appearance – Habitat diversity and forest area both were significantly related to virus appearance, with habitat diversity (300m) again showing a negative effect, such that colonies in areas with a lower number of different land-cover types showed a more frequent prevalence of viruses that were not yet present at the beginning of the field placement. Increasing forest area within the wider surroundings of a colony (500m) was associated with a higher rate of new viruses present (Table 2D; SI Fig. S5d, e).

No effect of the number of known honey bee apiaries or nearest distance to them was detected in any of the models, and neither was there a significant link between virus response variables and vineyard flower resources (flower species richness and flower coverage).

3.4 Colony development and landscape structure

We found that connectivity of residential and farmland areas, the area covered by farmland and vineyards and the number of honey bee apiaries were the most important predictors for the number of workers, number of cells and number of queens (Table 3). For the number of workers, the greater the distance between residential patches within a 500m radius, the lower the number of workers at the end of the field placement, while area of vineyard cover within 100m radius was positively related to worker numbers (Table 3A). The number of produced queens

increased in areas with a higher farmland cover within 500m, but also in areas with more honey bee apiaries within 1500m (Table 3B). Similarly, the more isolated the farmland patches were within 750m radius, the smaller the colony in terms of number of cells in total (Table 3C). Regarding the infestation of colonies with parasitic larvae or pupae after field placement, habitat diversity and the isolation of forest and grove patches were the strongest predictors. In areas with a higher landscape heterogeneity within 750m and larger distances between forest and grove patches within 500m (higher isolation), colonies showed lower numbers of parasites (Table 3D).

DISCUSSION

In this study we investigated changes in the bumble bee virome under field conditions in relation to habitat composition and configuration. Our results show that viral loads and the number of different viruses present increased after field placement and shifted in favor of a higher number of viruses recently associated with bumble bees. The spatial structure of the surrounding landscape was related to the changes in viral patterns at fine spatial scales, with habitat Shannon diversity having a positive influence such that colonies contained fewer viruses in spatially diverse habitat mosaics, and colonies in areas with well-connected farmland patches decreased in their viral load during field placement. Colony development parameters were not directly negatively affected by viruses but were influenced by the same or similar landscape parameters that drove viral changes. Our results emphasize the importance of landscape heterogeneity and connectivity for wild pollinator health (Senapathi et al. 2017) and that these influences predominate at fine spatial scales (e.g. less than 500m radii).

4.1 Change in virus composition before and after placement in the field

We demonstrate that initially, viruses such as DWV-B, BQCV and BcDV were highly prevalent in the bumble bee colonies, whereas after placement in the field bumble bee associated viruses (CBV and MV 1) were also among the most prevalent viruses in the virome. Regarding DWV-B, the high initial loads detected in some colonies (107-108 GE), which in honey bees are indicative of clinical symptoms and hence serve as reliable

proxies for overt infections (Zioni et al. 2011; Cilia et al. 2021), substantially decreased after placement in the field (102 -103 GE). This result was unexpected as DWV is regarded as an emerging infectious disease (Genersch & Aubert 2010; Tehel et al. 2016) commonly responsible for the elevated viral loads found in honey bees ("the reservoir host", Tehel et al. 2020), from where it is assumed to spill-over to other wild bees (Fürst et al. 2014). It could be argued that the low DWV prevalence in the colonies after field placement is due to the seasonal peak of DWV in honey bees being reached in late summer (Manley et al. 2023). However, recent DWV seasonal surveys in honey bees found that DWV loads are already high in spring (Kevill et al. 2019). Hence, DWV spill-over could occur even early in the season. On the other hand, the lower DWV prevalence could be related to bumble bees being inefficient hosts, as laboratory DWV inoculations revealed lower viral replication in *B. terrestris* than in honey bees (Tehel et al. 2022). Moreover, *B. terrestris* showed considerable resistance to DWV when fed orally (Gusachenko et al. 2020; Streicher et al. 2023). Since the oral-fecal route is the main virus transmission route between bees (Chen et al. 2006; Yañez et al. 2020), limited oral transmission would mitigate rapid transmission of this virus among the colonies and may help to explain the low DWV prevalence. Interestingly, DWV transmission among bumble bees occurs rarely (Tehel et al. 2022). Highly infected individuals could simply die, in which case the infection would be self-limiting. Alternatively, self-removing behavior as reported in honey bees (Rueppell et al. 2010) along with other collective disease defense mechanisms that grant colonies social immunity (Cremer et al. 2018), may explain why DWV infections did not progress (or even vanished), even in colonies with initial viral loads indicative of overt infections.

In contrast, bumble bee associated viruses (BcDV, CBV and MV 1) showed elevated prevalence after field placement. Those viruses have been reported in several bumble bee species such as *Bombus cryptarum*, *B. terrestris*, *B. pratorum*, and *B. pascuorum* (Schoonvaere et al. 2018; Pascall et al. 2021) suggesting their close association with the taxon. In addition, the capacity of viruses to exploit hosts is shaped by interactions with other co-infecting parasites (Cressler et al. 2016). If *B. terrestris* is indeed not an ideal host for DWV and other honey bee associated viruses, out-competition by better adapted viruses would be a plausible explanation for the observed shift (Remnant et al. 2019). However, predicting the outcome in multi-infection scenarios is

challenging and heavily context specific (Durand et al. 2023). Notably, the true host range of those viruses remains unknown, and many viruses were detected at low titers. It is uncertain whether these low titers reflect genuine covert latent infections, as frequently observed in honey bees (Zioni et al. 2011), or if they result from contamination by inactive viral particles, such as pollen carrying viral RNA fragments. Further experiments on infectivity would be required to confirm viability of these viruses. Moreover, with commercially reared bumble bees being fed sterilized pollen sourced from honey bees at the company, contaminated pollen could account for low levels of viruses detected at the initial screening (i.e. before placement in the field). Conversely, access to preferred floral resources in the field may enhance the transmission of those viruses more closely associated with bumble bees. Nonetheless, the decreasing trends in prevalence and viral loads of honey bee associated viruses suggests that impacts of virus host shifts from managed honey bees to bumble bees under field conditions may be less important than previously claimed. Further, our results did not reveal any discernible effects of nearby honey bee apiaries on viral patterns observed in bumble bee colonies.

In general, based on our correlative study, virus presence was mostly inconsequential to the performance or health of the colonies. The number of workers, queens, brood cells and hatched pupal cells, as well as weight gain, remained unaffected. Interestingly, elevated initial DWV-B loads were associated with heightened rates of moth parasitism, whereas higher initial BcDV loads coincided with decreased moth infection rates. Hence, our results remain inconclusive regarding whether virus presence modulates susceptibility for non-viral parasites. While quantification of fitness is straightforward in mathematical models, it remains exceedingly challenging to quantify empirically. Since measuring the production of drones as well as hibernation and colony initiation success of gynes was not possible within the scope of this study, carry-over effects and long-term impacts on populations cannot be excluded. Nonetheless, the used colony performance parameters are generally assumed to correlate with fitness and hence provide a suitable proxy (Maurer et al. 2020; Siviter et al. 2021).

4.2 Landscape structure influences viral patterns and colony development

Our data show that colonies located in areas with more and better-connected farmland or residential areas generally have lower viral loads and better colony development (Fig. 3a, c-e). Farmland patches, mainly

consisting of vegetated vineyards, orchards, and grasslands, along with residential areas, provide suitable floral resources (Maurer et al. 2020) as compared to forests, and aligns with previous research that has demonstrated a connection between higher amounts of floral resources and reduced pathogen loads in pollinators (Figueroa et al. 2020; Graystock et al. 2020) or bumble bee colony fitness (Maurer et al. 2020). Notably, our study revealed no discernable effects of floral resources in vineyards on the bumble bee virome.

Interestingly, our results show that colonies located in areas with a higher forest cover and connectivity contained a higher number of different viruses and showed increased virus turnover and appearance of new viruses. If forests do not provide suitable foraging grounds or even constitute landscape barriers to foraging bumble bees (Kreyer et al. 2004), a higher forest proportion in the landscape may reduce foraging resources and lead to lower colony fitness through poorer nutrition (Filipiak 2018) and/or higher viral spillover rates as foraging bees are concentrated in the remaining suitable patches (Piot et al. 2021). However, a growing body of evidence underscores the importance of forests for bee populations, with higher habitat area and connectivity of forests increasing foraging and nesting opportunities for various bee species (Mola et al. 2021) potentially increasing interspecific virus transmission via shared floral resources. Naturally, the value of forests and their influence on transmission dynamics is heavily context-dependent and will vary based on forest type and quality, as well as the bee species in question. Here, we did not differentiate between forest types, nor was forest habitat quality evaluated, which could explain the contrasting findings compared to previous research indicating viral loads in bumble bees to be lowest in forests (e.g. Gratton et al., 2023).

Yet, when considering the entire landscape mosaic, colonies situated in more heterogeneous areas (i.e. more diverse LULC) had a lower number of different viruses (Fig. 3b) as well as a lower probability to contain new viruses (i.e. virus appearance), suggesting that high habitat diversity might result in a higher chance of finding suitable and diverse floral resources, providing resource complementation (Dunning et al. 1992; Fahrig et al. 2011). Consequently, habitat heterogeneity might reduce virus transmission via three non-mutually exclusive mechanisms shaping fitness and foraging patterns of bees: (i) High floral resource availability and resource complementarity due to landscape heterogeneity increases general colony performance and health

(Maurer et al. 2020; Jachuła et al. 2021) and thereby decreases their susceptibility to pathogens (Roger et al. 2017), (ii) high floral abundance within a landscape may decrease the contact between pollinators simply because pollinators are not concentrated on the few available flower patches (dilution/amplification effect; Piot et al. 2019; Civitello et al. 2015), and (iii) high habitat/floral diversity can modify a species diet breath (Gómez-Martínez et al. 2022) with consequences on virus transmission (Figueroa et al. 2020; Manley et al. 2023).

Overall, the landscape structure factors operated at rather fine scales, ranging from 100 up to a 400-meter radius around the bumble bee colonies, indicating that the habitat structure of the immediate surroundings is more important than meso- or large-scale conditions (Saturni et al. 2016). Even though *B. terrestris* have been shown to forage over long distances (Redhead et al. 2016), their main foraging activity occurs within 70-600m around the colonies (Osborne et al. 1999), supporting the findings in this study.

4.3 Conclusions

This study shows that even though commercially raised bumble bees carry several viruses, the viral composition generally changes towards bumble bee associated viruses after field placement, with initial virus load having a neglectable impact on colony development. Our findings underline the importance of extending research beyond the mere detection of viruses and identification of transmission between different host species. It is crucial to encompass investigation of clinical symptoms and impacts within affected populations to predict potential ecological consequences of frequent spillover events. Furthermore, our results emphasize the potential of landscape structure in shaping viral patterns. Habitat heterogeneity and well-connected farmland patches in areas with little forest cover led to lower viral loads, fewer viruses, and lower appearance of new viruses in bumble bee colonies. We thus stress the importance of habitat diversity (Fahrig et al. 2011; Sirami et al. 2019) and underscore the need of maintaining existing heterogeneous agricultural landscapes.

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Declaration of competing interest

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

TABLES

Table 1. All initial covariates, their description, data types, scales, and mean±standard deviation SD. For variables measured at the nine spatial scales (mean±SD) are given for the intermediate 750-meter radius scale. PLAND = percentage of landscape; PD = patch density; NND=Nearest-Neighbor-Distance; BB = Bumble bee colonies; HB = Honey bee colonies; MS = Multiscale, meaning that those variables were measured across the nine spatial scales. See SI Table S2 and S3 for colony-level values per variable.

Covariate	Description	Data type/source	Scale	Mean±SD
Vineyard field				
Vegetation	Ground vegetation density (%)	Field estimate*	Field	68.33±10.19
Flower species richness	Mean number of flowering plant species	Field estimate*	Fields within 250m buffer	5.87±1.58
Flower coverage	Mean coverage of flowering plants	Field estimate*	Fields within 250m buffer	6.53±4.37
Field size	Area of vineyard field (m^2)	QGIS	Field	2810.2±4728.2
Landscape composition/ configuration				
Vegetated vineyard area	PLAND of vegetated vineyards (%)	FRAGSTATS	MS landscape	33.02±16.03
Vegetated vineyard fragmentation	PD of vegetated vineyards	FRAGSTATS	MS landscape	107.76±54.82
Vineyard area	PLAND of vineyards (%)	FRAGSTATS	MS landscape	36.38±19.65
Residential area	PLAND of residential areas (%)	FRAGSTATS	MS landscape	8.95±8.8
Mean distance residential	NND between residential areas (m)	FRAGSTATS	MS landscape	14.29±6.0
Forest area	PLAND of forests and groves (%)	FRAGSTATS	MS landscape	15.45±11.49
Mean distance forests	NND between forests and groves (m)	FRAGSTATS	MS landscape	25.13±11.33
Farmland area	PLAND of farmland (%)	FRAGSTATS	MS landscape	21.41±12.13
Mean distance farmland	NND between farmland patches (m)	FRAGSTATS	MS landscape	12.72±8.51
Habitat diversity	Shannon diversity of habitat types within the landscape	FRAGSTATS	MS landscape	1.53±0.24
Bumble bee colonies				

Nr workers	Number of workers per colony	Lab dissection*	Colony	67.97±66.82
Nr queens	Number of queens per colony	Lab dissection*	Colony	9.06±12.69
Nr cells	Number of total cells per colony	Lab dissection*	Colony	532.71±301.52
Nr pupal cells	Number of hatched pupal cells per colony	Lab dissection*	Colony	153.4±123.78
Colony weight change	Weight gain per colony (mg)	Lab dissection*	Colony	202.4±245.2
Moth infestation rate	Severity of moth infestation in the colony	Lab dissection*	Colony	1.51±1.12
Nr parasitic larvae or pupae	Number of parasitic larvae or pupae per colony	Lab dissection*	Colony	290.83±303.13
Honey bee colonies				
Distance to HB	Nearest-neighbor distance to known honey bee apiaries (m)	QGIS	Landscape	508.47±284.41
Nr HB colonies	Number of known honey bee apiaries around the bumble bee colonies	QGIS	MS landscape	2.89±3.03

** from Maurer et al. 2020*

Table 2. Model outputs of best linear models for A) change in total viral load, B) change in virus richness (number of viruses present), C) turnover of viruses from start to end of the experiment, D) appearance of new viruses from start to the end of the experiment. Estimates, standard errors (SE), p-values, and confidence intervals (CI) from linear models (lm) are given. N = 35 observations (1 observation per bumble bee colony) for all models. NND = nearest-neighbor distance (mean distance between patches of same land-cover type); PLAND = percentage of landscape (relative area covered by land-cover type); PD = patch density (number of separate patches per 100 ha of same land-cover type).

Variable	**Estimate**	**SE**	**p-value**	**2.5% CI**	**97.5% CI**
A) Change of total viral load (log) (R^2 = 0.298)					
Intercept	**1.335**	**0.312**	**p<0.001**	**0.699**	**1.972**
NND forests 1250 m	0.591	0.320	0.074	-0.062	1.244
NND farmland 400 m	**1.059**	**0.320**	**0.002**	**0.406**	**1.712**
B) Change in virus richness (R^2 = 0.280)					
Intercept	**1.457**	**0.252**	**p<0.001**	**0.944**	**1.970**

Habitat diversity 300 m	**-0.721**	**0.285**	**0.017**	**-1.302**	**-0.141**
PLAND residential 100 m	-0.318	0.279	0.263	-0.887	0.251
PLAND forest 100 m	**0.667**	**0.262**	**0.016**	**0.133**	**1.201**
C) Turnover of viruses (R^2 = 0.479)					
Intercept	**0.702**	**0.017**	**p<0.001**	**0.667**	**0.737**
PD vegetated vineyards 400 m	**-0.057**	**0.017**	**0.002**	**-0.092**	**-0.022**
PLAND forests 100 m	**0.071**	**0.017**	**p<0.001**	**0.036**	**0.107**
NND forests 400 m	**0.058**	**0.018**	**0.003**	**0.022**	**0.094**
D) Appearance of viruses (R^2 = 0.461)					
Intercept	**0.467**	**0.021**	**p<0.001**	**0.424**	**0.510**
Habitat diversity 300 m	**-0.074**	**0.024**	**0.004**	**-0.122**	**-0.025**
PLAND residential 100 m	-0.043	0.023	0.074	-0.091	0.005
PLAND forests 100 m	**0.088**	**0.023**	**0.001**	**0.042**	**0.134**
NND forests 500 m	0.034	0.022	0.140	-0.012	0.080

Table 3. Model outputs for the influence of field and landscape variables (Table 1) on A) Nr. of workers (negative binomial distribution; glm.nb), B) Nr. of queens (negative binomial distribution; glm.nb), C) Nr. of cells (normal distribution, lm), and D) Nr. of parasitic pupae and larvae per colony (negative binomial distribution; glm.nb). N = 35 observations (1 observation per bumble bee colony) for all models. Significant responses are presented in bold.

Variable	Estimate	SE	P-value	2.5% CI	97.5% CI
A) Nr. of workers per colony (R^2 = 0.395)					
Intercept	4.058	0.157	0.000	3.765	4.382
Residential NND 500 m	**-0.639**	**0.165**	**0.000**	**-1.062**	**-0.172**
Vineyards PLAND 100 m	**0.372**	**0.163**	**0.023**	**0.049**	**0.668**
B) Nr. of queens per colony (R^2 = 0.485)					
Intercept	1.893	0.193	0.000	1.528	2.289
Farmland PLAND 500 m	**0.645**	**0.201**	**0.001**	**0.269**	**1.032**
Nr. HB apiaries 1500 m	**0.565**	**0.200**	**0.005**	**0.225**	**0.933**
C) Nr. of cells in total per colony (R^2 = 0.298)					
Intercept	532.714	46.257	0.000	438.491	626.937
Forest PLAND 100 m	-95.486	47.059	0.051	-191.343	0.371
Farmland NND 750 m	**-113.604**	**47.059**	**0.022**	**-209.461**	**-17.748**
D) Nr. of parasitic larvae and pupae per colony (R^2 = 0.551)					
Intercept	5.315	0.182	0.000	4.978	5.694
Habitat diversity 750 m	**-0.623**	**0.197**	**0.002**	**-1.116**	**-0.171**
Forest NND 500 m	**-0.464**	**0.187**	**0.013**	**-0.774**	**-0.077**
Farmland PLAND 100 m	-0.281	0.196	0.152	-0.796	0.299
Flower species richness	0.361	0.186	0.052	-0.076	0.791

FIGURES

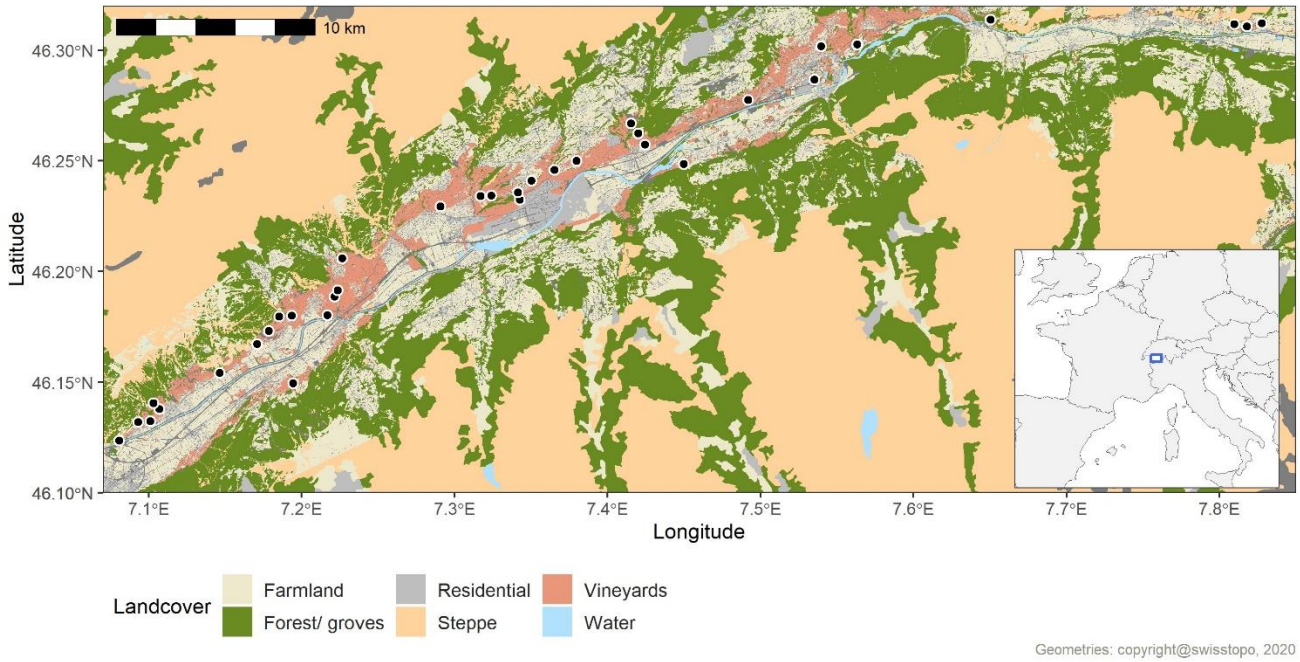


Fig. 1. Study area in Switzerland with 35 bumble bee colony locations (black dots) placed in the mosaic landscape of vineyards, other farmland, residential areas, forests, and steppe.

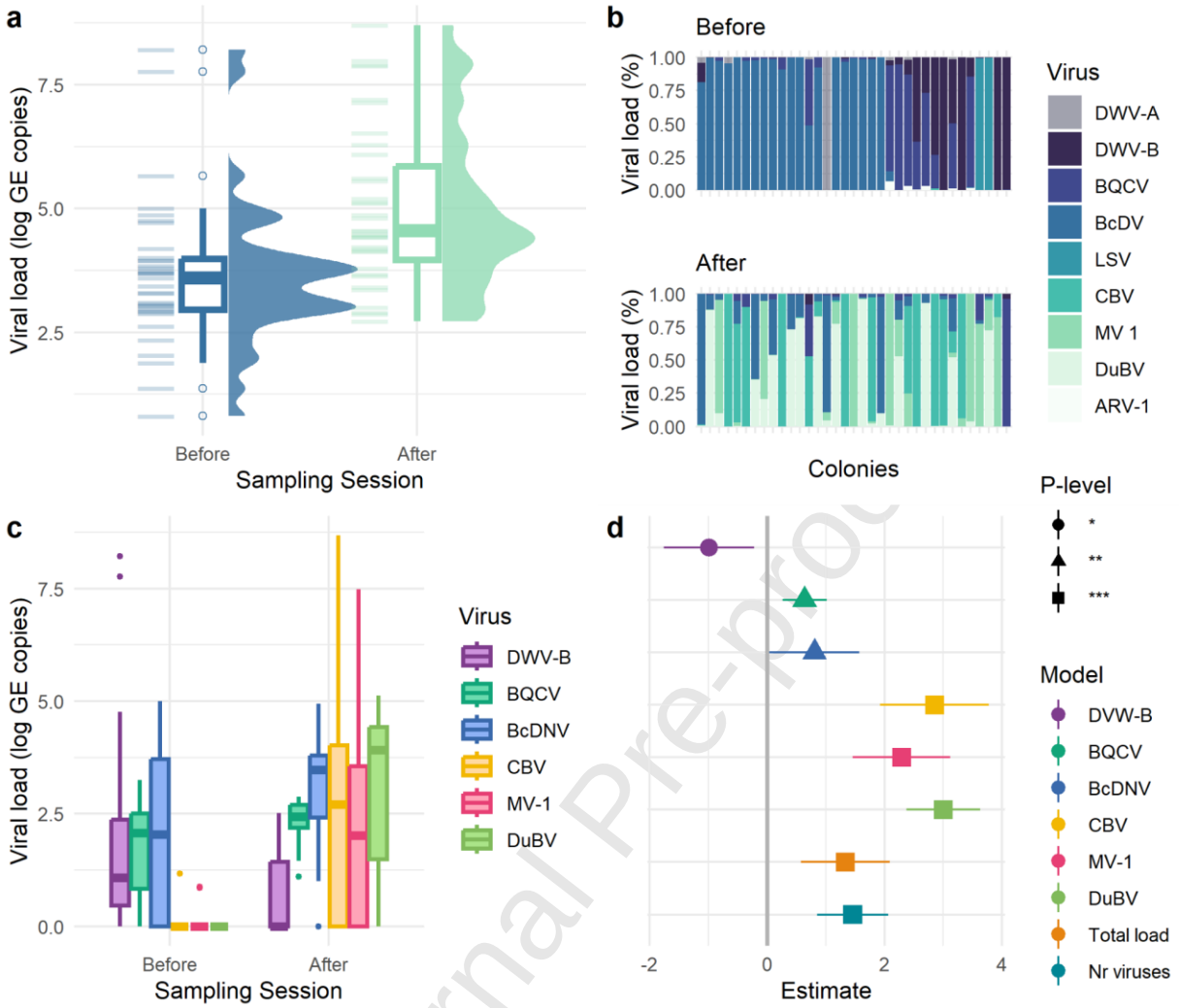


Fig. 2. Change in viral patterns from before field placement (Sampling session before) to after field placement (sampling session after) for a) the total viral load per colony across all nine screened viruses; b) the relative viral loads per bumble bee colony (x-axes) for the six most prevalent viruses present at the beginning and the end of the experiment; c) change in log-transformed viral load for the six most abundant viruses; and d) estimates from paired t-tests for each virus separately, the total virus load and number of different viruses present, where negative values mean a decrease in viral loads, positive values an increase in viral loads during field placement. None of the error bars cross zero, meaning that all changes (increase or decrease) were significant. DWV-A: Deformed wing virus A, DWV-B: Deformed wing virus B, BQCV: Black queen cell virus, LSV: Lake Sinai virus, ARV-1: *Apis* Rhabdovirus-1, BcDV: *Bombus Cryptarum* densovirus, CBV: Castleton Burn virus, MV 1: Mayfield virus 1, DuBV: Duke bunyavirus.

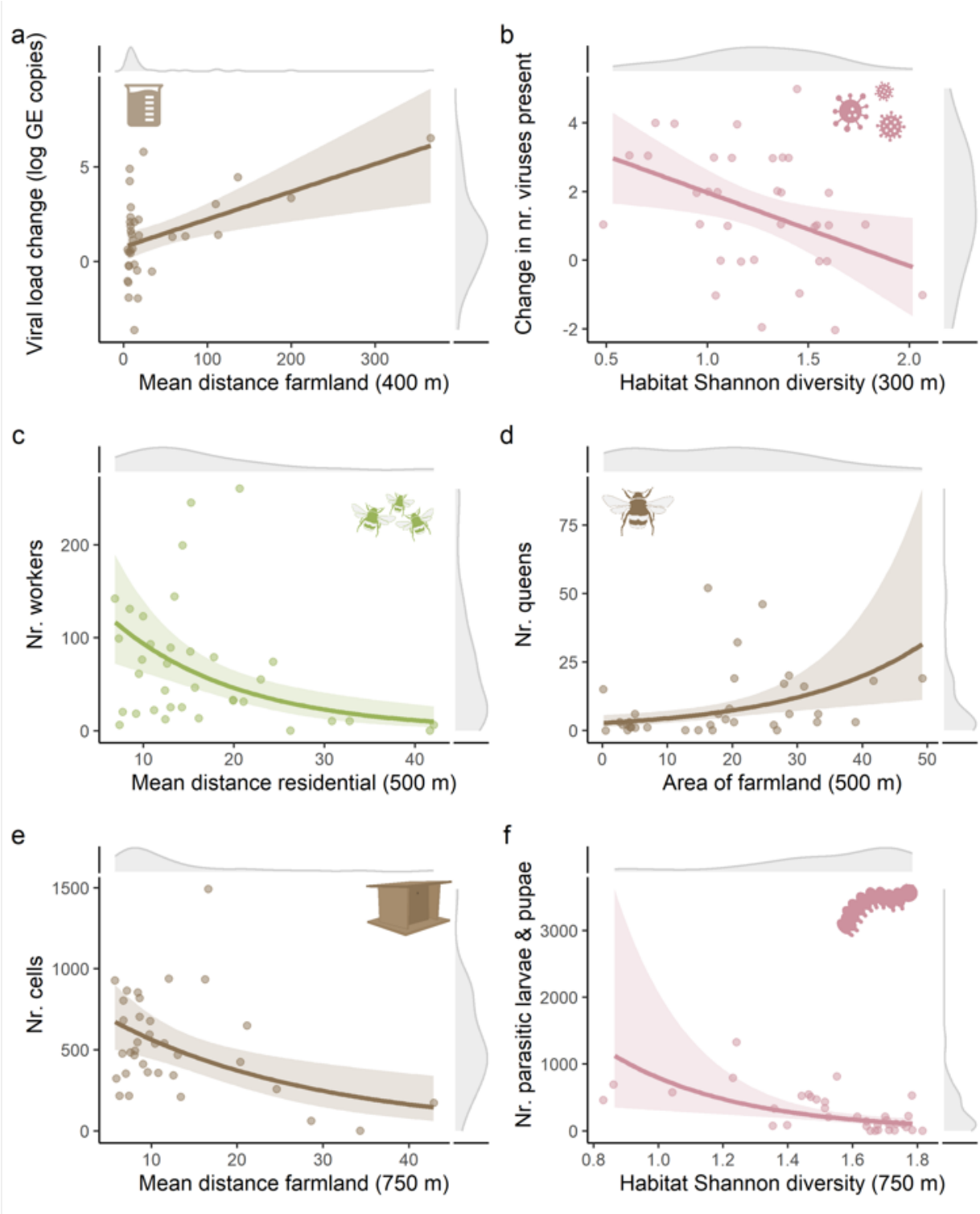
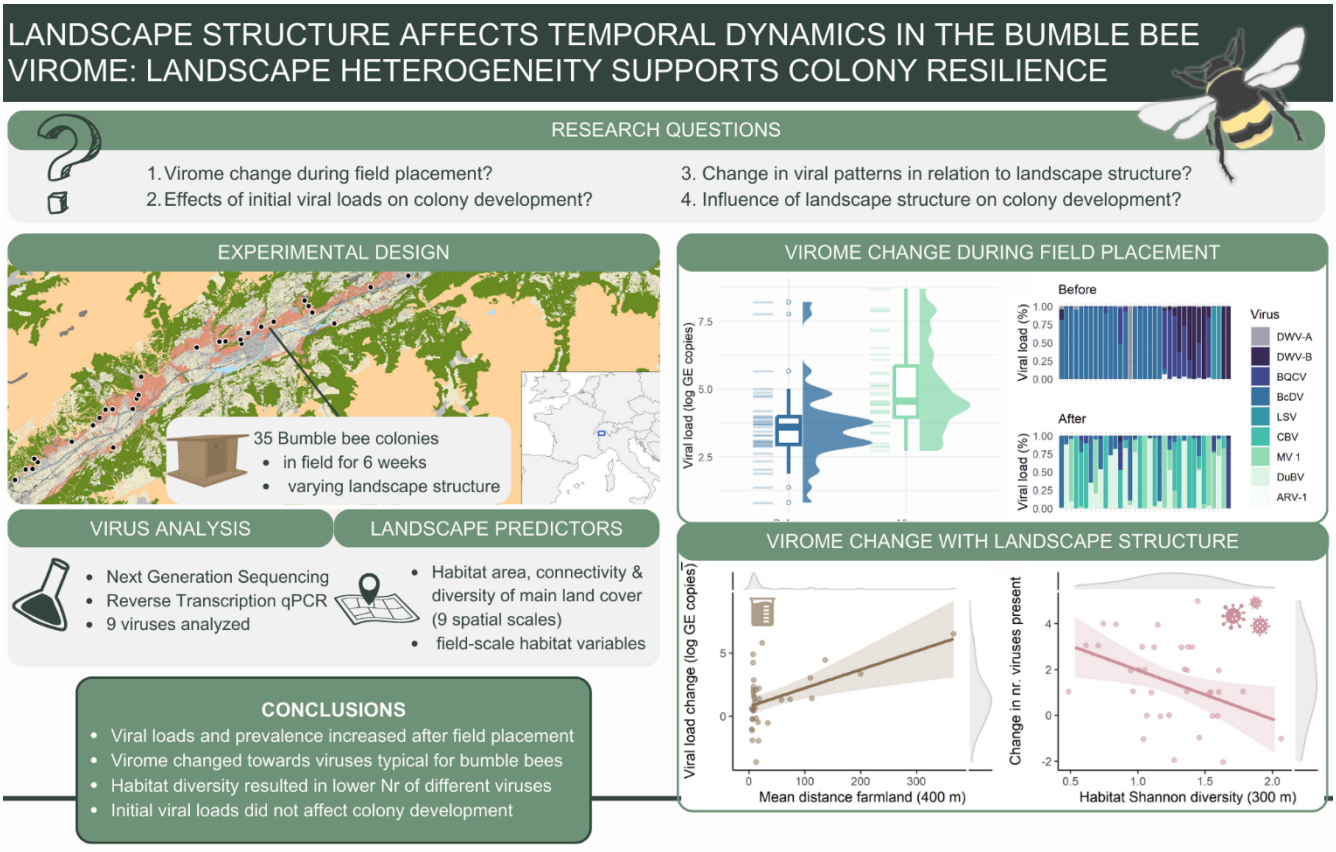


Fig. 3. Linear relationships between the landscape structure and changes in the bumble bee virome (a, b), and colony development parameters at the end of field placement(c-f). a) Change of total viral load (GE copies on log10 scale) and mean nearest-neighbor distance among farmland patches within 400m radius, b) change in number of different viruses present and habitat Shannon diversity within 300m radius around the bumble bee colonies, c) number of workers per colony and the mean nearest-neighbor distance of residential areas within

500m, d) number of queens per colony and the relative area covered by farmland within 500m, e) number of all cells per colony and mean nearest-neighbor distance among farmland patches within 750m, and f) number of parasitic larvae and pupae per colony and habitat Shannon diversity within 750m. Estimates are derived from the respective best model per response variable where other terms present in the model were set at their mean. Solid lines depict the estimated mean, shaded area the confidence interval and the points and density distributions at the right and top of the plot show the distribution of the raw data.

Graphical abstract



Highlights

- Virome composition shifts significantly in bumble bee colonies in field settings
- Virus presence was mostly inconsequential for colony development
- Habitat Shannon diversity alleviates viral loads in bumble bees
- Landscape heterogeneity and connectivity support bumble bee colony development