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Risk of plant protection products to wild bees in vineyards with sown wildflower strips compared to vineyards with natural vegetation

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Abstract

The vineyard habitat has faced considerable challenges over the past century, primarily due to the intensification of viticulture. Fungal diseases and pests have necessitated an increased reliance on plant protection products (PPPs), particularly fungicides, posing a growing threat to the flora and fauna of the vineyards. The resource project *Promotion of endangered flora in vineyards* pursues the goal of restoring the value of vineyards as habitats. One measure within this project involves the sowing of native wildflowers in vineyard inter-rows. However, this raises questions regarding the impact of PPPs on the pollinator populations that get attracted to these enhanced floral resources. Especially the effect of the widely used fungicides on pollinators is still largely unknown and not well investigated. This study focused on assessing the potential risks of PPPs on wild bees - an invaluable group of pollinators. We examined the exposure of wild bees to PPPs in vineyards, considering whether the quantities applied had the potential to cause toxic effects. Additionally, we investigated whether the abundance and diversity of wild bees in vineyards with flower strips were more negatively affected by increasing toxic risks resulting from PPP applications compared to vineyards with spontaneous flora. A crucial aspect of this investigation is to determine if the sown inter-rows, while being beneficial in terms of food resources, could inadvertently serve as ecological traps for wild bees. Furthermore, we assessed the reliability of vane traps in accurately representing wild bee abundance in vineyards, as well as the transferability of the vane trap method used for wild bee surveys to other non-bee pollinator groups. Our findings suggest to rule out low toxic risk for many of the applied PPPs. Taking into account the toxic risk from oral exposure, the occurrence of bumblebees tends to be affected by an ecological trapping effect posed by the sown vineyard rows. We did not find clear evidence of an ecological trap in relation to solitary bee diversity, but we do not rule it out, as it seems reasonable that immigration may have had an impact. Our findings are specific to wild bees and should be transferred to non-bee pollinators only with caution, given the observed discrepancies in the abundance of non-bee pollinators between vane traps and transect walks. Or results highlight the complexity of the vineyard habitat, as many factors need to be considered in order to examine the risk of PPPs to pollinators in sown vineyard inter-rows. We propose that, in the context of investigating potential ecological traps, it is crucial to include flower supply and diversity. These elements, among others, may offer insights into how the adverse effects of PPPs can potentially be offset or mitigated.

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List of Abbreviations

Abbreviation	Definition
AR	Application rate
BBA	Label of statistical model with bumblebee abundance as dependent variable
BBS	Label of statistical model with bumblebee Shannon index as dependent variable
Ef	Exposure factor
EFSA	European Food Safety Authority
FOAG	Federal Office for Agriculture
FSVO	Federal Food Safety and Veterinary Office
HQ	Hazard quotient
LD_{50}	Lethal dose
PPP	Plant protection product
RI	Risk index
SBA	Label of statistical model with solitary bee abundance as dependent variable
SBS	Label of statistical model with solitary bee Shannon index as dependent variable
SV	Shortcut value

1. Introduction

Since the turn of the decade between 1940 and 1950, the use of pesticides, hereafter also referred to as plant protection products (PPP)[1], has increased worldwide (Pimentel, 1996). In viticulture, the wine-growing crisis at the end of the 19th century led to a breakdown and a subsequent increase in PPP use. The crisis was caused by diseases and pests that were introduced in Europe and thus also in Switzerland, which permanently changed the entire Swiss viticulture (Flutsch/MI & Lüdi; Viret et al., 2019). The area of Switzerland covered by vinyards declined to about half its size of the 1900's (Historische Statistik der Schweiz HSSO, 2012). Today it spans 14'606 hectares (Federal Office for Agriculture (FOAG), 2023a). The introduced plagues included powdery and downy mildew, which are two fungal diseases, as well as the grape berry moth and the grape phylloxera (Viret et al., 2019). The fungal diseases prompted wine farmers to use fungicides, the Bordeaux mixture (based on calcium hydroxide and copper sulfate (Federal Food Safety and Veterinary Office (FSVO), 2023)) being one of the first and still most intensively used. Today, 7 of the 10 most sold PPPs in Switzerland are fungicides (FOAG, 2023b). These are: sulphur (555.585 t), folpet (104.404 t), copper (total) (92.682 t), mancozeb (86.174 t), potassium bicarbonate (77.161 t) and copper as copper oxychloride (40.041 t). These statistics refer to total sales, not just those for viticulture (FOAG, 2023b). Nevertheless, fungicides are intensively used in grape vine production, representing up to 90% of the applied PPPs in vineyards (Rossberg, 2009). Besides fungicides, other PPPs are used in viticulture, such as insecticides against *Drosophila suzukii*, an introduced and invasive dipteran species in vineyards (Knapp et al., 2019). Unlike insecticides, fungicides are not designed to harm insects and are not considered harmful to bees (Kubik et al., 1999). However, there are studies demonstrating lethal and sublethal effects of fungicides on different non-target organisms such as bees (Heard et al., 2017; Iverson et al., 2019, Park et al., 2015). Moreover, there is evidence of synergistic effects in the toxicity of these PPPs to non-target organisms when insecticides and/or herbicides are applied together with fungicides (Almasri et al., 2021; Belsky & Joshi, 2020; Iverson et al. 2019). There is still considerable uncertainty regarding the harmful effects of fungicides on insects, but pollinating insects can come into direct contact with the sprayed PPP through various ways. Either they come into contact directly with spray drift, dust deposits or plant surfaces, or indirectly through ingestion of contaminated soil, water or plant matrices (European Food Safety Authority (EFSA), 2013; Kopit & Pitts-Singer, 2018). The EFSA (2013) states, that contact exposure can lead to lethal effects whereas dietary exposure can lead to lethal or sublethal effects and can deteriorate brood development. The effects through contact exposure are acute, the effects through dietary exposure can be either acute or chronic (EFSA, 2013).

Bees are the most important of all pollinating insects worldwide, as they do not only feed themselves but also their larvae with pollen and nectar (Pfiffner & Müller, 2016; Stanley et al., 2015; Westrich, 2013). Therefore, they forage more often on flowering plants than other pollinating insects and even their larvae are already exposed to the PPPs (Stanley et al., 2015). The managed honeybee *Apis mellifera* is the most investigated species in terms of effects of PPPs on insects (Cullen et al., 2019; EFSA, 2013). However, sensitivity to PPPs varies between different bee species (Arena & Sgolastra, 2014; Heard et al., 2017) and it is important to investigate the impact of PPPs on wild bees as well. After all, wild bees represent a group of at least 20'000 species that are very diverse in their foraging behavior (Michener, 2007). Thus,

[1] In this paper, the term *PPP* refers to the active substances (e.g., copper oxychloride). The term *PPP product* refers to the product marketed by a manufacturer (e.g., Airone). This distinction is important because PPP products do not always contain only one active substance, but are sometimes composed of several active substances, i.e., PPPs.

different species forage at different times during the day or visit different plants. Some species are highly specialized and feed on only one plant species (Michener, 2007; Pfiffner & Müller, 2016). In addition, wild bees play an indispensable role in crop pollination. This is shown by several studies, such as the one by Garantonakis et al. (2016), which demonstrated the importance and high pollination efficiency of wild bees on watermelons. According to Button & Elle (2014), the pollination potential of crops can only be fully achieved by wild bees.

To assess the acute risk to which bees are exposed by PPPs, the hazard quotient (HQ) approach was developed about 40 years ago (Felton et al., 1986). The HQ is normally calculated by dividing the application rate (AR) of a PPP spray application by the lethal dose (LD_{50}) (Felton et al., 1986). This value describes the contact or oral dose of a toxic compound at which 50% of the target organisms die (EFSA, 2013). The HQ approach has been applied in many studies – including on wild bee species – and showed negative effects of PPPs on pollinators with regard to their abundance and species richness (Bloom et al., 2021; Rundlöf et al., 2022 and Stoner & Eitzer, 2013). Based on their findings, Bloom et al. (2021) conclude that a change in agriculture is needed, from the use of PPPs towards more sustainable pest and disease control.

An alternative to the intensive use of PPPs to reduce pest and disease damage is the use of integrated pest management measures; for example, with the promotion of beneficial insects by maintaining vineyards rich in habitat structures like hedges, walls and flower strips (Ganser et al., 2019; Pfiffner et al., 2018). As grape vines are self-pollinators, they are not typically attended by insect pollinators (Rondeau & Raine, 2022). Therefore, a permanent food supply through flowering plants in the vineyards promotes the conservation of beneficial organisms depending on floral food resources. One such group of beneficial insects are the *Tachinidae spp.,* a family of the Diptera (Reineke & Thiéry, 2016). They contribute to the reduction of harmful butterfly species such as the grape berry moth in vineyards. Besides the promotion of beneficial organisms, the above-mentioned management measures also support the preservation of the vineyard as a habitat for many other organisms that have no direct beneficial impact to the vineyards (Agroscope Changins, n.d). The intensification of agriculture and thus also of viticulture led to a threat to the vineyards as habitats and hence to a reduction of many groups of organisms such as reptiles, birds, mammals, insects and spiders, due to the loss of habitat structures and food sources (Agroscope Changins, n.d; Kestenholz, n.d.; Linder & Kehrli, 2021; Mermod et al., 2009; Verein biodivers et al., 2022).

To promote a high diversity of floral resources, Steinemann et al. (2022) developed and recommended a native species mixture that attracts a wide variety of pollinators and has positive effects on soil properties. The species mixture "*Nützlingsstreifen Reben mehrjährig"* for Swiss viticulture is published by the Federal Office for Agriculture (FOAG) and is subject to the Direct Payment Ordinance of Switzerland (FOAG, 2023c). The resource project *Promotion of endangered flora in vineyards*, launched in 2020 by Agrofutura and Hintermann & Weber in cooperation with Agroscope (Moser et al. 2023), implements such measures of vineyard enhancement using an adapted version of the species mixture recommended by Steinemann et al. (2022). The project is jointly supported by the nature conservation agencies and the agricultural offices with the vineyard commissioners of the cantons Aargau, Basel District, Bern, Schaffhausen and Zurich (FOAG, 2022). The project includes the sowing of species-rich flower strips in the inter-rows of the vineyards and an adapted management of these strips, so that the valuable plants can become established. One learning objective of the resource project is to investigate the impact of flower strips on pollinators with regard to PPPs (FOAG, 2022). As sowing is done directly in the inter-rows, the flowers and thus the pollinators they attract, are directly exposed to management practices such as the spraying of PPPs. As mentioned above, the exposure to PPPs may affect the pollinators negatively (EFSA, 2013). If

the negative effects of the PPPs on pollinators predominate over the positive effects of the flower strips, the measure of promoting flowering plants in agricultural areas may rather be harmful than beneficial to species attracted by these strips (Botías et al., 2015; Mogren & Lundgren, 2016). This is called ecological trap and refers to species that prefer a habitat over another, even though the chosen habitat has characteristics that lean to reduced fitness and subsequently to declining numbers of individuals of these species or lower species diversity (Battin, 2004; Horstmann, 2021; Schmied et al., 2022). Wild bees as well as other insects may be harmed by this ecological trapping effect through sowings in vineyards: they are directly attracted by the additional food resources provided by the flower strips and can be negatively affected by the PPPs at the same time (Botías et al., 2015; Rundlöf et al., 2022).

1.1 Hypotheses and research questions

The aim of this study is to find out whether the application of plant protection products in vineyards has negative effects on pollinators foraging in the flower strips of the inter-rows of the vineyards. As studied pollinator group, the focus is set on wild bees. The study is structured in three parts. In the first part, we focus on individual vineyard PPPs and their potential toxic risk[2] to wild bees. The second part addresses the effects of an increasing risk index (RI), which is based on accumulated PPP applications. This part examines the impact of the RI on the abundance and diversity of wild bees in vineyards with and without flower strips. If the abundance and/or diversity in the sown inter-rows decrease more than in the control inter-rows as the RI increases, an ecological trapping effect of the sown vineyard inter-rows should be assumed. Since wild bees represent only one group of pollinating insects, it will further be investigated whether the bee surveys used in this study can also be used to study other pollinator groups than bees. The study poses three main questions:

Part 1: Risk analysis

- Which plant protection products are wild bees exposed to in vineyards, and do the applied doses of PPPs have the potential to cause acute toxic risk to wild bees?

Part 2: Ecological trap

- Does abundance and/or species diversity of wild bees decrease more in vineyards with inter-row flower strips than in vineyards without flower strips as the toxic risk through the accumulation of applied PPP increases?

Part 3: Pollinator community

- Do the wild bee surveys of the vane traps correspond to the situation of the wild bees in the field?
- Can the abundance of non-bee pollinators caught in vane traps be transferred to the abundance of non-bee pollinators in the field?

We do not expect fungicides to be applied at levels that pose a toxic risk to wild bees. For insecticides, we expect high toxic risks, since they are intended to harm insects. As fungicides are mainly used in vineyards, we assume that the abundance and diversity of wild bees is not negatively affected by PPPs, neither in vineyards with nor without flower strips. Therefore, no evidence for the presence of an ecological trap is expected. Lastly, it is assumed that wild bees are over-represented in the traps and that the results obtained by the vane trap surveys are not transferable to pollinators other than bees.

[2]"Toxicity is the inherent property of a chemical to cause adverse biological effects at adequate dosages. The toxicity of pesticides to honeybees can be defined by the laboratory tests […]. Hazard is the possibility of producing an adverse effect in specific circumstances. The hazard of pesticides usage for […]bees can be assessed by the field and cage tests […]" (Felton et al., 1986, page 118). The term toxic risk is used in this study synonymously with the term hazard as defined by Felton et al. (1986).

2. Methods

2.1 Study sites

The study sites consisted of 10 vineyards with flower strips in their inter-rows, which were sown in spring 2020 and 2021. The vineyards with flower strips sown in 2020 were part of the project *Flowering habitats* of Agroscope and the Research Institute of Organic Agriculture (FiBL), whereas those sown in 2021 were part of the resource project *Promotion of endangered flora in vineyards* of Agrofutura and Hintermann & Weber. Both flowering strip mixtures are based on the perennial *beneficial strip for vines* with minor modifications (FOAG, 2023c). At least four inter-rows were sown at each treatment site, usually leaving an inter-row with spontaneous vegetation in between. Vineyards were only qualified as sown treatment sites for the study if their flower strips had a medium to good quality, if the vineyards were without terracing and if there was an available control site nearby (Moser et al., 2023). The sites were located from the lake of Geneva up to Schaffhausen in northern Switzerland, see Figure 1. The smallest distance between two sown sites was 400 meters to minimize bee flight, as the normal flight distance of wild bees is thought to be 350 meters (Pfiffner & Müller, 2016). Each sown treatment site was paired up with a nearby, unique control site, 10 in total. The control sites were also not allowed to have terracing. In addition, there couldn't be seedlings on the plot, the vegetation had to be characterized by spontaneous vine flora. The control sites should be cultivated by the same wine farmer or be subject to comparable cultivation and be located in the same region as their corresponding vineyard with flower strip. A distance to minimize bee flights between control and sown vineyards, could not be met as many wine farmers only cultivate a few plots that are close to each other. All sites were alternately mown or mulched by the wine farmers. Depending on the weather and the wine farmer, an individual inter-row was mulched zero to three times on average per growing season. Some inter-rows without sown wildflowers were partially opened annually.

Figure 1: Locations of the study sites. Blue labels indicate locations of vineyards with flower strips sown in 2020, red the ones with flower strips sown in 2021. 1: Echandens/Denges, 2: Spiez, 3: Auvernier, 4: Twann, 5: Aesch BL, 6: Bözen/Hornussen, 7: Schinznach, 8 and 9: Volken (two locations of two different vineyards), 10: Dörflingen (Moser et al., 2023).

2.2 Risk analysis

Initially, the risk assessment procedure was designed to collect wild bees from the study sites and subsequently analyse them for PPP residues in an analytical laboratory. However, many laboratories were already operating at full capacity during the period relevant to our study. In addition, due to the limit of quantification for PPPs, it would have been a challenge for some laboratories to detect the small amounts of residues in the bees. As the search for a suitable laboratory was not successful, our assessment was based on original spray application data rather than on detected PPP residues in wild bees.

The data on the AR of the PPP products of the years 2022 and 2023 was collected from the spraying schedules of the wine farmers for each study site. To calculate the AR of the active PPP substances, the index of phytosanitary products was used (FSVO, 2023).

The risk estimations in this study were based on the acute contact and acute dietary exposure scenarios from spray applications and were evaluated using the HQ approach. Risk estimations using the HQ approach were carried out in several other studies, such as Böhme et al. (2018), Drummond et al. (2018), Stoner & Eitzer (2013) or Tong et al. (2018). To determine the acute contact risk of each PPP per application, we calculated HQ values for each substance according to the European Food Safety Authority (EFSA) (EFSA, 2013) using equation 1:

$$HQcontact = \frac{AR\ \left(\frac{g}{ha}\right) * \%\ active\ ingredient}{LD50\ contact\ acute\ (\frac{\mu g}{bee})} \quad (1)$$

To calculate the acute dietary risk, the guidance proposes a slightly different approach (EFSA, 2013) than the classic HQ approach, shown in equation 2:

$$HQoral = \frac{AR\ \left(\frac{kg}{ha}\right) * \%\ active\ ingredient * Ef * SV}{LD50\ oral\ acute\ (\frac{\mu g}{bee})} \quad (2)$$

Standard LD_{50} values for both equations derived from the Pesticide Properties Data Base (PPDB) of the University of Hertfordshire (Lewis et al., 2016). The LD_{50} values for honeybees were used for the calculations of all substances, since the values for wild bees were only available for a small number of PPPs. PPPs without LD_{50} data for honeybees were excluded from the calculations. This was the case for 6.25% of contact LD_{50} values and 3.13% of oral LD_{50} values. The available LD_{50} values were then extrapolated by dividing them by a factor of 10, according to Arena & Sgolastra (2014) and the EFSA (2013), which suggest this procedure when using honeybee LD_{50} values for bumble- and solitary bees. In the second equation, Ef is an exposure factor[3] and SV are shortcut[4] values (EFSA, 2013). The exposure of the flower strips in the vineyards corresponded to the *weeds in the field* scenario (EFSA, 2013), which proposes an Ef of 0.3 if all vines are at a plant development stage (BBCH) above 50 at the time of spraying. This was the case for the vines of this study. For the herbicides, on the other hand, the EFSA (2013) recommends an Ef of 1, as herbicides are not sprayed on the vines, but directly on the plants below vines. The SVs used in the calculation were 6.6 for bumblebees and 2.3 for solitary bees. SVs for those two groups differ due to their distinct feeding strategy. The detailed derivations of the Ef and the SV are described in Appendix X and Appendix J of the Guidance Document on the risk assessment of plant protection products on bees (EFSA, 2013).

The calculated HQ values were then compared to the trigger values[5] proposed by the EFSA (2013) for up- and sideward spray applications because spray applications in viticulture are done sidewards (Syngenta Global, 2023). For acute contact exposure, these values were 14 for

[3] Exposure factors are values estimating the exposure for different scenarios, e.g., weeds in the field, treated crop, etc. (EFSA, 2013)

[4] Shortcut values are factors considering sugar content, consumption behaviour and pesticide residues for the worst-case scenario (EFSA, 2013).

[5] Trigger values are protection values that define if the level of harm is still acceptable and therefore the protection goals defined by the EFSA are met (the HQ does not exceed the trigger value) or if the harm is unacceptable (the HQ exceeds the trigger value) (EFSA, 2013).

bumblebees and 16 for solitary bees. For acute oral exposure, they were 0.036 for bumblebees and 0.04 for solitary bees (EFSA, 2013). A HQ value exceeding the trigger value, signals the exclusion of a low-risk scenario and highlights the need for further investigation of that PPP; However, this scenario does not necessarily indicate a high risk (EFSA, 2013; Thompson, 2021). For details on the trigger values, we refer to the Chapter 7 and Appendix M of the Guidance Document on the risk assessment of plant protection products on bees (EFSA, 2013).

2.2 Ecological trap

2.2.1 Wild bee abundance and diversity

The data on wild bee abundance and diversity were collected as part of the resource project *Promotion of endangered flora in vineyards* (Moser et al., 2023). To gain data on wild bee abundance and diversity, blue and yellow vane traps were used as in Hall & Reboud (2019) and Prendergast et al. (2020), which are designed to catch bees. The vane traps used were replicated by Agroscope and adapted so they can be set up and taken down quickly. At each of the 20 sites, two blue and two yellow traps were placed in two inter-rows and exposed for 72 hours. The trapping was conducted twice (2023: three surveys at Echandens/Denges, location number 1 in Figure 1) during the growing season of the flower strips. In 2022, the surveys began on the 6th of May and ended on the 18th of July. In 2023, because the weather in April and May was exceptionally cold, cloudy and too wet, the surveys were delayed until 19th of May and lasted until the 17th of August. The captured wild bees were then stored in ethanol until they were counted and determined to species level by the entomologist Andreas Müller. The location Hornussen (control site, location number 6 in Figure 1) had to be excluded because a few years ago this site received a special seed mixture and could therefore no longer be considered as control. Furthermore, there was no second bee survey in 2023 for the location Twann (sown and control site, location number 4 in Figure 1), because the flowering plants in the inter-rows did not grow back after mowing due to the extreme drought in June and July. We resulted in 10 and 9 different study sites in 2022 for sown and control vineyards respectively, and in 9 and 8 different study sites in 2023 for sown and control vineyards respectively. Mean daily temperature values of the 72 hours during survey were collected from Agrometeo stations located near the study sites.

2.2.2 Calculation of the risk index

For each vineyard site, a RI for acute contact and acute dietary exposure was calculated according to equation 3. The RIs were based on the sum of the $HQ_{contact}$ and HQ_{oral} values respectively for a specific time period. This time period lasted from the beginning of the first spraying to the last spraying (n) until the last day of the second round of bee survey with the vane traps. The RIs were only calculated for the second round of vane trapping for the years 2022 and 2023 since there were too few PPP applications before the first round. As the bee catches did not take place at all locations at the same time, the sum of the HQs was averaged over the number of days (d) from the start of spraying until the trapping of the bees.

$$RI\ (per\ study\ site\ and\ year) = \frac{(\sum_{n=1}^{n} HQ)}{d} \quad (3)$$

2.2.3 Statistical analysis

To test the impact of the toxic risk in every vineyard on wild bee abundance and diversity as response variables, six linear mixed effect models were built as listed in Table 1. Besides risk values, four other explanatory variables were included in the models: treatment (sown vs. spontaneous vegetation), the interaction between risk and treatment, year (2022 and 2023) and the mean temperature. Precipitation as climatic factor was excluded, because the sampling

mainly took place on days without rainfall. The location and the day of the year at which the bee survey occurred were included as random variables. Before running the statistical models, the variables were checked for multicollinearity. Data on wild bee diversity were calculated as Shannon index, using the vegan package in R 4.3.1 (R Core Team, 2023) and log1p transformed to ensure normal distribution of residuals. Data collection on abundance of wild bees was summed per study site (four traps) and log1p transformed to ensure normal distribution of residuals. Significance of the interaction variable treatment:risk would demonstrate a treatment dependent response of wild bee (or bumble- and solitary bee) abundance and diversity to the toxic risk caused by PPPs. If the treatment:risk interaction showed a significant effect it was further checked whether the change in the dependent variable was influenced by the toxic risk, independent of the treatment. To do this, the dataset was split into data of sown inter-rows and data of the control inter-rows. A linear mixed model with the same fixed and random effects as mentioned above, except for the interaction and treatment, was applied. All statistical analyses were performed in R 4.3.1 with the lmer function of the lm4 package (R Core Team, 2023).

Table 1: Overview of statistical linear mixed effect models used to analyse the effect of toxic risk to wild bees. Wild bees were expressed in six different response variables (middle). RI as one of the explanatory variables is listed (right), further fixed and random effects are not listed. For simplicity, the models were labelled (left).

Model label	Response variable	Explanatory variable
WBA	Wild bee abundance	Contact RI
WBS	Wild bee Shannon index	Contact RI
BBA	Bumblebee abundance	Oral RI for bumblebees
BBS	Bumblebee Shannon index	Oral RI for bumblebees
SBA	Solitary bee abundance	Oral RI for solitary bees
SBS	Solitary bee Shannon index	Oral RI for solitary bees

2.3 Pollinator community

2.3.1 Transect walks

In 2023, pollinator observations were conducted twice at every treatment and control site at the same time as the vane traps were set, between 9am and 6pm. This allowed to observe at different conditions regarding the weather and insect activity which changes over time (Pfiffner & Müller, 2016). Observations were made by four-minute transect walks on days with low wind (< 6m/s), no precipitation and a minimum temperature of 13°C according to the criteria of Griffiths-Lee et al. (2023). Each transect was 40 meters long and the whole width (1.5 meter) of the flower strip was included. Observed pollinators were identified according to the following groups: Hymenoptera (*Apis mellifera*, wild bees, Formicidae, Vespidae), Diptera (Syrphidae, other Diptera), Lepidoptera, Coleoptera (Coccinellidae, other Coleoptera), Hemiptera (Prosorryncha, Cicadas), Orthoptera, Ephemeroptera, Trichoptera, Plecoptera, Neuroptera, Dermaptera and Mecoptera.

2.3.2 Statistical analysis

We assessed the correlation between wild bees captured using vane traps and those observed during transects. Similarly, we examined the correlation between all non-bee pollinators captured with vane traps and those observed during transects, using Pearson's correlation coefficient. Further, we used a linear mixed effects model to analyse if the abundance of the observed wild bees can explain the abundance of the wild bees caught with vane traps. In a second linear mixed effects model we analysed for the abundance of the non-bee pollinators.

In both models, the vane trap abundances were dependent variables, the transect abundances were independent variables and the treatment was incorporated as a fixed effect. Additionally, site number, which signifies an individual study site, and the day of the year on which the survey took place were included as random factors. In our initial analysis, we included an interaction term between treatment and transect wild bee abundance. However, given its lack of significance and considering that this interaction was not the primary focus of our research question, we proceeded with the analysis without this interaction term, thereby simplifying the model and reducing potential overfitting. While correlation measures the strength and direction of the relationship between the vane trap and transect walk methods, it doesn't necessarily imply agreement (Giavarina, 2015). To further evaluate the level of agreement between the two methods, especially for non-bee pollinators, we used the Bland-Altman analysis. This analysis assesses whether the vane trap method produces similar quantitative results to the transects. Data from the first and second round of bee captures in 2023 were used, but not data of 2022, since no transect walks were conducted then. Statistical analyses were performed in R 4.3.1 with the lmer function of the lm4 package for the linear mixed effects models (R Core Team, 2023).

3. Results

3.1 Risk analysis

The examination of the spraying schedules revealed that a total of 68 different PPP products (59 fungicides, 2 insecticides, 3 herbicides and 4 stimulators of natural defense against fungal diseases) were used over the 2 years, which contained 49 PPPs. Table AT1 in the Appendix shows all used PPP products and their active substances. The fertilizers as well as the adhesive and wetting agents were not listed since they were not of interest in this study. At 7 of 20 study sites (Echandens (sown), Denges (control), Auvernier (sown and control), Twann (sown and control) and Aesch (control)), only PPP products with approval for organic agriculture were applied. All study sites were sprayed with fungicides between May and the beginning of August. On average, the spraying took place on 9 days in 2022 and on 11 days in 2023. The applied herbicides were sprayed in April and the insecticide kaolin in September. Between May and the end of June, the spraying interval was at roughly one week, after which it got increased to roughly two weeks. On average, 3 to 4 PPP products were applied at the same time. The herbicides and the insecticide kaolin were each applied alone. Only the insecticide calcium hydroxide was applied simultaneously with fungicides.

The most frequently applied substances in both years in terms of vineyards and individual PPP applications were sulphur and folpet. Sulphur was applied in all 20 study sites (100%) and was present in 19.97% (2022) and 23.02% (2023) of total PPP applications. The majority of sulphur applications were made in the organic vineyards: 31.17% (2022) and 31.02% (2023) of all spray applications in the organic vineyards contained sulphur, compared to 16.48% (2022) and 20.24% (2023) in the conventional vineyards. Folpet, a synthetic fungicide, was applied in 92.31% of all conventionally managed vineyards, which corresponds to 60.00% of all vineyards. Folpet was sprayed in 14.31% (2022) and 10.54% (2023) of total PPP applications. Figure 2 shows the application frequencies summed over all vineyards for each PPP in 2022 and 2023 as well as the proportion of vineyards that used a particular PPP at least once. There was a tendency for fungicides of natural origin, such as sulphur, copper compounds and potassium compounds, to be used more frequently than synthetic fungicides, with folpet being an exception. No synthetic insecticides were used; the two insecticides used were also of natural origin.

3.1.1 Contact risk

Acute LD_{50} data for contact exposure was not available for the substance disodium phosphonate, potassium phosphonate, oleum foeniculi, calcium hydroxide and all stimulators of natural defense against fungal diseases; therefore, they were not considered in the HQ calculations for acute risk. Furthermore, 64.10 % of the PPPs had at least one application where the corresponding $HQ_{contact}$ value exceeded the trigger value for bumblebees, and about three quarters of them exceeded the trigger value in 100% of their applications. The trigger value for solitary bees was exceeded at least once by 61.54 % of the PPPs, of which three quarters exceeded the value for all of their applications. Mean $HQ_{contact}$ values ranged from 2.095 for cyflufenamid to 2117.143 for kaolin. Table 2 lists the five highest PPPs in terms of their mean $HQ_{contact}$ value. The mean $HQ_{contact}$ values of all applied PPPs are listed in Table AT2 in the Appendix.

Table 2: PPPs with the five highest mean $HQ_{contact}$ values (± se) across all study sites and both study years. The columns on the right show the mean AR (g/ha) (± se) across all study sites and both study years, and the LD_{50} value (µg/bee) for honeybees (Lewis et al., 2016), which was extrapolated for wild bees by dividing the value by a factor of 10 according to the EFSA (2013).

Substance	Group	Mean $HQ_{contact}$	Mean AR (g/ha)	extrapolated LD_{50} (µg/bee) contact
Kaolin	Insecticide	2117.143±398.917	21'171±1507.765	10
Spiroxamine	Fungicide	795.455±190.396	334±24.111	0.42
Sulphur	Fungicide	226.792±78.319	2267±46.312	10
Fosetly-Al	Fungicide	111.103±36.156	1111±82.948	10
Potassium bicarbonate	Fungicide	102.719±31.058	3780±145.153	36.8

3.1.2 Oral risk

Acute oral toxicity data was not available for calcium hydroxide, oleum foeniculi and all stimulators of natural defense against fungal diseases, so they were not included in the calculations. 65.85% of the PPPs had at least one application where the corresponding HQ_{oral} value exceeded the trigger value for bumblebees, and 81.48% of these PPPs exceeded the trigger value in all of their applications. Less than half of the PPPs exceeded the value for solitary bees (41.46%), of which 41.18% had a HQ_{oral} value above the trigger value for each application. Mean HQ_{oral} values were lowest for cyflufenamid (0.004 for bumblebees and 0.001 for solitary bees) and highest for kaolin (4.128 and 1.461 respectively). The PPPs with the five highest mean HQ_{oral} values are listed in Table 3. All calculated mean HQ_{oral} values are listed in Table AT2 in the Appendix.

Table 3: PPPs with the five highest mean HQ_{oral} values (± se) for bumblebees and solitary bees across all study sites and both study years. The columns on the right show the mean AR (kg/ha) (± se) across all study sites and both study years, and the LD_{50} value (µg/bee) for honeybees (Lewis et al., 2016), which was extrapolated for wild bees by dividing the value by a factor of 10 according to the EFSA (2013).

Substance	Group	Mean HQ_{oral} bumblebees	Mean HQ_{oral} solitary bees	Mean AR (kg/ha)	extrapolated LD_{50} (µg/bee) oral
Kaolin	Insecticide	4.128±0.778	1.461±0.275	21.171±1.508	10
Potassium bicarbonate	Fungicide	3.071±0.929	1.087±0.329	3.780±0.145	2.4
Fenpropidin	Fungicide	0.638±0.000	0.226±0.000	0.327±0.000	1
Glyphosate	Herbicide	0.531±0.000	0.188±0.000	0.849±0.000	10.4
Sulphur	Fungicide	0.414±0.143	0.150±0.116	2.267±0.046	10.68

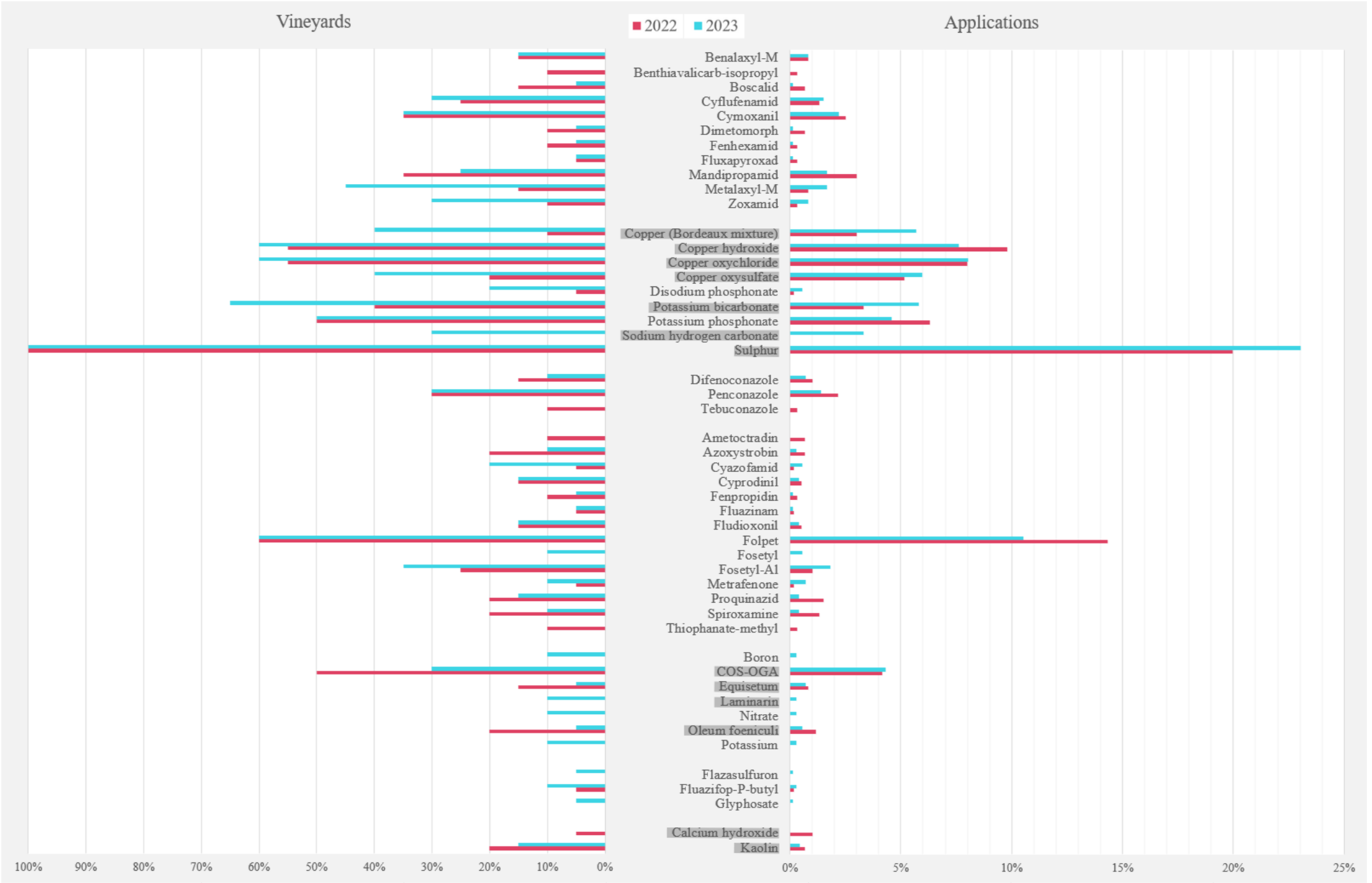

Figure 2: The used PPPs are listed in the middle according to chemical groups (top down): Amide fungicides / inorganic fungicides / Azole fungicides / fungicides belonging to various other chemical groups / stimulators of natural defense against fungal diseases / herbicides / insecticides. PPPs in grey are permitted in organic agriculture according to the list of inputs for organic farming in Switzerland (Speiser et al., 2022). Left: proportion of vineyards (%) that used a particular PPP at least once in 2022 and 2023. Right: application frequencies (%) summed over all vineyards for each PPP in 2022 and 2023.

3.2 Ecological trap

Over the two-year period, an average of 38±6.93 wild bees were caught in the sown inter-rows, and 32±5.29 in the control inter-rows. Of these, about one fifth (sown) and one eight (control) were bumblebees, the rest were solitary bees. Mean Shannon index was highest for solitary bees in the sown inter-rows (1.73±0.01), followed by the mean Shannon index of solitary bees in control inter-rows (1.43±0.08) and the mean Shannon index for bumblebees (0.90±0.15 in sown and 0.71±0.14 in control inter-rows).

In 7 out of 20 study sites the PPP applications considered for the RI calculation included only organically approved PPP products (Echandens (sown), Denges (control), Auvernier (sown and control), Twann (sown and control) and Aesch (control)). The PPP applications considered for the calculation of the RI in the conventional managed study sites contained on average 40.73±4.87 % (2022) and 54.49±3.65 % (2023) organically approved PPP products. The mean RIs for organically managed study sites and for the conventional study sites are listed in Table AT3 in the Appendix.

3.2.1 Contact Risk

Wild bee abundance and diversity were not significantly affected by treatment (sown/control), toxic risk or the treatment:risk interaction. Temperature had no effect at all, but year significantly affected total wild bee diversity ($p = 0.045$), with lower values in 2023, which is shown in Figure 3. Estimates, standard errors, significance levels and R^2 of both models are presented in Table AT4 in the Appendix.

3.2.2 Oral Risk

The BBA and BBS models for bumblebees showed a significant effect of treatment ($p = 0.004$ and 0.050) with on average 3±0.98 more bumblebees and a higher Shannon index of 0.19±0.13 on average, in the sown inter-rows compared to the control inter-rows. The treatment:risk interaction was significant in the BBA model ($p = 0.015$, Figure 4) and marginally significant in the BBS model ($p = 0.072$), but the 95% confidence interval of BBS rejected significance ([-4.36:0.11]). The SBS model showed significance of risk ($p = 0.036$) and of treatment:risk interaction ($p = 0.044$, Figure 5). The significance of the interaction means that toxic risk has different effects on solitary bee diversity and on bumblebee abundance, depending on the treatment. Further treatment-separated analysis of the BBA and SBS models, which were significant in the interaction, examined that risk was not significant for either the sown ($p = 0.280$) nor the control ($p = 0.410$) treatments in BBA, and the SBS showed no effect of risk in the sown treatment ($p = 0.369$), but an effect of risk in the control ($p = 0.023$) with decreasing solitary bee diversity as risk increased. Solitary bee abundance was not significantly affected by treatment, toxic risk or interaction between the two on the corresponding dependent variable.

While temperature had no effect at all, the years in which we collected data significantly affected bumblebee diversity ($p = 0.002$) and abundance of bumblebees ($p = 0.006$), with lower values in 2023, which is shown in Figure 3. Estimates, standard errors, significance levels and R^2 of all models are presented in Table AT4 in the Appendix.

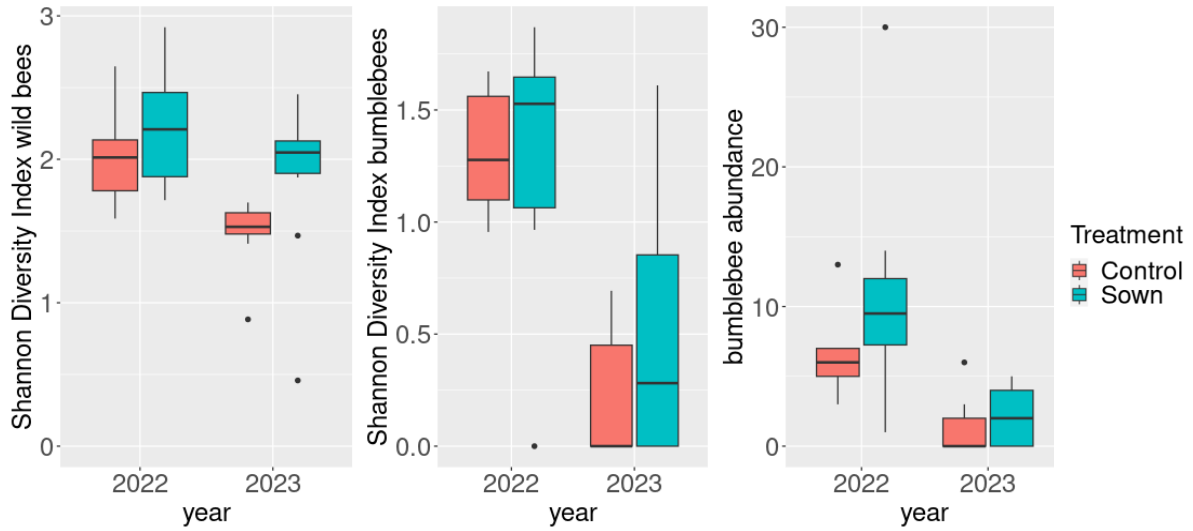

Figure 3: Total wild bee diversity shown as Shannon index (left), bumblebee diversity as Shannon index (middle) and bumblebee abundance (right) were significantly lower in 2023 (sown: n=11, control: n=10) than in 2022 (sown: n=10, control: n=9) (p = 0.045, 0.002 and 0.006 respectively).

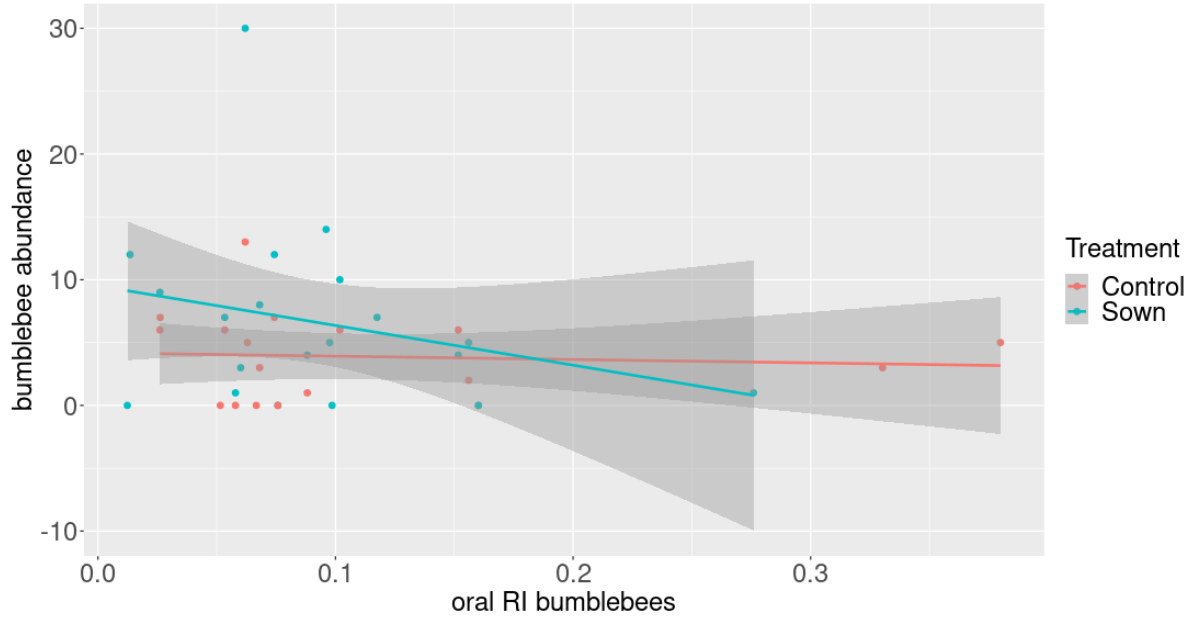

Figure 4: Change in bumblebee abundance as a function of the oral RI (sown: n=21, control: n=19) during 2022 and 2023. Depending on the treatment, the RI influences bumblebee abundance significantly differently (p = 0.015). Subsequent treatment-separated analysis showed no significant effect of the toxic risk on bumblebee abundance neither in the sown inter-rows (p = 0.280) nor in the control inter-rows (p = 0.410).

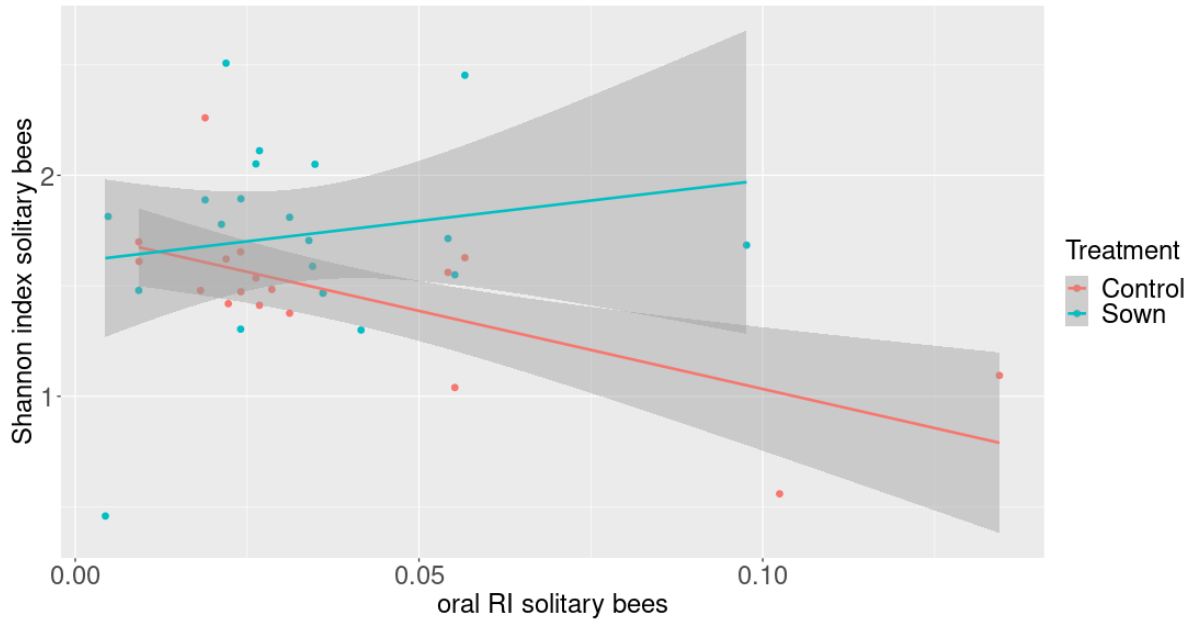

Figure 5: Change in Shannon index for solitary bees as a function of the oral RI (sown: n=21, control: n=19) during 2022 and 2023. Depending on the treatment, the RI influences solitary bee diversity significantly differently (p = 0.044). Subsequent treatment-separated analysis showed no significant effect of the toxic risk on solitary bee diversity (p = 0.369) in the sown inter-rows, but had a significant effect in the control inter-rows (p = 0.023).

3.3 Pollinator community

During the transect walks, across all locations and survey rounds, the highest number of observations were made in the Hymenoptera order with a total of 537 wild bees, 154 honeybees (*Apis mellifera*), 212 *Formidae* and 57 *Vaspoidae*. The order of Coleoptera counted 694 individuals, of which 34 belonged to the *Coccinellidae*. Among the Diptera, 588 observations were made, including 315 hoverflies. There was at least one observation in all defined groups except Plecoptera, Dermaptera and Mecoptera. Counts of all pollinator groups are shown in Figure AF1 in the Appendix.

Wild bees of vane traps and transects showed a positive correlation ($r = 0.50$, $p = 0.002$). Non-bee pollinators showed a weaker positive correlation of vane traps and transects than the wild bees ($r = 0.37$, $p = 0.020$). Both linear mixed effect models showed no significance for either transect abundance nor treatment on vane trap abundance. R^2 for the wild bee model was 0.66 and for the non-bee pollinator model 0.27. Figures 6 and 7 show that there were more sites where low numbers of wild bees or non-bee pollinators were trapped and observed, and that fewer data points were seen at higher abundances (transect abundance > 15 for wild bees and >75 for non-bee pollinators).

The mean difference between the values of the transect walk and the vane trap method was -4.13±1.83 for wild bees and -37.29±7.22 for non-bee pollinators, visualized by the blue dashed line in Figures AF2 and AF3 in the Appendix. The limits of agreement (red dashed lines Figures AF2 and AF3 in the Appendix) were 18.90 and -26.15 for wild bees and 48.38 and -126.53 for non-bee pollinators. These limits indicate whether the values that deviate from the mean difference are within an acceptable range. All data points, for wild bees as well as for non-bee pollinators, lied between the limits of agreement or in their 95% confidence interval (Figures AF2 and AF3 in the Appendix).

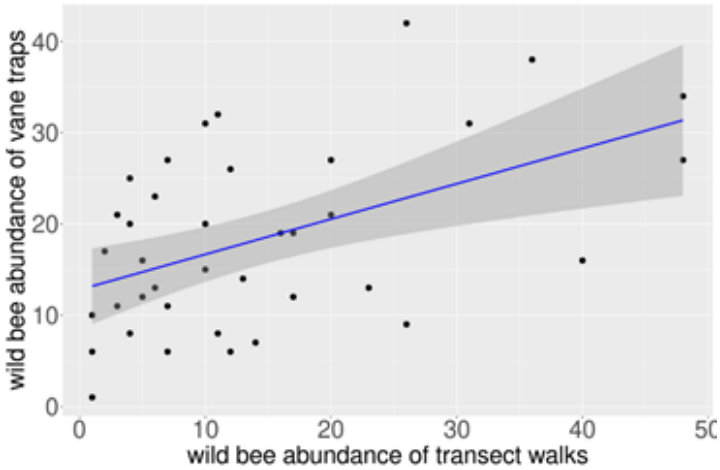

Figure 7: Positive correlation between wild bee abundance of transect walks and vane traps. Less wild bees were trapped and observed at higher transect abundances (> 15).

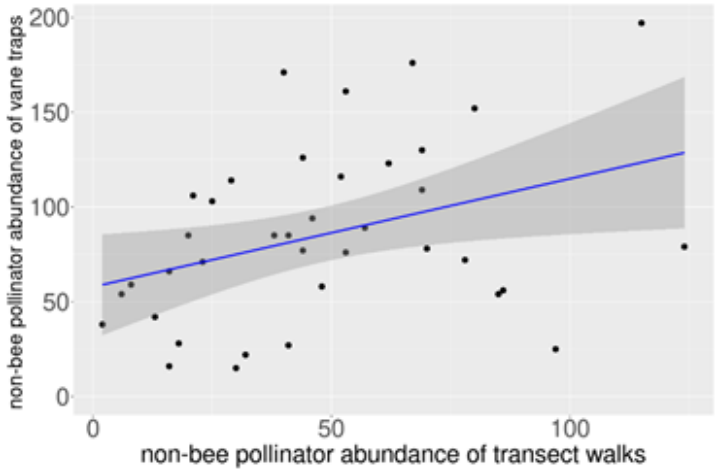

Figure 8: Weak positive correlation between non-bee pollinator abundance of transect walks and vane traps. Less non-bee pollinators were trapped and observed at higher transect abundances (> 75).

4. Discussion

4.1 Risk analysis

Wild bees in vineyards were exposed to a variety of PPPs over a short period of a few months, of which sulphur was the most regularly and widely applied. About half of the PPPs used may have the potential to pose an acute toxic risk to wild bees, as a small toxic risk cannot be excluded for these PPPs, due to the level of the calculated HQ. In the subsequent discussion, the PPPs with the five highest HQ values (Tables 2 and 3) will be examined in detail.

A comparison of the ARs can explain the high mean $HQ_{contact}$ values observed for kaolin, sulphur, fosetyl-Al and potassium bicarbonate. Besides sodium hydrogen carbonate, these four PPPs had the highest mean AR across both years (see Table AT2). Sodium hydrogen carbonate, despite its high AR, had a low HQ value. This is due to its exceptionally low oral and contact toxicity which is reflected in its high LD_{50} values (see Table AT2). In terms of toxic contact risk, spiroxamine was among the PPPs with the highest mean HQ value, as it has an exceptionally low LD_{50} contact value compared to all substances studied (see Table AT2) and at the same time has a moderate AR. The high mean HQ_{oral} values observed for kaolin, potassium bicarbonate, glyphosate and sulphur can also be associated with their substantial mean AR over the two-year period (see Table AT2). Although other substances such as folpet and sodium hydrogen carbonate were applied at similar levels, these two PPPs have much higher LD_{50oral} values (see Table AT2). In the case of glyphosate, in addition to its high AR, the increased HQ_{oral} could also be partly due to a higher Ef attributed to herbicides. These PPPs are applied more directly to the weeds, increasing the exposure of the sowings in the inter-rows. The high HQ_{oral} for fenpropidin can clearly be explained by its low LD_{50oral} value (see Table AT2), making it one of the most toxic PPP examined in this study.

Kaolin, a clay-based, non-degradable substance classified as an insecticide has no pesticidal properties (Karise et al., 2016; Lewis et al., 2016). The effect of kaolin is purely physical and prevents the oviposition of pests such as the cherry vinegar fly *Drosophila suzukkii*. Despite the permission of kaolin (as an ingredient of the product Surround) in organic viticulture (Speiser et al., 2022), the mean HQs exceeded the trigger values by more than a thousand-fold and thus, low acute contact and oral risk should be excluded. This conclusion is partially supported by the EFSA (2022), which ruled out a low acute contact risk of kaolin to honeybees. However, they gave no statement about wild bees. Furthermore, they argued that there is no need to examine the acute oral risk of kaolin, since it acts as a physical barrier and does not penetrate into plants and therefore pollen or nectar. However, a study conducted by Karise et al. (2016) provides further evidence of potential risks associated with kaolin. They demonstrated decreased lifetimes and sublethal effects on bumblebees after kaolin treatment, characterized by increased cuticular water loss of the bumblebees due to the abrasive and absorbent properties of kaolin. We assume that wild bees in the vineyards were exposed to kaolin even if its application did not occur until September. This is based on the observations that all subfamilies of wild bees found in Switzerland include species that are remain active until September or October (beeworld.ch, n.d.). Besides kaolin, the herbicide glyphosate showed concerning HQ_{oral} values. Herbicides are PPPs designed to control unwanted weeds, however, negative effects on wild bees are to be expected, as various studies confirm: A meta-analysis of Battisti et al. (2021) summarized that in several studies, glyphosate led to lethal and sublethal effects on different bee species even when glyphosate was applied at recommended ARs. Moreover, mainly the toxic effects of glyphosate through oral exposure on bees had been investigated (Cullen et al., 2019), and was shown to lead to a weakened immune system due to disturbed intestinal flora (Ledoux et al., 2020; Motta & Moran, 2023), as well as cognitive disorders (Balbuena et al., 2015) and an impaired olfactory sense (Mengoni Goñalons & Farina,

2018). It is likely that wild bees foraging in the vineyards of our study have been exposed to glyphosate, even if glyphosate was sprayed in early spring, as Duchenne et al. (2020) were able to show that warm years, such as 2022 (MeteoSchweiz, 2023), cause wild bees to become active earlier in the year (Duchenne et al., 2020).

There is a general lack of data concerning the risks posed by PPPs to wild bees. This is particularly evident for certain fungicides, namely sulphur, potassium bicarbonate, spiroxamine, fenpropidin, and fosetyl-Al. In our study, these fungicides were classified into the group with the highest mean HQ values for both oral and contact exposure. Although the EFSA reported a low acute oral or contact risk to honeybees for sulphur and potassium bicarbonate, both approved for organic farming, as well as for spiroxamine (EFSA, 2009; EFSA, 2010; EFSA et al., 2021), there are no data or definitive conclusions regarding their effects on wild bees. The European Commission (2021) also reported low acute contact and oral toxicity of fenpropidin to honeybees, but lacks corresponding information about wild bees. It is worth noting that a single study demonstrated the absence of toxic effects of potassium bicarbonate on a specific bumblebee species (Gradish et al., 2009). However, that study of Gradish et al. (2009) did not evaluate direct mortality as a result of the fungicide. They tested sublethal effects using an AR of 560 g/ha, which was lower than the average AR in the vineyards evaluated in our study. It is important to emphasize that studies on the toxicity of individual PPPs are predominantly conducted under controlled laboratory conditions, and synergistic effects with other PPPs are often overlooked. As a result, the potential hazards posed by fungicides are often underestimated (Park et al., 2015).

As mentioned at the beginning of this chapter, the HQ approach has its limitations as it can only provide an assessment of whether low risk can be excluded by comparing the HQ values to specific trigger values. It is important to recognize that different studies proposed and used different trigger values (Thompson, 2021), which makes direct comparison between studies difficult. Although the HQ approach was originally developed for spray applications, as employed in our study, it is often utilized in the context of PPP residues found in pollen or bee products, which was strongly criticized by Thompson (2021). Furthermore, it is important to note that the approach is a worst-case scenario, assuming that everything sprayed reaches the bees and equating the AR directly with PPP exposure. This might lead to an overestimation of potential risks. In addition, the SVs for the calculation of HQ_{oral} and the trigger values, both proposed by the EFSA (2013), are also based on a worst-case scenario. However, this methodology does not encompass all potential routes of PPP exposure. Besides contact through spray application or drift, there are other routes of PPP exposure which can also lead to chronic risk and risk posed to bee larvae (EFSA, 2023). Furthermore, the current approach does not take into account the potential synergistic effects of simultaneously applied PPPs, which could be shown e.g., by Belsky & Joshi (2020). Consequently, while there is a risk of overestimating effects due to its worst-case nature, there's also a substantial risk of underestimation by not accounting for all exposure avenues and synergistic interactions.

In summary, the calculated HQ values led to the exclusion of a low toxic risk for wild bees for about half of the applied PPPs, including fungicides. Our results show that fungicides may not be as harmless to bees as initially thought. This is supported by Park et al. (2015), which were able to show that fungicides applied under field conditions have negative impacts on wild bee communities. Their explanation for this was the use of high doses and the regular applications of fungicides in agriculture. However, our results must be interpreted with caution, as LD_{50} values for wild bees are largely missing. The PPDB, which served as source of LD_{50} values in our study, provides verified LD_{50} values for bumble- or solitary bees for only six of the PPPs used (Lewis et al., 2016). Therefore, the use of extrapolated LD_{50} values for honeybees, as

proposed by the EFSA (2013) provides a suitable approximation, as also confirmed in a study by Arena & Sgolastra (2014). More accurate LD_{50} values for wild bees would allow a more precise assessment of the risk of PPPs.

4.2 Ecological trap

The aim of our study was to determine if wild bee abundance and diversity decrease with increasing toxic risk induced by cumulative PPP applications and if this is more pronounced in sown vineyards than in control vineyards. We found no significant effect of toxic contact risk in interaction with treatment on wild bee abundance and diversity and no significant effect of oral toxic risk in interaction with treatment on bumblebee diversity and solitary bee abundance. However, there was a significant impact of oral toxic risk in interaction with treatment on both bumblebee abundance and solitary bee diversity. In subsequent treatment-separated analyses, we observed that toxic risk affected solitary bee diversity only in the control vineyards. There was no effect in sown vineyards or on bumblebee abundance in either type of vineyard.

The interaction between treatment and toxic oral risk significantly affected both bumblebee abundance and solitary bee diversity. This highlights the differential impact of toxic oral risk on these bee populations depending on the treatment applied. In the case of solitary bee diversity, there was no evidence of an ecological trapping effect since diversity declined more in control vineyards than in sown vineyards. The observed pattern in bumblebee abundance could indicate an ecological trapping effect. Specifically, there was a steeper decline in abundance in the sown inter-rows as the RI increased, compared to the control inter-rows (Figure 4). Contrastingly, a study by Rundlöf et al. (2022) reported that negative effects of PPPs on *Bombus vosnesenskii* can be mitigated by additional flower supply. However, they investigated the effects with regard to reproduction, to lethal effects. The subsequent treatment-separated analysis for bumblebee abundance and solitary bee diversity, which were significant for the interaction of treatment and toxic risk showed that the effect of the toxic risk seemed to be cancelled in the case of bumblebees, but not in the case of solitary bees: Unlike bumblebee abundance, toxic risk had an effect on solitary bee diversity in the control inter-rows. Diversity decreased with increasing toxic risk, whereas toxic risk had no effect in the sown inter-rows. This may suggest that the negative effects of the PPPs on solitary bee diversity were mitigated by the additional flower supply or attenuated by immigration into the sown vineyards. These different results in bumblebees and solitary bees can be explained by their different sensitivity to PPPs. There are some other studies that confirm that solitary bees are more sensitive to PPPs than bees of the genus *Bombus*, which can be attributed to physiological differences such as the higher body weight of bumblebees, or differences in social behavior (Arena & Sgolastra, 2014; Devillers et al., 2003; Linguadoca et al., 2022). Our results regarding the ecological trap on bumblebee abundance should therefore be interpreted with caution, as the observed decline due to increased toxic risk in sown vineyards was not statistically significant. Furthermore, overall bumblebee abundance was low and only one sown location had an elevated oral RI (>0.2) (Figure 4). More data would be needed, to confirm or decline the declination trend.

A study that clearly excluded a trapping effect of flower strips in agricultural areas on pollinators, as well as the immigration of pollinators from the surrounding landscape, was conducted by Schmied et al. (2022). They found that abundance and diversity of wild bees in annually mulched wildflower strips increased over time, which was attributed to the additional food resources and nesting sites that the wildflower strips provided (Schmied et al., 2022). Similarly, Kratschmer et al. (2019) reported an increase in wild bee diversity and abundance in sown vineyard inter-rows due to increased flower availability and extensive soil management. However, neither study addressed the potential hazards of PPPs. Immigration of wild bees into the vineyards with sown inter-rows, attracted by the additional food supply, could be one reason

why we did not find clear evidence of an ecological trap in the case of solitary bee diversity. Battin (2004) has addressed this effect of immigration in the context of ecological trapping: Habitats that are more attractive than others can lead to immigration into that habitat, leaving the population decline (due to a negative effect) and thus the ecological trap undetected. Schmied et al. (2022) consider an immigration effect in agricultural areas unlikely or not strong enough, since species abundance and diversity in such landscapes are at low levels anyway. However, we assume that the statement of Schmied et al. (2022) does not apply to Swiss agricultural areas. On the one hand, farmers must have biodiversity areas if they want to receive direct payments (Birrer, n.d.; Federal Statistical Office (FSO), 2015). On the other hand, Swiss farms tend to be small compared to other European countries, which could leave more space for biodiversity-enhancing landscape structures within farmland (Birrer, n.d.; FSO, 2015). Therefore, we do not exclude the possibility of immigration. Moreover, we assume that the negative effects of PPPs on solitary bee diversity are compensated in vineyards with sown inter-rows due to the additional floral resources, but remain in control inter-rows. Similar results were reported by Rundlöf et al. (2022) and Park et al. (2015). Rundlöf et al. (2022) showed that flower supply was able to reduce the negative effects of PPPs on wild bee reproduction rates. Furthermore, Park et al. (2015) demonstrated that the negative effects of PPPs on solitary bee diversity can be mitigated by a higher proportion of natural habitats surrounding intensively managed apple orchards. In contrast to the results of our study, Park et al. (2015) demonstrated this mitigation not only for solitary bee abundance but also for total abundance and diversity of wild bees. However, they used a different approach to calculate an index for evaluating PPP use, and their calculated PPP use intensity index was the same for total wild bee, bumblebee and solitary bee data. Our study considered total wild bees in terms of contact risk, whereas our approach calculating oral risk required a distinction between bumblebees and solitary bees, thus more accurately accounting for differences between bumblebees and solitary bees.

Szitár et al. (2022) reported on the potential of wildflower strips in agricultural landscapes to positively influence and maintain pollinator populations. However, they did not investigate direct effects on pollinator populations. Instead, they examined the effects of a sown wildflower strip on the floral resources, which they expected to correlate positively with pollinators (Szitár et al., 2022). They compared the flower abundance and diversity of a field margin of an organically managed area with two field margins of conventionally managed areas, one of which was a wildflower strip, and argue for a combination of organic farming and the sowing of wildflower strips. However, they focused only on flower data and not on pollinator abundance and therefore did not consider the actual risk to wild bees posed by PPPs. Nevertheless, this would be important with regard to our results. As our study shows, PPPs occur also in organic farming and the RIs of our purely organically managed vineyards were, with one exception, even higher than in our conventionally managed vineyards (Table AT3). This could be due to the fact that significantly more sulphur was sprayed in the organically managed vineyards and that we had a high mean HQ_{oral} and $HQ_{contact}$ for sulphur at the same time (see chapter 3.1).

In summary, our results suggest that the sown inter-rows in the vineyards may have an ecological trapping effect on the abundance of bumblebees, as the decrease in abundance in the sown inter-rows was greater than in the control rows. An ecological trapping effect on solitary bee diversity should not be ruled out, as toxic risk had a significant effect in the control inter-rows and the increase in diversity in the sown inter-rows could be due to immigration. Our results showed the complexity of the interaction between sowings and toxic risk, which challenges the detection of ecological traps by sown vineyard inter-rows.

4.3 Pollinator community

The objective of this study was to evaluate whether the numbers of pollinators caught in vane traps, encompassing both wild bees and non-bee pollinators, corresponded with their field abundances. In addition, the suitability of the vane trap method to depict non-bee pollinators was tested, to determine if our results for wild bees also apply to them. Our findings revealed a positive correlation between vane trap abundances and those observed during transect walks for wild bees. However, this correlation was less pronounced for non-bee pollinators. Subsequent analysis using a linear mixed effects model, which considered various factors, demonstrated that the abundances observed during transect walks did not significantly impact the abundances recorded by vane traps, both for wild bees and non-bee pollinators.

The relationship between vane traps and transect walks appeared complex due to factors like treatment, site number and survey day, resulting in no correlation between the methods. It is also possible that not all influencing factors have been considered in the analysis. The differing durations of vane trap deployment (72 hours) and transect walks (16 minutes) may contribute to this complexity. Nonetheless, our study highlights a positive trend between vane traps and transect abundances when considering these two variables alone, especially in the case of wild bees. This observation can be attributed to the fact that vane traps are a well-established method for selectively trapping wild bees (Prendergast et al., 2020). According to the findings presented in Figure 7, it appears that at low transect walk abundances, there is even an over-representation of wild bees in the vane traps. This can be confirmed by the negative difference we found between the transect and vane trap wild bee abundances. Especially blue vane traps are particularly suitable for catching wild bees and prone to oversample wild bees (Hall, 2018; Joshi et al., 2015). Abundances of non-bee pollinators caught with vane traps should be interpreted with caution since their correlation to field observed abundances was weak. Nevertheless, Hall & Reboud (2019) have shown that blue and yellow vane traps are also a good method to catch other pollinators such as flies or wasps very efficiently. That outcome is also shown in Figure AF3 in the Appendix, as all differences in the abundance of the two methods lied between the limits of agreement. However, our results also show that high abundances of wild bee and non-bee pollinators were recorded less frequently. The observation of decreased data points at higher abundances suggests several possibilities. Firstly, in situations of high pollinator abundance in the field, there is an increased likelihood of multiple flower visits occurring simultaneously, potentially leading to underrepresentation in the collected data. Secondly, the attractiveness of vane traps might decrease with increasing pollinator abundance, possibly related to the high flower diversity observed in areas with abundant pollinators (Kratschmer et al., 2019; Schmied et al., 2022; Steinemann et al., 2022). To validate the influence of floral resources, additional data related to flower diversity should be considered.

Our results show that vane traps tend to oversample wild bees when their abundance is low, but correlate with transect observations across all observations. Although the vane trap method appears to be acceptable for catching non-bee pollinators, their abundance obtained by vane trapping correlates only slightly with their abundance observed in the field. To obtain a more comprehensive view of the pollinator community, Hall and Reboud (2019) point out the necessity to include other trap types such as e.g., Malaise traps, which are better suited for trapping beetles and butterflies (Hall & Reboud, 2019). However, as there are carnivorous species in non-bee insect groups that are not dependent on pollen and nectar (Raupp et al., 2023), non-bee insect groups may include both pollinating and non-pollinating species. Therefore, we consider it useful to focus on wild bees as pollinators, as all wild bees depend on floral resources (Westrich, 2013).

5. Conclusion

In conclusion, our analysis of the plant protection products (PPPs) applied in vineyards has yielded unexpected results. Contrary to our initial expectations, the possibility that certain PPPs, including fungicides, may pose an increased risk must not be disregarded. In order to conduct comprehensive risk assessments for different bee species beyond honeybees, investment in toxicity studies with wild bees is essential. As the PPP analysis showed concerning HQ values, the basis for a trapping effect may be given, and in the case of bumblebee abundance we could showed indications that sown vineyard inter-rows may pose an ecological trap. No such effect could be confirmed for total wild bee and solitary bee abundance and diversity. Nevertheless, the possibility of such an effect should not be completely excluded. In the dynamic environment of the vineyard, several influencing factors, including immigration dynamics and management practices, complicate the detection of trapping effects. Due to this complexity, we propose to include data over several years of study and data on flower abundance and diversity in order to investigate the effects of sown vineyard rows in more detail.

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8. Appendix

Table AT1: List of all used PPP products across all study sites and across the years 2022 and 2023, with its active PPP substances, their percentage, the corresponding PPP group and if the PPP product is permitted in organic agriculture according to the list of inputs for organic farming in Switzerland (Speiser et al., 2022).

PPP product	Active PPP substance	Content (%)	Group	Source	Organic approved
Airone	Copper hydroxide	14	Fungicide	FSVO (2023)	Yes
	Copper oxychloride	14			
Alginure	Potassium phosphonate	13.36	Fungicide	FSVO (2023)	No
Amaline Flow	Copper oxysulfate	19.2	Fungicide	FSVO (2023)	No
	Zoxamid	2.9			
Amarel Folpet DF	Folpet	53.5	Fungicide	FSVO (2023)	No
	Cymoxanil	8			
Ampexio	Mandipropamid (25)	25	Fungicide	FSVO (2023)	No
	Zoxamid	24			
Armicarb	Potassium bicarbonate	85	Fungicide / Insecticide	FSVO (2023)	Yes
Astor	Fenpropidin	81.8	Fungicide	FSVO (2023)	No
Auralis	COS-OGA	1.02	Stimulator of natural defense against fungal diseases	FSVO (2023)	Yes
Avatar	Cyprodinil	37.5	Fungicide	FSVO (2023)	No
	Fludioxonil	25			
Bacchus	Copper oxysulfate	14.8	Fungicide	FSVO (2023)	No
	Cymoxanil	2.7			
Baxoda	Sodium hydrogen carbonate	99	Fungicide	Agroline (2021)	Yes
Bordeaux mixture	Copper	20	Fungicide / Bactericide	FSVO (2023)	Yes
Cantus	Boscalid	50	Fungicide	FSVO (2023)	No
Cercobin	Thiophanat-methyl	41.91	Fungicide	BASF (2021)	No
Chikara 25 WG	Flazasulfuron	25	Herbicide	FSVO (2023)	No
Cuprofix fluid	Copper oxychloride	25.42	Fungicide	FSVO (2023)	Yes
Cuproxat	Copper oxysulfate	14.84	Fungicide / Bactericide	FSVO (2023)	Yes
Cyflamid	Cyflufenamid	5	Fungicide	FSVO (2023)	No
Cymbal WG	Cymoxanil	45	Fungicide	FSVO (2023)	No
Cyrano	Fosetyl-Al	50	Fungicide	FSVO (2023)	No
	Folpet	25			
	Cymoxanil	4			
Difcor	Difenoconazole	23.6	Fungicide	FSVO (2023)	No
Dominator	Ametoctradin	27	Fungicide	FSVO (2023)	No
	Dimetomorph	20.3			
Dynali	Difenoconazole	5.6	Fungicide	FSVO (2023)	No
	Cyflufenamid	2.8			
Eleto	Dimetomorph	16.3	Fungicide	FSVO (2023)	No
	Zoxamid	16.3			
Escort	Cymoxanil	33	Fungicide	FSVO (2023)	No
	Zoxamid	33			
Fantic F	Folpet	48	Fungicide	FSVO (2023)	No
	Benalaxyl-M	3.75			
Fenicur	Oleum foeniculi	23	Fungicide	FSVO (2023)	Yes
Fezan	Tebuconazole	24.2	Fungicide	FSVO (2023)	No
Filan	Boscalid	50	Fungicide	FSVO (2023)	No
Folpet 80 WG	Folpet	80	Fungicide	FSVO (2023)	No
Funguran Flow	Copper hydroxide	22.7	Fungicide / Bactericide	FSVO (2023)	Yes

Fusilade Max	Fluazifop-P-butyl	13.4	Herbicide	FSVO (2023)	No
FytoSave	COS-OGA	1.02	Stimulator of natural defense against fungal diseases	FSVO (2023)	Yes
Heliosoufre S	Sulphur	51.1	Fungicide / Acaricide	FSVO (2023)	Yes
Kocide 2000	Copper hydroxide	35	Fungicide / Bactericide	FSVO (2023)	Yes
Kocide Opti	Copper hydroxide	30	Fungicide	FSVO (2023)	Yes
Labifol	Boron	2	Stimulator of natural defense against fungal diseases	Vitistim (n.d.)	No
	Nitrogen	1			
	Potassium	2			
Nekapur 2	Calcium hydroxide	98	Insecticide	Kalkfabrik Netstal (2022)	Yes
Mapro	Fluazinam	38.8	Fungicide	FSVO (2023)	No
Microthiol LG	Sulphur	57.3	Fungicide	FSVO (2023)	Yes
Mikal	Fosetyl	23.3	Fungicide	FSVO (2023)	No
	Fosetyl Al	23.3			
	Folpet	25			
Mildicut	Disodiumphosphonate	20.5	Fungicide	FSVO (2023)	No
	Cyazofamid	2.05			
Netzschwefel Stulln	Sulphur	80	Fungicide / Acaricide	FSVO (2023)	Yes
Oxykupfer 35	Copper oxychloride	35	Fungicide	FSVO (2023)	Yes
Pergado	Folpet	40	Fungicide	FSVO (2023)	No
	Mandipropamid	5			
Prosper	Spiroxamine	50	Fungicide	FSVO (2023)	No
Quadris Max	Folpet	39.2	Fungicide	FSVO (2023)	No
	Azoxystrobin	7.33			
Quartet Lux	Potassium phosphonate	51.7	Fungicide	FSVO (2023)	No
Revus	Mandipropamid	23.4	Fungicide	FSVO (2023)	No
Ridomil Vino	Folpet	40	Fungicide	FSVO (2023)	No
	Metalaxyl-M	4.85			
Rondo Sky	Fluxapyroxad	26.5	Fungicide	FSVO (2023)	No
Schachtelhalm Equi Bio	Equisetum extract	NA	Stimulator of natural defense against fungal diseases	Andermatt Biocontrol Suisse (2022)	Yes
Sico	Difenoconazole	23.5	Fungicide	FSVO (2023)	No
Soufre 80 WG	Sulphur	80	Fungicide / Acaricide	FSVO (2023)	Yes
Stamina S	Potassium phosphonate	51.7	Fungicide	FSVO (2023)	No
Sufralo	Sulphur	80	Fungicide / Acaricide	FSVO (2023)	Yes
Surround	Kaolin	95	Insecticide	FSVO (2023)	Yes
Switch	Cyprodinil	37.5	Fungicide	FSVO (2023)	No
	Fludioxonil	25			
Talendo	Proquinazid	20.53	Fungicide	FSVO (2023)	No
Teldor	Fenhexamid	51	Fungicide	FSVO (2023)	No
Thiovit	Sulphur	80	Fungicide / Acaricide	FSVO (2023)	Yes
Thiovit Jet	Sulphur	80	Fungicide / Acaricide	FSVO (2023)	Yes
Topas Vino	Penconazole	10.2	Fungicide	FSVO (2023)	No
Touchdown System 4	Glyphosat	28.3	Herbicide	FSVO (2023)	No
Vacciplant	Laminarin	4.3	Stimulator of natural defense against fungal diseases	FSVO (2023)	Yes
Vincare	Folpet	50	Fungicide	FSVO (2023)	No
	Benthiavalicarb-isopropyl	1.75			
Vitisan	Potassium bicarbonate	99.6	Fungicide	FSVO (2023)	Yes
Vivando	Metrafenone	42	Fungicide	FSVO (2023)	No

Table AT2: Mean (± se), minimum and maximum values of the $HQ_{contact}$ and HQ_{oral} across 2022 and 2023 per PPP. PPPs are sorted by chemical groups. Grey: HQ_{oral} bumblebees, blue: HQ_{oral} solitary bees, green: $HQ_{contact.}$ Columns at the right show the mean AR (kg/ha) (± se) across 2022 and 2023, as well as the oral and contact LD_{50} values (µg/bee) for honeybees (Lewis et al., 2016) extrapolated for wild bees by dividing them by a factor of 10 according to the EFSA (2013).

Substance	Mean HQ			Min. HQ			Max. HQ			Mean AR (kg/ha)	extrapolated LD_{50} (µg/bee) oral	extrapolated LD_{50} (µg/bee) contact
Amide Fungicides												
Benalaxyl-M	0.018±0.001	0.006±0.000	9.409±0.642	0.011	0.004	6.000	0.023	0.008	12.000	0.094±0.006	10.4	10
Benthiavalicarb-isopropyl	0.010±0.000	0.004±0.000	5.250±0.247	0.010	0.003	4.900	0.011	0.004	5.600	0.052±0.002	10	10
Boscalid	0.070±0.000	0.025±0.000	30.000±0.000	0.070	0.025	30.000	0.070	0.025	30.000	0.600±0.000	16.6	20
Cyflufenamid	0.004±0.000	0.001±0.000	2.095±0.098	0.002	0.001	1.250	0.005	0.002	2.400	0.021±0.001	10	10
Cymoxanil	0.021±0.001	0.007±0.000	9.131±0.524	0.006	0.002	2.700	0.029	0.010	12.800	0.091±0.005	8.53	10
Dimetomorph	0.176±0.017	0.062±0.006	28.671±2.838	0.098	0.035	15.980	0.195	0.069	31.843	0.292±0.029	3.24	10.2
Fenhexamid	0.146±0.000	0.052±0.000	36.957±0.000	0.146	0.052	36.957	0.146	0.052	36.957	0.765±0.000	10.207	20.7
Fluxapyroxad	0.007±0.000	0.002±0.000	3.975±0.000	0.007	0.002	3.975	0.007	0.002	3.975	0.039±0.000	11.09	10
Mandipropamid	0.014±0.000	0.005±0.000	6.963±0.217	0.009	0.003	4.675	0.016	0.006	8.000	0.139±0.004	20	20
Metalaxyl-M	0.022±0.001	0.008±0.000	10.870±0.683	0.017	0.006	8.730	0.035	0.012	17.460	0.108±0.007	9.73	10
Zoxamid	0.017±0.004	0.006±0.001	12.905±2.755	0.008	0.003	6.090	0.044	0.015	33.000	0.129±0.028	14.7	10
Inorganic Fungicides												
Copper (Bordeaux mix)	0.184±0.011	0.065±0.004	87.159±5.349	0.042	0.015	19.841	0.670	0.237	317.460	0.219±0.013	2.33	2.52
Copper hydroxide	0.055±0.003	0.019±0.001	30.209±1.598	0.017	0.006	9.447	0.139	0.049	78.722	0.134±0.007	4.9	4.446
Copper oxychloride	0.195±0.006	0.068±0.002	27.245±0.815	0.068	0.015	9.481	0.410	0.145	57.381	0.120±0.004	1.21	4.43
Copper oxysulfate	0.254±0.014	0.091±0.005	67.152±3.746	0.062	0.022	16.419	0.866	0.307	228.766	0.157±0.009	1.21	2.35
Disodium phosphonate	0.030±0.001	0.010±0.000	NA	0.025	0.009	NA	0.031	0.011	NA	0.791±0.026	52	NA
Potassium bicarbonate	3.071±0.118	1.087±0.042	102.719±3.944	1.619	0.573	54.130	4.046	1.432	135.326	3.780±0.145	2.4	36.8
Potassium phosphonate	0.097±0.005	0.034±0.002	NA	0.022	0.008	NA	0.174	0.062	NA	0.725±0.034	14.5	NA
Sodium hydrogen carbonate	0.166±0.004	0.059±0.001	81.895±1.750	0.144	0.051	70.828	0.180	0.064	88.535	4.578±0.098	53.74	55.91
Sulphur	0.414±0.008	0.150±0.007	226.792±4.631	0.112	0.023	61.320	0.730	1.369	400.000	2.267±0.046	10.68	10
Azole (Triazole) Fungicides												
Difenoconazole	0.004±0.000	0.002±0.000	3.984±0.184	0.003	0.001	3.055	0.005	0.002	4.720	0.039±0.002	17.7	10
Penconazole	0.052±0.002	0.018±0.001	99.043±3.862	0.036	0.013	68.000	0.071	0.025	136.000	0.029±0.001	1.12	0.3
Tebuconazole	0.011±0.000	0.004±0.000	2.420±0.000	0.011	0.004	2.420	0.011	0.004	2.420	0.048±0.000	8.305	20
Fungicides belonging to various other chemical groups												
Ametoctradin	0.076±0.000	0.027±0.000	43.200±0.000	0.076	0.027	43.200	0.076	0.027	43.200	0.432±0.000	11.15	10
Azoxystrobin	0.132±0.022	0.047±0.008	8.430±1.379	0.069	0.024	4.398	0.182	0.065	11.728	0.168±0.028	2.5	20
Cyazofamid	0.009±0.001	0.003±0.001	6.970±1.100	0.003	0.001	2.050	0.011	0.004	8.200	0.069±0.011	15.17	10
Cyprodinil	0.078±0.000	0.028±0.000	60.000±0.000	0.078	0.028	60.000	0.078	0.028	60.000	0.450±0.000	11.25	7.5
Fenpropidin	0.638±0.000	0.226±0.000	71.130±0.000	0.638	0.226	71.130	0.638	0.226	71.130	0.327±0.000	1	4.6
Fluazinam	0.053±0.005	0.019±0.002	13.580±1.372	0.045	0.016	11.640	0.061	0.021	15.520	0.271±0.027	10	20
Fludioxonil	0.059±0.000	0.021±0.000	30.000±0.000	0.059	0.021	30.000	0.059	0.021	30.000	0.300±0.000	10	10
Folpet	0.080±0.002	0.035±0.004	48.676±1.275	0.017	0.006	10.400	0.132	0.365	80.000	0.973±0.026	23.6	20
Fosetyl	0.078±0.006	0.027±0.002	65.240±4.660	0.066	0.024	55.920	0.089	0.031	74.560	0.652±0.047	16.4	10
Fosetyl-Al	0.200±0.015	0.071±0.005	111.103±8.295	0.101	0.036	55.920	0.288	0.102	160.000	1.111±0.083	10.85	10
Metrafenone	0.018±0.002	0.006±0.001	10.640±0.943	0.011	0.004	6.720	0.023	0.008	13.440	0.106±0.009	11.4	10

Proquinazid	0.008±0.001	0.003±0.000	2.762±0.249	0.006	0.002	2.084	0.013	0.005	4.169	0.054±0.005	12.5	19.7
Spiroxamine	0.065±0.005	0.023±0.002	795.455±57.407	0.039	0.014	476.190	0.078	0.028	952.381	0.334±0.024	10	0.42
Thiophanate-methyl	0.143±0.000	0.050±0.000	83.820±0.000	0.143	0.050	83.820	0.143	0.050	83.820	0.838±0.000	11.47	10
Herbicides												
Flazasulfuron	0.024±0.000	0.009±0.000	3.750±0.000	0.024	0.009	3.750	0.024	0.009	3.750	0.037±0.000	10	10
Fluazifop-P-butyl	0.065±0.000	0.023±0.000	10.050±0.000	0.065	0.023	10.050	0.065	0.023	10.050	0.201±0.000	20	20
Glyphosate	0.531±0.000	0.188±0.000	84.900±0.000	0.531	0.188	84.900	0.531	0.188	84.900	0.849±0.000	10.4	10
Insecticides												
Kaolin	4.128±0.294	1.461±0.104	2117.143±150.776	2.223	0.787	1140.000	4.446	1.573	2280.000	21.171±1.508	10	10
Calcium hydroxide	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.076±0.056	NA	NA

Table AT3: Mean RI values (± se) and standard errors of both study years for the study sites which used only organically approved PPP products and for the study sites which used organically approved and conventional PPP products.

PPP products used	Year	Mean RI oral bumblebees	Mean RI oral solitary bees	Mean RI contact
Organically approved	2022	0.122±0.04	0.043±0.01	37.158±5.43
Organically approved + conventional	2022	0.074±0.02	0.026±0.01	38.000±3.67
Organically approved	2023	0.163±0.03	0.056±0.01	47.119±2.47
Organically approved + conventional	2023	0.080±0.01	0.029±0.00	31.154±3.60

*Table AT4: Coefficient of determination (R^2) for linear mixed effect models (at the left) analysing the impact of the RI on bee data, as well as estimates (± se) (grey) and significance levels (white) for each fixed effect included in the models. Levels of significance are indicated as: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.*

	treatmentSown	**risk**	**treatmentSown:risk**	**temperature**	**Year2023**
WBA R^2 = 0.76	-0.22 ± 0.42	-0.01 ± 0.01	0.01 ± 0.01	0.09 ± 0.06	-0.20 ± 0.33
WBS R^2 = 0.50	-0.03 ± 0.15	-0.003 ± 0.003	0.004 ± 0.004	-0.02 ± 0.02	-0.19 ± 0.08 *
BBA R^2 = 0.80	0.92 ± 0.30 **	0.91 ± 1.44	-6.11 ± 2.30 *	-0.001 ± 0.07	-1.33 ± 0.39 **
BBS R^2 = 0.65	0.29 ± 0.14 *	0.55 ± 0.68	-2.19 ± 1.16 .	0.01 ± 0.03	-0.56 ± 0.14 **
SBA R^2 = 0.78	-0.0002 ± 0.24	-3.75 ± 4.20	1.73 ± 5.64	0.10 ± 0.07	0.0002 ± 0.39
SBS R^2 = 0.51	-0.03 ± 0.08	-2.27 ± 1.03 *	3.70 ± 1.75 *	-0.01 ± 0.02	-0.10 ± 0.08

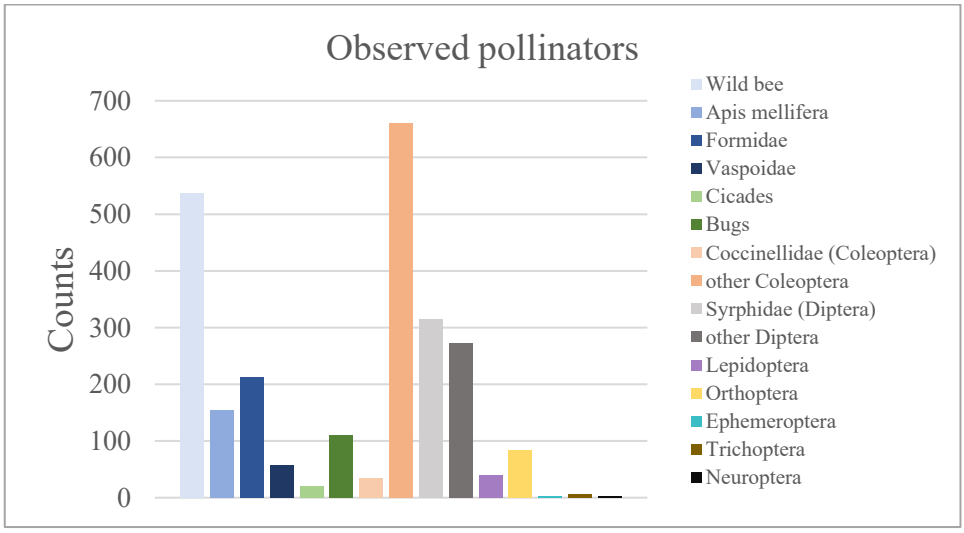

Figure AF1: Counts of observed pollinators of transect walks in 2023 across all locations (sum of sown and control sites and both survey rounds).

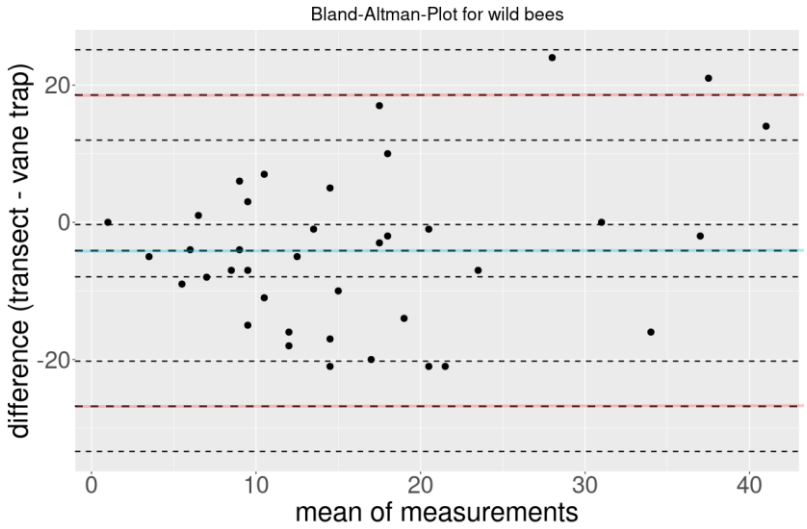

Figure AF2: Bland-Altman plot for wild bee abundances surveyed by vane traps and transect walks. The X-axis shows the mean wild bee abundances of both methods for each sampling site. The Y-axis shows the measurement differences between the two methods for each sampling site. The blue dashed line indicates the mean difference (-4.13±1.83). Red dashed lines show the limits of agreement (upper: 18.90, lower: -26.15). Black dashed lines show the 95% confidence intervals of the mean and the limits of agreement. There are less measures of large (>20) mean abundances. The larger the mean measurements, the more differently the two methods measure wild bee abundance.

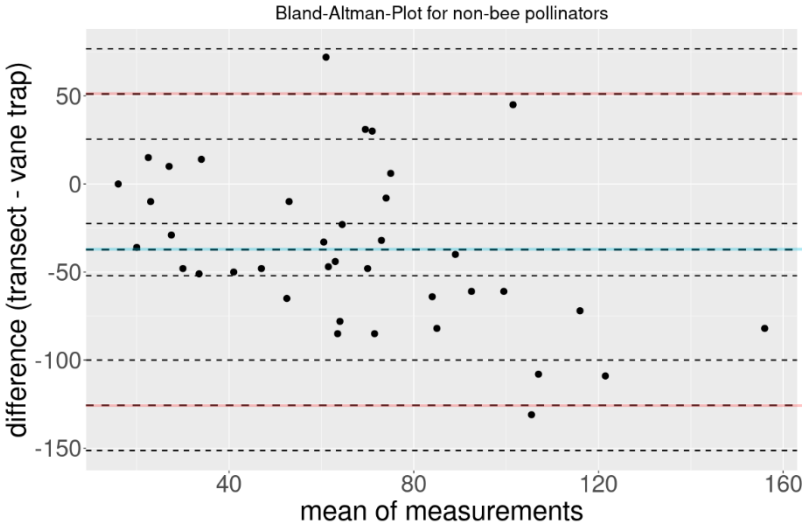

Figure AF3: Bland-Altman plot for non-bee pollinator abundances surveyed by vane traps and transect walks. The X-axis shows the mean non-bee pollinator abundances of both methods for each sampling site. The Y-axis shows the measurement differences between the two methods for each sampling site. The blue dashed line indicates the mean difference (-37.29±7.22). Red dashed lines show the limits of agreement (upper: 48.38, lower: -126.53). Black dashed lines show the 95% confidence intervals of the mean and the limits of agreement. There are less measures of large (>80) mean abundances. The larger the mean measurements, the more differently the two methods measure non-bee pollinator abundance.

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Risk of plant protection products to wild bees in vineyards with sown wildflower strips compared to vineyards with natural vegetation

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