SAFEGUARD

Report on under-researched pressures and mechanisms impacting pollinators and plant reproduction

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Preface

This deliverable (D2.6) reports on studies conducted, as per the Safeguard Grant Agreement, to investigate under-researched pressures and mechanisms impacting pollinators and plant reproduction. Specifically, following Task 2.6, it focuses on (i) nitrogen deposition, (ii) traffic, and (iii) pathogen spillover as emerging pressures on pollinators and plant reproduction.

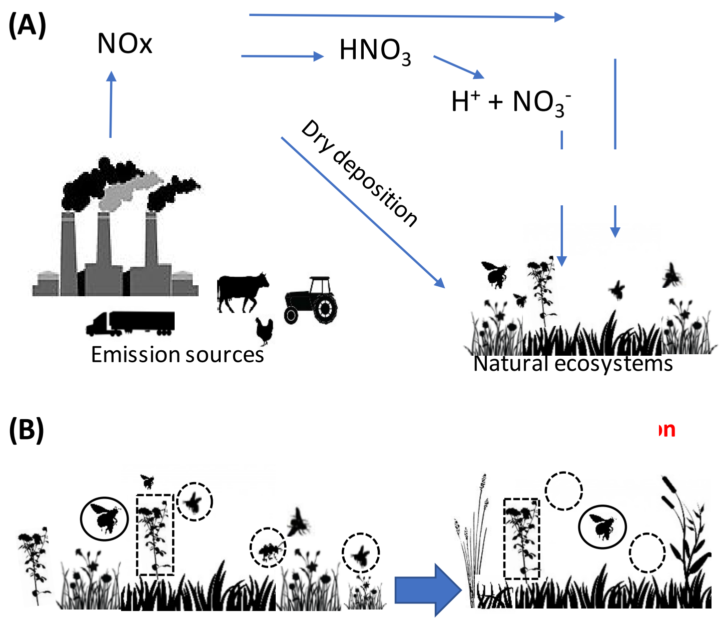
Summary

While land-use and climate change are recognised drivers of pollinator declines, a range of other factors that could drive declines remain under-researched. In the absence of this understanding, it is difficult to prioritise resources and interventions to halt and reverse pollinator declines. Here we report on studies that address three key priority knowledge gaps: the role of nitrogen deposition, traffic, and pathogen spillover as potential emerging drivers of pollinator declines. Semi-natural grasslands are a key habitat for pollinator diversity in Europe, but pollinator populations in these habitats could be impacted by nitrogen deposition and pathogen spillover. In contrast, road-side verges provide a potential habitat for pollinators that could be enhanced by targeted interventions, but could also be negatively impacted by traffic. Using a network of sites across Europe, we measured a gradient in nitrogen deposition that matched predictions from modelled deposition data. We found an unanticipated positive correlation of N deposition with the diversity of plant-pollinator networks and considerable spatial heterogeneity in plant-pollinator community composition (within and between subnational regions) that was only weakly associated with N-deposition levels. From these data it seems that N deposition may not have major impacts on pollinators, at least in semi-natural grassland habitats, although the potential that site management might be influencing our conclusions needs to be considered. Using a similar landscape of largely protected semi-natural grasslands across Europe, we screened wild and managed pollinators for Deformed Wing Virus B. This pathogen was found at high levels across both wild and managed bees, and the data suggest that pathogen spillover from honey bees to wild bees is driving virus prevalence and intensity in wild bees. Such pathogen spillover could undermine the value of these protected areas for pollinators. Actions that support beekeepers in maintaining health honey bee hives, and controls over the deployment of honey bee hives near protected areas could both mitigate the threat of spillover. Finally, using a network of roads across Europe, we assessed the relationship between floral abundance and diversity in road verges and pollinator populations, the impact of turbulence on pollinator foraging, the direct impact of collisions on pollinators. Pollinators were more abundance and diverse when floral resources were abundant and diverse, as would be expected from studies in other habitats. However, turbulence, caused by fast-moving traffic, made it difficult for pollinators to forage on road verges. In contrast, there appeared to little risk of mortality by collision with moving traffic. Consequently, floral enhancement on low-speed road verges should be prioritized to enhance pollinator populations. Overall, our results have identified the relevance of three under-explored threats to pollinators, and produced guidance for interventions that could enhance pollinator populations across Europe.

1. Nitrogen deposition as an emerging pressure

1.1. Introduction

Semi-natural grasslands are nitrogen (N) sensitive ecosystems of conservation value with documented declines in plant diversity in response to N deposition (e.g., Dupre et al 2010; Stevens et al. 2010a). The biogeochemical atmospheric processes leading to pollution of natural grassland and heathland ecosystems are well known to modify the diversity and composition of vegetation (Box 1.1A). However, effects of N-deposition on pollinator forage plant species and pollinator biodiversity remain unresolved hypotheses to be tested by Safeguard (Box 1.1B). Moreover, the degree that N deposition is a major pressure on pollinators relative to other well-established threats (e.g., land management intensity, landscape simplification remains to be understood.



Box 1 Biogeochemical atmospheric processes (A) leading to pollution of natural grassland and heathland ecosystems are known to modify the diversity and composition of vegetation. The consequences for the pollinator forage plant species and pollinator biodiversity are hypotheses to be tested by Safeguard (B).

Hypotheses

H1: Reduction in diversity & abundance of forage plants ➡

H2: Surviving forage plants are of lower nutritional quality/less visited ⬚

H3: Lower pollinator diversity especially of specialists ◌

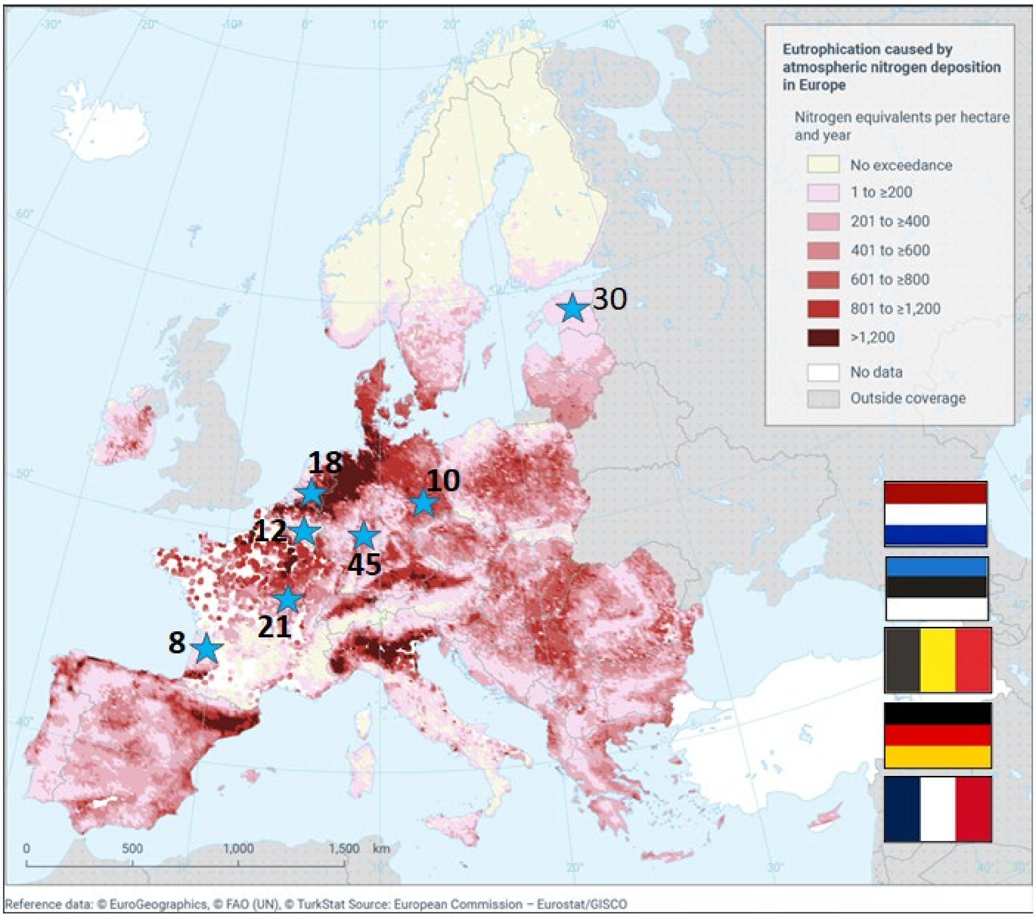
H4: Social, generalist bees dominate communities ○

Here we address these knowledge gaps through research testing several hypotheses on the role of N deposition in modifying floral resources, plant-pollinator community networks and pollinator flower visitation rates (Box 1). We focused on semi-natural grasslands (mostly calcareous) that are a threatened and protected habitat in the EU and that can provide important habitat and floral resources for pollinating insects. Our overall expectation was that long-term (multi-year) accumulation of N through atmospheric deposition in the grassland ecosystem would have altered plant community dynamics leading to changes in the composition and diversity of forage plant resources supporting pollinating insects (Box 1 H1-2). We also expected that the changes in the forage plant resources would have led to shifts in the community of pollinators (Box 1 H3-4). To test these hypotheses in field settings, we performed field sampling of plant and pollinator communities in a selection of grassland sites spanning a gradient of N deposition rates across Europe.

1.2 Methods

1.2.1 Nitrogen loading of the grassland system

A group of Safeguard partners (INRAE, UFZ, UMons, UWUE, WUR, EMU) in 7 regions spread over 5 EU member states (FR/DE/NL/BE/EE) implemented a specific protocol to test our hypotheses. We took the approach of sampling grassland sites along gradients of N deposition to allow comparison of plant-pollinator assemblages in sites under different levels of ecosystem N loading (Figure 1). Spatial N-deposition maps allowed for the selection of regions at EU scales that were under high or low levels of chronic N enrichment. But to obtain precise quantification of direct estimates of local N deposition loading site-specific measurements were necessary. These estimates followed a monitoring protocol of ICP Vegetation (allowing the future transfer of these data to the international monitoring network on air pollution) and are derived from elemental analysis (%N) of tissue samples taken from moss species present in the sites. The mosses function as an empirically-validated bio-indicator of the spatial distribution of atmospheric nitrogen deposition (Harmens et al. 2011). This is because the N content of moss tissues is solely derived from wet or dry aerial deposition of N and not from root uptake from soils (as is the case for vascular plants), thereby providing an estimation of the level of atmospheric N deposition independent of other anthropogenic inputs (e.g. fertilisers, livestock excreta).



Reference data: © EuroGeographics, © FAO (UN), © TurkStat Source: European Commission – Eurostat/GISCO

Figure 1.1 Distribution of study areas (stars) and numbers of sites (numbers) in five European Union member states covering zones of differential ecosystem loading of nitrogen across Europe. In total 130 landscapes were sampled for plants and pollinators.

1.2.2 Pollinator and plant sampling

In each site, we collected data on flower-pollinator interactions (in 2022 or 2023) using transect sampling methods (30 minute variable transects per visit, capturing on-site plant diversity as much as possible, 2-4 visits per site depending on region), which allowed us to quantify species composition and interspecific interactions in habitat under different levels of pressure from N deposition. Pollinators targeted were wild bees, syrphids, and butterflies, but data on other important taxa (e.g. bombyliid flies, beetles) was also recorded where they occurred (Figure 1.2). An interaction between a flower and insect individual was defined as when it is in active contact with the flower during feeding on nectar or collecting pollen (rather than resting on a petal or the calyx) (Figure 1.2). Each individual insect was identified to as fine a taxonomic resolution (species, genus) as possible, either in the field or in the lab.

For botanical responses to N deposition, we applied a two-step approach. First, we carried out a floral resource survey (quadrats) to provide data on the spatial and phenological turnover in flowering resources at three time points over the season (April-May/May-June/July-August). Floral resources were defined as a single individual or composite flower or an umbel, spike or capitulum on multi-flowered stems that an insect must crawl between or fly to access nectar/pollen. This floral resource survey was done at the same time (or within a 2-week window) as the pollinator transects. For each quadrat, the identity of plant species (dicotyledons) in flower was determined and the number of flowers/inflorescences per flowering species counted (up to a 100) or estimated using an ordinal scale (100–200, 200-300, etc.) per quadrat. Secondly, we carried out a complete botanical survey of each site at the point of maximal plant diversity to produce a detailed ‘snapshot’ of the taxonomic identity and % cover (visually estimated) of all dicot and monocot species in each site.

Figure 1.2 Photos of a calcareous grassland fragment, flora, and fauna (Burgundy, France)

1.2.3 Statistical analysis

Plant and pollinator species number were strongly correlated (r = 0.77), so we used overall network size (calculated as the geometric mean of the number of plant and pollinator species) as a simple response variable describing the community diversity. We tested whether plant-pollinator network size was related to the N content of the moss bioindicator of site-specific N deposition. We ran a Bayesian general linear mixed model with plant-pollinator network size (log-transformed) as the response variable and moss N content as a fixed effect and sub-national region as a random effect to control for variation between the study areas. Both network size and moss % N content were scaled by subtracting the mean and dividing by standard deviation. For network metrics – connectance, network asymmetry and nestedness (NODF) – we ran similar models, but including plant-pollinator network size as a covariate to account for its effect on network metrics.

1.3 Results and Discussion

We present results and interpretation of patterns in N loading in the sites sampled across Europe (Figure 1.3) and the overall plant-pollinator community response to N deposition in those sites (Figures 1.4-1.6).

1.3.1 Moss nitrogen content

In all grassland sites, mosses were sampled to analyse the nitrogen content of their tissues, which indicates the level of ecosystem N deposition at the level of each specific site. Moss species were sampled according to their availability within a location and country. The most sampled species were *Pseudoscleropodium purum* (N = 77 sites), *Hypnum cupressiforme* (N = 37) and *Hylocomium splendens* (N = 22). Both between and within European countries, we found variability in the percentage N of moss tissues, with values between 0.62 and 1.87% (and one outlier of 2.25%) indicating a gradient in N deposition (Figure 1.3). Between European countries, the average percentage N within moss tissue was consistent with large-scale modelled N deposition data (e.g. Figure 1.1) and these percentage estimates are consistent with earlier Europe-scale data on the percentage N content of mosses (e.g. 0.8 and 1.6%; Harmens et al. 2011). This analysis of pooling all moss species gives an indication of the variability in N deposition detected among the different regions within Europe, which can be used as a predictor of patterns in plant-pollinator communities (1.3.2).

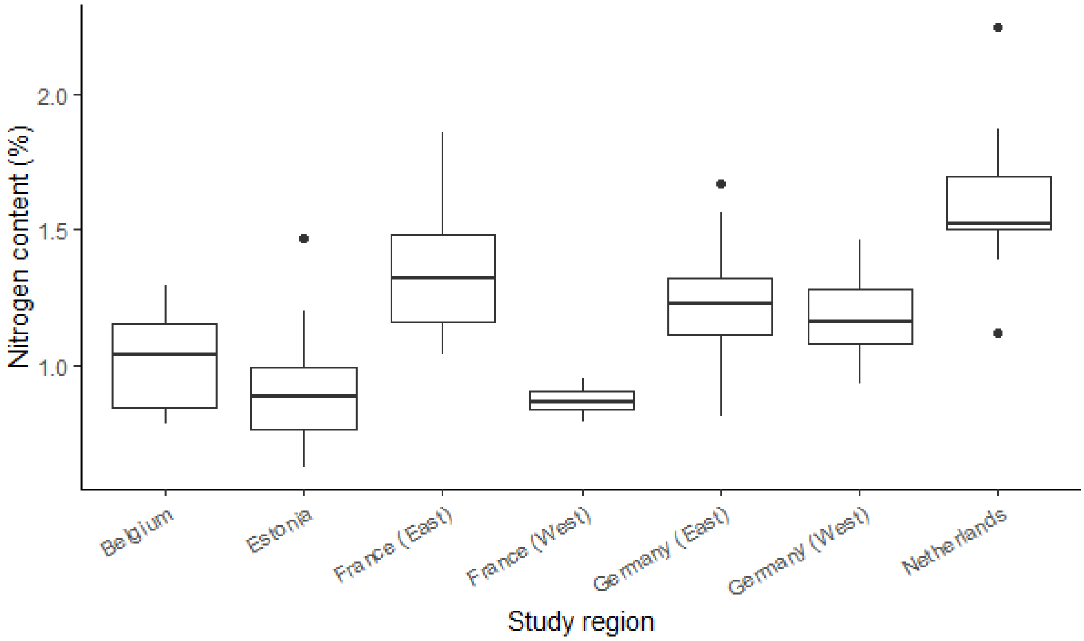


Figure 1.3 Boxplots of the moss nitrogen content per region. In the analysis, all moss species are pooled together, although the final analysis will estimate nitrogen deposition based on calibrated nitrogen content per species

1.3.2 Effect of nitrogen deposition on plant-pollinator communities

Using the data on plant and pollinator species recorded in the field sites (pooling all sampling periods per site) we assessed at the site-level the plant-pollinator network characteristics.

We did not find a strong effect of nitrogen deposition on network metrics, although nitrogen deposition increased network symmetry by increasing the relative amount of pollinator species compared to the number of plant species (β = 0.17, SD = 0.11, 95% CI = -0.05 – 0.38). Network size was correlated positively with increasing moss N content (β = 0.26, SD = 0.10, 95%CI = 0.07 – 0.46, Figure 1.4). This confirmed our expectation that N-deposition would produce a shift in plant-pollinator communities, but was surprisingly opposite to our specific hypotheses of declines in both plant and pollinator species with increased nitrogen deposition (Box 1.1: H1 & H3).

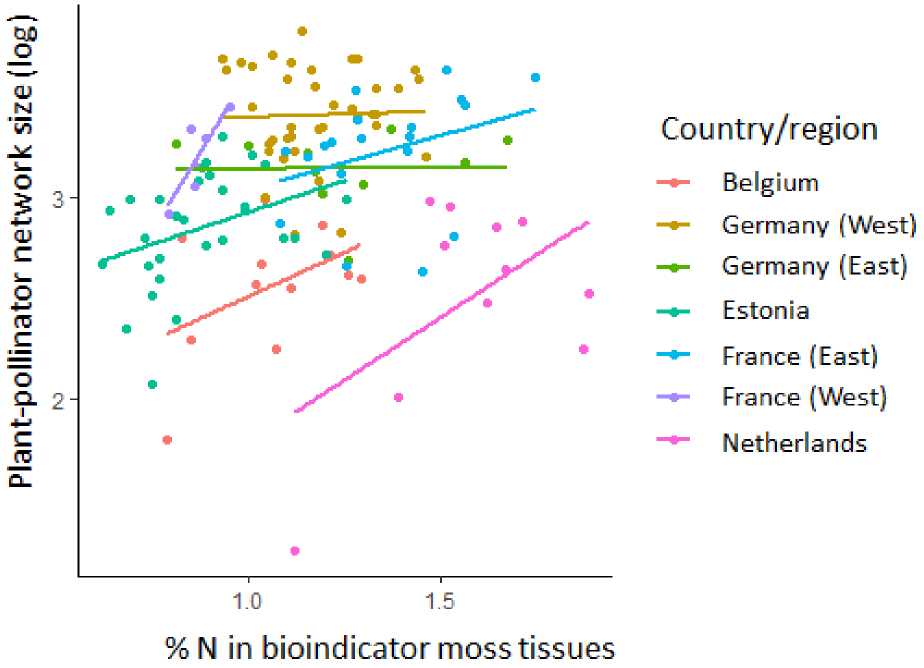


Figure 1.4 Relation of nitrogen content in bioindicator mosses and plant-pollinator network size

Ordination analyses (unconstrained PCA) were performed on the community composition of plants (genus-resolution) and insects (species-resolution) for each sub-national region. This produced some evidence of clustering in ordination space indicating that there was some association of plant community composition with the level of N deposition in the site (each point in the Figures 1.5 and 1.6). This was more marked in the sites sampled in Eastern France, Belgium, the Netherlands and Estonia, while the western France site (albeit few data points) and German sites did not produce strong gradients of association between plant community composition and site-specific N deposition (Figure 1.5). For the insect pollinators, there was only a degree of community structuring associated with N deposition in the Belgian sites, whereas the association with N deposition was heterogenous, weaker and equivocal in the other subnational regions (Figure 1.6).

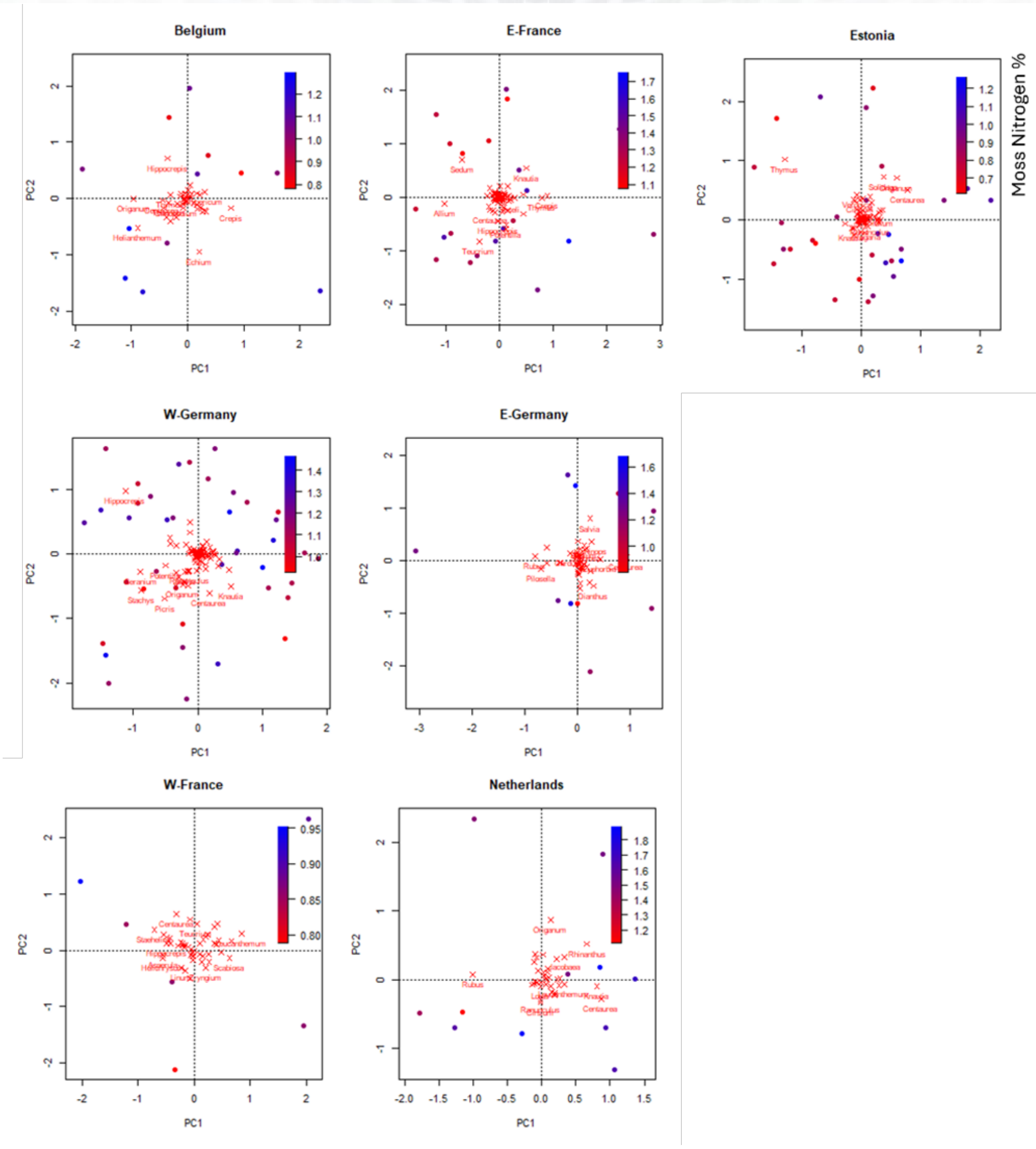


Figure 1.5 Unconstrained PCA of the plant community composition at the genus level in each of seven subnational European regions. Each point represents a site score for the plant community with the colour indicating a low (red) or high (blue) level of N deposition (% N content of moss bioindicator) at that site. X indicates the score and associated identity of flowering plant genus.

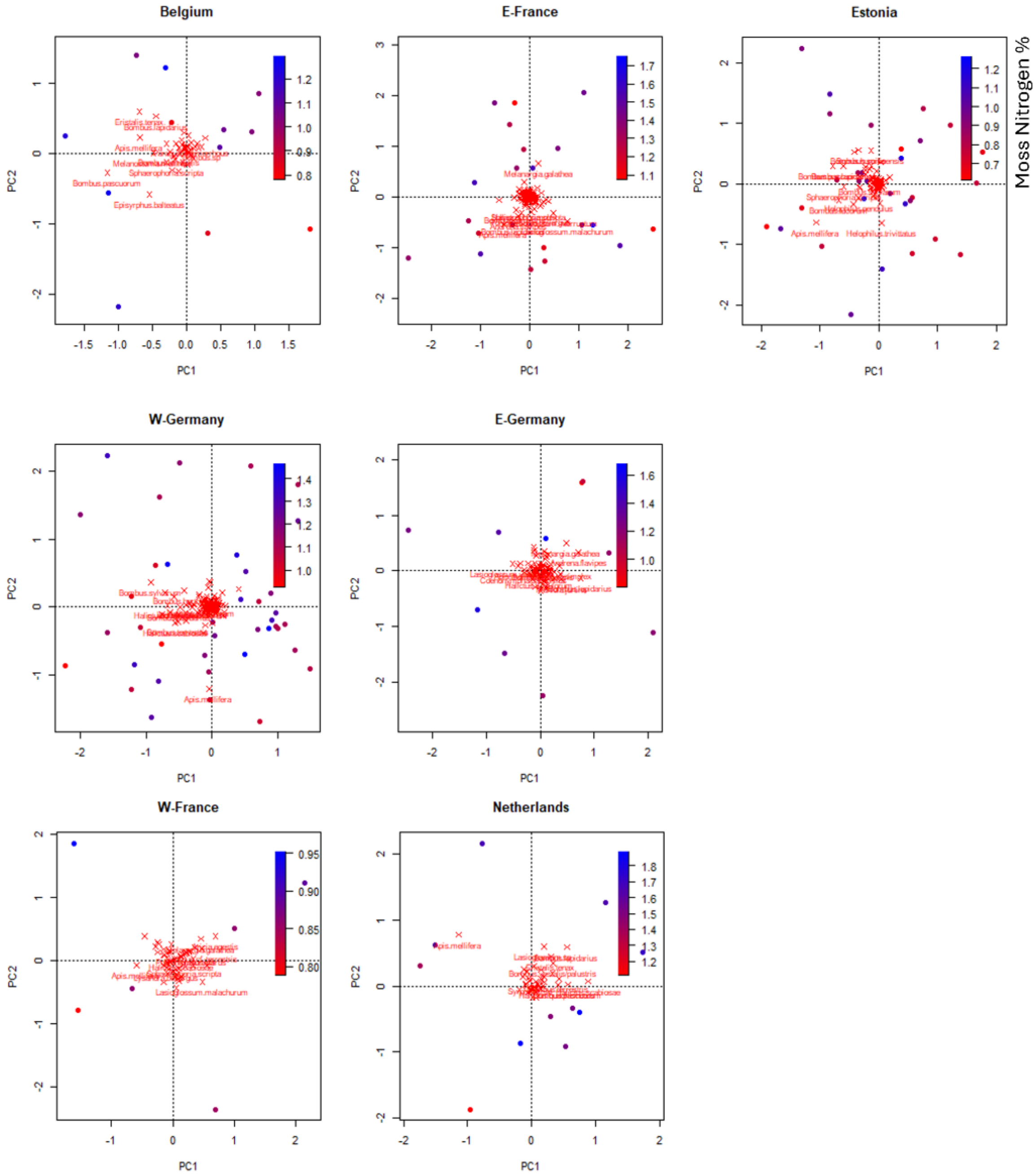


Figure 1.6 Unconstrained PCA of the pollinator species composition in communities sampled in each of seven subnational European regions. Each point represents a site score for the insect pollinator community, with the colour indicating a low (red) or high (blue) level of N deposition (% N content of moss bioindicator) at that site. X indicates the score and associated identity of an insect pollinator species.

Overall, there was a noticeable association between levels of site-specific N deposition and the diversity and community structure of plant and pollinator communities. Furthermore, the species richness of plant-pollinator networks was positively correlated with N-deposition (using mosses as a bioindicator to establish atmospheric N loading of the system) (Figure 1.4). A possible explanation of this unexpected finding is that because these naturally nutrient poor and well-buffered calcareous grasslands were less sensitive to nitrogen deposition than other grassland habitats (e.g. dunes, acid grassland and heathlands) with poorly buffered soils and documented plant declines (Field et al. 2014; Maskell et al. 2010; Stevens et al. 2010ab).

An extensive study on British calcareous grasslands found little direct effect of nitrogen on spatial differences in plant species richness, although in sites with high nitrogen deposition, species diversity declined over time (Van den Berg et al. 2010). Alternatively, terrain management could confound our analyses, as grasslands in more densely populated areas, while being exposed to higher levels of nitrogen deposition, are also more actively managed in the context of biodiversity conservation. High levels of N deposition and its impact on biodiversity could even be direct reasons to adapt management, e.g. by increasing mowing frequency, hence reversing or even overcompensating the adverse effects of N deposition on species richness.

Without dramatic changes in plant-pollinator species richness, chronic nitrogen deposition might have had subtle effects on the flora of the calcareous grassland fragments affecting the local nutritional landscape for pollinators (Box 1.1). One mechanism might be that chronic N-deposition leads to specific taxonomic shifts in floral community composition (Dupre et al. 2010, Maskell et al. 2010), for example, the presence or abundance of particular plant taxa (genera) some of which may provide important pollen and nectar sources to pollinators. Any such N-deposition driven shifts in the availability of pollen/nectar sources would be expected in turn to filter the species of pollinators foraging in those sites that depend on those plant sources, and therefore the pollinator community composition.

1.4 Conclusions

Our study detected an unanticipated positive correlation of N deposition with the diversity of plant-pollinator networks and considerable spatial heterogeneity in plant-pollinator community composition (within and between subnational regions) that was only weakly associated with N-deposition levels. It is therefore probable that N deposition effects on pollinator communities are either masked by secondary or other known pressures on pollinators, such as the habitat size and connectivity, land management intensity (e.g. grazing intensity) and the influence of the surrounding landscape.

2. Traffic as an emerging pressure

Roads are vital for human societies, yet they can also have negative impacts on the ecological communities that live in close proximity to them (and to associated ecosystem service provision). Insect pollinators, which nest and forage in verges running alongside roads are a group of particular importance. It is possible that these verges act as an “ecological trap”, drawing insect pollinators into contact with traffic, thereby increasing the risk of pollinator-traffic collisions.

Across six European regions, we evaluated the complex relationships between traffic, road verge floral composition and surrounding land use, to understand how these factors influence abundance and richness of bees, butterflies and hoverflies sampled within road verges. Using car-mounted sticky traps, we also assessed the relationships between traffic, road verges, and surrounding land use with insect-traffic collisions.

2.1. Methods

2.1.1. Site Selection and Characterisation

Six regions, across five European countries, including regions in Belgium (UMONS), Estonia (EMU), Serbia (UNSPMF), the United Kingdom (UREAD) and two regions in Germany (UFZ and UWUE) were sampled, capturing a diversity in road types and land uses (Figure 2.1). Each partner contributed to the experimental design and methods and carried out a full scale study in their country or region. SLU provided technical input into the methodology and sampling design. The overall coordination, data collation and analysis was led by UREAD.

Figure 2.1 Locations of the six study regions across Europe. 1. UK, 2. Belgium, 3. Germany (Bavaria), 4. Germany (Sachsen-Anhalt), 5. Serbia, 6. Estonia (study countries in white)

In each region, 24 study sites were selected as follows (Figure 2.2). Each site was surveyed three times, between April and September 2023.

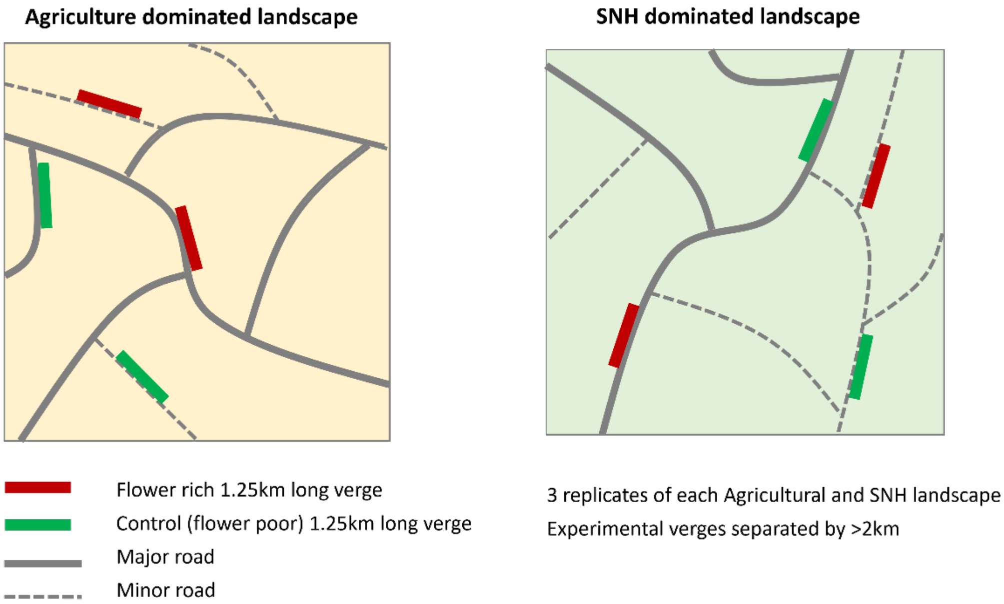


Figure 2.2 Schematic showing site selection criteria for a hypothetical landscape block (SNH = Semi-Natural Habitat)

Landscape Block: Three separate landscape blocks were selected, each approximately 10 km² in size. Within each landscape block, 8 sites were selected, where possible at least 2 km from other sites, each containing a unique combination of the following characteristics:

1) **Landscape Type:** Within each landscape block, two landscapes were selected containing contrasting amounts of cropland and semi-natural habitat. Land use data used to quantify the amount of cropland and semi-natural area was obtained from the ESRI Sentinel-2 10-Meter Land Use/Land Cover map for 2023 (Karra et al., 2021). Land use within a 1km radius of the centre of the site was extracted and the area of both cropland and semi-natural land use was calculated.

2) **Road Type:** Within each landscape type, a pair of roads, 1.25 km long, and expected to have contrasting traffic intensities were selected. Each road was single carriageway, with one lane of traffic in each direction (multi-lane roads were excluded on the basis of safety concerns for surveyors). Within each pair, one road was classified as “minor” with an expected low traffic intensity, and the other classified as “major” with an expected high traffic intensity. This classification was converted to a continuous variable “Seasonal traffic density” using additional data from the TomTom Move platform (https://move.tomtom.com), with traffic volume for the whole study period (April-September) extracted at each site. Traffic speed at each road was also extracted from the TomTom Move platform.

3) **Verge Type:** Within each landscape type, and for each road type, two road verges (Figure 2.3) with contrasting floral availability were selected. “Flower” margins consisted of verges either sown with flowering plants or managed in such a way as to promote floral resources. “Grass” margins consisted of verges that primarily contained grass species. This was also converted to a continuous variable following Baude et al. (2016), who used floral traits to model nectar availability for a wide range of species.

Figure 2.3 Two road verges from Estonia that were used in the study.

2.1.2 Surveys

A 250m transect was established to survey plant-pollinator networks within road verges. Initially, a 15 minutes butterfly transect was walked using a modified pollard walk technique (Pollard, 1977). All butterflies observed were recorded, and the plant species was also recorded for individuals observed visiting a plant. Subsequently, a bee and hoverfly transect was walked, over the same 250m, using the same method. Either immediately before or after the survey, traffic counts were also carried out, with all vehicles observed at one end of the 1.25km section of road for a 15-minutes period recorded.

The flowering plant community was surveyed every 50m along the 250m pollinator transect using a 1m2 quadrat. All plants in flower were identified, and the percentage cover of flowers of each species was estimated. From these data, the categorical variable Verge Type (“Flower” or “Grass”) was converted to a continuous variable (“sugar nectar availability”). Sugar nectar availability on the date of each transect was calculated using a model developed for British plant species by Baude et al., (2016), who collected data on annual nectar sugar productivity (kg/ha/year) for a range of plant species.

To measure insect-traffic collisions, sticky traps measuring 10cm x 25cm were attached to a car, which was driven along the whole length of the 1.25km stretch of road in both directions at a constant speed of 60km/h (although in some cases this differed depending on road conditions). Individuals that were caught on the sticky traps were identified to the most detailed taxonomic rank possible, and subsequently grouped into orders (Figure 2.4).

Figure 2.4 Photographs of the front of one of the survey cars with sticky traps attached, and close up of sticky traps with captured insects.

2.1.3 Statistical Analysis

To test whether nectar sugar availability (converted from floral abundance as described previously) in a road verge, surrounding land management, and traffic density and speed influence pollinator abundance in road verges, negative binomial generalized linear mixed effects models were run. Each pollinator guild (bee, butterfly and hoverfly) was modelled separately, to account for possible differential responses to the predictors. A model averaging procedure was used to refine the global model. Models containing each possible combination of predictor variables were created, and the average of all models within 2 AICc units of the “best” model (i.e. lowest AICc value) was used to generate a set of model-averaged parameters.

To test effects on pollinator richness in road verges, a similar approach was taken, with the following differences: Species richness (number of unique species) was pooled over the season to reduce collinearity with pollinator abundance. For each guild, the species richness observed in a verge was treated as the dependent variable. Instead of nectar sugar availability, number of unique plant species in flower at the time of the survey was used to test for the impact of floral richness on pollinator richness.

To test whether floral resources in a road verge, surrounding land management, and traffic intensity influence the number of insects killed in traffic collisions, another negative binomial generalized linear mixed effects model was run. The number of insects killed on sticky traps per transect was taken as the dependent variable, with nectar sugar content in the verge, proportion of cropland surrounding the verge, daily and seasonal traffic density, average traffic speed in the road alongside the verge, time of day, and day of year were treated as fixed effects. Again, a model averaging procedure was implemented to generate a set of model-averaged parameters.

2.2 Results

2.2.1 Summary of pollinator communities

10,960 pollinators belonging to 293 species were recorded across the study, including 4,735 bees (43.2%, 152 species), 2,705 butterflies (24.6%, 65 species) and 3,520 hoverflies (32.1%, 76 species). Honeybees (*Apis mellifera*) were the most frequently recorded pollinator (2,444 records, 22.3%), followed by the hoverflies *Sphaerophoria scripta* (1,067 records, 9.8%) and *Episyrphus balteatus* (1,016 records, 9.3%). Geographically, the most pollinators were recorded in Serbia (3,836, 35.0%), with all other locations recording between 1,102 (10.1%) and 1,695 (15.4%) pollinators.

2.2.2 Factors Influencing Pollinator Abundance and Richness

Nectar availability was the most important predictor of pollinator abundance for all three pollinator guilds, with more pollinators observed in verges with more available nectar. Traffic speed had the opposite effect, with faster traffic speeds reducing the abundance of bees and butterflies (but not hoverflies; Figure 2.5A).

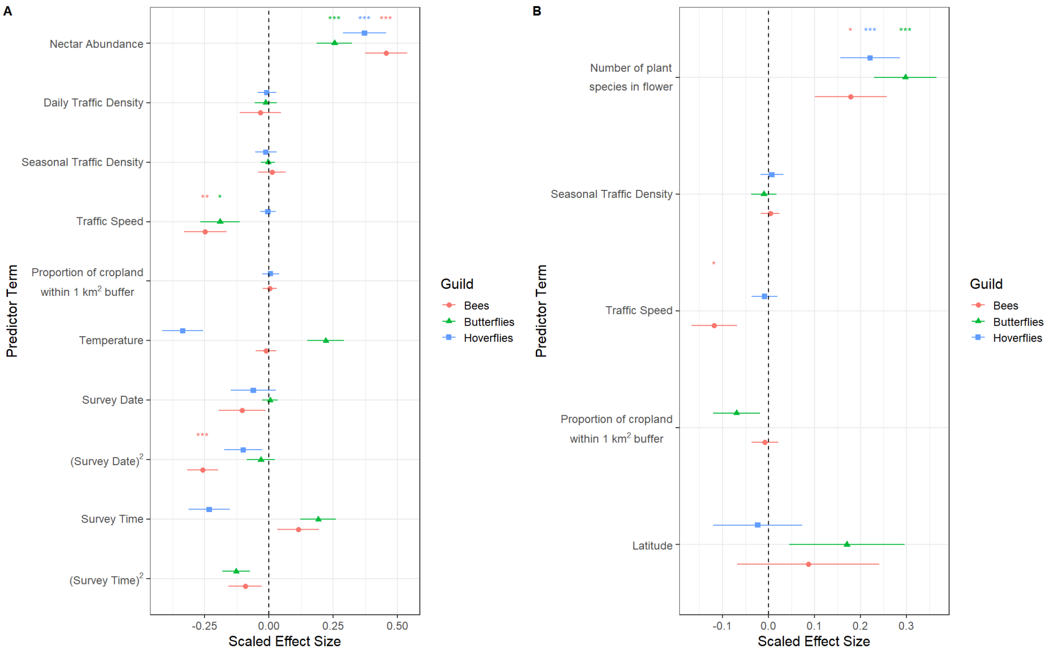


Figure 2.5 Scaled model plots showing the effect of predictor variables on A) pollinator abundance and B) pollinator richness. Absent points indicate absence of the environmental variable from the final averaged model. Error bars indicate standard error. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Plant species richness was the most important predictor of pollinator diversity for all three guilds, with more pollinator species observed in verges containing more species of flowering plant. Faster traffic speeds reduced the number of bee species (but not butterflies or hoverflies; Figure 2.5B).

2.2.3 Factors Influencing Insect-Traffic Collisions

Across the five regions participating in the insect-traffic collision surveys, a total of 3,259 insects were collected on sticky traps at a rate of 0.029 insects/km/cm². Most insects (86.1%) were smaller than 2mm in length, with only 4.5% greater than 5mm. 40.7% of insects killed were Thysanoptera (thrips), and a further 30.7% were Diptera. Only 33 bees, butterflies and hoverflies were caught (1.0%). The greatest number of insects were caught on sticky traps at the Germany (Sachsen-Anhalt) region (38.3%), with 20.4%, 16.2%, 13.4% and 11.5% caught on sticky traps in Belgium, Estonia, Serbia and the UK respectively.

Traffic, both at the time of the survey (“Daily”) and average between April and September (“Seasonal”) had a significant impact on the number of insects caught on sticky traps, with seasonal traffic having a large, negative impact (i.e. reduction) on the numbers of insects caught on sticky traps, whereas daily traffic density had a smaller, positive impact (i.e. increase) on the numbers of insects caught (Figure 2.6).

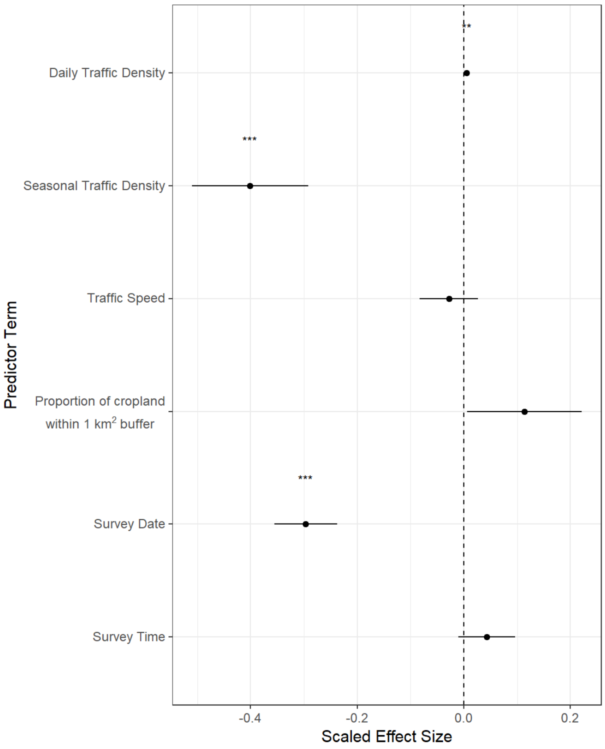


Figure 2.6 Scaled model plots showing the effect of predictor variables on insect-traffic collisions. Absent points indicate absence of the environmental variable from the final averaged model. Error bars indicate standard error. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001

2.3 Summary and Recommendations

- Florally abundant and diverse road verges harbour more abundant and diverse pollinator populations.
- Turbulence, caused by fast-moving traffic makes it difficult for pollinators to forage within road verges.
- The low number of pollinators caught on sticky traps indicates relatively low risk of pollinator-traffic collisions, suggesting that road verges can be a net positive if managed appropriately.
- Floral enhancement work should be carried out, initially targeted on low-speed roads.

A manuscript based on the study is currently in review with the Journal of Applied Ecology.

Chris Wyver, Andrijana Andrić, Maja Arok, Carolin Biegerl, Sofia Blomqvist, Christophe Dominik, Noah Feldmann, William Fiordaliso, Mike Garratt, Andrea Holzschuh, Hanna Honchar, Reet Karise, Hanno Korten, Sarah Lescot, Egle Liiskmann, John MacArthur, Marika Mänd, Denis Michez, Erik Öckinger, Oliver Schweiger, Tea Skendžić, Henrik G. Smith, Ingolf Steffan-Dewenter, Louise Truslove, Sanja Veselić, Dušanka Vujanović, Deepa Senapathi, Simon G. Potts (2025) Impacts of road verge floral composition and adjacent road traffic on European pollinators

3. Pathogen spillover as an emerging pressure

3.1 Introduction

Bees provide important pollinating services to natural and agricultural ecosystems (Klein et al., 2007; Ollerton, 2017; Tong et al., 2023). Therefore, general declines in populations of pollinators are raising concerns for ecosystem health and food security (Vanbergen & The Insect Pollinators Initiative, 2013). One driver implicated in these declines is emerging diseases (Brown & Paxton, 2009; Goulson et al., 2008; Potts et al., 2010).

Emerging disease can occur from cross-species transmission. Such movement of disease between species is known as pathogen spillover and occurs when pathogens are transmitted from infected reservoir species to sympatric species that are not the natural host (Daszak et al., 2000). Pathogen spillover can occur directly, from contact when foraging, or indirectly, via shared floral resources contaminated by oral regurgitant or faecal deposition (Adler et al., 2020; Burnham et al., 2021; Durrer & Schmid-Hempel, 1994). Pathogen spillover of RNA viruses has since been identified between managed honeybees and sympatric wild bees (Fürst et al 2014).

One virus which has frequently been found to spillover is deformed wing virus (DWV). DWV is a virus that principally infects honeybees, *Apis mellifera*. However, DWV has been shown to spillover into wild populations of bees. The impact of DWV infections in wild bees include the development of wing deformities (Genersch et al., 2006) and lower survival (Fürst et al. 2014).

Spillover of DWV-B is thought to be more likely in agricultural landscapes because of high pathogen pressure (which includes honeybee abundance, prevalence and viral load of DWV-B) and high contact with honeybees (Bartlett et al., 2021; Dalmon et al., 2021; Fürst et al., 2014). However, the use of managed colonies of honeybees and the presence of shared floral resources is not limited to agricultural ecosystems, and pathogen spillover could be present in other landscapes such as protected areas (Henry & Rodet, 2018, 2018), which play a key role in maintaining biodiversity (Hoffmann et al., 2018).

Here we ask whether pathogen spillover is occurring in protected grasslands by quantifying the prevalence of a key honeybee virus (DWV-B) in four wild bee species in protected grasslands across five European countries.

3.2 Methods

To determine whether pathogen spillover occurs in protected areas, samples of honeybees and abundant wild bee species were collected from protected grasslands across Europe. Sampling occurred in five European countries: UK, Germany, Hungary and Switzerland in 2022 and France in 2023.

All sites were grassland sites that represented important habitats for pollinators within each country, the majority of which were protected sites (Figure 3.1). Prior to collection of samples honeybee density, floral species richness, floral abundance, and floral diversity were assessed as potential drivers of spillover (see Task 2.3, Deliverable 2.2).

From each site, honeybee specimens and respective wild species were caught. The wild bee species caught were *Bombus lapidarius* in the UK and Germany, *Bombus terrestris* in Hungary, *Bombus pascuorum* in Switzerland, and *Halictus scabiosae* in France.

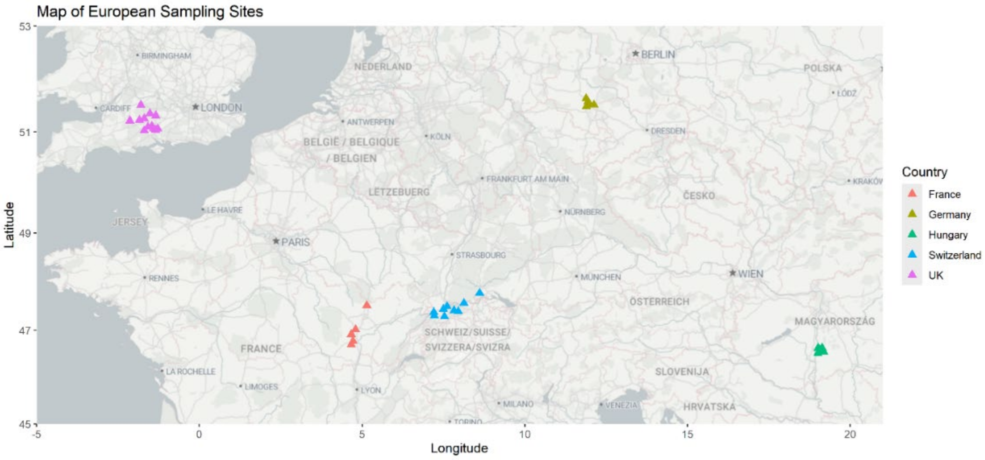


Figure 3.1 Map of grassland sites sampled, coloured by the country.

All samples were dissected and RNA was extracted. Extracted RNA was then used to synthesise cDNA and amplify regions of DWV viral RNA present. A housekeeping gene was also amplified to verify the effectiveness of RNA extraction. The number of DWV viral copies was quantified for honeybees and wild bees (*Bombus lapidarius*) from the UK using qPCR (this was done as additional work, not funded by Safeguard). The viral load Is presented as log10 Genome Equivalents (GE). Data were analysed using GLMMs to determine the significant predictors of viral prevalence and intensity.

3.3 Results

The prevalence of DWV-B at individual sites ranged from 20% to 100% in honeybees and 0% to 100% in wild bees (Figure 3.2). The prevalence of DWV-B was significantly higher in honeybees than all of the wild bee species.

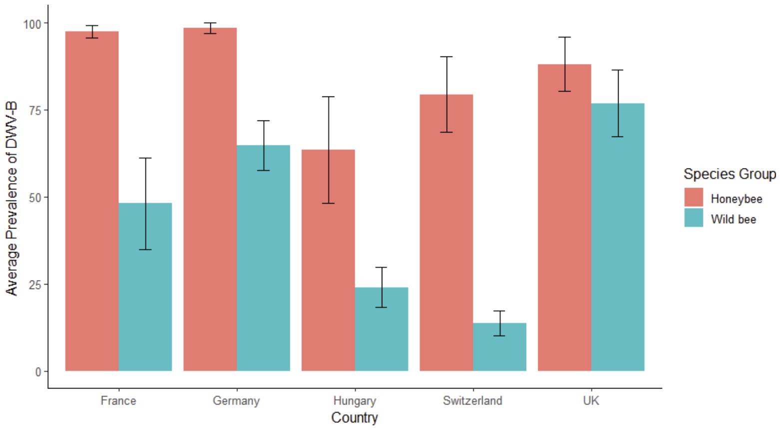


Figure 3.2 Prevalence of DWV-B in honeybee workers and wild bee foragers showing prevalence at sites and 95% CIs.

In the UK, the average viral load of DWV-B in honeybees was 5.38 (1.95-11.52) log10 genome equivalents and in *Bombus lapidarius* was 4.40 (2.14-7.14) log10 genome equivalents across 7 sites within the UK (Figure 3.3). The viral load of DWV-B was significantly higher in honeybees than Bombus lapidarius (beta = 0.85, 95% CI [0.53, 1.17]).

The likelihood of DWV-B infection in wild bees across Europe was higher in sites with higher prevalence of DWV-B in honeybees (beta = 0.029, 95% CI [0.008, 0.049]). Additionally, in the UK, the viral load of DWV-B was higher in sites with more honeybees (beta = 0.44, 95% CI [0.13, 0.75]). Further, in the UK, the viral load of DWV-B was higher in sites with a greater average honeybee DWV-B viral load (beta = 0.91, 95% CI [0.46, 1.13]).

The prevalence of DWV-B infections in wild bees across Europe increased with the number of bumblebees present at a site (beta = 0.038, 95% CI [0.015, 0.066]). However, the DWV-B viral load was lower in the UK in sites with higher bumblebee abundance (beta = -0.02, 95% CI [-0.04, -0.01]). Floral species richness did not drive DWV-B prevalence at the European level, or viral load in wild bees in the UK.

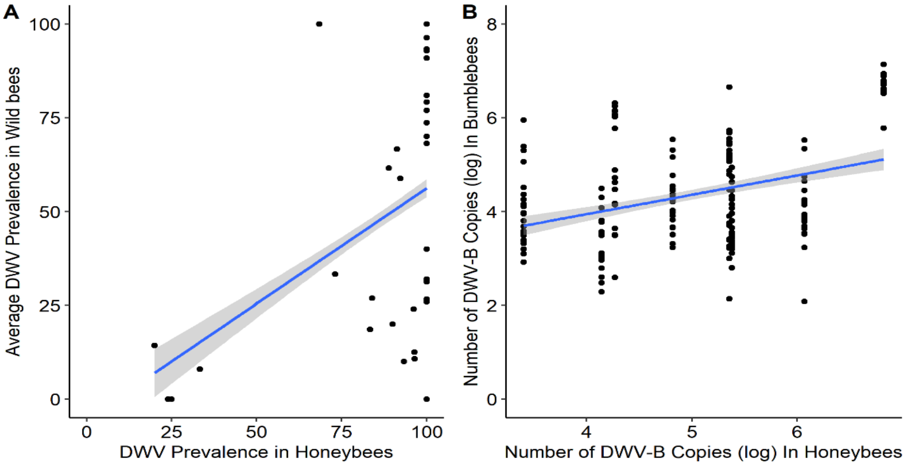


Figure 3.3 Plot (A) shows the effect of prevalence of DWV (%) in honeybees at a given site on the prevalence of DWV in wild bees (%). Plot (B) shows the effect of viral load in honeybees (as log10 Genome Equivalents) on viral load in wild bees.

3.4 Discussion

We have found evidence that pathogens are spilling over in 31 grassland sites in five European countries, the majority of which were protected areas. DWV-B was present in all four wild bee species with a prevalence of 46% across all wild bee species. Within the UK, 14% of wild bees had a viral load indicative of a symptomatic infection in honeybees (≥106 Genome Equivalent) (Chen et al., 2006; Cilia et al., 2023; Mazzei et al., 2014). Such infections could cause deformed wings (Genersch et al., 2006) or reduce lifespan in wild bumblebees, based on the results of laboratory experiments (Fürst et al., 2014).

There was evidence of spillover at most sites, with DWV-B prevalence and viral loads being significantly higher in honeybees than in wild bee species. However, there was significant variation in the prevalence of DWV-B seen across the four species of wild bee. Such differences could be attributed to differences in species' susceptibilities to DWV-B or the extent of niche overlap with honeybees.

Prevalence of DWV-B in wild bees was not higher in sites with higher abundance of honeybees, however, viral loads of DWV-B in wild bees were higher in sites with higher honeybee abundance. This goes against the general theory that the likelihood of spillover increases with host density (Daszak et al., 2000; Plowright et al., 2017). However, it is in line with previous studies where abundance of honeybees has also had no effect on spillover (Piot et al., 2019).

The prevalence of DWV-B in honeybees increased its prevalence in wild bees. This matches patterns from previous studies, where the prevalence of viruses in honeybees has been shown to drive the prevalence in wild bees of several honeybee associated viruses (DWV; (Fürst et al., 2014; Manley et al., 2019), BQCV; (Fleites-Ayil et al., 2023; McMahon et al., 2015) and ABPV; (McMahon et al., 2015)).

Additionally, the viral load in wild bees was higher in sites with a higher viral load of DWV-B in honeybees. Previous studies on the likelihood of spillover have shown similar effects of viral load in DWV (Manley et al., 2019) and BQCV (Maurer et al., 2024). However, this was not consistently seen for DWV (Maurer et al., 2024). One previous study has shown no effects of prevalence and viral loads of DWV on wild bee prevalences and viral load but, in contrast, presented effects of high floral resource overlap with honeybees (Maurer et al., 2024). Therefore, variable effects of honeybee prevalences and viral loads may be seen between species and predominantly affect species with high floral resource overlap (Proesmans et al., 2021).

Interestingly, the prevalence of DWV-B was higher in sites with high bumblebee abundance. These results are indicative of an amplification effect caused by bumblebee abundance (Mitchell et al., 2002; Schmidt & Ostfeld, 2001). Laboratory experiments suggest that bumblebee-bumblebee transmission of DWV-A is unlikely (Tehel et al., 2022). However, during their foraging activity, bumblebees could be moving honeybee-deposited viral particles between flowers (Graystock et al., 2015) and subsequently increasing the number of contaminated flowers or increasing the risk of transmission within nests. This mechanism is in line with our results, which showed that viral load of DWV-B in wild bees was lower in sites with high bumblebee abundance.

In this study, no effect of floral cover and floral species richness was found on DWV-B prevalence or viral load in wild bees. Pathogen spillover and transmission of virus between pollinator species commonly occurs on shared floral resources, and consequently a lack of effect of floral cover or species richness was unexpected. In contrast, previous studies on the effects of supplementing floral resources in the form of agri-environmental schemes have shown decreased virus prevalence with more floral resources, probably due to the dilution effect (Fearon et al., 2023; Manley et al., 2023; McNeil et al., 2020).

To conclude, this study shows that pathogen spillover from honeybees to wild bees in protected European grasslands is widespread, with a significant proportion of wild bees carrying symptomatic infections. Honeybee abundance, prevalence of infection and intensity of infection were key drivers of prevalence and intensity of infection in wild bees. As emerging diseases are linked to declines in wild bees, these drivers can be targeted and used to reduce the extent and subsequent impacts of pathogen spillover.

3.5 Summary and Recommendations

- DWV is spilling over into wild bee populations in protected grasslands

- This spillover is associated with viral prevalence and intensity in honey bees
- Honey bee keepers should be supported in effective disease management within their hives, to reduce the pressure of spillover on wild bees
- Managers of florally-rich protected areas should consider the potential impact from spillover before approving co-location of managed honey bees

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