The effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture

by

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ABSTRACT

In recent decades, there has been a strong global decline in biodiversity which is attributed, among other reasons, to intensified agriculture and the loss of habitats. Due to the significant ecological impacts it is crucial to comprehensively understand how management practices and the surrounding landscape affect species, as well as how these factors influence their populations over the long term. We studied the influence of weather and trapping effort on multi-day Malaise trap sampling, examining their effects on long-term monitoring data. We further explored how vineyard management and the presence of semi-natural habitats (SNH) affect arthropods in the wine-growing region Palatinate in southwest Germany.

We evaluated the impact of ambient weather conditions and trapping effort during Malaise trap exposure on biomass and taxa richness using metabarcoding. Insect activity was highest when the weather was warm and dry. Taxa accumulation increased fourfold from three days of monthly trapping to continuous trap exposure and nearly sixfold from sampling at a single site to 32 sites. Common species are likely to be captured with short trapping durations and a small number of sampling sites, while it remains challenging to comprehensively sample rare species. Metabarcoding provides a valuable method for long-term monitoring. However, additional sequencing efforts are required to establish more comprehensive DNA databases.

Furthermore, we investigated how organic and conventional management, reduction of pesticides, and SNH in the surrounding landscape affect arthropod diversity in vineyards. Biodiversity was assessed in 32 vineyards in a crossed design of management (organic vs. conventional) and pesticide use (regular vs. reduced in fungus-resistant grape varieties). The pairs of vineyards were located in 16 landscapes, with increasing proportions of SNH in the surrounding area of the vineyards. We measured the biomass of captured specimens and used metabarcoding to assess the general arthropod biodiversity. Furthermore, we used morphological and acoustic species identification to investigate effects on wild bees and orthopterans. Biomass was almost one-third higher in conventional compared to organic vineyards, while organic vineyards had almost 50 % more bees. Densities of herb-dwelling orthopterans were 2.9 times higher in fungus-resistant compared to classic grape varieties under organic management. Higher proportions of SNH increased arthropod richness as well as abundance and richness of above-ground-nesting bees and further changed community composition of arthropods, including wild bees and orthopterans. Increased inter-row vegetation had positive effects on various groups of organisms. Our studies on the influence of vineyard management show that reducing pesticide use, particularly under organic management, can enhance sustainability in viticulture and promote biodiversity. Moreover, further species benefit from diverse inter-row vegetation and SNH in the surrounding landscape. We conclude that the cultivation of fungus-resistant grape varieties is of importance to minimize the need for non-specific pesticides, while it is also important to provide diverse vegetation in inter-rows and create a structurally rich environment with suitable SNH to conserve biodiversity in viticulture.

ZUSAMMENFASSUNG

In den letzten Jahrzehnten ist die biologische Vielfalt weltweit deutlich zurückgegangen, was unter anderem auf die Intensivierung der Landwirtschaft und den Verlust von Lebensräumen zurückgeführt wird. Wir untersuchten den Einfluss des Wetters und des Fangaufwands auf mehrtägige Malaise-Fallenbeprobungen und deren Auswirkungen auf Monitoringdaten. Wir analysierten weiterhin, wie sich die Weinbergsbewirtschaftung und das Vorhandensein naturnaher Habitate (SNH) auf Arthropoden in der Weinbauregion Pfalz in Deutschland auswirken.

Wir bewerteten die Auswirkungen der Witterung und des Fangaufwands während Malaise-Fallenbeprobungen auf die Biomasse, sowie auf den Taxa-Reichtum mittels Metabarcoding. Die Insektenaktivität war bei warmem und trockenem Wetter am höchsten. Die Taxa-Akkumulation erhöhte sich um das Vierfache von dreitägiger monatlicher Beprobung zu kontinuierlicher Beprobung und um das Sechsfache von einer Beprobungstelle zu 32 Stellen. Häufige Arten können mit kurzen Fangzeiten und geringer Anzahl von Beprobungsstellen erfasst werden, während sich das umfangreiche Erfassen seltener Arten als Herausforderung gestaltet. Metabarcoding zeigt sich als geeignete Methode für langfristige Monitorings, jedoch ist ein Aufbau umfassender DNA-Datenbanken essenziell.

Weiterhin untersuchten wir, wie sich ökologische und konventionelle Bewirtschaftung, Pestizidreduzierung und SNH in der umgebenden Landschaft auf die Arthropodenvielfalt in Weinbergen auswirken. Die Biodiversität wurde in 32 Weinbergen in einem gekreuzten Design aus Bewirtschaftung (ökologisch vs. konventionell) und Pestizideinsatz (regulär vs. reduziert bei pilzwiderstandsfähigen Rebsorten) bewertet. Die Weinbergspaare befanden sich in 16 Landschaften mit zunehmenden Anteilen von SNH in der Umgebung. Wir haben die Biomasse der gefangenen Exemplare gemessen und die allgemeine Arthropodenvielfalt mittels Metabarcoding bewertet. Darüber hinaus untersuchten wir die Auswirkungen auf Wildbienen und Heuschrecken. Die Biomasse war in konventionellen Weinbergen um fast ein Drittel höher als in ökologischen, während in ökologischen Weinbergen fast 50 % mehr Bienen vorhanden waren. Die Dichte bodenbewohnender Heuschrecken war bei ökologischer Bewirtschaftung bei pilzwiderstandsfähigen Rebsorten 2,9-mal höher als bei klassischen Rebsorten. Zunehmender SNH-Anteil erhöhte den Reichtum an Arthropoden sowie die Abundanz und den Reichtum an oberirdisch nistenden Bienen und veränderte die Artgemeinschaften von Arthropoden, darunter die von Wildbienen und Heuschrecken. Eine stärkere Gassenvegetation hatte positive Auswirkungen auf verschiedene Organismengruppen. Unsere Studien über den Einfluss der Weinbergsbewirtschaftung zeigen, dass eine Verringerung des Pestizideinsatzes, insbesondere bei ökologischer Bewirtschaftung, die Nachhaltigkeit im Weinbau verbessern und die biologische Vielfalt fördern kann. Außerdem profitieren Arten von einer vielfältigen Gassenvegetation und SNH in der umgebenden Landschaft. Zusammenfassend stellen wir fest, dass die Artenvielfalt im Weinbau zum einen durch den Anbau pilzwiderstandfähiger Rebsorten und der daraus resultierenden Reduktion unspezifischer Pestizide, als auch durch eine vielfältige Vegetation in den Gassen und eine strukturreiche Umgebung mit geeigneten SNH gefördert und erhalten werden kann.

CHAPTER 1

General introduction

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1.1 General introduction

1.1.1 Decline of arthropods

In recent decades, there has been a strong and global decline of arthropods with consequences for the functioning of ecosystems worldwide (Hochkirch 2016; Hallmann *et al*. 2017; Seibold *et al*. 2019). Alongside climate change, habitat destruction, and invasive species, the intensification of modern agriculture, with characteristics such as the clearance of semi-natural habitats (SNH), the scarcity of food resources, and the extensive use of pesticides and fertilizers is considered one of the main factors driving this significant loss of biodiversity and biomass (Sánchez-Bayo and Wyckhuys 2019). Policy measures include actions such as increasing the share of organic agriculture, reducing pesticide use, and increasing the proportion of SNH in agricultural landscapes (European Commission 2019). These measures are assumed to enhance biodiversity and counteract the decline of arthropods observed in recent years (Bengtsson *et al*. 2005). Given that the effects of agricultural practices vary both between organism groups and across different cropping systems, it is crucial to comprehensively understand the differential impacts and specific threats posed to arthropod diversity (Bengtsson *et al*. 2005; Hole *et al*. 2005). Systematic long-term monitoring plays a vital role in quantifying ongoing trends of biodiversity loss and evaluating the effectiveness of policy measures.

1.1.2 Monitoring of arthropods

To monitor arthropod biomass, abundance, richness, and composition, a wide range of trap types can be utilized, including bait traps, light traps, Malaise traps, pan traps, and pitfall traps (Yi *et al*. 2012; Henderson and Southwood 2021). However, the vast diversity of insects, the high number of trapped individuals, and the time-consuming process of species identification often result in laborious work, necessitating limitations on research studies (Thomas 2005; Hausmann *et al*. 2020; Karlsson *et al*. 2020). Therefore, especially when relying on morphological species identification, studies may either focus on specific trapping methods, with the choice of trap type inherently emphasizing certain species groups (Skvarla *et al*. 2021), or focus on indicator groups. For example, Malaise traps are highly effective in capturing flying insects, while pan traps are primarily used for capturing pollinators (Malaise 1937; Hausmann *et al*. 2020; Skvarla *et al*. 2021). Moreover, the arthropod activity and consequently the biodiversity recorded during trapping are influenced by seasonal variations and ambient weather conditions (Juillet 1964; Bergman *et al*. 1996; Kirse *et al*. 2021). Warm, dry weather generally enhances arthropod activity, while cold and rainy weather reduces it (Kasper *et al*. 2008; Welti *et al*. 2012; Kirse *et al*. 2021). Therefore, it is important to precisely understand the impact of trapping methods and trapping effort as well as environmental conditions on the biodiversity being recorded during sampling to evaluate long-term monitoring data.

Time-consuming morphological identification of species often depends on the few experts available, which is a problem known as the taxonomic impediment (Giangrande 2003). To address this issue, metabarcoding presents a solution by identifying large bulk samples time- and cost-efficiently (deWaard *et al*. 2019). Through high-throughput sequencing, metabarcoding analyzes DNA sequences in the cytochrome c oxidase I (COI) gene, which can

then be assigned to taxa based on reference sequences in DNA databases such as the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2013). This approach enables the study of entire arthropod communities and can serve as a valuable tool for long-term monitoring, as data can be efficiently stored and shared for re-analysis (deWaard *et al*. 2019). However, monitoring and research studies depend on extensive DNA databases that can reliably identify captured specimens. How well databases cover the diversity of species varies by region and the effectiveness of metabarcoding should therefore be assessed separately for specific regions and, in agriculture, for different cropping systems. Furthermore, despite promising approaches to estimate the relative abundance of a species based on the reads of DNA sequences in a bulk sample (Krehenwinkel *et al*. 2017; Piñol *et al*. 2019; Kennedy *et al*. 2020), metabarcoding does not yet allow for the evaluation of quantified species abundances (Liu *et al*. 2020). Therefore, in addition to metabarcoding, focusing on specific ecological indicator groups, such as wild bees and orthopterans, and measuring their abundances with taxonomic species identification can provide valuable insights into how distinct species respond to agricultural influences and environmental changes.

1.1.3 Pesticide management and the cultivation of fungus-resistant grape varieties

Organic farming that avoids insecticides, herbicides, and inorganic fertilizers clearly promotes biodiversity (Bengtsson *et al*. 2005; Hole *et al*. 2005; Holzschuh *et al*. 2008), as species richness increases by about 30 % compared to conventional farming (Tuck *et al*. 2014). In viticulture, however, the prevalence of fungal diseases such as powdery and downy mildew leads to an extensive use of pesticides, especially fungicides, in both conventional and organic managed vineyards, making it one of the cropping systems with the highest utilization of pesticides (Pertot *et al*. 2017). Organic viticulture promotes biodiversity in regions where insecticides are used in conventionally managed vineyards, such as against *Scaphoideus titanus*, the vector of Flavescence dorée (Beaumelle *et al*. 2023). However, in regions where insecticide use is generally absent, the positive impact of organic management appears to be less pronounced compared to other cropping systems, as both positive and negative impacts have been reported (Caprio *et al*. 2015; Döring *et al*. 2019; Paiola *et al*. 2020; Ostandie *et al*. 2021; Schirmel *et al*. 2022). Both synthetic chemicals in conventional viticulture and inorganic compounds like copper and sulfur in organic viticulture are used to prevent yield losses, but these substances can potentially negatively impact non-target organisms through surface contact and ingestion (Ingrisch and Köhler 1998; Pedneault and Provost 2016; Pertot *et al*. 2017; Vogelweith and Thiéry 2018; Biondi *et al*. 2012; Nash *et al*. 2010). For example, in addition to the actual spraying event, the accumulation of copper in the soil poses potential long-term risks to the environment (Komárek *et al*. 2010). Duque *et al*. (2023) found that copper concentrations, such as those found in Palatinate vineyards, can have lethal effects on soil organisms such as earthworms. Sulfur, which is used as a fungicide but has broad-spectrum pesticide properties, has also been shown to have negative effects on non-target organisms (Hanna et al. 1997; Jepsen et al. 2007; Tacoli et al. 2020). However, the effects of organic versus conventional viticulture vary among groups of organisms (Ostandie *et al*. 2021), depending on their sensitivity and exposure to pesticides. Therefore, studying the diverse effects of vineyard management on various organism groups is crucial for comprehending their specific responses and formulating appropriate conservation measures.

Considering the extensive use of pesticides in viticulture, it is imperative to reduce their use to mitigate impacts on non-target organisms. However, winegrowers heavily rely on pesticides for wine production due to the spread of the major fungal diseases. Nevertheless, in the late 19th and early 20th centuries, the first grape cultivars with resistances to fungal diseases were developed through breeding of *Vitis vinifera* with American and Asian *Vitis* species (Töpfer and Trapp 2022). By the end of the 20th century, the first cultivars with convincing wine qualities had been developed, and today approximately 40 cultivars of fungus-resistant grape (FRG) varieties are available to winegrowers (Töpfer and Trapp 2022). Some multi-resistant varieties have the potential to reduce pesticide use by up to 80 %, thereby enhancing the sustainability of viticulture (Töpfer and Trapp 2022). Recent studies have reported positive effects of the cultivation of FRG varieties on different groups of non-target organisms (Pedneault and Provost 2016), such as predatory mites and certain spider families (Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021a; Reiff *et al*. 2023). Furthermore, reduced pesticide use in FRG varieties promotes the presence of predatory mites over pest mites, thereby promoting the abundance of these beneficial species (Reiff *et al*. 2021a). This can have a positive impact on trophic interactions that can be of importance to winegrowers by enhancing natural pest control (Winkler *et al*.2017; Reiff *et al*. 2021b). Despite the significant reduction in pesticide use and the positive impact on biodiversity, only about 2.7 % of the area used for wine production in Palatinate is planted with FRG varieties (Statistisches Bundesamt (Destatis) 2023). Moreover, by reducing the necessity for pesticide applications, there is a subsequent reduction in tractor passages. This, in turn, reduces the disturbance of ground vegetation and soil compaction (Bruggisser *et al*. 2010). Consequently, there is still a high potential to increase sustainability in viticulture through the cultivation of FRG varieties, which represents a promising approach to promote biodiversity. Yet, to date, research on this topic is sparse and limited (Pedneault and Provost 2016; Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021b). Thus, more studies are needed to better understand the specific effects of reduced pesticide use in vineyards on the various groups of organisms.

1.1.4 Landscape heterogeneity and vegetation management

In addition to more sustainable pesticide management, improving ground vegetation and tillage practices can also promote biodiversity (Winter *et al*. 2018; Paiola *et al*. 2020; Ortis *et al*. 2021; Blaise *et al*. 2022). Diverse vegetation cover, including flower-rich cover crops, facilitates resources like nectar and pollen for various organisms, especially pollinators (Westrich 2018). Understanding how different vegetation management practices support arthropod diversity is vital for biodiversity conservation. Moreover, landscape composition plays a significant role in shaping species diversity (Bengtsson *et al*. 2005). The loss and fragmentation of SNH within intensively managed agricultural landscapes pose threats to biodiversity (Brown and Paxton 2009). A heterogeneous landscape with large proportions of SNH near agricultural areas promotes various organisms (Martin *et al*. 2019; Kolb *et al*. 2020; Paiola *et al*. 2020; Ostandie *et al*. 2021; Tscharntke *et al*. 2021). Habitats like forests, hedges, shrubs, and grasslands offer shelter, overwintering sites, and food resources for species that may not find these within vineyards (Holland *et al*. 2017). Therefore, investigating how a high proportion and connectivity of SNH enhance species becomes relevant. This is especially important for promoting beneficial insects in viticulture like parasitoids, predators,

antagonists, and pathogens of pests that can enhance natural pest control (Tscharntke *et al*. 2021).

1.1.5 Research objectives and outline

The first objective of my dissertation thesis is to examine the potential of metabarcoding as a powerful tool for ecological studies and long-term monitoring in viticulture. This method may offer a solution to the time-consuming taxonomic identification of diverse arthropod communities. To accurately evaluate long-term monitoring data, however, it is essential to understand the influences that affect the biodiversity recorded during sampling and thus to be able to differentiate long-term trends from short-term influences. Therefore, in **Chapter 2** of this thesis, we investigate the impact of ambient weather conditions during Malaise trap exposure and trapping effort on biomass and taxonomic richness in vineyards. We evaluate the trapping duration and the number of traps needed to comprehensively record the arthropod diversity. Additionally, because the effectiveness of metabarcoding depends on the coverage of taxa in DNA databases, we evaluate how well they cover viticultural biodiversity.

In the following three chapters of this thesis, we assess the effects of major factors that influence arthropod diversity in viticulture, a cropping system characterized by extensive pesticide use. The impacts of pesticide management vary among organism groups, depending on their sensitivity and exposure to pesticides. Similarly, vegetation management practices and the presence of SNH in the surrounding landscape have varying effects on different organism groups. Thus, a thorough understanding of the impacts of pesticide and vegetation management as well as landscape heterogeneity on vineyard biodiversity necessitates to investigate the differential effects on various organism groups. From this understanding, adapted measures can be derived that subsequently contribute to biodiversity conservation.

Our research involves a crossed design with both FRG and classic grape varieties either cultivated in organically or conventionally managed vineyards. The pairs of vineyards were located along a gradient of landscape composition with increasing proportions of SNH. This design allows for a comprehensive assessment of the effects of viticulture on biodiversity.

Some recent studies have investigated the effects of vineyard management and landscape heterogeneity on ground-dwelling and less mobile species, such as spiders, beetles, and mites. In **Chapter 3**, we complement these studies by focusing on more mobile species, primarily capturing flying insects using Malaise traps, and utilizing metabarcoding for taxa identification. Using our crossed study design, we investigate how vineyard management and the surrounding landscape affect arthropod biomass, taxa richness, and community composition. We differentiate between the effects of the studied factors on the orders Diptera, Coleoptera, Hemiptera, Hymenoptera, and Lepidoptera.

Because metabarcoding does not yet provide the ability to examine abundances of individual species, in **Chapter 4** of this thesis, we focus on wild bees as an ecological indicator group among the highly mobile organism groups. We used yellow pan traps to sample wild bees in the studied vineyards and investigate the influence of viticulture on their abundance, richness, and composition.

Furthermore, in **Chapter 5**, we focus on herb- and vine-dwelling orthopterans as additional ecological indicators representing a less mobile group of organisms. We used box quadrats to assess the density of Caelifera in the inter-rows of the vineyards and conducted transect walks with species-specific song detection for the identification of Ensifera within the vines. Like the previous two chapters, our crossed study design enabled us to examine the effects of vineyard management and the surrounding landscape on the abundance and composition of orthopterans.

Chapter 6 comprises the results and conclusions of the previous chapters in a general discussion and outlook.

The chapters of this thesis consist of four scientific manuscripts (Figure 1.1), with three already published and one intended for future publication. These manuscripts have been written with co-authors, and the individual contributions of each author are detailed in the thesis appendix.
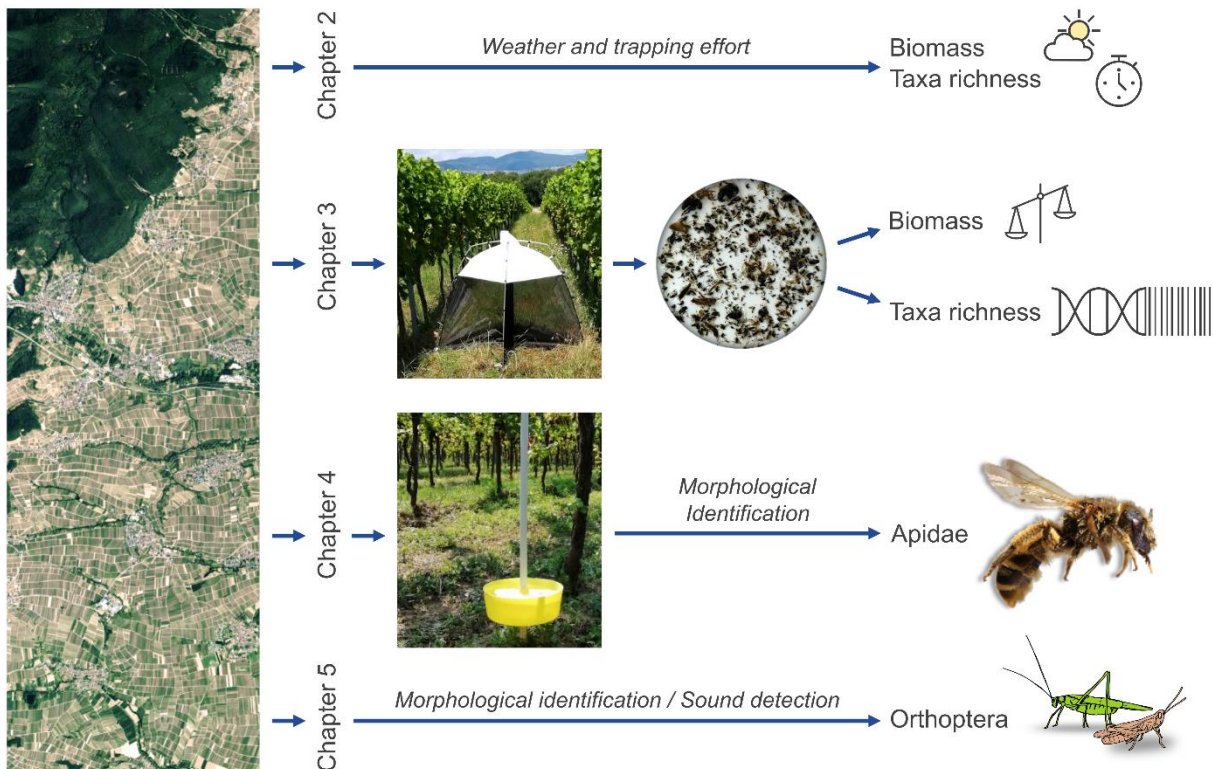
Figure 1.1 Outline of the thesis with the methods used in chapters 2 to 5. Map on the left by ©GeoBasis-DE/LVermGeoRP (2022).

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CHAPTER 2

Using Malaise Traps and Metabarcoding for Biodiversity Assessment in Vineyards: Effects of Weather and Trapping Effort

Marvin Kaczmarek, Martin H. Entling & Christoph Hoffmann

Abstract

Metabarcoding is a powerful tool for ecological studies and monitoring that might provide a solution to the time-consuming taxonomic identification of the vast diversity of insects. Here, we assess how ambient weather conditions during Malaise trap exposure and the effort of trapping affect biomass and taxa richness in vineyards. Biomass varied by more than twofold with weather conditions. It increased with warmer and drier weather but was not significantly related with wind or precipitation. Taxa richness showed a saturating relationship with increasing trapping duration and was influenced by environmental and seasonal effects. Taxa accumulation was high, increasing fourfold from three days of monthly trap exposure compared to continuous trapping and nearly sixfold from sampling at a single site compared to 32 sites. The limited saturation was mainly due to a large number of singletons, such as rare species, in the metabarcoding dataset. Metabarcoding can be key for long-term insect monitoring. We conclude that single traps operated for up to ten days per month are suitable to monitor the presence of common species. However, more intensive trapping is necessary for a good representation of rare species in biodiversity monitoring. The data collected here can potentially guide the design of monitoring studies.

2.1 Introduction

Terrestrial insects have strongly declined during recent decades, with important consequences for the functioning of the world's ecosystems (Hochkirch 2016; Hallmann *et al*. 2017; Sánchez-Bayo and Wyckhuys 2019; Seibold *et al*. 2019; Wagner 2020). A significant loss of biodiversity and biomass is reported, the reasons for which are attributed to habitat destruction, intensified agriculture, invasive organisms, and climate change (Sánchez-Bayo and Wyckhuys 2019). To be able to record the further course of these trends and the reasons responsible for them, systematic monitoring of terrestrial insects, as it is performed in only a few monitoring programs (Welti *et al*. 2012; Geiger *et al*. 2016; Karlsson *et al*. 2020; Lehmann *et al*. 2021), is essential. However, in addition to the long-term influences of, e.g., agriculture and climate change, environmental and methodological conditions during sampling could have a direct effect on the insect diversity collected and should thus be considered when evaluating data from ecological surveys (Skvarla *et al*. 2021).

Insect activity depends on the season and ambient weather conditions (Juillet 1964; Bergman *et al*. 1996; Kirse *et al*. 2021). While warm, dry weather can promote activity, especially in the summer months, it can be reduced in cold and rainy weather (Kasper *et al*. 2008; Welti *et al*. 2012; Kirse *et al*. 2021). Most flying insects are trapped at hot and sunny conditions after it has rained, although there are differences among taxa (Juillet 1964; Matthews and Matthews 1970). Nevertheless, short-term weather conditions only affect activity during the event. Long-term changing temperature or precipitation patterns due to climate change, however, have a lasting impact on insect populations (Halsch *et al*. 2021). For example, flight activity can decrease at above-average temperatures in summer (Kasper *et al*. 2008; Welti *et al*. 2012).

Long-term insect monitoring can quantify trends in biomass, species richness, species composition, and species abundance and allow conclusions about the reasons for changes by including environmental parameters (Conrad *et al*. 2007). Because of the vast diversity of

insects, the large number of trapped individuals, and the thus time-consuming and costly identification of species, long-term studies, especially when based on morphological species identification, usually either depend on adequate funding and high effort or are limited to some of these proxies or focus on indicator groups (Thomas 2005; Hausmann *et al.* 2020; Karlsson *et al.* 2020). Additionally, reducing the monthly effort, i.e., shortening the sampling period, may be useful from both a conservation and economic perspective to reduce environmental impacts and costs (Conrad *et al.* 2007). In any case, the selection of the trapping method already turns the focus on certain species groups (Skvarla *et al.* 2021).

Arthropods can be trapped with a large variety of trap types, including pitfall traps, suction traps, window traps, pan traps, bait traps, light traps, and Malaise traps (Yi *et al.* 2012; Henderson and Southwood 2021). Malaise traps are a widely used trap type in biodiversity surveys and monitoring because they are easy to handle and capture a huge variety of flying insects and also wingless arthropods, with Diptera and Hymenoptera being by far the most-collected taxa (Malaise 1937; Hausmann *et al.* 2020; Skvarla *et al.* 2021). The large quantity of insects caught in Malaise traps, however, makes it laborious to process bulk samples (Karlsson *et al.* 2020). Time-intensive species identification often relies on the few available experts, a problem known as the taxonomic impediment (Giangrande 2003). Metabarcoding can be a solution to the challenge of the high time required for taxonomic identification by identifying taxa in a time- and cost-efficient way (deWaard *et al.* 2019).

Using high-throughput sequencing, metabarcoding combines DNA sequences in the region of the cytochrome c oxidase I (COI) gene of similar specimens into operational taxonomic units (OTUs; Ratnasingham and Herbert 2013). OTUs can be assigned to barcode index numbers (BINs) by comparison with reference sequences in the Barcode of Life Data System (BOLD; Ratnasingham and Herbert 2013). BINs allow a taxonomic assignment based on reference sequences. The proportion of OTUs that can be assigned to BINs or species depends on the coverage of species in the databases. Even though not all BINs are assigned to Linnean names, they still often correspond well to the species level (deWaard *et al.* 2019). Thus, BINs can be a good proxy for species diversity to derive trends also for arthropod groups that are not well covered in the BOLD library (Morinière *et al.* 2019). If BINs have an assignment to species, the comparison with red lists also allows short-term conclusions about the occurrence of endangered or invasive species (Svenningsen *et al.* 2021). A disadvantage of metabarcoding is that, in contrast to morphological species identification and counting of individuals, no quantified species abundance is recorded (Liu *et al.* 2020). Thus, no accurate conclusion can be made about the abundance of individual species in single samples (Elbrecht and Leese 2015). For replicate samples, the relative abundance can be derived from the frequency of species occurrence (Aizpurua *et al.* 2018; Morinière *et al.* 2019). In addition, there are promising approaches to estimate the relative abundance of a species based on the reads of DNA sequences in a sample (Krehenwinkel *et al.* 2017; Piñol *et al.* 2019; Kennedy *et al.* 2020).

In this study, we used Malaise traps and metabarcoding to collect and identify insects in vineyards in southwest Germany. As part of a larger effort to establish an insect monitoring program for viticulture, our first aim was to assess if biomass in southwest German viticulture

is affected in a comparable way by environmental conditions as it has already been demonstrated in other ecosystems (Juillet 1964; Matthews and Matthews 1970; Bergman *et al*. 1996; Kasper *et al*. 2008; Welti *et al*. 2012; Kirse *et al*. 2021). We tested the following hypothesis: (H1) (a) cool temperatures, (b) precipitation, and (c) wind reduce the biomass of trapped insects. The question if vineyards are saturated or unsaturated ecosystems is tested in the two further hypotheses: (H2) (a) taxa richness and (b) cumulative taxa richness show a saturating relationship with trapping duration; and (H3) a larger number of trapping sites increase cumulative taxa richness.

2.2 Materials and methods

2.2.1 Study area

Our study area is located in the German wine-growing region Palatinate (Figure 2.1), which has a warm temperate climate with warm summers, an average annual temperature of 11.1 °C, and a total annual precipitation of 677.7 mm (Beck *et al*. 2018; Agrarmeteorologie Rheinland-Pfalz 2022). We sampled locally in the vineyards of the Julius Kühn Institute (JKI) in Siebeldingen (49.218350° N, 8.045650° E, Rhineland-Palatinate, Germany) and regionally in 32 vineyards in the surrounding area (49.273280° N, 8.020602° E/49.147516° N, 8.175736° E, Rhineland-Palatinate, Germany).

Figure 2.1 Study area with the location of the Julius Kühn institute (JKI) and the 32 regional sampling sites (left, map data by OpenStreetMap, under ODbL) and the institutes area with the location of the four local sampling sites (right, image data by © GeoBasis-DE/LVermGeoRP (2022)). The arrow indicates the study area in Rhineland-Palatinate on the map of Germany.

2.2.2 Sampling

We conducted the local sampling in four vineyards in the institute area. In each vineyard, we installed one Malaise trap (standard SLAM trap, MegaView Science Co., Ltd., Taichung, Taiwan) from 4 June to 2 October 2021. We filled collecting bottles with 300 ml ethanol denatured with about 1 % methyl ethyl ketone (EtOH MEK) and changed them at least every

five days to preserve already-trapped material. Ethanol was subsequently replaced in all samples. The sampling period was divided into four 30-day cycles with four trapping intervals each. Each cycle, we collected insect material on the 3rd, 8th, 16th, and 30th day, resulting in intervals of 3, 5, 8, and 14 trapping days per month, respectively. For each of the four vineyards, we pooled the material from the four 30-day cycles for each duration of trapping, resulting in four bulk samples with a total trapping duration of 12, 20, 32, and 56 trapping days, respectively.

We conducted the regional sampling in 32 vineyards and sampled two years from April to September in 2020 and 2021. Each month, we installed one Malaise trap (first three months Malayse traps with a combination of black and white net, ENTO SPHINX s.r.o., Pardubice, Czech, from then on standard SLAM traps) for three consecutive days in each vineyard, resulting in a total of 36 trapping days per site. Collecting bottles were filled with 300 ml of 70 % EtOH MEK, and collected material was stored in undiluted EtOH MEK. We pooled the material for each site of each year, resulting in two bulk samples per vineyard and 64 samples in total.

2.2.3 Environmental conditions
We retrieved daily environmental data for temperature, radiation, precipitation, air humidity, and wind speed from a weather station, which is located in the institute area (Agrarmeteorologie Rheinland-Pfalz 2022). We then calculated the mean of the variables for each trapping interval of the local sampling using the daily mean for temperature, air humidity, and wind speed and the daily total for radiation and precipitation (Table 2.1).

Table 2.1 Mean of daily meteorological conditions at trapping intervals during the experiment. Minimal, maximal, mean value, and SD for the environmental variables.

Variable	Unit	Min	Max	Mean	SD
Temperature	Degrees Celsius (°C)	13.9	22.1	18.2	1.7
Radiation	Watt hours per square meter (Wh/m^2)	2,956	7,822	4,918	1,240
Precipitation	Millimeters (mm)	0	9	2.9	2.6
Air humidity	Percent (%)	63.4	88.7	79.3	6.2
Wind speed	Meters per second (m/s)	0.4	1.5	1.1	0.3

2.2.4 Biomass
For the local sampling, we weighed the wet biomass material of each trap for each interval after placing it in a sieve and letting the liquid drip off (Table S2.1). Liquid at the bottom of the sieve was additionally dapped on a paper tissue.

2.2.5 Taxa richness
DNA metabarcoding and bioinformatics (using VSEARCH *v.2.9.1* (Rognes *et al*. 2016), Cutadapt *v.1.18* (Martin 2011), and Geneious *v.10.2.5* (Biomatters, Auckland, New Zealand)) of the 16 bulk samples of the local sampling and the 64 bulk samples of the regional sampling were conducted by AIM (Advanced Identification Methods GmbH) following the methods of Hausmann *et al*. (2020) and Morinière *et al*. (2016) (Supplementary methods), with species identification based on high-throughput sequencing (HTS) data grouped to genetic clusters

(OTUs), blasted, and assigned to BINs and species. We filtered the results table for OTUs with a Hit-%-ID value in BOLD ≥ 97 % and an assignment to a BIN and condensed BINs that occurred more than once into one entry. We then filtered the results table for BINs with an assignment to a species and condensed species that occurred more than once into one entry. For the regional sampling, we condensed BIN lists of the two years for each of the 32 vineyards. The numbers of BINs were used as a value for taxa richness (Table S2.2, Table S2.3). According to their occurrence in the four vineyards of the local sampling and the 32 vineyards of the regional sampling, respectively, BINs were classified into subsets with taxa with high (caught at more than three-quarters of the sites), medium (caught at more than one and up to three-quarters of the sites), and low (caught at up to one-quarter of the sites).

2.2.6 Data analysis

All analyses were conducted using R *v.4.0.4* (R Core Team 2021) and RStudio *v.1.2.5033* (RStudio Team 2019) with the R packages car (Fox and Weisberg 2019) for performing linear regressions and ggplot2 (Wickham 2016) and vegan (Oksanen *et al*. 2020) for creating figures. Additionally, we used Inkscape *v.1.0.2-2* (Inkscape 2020) for creating figures. We explored the data for distribution patterns. We investigated the effect of the environmental variables on the daily biomass and the effect of the trapping duration on the taxa richness and the accumulated taxa richness, including the presence subsets, by performing linear regression analyses with type III ANOVA using a significance level of $P < 0.05$. Due to correlations between temperature, radiation, and air humidity as well as precipitation and wind speed, we used separated models for each environmental variable (Figure S2.1).

2.3 Results

2.3.1 Biomass

Daily biomass was influenced by temperature ($F = 7.5$, $df = 1$, $P = 0.016$, Table 2.2), radiation ($F = 15.8$, $df = 1$, $P = 0.001$), and air humidity ($F = 11.1$, $df = 1$, $P = 0.005$), with temperature and radiation positively associated and air humidity negatively associated with daily biomass (Figure 2.2). Precipitation ($F = 0.0$, $df = 1$, $P = 0.884$) and wind speed ($F = 0.0$, $df = 1$, $P = 0.846$) had no significant effect on daily biomass.

2.3.2 Taxa richness

We obtained a total of 1,494 OTUs from metabarcoding of the local sampling, which were assigned to 836 BINs (Table 2.3). BINs were assigned to 18 orders, 157 families, and 461 species (Table S2.4). The orders Diptera (43.2 %), Hymenoptera (14.5 %), Coleoptera (13.9 %), Lepidoptera (11.5 %), and Hemiptera (10.6 %) accounted for the largest proportions of BINs. The duration of monthly trapping affected the number of total BINs ($F = 7.2$, $df = 1$, $P = 0.018$, Table 2.2) and taxa with high ($F = 9.5$, $df = 1$, $P = 0.008$) and medium presence ($F = 7.2$, $df = 1$, $P = 0.018$). For low presence taxa, we observed no significant effect of the monthly trapping duration ($F = 3.7$, $df = 1$, $P = 0.075$). Taxa richness increased by twofold from three to eight days of monthly trapping with no further increase to 14 days (Figure 2.3A), and higher proportions of total BINs were captured for high presence taxa compared to medium and low presence taxa (Figure 2.3B–D).

Table 2.2 Results table of linear regression analyses with type III ANOVA for the effect of the environmental variables temperature, radiation, precipitation, air humidity, and wind speed on daily biomass and for the effect of monthly trapping duration on taxa richness and accumulated taxa richness with *F*-value, degrees of freedom (*df*), and *P*-value. The effects on taxa richness and accumulated taxa richness are also given for subsets of high (taxa caught at 4 sites), medium (taxa caught at 2 or 3 sites), and low presence (taxa caught at 1 site). Bold letters indicate significant effects.

	Dependent Variable	Explanatory Variable	*F*-value	*df*	*P*-value
Environment	Biomass/day	**Temperature**	7.5	1	**0.016**
	Biomass/day	**Radiation**	15.8	1	**0.001**
	Biomass/day	Precipitation	0	1	0.884
	Biomass/day	**Air humidity**	11.1	1	**0.005**
	Biomass/day	Wind speed	0	1	0.846
Taxa richness	BINs	**Trapping duration**	7.2	1	**0.018**
	BINs (High presence)	**Trapping duration**	9.5	1	**0.008**
	BINs (Medium presence)	**Trapping duration**	7.2	1	**0.018**
	BINs (Low presence)	Trapping duration	3.7	1	0.075
Accumulated richness	BINs	**Trapping duration**	71.4	1	**< 0.001**
	BINs (High presence)	**Trapping duration**	43.6	1	**< 0.001**
	BINs (Medium presence)	**Trapping duration**	85.1	1	**< 0.001**
	BINs (Low presence)	**Trapping duration**	47.7	1	**< 0.001**
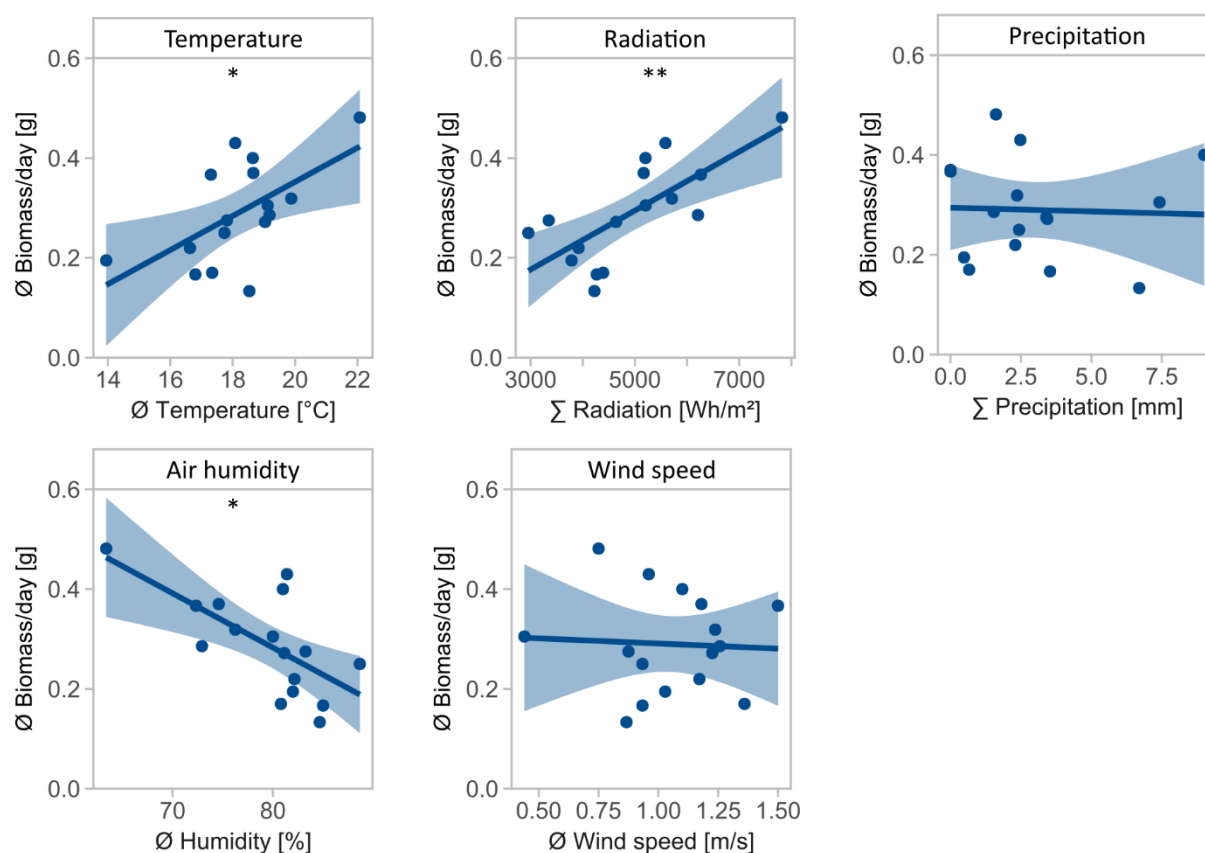
Figure 2.2 Mean daily biomass in g for environmental variables temperature in °C, radiation in Wh/m2, precipitation in mm, air humidity in %, and wind speed in m/s. Asterisks indicate significant effects of environmental variables on daily biomass (significance codes: **$P < 0.01$, *$P < 0.05$).

Table 2.3 Number of barcode index numbers (BINs) assigned to the 1,494 operational taxonomic units (OTUs) of the local and the 3,245 OTUs of the regional sampling and number of families and species assigned to BINs for the most common orders. Proportion of total in % is given in brackets.

Order	Local Sampling			Regional Sampling		
	BINs	Families	Species	BINs	Families	Species
Diptera	361 (43.2)	43 (27.4)	165 (35.8)	664 (38.0)	51 (22.5)	321 (31.5)
Hymenoptera	121 (14.5)	19 (12.1)	60 (13.0)	383 (21.9)	37 (16.3)	201 (19.7)
Coleoptera	116 (13.9)	25 (15.9)	94 (20.4)	262 (15.0)	36 (15.9)	211 (20.7)
Lepidoptera	96 (11.5)	24 (15.3)	68 (14.8)	198 (11.3)	41 (18.1)	143 (14.0)
Hemiptera	89 (10.6)	17 (10.8)	45 (9.8)	154 (8.8)	24 (10.6)	96 (9.4)
Others	53 (6.3)	29 (18.5)	29 (6.3)	87 (5.0)	38 (16.7)	48 (4.7)
Total	836	157	461	1748	227	1020
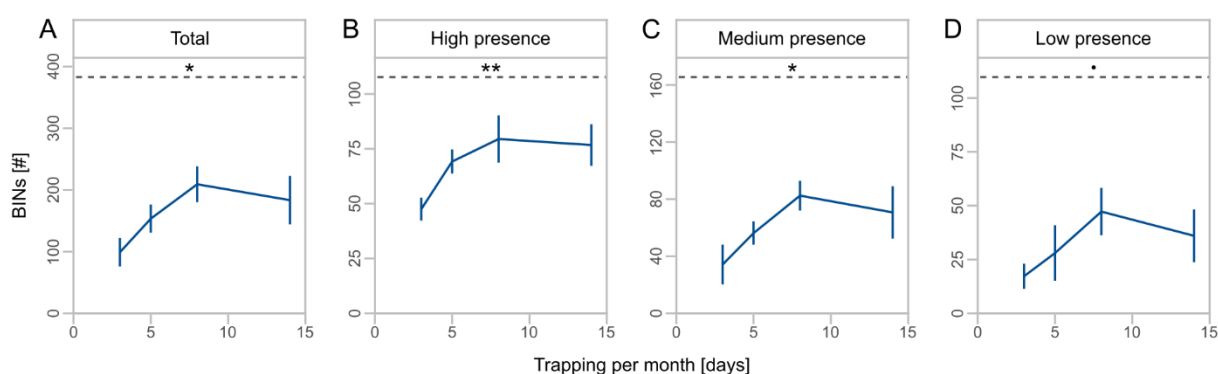
Figure 2.3 Mean number of barcode index numbers (BINs) ± SD for different days of monthly trapping for total BINs (A) and subsets (B–D) with high (taxa caught at 4 sites), medium (taxa caught at 2 or 3 sites), and low presence (taxa caught at 1 site). Asterisks indicate significant effects of monthly trapping duration on number of BINs (significance codes: **$P < 0.01$, *$P < 0.05$, •$P < 0.1$). Dashed lines indicate the mean total number of BINs. Note different scale of y-axes.

The cumulative number of BINs ($F = 71.4$, $df = 1$, $P < 0.001$) and taxa with high ($F = 43.6$, $df = 1$, $P < 0.001$), medium ($F = 85.1$, $df = 1$, $P < 0.001$), and low presence ($F = 47.7$, $df = 1$, $P < 0.001$) increased with increasing duration of trapping (Table 2.2). The number of BINs was nearly four times greater at 30 days of monthly trapping than at 3 days (Figure 2.4A) but differed for subsets with decreasing saturation from high to low presence taxa (Figure 2.4B–D).

We obtained a total of 3,245 OTUs from the metabarcoding of the regional sampling, which were assigned to 1,748 BINs (Table 2.3). BINs were assigned to 19 orders, 227 families, and 1,020 species, with the orders Diptera (38.0 %), Hymenoptera (21.9 %), Coleoptera (15.0 %), Lepidoptera (11.3 %), and Hemiptera (8.8 %) accounting for the largest proportions of BINs (Table S2.5). The cumulative number of BINs increased with the number of sites, but with a slight saturation effect (Figure 2.5A). BIN numbers for high presence taxa reached saturation at about three traps (Figure 2.5B) and for medium presence taxa at about eight traps (Figure 2.5C). For rare species, we observed almost no saturation effect (Figure 2.5D). In total, 75 % of BINs were recorded using at least 17 traps. For high, medium, and low presence taxa, 75 % of BINs were recorded using at least one, three, and 19 traps, respectively.
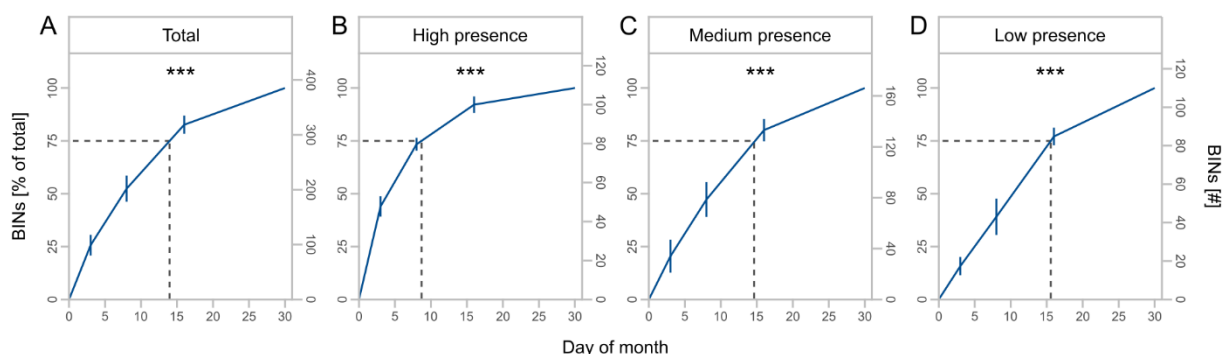
Figure 2.4 Mean cumulative proportion and number of BINs ± SD at different days of monthly trapping for total BINs (A) and presence subsets (B–D) with high (taxa caught at 4 sites), medium (taxa caught at 2 or 3 sites), and low presence (taxa caught at 1 site). Asterisks indicate significant effects of monthly trapping duration on number of BINs (significance code: *** $P < 0.001$). Dashed lines indicate the day of month at 75 % of the total BINs.
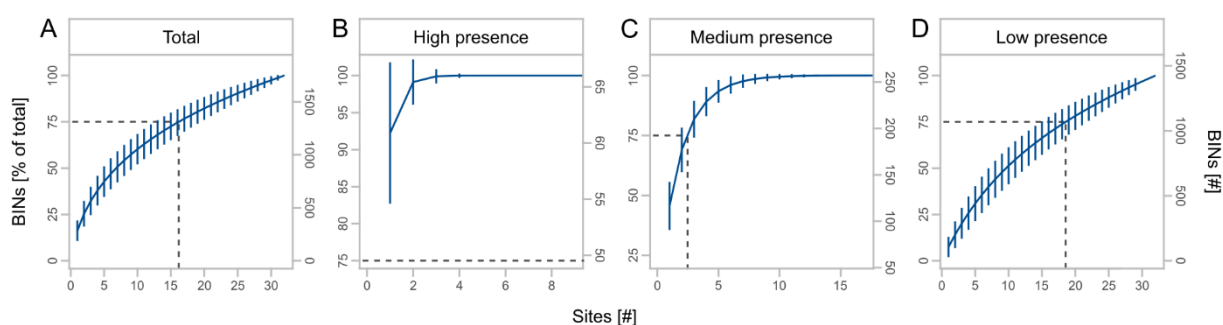
Figure 2.5 Mean cumulative proportion and number of BINs ± SD at different number of sites for total BINs (A) and subsets (B–D) with high (taxa caught at 25 to 32 sites), medium (taxa caught at 9 to 24 sites), and low presence (taxa caught at 1 to 8 sites). Dashed lines indicate the number of sites at 75 % of the total BINs. Note different scale of x- and y-axes.

2.4 Discussion

Biomass increased with higher temperature and radiation levels, which corresponds to our first hypothesis (H1a), where we expected biomass to decrease with cool temperatures. Temperature and radiation are strongly coupled, in particular during the summer period (Bristow and Campbell 1984; Makowski *et al*. 2009). Both were observed to positively influence insect activity rates in earlier studies (Bergman *et al*. 1996; Welti *et al*. 2012; Hallmann *et al*. 2017), with ambient temperature having a direct effect on body temperature and thus flight ability (Stone and Willmer 1989). However, other studies reported that at above-average temperatures in the summer months, the linear relationship breaks down and activity decreases (Kasper *et al*. 2008; Welti *et al*. 2012), so that a temperature optimum curve could actually have been expected. We did not observe such a decrease at high temperatures, presumably because temperatures in the study year of the local sampling were rather moderate compared to the three preceding years (Agrarmeteorologie Rheinland-Pfalz 2022).

In contrast to the second part of our first hypothesis but similar to Welti *et al*. (2012), biomass did not decrease with precipitation (H1b). Although rain events reduce flight activity in various insects (Kasper *et al*. 2008), the effect of short-term weather conditions becomes less

apparent when considering multi-day trapping intervals (Matthews and Matthews 1970). However, biomass decreased with increasing air humidity. While we have found a negative effect of air humidity on biomass, other studies have found both positive and negative effects for different groups or species of insects (Juillet 1964; Contreras *et al*. 2013). Air humidity, however, was not at extreme levels during data collection, which can cause a clear reduction in catches (Juillet 1964). In addition, air humidity correlates with temperature and radiation, so that the decrease of catches with increasing high air humidity in our study may be a consequence of reduced temperature or radiation at high air humidity rather than a direct effect of air humidity itself.

Contrary to what we hypothesized, wind speed was not affecting biomass (H1c). Mean wind speed was generally low in the present study, with daily means not exceeding 2 m/s throughout data collection of the local sampling and not affecting flight activity as it did in other studies (Juillet 1964). The daily maximum wind speed reached values of more than 10 m/s during sampling (Agrarmeteorologie Rheinland-Pfalz 2022). As with rain, however, we expect stronger wind to be a short-term event and therefore less apparent at multi-day trapping intervals.

Almost 60 % of the OTUs could be assigned to BINs, and more than 30 % could be assigned to species. The distribution of BINs among orders is comparable to that of other studies with Malaise traps (Skvarla *et al*. 2021). Despite several projects to record German insects in the DNA barcode libraries (Hausmann *et al*. 2011a; Hausmann *et al*. 2011b; Spelda *et al*. 2011; Morinière *et al*. 2014; Raupach *et al*. 2014; Hendrich *et al*. 2015; Schmidt *et al*. 2015; Wesener *et al*. 2015; Astrin *et al*. 2016; Raupach *et al*. 2016; Hawlitschek *et al*. 2017; Morinière *et al*. 2017; Rulik *et al*. 2017; Schmidt *et al*. 2017; Havemann *et al*. 2018; Raupach *et al*. 2018; Morinière *et al*. 2019; Schmid-Egger *et al*. 2019), these results underline that there still is a need for further sequencing work to provide more comprehensive databases to match OTUs to BINs and species and by that to increase the informative value of monitoring programs and insect surveillance. Noticeably, only about half of the BINs of Hymenoptera and Diptera could be assigned to a species, while the proportion is higher for other insect orders such as Lepidoptera and Coleoptera. In addition to species not yet recorded in DNA barcode libraries, this can also be attributed to a higher proportion of undescribed species and "dark taxa" in these hyperdiverse orders (Geiger *et al*. 2016; Page 2016; Morinière *et al*. 2019). Despite the current lack of species linked to reference sequences, the use of metabarcoding in long-term monitoring offers an advantage herein, as archived raw sequence data can be quickly reprocessed with updated databases. Today's undescribed species, "dark taxa" without scientific names in the databases, or species whose taxonomic classification will change can be included in future analyses.

We expected taxa richness to increase with increasing trapping duration (H2a), which was only the case up to a certain value. After a duration of eight days, there was no further increase of BINs. The lack of increase can be explained in part by a saturating effect of more common species that are caught in all trapping intervals. In addition, the environmental and seasonal conditions presumably reduced flight activity, especially in the 14-day interval compared to

the 8-day interval, as the average temperature was much lower in three of the four 30-day cycles (Figure S2.2). Seasonal conditions generally changed toward the end of data collection, and a decline in activity of many species toward fall and winter likely reduced the taxa richness in the later trapping intervals (Kirse *et al*. 2021), with trapping of rare species appearing to be more affected here.

Consistent with the second part of our second hypothesis (H2b), taxa accumulation was high with increasing monthly trapping duration. While a clear saturating effect can be seen for more common taxa, this effect increasingly weakens to a barely flattening accumulation curve for low presence taxa. As a result, complete saturation is not evident in the total number of BINs either, as it has similarly been reported in previous studies (Fraser *et al*. 2008; Geiger *et al*. 2016; Steinke *et al*. 2021). Common species are likely caught with short trapping durations, so a flattening of the accumulation curve was expected. However, some species are generally less likely to be caught, such as rare and transient or non-flying species, and are thus infrequently captured in traps (Steinke *et al*. 2021). In addition, Malaise traps mainly catch actively flying insects, mainly from the orders Diptera and Hymenoptera (Skvarla *et al*. 2021). The flight period of some species can be less than one month (Noordijk *et al*. 2008), which reduces the likelihood of catching these species during short trapping periods. Yet, the non-flattening accumulation curve for rare taxa shows that even with continuous trapping duration, not all of these species can be captured.

Cumulative taxa richness increased with a greater number of sampled sites, as expected in our last hypothesis (H3), but without a clear saturation effect on the total number of BINs. As with local sampling, rare species cannot be comprehensively detected even with excessive sampling effort, whereas for more common species sampling at three to ten sites can be sufficient to record regionwide-distributed species. However, due to the higher beta diversity in the region (Keil *et al*. 2012), the more common species accounted for only a small proportion of the total species, and species classified as low presence taxa may be common at one site but infrequently distributed across the landscape. Comprehensively detecting species classified as those with a higher presence at local scale can hence require a higher sampling effort at regional scale.

2.5 Conclusions

We showed how the sampling effort is affecting biomass and the recorded biodiversity through weather conditions, trapping duration, and sampled sites. Biomass is the highest on warm and dry days, which increased taxa richness within Malaise trap samples. More than three-quarters of species at a site can be detected by trapping for half a month, and sampling at only three to ten sites can be sufficient to capture regionwide-distributed species. While common species in vineyards can be extensively surveyed with less effort, a complete survey of rare taxa requires high effort due to low saturation. Metabarcoding can provide comprehensive species lists and thus be an answer to the problem of time-consuming morphological identification, especially for long-term monitoring where archived data can be reprocessed with updated DNA barcode libraries. Samples from Malaise traps, however, consist of a large extent of single taxa that can be, e.g., transient or low abundant species. As

recommended by Steinke *et al*. (2021), research on the origin of these singletons should be considered in future biodiversity surveys. Here, long-term monitoring could contribute to a better understanding by providing long-term data on the recurrent abundance of single taxa at a sampling site.

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CHAPTER 3

Differentiating the effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture

Marvin Kaczmarek, Martin H. Entling & Christoph Hoffmann

Abstract

Biodiversity loss is a main challenge for agricultural sustainability. Major drivers include local management and landscape simplification. Therefore, conservation measures aim to increase organic agriculture, reduce pesticide use, and increase the proportion of semi-natural habitats (SNH). Yet, it is important to understand the effects of such measures. We investigate how arthropod biomass, taxa richness, and community composition in Malaise trap samples are affected by organic management, pesticide use, and SNH in the landscape. The 32 studied vineyards were chosen in a crossed design of management (organic vs. conventional) and pesticide use (regular vs. reduced) along a gradient of landscape composition. Pesticide reduction by 55 % was obtained by including half of the vineyards with fungus-resistant grape (FRG) varieties. Malaise trap samples were weighed and arthropods identified using metabarcoding. Surprisingly, biomass was almost one-third higher in conventionally managed vineyards compared to organic ones. Taxa richness increased by more than one third when the proportion of SNH in a radius of 1,000 m in the surrounding landscape increased from zero to 50 %. Diptera richness tended to be 4 % higher in conventionally managed vineyards and the richness of Hymenoptera was 9 % higher in FRG varieties. Community composition changed with the proportion of SNH and differed between organic and conventional management. Overall, organic viticulture was not effective to enhance the arthropod community, which was dominated by flying insects in our study. Agricultural policies should therefore rather preserve and promote SNH in the surrounding in order to promote arthropod biodiversity in viticultural landscapes.

3.1 Introduction

In the past decades, arthropods have declined strongly in many landscapes (Hochkirch 2016; Hallmann *et al*. 2017; Seibold *et al*. 2019). One of the main drivers for the loss of biomass and biodiversity is considered to be intensive agriculture, including the use of pesticides and fertilizers (Sánchez-Bayo and Wyckhuys 2019). Policy measures, such as the European Green Deal, include increasing the share of organic agriculture, reducing pesticide use, and increasing the proportion of semi-natural habitats (SNH) in agricultural landscapes (European Commission 2019). It is assumed that these measures enhance biodiversity to counteract the strong decline in recent years (Bengtsson *et al*. 2005). However, effects may vary between and within organism groups and between crops (Bengtsson *et al*. 2005; Hole *et al*. 2005). So it is important to understand the differential impacts of such changes in agricultural practices.

Organic farming can promote biodiversity compared to conventional farming (Bengtsson *et al*. 2005; Hole *et al*. 2005; Caprio *et al*. 2015; Beaumelle *et al*. 2023). Hence, the European Green Deal's Farm to Fork Strategy targets, for example, to increase the share of organic farming to at least 25 % by 2030 (European Commission 2019). In viticulture, at least in regions with no insecticide use, the effects of organic management appear, however, to be less pronounced compared to other cropping systems (Döring *et al*. 2019; Paiola *et al*. 2020). The occurrence of fungal diseases, particularly powdery mildew and downy mildew, causes one of the highest use of plant protection products in agriculture (Pertot *et al*. 2017). Their presence thus necessitates a high use of either synthetic chemicals in conventional viticulture or

inorganic compounds such as copper and sulfur in organic viticulture, both of which may affect non-target organisms (Pedneault and Provost 2016; Vogelweith and Thiéry 2018).

Under the Farm to Fork Strategy, efforts are made to reduce the pesticide use in order to mitigate environmental pollution (European Commission 2019). The cultivation of fungus-resistant grape (FRG) varieties allows a reduction of pesticide use by more than 80 % due to resistance traits against fungal diseases (Pedneault and Provost 2016). It was recently reported that the cultivation of FRG varieties thus has positive effects on non-target organisms (Pedneault and Provost 2016), promotes the presence of predatory mites over pest mites, and positively affects certain spider families (Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021a). The promotion of such beneficial species may affect trophic interactions that are important to winegrowers by improving for example natural pest control (Winkler *et al*. 2017; Reiff *et al*. 2021a).

In addition to management practices, the landscape may play an important role for species diversity and composition (Bengtsson *et al*. 2005). A heterogeneous landscape with high proportions of SNH in the surrounding area of agricultural land is reported to promote various organisms (Martin *et al*. 2019; Kolb *et al*. 2020; Tscharntke *et al*. 2021). Therefore, it is recommended and targeted, e.g., by the Biodiversity Strategy of the European Green Deal (European Commission 2019), to increase the proportion of SNH (Martin *et al*. 2019). Furthermore, high cover and connectivity of non-crop habitat may improve conditions for beneficial organisms such as parasitoids, predators, antagonists, and pathogens of pests in agricultural landscapes (Tscharntke *et al*. 2021).

Arthropod communities can be comprehensively analyzed using metabarcoding by identifying specimens based on DNA sequences in the cytochrome c oxidase I (COI) gene and clustering similar sequences into operational taxonomic units (OTUs) that can be assigned to barcode index numbers (BINs) based on reference sequences in the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2013). In this way, metabarcoding allows the study of entire arthropod communities, including the highly diverse orders of Diptera and Hymenoptera that account for large proportion of species in, for example, Malaise trap samples (Skvarla *et al*. 2021). Furthermore, metabarcoding may be a valuable tool for long-term monitoring, as taxa can be identified in a time- and cost-efficient way and data effectively stored and shared for re-analysis (deWaard *et al*. 2019).

In this study, we aimed to distinguish the effects of three major drivers underlying agricultural biodiversity. We investigated how organic farming, reduced pesticide use, and the proportion of SNH in the landscape surrounding vineyards in southwest Germany affect the diversity of arthropods. We used Malaise traps and metabarcoding in a crossed design with FRG and classic varieties in either organically or conventionally managed vineyards along a gradient of landscape composition. In this way, our study design was suitable to assess the impact of key policies that address biodiversity loss in a cropping system with generally high pesticide use. Furthermore, the cultivation of FRG varieties allowed for the study of pesticide reduction in healthy crops under realistic cropping conditions.

We tested the following hypotheses: (H1) arthropod biomass and (H2) richness are higher in (a) organically compared to conventionally managed vineyards, (b) FRG compared to classic varieties, and (c) SNH-rich compared to vineyard-dominated landscapes; and (H3) community composition differs between (a) management types and (b) grape varieties, and (c) with the proportion of SNH in vineyard landscapes.

3.2 Materials and methods

3.2.1 Study area

We conducted our study in the German wine-growing region Palatinate (49.273280°N, 8.020602°E/49.147516°N, 8.175736°E; Figure 3.1). The region lies in the Upper Rhine Valley east of the Palatinate Forest and is characterized by a temperate climate with an average annual temperature of 11.1 °C and a total annual precipitation of 677.7 mm, and a widespread cultivation of grapevines (Beck *et al*. 2018; Agrarmeteorologie Rheinland-Pfalz 2022). The mean temperature was 11.9 °C and 10.4 °C with a total precipitation of 630.4 and 814.5 mm in 2020 and 2021, respectively (Agrarmeteorologie Rheinland-Pfalz 2022).
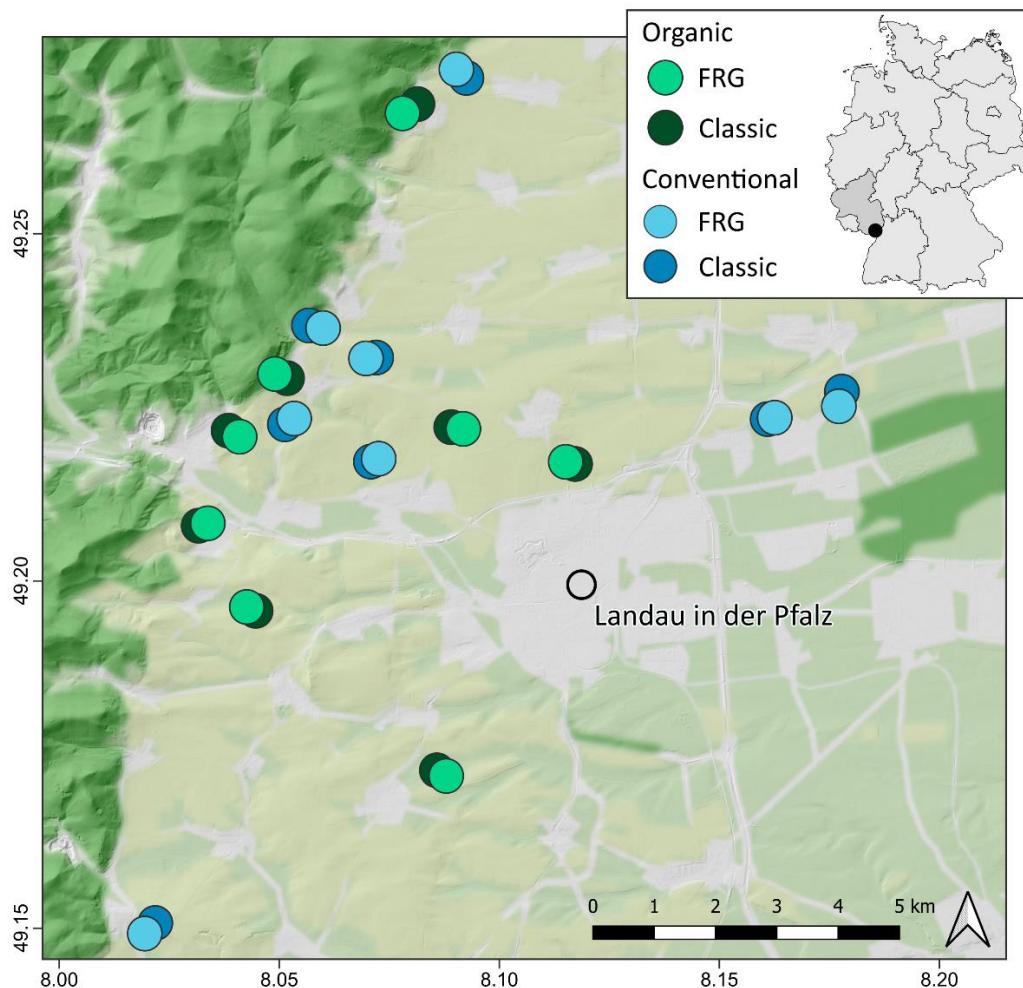
Figure 3.1 Study area with the location of the 32 vineyards. Each pair of either organically (blue) or conventionally (red) managed vineyards consisted of one fungus-resistant (FRG, brighter) and one classic (darker) grape variety (basic map data by ©GeoBasis-DE/LVermGeoRP (2022)).

3.2.2 Design and sampling

We chose 16 pairs of vineyards along a gradient of landscape composition, varying in the proportion of SNH in their surrounding (Figure 3.1, Table S3.1). Half of them were managed organically after the European Union regulation No 2092/91 and the other half conventionally. Each pair consisted of one vineyard with a fungus-resistant grape variety (FRG) and one with a classic variety. We conducted fieldwork from April to September in 2020 and 2021. In each vineyard, we placed a Malaise trap (first 3 months Malayse traps with a combination of black and white net, ENTO SPHINX s.r.o., Pardubice, Czech; then standard SLAM traps, MegaView Science Co., Ltd., Taichung, Taiwan) in a central inter-row for three consecutive days per month. If there were differences in tillage between adjacent inter-rows, we chose the more vegetated row. We filled the collecting bottles with 300 ml of 70 % ethanol denatured with about 1 % methyl ethyl ketone (EtOH MEK) and stored collected specimens in undiluted EtOH MEK.

3.2.3 Landscape and environmental variables

Within a radius of 1,000 m of each vineyard, the cover of SNH was calculated by using ATKIS data (Basis-DLM by ©GeoBasis-DE/BKG (2013); Table 3.1) with intersection of spatial data in an Oracle database 12c (Oracle 2017). We defined SNH as forests, hedges, shrubs, and grassland. Within each pair, we used the mean proportion of SNH of the two vineyards for further analyzes, resulting in a total of 16 landscapes.

Table 3.1 Landscape and environmental variables with minimum, maximum, mean value, and standard deviation.

Variable	Description	Unit	Min	Max	Mean	SD
SNH	Proportion of SNH in 1,000 m radius	Percent [%]	1.4	47.2	17.2	14.5
Spraying events	Number of annual applications	#	0	14	8	4
Vegetation cover	Proportion of ground covered by vegetation	Percent [%]	46	93	69	15
Plant species	Number of plant species having flowers during survey	#	0.9	3.5	2	0.7

We received the number of annual pesticide applications from the winegrowers. Predominantly fungicides were sprayed. Herbicides were used in three pairs of the conventional and none of the organic vineyards. To our knowledge, no insecticides were used, but mating disruption of grapevine moths (*Lobesia botrana* and *Eupoecilia ambiguella*) was done with pheromone dispensers in the whole study area. We measured vegetation cover and the number of plant species that had flowers during the survey three times each year (between first and second, third and fourth, and fifth and sixth Malaise trap sampling) in two plots per vineyard with two subplots each in adjacent inter-rows. Each subplot was 1 m² (2 m x 0.5 m) in size, covering a total of 4 m² per vineyard. For the vegetation cover, the proportion of ground covered by vegetation was measured visually in tens from 0 % to 100 %. For analyzes, we used the mean of the variables of all surveys and both years.

3.2.4 Biomass

To obtain arthropod biomass, we weighed the wet catch after placing it in a sieve and letting the liquid drip off. For each vineyard, we used the total biomass of both years for analyzes by summing all samples.

3.2.5 Diversity

We pooled the arthropod material of each year, resulting in two samples per vineyard. Orthopterans were sorted out beforehand and only one leg of each specimen was left in the samples for the DNA metabarcoding analysis in order to avoid the dominance of their DNA. Metabarcoding of a 313 base pairs long mini-barcode region in the CO1-5P target region and bioinformatics were conducted by AIM - Advanced Identification Methods GmbH, following the protocol and methods of Hausmann *et al*. (2020) and Morinière *et al*. (2016) and using the VSEARCH suite *v.2.9.2* (Rognes *et al*. 2016) and Cutadapt *v.1.18* (Martin 2011; Supplementary methods). In the bioinformatical process, similar DNA sequences were clustered into operational taxonomic units (OTUs) and assigned to barcode index numbers (BINs) based on reference sequences in the Barcode of Life Data System (BOLD). We filtered the results table for OTUs with a Hit-%-ID value in BOLD ≥ 97 % and an assignment to a BIN and condensed BINs that occurred more than once into one entry. For most analyzes, we combined the tables from the 2 years into an overall BIN list. The total number of BINs was used as a taxon richness value. Taxonomic information was used from BOLD.

3.2.6 Data analysis

All analyzes were conducted using R *v.4.1.2* (R Core Team 2021) and RStudio *v.2022.07.1* (RStudio Team 2022) with the R packages car (Fox and Weisberg 2019), lme4 (Bates *et al*. 2015), blmeco (Korner-Nievergelt 2015), MuMIn (Bartoń 2020), vegan (Oksanen *et al*. 2020), ggplot2 (Wickham 2016), ggpubr (Kassambara 2020), and dplyr (Wickham *et al*. 2022).

To investigate whether environmental variables differ between management types and grape varieties, we performed linear mixed model regressions (LMM) for spraying events and flowering plant species with management and grape variety as fixed factors and the vineyard pair as random factor. A general linear mixed model regression (GLMM) with negative binomial distribution and logarithmic link function (log link) was used for vegetation cover with the same fixed and random factors. To investigate the effects of management type, grape variety, their interaction, landscape, and environmental variables on biomass and BINs, we performed LMMs for BINs of Coleoptera, Hemiptera, and Lepidoptera and GLMMs with negative binomial distribution and log link for biomass, total BINs, and BINs of Diptera and Hymenoptera and included the vineyard pair as random factor. We rescaled and centered continuous variables. To obtain whole-numbers, we multiplied the values of biomass and vegetation cover by ten. We selected the best fitting model based on the lowest AICc value by using a backward elimination method with management, variety, and SNH as fixed factors. We used type III ANOVA to test the effects using a significance level of $P < 0.05$. We investigated differences in community composition between years and between management types and grape varieties by performing non-metric multidimensional scaling (NMDS) reduced to two dimensions and with the lowest stress out of 50 runs and tested effects using PERMANOVA with Binary Jaccard distances. Unlike the other analyzes, we did not use the

combined BIN list of both years to investigate the differences in community composition between years.

3.3 Results

3.3.1 Environmental variables

With two more spraying events compared to conventional managed vineyards (seven applications, SD = ±3), the number of annual applications tended to be higher under organic management (nine applications, SD = ±4; Table 3.2; Figure 3.2A). With five annual applications (SD = ±3 applications), FRG varieties received significantly fewer sprayings than classic varieties with 11 applications per year (SD = ±2 applications). Vegetation cover was 26 % higher in conventionally managed vineyards than in organically managed ones (Figure 3.2B). The plant species richness was unaffected by the studied factors (Figure 3.2C).

Table 3.2 Differences in management type and grape variety for the mean number of spraying events, the mean proportion of ground covered by vegetation, and the mean number of plant species of both years analyzed by using LMMs for spraying events and the number of plant species and GLMM with negative binomial error distribution for vegetation cover. Chi-square (χ^2), degrees of freedom (df), P-value, and the significance level (Sig.) are indicated. Significance codes: ***$P < 0.001$, **$P < 0.01$, • $P < 0.1$.

Variable	Spraying events				Vegetation cover				Plant species			
	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.
(Intercept)	63.1	1	< 0.001	***	11,518.1	1	< 0.001	***	70.2	1	< 0.001	***
Management	2.8	1	0.095	•	8.6	1	0.003	**	0.2	1	0.685	
Variety	85.1	1	< 0.001	***	1.8	1	0.179		0.8	1	0.380	
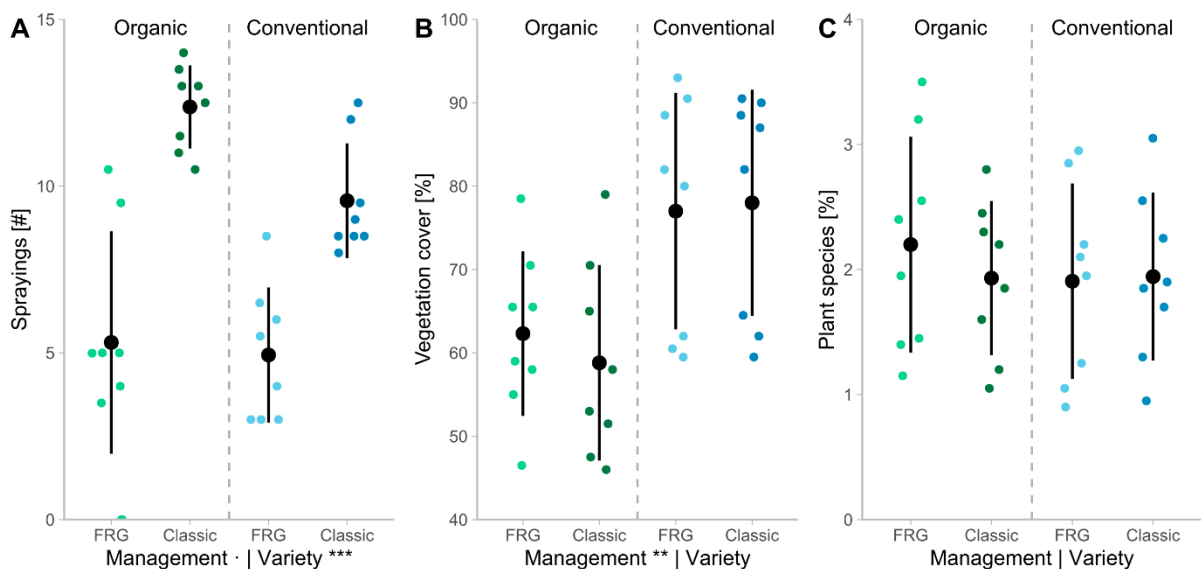
Figure 3.2 Mean number of spraying events (A), mean proportion of ground covered by vegetation (B), and mean number of plant species (C) of both years with mean and standard deviation for fungus-resistant (FRG, brighter) and classic (darker) grape varieties in organic (green) and conventional (blue) management. Significant effects are indicated in x-axis labels. Significance codes: ***$P < 0.001$, **$P < 0.01$, •$P < 0.1$.

3.3.2 Biomass

The cumulative wet biomass of arthropods per vineyard was on average 16.2 g (SD = ±5.3 g), with 9.8 g (SD = ±3.0 g) in 2020 and 6.4 g (SD = ±3.2 g) in 2021 (Table S3.2). We found a significant effect of the management type (Table 3.3; Figure 3.3A). On average, biomass was 31 % higher in conventional management (18.3 g, SD = ±5.3 g) compared to organic management (14.0 g, SD = ±4.4 g). Biomass increased significantly with increasing number of plant species (Figure 3.3C). However, we found no significant influence of the grape variety (Figure 3.3A), SNH (Figure 3.3B), and vegetation cover on biomass.

Table 3.3 Effects of management type, variety, their interaction (Man:Var), semi-natural habitats (SNH), and vegetation variables on the summarized biomass of both years analyzed by using GLMM with negative binomial error distribution and a backward elimination method. Chi-square (χ^2), degrees of freedom (*df*), *P*-value, and the significance level (Sig.) are indicated. Significance codes: ***$P < 0.001$, **$P < 0.01$.

Variable	Biomass			
	χ^2	*df*	*P-value*	Sig.
(Intercept)	3526.0	1	< 0.001	***
Management	7.7	1	0.005	**
Variety	0.0	1	0.982	
Man:Var	*Not included in reduced model*			
SNH	0.1	1	0.701	
Vegetation cover	*Not included in reduced model*			
Plant species	9.7	1	0.002	**
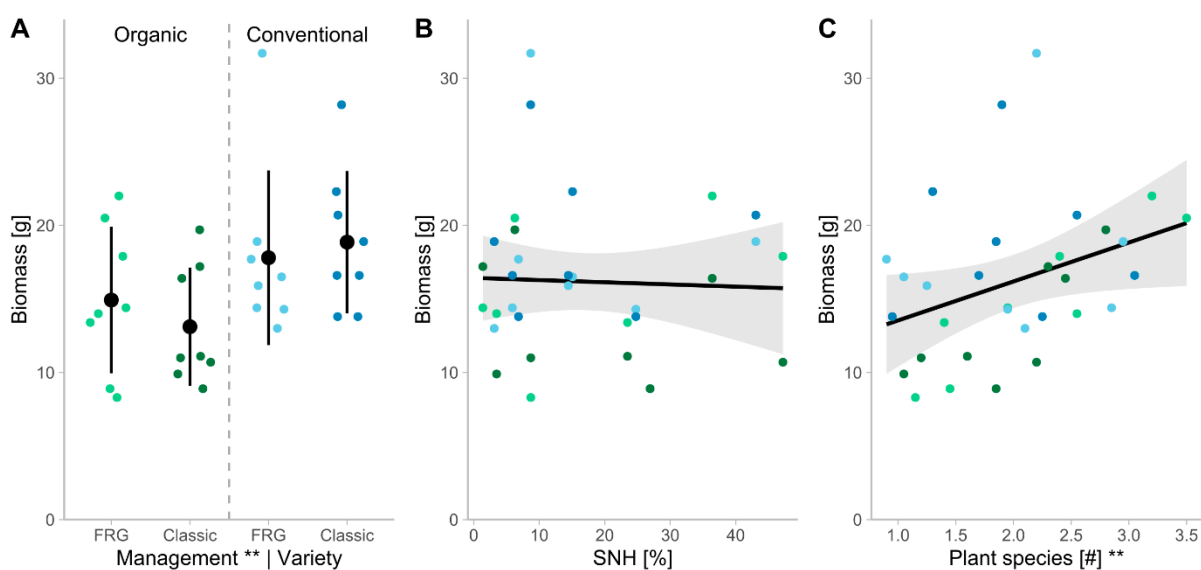
Figure 3.3 Summarized biomass in g of both years for fungus-resistant (FRG, brighter) and classic (darker) grape varieties in organic (green) and conventional (blue) vineyards with mean and standard deviation (A), proportion of semi-natural habitats (SNH) (B), and number of plant species (C). Shaded areas represent the 95 % confidence intervals. Significant effects are indicated in x-axis labels. Significance code: **$P < 0.01$.

3.3.3 Diversity

A total of 1,748 different BINs were recorded in this study (Table S3.1). 1,389 BINs were recorded in 2020 and 1,028 in 2021 (Table 3.4), with 38.3 % of the BINs recorded in both years and significant differences in the community composition between the two years ($F_{1,62} = 10.6$,

$R^2 = 0.146$, $P = 0.001$; Figure 3.4A). On average, we found 200 BINs per site (SD = ±43 BINs) in 2020 and 142 BINs per site (SD = ±20 BINs) in 2021. 705 BINs were recorded on only one site either in 2020 or in 2021. The orders with the most BINs were Diptera (664), Hymenoptera (375), Coleoptera (262), Lepidoptera (198), Hemiptera (154), and Araneae (35). We found community composition to be significantly different between organic and conventional management ($F_{1,30} = 1.6$, $R^2 = 0.050$, $P = 0.004$) but no difference between grape varieties ($F_{1,30} = 0.8$, $R^2 = 0.027$, $P = 0.870$; Figure 3.4B).

Table 3.4 Number of barcode index numbers (BINs) for the years 2020 and 2021 with the number of assigned species and families for the most common orders and the relative delta (Δ) for BINs in 2021 compared to 2020 in %. The proportion of the total per year in % is given in brackets.

Order	2020			2021			Δ BINs
	BINs	Species	Families	BINs	Species	Families	
Diptera	517 (37.2)	247 (30.6)	48 (24.0)	445 (43.3)	214 (36.5)	47 (26.6)	-13.9
Hymenoptera	338 (24.3)	181 (22.5)	33 (16.5)	163 (15.9)	86 (14.7)	24 (13.6)	-51.8
Coleoptera	187 (13.5)	148 (18.4)	29 (14.5)	160 (15.6)	128 (21.8)	28 (15.8)	-14.4
Lepidoptera	155 (11.2)	117 (14.5)	37 (18.5)	109 (10.6)	75 (12.8)	29 (16.4)	-29.7
Hemiptera	127 (9.1)	78 (9.7)	22 (11.0)	91 (8.9)	54 (9.2)	17 (9.6)	-28.3
Others	65 (4.7)	35 (4.3)	31 (15.5)	60 (5.8)	30 (5.1)	32 (18.1)	-7.7
Total	1,389	806	200	1,028	587	177	-26.0
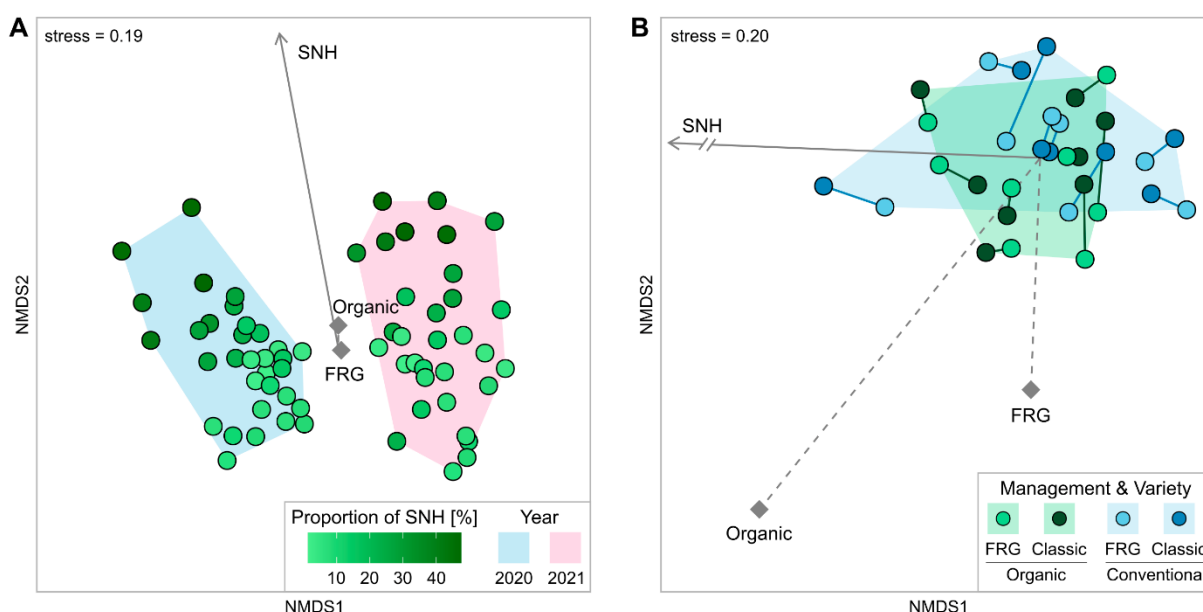
Figure 3.4 Non-metric multidimensional scaling analysis (NMDS) for arthropod communities based on the 1,748 barcode index numbers (BIN) at the 32 study sites for (A) 2020 (blue) and 2021 (pink) (2 dimensions, $n = 50$ runs, stress = 0.19, Binary Jaccard distance) and (B) total communities with fungus-resistant (FRG, brighter) and classic (darker) grape varieties in organic (green) and conventional (blue) management and the variables semi-natural habitats (SNH), organic management, and FRG variety (2 dimensions, n = 50 runs, stress = 0.20, Binary Jaccard distance). Dots in A are colored based on the proportion of SNH in the surrounding landscape of the vineyards. The dots of pairs of vineyards are connected by a line in B. The SNH vector in B is shown shortened by about 25 times to increase visibility of the other data points.

For the total number and for most orders, we found no significant effect of the management type on the number of BINs per vineyard (Table 3.5; Figure 3.5). Only for Diptera, richness tended to be 4 % higher in conventionally managed vineyards (Figure 3.5C). Similarly, we found no significant effect of the grape variety, except for a trend in Hymenoptera with 9 % more BINs in FRG varieties (Figure 3.5E). There was no significant interaction between management and grape variety. The cover of SNH in the surrounding landscape increased significantly total richness of BINs (Figure 3.6A) as well as BINs' richness within Diptera (Figure 3.6C), Hemiptera (Figure 3.6D), Hymenoptera (Figure 3.6E), and Lepidoptera (Figure 3.6F). For vegetation variables, total richness of arthropod BINs increased with the number of present plant species, whereas the amount of vegetation cover had no effect.

Table 3.5 Effects of management type, variety, their interaction (Man:Var), semi-natural habitats (SNH), and vegetation variables on the cumulative total number of barcode index numbers (BIN) and the number of BINs of Diptera and Hymenoptera of both years analyzed by using GLMM with negative binomial error distribution and the number of BINs of Coleoptera, Hemiptera, and Lepidoptera analyzed by using LMM. A backward elimination method was used in all models. Chi-square (χ^2), degrees of freedom (*df*), *P*-value, and the significance level (Sig.) are indicated. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, • $P < 0.1$.

Variable	Total				Coleoptera				Diptera			
	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.
(Intercept)	33,185.6	1	< 0.001	***	245.7	1	< 0.001	***	18,441.3	1	< 0.001	***
Management	0.5	1	0.461		0.0	1	0.986		3.2	1	0.072	•
Variety	2.1	1	0.147		0.0	1	0.876		1.984	1	0.159	
Man:Var	*Not included in reduced model*				2.0	1	0.154		*Not included in reduced model*			
SNH	36.4	1	< 0.001	***	0.2	1	0.668		37.7	1	< 0.001	***
Veg. cover	*Not included in reduced model*				*Not included in reduced model*				*Not included in reduced model*			
Plant species	4.0	1	0.045	*	2.7	1	0.102		*Not included in reduced model*			

Variable	Hemiptera				Hymenoptera				Lepidoptera			
	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.
(Intercept)	123.0	1	< 0.001	***	4,205.1	1	< 0.001	***	174.8	1	< 0.001	***
Management	0.0	1	0.824		0.4	1	0.501		0.6	1	0.444	
Variety	0.0	1	0.999		3.6	1	0.057	•	0.4	1	0.542	
Man:Var	1.6	1	0.172		*Not included in reduced model*				1.2	1	0.281	
SNH	10.1	1	< 0.001	**	16.2	1	< 0.001	***	6.5	1	0.011	*
Veg. cover	*Not included in reduced model*				*Not included in reduced model*				*Not included in reduced model*			
Plant species	1.6	1	0.201		*Not included in reduced model*				*Not included in reduced model*			

3.4 Discussion

We assessed how organic management, reduced pesticide use, and the proportion of SNH in the surrounding landscape affect arthropod biomass, richness, and community composition in viticulture. The results show that arthropods, mostly composed of flying insects in our study, are affected by both management practices within vineyards and by the surrounding landscape. Within vineyards, this is particularly reflected by reduced biomass under organic management, but also by changes in the community composition rather than species richness.
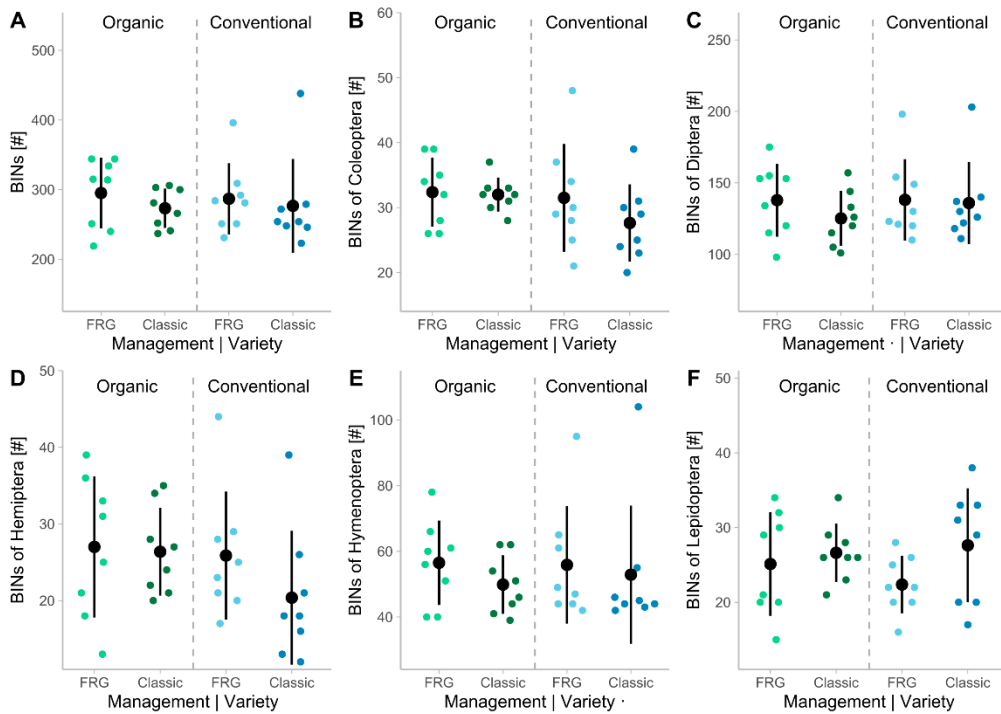
Figure 3.5 Cumulative number of barcode index numbers (BIN) of both years with mean and standard deviation for fungus-resistant (FRG, brighter) and classic (darker) grape varieties in organic (green) and conventional (blue) management for (A) total, (B) Coleoptera, (C) Diptera, (D) Hemiptera, (E) Hymenoptera, and (F) Lepidoptera. Effects with a trend are indicated in x-axis labels. Significance code: •$P < 0.1$.
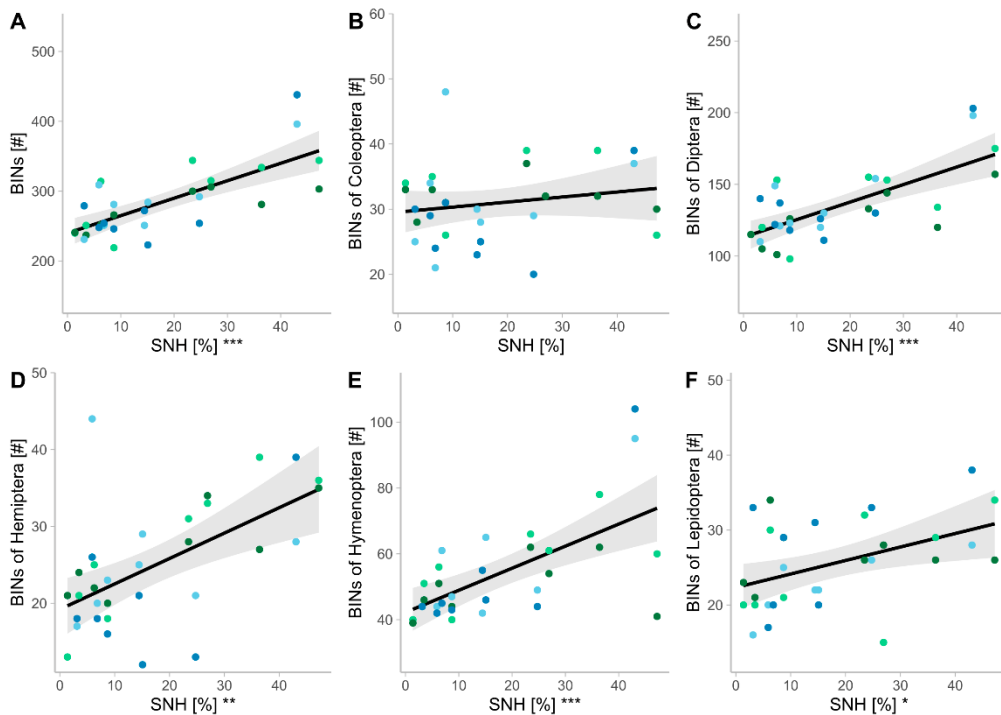
Figure 3.6 Cumulative number of barcode index numbers (BIN) of both years for the proportion of semi-natural habitats (SNH) in the surrounding landscape for (A) total, (B) Coleoptera, (C) Diptera, (D) Hemiptera, (E) Hymenoptera, and (F) Lepidoptera. Colors indicate grape variety and management, with fungus-resistant (FRG, brighter) and classic (darker) grape varieties in organic (green) and conventional (blue) management. Shaded areas represent the 95 % confidence intervals. Significant effects are indicated in x-axis labels. Significance codes: ***$P < 0.001$, **$P < 0.01$.

Contrary to our first hypothesis (H1a), where we expected arthropod biomass to be higher in organically managed vineyards, biomass was lower in organic vineyards than in conventional ones. Thus, an increase in the share of organic farming, as targeted by the European Green Deal (European Commission 2019), may not be efficient in enhancing arthropod biomass in viticulture in our region. We also found no difference in arthropod richness between organic and conventional viticulture, contrary to our hypothesis (H2a) that arthropod richness is higher under organic management. Only for Diptera, taxa richness tended to be 4 % higher under conventional management. While a strong positive effect of organic management in agricultural landscapes has been demonstrated for other cropping systems (Bengtsson *et al*. 2005), the difference between management types in viticulture seems weaker with both positive and negative effects being reported (Döring *et al*. 2019; Kolb *et al*. 2020; Paiola *et al*. 2020). This may be due to the high use of pesticides, in particular fungicides, but a generally low use of insecticides in both management systems. However, large positive effects of organic viticulture over conventional are reported from regions where insecticide use is mandatory against *Scaphoideus titanus*, the vector of Flavescence dorée (Beaumelle *et al*. 2023). As we found an effect of management on biomass but only a trend for Diptera richness, the effects appear to vary among organism groups. Ostandie *et al*. (2021) observed an increased abundance of springtails and spiders in organic versus conventional viticulture, while pollinator abundance decreased and ground beetles as well as mites remained unaffected. Although such effects may also be explained by differences in tillage and plant cover, we did not find effects of vegetation cover on biomass or taxa richness. In the studied vineyards, on average, those managed organically had a lower vegetation cover compared to conventionally managed ones, but this does not necessarily mean that their overall vegetation was less diverse or structured. In fact, we surveyed the highest proportion of vegetation cover in conventionally managed vineyards with mostly dense grass covers, whereas other vineyards often had lower vegetation cover but more diverse plant communities. It is worth noting that newly tilled inter-rows may also have contributed to a temporary lower vegetation cover at the time of the survey. Arthropod biomass and richness did, however, increase with a higher number of plant species. Since there was no significant difference in the number of plant species between management types, the inter-row vegetation does not seem to explain the lower biomass and lower Diptera richness under organic management in our study. Möth *et al*. (2021) found pesticide toxicity for non-target organisms to be higher in organic vineyards due to the use of copper and sulfur instead of synthetic fungicides. Copper and sulfur are non-specific agents that can also affect non-target organisms (Nash *et al*. 2010; Pedneault and Provost 2016; Vogelweith and Thiéry 2018), which may explain the lower biomass in organic vineyards. Inorganic or synthetic products affect the abundance of diverse arthropod taxa to various extents (Nash *et al*. 2010; Vogelweith and Thiéry 2018), thereby favoring species that are less sensitive or less exposed to fungicides during applications over others and alter community composition and species interactions. This in turn may also enhance or reduce natural pest control (Nash *et al*. 2010; Reiff *et al*. 2021a). Along with hypothesis 3a, where we predicted community composition to differ between management types, this is reflected in our study results. Arthropod communities differed between organic and conventional

management, even though there was a high overlap in community composition over the two seasons.

In contrast to the second parts of our hypotheses (H1b, H2b & H3b) that predicted higher arthropod biomass and richness in vineyards with FRG varieties and different community composition compared to classic ones, biomass and taxa richness did not increase in FRG varieties and communities did not differ compared to classic varieties. Although FRG varieties had less than half as many fungicide applications and the overall pesticide toxicity was lower in those vineyards (Pedneault and Provost 2016), it seems that there was no clear effect on the arthropod community captured by our malaise traps. Yet, the richness of Hymenoptera tended to be 9 % higher compared to classic varieties. Even though no clear effects were found in our study, positive effects of FRG are reported in studies that sampled the grapevine canopy (Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021a) as well as on grasshoppers in the ground vegetation (Kaczmarek *et al*. 2023). Thus, it is highly likely that the cultivation of grapevine varieties with resistance traits to major fungal diseases may still be an important way to reduce the impact of pesticides on non-target organisms. In addition to the lower pesticide toxicity, differences in richness between FRG and classic grape varieties may also be caused by the lower number of pesticide applications with fewer tractor passages. To date research to this topic is sparse and limited (Pedneault and Provost 2016; Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021b), and consequently more studies are needed to better understand the effects of reduced pesticide use and disturbance in vineyards on biodiversity.

Similar to some earlier studies on biodiversity in viticulture, the effects of the type and the amount of pesticides, in particular fungicides, were rather weak on the arthropod community (Döring *et al*. 2019; Paiola *et al*. 2020). Besides the assumption that differences in pesticide use rather change community composition than species numbers, vineyards of our region are often small and neighboring vineyards are frequently managed differently. Species may therefore not only be affected by management practices in the focal vineyard itself, but also by pesticide drift from adjacent fields (Druart *et al*. 2011). This may explain the rather weak effect of pesticides in the present study. In addition, we captured mainly flying insects with our Malaise traps. The highly mobile species that are predominantly caught in these traps may be less affected because they may not be as exposed to pesticides during applications as species living only in grapevine canopies. Further, about two-thirds of captured species belonged to the highly mobile orders Diptera and Hymenoptera. Due to their mobility, effects of pesticides are likely to be less evident compared to less mobile species as species diversity and density tend to converge between differently managed vineyards. Similarly, the capturing of mostly flying species may explain why we did not find stronger effects of organic management or the local vegetation on arthropods. Earlier studies reported positive effects of organic management and local vegetation for ground-dwelling and less mobile species such as spiders, beetles, and mites (Ostandie *et al*. 2021; Reiff *et al*. 2021b; Blaise *et al*. 2022).

The proportion of SNH near vineyards had a strong influence on arthropod diversity. Consistent with hypothesis 2c and 3c and contrary to 1c, where we predicted that SNH-rich

landscapes have higher arthropod richness and different community composition but also have higher biomass, more species were found in SNH-rich landscapes and community composition changed with changing landscape composition, while we did not find any effect on biomass. Landscape structure is a major determinant of biodiversity patterns (Tscharntke *et al.* 2012). SNH-rich landscapes provide shelter and habitats for overwintering, as well as resources for feeding and reproduction to numerous species that are unable to complete their life cycle in agricultural fields alone (Holland *et al.* 2017). Positive effects of SNH have therefore also been found in previous studies for various taxonomic groups (Martin *et al.* 2019; Kolb *et al.* 2020; Tscharntke *et al.* 2021). In addition, SNH-rich landscapes may favor parasitoids and predators, thereby improving natural pest control (Holland *et al.* 2017).

In the second year of sampling, fewer arthropods were recorded, both in terms of biomass and richness. Arthropod activity, particularly of flying insects, is influenced by meteorological conditions with generally higher activity during warmer and drier weather (Goodwin *et al.* 2021; Kaczmarek *et al.* 2022). The year 2021 was overall colder and wetter than 2020 (Agrarmeteorologie Rheinland-Pfalz 2022). Therefore, differences in biomass and richness between the two sampling years can be primarily attributed to differences in species activities. Similar to Goodwin *et al.* (2021), we observed the largest change in Hymenoptera, where only about half as many species were recorded in 2021 compared to the previous year, while Diptera and Coleoptera were less affected. Also community composition differed strongly, with less than half of the BINs being recorded over both years. Hence, the large differences in biomass, richness, and composition between the two sampling years highlights the importance of environmental conditions on the number and community of species recorded (Williams 1961). However, the fact that less than half of the BINs are recorded in both years is also a consequence of a significant proportion of singleton species in metabarcoding studies, with high numbers of species recorded exclusively at single sites (Steinke *et al.* 2021; Kaczmarek *et al.* 2022).

3.5 Conclusions

Although targeted by agro-environmental policy, the conversion to organic farming may not be appropriate to promote arthropod diversity in viticulture in our region as it reduces their biomass. This may be the result of different types of pesticides used in organic and conventional management but could also be related to differences in tillage and ground cover management. Therefore, measures such as those under the European Green Deal should be implemented in viticulture with caution, as we have observed biodiversity effects deviating from other cropping systems and from viticultural areas with insecticide use. Arthropod species richness may rather be promoted by a more diverse landscape with a higher proportion of semi-natural habitats in the surrounding area. This result suggests that suitable Ecological Focus Areas such as hedgerows should be promoted in vineyard landscapes. We only found a small positive effect of pesticide reduction on Hymenoptera diversity. The cultivation of FRG varieties and thereby a limited use of fungicides, however, may still be an important approach, as it had positive effects on less mobile or more pesticide-exposed species in other studies (Pennington *et al.* 2017; Pennington *et al.* 2019; Reiff *et al.* 2021a). However, as management practices alter community composition, the extent to which

conversion to organic farming and reduction of pesticide use promote or diminish the occurrence of species of conservation concern should be studied more closely in the future. Further, it is important to consider the role of beneficial insects and pests in natural pest control and how they may be affected by these changes.

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CHAPTER 4

Wild bee conservation in viticulture: Effects of semi-natural habitats, organic management, and pesticide reduction

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Abstract

In agricultural landscapes, the clearing of semi-natural habitats (SNH) and the intensive use of pesticides contribute to declines of biodiversity, including pollinators. However, effects of pesticide use and landscape characteristics on pollinators have rarely been studied together. In this study, we investigated how SNH in the surrounding area, organic and conventional management, and the reduction of pesticides affect bee diversity in 32 vineyards in southwest Germany. We used yellow pan traps to sample wild bees in a crossed design of management (organic versus conventional) and pesticide use (reduced in fungus-resistant grape varieties versus regular) along a gradient with increasing proportions of SNH. Higher proportions of SNH influenced species composition of bees and increased the abundance and richness of above-ground-nesting species. Organic vineyards had a 49 % higher abundance of bees compared to conventional vineyards. This difference was primarily attributed to ground-nesting species that rely on bare ground areas for nesting sites, and we found lower vegetation cover in organic vineyards. The reduction of pesticides showed no significant effect on bees in our region, where insecticide use is generally low. Wild bees appear to benefit more from SNH and the provision of floral and nesting resources by diverse vegetation cover in inter-rows rather than relying on specific pesticide management. Our study underlines that providing diverse vegetation in inter-rows while maintaining bare ground areas for ground-nesting species and creating a structurally rich environment with suitable SNH is important for wild bee conservation in viticulture.

4.1 Introduction

Wild pollinators, including bees, are experiencing global declines, with diverse drivers affecting their populations (Potts *et al*. 2010). In agricultural landscapes, the clearing of semi-natural habitats (SNH) and the high use of pesticides and fertilizers are main drivers of biodiversity loss in recent decades (Hochkirch 2016; Hallmann *et al*. 2017; Sánchez-Bayo and Wyckhuys 2019). Europe is home to about 2,000 bee species, with more than 550 species recorded in Germany (Westrich 2018). About half of them are currently classified as endangered due to the loss of nesting structures, scarcity of nectar and pollen providing flowers, and exposure to pesticides (Potts *et al*. 2010; Westrich *et al*. 2011). For the conservation of wild bees and their pollination services in agricultural landscapes, it is therefore important to understand the specific threats to their diversity.

Viticultural areas, located in climatically favorable locations, provide habitats for a large number of mainly thermophilic bee species (Hentrich 2014; Krahner *et al*. 2018; Wersebeckmann *et al*. 2023). Loamy, sandy, and loess soils, that also can be found in Palatinate vineyards, provide suitable nesting conditions for many ground-nesting species (Westrich 2018). Additionally, bees rely on flowers that provide nectar and pollen and diverse inter-row vegetation with flower-rich cover crops can offer such valuable food resources (Westrich 2018). Although grape vines do not depend on bee pollination, viticultural areas can thus serve as crucial open land habitats for bee conservation, including for rare species (Kehinde and Samways 2014; Burger 2018). However, the loss and fragmentation of SNH in agricultural landscapes is one of the key factors threatening wild bees in intensive agriculture

(Brown and Paxton 2009), particularly because certain species depend on wooden nesting structures and diverse floral resources provided by SNH (Westrich 2018; Eckerter *et al*. 2022).

Viticulture is one of the cropping systems that heavily relies on pesticides due to the occurrence of fungal diseases such as powdery and downy mildew (Pedneault and Provost 2016). The presence of fungal diseases necessitates the extensive use of plant protection products, with conventional management using synthetic fungicides, while organic viticulture relying on inorganic compounds, primarily copper and sulfur (Pedneault and Provost 2016). Consequently, it can be expected that non-target organisms, including bees, are affected by the use of pesticide. Although organic farming is known to generally promote biodiversity compared to conventional farming (Bengtsson *et al*. 2005; Hole *et al*. 2005; Holzschuh *et al*. 2008; Tuck *et al*. 2014), its effects in viticulture are not as well-defined and vary between and within organism groups (Bruggisser *et al*. 2010; Döring *et al*. 2019; Ostandie et al. 2021; Paiola *et al*. 2020; Beaumelle *et al*. 2023; Kaczmarek *et al*. 2023a).

Given that the use of plant protection products is considered to impact non-target organisms, reducing pesticides may have an important part in counteracting the decline of bees and biodiversity in general. In viticulture, it is possible to achieve pesticide reduction of up to 80 % by cultivating fungus-resistant grape (FRG) varieties (Töpfer and Trapp 2022). Positive effects of the cultivation of FRG varieties on non-target organisms were reported for some groups such as predatory mites and certain spider families (Pennington et al. 2017; Pennington et al. 2019; Reiff et al. 2021; Reiff et al. 2023). However, despite the availability of 38 cultivars to winegrowers (Töpfer and Trapp 2022), in our study region, only about 2.7 % of the cultivated area is planted with FRG varieties (Statistisches Bundesamt (Destatis) 2023). Consequently, there is still a high potential to increase sustainability in viticulture. Additionally, reducing pesticide applications also leads to a decrease in tractor passages, resulting in fewer disturbances and less soil compaction (Bruggisser *et al*. 2010), which may benefit particularly ground-nesting bees.

In this study, we used yellow pan traps to sample bees and assess how SNH in the surrounding area, organic vineyard management, and pesticide reduction through the cultivation of FRG varieties affect their diversity in vineyards in southwest Germany. To achieve this, we used a crossed design that involved FRG and classic grape varieties grown in either organically or conventionally managed vineyards. This design allowed us to examine the impact of pesticide reduction in healthy crops under realistic cropping conditions. Our hypotheses were as follows: Bee diversity is higher in (H1) landscapes rich in SNH compared to vineyard-dominated landscapes, (H2) organic vineyards compared to conventional ones, and (H3) FRG varieties compared to classic grape varieties.

4.2 Material and methods

4.2.1 Study area and sites

We conducted sampling in the Palatinate wine-growing region of Germany (49.273280 °N, 8.020602 °E / 49.147516 °N, 8.175736 °E). The studied vineyards are located in the Upper Rhine Valley east of the Palatinate Forest, an area characterized by warm temperate climate (Beck *et al*. 2018). The average annual temperature measured during the last 15 years is

11.2 °C and the total annual precipitation is 677.7 mm with a mean temperature of 11.9 °C and a total precipitation of 630.4 mm in 2020 (Agrarmeterologie Rheinland-Pfalz 2022). We sampled 16 pairs of vineyards located along a landscape gradient with varying proportions of SNH in their surrounding landscapes (Figure 4.1). Half of them were managed organically after the European Union regulation No 2092/91, while the other half were managed conventionally. Each pair included one vineyard with a FRG variety and one with a classic grape variety.
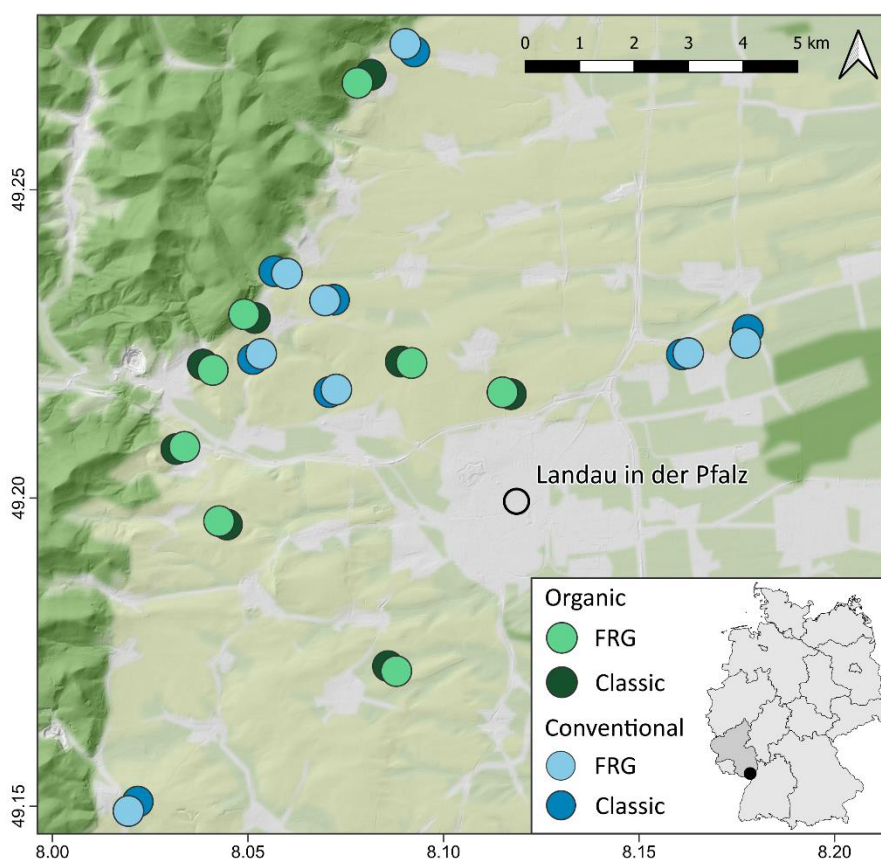
Figure 4.1 The study area with the location of the 16 pairs of vineyards, each managed either organically (green) or conventionally (blue) and consisting of one vineyard with a fungus-resistant grape variety (FRG, brighter) and one with a classic grape variety (darker). Basic map data by ©GeoBasis-DE/LVermGeoRP (2022).

4.2.2 Landscape and environmental variables

The cover of SNH within a 500 m radius of each vineyard was calculated by using ATKIS data (Basis-DLM by ©GeoBasis-DE/BKG (2013); Table 4.1) with intersection of spatial data in an Oracle database 12c (Oracle 2017; for details see Kaczmarek *et al*. 2023b). SNH was defined as forests, hedges, shrubs, and grassland. For each pair of vineyards, we averaged the proportion of SNH cover across the two vineyards, resulting in a total of 16 landscapes.

Information on the number of pesticide applications in 2020 was obtained from the winegrowers (Table 4.1; for details see Kaczmarek *et al*. 2023a). Fungicides were applied in all vineyards, while only three conventional vineyard pairs received herbicides and none of the studied vineyards was treated with insecticides. The grapevine moths *Lobesia botrana* and

Eupoecilia ambiguella were controlled by mating disruption with pheromone dispensers throughout the study area. To measure vegetation variables, we conducted three surveys during the season (late April, early July, and early September). At each survey, we recorded the proportion of ground covered by vegetation and the plant species that had flowers in two centrally located plots per vineyard (Table 4.1). Two subplots, each measuring 1 m² (2 m x 0.5 m), were surveyed in adjacent inter-rows, covering a total of 4 m² per vineyard. Vegetation cover was measured visually in tens from 0 % to 100 %. For each vineyard, we calculated the mean vegetation cover of the three surveys and the total number of plant species to use in further analyzes.

Table 4.1 Variables with minimum, maximum, mean value, and standard deviation.

Variable	Description	Min	Max	Mean	SD
SNH [%]	Proportion of SNH in a radius of 500 m	0	42.9	12.0	13.4
Spraying events [#]	Number of pesticide applications	0	14	7	4
Vegetation cover [%]	Proportion of ground covered by vegetation	22	94	65.5	18.9
Flowering plants [#]	Number of plant species having flowers	6	21	11	4

4.2.3 Sampling and identification of bees

In the center of each vineyard, we placed a yellow pan trap about 10 cm above ground between two vines. Traps were operated for three consecutive days every month from April to September 2020. We filled the traps with approximately 1.25 l of water with one drop of soap to reduce surface tension. After three days of exposure, we transferred collected material to 70 % ethanol and stored the samples in undiluted ethanol. The bees contained in the samples were pinned onto insect needles for subsequent species identification.

We used the identification keys of Amiet *et al*. (2001, 2004, 2007, 2010; 2014; 2017), Pauly (2015, 2021), Schmid-Egger and Scheuchl (1997), along with additional literature of Scheuchl and Willner (2016) and Westrich (2018), to identify the bees. We obtained information on the conservation status from the German national Red List (Westrich *et al*. 2011) and the Wildbienen-Kataster (Scheuchl *et al*. 2018) and information on the feeding, nesting, and social behavior from Westrich (2018).

4.2.4 Data analysis

We used R *v.4.2.3* (R Core Team 2023) and RStudio *v.2023.03.0* (RStudio Team 2023) with the R packages car (Fox and Weisberg 2019), lme4 (Bates *et al*. 2015), blmeco (Korner-Nievergelt 2015), MuMIn (Bartoń 2020), vegan (Oksanen *et al*. 2020), indicspecies (Cáceres and Legendre 2009), ggplot2 (Wickham 2016), ggpubr (Kassambara 2020), and dplyr (Wickham *et al*. 2022) for data analyzes.

We investigated differences in the environmental variables between organic and conventional management and between FRG and classic grape varieties by using linear mixed model regressions (LMM) for spraying events and flowering plants and a general linear mixed model

regression (GLMM) with negative binomial distribution and logarithmic link function (log link) for vegetation cover (multiplied by ten to obtain integers). Management and grape variety were defined as fixed factors and the pair of vineyards as random factor. We investigated the effects of SNH, management, grape variety, their interaction, vegetation cover, and flowering plants on the abundance (number of individuals) and richness (number of species) of bees by using a GLMM with negative binomial distribution and log link and a LMM, respectively. Management, grape variety, and SNH were defined as fixed factors and the pair of vineyards as random factor. Accordingly, we investigated effects on the abundance and richness of ground- and above-ground-nesting bees using GLMMs with negative binomial distribution and log link for abundance and richness of ground-nesting bees and abundance of above-ground-nesting bees and a LMM for richness of above-ground-nesting bees. We rescaled and centered continuous variables and excluded the honey bee *Apis mellifera* from all analyzes because its occurrence depends on the location of beehives. By using a backward elimination method, the best fitting model was selected based on the lowest AICc, for which we used type III ANOVA to test the effects with a significance level of $P < 0.05$.

We investigated species composition of bees based on species abundances using redundancy analysis (RDA) with SNH, management, grape variety, vegetation cover, and flowering plants as explanatory variables. To reduce the influence of highly abundant species, we used Hellinger standardization to transform species abundance data to relative values. The best fitting model was selected using a backward elimination method. Furthermore, we identified bee species related to either organic or conventional management using a species indicator analysis with the transformed data. We used the same species indicator analysis to identify flowering plant species that are related to either organic or conventional management.

4.3 Results

4.3.1 Environmental variables

With two more spraying events compared to conventionally managed vineyards (mean = 6.1, SD = ±3.4), organically managed vineyards (mean = 8.1, SD = ±4.8) had a significant higher number of pesticide applications (Table 4.2, Figure 4.2). FRG varieties received significantly fewer applications (mean = 3.8, SD = ±2.9) than classic varieties (mean = 10.4, SD = ±2.2). Vegetation cover was 43 % higher in conventionally managed vineyards (mean = 77.3 %, SD = ±15.5 %) compared to organically managed ones (mean = 53.8 %, SD = ±14.2 %, Table 4.2, Figure 4.2). The number of flowering plants was not affected by either management type or grape variety. Flowering plant species that were more common in organic vineyards were *Chenopodium album* agg., *Convolvulus arvensis*, *Fagopyrum esculentum*, *Malva sylvestris*, *Raphanus raphanistrum* agg., and *Trifolium incarnatum*, while *Bellis perennis*, *Ornithogalum umbellatum* agg., *Taraxacum* spp., and *Trifolium repens* were more common in conventional vineyards (Table S4.1).

Table 4.2 Differences in management type and grape variety for spraying events, vegetation cover, and flowering plants. Number of spraying events and flowering plants were analyzed using LMMs, while the proportion of vegetation cover was analyzed using a GLMM. Chi-square (χ^2), degrees of freedom (*df*), *P*-value, and the significance level (Sig.) are indicated. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Variable	Spraying events				Vegetation cover				Flowering plants			
	χ^2	*df*	*P-value*	Sig.	χ^2	*df*	*P-value*	Sig.	χ^2	*df*	*P-value*	Sig.
(Intercept)	39.3	1	<0.001	***	4,772.5	1	<0.001	***	417.7	1	<0.001	***
Management	4.2	1	0.039	*	9.6	1	0.002	**	0.5	1	0.491	
Variety	92.6	1	<0.001	***	0.6	1	0.443		0.3	1	0.587	
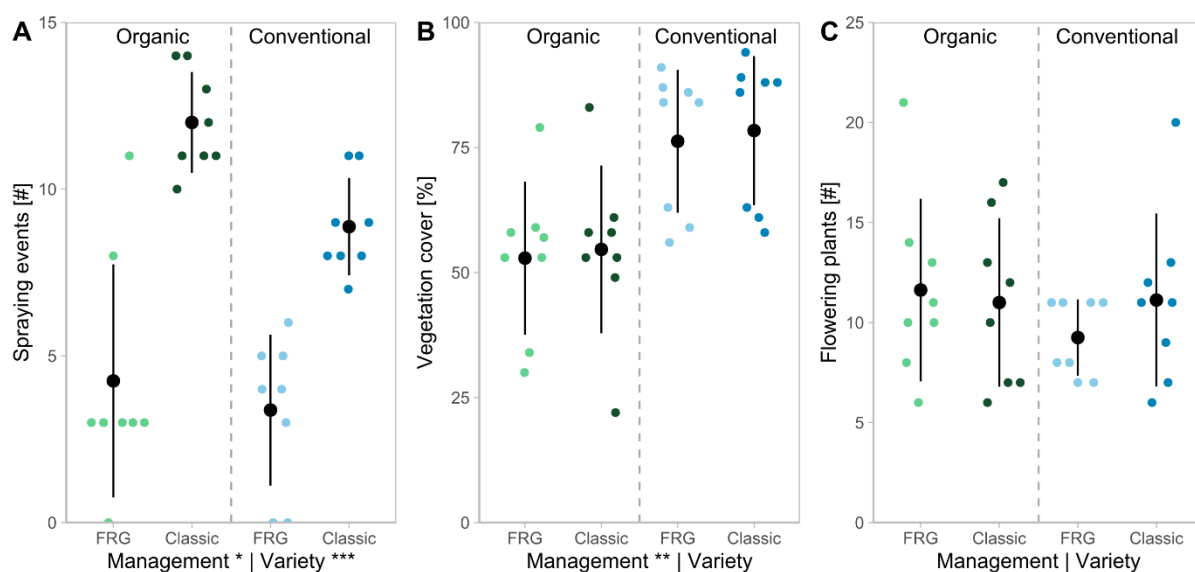
Figure 4.2 Number of spraying events (A), proportion of vegetation cover (B), and number of flowering plants (C) for fungus-resistant (FRG) and classic grape varieties under organic and conventional management. Significant effects are stated at x-axis labels. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

4.3.2 Bee diversity

We sampled 2015 bees of 14 genera and 89 species (Table 4.3, Table S4.2). The most diverse genera were *Andrena* (27 species) and *Lasioglossum* (23). We further found species of the genera *Apis* (1), *Bombus* (4), *Ceratina* (1), *Colletes* (1), *Eucera* (1), *Halictus* (9), *Hylaeus* (5), *Megachile* (1), *Nomada* (8), *Osmia* (5), *Sphecodes* (1), and *Stelis* (2). The most abundant genera were *Lasioglossum* (60.7 %) and *Andrena* (26.7 %). The most abundant species were *Lasioglossum malachurum* (21 %), *Lasioglossum glabriusculum* (12.4 %), *Lasioglossum lineare* (10.8 %), *Andrena dorsata* (7.8 %), and *Lasioglossum morio* (5 %).

Table 4.3 Sampled bee species with the total number of individuals and the number of individuals sampled in vineyards with fungus-resistant (FRG) and classic grape varieties under organic and conventional management. The conservation status (● for least concern, V for near threatened, G for threat of unknown extent, 3 for vulnerable, 2 for endangered, and NA for not listed) of each species is listed along with their behavioral traits, including their source of pollen (o for oligolectic, p for polylectic), nesting sites (g for ground-nesting bees, a for above-ground-nesting bees), and sociality (com for communal, soc for social, sol for solitary, par for parasitic).

| Species | Total | Organic | | Conventional | | Status | Behavior | | |
		FRG	Classic	FRG	Classic		Pollen	Nesting	Sociality
Andrena bicolor Fabricius, 1775	4	1	2	0	1	●	p	g	sol
Andrena bimaculata (Kirby, 1802)	3	1	1	0	1	V	p	g	sol
Andrena chrysosceles (Kirby, 1802)	3	0	2	1	0	●	p	g	sol
Andrena cineraria (Linnaeus, 1758)	77	9	51	9	8	●	p	g	sol
Andrena distinguenda Schenck, 1871	2	0	0	1	1	3	o	g	sol
Andrena dorsata (Kirby, 1802)	157	43	23	42	49	●	p	g	sol
Andrena flavipes Panzer, 1799	72	14	24	26	8	●	p	g	sol
Andrena fulva (Müller, 1766)	2	1	1	0	0	●	p	g	com
Andrena cf. *fulvago* (Christ, 1791)	1	1	0	0	0	3	o	g	sol
Andrena gravida Imhoff, 1832	19	1	15	2	1	●	p	g	sol
Andrena haemorrhoa (Fabricius, 1781)	33	4	9	9	11	●	p	g	sol
Andrena humilis Imhoff, 1832	1	1	0	0	0	V	o	g	sol
Andrena labialis (Kirby, 1802)	3	1	1	1	0	V	o	g	sol
Andrena labiata Fabricius, 1781	2	1	0	0	1	●	p	g	sol
Andrena lagopus Latreille, 1809	59	33	15	3	8	●	o	g	sol
Andrena lathyri Alfken, 1899	2	0	2	0	0	●	o	g	sol
Andrena minutula (Kirby, 1802)	63	8	20	20	15	●	p	g	sol
Andrena mitis Schmiedeknecht, 1883	1	1	0	0	0	V	o	g	sol
Andrena cf. *nitida* (Müller, 1776)	14	3	6	3	2	●	p	g	sol
Andrena niveata Friese, 1887	5	1	3	0	1	3	o	g	sol
Andrena ovatula (Kirby, 1802)	4	0	0	4	0	●	p	g	sol
Andrena scotica Perkins 1917	1	0	0	1	0	●	p	g	com
Andrena strohmella Stöckhert, 1928	1	1	0	0	0	●	p	g	sol
Andrena tibialis (Kirby,1802)	5	0	1	2	2	●	p	g	sol
Andrena vaga Panzer, 1799	1	0	0	1	0	●	o	g	sol
Andrena ventralis Imhoff, 1832	1	0	1	0	0	●	o	g	sol
Andrena viridescens Viereck, 1916	2	1	0	1	0	V	o	g	sol
Apis mellifera Linnaeus 1758	74	18	20	22	14	●	p	a	soc
Bombus hortorum agg. (Linnaeus, 1761)	7	3	4	0	0	●	p	g/a	soc
Bombus pascuorum (Scopoli, 1763)	1	0	0	1	0	●	p	g/a	soc
Bombus ruderarius (Müller, 1776)	3	1	0	2	0	3	p	a	soc
Bombus terrestris agg. (Linnaeus, 1758)	13	4	7	1	1	●	p	g	soc
Ceratina cyanea (Kirby, 1802)	1	0	1	0	0	●	p	a	sol
Colletes cunicularius (Linnaeus, 1761)	3	0	2	0	1	●	p	g	sol
Eucera nigrescens Pérez, 1879	8	3	2	1	2	●	o	g	sol
Halictus cf. *eurygnathus* Blüthgen, 1931	1	0	0	0	1	●	p	g	sol
Halictus cf. *langobardicus* Blüthgen, 1944	2	0	1	1	0	●	p	g	sol
Halictus leucaheneus Ebmer, 1972	1	0	1	0	0	3	p	g	sol
Halictus quadricinctus (Fabricius, 1776)	1	0	0	1	0	3	p	g	sol
Halictus scabiosae (Rossi, 1790)	12	5	3	1	3	●	p	g	sol
Halictus sexcinctus (Fabricius, 1775)	1	0	0	0	1	3	p	g	sol
Halictus simplex agg. Blüthgen, 1923	7	3	3	1	0	●	p	g	sol
Halictus subauratus (Rossi, 1792)	6	2	2	1	1	●	p	g	soc
Halictus tumulorum agg. (Linnaeus, 1758)	27	5	6	8	8	●	p	g	soc
Hylaeus angustatus (Schenck, 1861)	9	3	0	2	4	●	p	a	sol

Hylaeus brevicornis Nylander, 1852	4	0	3	0	1	●	p	a	sol
Hylaeus dilatatus (Kirby, 1802)	1	0	1	0	0	●	p	a	sol
Hylaeus cf. *hyalinatus* Smith, 1842	1	0	1	0	0	●	p	a	sol
Hylaeus pictipes Nylander, 1852	1	0	1	0	0	●	p	a	sol
Lasioglossum aeratum (Kirby, 1802)	1	1	0	0	0	3	p	g	
Lasioglossum cf. *bluethgeni* Ebmer, 1971	9	3	4	0	2	G	p	g	
Lasioglossum calceatum (Scopoli, 1763)	11	4	1	5	1	●	p	g	soc
Lasioglossum glabriusculum (Morawitz, 1872)	249	63	60	59	67	●	p	g	soc
Lasioglossum cf. *laevigatum* (Kirby, 1802)	1	1	0	0	0	3	p	g	sol
Lasioglossum laticeps (Schenck, 1868)	51	18	11	13	9	●	p	g	soc
Lasioglossum lativentre (Schenck, 1853)	11	2	2	4	3	V	p	g	sol
Lasioglossum leucopus (Kirby, 1802)	1	0	0	0	1	●	p	g	sol
Lasioglossum leucozonium (Schrank, 1781)	6	1	0	3	2	●	p	g	sol
Lasioglossum lineare (Schenck, 1868)	218	64	36	69	49	3	p	g	soc
Lasioglossum malachurum (Kirby, 1802)	424	196	153	55	20	●	p	g	soc
Lasioglossum minutissimum (Kirby, 1802)	13	0	4	6	3	●	p	g	sol
Lasioglossum morio (Fabricius, 1793)	100	22	28	32	18	●	p	g	soc
Lasioglossum pallens (Brullé, 1832)	2	0	0	1	1	●	p	g	sol
Lasioglossum cf. *pauperatum* (Brullé, 1832)	10	6	2	1	1	2	p	g	
Lasioglossum pauxillum (Schenck, 1853)	38	4	6	19	9	●	p	g	soc
Lasioglossum politum (Schenck, 1853)	33	15	8	3	7	●	p	g	soc
Lasioglossum punctatissimum (Schenck, 1853)	7	1	2	2	2	●	p	g	
Lasioglossum puncticolle (Morawitz, 1872)	3	1	1	0	1	3	p	g	sol
Lasioglossum quadrinotatum (Kirby, 1802)	1	1	0	0	0	3	p	g	sol
Lasioglossum cf. *subhirtum* (Lepeletier, 1841)	3	1	2	0	0	3	p	g	
Lasioglossum villosulum (Kirby, 1802)	30	10	12	4	4	●	p	g	sol
Lasioglossum xanthopus (Kirby, 1802)	1	0	0	1	0	●	p	g	sol
Megachile cf. *centuncularis* (Linnaeus, 1758)	3	1	0	0	2	V	p	g/a	sol
Nomada distinguenda Morawitz, 1873	1	1	0	0	0	G			par
Nomada fabriciana (Linnaeus, 1767)	5	2	3	0	0	●			par
Nomada flavoguttata (Kirby 1802)	15	5	6	3	1	●			par
Nomada fucata Panzer, 1798	1	0	1	0	0	●			par
Nomada marshamella (Kirby, 1802)	1	0	1	0	0	●			par
Nomada cf. *minuscula* Noskiewicz, 1930	1	0	0	1	0	NA			par
Nomada striata Fabricius, 1793	1	0	1	0	0	●			par
Nomada zonata Panzer, 1798	1	0	0	1	0	V			par
Osmia adunca (Panzer, 1798)	3	1	2	0	0	●	o	a	sol
Osmia bicornis (Linnaeus, 1758)	31	8	10	9	4	●	o	a	sol
Osmia brevicornis (Fabricius, 1798)	2	1	1	0	0	G	o	a	sol
Osmia cornuta (Latreille, 1805)	1	1	0	0	0	●	p	a	sol
Osmia niveata (Fabricius, 1804)	1	0	0	0	1	3	o	a	sol
Sphecodes cf. *crassus* Thomson, 1870	1	0	0	1	0	●			par
Stelis minuta Lepeletier & Serville, 1828	1	1	0	0	0	●			par
Stelis ornatula (Klug, 1807)	1	0	0	0	1	●			par
Total	2015	608	592	460	355				

With 49 % more bees on average, organically managed vineyards (mean = 73 bees, SD = ±41) had a significantly higher bee abundance compared to conventionally managed vineyards (mean = 49 bees, SD = ±28; Table 4.3, Figure 4.3C), while the richness was not significantly affected by the management type (Figure 4.3D). Bee abundance increased significantly with increasing vegetation cover, and the richness, as a trend, increased as well (Figure 4.3B). SNH, the grape variety, and the number of flowering plant species did not have any significant effect

on the abundance or richness of bees (Figure 4.3A–D). The abundance of ground-nesting bees tended to be 48 % higher in organic vineyards and the richness tended to be higher with increasing vegetation cover (Table 4.4, Figure 4.4B–C, Figure S4.1). The abundance and richness of above-ground-nesting bees was higher with increasing proportions of semi-natural habitats (Table 4.4, Figure 4.4E).
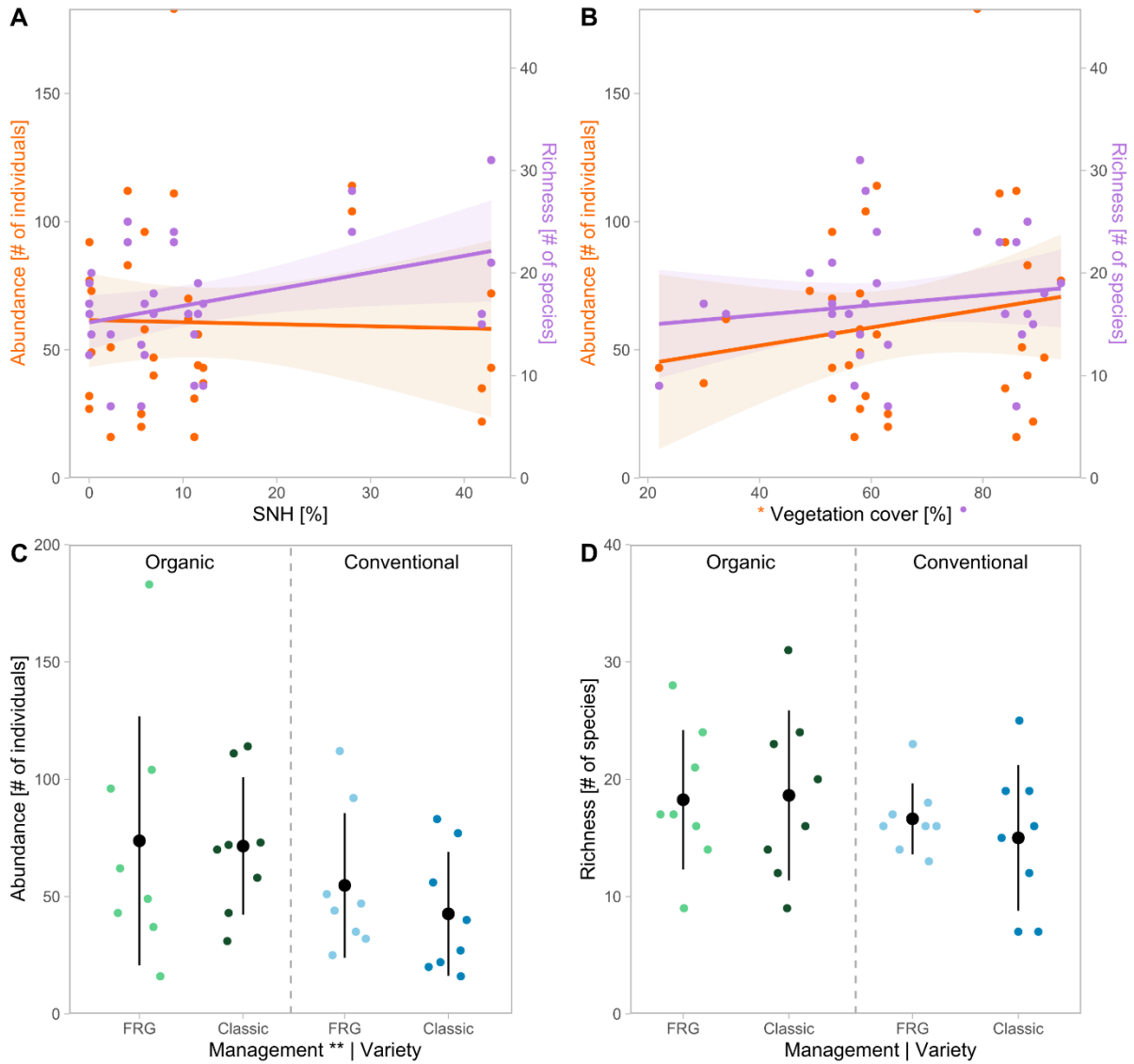
Figure 4.3 Abundance (orange) and richness (purple) of bees for proportion of semi-natural habitat (SNH; A) and vegetation cover (B), and abundance (C) and richness (D) for fungus-resistant (FRG, brighter) and classic (darker) grape varieties under organic (blue) and conventional (green) management. Shaded areas represent the 95 % confidence intervals. Significant effects are indicated in x-axis labels. Significance code: **$P < 0.01$, *$P < 0.05$, ●$P < 0.1$.

Table 4.4 Effects of semi-natural habitat (SNH), management type and grape variety, their interaction, vegetation cover, and flowering plants for abundance and richness of ground-nesting and above-ground-nesting bees. Abundance and richness of ground-nesting bees as well as abundance of above-ground-nesting bees was analyzed using a GLMM, while the richness of above-ground-nesting bees was analyzed using a LMM. Chi-square (χ^2), degrees of freedom (df), P-value, and the significance level (Sig.) are indicated. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ●$P < 0.1$.

	Ground-nesting bees								Above-ground-nesting bees							
	Abundance				Richness				Abundance				Richness			
Variable	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.	χ^2	df	P-value	Sig.
(Intercept)	467.9	1	<0.001	***	85.1	1	<0.001	***	10.0	1	0.001	**	1.7	1	0.192	
SNH	0.5	1	0.467		1.1	1	0.287		5.8	1	5.823	*	4.2	1	0.041	*
Management	2.8	1	0.097	●	1.3	1	0.254		1.7	1	0.193		2.6	1	0.107	
Variety	1.1	1	0.302		0.0	1	0.843		0.0	1	0.975		0.2	1	0.637	
Management:Variety	*Not included in best-fit model*				0.2	1	0.631		*Not included in best-fit model*				*Not included in best-fit model*			
Vegetation cover	*Not included in best-fit model*				3.4	1	0.063	●	*Not included in best-fit model*				*Not included in best-fit model*			
Flowering plants	*Not included in best-fit model*				*Not included in best-fit model*				*Not included in best-fit model*				*Not included in best-fit model*			
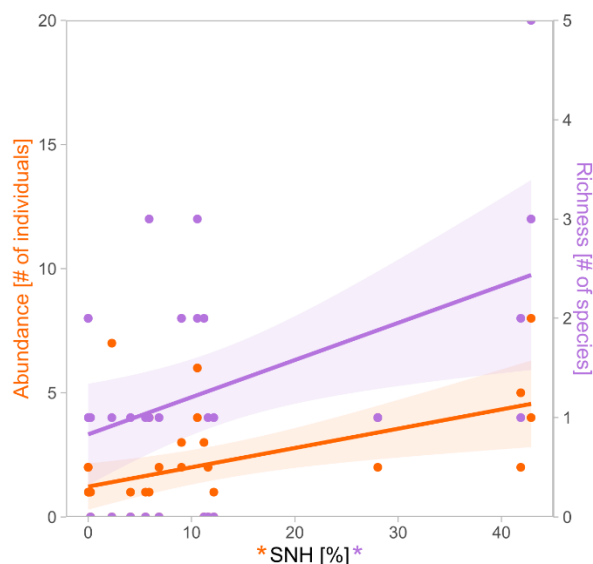
Figure 4.4 Abundance (orange) and richness (purple) of above-ground-nesting bees for proportion of semi-natural habitat (SNH). Shaded areas represent the 95 % confidence intervals. Significant effects are indicated in x-axis labels. Significance code: *$P < 0.05$.

The species composition of bees was influenced by SNH, vegetation cover, and management (Table 4.5, Figure 4.5). Most species (particularly *Osmia bicornis*, *A. dorsata*, *Lasioglossum bluethgeni*, *Andrena falvipes*, and *Lasioglossum pauxillum*) were related to vineyards with higher proportions of SNH in the surrounding landscape, while fewer species (particularly *L. lineare*, *L. glabriusculum*, and *L. malachurum*) were more abundant in vineyards with less SNH. Species were rather more abundant in vineyards with higher proportions of vegetation cover (particularly *L. lineare*, *Andrena haemorrhoa*, *L. pauxillum*, *L. morio*, and *Halictus tumulorum*), while some (particularly *L. malachurum*, *Andrena cineraria*, *Andrena lagopus*, *Lasioglossum villosulum*, and *Andrena gravida*) were related to vineyards with less vegetation cover. Indicator species related to organic vineyards were *Andrena lagopus*, *Bombus horturum*, *Bombus terrestris*, *L. malachurum*, and *Nomada fabriciana*, while *A. dorsata*, *A. haemorrhoa*, *Lasioglossum leucozonium*, and *L. pauxillum* were related to conventional vineyards.

Table 4.5 Effects of semi-natural habitat (SNH), management type, grape variety, vegetation cover, and flowering plants on the species composition of bees analyzed using redundancy analysis on species abundances. The variance, *F*-value, *P*-value, and the significance level (Sig.) are indicated. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Variable	Variance	*F*	*P-value*	Sig.
SNH	0.056	4.897	0.001	***
Management	0.026	2.309	0.006	**
Variety	*Not included in best-fit model*			
Vegetation cover	0.019	1.691	0.040	*
Flowering plants	*Not included in best-fit model*			
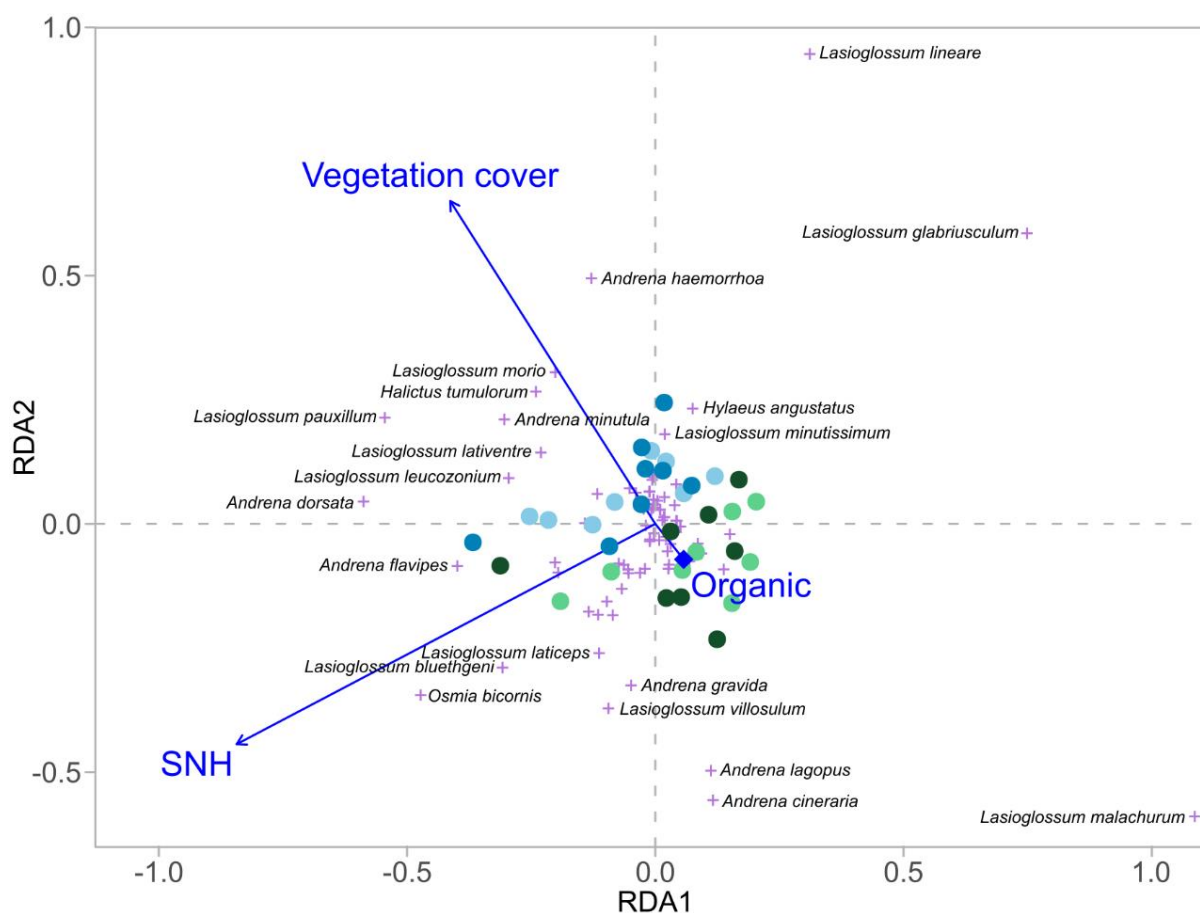
Figure 4.5 Effect of management (organic and conventional), proportion of semi-natural habitat (SNH), and vegetation cover on the species composition of bees in vineyards analyzed using redundancy analysis (based on species abundances with scaling 1). The first axis explains 56.89 % of the fitted and 14.56 % of the total variation and the second axis 29.17 % of the fitted and 7.46 % of the total variation.

4.4 Discussion

We investigated how SNH in the surrounding landscape, local ground vegetation, organic vineyard management, and pesticide reduction affect wild bees in Palatinate vineyards using yellow pan traps. Our main findings were that the proportion of SNH in the surrounding landscape influenced species composition and significantly increased the abundance and richness of above-ground-nesting bees. Organic vineyards had a 49 % higher abundance of bees, driven by some ground-nesting species that prefer low vegetation cover with bare ground areas. Pesticide reduction with the cultivation of fungus-resistant grape varieties, however, showed no significant effects.

The landscape context is known to be an important determinant of biodiversity patterns (Tscharntke *et al*. 2021). Surprisingly, we did not find a significant positive effect of SNH on the total abundance and richness of bees, which contradicts our first hypothesis, where we expected bee diversity to increase with increasing proportions of SNH in the surrounding. This finding deviates from the strong positive effects of landscape structure reported in various studies (Martin *et al*. 2019; Kolb *et al*. 2020; Barbaro *et al*. 2021; Kaczmarek *et al*. 2023a). The diversity of bees in our study predominantly comprised species from the genera *Lasioglossum*

and *Andrena*, which nest in the ground (Westrich 2018). Since they build their nests in bare ground areas also found in and near vineyards, they may not rely on nesting structures provided by SNH within their habitat range. Similarly, Kaczmarek *et al*. (2023b) found rather small effects of SNH on orthopterans, some of which complete the entire life cycle within vineyards and do therefore not depend on SNH in the surrounding landscape. Nonetheless, SNH supply abundant floral resources especially early in the season, which could also support the presence of some ground-nesting species (Eckerter *et al*. 2022). However, SNH were significantly increasing the abundance and richness of above-ground-nesting bees, similar to the positive effect of increasing cover of SNH on cavity-nesting bees observed by Uzman *et al*. (2020) and Wersebeckmann *et al*. (2023). *O. bicornis* was strongly related to SNH in our study. Contrary to the species from the genera *Lasioglossum* and *Andrena*, *O. bicornis* depends on woody structures to build cavity nests for breeding and thus relies on SNH in the surrounding area of vineyards (Westrich 2018), as vineyards do not provide such nesting resources. The abundance of other cavity-nesters including the genera *Osmia*, *Hylaeus*, and *Ceratina* was low in our study, which may reflect a lack of suitable nesting opportunities within vineyards in our region. The diversity of above-ground-nesting bees in viticultural areas can thus be promoted by a more diverse landscape with suitable SNH such as hedgerows providing nesting opportunities.

For other cropping systems, organic management clearly benefits biodiversity compared to conventional management and bees are among the most strongly favored organisms in organic arable crops, likely due to the strongly enriched flowering weeds (Bengtsson *et al*. 2005). In viticulture, however, effects of organic management on biodiversity are not as clear, and both positive and negative effects have been reported (Döring *et al*. 2019; Kolb *et al*. 2020; Paiola *et al*. 2020; Ostandie *et al*. 2021; Beaumelle *et al*. 2023; Kaczmarek *et al*. 2023a). Consistent with our second hypothesis, where we expected a higher diversity of wild bees in organically managed vineyards, the abundance of bees was higher in organic vineyards. A major difference between the two management systems is the use of synthetic pesticides in conventional management, while organic viticulture relies solely on inorganic compounds, mostly copper and sulfur (Pedneault and Provost 2016). While insecticide use is low in our study region, fungicides, including copper and sulfur, can still affect non-target organisms (Nash *et al*. 2010; Biondi *et al*. 2012; Vogelweith and Thiéry 2018). Kaczmarek *et al*. (2023a) reported reduced arthropod biomass in organic compared to conventional viticulture. To find opposing effects on the abundance of bees may indicate, that bees are not as affected by the use of copper or sulfur, and that other factors determine their presence. Considering bees' high mobility, they may be less exposed to fungicides if they are able to avoid them during application. On the other hand, they are likely influenced by diverse management practices within their habitat range, which may have weakened any effects of pesticide use. The vineyards within our study region are often relatively small in size, and are likely affected by pesticide drift from adjacent vineyards that are frequently managed in different ways (Druart *et al*. 2011). In addition, differences between management systems may be caused by differences in vegetation and tillage practices. In our study, the higher abundance of bees in organic vineyards was mainly caused by some ground-nesting species. Although organic vineyards had significantly less vegetation cover in the studied year, ground-nesting species seemed to benefit from this, as it increased the availability of bare ground areas for nesting sites, which we will discuss further below. Therefore, negative effects on arthropod biomass,

as reported by Kaczmarek *et al.* (2023a), may be more related to species that are more exposed to pesticides or, unlike ground-nesting bees, do not depend on bare ground areas.

Positive effects of reduced pesticide use in FRG varieties were shown for some groups of non-target organisms, e.g., predatory mites, spiders, and herb-dwelling orthopterans (Pennington *et al.* 2017; Pennington *et al.* 2019; Reiff *et al.* 2021; Kaczmarek *et al.* 2023b; Reiff *et al.* 2023). Interestingly, and contrasting to our third hypothesis where we expected such an effect, we found no effect of reduced pesticide applications in FRG varieties on bees, even though they received less than half as many applications compared to classic grape varieties. This supports the previously mentioned assumption that bees, at least in our study region with no insecticide use, are not as affected by pesticide management as vine-dwelling organisms due to their mobility or effects are not as clear due to pesticide drift from adjacent vineyards. Besides negative effects of pesticides, however, also reduced disturbance and soil compaction along with fewer pesticide applications appear to have no strong effects on bee diversity. Therefore, other factors, such as the inter-row vegetation, may play a more prominent role in determining their presence.

Extensive vegetation management with diverse vegetation cover contributes to biodiversity conservation in vineyards (Winter *et al.* 2018). According to our study results, bees benefit from increased vegetation cover in inter-rows. Since in the year of sampling the average vegetation cover was higher in conventional vineyards than in organically managed ones, high vegetation cover alone does not seem to account for the higher abundance of bees in organic vineyards. Ground-nesting bees, including the most common species in our study (*L. malachurum*), benefit from bare ground areas without vegetation cover because they rely on it for nesting (Potts *et al.* 2005). In the year of sampling, we observed lower vegetation cover in organic vineyards. While this is not a general characteristic of organic management in our region, it appeared beneficial for ground-nesting bees in our study. Furthermore, it is important to note that high vegetation cover does not necessarily indicate a structurally diverse and resource-rich vegetation in the inter-rows of the vineyards. Conventional vineyards tended to have a high cover of grasses, which are unattractive to bees. On the other hand, although the number of flowering plants did not differ between management types and did not significantly influence bee diversity, some nectar- and pollen-rich plants (e.g., *Convolvulus arvensis*, *Fagopyrum esculentum*, *Malva sylvestris*, and *Trifolium incarnatum*) were typical of organic vineyards. These species are included in seed mixtures for the greening of inter-rows. Although flower-rich mixtures are not specific for organic farming, they are more commonly used in organic than in conventional viticulture in our region. This characteristic may have contributed to the increased abundance of bees in organic vineyards, which may benefited from diverse vegetation cover (Sutter *et al.* 2017; Winter *et al.* 2018). Therefore, an inter-row consisting of both diverse and flower-rich vegetation with high cover, as well as bare ground, may enhance the presence of such species. Differences in vegetation diversity and structure could therefore also account for the differences in species composition in our study.

Notably, our study identified the presence of *Lasioglossum* cf. *subhirtum*, which was recently observed in the region for the first time in about 70 years (Burger 2018). Additionally, we found a high abundance of *L. lineare*, along with other vulnerable bee species, emphasizing the potential of viticultural areas as important habitats for wild bee conservation, provided that species specific floral and nesting resources are abundant.

4.5 Conclusions

Our study revealed a higher abundance of wild bees in organic viticulture, but we observed no effect of reduced pesticide use. Furthermore, abundance and richness of above-ground-nesting bees were positively affected by SNH. This suggests that wild bee communities in our study region are primarily influenced by SNH in the surrounding area and ground vegetation within vineyards rather than the type and amount of pesticides used. Additionally, the low abundance of above-ground-nesting bees may indicate a lack of suitable nesting opportunities in the region. We conclude that wild bees would benefit mostly from the presence of diverse vegetation cover in both the vineyard and the surrounding landscape, as they can both offer abundant floral and nesting resources. Therefore, it is important to provide diverse vegetation in inter-rows while maintaining bare ground areas and create a structurally rich environment with suitable SNH to conserve wild bees in viticulture. Overall, viticultural landscapes in the area have the potential to serve as valuable habitats for wild bee conservation.

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CHAPTER 5

Differential responses of Orthoptera in vineyards to organic farming, pesticide reduction, and landscape heterogeneity

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Abstract

Conservation measures such as those under the European Green Deal aim to counteract the biodiversity loss by increasing the share of organic farming and reducing pesticide use, as well as increasing the proportion of semi-natural habitats (SNH) in agricultural landscapes. Given the large environmental impacts of agriculture, it is important to thoroughly understand effects of such measures on organisms to provide evidence-based and effective implications for conservation. In this study, we analyzed how vineyard management, pesticide reduction, and landscape composition affect Orthoptera densities and species composition. Therefore, we sampled herb- and vine-dwelling orthopterans in a paired design of classic and fungus-resistant grape (FRG) varieties in conventionally and organically managed vineyards along a landscape heterogeneity gradient. Here, FRG varieties allowed us to study the effect of 44 % reduced pesticide applications under real-world conditions. Total densities of herb-dwelling Orthoptera did not differ between grape varieties in conventional vineyards, but were 2.9 times higher in FRG varieties under organic management. In contrast, total densities of vine-dwelling Orthoptera, mainly driven by the dominant species *Phaneroptera falcata*, were similar between grape varieties in organic vineyards, but tended to be 1.4 times higher in classic grapes under conventional management. Furthermore, the management system and SNH in a radius of 500 m in the surrounding landscape influenced species composition.

Implications for insect conservation

Our work shows that the cultivation of FRG varieties, at least in organic viticulture, clearly benefits some orthopteran species. It appears that the reduction of non-specific pesticides such as copper and sulfur is important to mitigate negative effects and promote Orthoptera in viticulture.

5.1 Introduction

Together with the impact of climate change, habitat destruction, and invasive species, intensified agriculture is considered one of the main drivers of the serious loss of biodiversity in recent decades, harming the environment through regular disturbance, clearing of semi-natural habitats (SNH), and high use of pesticides and fertilizers (Hochkirch 2016; Hallmann *et al*. 2017; Seibold *et al*. 2019; Wagner 2020). To counteract the negative impacts of agriculture on biodiversity, measures such as those under the European Green Deal, including the Farm to Fork Strategy, are intended to increase the proportion of organic farming in the upcoming years, as well as reducing the use of pesticides (European Commission 2019). Given the large environmental impacts of agriculture, it is important to thoroughly understand the effects of intensive crop management on organisms as well as the impacts of such measures.

In viticulture, where the use of pesticides, especially fungicides, is particularly high due to introduced fungal diseases such as powdery and downy mildew, large non-target effects, e.g., on arthropods, can be expected (Pertot *et al*. 2017). While organic farming promotes biodiversity in many cropping systems compared to conventional farming (Bengtsson *et al*. 2005; Hole *et al*. 2005; Tuck *et al*. 2014), the effect seems to be less clear in viticulture and vary between and even within organism groups (Bruggisser *et al*. 2010; Döring *et al*. 2019;

Paiola *et al*. 2020; Ostandie *et al*. 2021; Schirmel *et al*. 2022; Kaczmarek *et al*. 2023). Since the use of synthetic chemicals is prohibited in organic management, non-synthetic components such as copper and sulfur are used in high quantities in viticulture to control pests, which, however, can also negatively impact non-target organisms (Nash et al. 2010; Biondi *et al*. 2012; Vogelweith and Thiéry 2018). The copper accumulates in the soil and thus may have long-term risks on the environment (Komárek *et al*. 2010). Further, an increased number of pesticide applications in organic viticulture leads to more tractor passages, and may affect non-target organisms by higher disturbance of the ground vegetation and increased soil compaction (Bruggisser *et al*. 2010).

Regardless of conventional or organic vineyard management, the impact of pesticides on biodiversity can be reduced by cultivating fungus-resistant grape (FRG) varieties, which are characterized by resistance traits to the major fungal diseases (Töpfer *et al*. 2011). While the first resistant grape varieties were developed in the late 19th and early 20th centuries, the first cultivars with convincing wine qualities were only developed by the end of the 20th century and nowadays, over 38 cultivars are available for winegrowers (Töpfer and Trapp 2022). However, despite that the resistances can reduce the need for pesticides by up to 80 % for some multi-resistant varieties and increase the sustainability of viticulture (Töpfer and Trapp 2022), only about 2.7 % of the area under cultivation is planted with FRG varieties in our study region (Statistisches Bundesamt (Destatis) 2023). Positive effects of the cultivation of such varieties are recently reported to benefit non-target organisms, including predatory mites and some spider families (Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021a; Reiff *et al*. 2023), making the cultivation of FRG varieties a promising approach to promote biodiversity in viticulture. While this is of importance in the context of, e.g., the European efforts to reduce pesticides, evidence is lacking as there are only few recent research studies on the effects of the cultivation of FRG varieties on biodiversity.

Although organisms are usually exposed to high levels of pesticides due to the intensive management in viticulture, vineyards can provide a suitable habitat for species through the vines themselves, but especially through the vegetation in the inter-rows. Improved ground vegetation management may promote biodiversity by higher vegetation cover and species-rich cover crops providing habitat especially for typical grassland species (Winter *et al*. 2018; Paiola *et al*. 2020; Ortis *et al*. 2021; Blaise *et al*. 2022). In addition, biodiversity is usually enhanced by heterogeneous landscapes with a high proportion of SNH (Martin *et al*. 2019; Paiola *et al*. 2020; Ostandie *et al*. 2021). Forests, hedges, shrubs, and grasslands can provide habitat for shelter and overwintering as well as food and breeding resources for species for which such resources are not available in vineyards (Holland et al. 2017).

Orthopterans are an important arthropod group in terrestrial food webs and provide a food source for various predatory species (Belovsky and Slade 1993). However, agricultural land use intensification, including the use of pesticides, is considered one of the major threats to Orthoptera species (Zuna-Kratky *et al*. 2016). They may be highly exposed to pesticides through surface contact, their feeding behavior, and the egg-laying substrate (Ingrisch and Köhler 1998; Bundschuh *et al*. 2012), making them sensitive to environmental changes caused

by management practices. In viticulture, the orthopteran fauna is further determined by tillage, cover crop management, the presence of habitat structures in the surrounding area, and the location of the vineyard in the landscape context (Detzel 1998). In Central Europe, vineyards can mainly be found in climatically favorable regions (Bruggisser *et al*. 2010) and can provide habitat for several Orthoptera species including both shrub-dwelling orthopterans (mainly species of the order Ensifera) in the vines and herb-dwelling orthopterans (mainly of the order Caelifera) in the inter-row vegetation (Detzel 1998). Hence, Orthoptera are appropriate indicators of the effects of pest, soil, and cover crop management practices and landscape heterogeneity in viticulture as they meet many of the criteria for effective ecological indicators (Noss 1990). In addition, density and species composition can be easily measured (Gardiner *et al*. 2005), which is why orthopterans are widely used as indicator species in ecological studies (Bazelet and Samways 2011; Alignan *et al*. 2018; Dvořák *et al*. 2022).

To assess how organic vineyard management, pesticide reduction, and landscape heterogeneity affect orthopteran densities and species composition, we sampled herb- and vine-dwelling orthopterans using box quadrats and transect walks with song detection, respectively. For this purpose, we used a paired design with classic and FRG varieties in either conventionally or organically managed vineyards along a gradient of landscape heterogeneity. In particular, we expected that (1) orthopterans are promoted by organic compared to conventional farming and (2) that they benefit from the cultivation of FRG varieties. Lastly, we investigated whether (3) SNH-rich compared to vineyard-dominated landscapes favor orthopterans.

5.2 Materials and methods

5.2.1 Study area and site selection
We conducted the study in the district Südliche Weinstraße and the district-free city Landau in der Pfalz (49.273280 °N, 8.020602 °E / 49.147516 °N, 8.175736 °E). The area is located in the wine-growing region Palatinate in the south of Rhineland-Palatinate in southwestern Germany with a temperate climate with an average annual temperature of 11.1 °C and a total annual precipitation of 687.5 mm (Beck *et al*. 2018; Agrarmeteorologie Rheinland-Pfalz 2022).

We chose 16 different landscapes along a gradient of landscape heterogeneity in the study region that differed in their proportion of SNH in the surrounding landscape (Figure 5.1). In each landscape, we sampled in two vineyards planted with a FRG and a classic grape variety, respectively, while the management system was organic in half of the pairs of vineyards and conventional in the other half. Of the vineyards studied, those managed conventionally had 10 pesticide applications in classic grape varieties (SD = ±2) and 7 in FRG varieties (SD = ±3), while those managed organically had 13 pesticide applications in classic grape varieties (SD = ±1) and 6 in FRG varieties (SD = ±3; Table S5.1).

5.2.2 Sampling of herb-dwelling Orthoptera
In mid-August 2021, during dry and warm weather with temperatures ranging from 20 to 30 °C, a 40 m section was sampled in two randomly selected inter-rows in the center of each vineyard using a 1.96 m box quadrat to assess the density of herb-dwelling Orthoptera. We
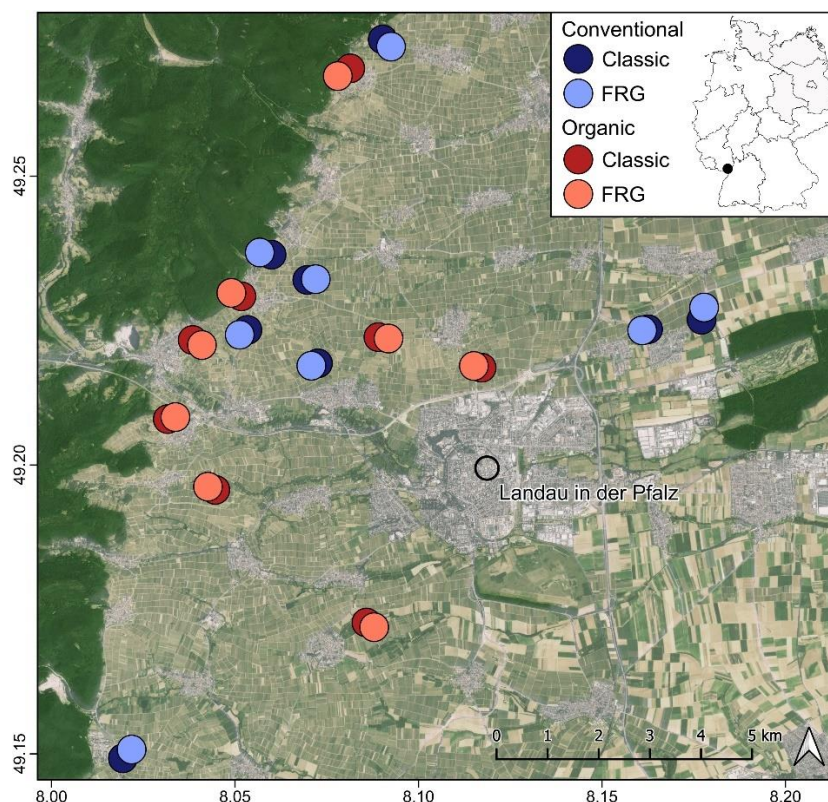
Figure 5.1 Locations of the 16 landscapes in the south of Rhineland-Palatinate, Germany, with organic (red) and conventional (blue) management and fungus-resistant (FRG, brighter) and classic grape varieties (darker). Basic map data by © GeoBasis-DE/LVermGeoRP (2022).

sampled in two adjacent, differently tilled inter-rows and placed the isolation square on the ground seven times per inter-row. We identified orthopterans within the box quadrat (according to Fischer *et al*. 2020) and released individuals afterwards. Only adult individuals were analyzed.

5.2.3 Sampling of vine-dwelling Orthoptera
In early September 2021, after sunset and during dry weather with temperatures above 10 °C, we walked through two randomly selected inter-rows in the center of each vineyard to assess the density of vine-dwelling Orthoptera in the adjacent rows of grape vines. We detected individuals by their species-specific songs (according to Orthoptera.ch 2021) and additionally used a bat detector (Observer 2 HD², CIEL-electronique) to make calls in the high frequency range audible. We calculated the number of individuals per 100 m inter-row.

5.2.4 Vegetation and landscape parameters
We measured the vegetation height in two randomly selected inter-rows per vineyard by using a cardboard-disc with a diameter of 30 cm and a measuring stick. Further, we visually assessed the proportion of ground covered by vegetation in the two whole inter-rows. A mean value for vegetation height and cover was calculated out of all measurements for each vineyard (Table S5.1). For each landscape, we calculated the mean proportion of SNH, which we defined as forests, hedges, shrubs, and grassland, within a radius of 500 m of each vineyard using ATKIS data (Basis-DLM by ©GeoBasis-DE/BKG (2013)) with intersection of spatial data in an Oracle database 12c (Oracle 2017).

5.2.5 Data analysis

We used R *v.4.0.5* (R Core Team 2021) for statistical analyses and the R package ggplot2 (Wickham 2016) for creating figures.

We used an information-theoretic approach to multi-model inference (Burnham and Anderson 2002) to analyze the effects of vineyard management and vegetation and landscape parameters on Orthoptera densities. We conducted linear mixed models (R command *lmer* in the R package lme4, Bates *et al*. 2015) and used total density of herb-dwelling Orthoptera, total density of vine-dwelling Orthoptera, and densities of the three most frequent herb- and vine-dwelling Orthoptera, respectively, as dependent variables. In order to meet model assumptions, densities were log(x+1) transformed. We standardized the regression predictors using the *standardize* function (R package arm, Gelman and Su 2016). As explanatory variables we included vineyard management (factor with the two levels 'organic' and 'conventional'), grape variety (factor with the two levels 'FRG' and 'classic'), vegetation cover (continuous), vegetation height (continuous), and the amount of SNH in the surrounding landscape (continuous) in the full models. To assess whether the effect of grape variety differs among organic and conventional vineyards, we further included their interaction in the models. Due to our paired design, we included the site ID as a random effect in the models. For automated model selection, we used the *dredge* function (R package MuMln, Bartoń 2020) and selected those top-ranked models within Δ AICc < 4. We used the AICc for small sample sizes. Conditional averaged parameter estimates from this top set of models were then produced using the *model.avg* function. To check for correlations among the explanatory variables we calculated the variation inflation factors (VIF). In cases where an explanatory variable had a VIF > 2 (which was either vegetation height or vegetation cover), we excluded this variable in the full model which resulted in VIF < 2 of all remaining variables.

The species composition of Orthoptera was analyzed using redundancy analysis (command *rda* in package vegan, Oksanen et al. 2020). For the multivariate analysis, we used binary data (presence/absence) because densities of herb- and vine-dwelling Orthoptera are not comparable due to the differently used sampling methods. We used the same explanatory variables as in the univariate models.

5.3 Results

5.3.1 General results

With the box quadrat, we sampled 271 adult individuals of five herb-dwelling Orthoptera species (Table S5.2). Most frequent species were *Chorthippus brunneus* (Thunberg, 1815; 126 individuals) followed by *C. biguttulus* (Linnaeus, 1758; 91 individuals) and *Pseudochorthippus parallelus* (Zetterstedt, 1821; 50 individuals). Only one Ensifera individual of *Roeseliana roeselii* (Hagenbach, 1822) was found while all others belonged to Caelifera.

With the sound detection, we sampled 270 individuals of four vine-dwelling species (Table S5.3). By far the most frequent species was *Phaneroptera falcata* (Poda, 1761; 209 individuals) followed by *Leptophyes punctatissima* (Bosc, 1792; 31 individuals) and *Tettigonia viridissima* (Linnaeus, 1758; 23 individuals).

5.3.2 Effects of vineyard management, grape variety, and local and landscape parameters
Total density of herb-dwelling Orthoptera was significantly affected by grape variety and the interaction of management system and grape variety (Table 5.1). This indicates that total densities were not different between classic and FRG varieties in conventional vineyards, but were almost 3 times higher in FRG varieties under organic management (Figure 5.2A). Management, vegetation cover, and the amount of SNH in the surrounding were included in the final model of total density of herb-dwelling Orthoptera but had no significant effects (Table 5.1). The two most common herb-dwelling species *C. brunneus* and *C. biguttulus* showed similar responses: Both species had higher densities in FRG varieties under organic management, while no such differences were observed in conventional vineyards. Furthermore, *C. brunneus* densities significantly increased with increasing vegetation height (Figure 5.2B–C). Management, grape variety, and the amount of SNH in the surrounding were all not significantly related to densities of *C. brunneus* and *C. biguttulus* (Table 5.1). For *P. parallelus* we found a significant influence of the grape variety while all other parameters had no significant effect (Table 5.1). Densities were on average about two times higher in FRG compared to classic varieties (organic vineyards: three times higher, conventional vineyards: 1.7 times higher; Figure 5.2D).
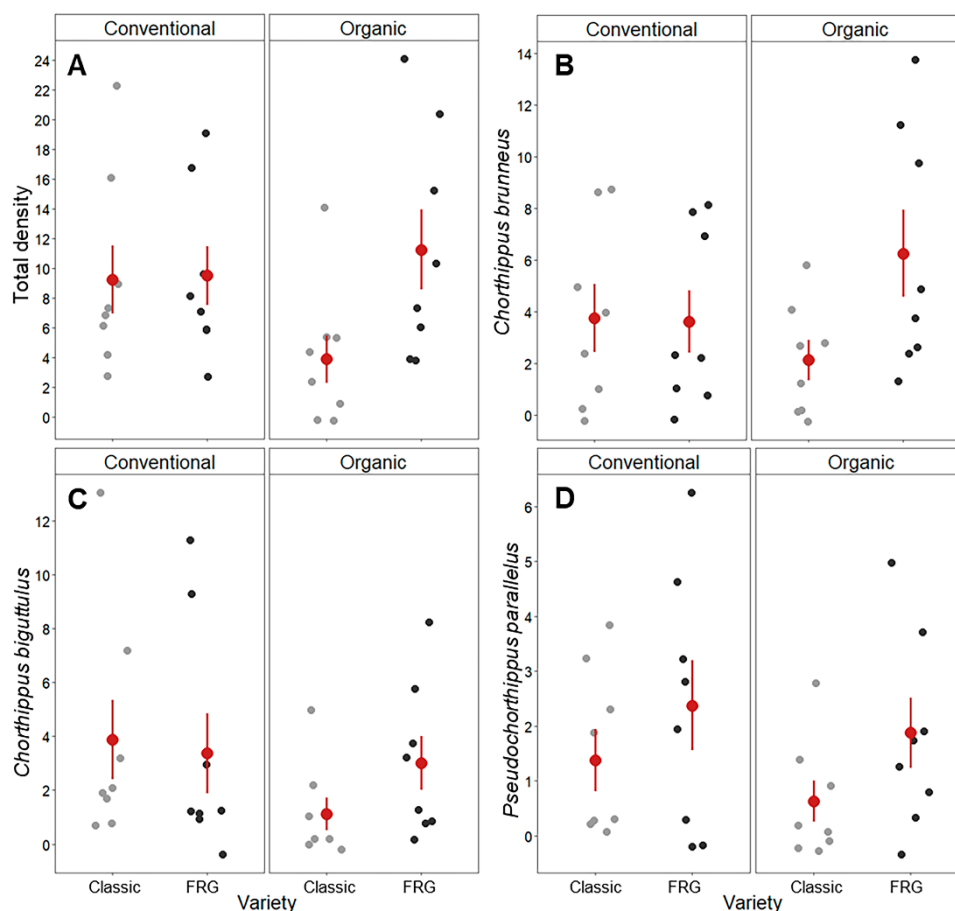
Figure 5.2 Densities (mean ± SE and raw data points) of herb-dwelling Orthoptera (total and the three most common species) for classic and fungus-resistant grape (FRG) varieties under conventional and organic management.

As a trend, total density of vine-dwelling Orthoptera was affected by the interaction of management and grape variety (Table 5.2). Densities were similar between classic and FRG varieties in organic vineyards, but 1.4 times higher in classic grapes under conventional management (Figure 5.3A). Further, their density tended to increase with increasing vegetation cover. All other parameters included in the final model of total density of vine-dwelling Orthoptera had no significant effects (Table 5.2). For the density of the most frequent vine-dwelling Orthoptera *P. falcata*, we found no significant effects of the explanatory variables (Table 5.2). *P. falcata* densities were higher in conventional than in organic vineyards, but this pattern was statistically not significant and driven by two extreme values in classic and FRG varieties, respectively (Figure 5.3B; Table 5.2). Organic management had a significant negative effect on *T. viridissima* and densities were on average three times higher in conventional than in organic vineyards (Table 5.2; Figure 5.3C). The amount of SNH in the surrounding landscape, grape variety, and its interaction with management as well as vegetation height had all no significant influence on the density of *T. viridissima* (Table 5.2). *L. punctatissima* densities increased significantly with increasing amount of SNH while no other explanatory variable had a significant effect (Table 5.2; Figure 5.3D).
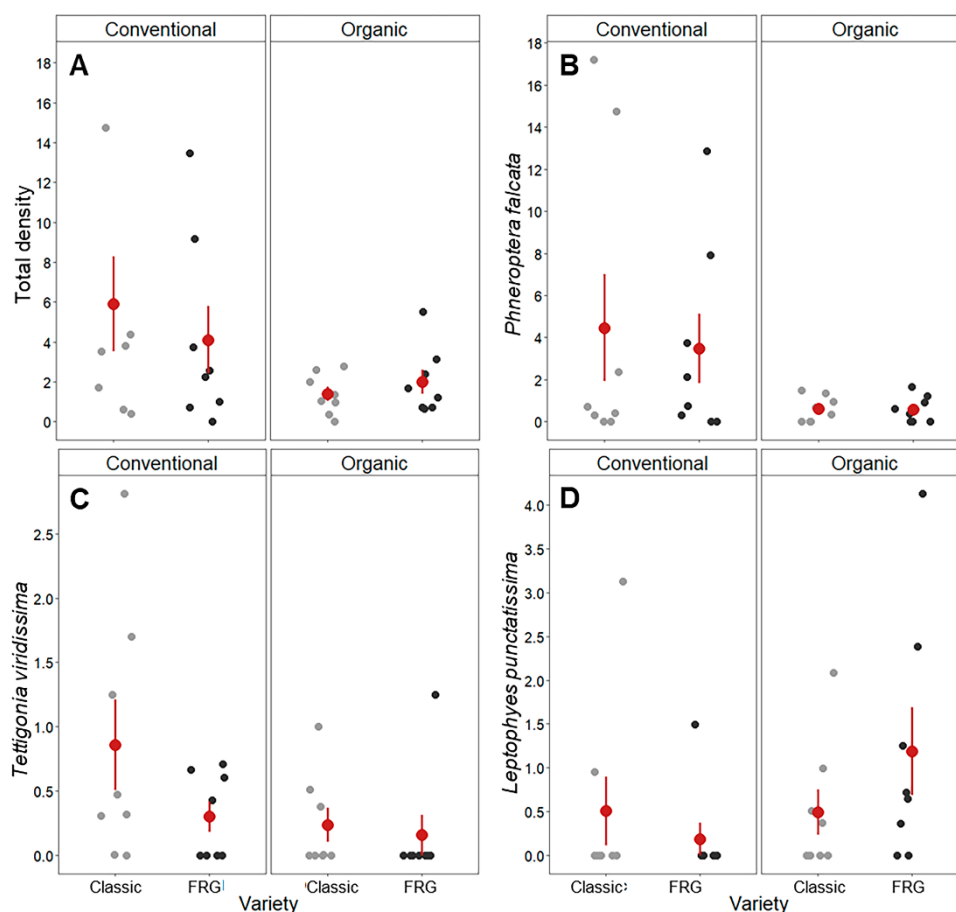
Figure 5.3 Densities (mean ± SE and raw data points) of vine-dwelling Orthoptera (total and the three most common species) for classic and fungus-resistant grape (FRG) varieties under conventional and organic management.

Table 5.1 Model-averaging results of the top-ranked models for total densities of herb-dwelling Orthoptera and the three most common species in vineyards with different management (conventional, organic) and grape varieties (classic, fungus-resistant). Indicated are the standardized coefficient (Coeff.), adjusted standard error (SE), *z*-score (*z*), and *P*-value (*P*) for management, grape variety, their interaction (Man:Var), semi-natural habitats (SNH), and vegetation cover and height. Significant *P*-values are in bold, *P*-values with a trend are in italics.

Predictor	Total density				*Chorthippus brunneus*				*Chorthippus biguttulus*				*Pseudochorthippus parallelus*			
	Coeff.	SE	z	P	Coeff.	SE	z	P	Coeff.	SE	z	P	Coeff.	SE	z	P
Intercept	0.863	0.069	12.586	**< 0.001**	0.557	0.067	8.364	**< 0.001**	0.451	0.074	6.127	**< 0.001**	0.306	0.060	5.107	**< 0.001**
Management	-0.186	0.138	1.343	0.179	0.030	0.137	0.218	0.828	-0.166	0.146	1.139	0.255	-0.083	0.120	0.691	0.489
Grape variety	0.254	0.053	4.809	**< 0.001**	0.179	0.120	1.493	0.135	-0.081	0.068	1.201	0.230	0.189	0.090	2.101	**0.036**
Man:Var	0.456	0.106	4.285	**< 0.001**	0.374	0.195	1.916	*0.055*	0.363	0.128	2.830	**0.005**	*Not included in top-ranked model*			
SNH	0.093	0.141	0.659	0.510	0.027	0.136	0.026	0.844	0.037	0.161	0.277	0.820	0.101	0.121	0.836	0.403
Veg. cover	0.099	0.085	1.155	0.248	*Not included in full model*				-0.151	0.117	1.294	0.196	*Not included in full model*			
Veg. height	*Not included in full model*				0.272	0.129	2.104	**0.035**	*Not included in full model*				-0.117	0.111	1.058	0.290

Table 5.2 Model-averaging results of the top-ranked models for total densities of vine-dwelling Orthoptera and the three most common species in vineyards with different management (conventional, organic) and grape varieties (classic, fungus-resistant). Indicated are the standardized coefficient (Coeff.), adjusted standard error (SE), *z*-score (*z*), and *P*-value (*P*) for management, grape variety, their interaction (Man:Var), semi-natural habitats (SNH), and vegetation cover and height. Significant *P*-values are in bold, *P*-values with a trend are in italics.

Predictor	Total density				*Phaneroptera falcata*				*Tettigonia viridissima*				*Leptophyes punctatissima*			
	Coeff.	SE	z	P	Coeff.	SE	z	P	Coeff.	SE	z	P	Coeff.	SE	z	P
Intercept	0.497	0.075	6.615	**< 0.001**	0.311	0.085	3.673	**< 0.001**	0.111	0.026	4.214	**< 0.001**	0.144	0.032	4.537	**< 0.001**
Management	-0.225	0.146	1.536	0.125	-0.258	0.164	1.575	0.115	-0.108	0.053	2.045	**0.041**	0.074	0.063	1.171	0.242
Grape variety	-0.018	0.056	0.331	0.741	0.008	0.060	0.129	0.898	-0.081	0.053	1.527	0.127	0.035	0.060	0.588	0.556
Man:Var	0.200	0.103	1.940	*0.053*	*Not included in top-ranked model*				0.089	0.100	0.864	0.386	*Not included in top-ranked model*			
SNH	0.012	0.165	0.678	0.498	-0.191	0.174	1.100	0.271	0.071	0.055	1.305	0.192	0.241	0.064	3.701	**0.002**
Veg. cover	0.149	0.091	1.650	*0.099*	0.100	0.099	1.009	0.313	*Not included in full model*				*Not included in full model*			
Veg. height	*Not included in full model*				*Not included in full model*				0.033	0.060	0.547	0.584	0.034	0.064	0.533	0.594

The Orthoptera species composition, based on presence-absence data, was significantly influenced by vineyard management and the amount of SNH in the surrounding of the vineyard (Table 5.3). However, it was not affected by the grape variety. Species related to conventional vineyards were *T. viridissima* and *C. biguttulus*, while *L. punctatissima* were more common in organic vineyards (Figure 5.4). *L. punctatissima* was also related to vineyards with a higher amount of SNH in the surrounding landscape, while *P. falcata* was more common in vineyards with less SNH (Figure 5.4).

Table 5.3 Effect of vineyard management (conventional, organic), grape varieties (classic, fungus-resistant), vegetation height and cover, and the proportion of semi-natural habitats in the surrounding landscape on the species composition of Orthoptera in vineyards analyzed using redundancy analysis on presence-absence data. Indicated are the *F*-value (*F*) and *P*-value (*P*). Significant *P*-values are in bold.

Predictor	F	P
Management	2.576	**0.009**
Grape variety	1.265	0.267
Vegetation height	1.463	0.159
Vegetation cover	1.615	0.122
Semi-natural habitats	2.177	**0.028**
Management:Grape variety	0.708	0.670
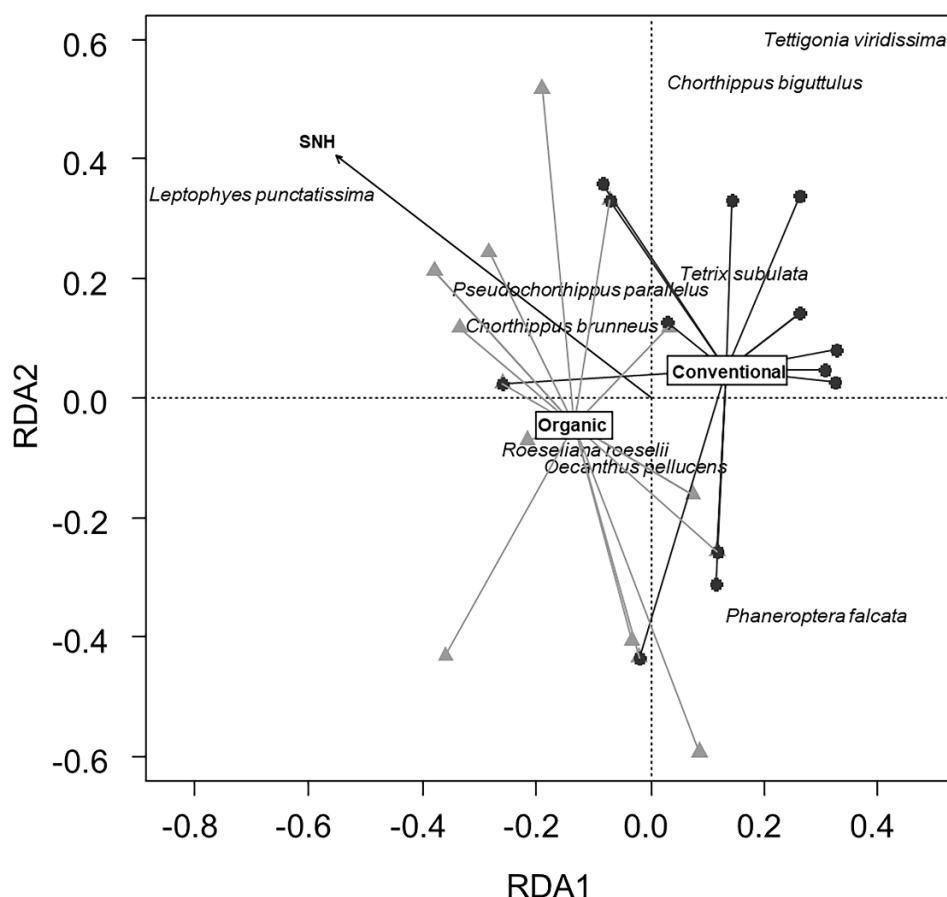
Figure 5.4 Biplot based on redundancy analysis (presence-absence data) of the species composition of Orthoptera in vineyards. Species composition was significantly affected by vineyard management (organic, conventional) and the proportion of semi-natural habitats in the surrounding landscape. For statistics see Table 5.3.

5.4 Discussion

We assessed how organic and conventional viticulture, reduced pesticide application by using FRG varieties, and the proportion of SNH in the landscape around vineyards affected the density and species composition of herb- and vine-dwelling Orthoptera. Our main findings were that total densities of herb-dwelling Orthoptera were greatly enhanced in FRG varieties under organic management, while total densities of vine-dwelling Orthoptera tended to be higher in classic grapes under conventional management. Further, the management system and SNH in the surrounding landscape influenced species composition.

In contrast to our first hypothesis, we did not find any general effects of organic viticulture on total densities of Orthoptera. The only species where we found an effect was *T. viridissima*, being even three times less abundant in organically managed vineyards than in conventional ones. Thus, a conversion from conventional to organic viticulture, such as that aimed for by the European Green Deal to counteract the loss of biodiversity (European Commission 2019), does not seem to increase Orthoptera densities in our study. However, it is important to note that the diversity and conservation value of orthopterans that we found was rather low compared to some areas with different soil and relief, such as in the Middle Rhine valley (Wersebeckmann *et al*. 2023). Similarly, other studies showed that general effects of organic farming in viticulture on biodiversity are less clear then in other cropping systems, where both positive and negative effects were reported (Bengtsson *et al*. 2005; Bruggisser *et al*. 2010; Paiola *et al*. 2020; Ostandie *et al*. 2021). In organic viticulture, non-synthetic compounds (mainly copper and sulfur) are used instead of synthetic chemicals, but they can have strong effects on non-target organisms, too (Nash *et al*. 2010; Biondi *et al*. 2012; Vogelweith and Thiéry 2018). Möth *et al*. (2021) found higher toxicity levels in organic vineyards, where high concentrations of copper accumulate in the soil (Mackie *et al*. 2012). The effects of management and fungicide reduction observed in our study could have been weakened by pesticide drift from neighboring fields (Druart *et al*. 2011), because vineyards of our region are often small and adjacent vineyards are frequently managed differently. However, the observed differences in species abundances and species composition confirm that our study design was suitable to detect local management effects on Orthoptera. Vineyard management influenced species and taxonomic groups differently, and thus, may affect species interactions (Caprio *et al*. 2015; Pedneault and Provost 2016; Vogelweith and Thiéry 2018; Ostandie *et al*. 2021). Further research is needed to determine the extent to which species relevant to nature conservation or beneficial insects are harmed or benefited by conversion to organic viticulture.

Herb-dwelling orthopterans were, in accordance with our second hypothesis, more common in FRG varieties with fewer pesticide applications compared to classic grape varieties. However, a positive effect of reduced pesticide application, with the exception of *P. parallelus*, was only present in organically managed vineyards, where copper and sulfur are used for plant protection. Since both the herb-dwelling Orthoptera and *T. viridissima*, which was affected by organic management as we discussed before, lay their eggs in the soil (Ingrisch and Köhler 1998), the eggs and hatching larvae, respectively, may be comparatively affected by accumulating copper in the topsoil (Karimi *et al*. 2021), which could explain the stronger

negative effect. Furthermore, orthopterans can be exposed to copper through surface contact and their feeding behavior (Ingrisch and Köhler 1998). In a microcosm experiment, however, Karimi *et al*. (2021) have determined that the effects of copper on soil biodiversity are only measurable at annual concentrations far above those authorized by the European commission, while in toxicity tests, Duque *et al*. (2023) found that concentrations also found in regional vineyards could have lethal effects on earthworms. However, Karimi *et al*. (2021) also point out that there is still a lack of field experiments that provide information on community dynamics under in situ conditions. In addition to the negative effects of copper, the use of dusting sulfur, which is used particularly in organic viticulture as fungicide but acts as a broad-spectrum pesticide, may also negatively affect non-target organisms in the inter-rows, potentially leading to a positive effect of reduced plant protection on herb-dwelling orthopterans. A negative effect of sulfur on non-target organisms such as parasitoids, predatory thrips and mites, and grapevine moths has been shown in earlier studies (Hanna *et al*. 1997; Jepsen *et al*. 2007; Tacoli *et al*. 2020). In addition to negative effects of pesticides in organic and conventional viticulture, the fact that *P. parallelus* is similarly affected by the cultivation of FRG varieties in both organic and conventional management may be also due to a lower overall workload in those vineyards. A reduced number of pesticide applications leads to less tractor traffic, resulting in less disturbance of the ground vegetation and reduced soil compaction likely enhancing orthopterans (Bruggisser *et al*. 2010). This could be particularly relevant in flightless species such as *P. parallelus*. Less intensive tillage may also be beneficial for egg pods development (Detzel 1998).

Vine-dwelling Orthoptera had similar densities in organic managed vineyards, but tended to be 1.4 times more abundant in classic than in FRG varieties in conventional managed vineyards. However, the total density of vine-dwelling Orthoptera was driven by high densities of *P. falcata*. *T. viridissima* seems to be more affected by pesticides used under organic management, where it occurs generally less common compared to conventional management. This may be because the larval stage lives in the herb layer (Detzel 1998), where it may come into contact with copper more intensively. Although copper also accumulates in the leaves of grapevines throughout the season (Angelova *et al*. 1999), species appear to be less affected here. One reason for this could be that copper on leaves is washed off by precipitation (Angelova *et al*. 1999). Furthermore, the use of sulfur as a widely used pesticide in organic viticulture may play a role here, too. Thus, reducing high toxicity levels in organic viticulture by cultivating FRG varieties can have a particularly large effect on promoting such affected species and may be a promising approach to reduce the pressure of intensified agriculture on biodiversity, as it was also reported to have positive effects on mites, spiders, and certain insects (Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021a; Kaczmarek et al. 2023; Reiff *et al*. 2023). Furthermore, the trend for higher densities of vine-dwelling Orthoptera in classic grape varieties compared to FRG varieties in conventionally managed vineyards, driven by *P. falcata*, could possibly be explained by changes in species composition. For example, vine-dwelling Orthoptera could benefit from increased pesticide use if their predators or competitors are affected negatively. Further research would be needed to substantiate such possible indirect effects. Regardless of the exact mechanisms,

our results highlight how the use of pesticides can affect species abundance differently and how these differential effects can alter species composition.

While a strong positive effect of landscape heterogeneity on biodiversity has been reported in various studies (Schmidt *et al*. 2005; Martin *et al*. 2019; Kolb *et al*. 2020; Barbaro *et al*. 2021) the influence of landscape is comparatively low in our study. One reason for this rather low positive effect on Orthoptera could be that orthopterans tend to be sedentary species with a small range of action and the entire life cycle of the observed herb- and vine-dwelling species can take place in the vineyards or the immediate surroundings (Detzel 1998; Ingrisch and Köhler 1998). Only the species *L. punctatissima* occurs more frequently with a higher proportion of SNH. *L. punctatissima* prefers forest edges and is dependent on woody structures with adjacent grass and herbaceous areas (Detzel 1998; Schlumprecht 2003), so a higher abundance is expected here, as a higher proportion of SNH in our study is often associated with a higher proportion of forest in the surrounding area and thus a shorter distance to forest edges (Figure S5.1).

Biodiversity is usually influenced by local practices besides pesticide use, such as tillage and cover crop management (Ostandie *et al*. 2021; Reiff *et al*. 2021b; Blaise *et al*. 2022). In line with this, we found that vegetation positively affected *C. brunneus*, which became more abundant with increasing vegetation height. Additionally, the total density of vine-dwelling Orthoptera tended to increase with higher ground vegetation cover, possibly being influenced by the inter-row vegetation during larval stages that live in the herb layer (Detzel 1998). Increased densities of Orthoptera with increasing vegetation height and vegetation cover might be linked to higher food supply, suitable microclimate, and the provision of hiding places. Further, more intensive tillage may harm egg pods development (Detzel 1998). Adapted management of vineyard inter-rows, such as grazing or less intensive mowing and tillage, can thus be another effective measure to promote the biodiversity of herb-dwelling species, in addition to the measures previously discussed (Detzel 1998; Blaise *et al*. 2022; Bosco *et al*. 2022).

5.5 Conclusions

According to our results, organic viticulture had no general positive effect on Orthoptera. Rather, reducing the number of pesticide applications and associated tractor passages, at least in organic viticulture, is important and shows a clear benefit to some orthopterans. Effects of the proportion of semi-natural habitats in the surrounding landscape on Orthoptera in vineyards were weak, while other studies showed strong positive effects for other taxonomic groups of insects as well as for spiders and birds. We conclude that under organic viticulture, reducing the use of non-specific pesticides is important to promote biodiversity and that the cultivation of fungus-resistant grape varieties can have a significant part to this progress.

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CHAPTER 6

General discussion

Marvin Kaczmarek

6.1 General discussion

Vineyards provide habitat for a wide range of organisms but are characterized by intensive pesticide management (Pertot *et al*. 2017). To investigate the impact of viticulture on biodiversity, we assessed its influence on arthropods in the Palatinate region of southwestern Germany. We studied the influence of weather and trapping effort on multi-day Malaise trap samples, examining their effects on long-term monitoring data (Chapter 2; Kaczmarek *et al*. 2022). Additionally, we explored how pesticide management in viticulture and the surrounding landscape affect arthropods in general using metabarcoding (Chapter 3; Kaczmarek *et al*. 2023a), and how they affect wild bees (Chapter 4; Kaczmarek *et al*., unpublished data) and orthopterans (Chapter 5; Kaczmarek *et al*. 2023b) in particular.

6.1.1 Monitoring of arthropods

Systematic monitoring of species is crucial for recording the continuing decline in biodiversity and biomass, attributed to habitat destruction, intensified agriculture, invasive organisms, and climate change (Sánchez-Bayo and Wyckhuys 2019). However, besides the long-term influences of these factors, ambient weather conditions during sampling and the sampling methodology itself can directly affect the biodiversity being recorded (Skvarla *et al*. 2021). Therefore, these effects should be considered when evaluating data from monitoring programs. By measuring biomass and biodiversity of multi-day Malaise trap samples, we found influences of both weather conditions and trapping effort on the species being recorded (Kaczmarek *et al*. 2022).

As ambient temperatures and radiation increased, biomass also increased (Kaczmarek *et al*. 2022). Higher temperatures directly affect flight ability and, as a result, positively influence insect activity (Bergman *et al*. 1996; Welti *et al*. 2012; Hallmann *et al*. 2017). However, at above-average temperatures, which are increasingly expected with climate change, the linear relationship breaks down and activity decreases (Kasper *et al*. 2008; Welti *et al*. 2012). Contrary to temperature and radiation, air humidity negatively affected biomass in our study (Kaczmarek *et al*. 2022), while both positive and negative effects are reported for different groups of insects (Juillet 1964; Contreras *et al*. 2013). The negative effect of air humidity in our study may be a consequence of reduced temperature or radiation at high air humidity, rather than a direct effect of air humidity itself, due to its correlation with these factors. However, extreme levels of air humidity can cause a clear reduction in insect activity (Juillet 1964). Although both rain events and wind speed are known to decrease insect activity (Kasper *et al*. 2008; Juillet 1964), they did not affect biomass in our study (Kaczmarek *et al*. 2022). It is important to note that both stronger rain and wind are rather short-term events, and therefore, appear to be less apparent at multi-day trapping intervals (Matthews and Matthews 1970). The effect of weather conditions on the recorded biodiversity was also reflected in differences of arthropod biomass and richness during our two-year-long sampling in the region. We observed fewer arthropods in the overall colder and wetter year 2021 compared to 2020 (Kaczmarek *et al*. 2023a).

Besides ambient weather conditions, the sampling methodology also affects the biodiversity being recorded. On one hand, the type of trap emphasizes certain species groups while others

are not represented in the collected data (Skvarla *et al*. 2021). Malaise traps, for example, primarily catch flying insects (Skvarla *et al*. 2021). Furthermore, the trapping duration and number of sampling sites influences the results. Increased trapping duration increased the richness of detected taxa and, thus, enables the detection of more species (Kaczmarek *et al*. 2022). We observed a clear saturating effect with increasing trapping duration for more common taxa. However, this effect weakens with almost no saturation for low presence taxa, as also reported in other studies (Fraser *et al*. 2008; Geiger *et al*. 2016; Steinke *et al*. 2021). Similarly, cumulative taxa richness increased with a greater number of sampling sites with no saturating effect (Kaczmarek *et al*. 2022). Consequently, common species are likely caught with short trapping durations and few sampling sites, while rare and transient species are infrequently captured and cannot be comprehensively detected even with excessive sampling effort (Kaczmarek *et al*. 2022; Steinke *et al*. 2021).

Our study on monitoring shows that weather conditions during sampling have a clear impact on the captured species diversity and, at the same time, the methodology affects the results through the duration of trapping and number of sampling sites. Ambient weather conditions should therefore be documented during long-term monitoring and the methodology, if possible, coordinated with other monitoring programs to enable comparable data collection. Furthermore, in our studies, a large proportion of operational taxonomic units (OTUs) could not be assigned to barcode index numbers (BINs), and even fewer were matching with species in the DNA barcode libraries (Kaczmarek *et al*. 2022; Kaczmarek *et al*. 2023a). Hence, further sequencing work is needed to provide more comprehensive databases to increase the informative value of monitoring programs. However, metabarcoding studies show that there is also a high proportion of undescribed species in the databases, particularly in diverse orders like Diptera and Hymenoptera (Geiger *et al*. 2016; Page 2016; Morinière *et al*. 2019). One advantage of long-term monitoring, besides its cost and time efficiency, is the ability to archive data that can be reprocessed with updated DNA barcode libraries. Therefore, species that are undescribed today and changes in taxonomic classification can be taken into account when data is re-evaluated in future analyses.

Particularly concerning the use of mass collection methods in long-term monitoring programs (e.g., Malaise traps and yellow pan traps, as employed in our studies (Kaczmarek *et al*. 2022; Kaczmarek *et al*. 2023a; Kaczmarek *et al*., unpublished data)), lethal sampling methods result in the mass killing of arthropods (Lövei *et al*. 2023). Although currently non-lethal methods are not available for all groups of organisms, the long-term goal should aim to reduce the use of lethal methods and instead conduct data collection using non-lethal ones to maintain public support for arthropod conservation (Lövei *et al*. 2023). While we sampled orthopterans in a non-destructive manner (Kaczmarek *et al*. 2023b), methods such as box quadrats and transect walks with song detection may often be too time-consuming and costly for broad monitoring efforts. Therefore, it is important to continue developing innovative methods, such as the use of environmental DNA for non-destructive sampling of terrestrial biodiversity, as demonstrated recently (Roger *et al*. 2022; Newton *et al*. 2023).

6.1.2 Pesticide management and the cultivation of fungus-resistant grape varieties

In viticulture, the occurrence of fungal diseases like powdery and downy mildew necessitates the frequent use of pesticides, particularly fungicides (Pertot *et al*. 2017), with both positive and negative effects on biodiversity being reported (Döring *et al*. 2019; Kolb *et al*. 2020; Paiola *et al*. 2020; Ostandie *et al*. 2021; Beaumelle *et al*. 2023; Reiff *et al*. 2023). In the context of this dissertation thesis, we investigated the influence of vineyard management on arthropod diversity (Kaczmarek *et al*. 2023a), particularly on wild bees (Kaczmarek *et al*., unpublished data) and orthopterans (Kaczmarek *et al*. 2023b).

In line with the less pronounced effects of organic viticulture on biodiversity reported in previous studies (Döring *et al*. 2019; Kolb *et al*. 2020; Paiola *et al*. 2020; Ostandie *et al*. 2021; Beaumelle *et al*. 2023; Reiff *et al*. 2023), we found no significant differences in general arthropod richness between organic and conventional vineyards (Kaczmarek *et al*. 2023a). The relatively small effect of the management system on taxa richness could be attributed to the generally low use of insecticides in Germany and in the Palatinate region. In contrast, in regions where insecticide use is mandatory in conventional vineyards, organic viticulture shows significant positive effects (Beaumelle *et al*. 2023). Moreover, the relatively small size of vineyards in our study region and the varying plant protection management of adjacent vineyards, with pesticide drift affecting the surrounding areas (Druart *et al*. 2011), suggest that species are likely influenced by diverse management practices. This could make the effects of pesticide use less evident between differently managed vineyards, with a major difference between pesticide management systems being the use of synthetic pesticides in conventional management or inorganic compounds in organic viticulture (Pedneault and Provost 2016). Additionally, using Malaise traps, we captured mainly flying insects, particularly from the orders Diptera and Hymenoptera that include highly mobile species (Kaczmarek *et al*. 2023a). They may be influenced by varying plant protection practices due to a wider habitat range. Furthermore, such species may have the ability to avoid pesticides during applications, resulting in reduced exposure compared to less mobile species, particularly compared to those living in grapevine canopies.

Nevertheless, fungicides can still have an impact on non-target organisms (Nash *et al*. 2010; Biondi *et al*. 2012; Vogelweith and Thiéry 2018). Although taxa richness was not significantly reduced in organic compared to conventional vineyards, biomass was almost one-third higher under conventional management (Kaczmarek *et al*. 2023a). The use of copper and sulfur for plant protection, instead of synthetic fungicides, was found to result in higher pesticide toxicity for non-target organisms in organic vineyards (Möth *et al*. 2021), which may explain the lower biomass observed under organic management. Besides the actual spraying event, copper accumulates in the topsoil (Karimi *et al*. 2021), and its concentrations in Palatinate vineyards were found to have lethal effects on soil organisms such as earthworms (Duque *et al*. 2023). The use of sulfur, acting as a fungicide but with broad-spectrum pesticide properties, was also shown to have negative effects on non-target organisms such as parasitoids, predatory thrips and mites, and grapevine moths (Hanna *et al*. 1997; Jepsen *et al*. 2007; Tacoli *et al*. 2020).

The effects of plant protection products vary among organism groups, and community composition changes between different management systems (Kaczmarek et al. 2023a; Kaczmarek et al. 2023b). Pesticide use affects the abundance of diverse arthropod taxa to varying extents (Nash *et al*. 2010; Vogelweith and Thiéry 2018), favoring some species that are less sensitive or less exposed to pesticides during applications over others. Ostandie *et al*. (2021) observed varying effects on different taxonomic groups. They found that the abundance of springtails and spiders increased in organic versus conventional viticulture, while pollinator abundance decreased, and ground beetles and mites remained unaffected. We found that orthopterans were affected by the plant protection management (Kaczmarek *et al*. 2023b), presumably due to the compounds used in organic viticulture. Orthopterans are generally sedentary species with a limited range of action (Detzel 1998; Ingrisch and Köhler 1998). Therefore, they are likely more influenced by local vineyard management compared to mobile species such as Diptera and Hymenoptera. For instance, *Tettigonia viridissima* was three times less abundant in organically managed vineyards than in conventional ones (Kaczmarek *et al*. 2023b). Herb-dwelling orthopterans were more common in fungus-resistant grape (FRG) varieties that received fewer than half as many fungicide applications, resulting in overall lower pesticide toxicity (Pedneault and Provost 2016). However, this positive effect of FRG varieties was observed only in organic vineyards, where copper and sulfur are used for plant protection. Since both the herb-dwelling orthopterans and *T. viridissima* lay their eggs in the soil (Ingrisch and Köhler 1998), their eggs and hatching larvae may be more affected by the accumulation of copper in the topsoil (Karimi *et al*. 2021). Moreover, they can be exposed to copper through surface contact and their feeding behavior (Ingrisch and Köhler 1998).

Apart from a trend of Hymenoptera richness tending to be 9 % higher in FRG varieties compared to classic ones, we found no effect of reduced pesticide use in FRG varieties for the Malaise trap sampling and for wild bees (Kaczmarek *et al*. 2023a; Kaczmarek *et al*., unpublished data). This might be attributed to the higher mobility of the observed species and wider habitat range, as discussed before. However, positive effects of reduced pesticide use in FRG varieties, in addition to the positive effects on orthopterans in our study, were recently shown for other less mobile groups, such as predatory mites and spiders (Pennington *et al*. 2017; Pennington *et al*. 2019; Reiff *et al*. 2021a; Reiff *et al*. 2023). Although research on this topic is sparse and limited, reducing the use of non-specific pesticides by cultivating FRG varieties appears to be important to increase sustainability in viticulture and promote biodiversity. Along with fewer pesticide applications, subsequently reducing tractor passages and by that reducing disturbance and soil compaction, further reduces the disturbance of biodiversity within vineyards (Bruggisser *et al*. 2010).

The abundance of wild bees was higher in organic vineyards, primarily due to some ground-nesting species (Kaczmarek *et al*., unpublished data). However, they did not appear to be as affected by pesticides, as we found no effect of reduced pesticide use in FRG varieties. Other factors, such as the inter-row vegetation, may play a more prominent role in determining their presence, as differences between management systems could also be attributed to variations in vegetation and tillage practices. Therefore, negative effects of pesticides that we found on arthropod biomass and orthopterans (Kaczmarek *et al*. 2023a; Kaczmarek *et al*. 2023b), may

be generally more related to species that are more exposed to pesticides compared to wild bees.

6.1.3 Landscape heterogeneity and vegetation management

The landscape heterogeneity, known to be an essential determinant of biodiversity patterns (Tscharntke *et al.* 2021), has been reported to exert a strong positive effect on biodiversity in various studies (Schmidt *et al.* 2005; Martin *et al.* 2019; Kolb *et al.* 2020; Barbaro *et al.* 2021). Accordingly, landscapes rich in semi-natural habitats (SNH) significantly increased arthropod diversity in our studies. Overall, we found positive effects on total biodiversity, particularly on the diversity of Diptera, Hemiptera, Hymenoptera, and Lepidoptera (Kaczmarek *et al.* 2023a). SNH, such as forests, hedges, shrubs, and grasslands, offer abundant feeding resources (Eckerter *et al.* 2022). Furthermore, they provide shelter and habitats for overwintering, as well as resources for reproduction for numerous species unable to complete their life cycle in vineyards alone (Holland *et al.* 2017).

For instance, the abundance and richness of above-ground-nesting bees increased significantly with higher proportions of SNH in the surrounding landscape of vineyards (Kaczmarek *et al.*, unpublished data). In our study, *Osmia bicornis* was strongly related to SNH. Like other above-ground-nesting bees, this species relies on woody structures to build cavity nests for breeding (Westrich 2018) and, therefore, depends on SNH in the area. Similarly, Uzman *et al.* (2020) and Wersebeckmann *et al.* (2023) reported positive effects of increasing SNH cover on cavity-nesting bees. The bush-cricket *Leptophyes punctatissima* prefers forest edges and is dependent on woody structures with adjacent grass and herbaceous areas (Detzel 1998; Schlumprecht 2003) and showed a positive response to increasing SNH cover in our study (Kaczmarek *et al.* 2023b). Consequently, a more diverse landscape with suitable SNH providing feeding resources and nesting opportunities can promote the diversity of such species that rely on resources not available within vineyards.

On the other hand, we observed no or relatively small effects of SNH on ground-nesting bees and other orthopterans (Kaczmarek *et al.*, unpublished data, Kaczmarek *et al.* 2023b). Bee species from the genera *Lasioglossum* and *Andrena* nest in bare ground areas (Westrich 2018). As such areas can also be found in and near vineyards, these bee species do not rely on nesting structures provided by SNH within their habitat range. Similarly, the entire life cycle of the observed herb- and vine-dwelling orthopterans can take place in the vineyards or the immediate surroundings (Detzel 1998; Ingrisch and Köhler 1998).

Our results demonstrate that the presence of SNH in viticultural landscapes affects different organism groups differently, depending on their preferences or needs. This is also reflected in changes in community composition with changing landscape in our studies, for species sampled using Malaise traps (Kaczmarek *et al.* 2023a), for wild bees (Kaczmarek *et al.*, unpublished data), and for orthopterans (Kaczmarek *et al.* 2023b). How landscape heterogeneity affects whole arthropod community composition and its impact on viticulture should be studied in more closely. SNH-rich landscapes may favor parasitoids and predators, thereby enhancing natural pest control (Holland et al. 2017). Therefore, maintaining SNH in

the area to improve natural pest control and reduce the need for pesticides could also benefit winegrowers themselves.

Furthermore, local practices within vineyards, such as inter-row vegetation management and tillage, have a significant impact on biodiversity (Ostandie *et al*. 2021; Reiff *et al*. 2021b; Blaise *et al*. 2022), and extensive vegetation management can contribute to biodiversity conservation in vineyards (Winter *et al*. 2018). In our studies, we observed that a higher number of plant species in inter-rows led to an increase in arthropod biomass and richness (Kaczmarek *et al*. 2023a). Additionally, we found that wild bees benefited from increased vegetation cover (Kaczmarek *et al*., unpublished data). Moreover, the abundance of the grasshopper *Chorthippus brunneus* increased with increasing vegetation height, and the density of vine-dwelling orthopterans, possibly affected during the larval stages living in the herb layer, tended to increase with higher ground vegetation cover (Kaczmarek *et al*. 2023b). However, it is important to note that a high vegetation cover does not necessarily indicate a structurally diverse and resource-rich vegetation in the inter-rows of the vineyards, as it might comprise mostly grasses that could be unattractive for feeding. Furthermore, during our research, we observed lower vegetation cover in organic vineyards. However, we also found that some nectar- and pollen-rich plants, which are frequently included in seed mixtures for inter-row greening, were more common in organic vineyards. While lower vegetation cover is not necessarily a characteristic of organic management in our region, the utilization of flower-rich mixtures is more common in organic viticulture compared to conventional management.

The positive effects of inter-row vegetation on biodiversity, which includes diverse and flower-rich vegetation, seem to be linked to a higher food supply, a suitable microclimate, and the provision of hiding places. Simultaneously, maintaining patches without vegetation cover can enhance ground-nesting species, such as wild bee species that nest in bare ground areas (Kaczmarek *et al*., unpublished data). Furthermore, tillage practices can influence biodiversity, as more intensive tillage may harm, for example, the development of orthopterans' egg pods (Detzel 1998). To promote arthropod diversity, adapted vegetation management in vineyard inter-rows, such as using seed mixtures including nectar- and pollen-rich plants, grazing, or implementing less intensive mowing and tillage, can be effective measures (Detzel 1998; Blaise *et al*. 2022; Bosco *et al*. 2022). These practices contribute to the conservation of biodiversity within vineyards and can have positive impacts on various species of plants and animals that inhabit these ecosystems. Our studies showed that vineyards can provide a habitat for rare species, such as *Lasioglossum* cf. *subhirtum*, which was recently observed in the region for the first time in about 70 years (Burger 2018, Kaczmarek *et al*., unpublished data). This emphasizes the potential of viticultural areas as important habitats for arthropod conservation.

6.2 General conclusions

Our study has shown that the biodiversity recorded in monitoring programs and ecological surveys is influenced by both weather conditions and sampling effort. Insect activity was highest during warm and dry weather. While common species are likely to be captured with short trapping durations and a small number of sampling sites, it remains challenging to

comprehensively sample rare and transient species, even with extensive sampling effort. The results highlight the importance of documenting ambient weather conditions during surveys and employing a methodology that enables the collection of comparable data. Metabarcoding provides comprehensive biodiversity data in a cost- and time-efficient manner, making it a valuable method for long-term monitoring. However, additional sequencing efforts are necessary to establish more comprehensive DNA databases and enhance the informative value of monitoring programs using metabarcoding.

Our studies on the impact of vineyard management on biodiversity have revealed variations among different organism groups. The effects of pesticide use were more pronounced in less mobile species, while mobile species appeared to be less sensitive or less exposed to pesticides. We conclude that reducing pesticide use, particularly under organic management that uses copper and sulfur for plant protection, has a positive impact on arthropods. To enhance sustainability in viticulture and promote biodiversity, therefore, the cultivation of FRG varieties is of importance to minimize the need for non-specific pesticides, and subsequently reducing disturbances caused by tractor passages. We further conclude that while certain species benefit more from sustainable pesticide management, others, e.g., wild bees, are enhanced more by the provision of abundant floral and nesting resources in the inter-row vegetation and SNH in the surrounding landscape. Therefore, it is also important to provide diverse vegetation in inter-rows and create a structurally rich environment with suitable SNH to conserve biodiversity in viticulture. However, more research is needed to distinguish the separate impacts of pesticide management and landscape heterogeneity on the diverse organism groups and how these factors promote or diminish the occurrence of species of conservation concern.

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Importantly, I want to express my heartfelt thanks to all the friends who supported me and made my time in Landau such an enjoyable phase of my life.

I am deeply grateful to my family for giving me the opportunity to pursue my studies. Their steady support has been an essential driving force on my academic path.

APPENDIX

Status and author contributions of publications included in the thesis

Curriculum vitae

Declaration

Supplementary materials

Status and author contributions of publications included in the thesis

Chapter 2

Kaczmarek M[1,2], Entling MH[2], Hoffmann C[1] (2022) Using Malaise Traps and Metabarcoding for Biodiversity Assessment in Vineyards: Effects of Weather and Trapping Effort. Insects, 13(6):507. https://doi.org/10.3390/insects13060507

MK, MHE, and CH conceptualized the study and designed methodology. MK collected and analyzed the data and led the writing of the manuscript under supervision of MHE and CH. CH administered the project and acquired funding. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 3

Kaczmarek M[1,2], Entling MH[2], Hoffmann C[1] (2023) Differentiating the effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture. Biodiversity and Conservervation, 32:2637–2653. https://doi.org/10.1007/s10531-023-02621-y

MK, MHE, and CH conceptualized the study and designed methodology. MK collected and analyzed the data and led the writing of the manuscript under supervision of MHE and CH. CH administered the project and acquired funding. All authors contributed critically to the drafts and gave final approval for publication.

Chapter 4

Kaczmarek M[1,2], Entling MH[2], Hoffmann C[1] (Manuscript in preparation) Wild bee conservation in viticulture: Effects of semi-natural habitats, organic management, and pesticide reduction.

MK, MHE, and CH conceptualized the study and designed methodology. MK collected and analyzed the data and led the writing of the manuscript under supervision of MHE and CH. CH administered the project and acquired funding. All authors contributed critically to the drafts and agreed to the final version of the manuscript.

Chapter 5

Kaczmarek M[1,2], Gillich M[2], Entling MH[2], Hoffmann C[1], Schirmel J[2] (2023) Differential responses of Orthoptera in vineyards to organic farming, pesticide reduction, and landscape heterogeneity. Journal of Insect Conservation, 27:729–741. https://doi.org/10.1007/s10841-023-00493-9

MK, MG, MHE, CH, and JS conceptualized the study and designed methodology. MG collected data of herb-dwelling orthopterans while MK collected data of vine-dwelling orthopterans. JS analyzed the data. MK led the writing of the manuscript with MG and JS under supervision of MHE and CH. CH administered the project and acquired funding. All authors contributed critically to the study and gave final approval for publication.

AI programmes (DeepL, Grammarly, ChatGPT) were used throughout the thesis to improve the language.

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Curriculum vitae

Marvin Kaczmarek

Education

08/2019 – 10/2023	Ph.D. student in Environmental Sciences Institute for Plant Protection in Fruit Crops and Viticulture, Julius Kühn Institute, Siebeldingen, Germany iES Landau, RPTU Kaiserslautern-Landau, Germany Thesis: *The effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture*
12/2018	Master of Science in Evolution and Ecology Eberhard Karls Universität Tübingen, Germany Thesis: *Influence of habitat on foraging behavior in Golden-Headed Lion Tamarins (Leontopithecus chrysomelas) in Bahia, Brazil*
11/2015	Bachelor of Science in Biology Eberhard Karls Universität Tübingen, Germany Thesis: *Untersuchung der Unterkieferknochen bei Varanus glauerti (Squamata, Varanidae)*
06/2012	General qualification for university entrance (Abitur) Berufsbildende Schule Wirtschaft Koblenz, Germany

Experience

Since 2020	Scientific assistant: AMAP e. V. Assistance of scientific area of AMAP e. V.
2018	Research assistant: Plant Ecology Experimental setup of common garden experiment Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology
2016	Research assistant: Fieldwork with Northern Crested Newts Assessment of population by capturing individuals and taking tissue samples Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology
2015 – 2018	Research assistant: Comparative Zoology Behavioral and sexual selection studies with Alpine Newts Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology

2015	Research assistant: Fieldwork with Corn Buntings
	Observational study of feeding behavior
	Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology
2014 – 2018	Teaching assistant: Practical courses in Zoology and Ecology
	Native animal species identification courses and ecology courses
	Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology
2014 – 2015	Field trip leader: Zoological field trips for bachelor students
	Field trips about primates and ungulates in the zoological-botanical garden Wilhelma
	Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology
2013 – 2019	Field trip leader: Ornithological field trips for bachelor students
	Native bird species field trips
	Eberhard Karls Universität Tübingen, Germany, Institute for Evolution and Ecology

Publications

Kaczmarek M, Entling MH, Hoffmann C (2022) Using Malaise Traps and Metabarcoding for Biodiversity Assessment in Vineyards: Effects of Weather and Trapping Effort. Insects, 13(6):507. https://doi.org/10.3390/insects13060507

Kaczmarek M, Entling MH, Hoffmann C (2023) Differentiating the effects of organic management, pesticide reduction, and landscape diversification for arthropod conservation in viticulture. Biodiversity and Conservation, 32:2637–2653. https://doi.org/10.1007/s10531-023-02621-y

Kaczmarek M, Gillich M, Entling MH, Hoffmann C, Schirmel J (2023) Differential responses of Orthoptera in vineyards to organic farming, pesticide reduction, and landscape heterogeneity. Journal of Insect Conservation. https://doi.org/10.1007/s10841-023-00493-9

Conference presentations

Kaczmarek M, Krahner A, Entling MH, Hoffmann C (2021) Insektendiversität im Weinbau – Einfluss von Bewirtschaftung und Landschaft. 62. Deutsche Pflanzenschutztagung. 21-23 September, Virtual Conference (Poster presentation). https://doi.org/10.5073/20210817-121649

Kaczmarek M, Entling MH, Hoffmann C (2022) Biodiversity of arthropods in viticulture – Influence of management and landscape. 14th Young Scientists Meeting Conference. 9-11 November, Berlin (Oral presentation).

Kaczmarek M, Entling MH, Hoffmann C (2022) Biodiversity of arthropods in viticulture – Influence of management and landscape. SFE² GfÖ EEF Joint meeting, International Conference on Ecological Sciences. 21-25 November, Metz (Oral presentation).

Kaczmarek M, Entling MH, Hoffmann C (2023) Erhebungen zur Arthropodenbiodiversität in Weinbergen mit Hilfe von Metabarcoding - Einfluss von Managementsystem, Pflanzenschutz und Landschaft. 63. Deutsche Pflanzenschutztagung. 26-29 September, Göttingen (Oral presentation).

Declaration

according to §8 of the Promotionsordnung des Fachbereichs 7: Natur- und Umweltwissenschaften der Universität Koblenz-Landau, Campus Landau vom 14.06.2013 i.d.F. vom 19.08.2014

Declaration that

I have drawn up the submitted dissertation independently. All aids and sources have been clearly specified, and the contribution of other scientists or authors have been clearly highlighted;

I had no paid assistance by any sort of PhD service agencies;

the dissertation has neither identically nor in a similar form been submitted for any scientific examination in Germany or in another country;

the dissertation has neither earlier nor simultaneously been submitted at any other university or faculty;

I am aware that the PhD title can be revoked as a result of failure regarding any of the aforementioned points and that legal ramifications are also possible.

Landau, 31 October 2023

Marvin Kaczmarek

Supplementary materials – Chapter 2
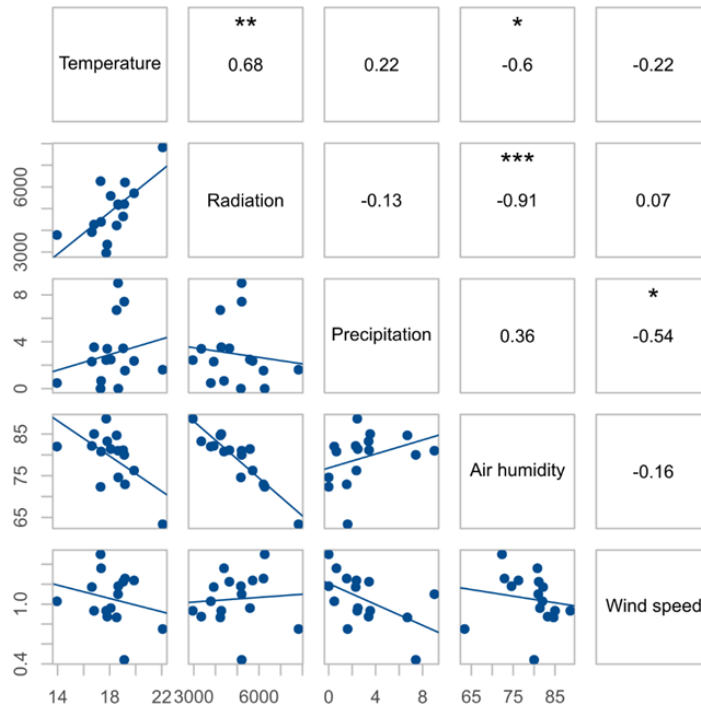
Figure S2.1 Correlation matrix with *R*-value for environmental variables temperature, radiation, precipitation, air humidity, and wind speed. Asterisks indicate significant correlation between variables based on *P*-values. Significance codes: ****P* < 0.001, ***P* < 0.01, **P* < 0.05.
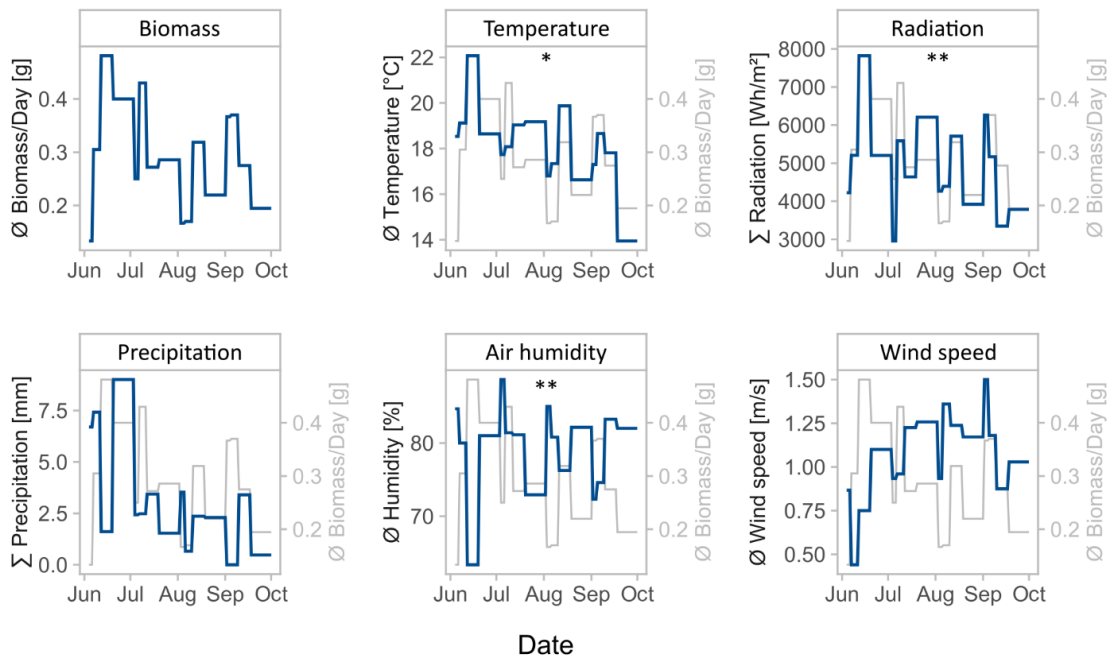
Figure S2.2 Course of mean daily biomass in g and the environmental variables temperature in °C, radiation in Wh/m², precipitation in mm, air humidity in %, and wind speed in m/s for each trapping interval throughout data collection. Asterisks indicate significant effects of environmental variables on daily biomass. Significance codes: ***P* < 0.01, **P* < 0.05.

Table S2.1 Biomass for trap A-D of the local sampling and the mean environmental variables temperature in °C, radiation in Wh/m², precipitation in mm, air humidity in %, and wind speed in m/s for each trapping interval.

Cycle	Interval	Days of trapping	Biomass						Temperature	Radiation	Precipitation	Air humidity	Wind
			A	B	C	D	Mean	Mean/Day					
1	i1	3	0.4	0.6	0.3	0.3	0.4	0.1	18.53	4,223	6.70	84.67	0.87
1	i2	5	1.3	1.6	1.1	2.1	1.5	0.3	19.12	5,206	7.42	80.00	0.44
1	i3	8	2.3	5.3	3.3	4.5	3.9	0.5	22.08	7,822	1.61	63.38	0.75
1	i4	14	2.8	8.4	5.4	5.8	5.6	0.4	18.64	5,203	9.01	81.00	1.10
2	i1	3	0.2	1.0	0.8	1.0	0.8	0.3	17.73	2,956	2.43	88.67	0.93
2	i2	5	1.2	3.0	2.7	1.7	2.2	0.4	18.08	5,586	2.48	81.40	0.96
2	i3	8	2.3	1.1	2.5	2.8	2.2	0.3	19.04	4,638	3.44	81.13	1.23
2	i4	14	7.3	4.5	2.3	1.9	4.0	0.3	19.18	6,209	1.54	72.93	1.26
3	i1	3	0.3	0.5	0.4	0.8	0.5	0.2	16.80	4,266	3.53	85.00	0.93
3	i2	5	0.9	0.9	0.6	1.0	0.9	0.2	17.34	4,391	0.66	80.80	1.36
3	i3	8	2.4	2.6	2.9	2.3	2.6	0.3	19.88	5,709	2.36	76.25	1.24
3	i4	14	2.7	3.2	2.4	4.0	3.1	0.2	16.63	3,919	2.30	82.14	1.17
4	i1	3	1.7	0.8	0.8	1.1	1.1	0.4	17.30	6,265	0.00	72.33	1.50
4	i2	5	2.4	1.8	2.0	1.2	1.9	0.4	18.66	5,169	0.00	74.60	1.18
4	i3	8	3.1	2.3	2.2	1.2	2.2	0.3	17.81	3,346	3.40	83.25	0.88
4	i4	14	2.9	2.9	2.7	2.4	2.7	0.2	13.94	3,785	0.48	82.00	1.03

Table S2.2 Numbers of BINs, OTUs, species, and accumulated BINs (BINsAcc) for each site and trapping interval of the local sampling. The numbers of BINs and BINsAcc are also given for subsets of high (taxa caught at 4 sites), medium (taxa caught at 2 or 3 sites), and low presence (taxa caught at 1 site).

Site	Interval	Days of trapping	BINs	OTUs	Species	BIN subsets			BINsAcc	BINAcc subsets		
						Low	Medium	High		Low	Medium	High
A	i1	3	130	232	78	25	52	53	130	25	52	53
A	i2	5	176	292	102	38	66	72	243	61	99	83
A	i3	8	191	323	107	37	80	74	332	94	141	97
A	i4	14	184	295	101	36	67	81	399	120	171	108
B	i1	3	75	120	39	16	18	41	75	16	18	41
B	i2	5	131	231	74	12	47	72	163	27	59	77
B	i3	8	233	403	116	49	95	89	311	73	134	104
B	i4	14	134	210	76	21	48	65	352	89	155	108
C	i1	3	100	171	53	17	33	50	100	17	33	50
C	i2	5	170	292	102	39	59	72	215	54	80	81
C	i3	8	235	383	122	62	85	88	343	106	135	102
C	i4	14	230	401	124	51	92	87	426	140	178	108
D	i1	3	91	159	53	11	34	46	91	11	34	46
D	i2	5	137	235	81	23	53	61	186	33	77	76
D	i3	8	178	287	97	41	70	67	281	65	121	95
D	i4	14	186	309	110	36	76	74	358	90	160	108

Table S2.3 Number of BINs for each site of the regional sampling. The numbers of BINs and BINsAcc are also given for subsets of high (taxa caught at 4 sites), medium (taxa caught at 2 or 3 sites), and low presence (taxa caught at 1 site).

Site	BINs	BIN subsets		
		Low	Medium	High
1	344	153	131	60
2	334	140	133	61
3	344	130	151	63
4	240	68	106	66
5	251	91	99	61
6	315	131	122	62
7	219	64	100	55
8	314	128	124	62
9	303	127	120	56
10	281	99	121	61
11	300	118	118	64
12	241	67	109	65
13	237	67	109	61
14	306	128	122	56
15	266	90	113	63
16	252	79	114	59
17	292	119	109	64
18	396	190	146	60
19	231	64	105	62
20	251	79	109	63
21	251	81	111	59
22	309	137	113	59
23	281	113	109	59
24	284	95	126	63
25	254	81	118	55
26	438	243	137	58
27	279	89	124	66
28	272	85	121	66
29	254	80	110	64
30	248	84	107	57
31	246	90	95	61
32	223	68	98	57

Table S2.4 BINs recorded in the local sampling with assigned taxonomy and presence subsets with high (taxa caught at 4 sites), medium (taxa caught at 2 or 3 sites), and low presence (taxa caught at 1 site).

BIN	Taxonomy						Presence
	Phylum	Class	Order	Family	Genus	Species	
BOLD:AAG8519	Arthropoda	Arachnida	Araneae	Araneidae	*Nuctenea*	*Nuctenea umbratica*	Medium
BOLD:ABU7235	Arthropoda	Arachnida	Araneae	Cheiracanthiidae	*Cheiracanthium*	*Cheiracanthium punctorium*	Low
BOLD:AAP3543	Arthropoda	Arachnida	Araneae	Dictynidae	*Dictyna*	*Dictyna uncinata*	Medium
BOLD:ACF0172	Arthropoda	Arachnida	Araneae	Gnaphosidae	*Drassodes*	*Drassodes lapidosus*	Low
BOLD:AAE5234	Arthropoda	Arachnida	Araneae	Linyphiidae	*Agyneta*	*Agyneta rurestris*	Medium
BOLD:ADJ6866/ BOLD:ADK5830	Arthropoda	Arachnida	Araneae	Philodromidae	*Philodromus*	*Philodromus cespitum*	Low
BOLD:ACR4121	Arthropoda	Arachnida	Araneae	Philodromidae	*Philodromus*	*Philodromus praedatus*	Low
BOLD:AAE4245	Arthropoda	Arachnida	Araneae	Pisauridae	*Pisaura*	*Pisaura mirabilis*	Medium
BOLD:AAC9044	Arthropoda	Arachnida	Araneae	Salticidae	*Salticus*	*Salticus scenicus*	Low
BOLD:AAY7917	Arthropoda	Arachnida	Araneae	Salticidae	*Synageles*	*Synageles venator*	Medium
BOLD:AAK2576	Arthropoda	Arachnida	Araneae	Theridiidae	*Phylloneta*	*Phylloneta impressa*	Medium
BOLD:AAP2437	Arthropoda	Arachnida	Araneae	Thomisidae	*Xysticus*		Low
BOLD:AAZ7526	Arthropoda	Arachnida	Araneae	Thomisidae	*Xysticus*		Medium
BOLD:AAJ1655	Arthropoda	Arachnida	Araneae				Medium
BOLD:AAE6136	Arthropoda	Arachnida	Araneae				Low
BOLD:AAA7188	Arthropoda	Arachnida	Araneae				Low
BOLD:AAF9222	Arthropoda	Arachnida	Mesostigmata	Parasitidae	*Parasitus*	*Parasitus fimetorum*	Medium
BOLD:ACN5817	Arthropoda	Arachnida	Mesostigmata	Parasitidae			Low
BOLD:ACV9793	Arthropoda	Arachnida	Opiliones	Phalangiidae	*Odiellus*	*Odiellus spinosus*	Medium
[...]	[...]	[...]	[...]	[...]	[...]	[...]	[...]

The whole table with all 830 BINs can be downloaded at https://www.mdpi.com/article/10.3390/insects13060507/s1.

Table S2.5 BINs recorded in the regional sampling with assigned taxonomy and presence subsets with high (taxa caught at 25 to 32 sites), medium (taxa caught at 9 to 24 sites), and low presence (taxa caught at 1 to 8 sites).

BIN	Taxonomy						Presence
	Phylum	Class	Order	Family	Genus	Species	
BOLD:AAA2504	Annelida	Clitellata	Haplotaxida	Lumbricidae	*Lumbricus*		Low
BOLD:AAJ1249	Arthropoda	Arachnida	Araneae	Agelenidae	*Allagelena*	*Allagelena gracilens*	Low
BOLD:AAF8740	Arthropoda	Arachnida	Araneae	Agelenidae	*Eratigena*	*Eratigena agrestis*	Low
BOLD:AAY7695	Arthropoda	Arachnida	Araneae	Araneidae	*Gibbaranea*	*Gibbaranea gibbosa*	Low
BOLD:ACF3690	Arthropoda	Arachnida	Araneae	Araneidae	*Mangora*	*Mangora acalypha*	Low
BOLD:AAG8519	Arthropoda	Arachnida	Araneae	Araneidae	*Nuctenea*	*Nuctenea umbratica*	Low
BOLD:ABU7235	Arthropoda	Arachnida	Araneae	Cheiracanthiidae	*Cheiracanthium*	*Cheiracanthium punctorium*	Low
BOLD:AAP3543	Arthropoda	Arachnida	Araneae	Dictynidae	*Dictyna*	*Dictyna uncinata*	Low
BOLD:AAL7655	Arthropoda	Arachnida	Araneae	Gnaphosidae	*Drassyllus*	*Drassyllus pusillus*	Low
BOLD:ADL9712	Arthropoda	Arachnida	Araneae	Gnaphosidae	*Haplodrassus*		Low
BOLD:AAO2286	Arthropoda	Arachnida	Araneae	Gnaphosidae	*Scotophaeus*	*Scotophaeus scutulatus*	Low
BOLD:AAE5234	Arthropoda	Arachnida	Araneae	Linyphiidae	*Agyneta*	*Agyneta rurestris*	Medium
BOLD:AAB2008	Arthropoda	Arachnida	Araneae	Linyphiidae	*Diplostyla*	*Diplostyla concolor*	Low
BOLD:AAI5655	Arthropoda	Arachnida	Araneae	Linyphiidae	*Frontinellina*	*Frontinellina frutetorum*	Low
BOLD:AAG5627	Arthropoda	Arachnida	Araneae	Linyphiidae	*Microlinyphia*		Low
BOLD:ABA3607	Arthropoda	Arachnida	Araneae	Linyphiidae	*Pelecopsis*	*Pelecopsis parallela*	Low
BOLD:AAG9172	Arthropoda	Arachnida	Araneae	Linyphiidae	*Tenuiphantes*		Low
BOLD:AAI2650	Arthropoda	Arachnida	Araneae	Lycosidae	*Pardosa*		Low
BOLD:ACR3732	Arthropoda	Arachnida	Araneae	Philodromidae	*Philodromus*	*Philodromus aureolus*	Low
BOLD:ADK5830	Arthropoda	Arachnida	Araneae	Philodromidae	*Philodromus*	*Philodromus cespitum*	Medium
[…]	[…]	[…]	[…]	[…]	[…]	[…]	[…]

The whole table with all 1,735 BINs can be downloaded at https://www.mdpi.com/article/10.3390/insects13060507/s1.

Supplementary methods

DNA Metabarcoding

Species identification of organic material in the Malaise traps was performed using DNA metabarcoding following the protocol published in Hausmann *et al*. (2020). Each single sample was dried in a 60 °C oven for at least eight hours and subsequently homogenized in a FastPrep96 machine (MP Biomedicals) using sterile steal beads to generate a homogeneous mixture of animal material. Prior to DNA extraction, a subsample of each homogenate was transferred into sample vials and processed using the DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. For amplification of the CO1-5P target region and preparation of the MiSeq libraries, a 2-step PCR was performed. First, a 313 bp long mini-barcode region was amplified by PCR, using forward and reverse HTS primers, equipped with complementary sites for the Illumina sequencing tails. In a subsequent PCR reaction, index primers with unique i5 and i7 inline tags and sequencing tails were used for amplification of indexed amplicons. Afterward, equimolar amplicon pools of 100 ng/µl each were created and size checked using preparative gel electrophoresis. The pooled DNA was purified using MagSi-NGSprep Plus beads (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany). A bioanalyzer (High Sensitivity DNA Kit, Agilent Technologies) was used for a final check of the bp distribution and concentration of the amplicons before the creation of the final library. High-throughput sequencing (HTS) was performed on an Illumina MiSeq using *v2* (2*250 bp, 500 cycles, maximum of 20mio reads) chemistry (Illumina) aiming for 250k raw reads for each sample.

Bioinformatics

The bioinformatics processing of raw FASTQ files from Illumina was carried out using the VSEARCH suite *v.2.9.1* (Rognes *et al*. 2016) and Cutadapt *v.1.18* (Martin 2011). Forward and reverse reads in each sample were merged using the VSEARCH program *fastq_mergepairs* with a minimum overlap of 10 bp, yielding approximately 313 bp long sequences. Forward and reverse primers were removed with Cutadapt, using the *discard_untrimmed* option to discard sequences for which primers were not reliably detected at ≥ 90 % identity. Quality filtering was done with the *fastq_filter* in VSEARCH, keeping sequences with zero expected errors (*fastq_maxee* 1). Sequences were dereplicated with *derep_fulllength*, first at the sample level and then concatenated into one FASTA file, which was subsequently dereplicated. Chimeric sequences were filtered out from the FASTA file using the VSEARCH program *uchime_denovo*. The remaining sequences were then clustered into OTUs at 97 % identity with *cluster_size*, a greedy centroid-based clustering program. OTUs were blasted against a custom Animalia database downloaded from BOLD in Q4 2021, including taxonomy and BIN information, by means of Geneious *v.10.2.5* (Biomatters, Auckland, New Zealand), and following methods described in Morinière *et al*. (2016). This local sequence database consists of the compiled data which are based on the DNA library with more than 23,000 barcoded German animal species assembled in two major DNA barcoding campaigns: "Barcoding Fauna Bavarica" (BFB, www.faunabavarica.de) and "German Barcode of Life" project (GBOL, www.bolgermany. de), with nearly 250,000 vouchers curated at the Zoological State Collection Munich, Germany

(www.barcoding-zsm.de). Data releases have been published for all major arthropod groups (Coleoptera (Hendrich *et al*. 2015; Rauprach *et al*. 2016; Rulik *et al*. 2017; Raupach *et al*. 2018), Diptera (Morinière *et al*. 2019), Ephemeroptera, Plecoptera, and Trichoptera (Morinière *et al*. 2017), Heteroptera (Raupach *et al*. 2014; Havemann *et al*. 2018), Hymenoptera (Schmidt *et al*. 2015; Schmidt *et al*. 2017; Schmid-Egger *et al*. 2019), Lepidoptera (Hausmann *et al*. 2011a; Hausmann *et al*. 2011b), Neuroptera (Morinière *et al*. 2014), Orthoptera (Hawlitschek *et al*. 2017), Araneae and Opiliones (Astrin *et al*. 2016), and Myriapoda (Spelda *et al*. 2011; Wesener *et al*. 2015)). The resulting CSV file which included the OTU ID, BOLD Process ID, BIN, Hit-%-ID value (percentage of overlap similarity (identical base pairs) of an OTU query sequence with its closest counterpart in the database), length of the top BLAST hit sequence, phylum, class, order, family, genus, and species information for each detected OTU was exported from Geneious and combined with the OTU table generated by the bioinformatic pipeline.

Supplementary materials – Chapter 3

Supplementary methods

DNA metabarcoding and bioinformatics were conducted by AIM - Advanced Identification Methods GmbH. Following the protocol published in Hausmann *et al*. (2020), species identification of insect material of the Malaise trap samples was performed using DNA metabarcoding. Each sample was dried in a 60°C oven for at least eight hours and subsequently homogenized in a FastPrep96 machine (MP Biomedicals) using sterile steal beads to generate a homogeneous mixture of animal material. A subsample of each homogenate was transferred into sample vials prior to DNA extraction and processed using the DNeasy 96 Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. A 2-step PCR was performed for amplification of the CO1-5P target region and preparation of the MiSeq libraries. First, using forward and reverse HTS primers, a 313 base pairs (bp) long mini-barcode region was amplified by PCR, equipped with complementary sites for the Illumina sequencing tails. Index primers with unique i5 and i7 inline tags and sequencing tails were used for amplification of indexed amplicons in a subsequent PCR reaction. Afterwards, equimolar amplicon pools of 100 ng/µl each were created and size checked using preparative gel electrophoresis. Using MagSi-NGSprep Plus beads (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany), the pooled DNA was purified. For a final check of the bp distribution and concentration of the amplicons, a bioanalyzer (High Sensitivity DNA Kit, Agilent Technologies) was used before the creation of the final library. Then, high-throughput sequencing was performed on an Illumina MiSeq using *v2* (2*250 bp, 500 cycles, maximum of 20mio reads) chemistry (Illumina) aiming for 250k raw reads for each sample.

The bioinformatics processing of raw FASTQ files from Illumina was carried out with the VSEARCH suite *v.2.9.1* (Rognes *et al*. 2016) and Cutadapt *v.1.18* (Martin 2011). In each sample, forward and reverse reads were merged using the VSEARCH program *fastq_mergepairs* with a minimum overlap of 10 bp, yielding approximately 313 bp long sequences. Forward and reverse primers were removed using Cutadapt with the *discard_untrimmed* option to discard sequences for which primers were not reliably detected at ≥ 90 % identity. Then, using the *fastq_filter* in VSEARCH, quality filtering was done, keeping sequences with zero expected errors (*fastq_maxee* 1). Sequences were dereplicated using *derep_fulllength*, first at the sample level and then concatenated into one FASTA file, which was subsequently dereplicated. Using the VSEARCH program *uchime_denovo*, chimeric sequences were filtered out from the FASTA file. The remaining sequences were clustered into OTUs at 97 % identity with *cluster_size*, a greedy centroid-based clustering program. Afterwards, OTUs were blasted against a custom Animalia database which was downloaded from BOLD in Q4 2021, including taxonomy and BIN information, by means of Geneious *v.10.2.5* (Biomatters, Auckland, New Zealand), and following methods described in Morinière *et al*. (2016). The resulting CSV file, including the OTU ID, BOLD Process ID, BIN, Hit-%-ID value (percentage of overlap similarity (identical bp) of an OTU query sequence with its closest counterpart in the database), length of the top BLAST hit sequence and phylum, class, order, family, genus, and species information for each detected OTU, was exported from Geneious and combined with the OTU table generated by the bioinformatics pipeline.

Supplementary Results

The initial analysis showed that reads had high sequence quality scores. The number of paired-end sequences that came from the sequencer was 3,714,061. Across all samples, a median of 95.0 % of reads merged (mean 94.5 %, minimum 53.9 %, maximum 99.1 %). Adapters were detected in a median of 99.6 % of the forward and 99.0 % of reverse reverse-complement reads. After quality filtering, 3,070,422 sequences were kept. There were 2,035,286 unique sequences in all samples, of which 146,456 unique non-singleton sequences were kept after dereplication. Of a total of 10,915 OTUs, 4,325 OTUs were kept after de novo chimera detection, 3,245 of which found matches in the databases following OTU table cleaning. 2,150 OTUs had a BOLD Hit-%-ID equal to or higher than 97 %, 1,809 of which were assigned to BINs. 61 BINs occurred doubled and were condensed to one entry.

Table S3.1.1 Proportion (%) of semi-natural habitat (SNH), mean vegetation cover (%), mean number of flowering plant species, number of spraying events, biomass (g), number of total barcode index numbers (BINs) and BINs of the most common orders, and BOLD ID for the 32 studied vineyards in the 16 pairs with either fungus-resistant (FRG) or classical grape varieties under organic or conventional management in 2020 and 2021.

Pair	Management	Variety	Year	SNH	Vegetation cover	Plant species	Sprayings	Biomass
1	Organic	FRG	2020	47.20	53	1.5	3	10
1	Organic	Classic	2020	47.20	58	1.1	11	4.4
2	Organic	FRG	2020	36.41	59	1.8	3	14
2	Organic	Classic	2020	36.41	61	2.2	13	11.6
3	Organic	FRG	2020	23.47	30	1	11	8.8
3	Organic	Classic	2020	23.47	22	1.3	14	6.9
4	Organic	FRG	2020	1.39	58	1	8	8
4	Organic	Classic	2020	1.39	49	1.2	11	11.7
5	Organic	FRG	2020	3.49	53	1.8	3	7.8
5	Organic	Classic	2020	3.49	58	1.2	14	7.2
6	Organic	FRG	2020	26.93	34	1.6	0	5.9
6	Organic	Classic	2020	26.93	53	2	11	5.7
7	Organic	FRG	2020	8.71	57	1.8	3	5.2
7	Organic	Classic	2020	8.71	53	1.8	12	7
8	Organic	FRG	2020	6.27	79	2.8	3	12.7
8	Organic	Classic	2020	6.27	83	2.3	10	13.1
9	Conventional	FRG	2020	24.75	91	0.7	3	9.7
9	Conventional	Classic	2020	24.75	88	1.7	7	7.9
10	Conventional	FRG	2020	43.06	84	2.3	5	12.9
10	Conventional	Classic	2020	43.06	89	1.9	9	13.8
11	Conventional	FRG	2020	3.13	59	1.5	0	9.2
11	Conventional	Classic	2020	3.13	58	1.8	8	12.3
12	Conventional	FRG	2020	14.44	63	1.2	0	12.4
12	Conventional	Classic	2020	14.44	63	1.7	8	10.9
13	Conventional	FRG	2020	6.83	84	1.3	4	10.2
13	Conventional	Classic	2020	6.83	94	0.9	8	7.4
14	Conventional	FRG	2020	5.90	87	2.1	6	7.3
14	Conventional	Classic	2020	5.90	86	2.7	11	7.9
15	Conventional	FRG	2020	8.69	86	2.3	5	15.9
15	Conventional	Classic	2020	8.69	88	1.5	11	10.4
16	Conventional	FRG	2020	15.08	56	0.8	4	10.6
16	Conventional	Classic	2020	15.08	61	1.3	9	14.2
1	Organic	FRG	2021	47.20	78	3.3	7	7.9
1	Organic	Classic	2021	47.20	83	3.3	10	6.3
2	Organic	FRG	2021	36.41	59	4.6	5	8
2	Organic	Classic	2021	36.41	55	2.7	13	4.8
3	Organic	FRG	2021	23.47	63	1.8	10	4.6
3	Organic	Classic	2021	23.47	70	1.9	13	4.2
4	Organic	FRG	2021	1.39	83	2.9	11	6.4

4	Organic	Classic	2021	1.39	81	3.4	14	5.5
5	Organic	FRG	2021	3.49	78	3.3	7	6.2
5	Organic	Classic	2021	3.49	48	0.9	14	2.7
6	Organic	FRG	2021	26.93	76	1.3	0	3
6	Organic	Classic	2021	26.93	42	1.7	11	3.2
7	Organic	FRG	2021	8.71	59	0.5	4	3.1
7	Organic	Classic	2021	8.71	50	0.6	14	4
8	Organic	FRG	2021	6.27	78	4.2	7	7.8
8	Organic	Classic	2021	6.27	75	3.3	13	6.6
9	Conventional	FRG	2021	24.75	95	3.2	3	4.6
9	Conventional	Classic	2021	24.75	92	2.8	9	5.9
10	Conventional	FRG	2021	43.06	76	3.6	6	6
10	Conventional	Classic	2021	43.06	75	3.2	10	6.9
11	Conventional	FRG	2021	3.13	60	2.7	6	3.8
11	Conventional	Classic	2021	3.13	61	1.9	9	6.6
12	Conventional	FRG	2021	14.44	61	1.3	6	3.5
12	Conventional	Classic	2021	14.44	61	1.7	9	5.7
13	Conventional	FRG	2021	6.83	80	0.5	8	7.5
13	Conventional	Classic	2021	6.83	80	1	9	6.4
14	Conventional	FRG	2021	5.90	94	3.6	11	7.1
14	Conventional	Classic	2021	5.90	91	3.4	14	8.7
15	Conventional	FRG	2021	8.69	91	2.1	8	15.8
15	Conventional	Classic	2021	8.69	93	2.3	13	17.8
16	Conventional	FRG	2021	15.08	65	1.3	4	5.9
16	Conventional	Classic	2021	15.08	68	1.3	9	8.1

The table is showing only the first 9 rows of Table S3.1. See the following table S3.1.2 for the next 7 rows.

Table S3.1.2 Proportion (%) of semi-natural habitat (SNH), mean vegetation cover (%), mean number of flowering plant species, number of spraying events, biomass (g), number of total barcode index numbers (BINs) and BINs of the most common orders, and BOLD ID for the 32 studied vineyards in the 16 pairs with either fungus-resistant (FRG) or classical grape varieties under organic or conventional management in 2020 and 2021.

Sum of BINs						BOLD ID
Total	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera	BOLD:AAA2504
251	16	133	26	45	22	0
176	16	87	21	32	15	0
227	28	82	27	63	18	0
209	22	89	19	53	18	0
249	24	101	25	59	29	0
222	25	95	21	52	21	0
177	22	92	9	30	14	0
176	18	89	14	33	15	0
173	18	82	15	41	9	0
178	15	81	19	43	12	0
239	26	109	30	55	9	0
207	15	101	17	48	17	0
139	15	65	12	24	14	0
171	14	79	14	34	20	0
202	19	98	19	42	16	0
191	22	83	16	37	24	0
209	19	118	15	38	12	0
181	12	95	9	36	22	0
300	24	153	22	79	14	0
340	27	153	32	87	27	0
148	15	75	10	31	10	0
210	18	106	14	37	25	0
179	16	92	16	35	12	0
199	13	92	15	45	25	0
149	12	63	15	42	12	0
151	15	80	13	31	8	0
207	19	98	34	31	14	0
174	18	88	19	33	8	0
225	39	98	16	44	19	0
175	21	83	10	35	19	0
212	17	105	20	52	10	0
155	12	84	7	33	13	0
168	12	89	18	21	18	0
178	17	101	19	17	13	0
163	15	80	17	25	15	0
142	21	64	16	17	14	0
162	20	88	15	17	6	0
150	19	77	16	20	9	0
119	19	54	10	17	9	0
119	19	56	11	15	12	0

129	12	68	12	17	13	0
101	16	51	8	8	11	0
135	11	76	14	11	8	0
148	21	76	20	9	15	0
129	16	57	11	24	11	0
154	19	78	13	19	15	0
177	25	83	13	26	18	0
127	18	51	12	22	17	0
145	12	79	11	18	17	0
135	12	71	6	20	15	0
159	20	83	12	26	15	1
164	13	92	12	27	14	0
139	15	68	11	19	11	0
142	18	76	11	16	14	0
128	21	62	11	12	14	0
131	12	68	9	20	10	0
155	13	84	10	32	10	0
163	16	89	11	26	14	0
157	20	83	16	19	7	0
123	15	60	13	17	12	0
107	11	54	12	10	12	0
117	14	60	9	15	15	0
147	13	71	17	22	17	0
115	16	58	6	17	12	0

The table is showing only rows 10 to 16 of table S3.1. The whole table with all 1,748 BOLD IDs can be downloaded at https://static-content.springer.com/esm/art%3A10.1007%2Fs10531-023-02621-y/MediaObjects/10531_2023_2621_MOESM2_ESM.xlsx.

Supplementary materials – Chapter 4
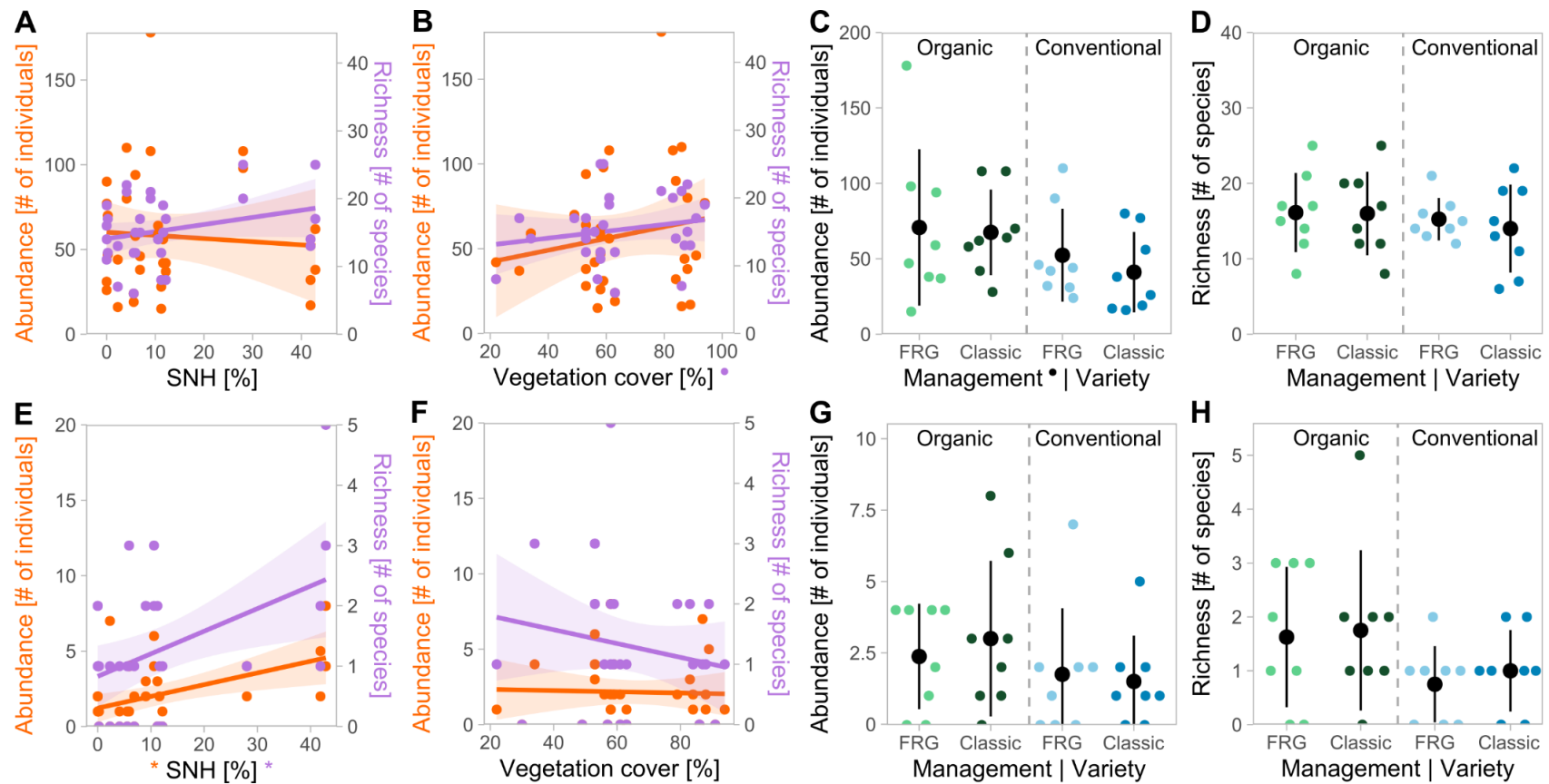
Figure S4.1 Abundance (orange) and richness (purple) of ground-nesting (A-D) and above-ground-nesting bees (E-H) for proportion of semi-natural habitat (SNH; A and E) and vegetation cover (B and F), and abundance (C and G) and richness (D and H) for fungus-resistant (FRG, brighter) and classic (darker) grape varieties under organic (blue) and conventional (green) management. Shaded areas represent the 95 % confidence intervals. Significant effects are indicated in x-axis labels. Significance code: *** P <0.001, ** P <0.01, * P <0.05, ● P <0.1.

Table S4.1 Number of flowering plants per species for the 32 studied vineyards in the 16 landscapes with either fungus-resistant (FRG) or classic grape varieties under organic or conventional management.

Landscape	Management	Variety	*Achillea millefolium* agg.	*Amaranthus retroflexus*	*Artemisia vulgaris* agg.	*Bellis perennis*	*Calendula officinalis*	*Capsella bursa-pastoris*	*Centaurea cyanus*	*Cerastium fontanum* agg.	*Chenopodium album* agg.	*Cirsium arvense*	*Convolvulus arvensis*	*Crepis capillaris*
1	Organic	FRG	0	1	0	0	0	0	0	0	2	0	0	0
1	Organic	Classic	0	0	0	0	0	0	1	0	1	0	0	0
2	Organic	FRG	0	4	0	0	0	0	0	0	1	0	1	0
2	Organic	Classic	0	0	0	0	0	0	0	0	1	0	5	0
3	Organic	FRG	0	2	0	0	0	0	0	0	4	0	0	0
3	Organic	Classic	0	2	0	0	0	0	0	0	2	0	1	0
4	Organic	FRG	0	0	0	0	0	0	0	0	3	0	2	0
4	Organic	Classic	0	0	0	0	0	0	0	0	2	0	2	0
5	Organic	FRG	0	0	0	0	0	0	0	0	1	0	0	0
5	Organic	Classic	0	0	0	0	1	1	0	0	1	0	1	0
6	Organic	FRG	0	2	0	0	0	1	0	0	2	0	2	0
6	Organic	Classic	0	0	0	0	0	0	0	0	0	0	3	0
7	Organic	FRG	0	0	0	0	0	1	0	0	5	0	0	0
7	Organic	Classic	0	1	0	0	1	0	0	0	1	1	1	0
8	Organic	FRG	0	0	0	0	0	0	0	0	3	0	3	0
8	Organic	Classic	2	1	0	0	0	0	0	0	3	0	1	0
9	Conventional	FRG	0	0	0	0	0	0	0	0	0	0	0	1
9	Conventional	Classic	0	0	0	3	0	4	0	0	0	0	0	0
10	Conventional	FRG	0	0	0	0	0	1	0	0	1	0	0	0
10	Conventional	Classic	0	1	0	0	0	0	0	0	0	1	0	0
11	Conventional	FRG	1	0	0	0	0	0	0	0	0	0	2	0
11	Conventional	Classic	0	0	0	3	0	0	0	0	2	0	0	0
12	Conventional	FRG	3	0	1	0	0	0	0	0	0	0	0	0
12	Conventional	Classic	1	0	0	0	0	0	0	1	0	0	0	0
13	Conventional	FRG	0	0	0	0	0	0	0	0	0	0	0	2
13	Conventional	Classic	0	0	0	2	0	1	0	1	0	0	0	0
14	Conventional	FRG	0	1	0	0	0	2	0	0	2	0	0	0
14	Conventional	Classic	0	1	0	0	0	0	0	1	1	0	0	0
15	Conventional	FRG	0	0	0	0	0	0	0	0	0	0	1	0
15	Conventional	Classic	0	0	0	1	0	0	3	1	0	0	0	1
16	Conventional	FRG	0	0	0	2	0	0	0	0	0	0	0	0
16	Conventional	Classic	0	1	0	0	1	0	0	0	3	0	0	0

Epilobium tetragonum	Erigeron annuus	Erigeron canadensis	Euphorbia helioscopia	Fagopyrum esculentum	Fumaria officinalis	Galium aparine agg.	Galium mollugo agg.	Geranium dissectum	Geranium molle agg.	Geranium pusillum	Geranium pyrenaicum	Geranium rotundifolium	Geum urbanum	Glechoma hederacea agg.
0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
0	0	0	1	1	0	0	0	0	0	0	0	3	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
0	0	0	0	1	0	0	0	0	0	1	0	3	0	0
0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	1	0	0	3	0	0	1	0	1
0	0	0	0	0	0	0	0	0	1	0	0	4	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	2	0	0	0	5	0	0
0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	1	2	0	0
0	1	1	0	0	0	0	0	0	0	4	0	0	0	0
0	3	1	0	0	0	0	0	2	0	0	0	0	0	2
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
0	0	2	0	0	0	0	1	0	0	2	0	1	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Hypericum perforatum	Hypochaeris radicata	Lamium purpureum	Lepidium draba	Linum usitatissimum	Lactuca serriola	Lotus corniculatus agg.	Malva pusilla	Malva sylvestris	Medicago lupulina	Meum athamanticum	Medicago sativa agg.	Melilotus officinalis	Mercurialis annua	Ornithogalum umbellatum agg.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	1	0	2	0	0	2	1	0	2	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	2	0	1	0	0	0	1	0	1	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	2	0	2	2	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	3	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	1	2	0	0	1	0	0
0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
0	0	3	0	0	0	0	0	0	0	0	0	0	0	4
0	0	1	0	0	0	0	0	0	0	0	0	0	0	4
0	0	1	0	0	0	0	0	0	0	0	0	0	0	4
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	2	1	0	1
1	0	0	0	0	0	0	0	0	5	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0	0	0	0	0	3
0	0	2	0	0	0	0	0	0	0	0	0	0	0	3
0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	2	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	1	0	0	1	0	0

Phacelia tanacetifolia	Papaver dubium agg.	Plantago lanceolata	Plantago major agg.	Polygonum aviculare agg.	Potentilla reptans	Prunella vulgaris	Ranunculus repens	Raphanus raphanistrum agg.	Rumex obtusifolius	Sanguisorba minor	Senecio vulgaris	Sisymbrium officinale	Sinapis alba
0	0	0	0	0	0	0	0	3	0	0	0	0	2
2	0	0	0	0	0	0	0	2	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	3	0	0	0	0	0	0	3	0	0
0	0	0	0	2	0	0	0	0	0	0	4	0	0
2	1	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	2	0	0	0	0	2
3	0	0	0	2	0	0	0	2	0	0	0	0	0
0	0	0	0	2	0	0	0	0	1	0	1	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	1	0	0	3	0	2
0	0	0	0	1	0	0	0	0	0	0	2	0	0
0	0	0	0	1	0	0	0	2	0	1	0	0	2
0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	2	0	0
0	0	0	0	0	0	0	0	0	0	0	2	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	3	0	0	0	0	0	0	0	0	0
0	0	0	0	4	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	3	0	1	0	0	0	0	0	0	0
0	0	0	0	4	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	2	0	0
0	0	0	0	0	0	0	1	0	0	0	2	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	4	0	0	0	0	0	0	0	0	0
0	0	0	0	4	0	4	0	0	2	0	0	0	0
0	0	1	0	1	0	0	0	0	0	0	0	0	0

Sonchus asper	Sonchus oleraceus	Stellaria media agg.	Taraxacum spp.	Trifolium hybridum	Trifolium incarnatum	Trifolium repens	Trifolium resupinatum	Valerianella locusta	Verbena officinalis	Veronica persica	Veronica polita	Veronica serpyllifolia	Vicia sativa agg.	Vicia sepium	Vicia villosa agg.
0	0	0	0	0	0	0	0	4	0	0	0	0	1	0	1
0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0
0	0	1	0	0	0	0	0	0	0	3	0	0	0	0	0
0	1	1	0	0	1	0	0	0	0	2	0	0	0	0	0
1	0	0	3	0	0	0	0	1	0	1	0	0	0	0	0
0	0	0	1	0	0	2	0	0	0	4	0	1	0	0	0
0	0	1	2	0	2	2	0	0	0	4	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
0	0	0	0	1	2	0	4	0	0	0	0	0	0	1	0
0	0	0	1	0	0	0	0	0	0	2	1	0	0	0	0
0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	3	0	2	0	0	0	0	4	0	0	0	0	0
0	0	1	2	0	0	2	0	0	0	3	0	1	0	0	0
0	0	0	3	0	0	1	0	0	0	2	0	0	0	0	0
0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0
0	0	1	3	0	0	2	0	2	0	1	0	0	0	0	0
0	0	1	1	0	0	0	0	2	0	3	0	0	0	0	0
0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0
0	0	0	4	0	0	0	0	0	0	5	0	0	0	0	0
0	0	0	4	0	0	6	0	0	0	0	0	0	0	0	0
0	0	0	3	0	0	8	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	4	0	0	0	0	0	0	0	0	0
0	0	0	2	0	0	6	0	0	0	0	0	0	0	0	0
0	0	1	2	0	0	0	0	1	0	0	0	0	0	2	0
0	0	0	3	0	0	0	0	1	0	4	0	0	0	0	0
0	0	1	2	0	0	0	0	0	0	4	0	0	0	1	0
0	0	1	4	0	0	0	0	0	0	1	0	0	0	0	0
0	0	1	3	0	0	6	0	0	1	1	0	0	1	0	0
0	0	0	4	0	0	6	0	0	0	0	0	0	0	0	0
0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0

Table S4.2 Number bees per species for the 32 studied vineyards in the 16 landscapes with either fungus-resistant (FRG) or classic grape varieties under organic or conventional management.

Landscape	Management	Variety	*Andrena bicolor*	*Andrena bimaculata*	*Andrena chrysosceles*	*Andrena cineraria*	*Andrena distinguenda*	*Andrena dorsata*	*Andrena flavipes*	*Andrena fulva*	*Andrena* cf. *fulvago*	*Andrena gravida*	*Andrena haemorrhoa*	*Andrena humilis*	*Andrena labialis*
1	Organic	FRG	1	0	0	1	0	6	2	0	0	0	0	0	0
1	Organic	Classic	2	1	0	2	0	14	3	1	0	0	1	0	0
2	Organic	FRG	0	1	0	1	0	29	4	1	0	0	1	0	0
2	Organic	Classic	0	0	0	32	0	0	11	0	0	8	0	0	0
3	Organic	FRG	0	0	0	4	0	1	2	0	0	1	1	0	0
3	Organic	Classic	0	0	0	15	0	1	0	0	0	0	6	0	0
4	Organic	FRG	0	0	0	1	0	2	0	0	1	0	0	1	0
4	Organic	Classic	0	0	0	2	0	0	1	0	0	0	3	0	0
5	Organic	FRG	0	0	0	0	0	0	1	0	0	0	0	0	0
5	Organic	Classic	0	0	0	0	0	0	2	0	0	0	0	0	0
6	Organic	FRG	0	0	0	1	0	2	1	0	0	0	0	0	0
6	Organic	Classic	0	0	0	0	0	2	3	0	0	0	0	0	0
7	Organic	FRG	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Organic	Classic	0	0	2	0	0	5	0	0	0	0	1	0	1
8	Organic	FRG	0	0	0	1	0	3	4	0	0	0	2	0	1
8	Organic	Classic	0	0	0	0	0	1	4	0	0	1	4	0	0
9	Conventional	FRG	0	0	0	1	0	10	4	0	0	0	2	0	0
9	Conventional	Classic	0	0	0	0	1	3	0	0	0	0	4	0	0
10	Conventional	FRG	0	0	0	0	0	2	3	0	0	1	0	0	0
10	Conventional	Classic	0	1	0	0	0	1	2	0	0	0	2	0	0
11	Conventional	FRG	0	0	0	1	1	3	1	0	0	0	1	0	0
11	Conventional	Classic	0	0	0	1	0	2	1	0	0	0	1	0	0
12	Conventional	FRG	0	0	0	0	0	6	3	0	0	0	1	0	0
12	Conventional	Classic	0	0	0	0	0	7	0	0	0	0	1	0	0
13	Conventional	FRG	0	0	0	7	0	0	2	0	0	0	0	0	0
13	Conventional	Classic	0	0	0	4	0	10	1	0	0	1	1	0	0
14	Conventional	FRG	0	0	1	0	0	2	0	0	0	0	2	0	0
14	Conventional	Classic	0	0	0	0	0	0	0	0	0	0	1	0	0
15	Conventional	FRG	0	0	0	0	0	5	11	0	0	0	2	0	1
15	Conventional	Classic	0	0	0	1	0	5	2	0	0	0	1	0	0
16	Conventional	FRG	0	0	0	0	0	14	2	0	0	1	1	0	0
16	Conventional	Classic	1	0	0	2	0	21	2	0	0	0	0	0	0

Andrena labiata	Andrena lagopus	Andrena lathyri	Andrena minutula	Andrena mitis	Andrena cf. nitida	Andrena niveata	Andrena ovatula	Andrena scotica	Andrena strohmella	Andrena tibialis	Andrena vaga	Andrena ventralis	Andrena viridescens	Apis mellifera	Bombus hortorum agg.	Bombus pascuorum
0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
0	2	1	10	0	0	0	0	0	0	1	0	0	0	2	1	0
1	9	0	3	1	0	0	0	0	0	0	0	0	0	3	0	0
0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0
0	5	0	1	0	0	0	0	0	1	0	0	0	0	5	0	0
0	0	0	0	0	3	0	0	0	0	0	0	0	0	3	0	0
0	5	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
0	4	0	1	0	1	0	0	0	0	0	0	0	0	2	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0
0	2	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0
0	6	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0
0	3	1	0	0	0	0	0	0	0	0	0	0	0	7	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0	0
0	2	0	0	0	2	1	0	0	0	0	0	0	1	4	1	0
0	3	0	5	0	0	3	0	0	0	0	0	1	0	2	2	0
0	1	0	5	0	0	0	0	0	0	0	1	0	0	3	0	0
1	1	0	1	0	1	0	0	0	0	0	0	0	0	4	0	0
0	0	0	2	0	0	0	1	0	0	0	0	0	0	7	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	1	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	2	0	0	0	0	0	0	0	0	0	0	5	0	0
0	0	0	0	0	0	0	0	0	0	2	0	0	1	4	0	0
0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0
0	0	0	2	0	1	0	0	0	0	0	0	0	0	1	0	0
0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	8	0	1	0	3	0	0	0	0	0	0	2	0	0
0	1	0	10	0	0	1	0	0	0	0	0	0	0	1	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0

Appendix

Bombus ruderarius	Bombus terrestris agg.	Ceratina cyanea	Colletes cunicularius	Eucera nigrescens	Halictus cf. eurygnathus	Halictus cf. langobardicus	Halictus leucaheneus	Halictus quadricinctus	Halictus scabiosae	Halictus sexcinctus	Halictus simplex agg.	Halictus subauratus	Halictus tumulorum
0	0	0	0	1	0	0	0	0	1	0	0	0	0
0	0	0	1	1	0	0	0	0	1	0	0	2	0
0	2	0	0	1	0	0	0	0	2	0	0	1	1
0	1	0	0	1	0	1	0	0	2	0	1	0	1
0	0	0	0	0	0	0	0	0	1	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	1	0	0	0	0	0	0	0	0	0	1	0	2
1	0	0	0	0	0	0	0	0	0	0	1	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	2	0	0	0	0	0	0	0	1	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	1	0	1	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	1	1	0
0	3	0	0	0	0	0	1	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	2
0	0	0	0	0	0	0	0	0	0	1	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0
0	1	0	0	2	0	0	0	0	2	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	1	0	0	0	1	0
0	0	0	0	0	1	0	0	0	1	0	0	0	2
2	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	1	0	0	0	0	0	0	0	0	0	0

Hylaeus angustatus	Hylaeus brevicornis	Hylaeus dilatatus	Hylaeus cf. hyalinatus	Hylaeus pictipes	Lasioglossum aeratum	Lasioglossum cf. bluethgeni	Lasioglossum calceatum	Lasioglossum glabriusculum	Lasioglossum cf. laevigatum	Lasioglossum laticeps	Lasioglossum lativentre	Lasioglossum leucopus	Lasioglossum leucozonium
0	0	0	0	0	0	3	0	1	0	5	0	0	0
0	3	0	0	0	0	3	0	0	0	2	1	0	0
0	0	0	0	0	0	0	1	4	0	0	1	0	1
0	0	0	0	0	0	0	0	19	0	3	1	0	0
0	0	0	0	0	0	0	1	4	0	2	0	0	0
0	0	0	0	0	0	0	0	2	0	1	0	0	0
1	0	0	0	0	0	0	0	9	0	1	0	0	0
0	0	0	0	0	0	0	1	8	0	2	0	0	0
1	0	0	0	0	1	0	1	23	1	3	0	0	0
0	0	0	0	0	0	0	0	26	0	0	0	0	0
0	0	0	0	0	0	0	0	4	0	1	0	0	0
0	0	0	1	0	0	1	0	1	0	1	0	0	0
0	0	0	0	0	0	0	0	6	0	2	0	0	0
0	0	1	0	0	0	0	0	3	0	1	0	0	0
1	0	0	0	0	0	0	1	12	0	4	1	0	0
0	0	0	0	1	0	0	0	1	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	2	3	0	1
2	0	0	0	0	0	0	0	17	0	0	1	0	0
0	0	0	0	0	0	0	1	0	0	1	0	0	1
0	0	0	0	0	0	1	0	0	0	1	1	0	1
1	0	0	0	0	0	0	0	8	0	2	0	0	0
0	1	0	0	0	0	0	0	7	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0	0	0
1	0	0	0	0	0	0	0	0	0	1	0	0	0
1	0	0	0	0	0	0	3	11	0	2	0	0	0
0	0	0	0	0	0	0	1	6	0	4	0	0	0
0	0	0	0	0	0	0	0	10	0	0	0	0	1
0	0	0	0	0	0	0	0	6	0	0	0	0	0
0	0	0	0	0	0	0	1	27	0	4	1	0	0
1	0	0	0	0	0	0	0	25	0	1	1	1	0
0	0	0	0	0	0	0	0	3	0	1	0	0	0
0	0	0	0	0	0	1	0	6	0	2	0	0	1

Lasioglossum lineare	Lasioglossum malachurum	Lasioglossum minutissimum	Lasioglossum morio	Lasioglossum pallens	Lasioglossum cf. pauperatum	Lasioglossum pauxillum	Lasioglossum politum	Lasioglossum punctatissimum	Lasioglossum puncticolle	Lasioglossum quadrinotatum	Lasioglossum cf. subhirtum	Lasioglossum villosulum
1	2	0	2	0	0	1	0	0	0	1	0	2
1	0	0	7	0	0	1	1	1	0	0	0	1
2	21	0	2	0	6	1	0	0	0	0	0	1
2	10	0	1	0	0	3	1	0	0	0	0	7
2	6	0	2	0	0	0	2	0	0	0	0	1
0	13	0	0	0	0	0	0	0	1	0	0	0
3	19	0	2	0	0	0	0	0	0	0	0	2
4	29	0	6	0	0	0	0	0	0	0	2	2
11	36	0	7	0	0	0	0	1	0	0	1	0
4	12	2	3	0	1	0	1	0	0	0	0	0
5	30	0	0	0	0	0	0	0	1	0	0	3
3	38	0	5	0	1	2	0	0	0	0	0	2
0	2	0	1	0	0	1	0	0	0	0	0	1
1	9	0	0	0	0	0	0	0	0	0	0	0
40	80	0	6	0	0	1	13	0	0	0	0	0
21	42	2	6	0	0	0	5	1	0	0	0	0
1	2	0	5	0	0	4	0	0	0	0	0	1
0	1	0	0	0	1	0	0	2	0	0	0	1
3	3	0	4	0	0	4	0	0	0	0	0	2
0	0	0	2	0	0	2	0	0	0	0	0	0
1	5	1	2	0	0	1	0	0	0	0	0	0
4	3	0	1	0	0	0	0	0	0	0	0	0
1	3	1	2	0	1	0	0	0	0	0	0	0
3	5	0	0	0	0	0	0	0	0	0	0	0
34	16	0	6	0	0	2	2	0	0	0	0	0
29	3	0	4	0	0	2	3	0	1	0	0	0
10	9	0	2	0	0	2	0	0	0	0	0	0
3	0	0	2	0	0	0	0	0	0	0	0	0
17	11	3	7	1	0	3	0	1	0	0	0	1
9	3	1	8	1	0	2	1	0	0	0	0	2
2	6	1	4	0	0	3	1	1	0	0	0	0
1	5	2	1	0	0	3	3	0	0	0	0	1

Lasioglossum xanthopus	Megachile cf. centuncularis	Nomada distinguenda	Nomada fabriciana	Nomada flavoguttata	Nomada fucata	Nomada marshamella	Nomada cf. minuscula	Nomada striata	Nomada zonata	Osmia adunca	Osmia bicornis	Osmia brevicornis	Osmia cornuta	Osmia niveata	Sphecodes cf. crassus	Stelis minuta	Stelis ornatula
0	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	1	0
0	0	0	1	2	0	0	0	0	0	2	1	1	0	0	0	0	0
0	0	0	1	1	0	0	0	0	0	0	4	0	0	0	0	0	0
0	0	0	1	2	1	0	0	0	0	0	2	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	4	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary materials – Chapter 5
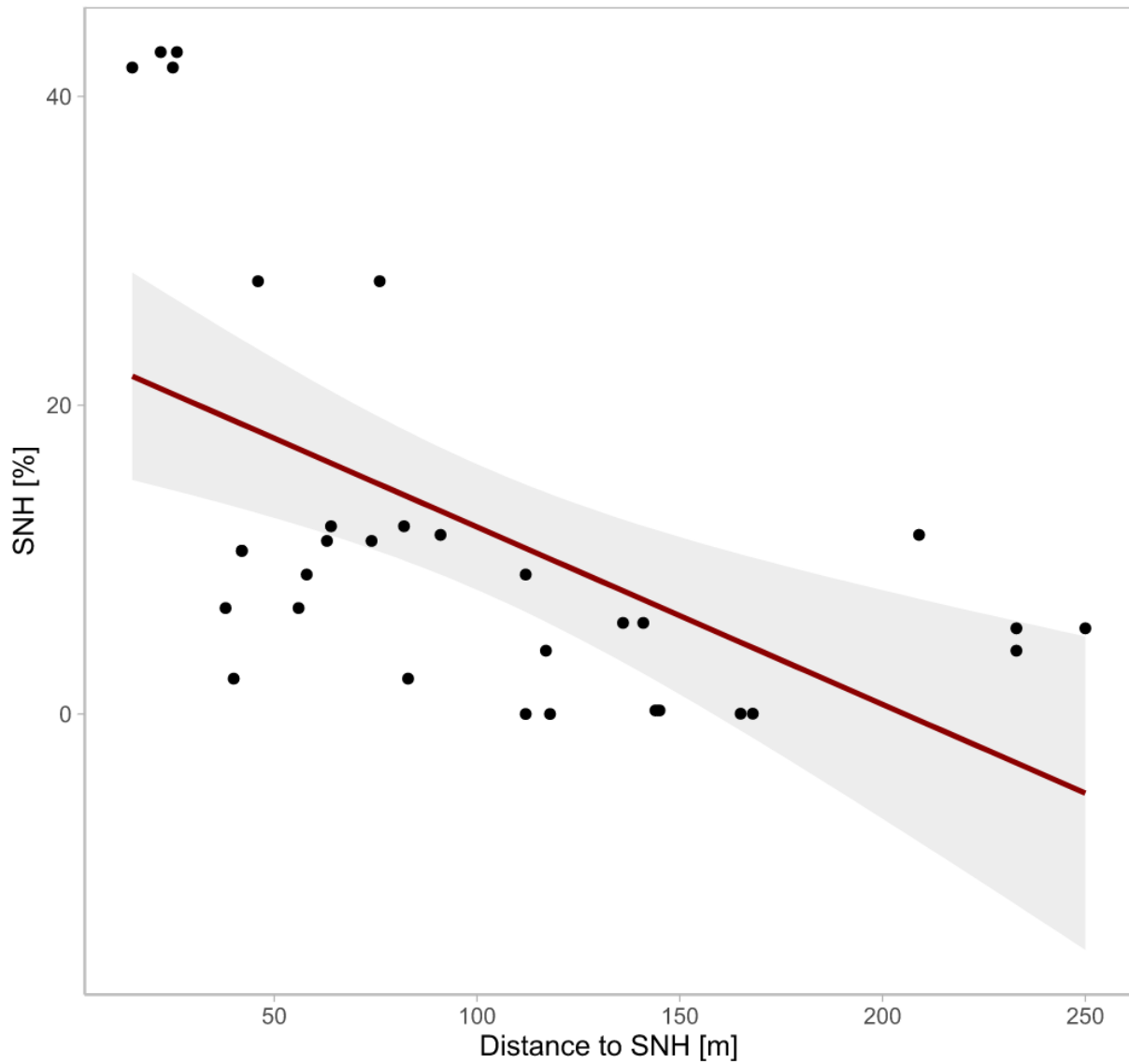
Figure S5.1 Proportion (%) of semi-natural habitats (SNH) in the surrounding landscape for the distance from the studied vineyard to the closest SNH. Shaded area represents the 95 % confidence intervals.

Table S5.1 Number of pesticide applications, mean vegetation height (cm), mean vegetation cover (%), and proportion (%) of semi-natural habitats (SNH) within a radius of 500 m for the 32 studied vineyards in the 16 landscapes with either fungus-resistant (FRG) or classic grape varieties under organic or conventional management.

Landscape	Management	Pesticide applications	Vegetation height (cm)	Vegetation cover (%)	SNH (%)
1	Organic, FRG	7	15	50	42.87
1	Organic, Classic	10	15	50	42.87
2	Organic, FRG	5	13	45	28.03
2	Organic, Classic	13	7.5	50	28.03
3	Organic, FRG	10	6.5	45	12.16
3	Organic, Classic	13	12	85	12.16
4	Organic, FRG	11	12	85	0.23
4	Organic, Classic	14	10.5	85	0.23
5	Organic, FRG	7	12	90	5.90
5	Organic, Classic	14	0	5	5.90
6	Organic, FRG	0	15.5	100	10.57
6	Organic, Classic	11	8	80	10.57
7	Organic, FRG	4	17	85	11.21
7	Organic, Classic	14	6	50	11.21
8	Organic, FRG	7	11.5	100	9.03
8	Organic, Classic	13	14	95	9.03
9	Conventional, FRG	3	10	100	6.86
9	Conventional, Classic	9	10	100	6.86
10	Conventional, FRG	6	3.75	40	41.87
10	Conventional, Classic	10	3.5	40	41.87
11	Conventional, FRG	6	15.5	60	0.00
11	Conventional, Classic	9	7.5	65	0.00
12	Conventional, FRG	6	8	65	5.55
12	Conventional, Classic	9	6	65	5.55
13	Conventional, FRG	8	23	95	0.02
13	Conventional, Classic	9	11	90	0.02
14	Conventional, FRG	11	10.5	90	2.29
14	Conventional, Classic	14	11.5	90	2.29
15	Conventional, FRG	8	11.5	100	4.10
15	Conventional, Classic	13	10	90	4.10
16	Conventional, FRG	4	13.5	45	11.60
16	Conventional, Classic	9	12.5	70	11.60

Table S5.2 Densities of Orthoptera sampled using box quadrats for the 32 studied vineyards in the 16 landscapes with either fungus-resistant (FRG) or classic grape varieties under organic or conventional management.

Landscape	Management	*Chorthippus brunneus*	*Chorthippus biguttulus*	*Chorthippus parallelus*	*Roeseliana roeselii*	*Tetrix subulata*
1	Organic, FRG	10	8	2	0	0
1	Organic, Classic	6	5	3	0	0
2	Organic, FRG	11	3	1	0	0
2	Organic, Classic	4	1	0	0	0
3	Organic, FRG	1	1	5	0	0
3	Organic, Classic	1	0	1	0	0
4	Organic, FRG	14	6	4	0	0
4	Organic, Classic	3	2	0	0	0
5	Organic, FRG	2	1	1	0	0
5	Organic, Classic	0	0	0	0	0
6	Organic, FRG	3	1	0	0	0
6	Organic, Classic	0	0	0	0	0
7	Organic, FRG	4	4	2	0	0
7	Organic, Classic	3	0	1	0	0
8	Organic, FRG	5	0	0	1	0
8	Organic, Classic	0	1	0	0	0
9	Conventional, FRG	2	1	5	0	0
9	Conventional, Classic	1	1	4	0	0
10	Conventional, FRG	1	0	6	0	0
10	Conventional, Classic	0	3	0	0	0
11	Conventional, FRG	0	1	2	0	0
11	Conventional, Classic	5	2	2	0	0
12	Conventional, FRG	2	1	3	0	0
12	Conventional, Classic	2	2	3	0	0
13	Conventional, FRG	8	11	0	0	0
13	Conventional, Classic	9	7	0	0	0
14	Conventional, FRG	7	3	0	0	0
14	Conventional, Classic	0	2	2	0	0
15	Conventional, FRG	8	9	0	0	0
15	Conventional, Classic	9	13	0	0	0
16	Conventional, FRG	1	1	3	0	1
16	Conventional, Classic	4	1	0	0	2

Table S5.3 Densities of Orthoptera sampled using transect walks with song detection for the 32 studied vineyards in the 16 landscapes with either fungus-resistant (FRG) or classic grape varieties under organic or conventional management.

Landscape	Management	*Tettigonia viridissima*	*Phaneroptera falcata*	*Leptophyes punctatissima*	*Oecanthus pellucens*
1	Organic, FRG	0	0	2	0
1	Organic, Classic	1	0	1	0
2	Organic, FRG	2	1	2	0
2	Organic, Classic	0	1	3	0
3	Organic, FRG	0	0	1	0
3	Organic, Classic	1	0	1	0
4	Organic, FRG	0	5	0	0
4	Organic, Classic	0	4	0	0
5	Organic, FRG	0	3	0	0
5	Organic, Classic	0	2	0	0
6	Organic, FRG	0	0	1	0
6	Organic, Classic	0	0	0	0
7	Organic, FRG	0	1	1	0
7	Organic, Classic	0	1	0	0
8	Organic, FRG	0	2	9	1
8	Organic, Classic	1	4	1	1
9	Conventional, FRG	0	1	2	0
9	Conventional, Classic	1	5	2	0
10	Conventional, FRG	0	3	0	0
10	Conventional, Classic	2	0	5	0
11	Conventional, FRG	2	1	0	0
11	Conventional, Classic	1	1	0	0
12	Conventional, FRG	1	0	0	0
12	Conventional, Classic	4	1	0	0
13	Conventional, FRG	1	5	0	0
13	Conventional, Classic	0	1	0	0
14	Conventional, FRG	0	19	0	3
14	Conventional, Classic	1	54	0	2
15	Conventional, FRG	2	43	0	0
15	Conventional, Classic	0	51	0	0
16	Conventional, FRG	0	0	0	0
16	Conventional, Classic	3	0	0	0