THE ROLE OF ALTERNATIVE RESOURCES FOR POLLINATORS AND APHID PREDATORS IN AGRICULTURAL LANDSCAPES

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Abstract

The world wide decline of insects is often associated with loss of natural and semi-natural habitat caused by intensified land-use. Many insects provide important ecosystem services to agriculture, such as pest control or pollination. To efficiently promote insects on remaining semi-natural habitat we need precise knowledge of their requirements to non-crop habitat. This thesis focuses on identifying the most important semi-natural habitats (forest edges, grasslands, and semi-open habitats) for pollinators and natural enemies of crop pests with respect to their food resource requirements. Special attention is given to floral resources and their spatio-temporal distribution in agricultural landscapes. Floral resource maps might get closer at characterizing landscapes the way they are experienced by insects compared to classical habitat maps. Performance of the two map types was compared on the prediction of wild bees and natural enemies that consume nectar and pollen, identifying habitats of special importance in the process. In wild bees, influences of spatio-temporal floral resource availability were analysed as well as habitat preferences of specific groups of bees. Understanding dietary needs of natural enemies of crop pests requires additional knowledge on prey use. To this end, ladybird gut contents have been analysed by means of high-throughput sequencing for insight into aphid prey-use.

Results showed, that wild bees were predicted better by floral resource maps compared to classical habitat maps. Forest edge area, as well as floral resources in forest edges had positive effects on abundance and diversity of rare bees and important crop pollinators. Similar patterns were retained for grassland diversity. Especially early floral resources seemed to have positive effects on wild bees. Crops and fruit trees produced a resource pulse in April that exceeded floral resource availability in May and June by tenfold. Most floral resources in forest edges appeared early in the season, with the highest floral density per area. Grasslands provided the lowest amount of floral resources but highest diversity, which was evenly distributed over the season.

Despite natural enemies need for floral resources, classical habitat maps performed better at predicting natural enemies of crop pests compared to floral resource maps. Classical habitat maps revealed a positive effect of forest edge habitat on the abundance of pest enemies, which translated into improved aphid control. Results from gut content analysis reveal high portions of pest aphid species and nettle aphids as well as a broader insight into prey spectra retained from ladybirds collected from sticky traps compared to individuals collected by hand. The aphid specific primer designed for this purpose will be helpful for identifying aphid consumption by ladybirds in future studies.

Findings of this thesis show the potential of floral resource maps for understanding interactions of wild bees and the landscape but also indicate that natural enemies are limited by other resources. I would like to highlight the positive effects of forest edges for different groups of bees as well as natural enemies and their performance on pest control.

Zusammenfassung

Der Verlust zahlreicher Insekten wird weitgehend in Verbindung gebracht mit dem Verlust von natürlichem und halbnatürlichem Lebensraum durch intensivierte Landnutzung. Viele Insekten liefern wichtige Ökosystemleistungen an die Landwirtschaft wie z.B. Bestäubung und Schädlingsbekämpfung. Um diese Insekten effizient auf den verbleibenden halbnatürlichen Flächen zu fördern, ist genaues Wissen über ihre Ansprüche an das Umland von Agrarflächen erforderlich. Der Fokus dieser Dissertation liegt auf der Suche nach den wichtigsten halbnatürlichen Habitattypen (Waldrand, Wiesen und halb-offene Habitate) zur Förderung von Nützlingen und Bestäubern aufgrund der Bedeutung von Nahrungsressourcen, welche sie dort nutzen. Besonderes Augenmerk liegt dabei auf Blütenressourcen und wie diese räumlich und zeitlich im Kulturland verteilt sind. Darauf basierte Ressourcenkarten versprechen eine Charakterisierung der Landschaft, welche der Relevanz für Insekten näher kommt als klassische Habitatkarten. In dieser These wurde deshalb verglichen, ob sich das Vorkommen von Wildbienen, sowie Nektar und Pollen konsumierenden Nützlingen besser mit klassischen Habitatkarten, oder mit Ressourcenkarten vorhersagen lässt und identifizierte Habitate besonderer Wichtigkeit. Bei Wildbienen wurde untersucht, inwiefern sich Präferenzen verschiedener Gruppen von Wildbienen unterscheiden und ob es zeitliche und räumliche Zusammensetzungen von Blühressourcen gibt, die besonders optimal sind. Da sich Nützlinge nebst der Nutzung von Blüten vor allem räuberisch ernähren, wurde des Weiteren deren Beutespektrum untersucht. Dazu wurde der Darminhalt von Marienkäfern mit genetischen Methoden mittels High Throughput Sequencing auf konsumierte Blattläuse analysiert.

Blütenbasierte Ressourcenkarten sagten Bienen besser voraus als klassische Habitatkarten. Der Waldrand war dabei von besonderer Bedeutung. Sowohl Flächenanteil als auch Blühangebot hatten positive Einflüsse auf Abundanz und Artenreichtum von wichtigen Kulturbestäubern und seltenen Arten. Ähnliche Muster zeigten sich für Wiesendiversität. Dabei schien besonders das frühe Blühangebot einen positiven Einfluss auf Wildbienen zu haben. Kulturen und Obstbäume verursachten im April einen Blütenpuls, der das Blühangebot vom Mai und Juni um mehr als das Zehnfache überstieg. Waldränder boten besonders Anfang Mai und im Juni ein Blühangebot, das im Verhältnis zur Fläche die weitaus höchste Dichte aufwies. Das Blühangebot von Wiesen war äusserst gering, zeigte aber die höchste Diversität, welche regelmässig über die Saison verteilt war.

Obwohl die untersuchten Nützlinge Blüten fürs Überleben benötigen, waren blütenbasierte Habitatkarten weniger geeignet, um die Nützlingsabundanz zu erklären, als herkömmliche Habitatkarten. Diese zeigten, dass Waldränder von besonderer Bedeutung für Nützlinge sind. Die Anzahl der Nützlinge wiederum führte zur Unterdrückung von Blattläusen. Die Resultate der Darmuntersuchungen zeigten zum einen, dass Marienkäfer einen relativ hohen Anteil an schädlichen Blattlausarten und Brennesselblattläusen konsumieren, zum anderen zeigen sie, dass mit Klebfallen gefangene Marienkäfer einen wesentlich breiteren Einblick in das Beutespektrum erlauben, als von Hand gesammelt. Der zu diesem Zweck entwickelte Blattlausprimer wird für kommende Studien bei der Identifizierung der Blattlausbeute von Marienkäfern hilfreich sein.

Unsere Resultate zeigen, dass Blütenkarten einen wichtigen Mehrwert für die Vorhersage von Wildbienen haben, nicht aber von Nützlingen, da für diese wohl andere Habitatfaktoren zusätzlich limitierend wirken. Der positive Einfluss von Waldrändern für unterschiedliche Gruppen von Wildbienen wie auch für Nützlinge und ihre Leistung als Schädlingsbekämpfer ist besonders hervorzuheben.

Chapter 1

General introduction

Lolita Ammann

Global food security, biodiversity and ecosystem services

Agricultural systems are challenged to feed the world's quickly growing population (Godfray et al. 2010; Ray et al. 2012). Pesticide and synthetic fertilizer application have allowed agriculture to keep pace with the increasing demand for food and fibre. Along chemical input, production area has been increasing, at cost of natural and semi-natural habitats (Matson et al. 1997; Foley et al. 2011). In many of the world's agricultural systems, yield optimisation has now reached a plateau allowing little improvements with conventional practices (Ray et al. 2012). To date, 37% of the terrestrial surface is used for agriculture, of which 10% is in arable use (Data of 2017 from FAOSTAT). Much of the land best suitable for crop production is already cultivated, leaving relatively little room for expansion (Fischer 2000). We should therefore use agricultural land as sustainably as possible, avoiding its loss through factors such as soil erosion resulting in soil depletion and drought (Timsina and Connor 2001) as well as pollution (China 2006). The fact that such a large portion of the earth's terrestrial surface is in agricultural use also asks for sustainable management from a biodiversity point of view (Tallis et al. 2009). The European Union estimates that about 50% of the continent's wild species spend at least part of their life cycle on farmland (Kristensen 2003). Indeed, agricultural practices associated with intensification such as pesticide input, landscape simplification and decreasing amounts of natural habitat are widely associated with species loss (Benton et al. 2003; Tscharntke et al. 2005; Potts et al. 2016a). Apart from the inherent value of biodiversity, this decrease should also be avoided for the sake of many ecosystem services provided by biodiversity such as nutrient cycling, soil formation, climate regulation, pollination or pest control, all being vital to crop yields in the end (final ecosystem service) (Bommarco et al. 2013). Biodiversity ensures redundancy as well as complementarity of species and their role in the ecosystem, making it more resilient against disturbance, reducing the likelihood of impairment of ecosystem functioning through loss of single players (Tilman 1996; Naeem and Li 1997; Cardinale et al. 2012). Ecological intensification aims at augmenting yields through protection and integration of biodiversity into agricultural systems by managing ecosystem services that biodiversity provides (Bommarco et al. 2013).

Pollinators and natural enemies of crop pests

Arthropods are the most diverse group of animals in agroecosystems and offer important ecosystem services. For example, they pollinate around 80% of the world's plants (Ollerton et al. 2011). Especially wild bees are a functional group more important for pollination than generally assumed: Wild bee pollination of crops has recently been estimated to resemble that of managed bees across the globe (Garibaldi et al. 2011; Kleijn et al. 2015). Pollination services in the United states alone are estimated worth more than 3 Billion USD on a total of 50 crops requiring insect pollination. But arthropods can also appear in agricultural landscapes as crop pests, however, they often also provide the suitable arthropod antagonists for pest control (Landis et al. 2005; Tschumi et al. 2015). Crop yield losses as a result of insect pests are estimated to probably exceed 10 % and are not decreasing worldwide despite growing insecticide use (Oerke 2006), while insect pest predators reduce losses through pests worth 4.49 billion USD in the United States alone (Losey and Vaughan 2006). Thus, wild bees and natural enemies of crop pests are an indispensable part of agricultural landscapes, which needs to be protected. The strong decline of insects reported over the last 25 years (Hallmann

et al. 2017) may lead to crop pollination and pest control being one of the most threatened ecosystem functions in agricultural landscapes (Tscharntke et al. 2005; Biesmeijer et al. 2006; Geiger et al. 2010; Meehan et al. 2011).

Bee decline is associated with loss and degradation of habitats providing nesting opportunities and in particular floral food resources (pollen and nectar) (Scheper et al. 2014; IPBES 2016; Kovács-Hostyánszki et al. 2017). Landscape-level spatio-temporal availability of floral resources is a main driver of bee communities (Baude et al. 2016; Woodard and Jha 2017). Wild bees comprise several groups with different functional importance to agriculture and different requirements to the landscape. Rare and endangered bees often have more specific habitat requirements due to specialized plant-pollinator interactions (Senapathi et al. 2015; Sutter et al. 2017). Important crop pollinators, on the other hand, are typically more generalized, and able to use resource pulses provided by SNH as well as crops (Williams et al. 2010; Scheper et al. 2014; Kleijn et al. 2015; Winfree et al. 2015). To synergistically promote different groups of bees, precise knowledge on floral resource needs, as well as on the distribution of floral resources in a given landscape is required (Kleijn et al. 2015; Senapathi et al. 2015). Synergistic landscape management may even comprise groups from different guilds; Previous findings indicate a common use of early woody floral resources not only in Bombus terrestris and Osmia bicornis but also in lacewings and ladybirds that was replaced by herbaceous resources later in the season (Bertrand et al. 2019).

Increased pest control is generally associated with large, diverse natural enemy populations (Letourneau et al. 2009; Veres et al. 2013) and complex landscapes (Andow 1991; Bianchi et al. 2006; Rusch et al. 2010). Thus many natural enemies of crop pests relate to their environment on a landscape-scale, similar to bees; Hoverflies, parasitoids and lacewings consume pollen and nectar during their adult live stages to enhance fecundity and longevity (reviewed in Wäckers and Van Rijn 2012). The nutritional supplementation by pollen can also enhance larval growth of ladybirds (Lundgren 2009) and serve as alternative resource during scarce prey supply (Triltsch 1997; Ricci et al. 2005). However, feeding morphology of most natural enemies is different from that of bees, as they lack long tongues. This is why the type of flowers required might be quite different (Wäckers and Van Rijn 2012). Furthermore, natural enemies require insect prey. Prey is scarce especially in early season, when ladybirds emerge from hibernation and is more likely found in the surroundings of cropping areas than the crops themselves. Food resources in SNH can be essential for population growth, and eventually the population size, that will match pests in crops later in the season (Symondson et al. 2002). However, not all types of SNH provide suitable alternative prey and floral resources and some habitats can even promote pests instead of natural enemies (Alejandro and Costamagna; Tscharntke et al. 2016; Turlure et al. 2019). It is therefore necessary to understand, what prey predators use from SNH. Identification of prey-use is still subject to important methodological challenges (Pompanon et al. 2012; Birkhofer et al. 2017; Alberdi et al. 2018). Prey availability in the landscape is more difficult to evaluate than floral resources, as prey is often mobile and less predictable than floral resources in its spatio-temporal distribution (Sequeira and Dixon 1997; Bahlai et al. 2010; Senior et al. 2020). This is why there are ongoing attempts to identify the actual prey consumed by predators through gut content analysis. Morphological identification of prey that has either been chewed or sucked is not straightforward. Therefore, molecular approaches have become

increasingly popular and high throughput sequencing in particular. The method is well adapted to feeding experiments in the laboratory (Chen et al. 2000; Gagnon et al. 2011). However, for field experiments, it imposes issues mainly related with (i) landscape-scale sampling and (ii) broad scale screening of the various potential prey species. Sampling methods that capture insects at the landscape-scale either compromise on sampling bias, trapping efficiency, DNA-recovery rate or risk of contamination (Hoogendoorn and Heimpel 2002; Stephens and Losey 2004; Greenstone et al. 2011; King et al. 2012). Broad scale screening for prey imposes further challenges related for example to the availability of reliable and comprehensive reference sequence databases that contain the prey species' sequence. For aphids, only around 10% of the global species are available yet (Lee et al. 2017).

Resources in semi-natural habitats

Enhancement of beneficial insects and their services through the conservation and restoration of semi-natural habitats (SNH) is often (Martin et al. 2019; Rusch et al. 2016; Sutter et al. 2018; Tscharntke et al. 2012), but not always sucessful (Karp et al. 2018; Tscharntke et al. 2016). To efficiently promote provision of pollinators and natural enemies of crop pests, we need to understand the versatile character of SNH and the interplay with its inhabitants (Holland et al. 2016; Rega et al. 2018; Bartual et al. 2019; Cole et al. 2020; Kheirodin 2020). SNH comprise many different habitats such as forests, hedgerows, meadows and fallows. The vegetation in these habitats differs, and as a consequence the primary resource composition, availability and phenology as well as structure, microclimate and species composition at higher tropic levels (Moonen et al. 2012; Martin et al. 2013). However, it is not only the type of SNH that characterises the resources provided by a landscape, it is also the management of SNH that plays a role. Management may comprise deliberate measures such as the provision of dead wood along forest edges creating nesting opportunities, or delayed mowing dates in grasslands leaving sufficient floral resources for pollinators (Ekroos et al. 2020). Thus, the type of SNH and its management offer different food resources and promote different plant and animal species which may be suitable for different conservation objectives such as rare species conservation, biodiversity conservation but also the promotion of different ecosystem services to crops (Senapathi et al. 2015; Sutter et al. 2017; Bertrand et al. 2019). Landscape management following the scope of ecological intensification but also conservation will ultimately imply provision of suitable habitats that are managed in a manner to offer the appropriate resources at the right time (Dennis et al. 2006; Bommarco et al. 2013). To do so in terms of food resources we require knowledge on the distribution of the food resources in different types of SNH and how they are influenced by different management measures, as well as how insects of interest relate to the resources in a landscape-context.

Resource maps

Functional resource maps inform on the spatial distribution of a set of resources, which are available to species in a given landscape. Resource maps have been suggested to predict target organisms for the scope of biodiversity conservation (e.g. Dennis et al. 2006; Moore et al. 2010; Turlure et al. 2019) and may as well help improving ecological intensification. The amount and type of floral resources provided by different habitat types influences natural enemy and wild pollinator abundance as well as

the quality and amount of ecosystem services they provide to crops (Wäckers and Van Rijn 2012; Tschumi et al. 2016; Carvell et al. 2017; Bartual et al. 2019). To date, we still lack floral resource maps that give spatio-temporal insights into floral resource availability across all major habitat types based on local patch quality (Cole et al. 2017). It is therefore unclear, how limiting floral resources are in comparison to other factors, what floral metric is the most suitable descriptor (diversity, floral abundance) and whether floral resource maps actually perform better at predicting functional groups compared to classical habitat maps. Findings may have implications on landscape, as well as habitat management and may give information on how we can improve the process of understanding relations between insects and the landscape.

Fig. 1. Insect trap setting for chapter 3 and the study by Bertrand et al. (2019).

Research objectives

In this thesis I investigate food resource and habitat requirements of different groups of pollinators and natural enemies of pest apids as well as spatio-temporal distribution of floral resources on a landscape-scale. The following questions were addressed:

1) What is the temporal distribution of flower abundance and diversity over all major habitat types of agricultural landscapes?
2) What is the importance of different habitat types for floral resource supply to different groups of wild bees at different times of the season?
3) Can floral resource maps improve the prediction of wild bees as well as natural enemies and pest control compared to classical habitat maps?
4) What are the food resource requirements of natural enemies in terms of flowers and aphid prey?
5) Is there a particular habitat type or floral resource that promotes natural enemies and pest control?

Chapter outline:

Chapter 1 addresses abundance and diversity in different groups of bees (social bees, solitary bees, rare bees and important crop pollinators) in response to landscape parameters. Classical habitat maps are compared with floral resource maps in their performance of predicting the diversity and abundance of bees. Landscape-scale floral resources as well as the importance of floral resources from different habitats and temporal subsets (early and late season) are assessed for different groups of bees.

Chapter 2 focuses on natural enemies and pest control and their relation to agricultural landscapes. Analogous to chapter 1, the performance of floral resource maps and classical maps is compared. Effects of specific habitat types and their floral resources on natural enemies and aphid control are assessed, as well as effects of natural enemy abundance on pest control.

Chapter 3 investigates ladybirds' predation on aphids. Information on aphid prey use is obtained from ladybird guts with high throughput sequencing. This chapter addresses methodological challenges related to high throughput sequencing of field samples and presents a new aphid primer as well as recommendations for sampling strategies.

Chapter 2

Spatio-temporal floral resource availability across different habitats drives wild bee communities in agricultural landscapes

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Abstract

1. Effective conservation of pollinators and pollination services in agroecosystems requires quantitative knowledge on the spatio-temporal contribution of major habitat types to landscape-scale floral resource availability and how this drives key groups of pollinators such as rare species or important crop pollinators.
2. We quantified spatio-temporal floral resource availability and wild bee communities across different habitat types in 20 agricultural landscapes in Switzerland.
3. Resource availability shifted from flower-rich woody vegetation early in the season to herbaceous vegetation such as grasslands and crops later in the season. This shift was associated with a ten-fold decline in overall flower availability. In contrast, the contribution of different habitat types to floral diversity was less variable, with high and continuous overall contributions of grasslands and highest average per-area contributions of forest edges in spring.
3. Total abundance and species richness of wild bees increased with landscape-level flower abundance, but not floral diversity. “Functional resource maps” considering floral resource provisioning by major habitat types early or late in the season predicted wild bee abundance (R^2=0.54) and species richness (R^2=0.61) better than traditional landscape descriptors such as proportion semi-natural habitat or the simple areal proportions of major habitat types.
4. All studied groups of wild bees (social bees, solitary bees, rare bees and important crop pollinators) were positively related to floral resource abundance or diversity contributed by forest edges, and floral resource diversity of grasslands. Social and rare bees also increased with the floral abundance of crops.
5. Synthesis and applications. Our study highlights the advantages of temporally resolved functional resource maps over classical habitat maps to predict wild bee communities in agricultural landscapes. Moreover, it reveals a pronounced temporal shift in the importance of woody towards herbaceous vegetation during the season and thus the importance of taking a landscape-scale perspective on pollinator conservation to ensure continuous floral resource availability in agricultural landscapes. Our findings highlight potential synergies in pollinator conservation measures for bee diversity, rare bees and important crop pollinators by promoting flower-rich forest edges and grasslands in European agricultural landscapes.

Introduction

Bees play a vital role as pollinators in agroecosystems and other terrestrial ecosystems (IPBES 2016). Globally, nearly 90% of wild flowering plant species depend, at least partly, on the transfer of pollen by animals for pollination (Ollerton et al. 2011). Moreover, approximately 75% of the worldwide most important crops and 85% of European crops benefit from insect pollination to some extent (Klein et al. 2007). The volume of production of pollinator-dependent crops has increased four-fold over the last five decades with an estimated economic value of at least €150 billion per year in 2005 (Aizen et al. 2009; Gallai et al. 2009). Wild bees are not only essential for the pollination of most wild plant species, but also give crucial contribution to crop pollination (Garibaldi et al., 2013; IPBES 2016). Although highly variable across regions and crops, crop pollination by wild bees has recently been estimated to be overall roughly equal to that of managed bees, such the Western honeybee (Kleijn et al. 2015). Beyond their functional importance for pollination, wild bees are of high intrinsic value as they contribute to agroecosystem's biodiversity, which is vulnerable to multiple anthropogenic stressors. Therefore, they have a high conservation priority (Potts et al. 2016). In fact, several studies have reported strong declines of wild bee populations, as well as bee diversity in several regions of Europe and North America during the last decades (Biesmeijer et al. 2006; Carvalheiro et al. 2013).

Loss and degradation of habitats providing nesting and in particular suitable floral food resources (pollen and nectar) is considered to be among the major drivers of bee decline (Scheper et al. 2014; IPBES 2016; Kovács-Hostyánszki et al. 2017). Among the most important divers of bee communities is landscape-level spatio-temporal floral resource availability (Baude et al., 2016; Woodard & Jha, 2017). Hence, a prerequisite for successful bee conservation in agroecosystems is (i) quantitative knowledge on floral resources provided by different habitat types in agricultural landscapes and their temporal dynamics during the season, and (ii) and improved understanding about the importance of different descriptors of floral resource availability (e.g., floral abundance and diversity) in their contribution to sustain wild bee pollinators (Dicks et al. 2010).

Management goals for entire agricultural landscapes to maximize biodiversity conservation and the potential for ecosystem service delivery is a central aim of agroecology and conservation (Tscharntke et al. 2012; Marini et al. 2019). Likely not at least due to the logistical challenges and efforts required to collect data on floral resource availability across major habitat types, previous studies have either mainly focused on floral resource use by bees in local habitat elements (Cole et al. 2017) or effects of landscape-level floral resources availability on a single bee species (Williams et al. 2012). However, different pollinator groups such as solitary bees or social bees (i.e., sociality), or different target groups, for example rare bees of high conservation concern and important crop pollinating wild bees, may vary considerably in their spatio-temporal requirements of floral resources offered by different habitat types; this may require tailored management strategies of the habitat types primarily contributing to their resource requirements (Senapathi et al. 2015; Sutter et al. 2017). In contrast, to promote the usually generalist and common crop pollinating bees, mass-flowering crops and other habitat types offering particularly high abundances of floral resources during peak activity might be most effective (Westphal et al. 2003; Bertrand et al. 2019). Alternatively, different groups of bee pollinators may overlap to a large extent in their reliance on floral resources provided by certain habitat types. An improved understanding of such potential trade-offs or synergies is essential to

enhance the effectiveness of targeted measures, and can inform management strategies for win-win situations: both for the conservation of rare pollinator species, and to promote important crop pollinators and pollination services (Ekroos et al. 2014; Kleijn et al. 2015; Senapathi et al. 2015). Such knowledge is highly relevant to guide future agricultural policy to better support wild pollinators in agroecosystems (Cole et al. 2020). Improving our understanding of the relationships between floral resource composition of landscapes across habitat types should also help to refine models to predict impacts of habitat change on pollinator communities (Lonsdorf et al. 2009). However, landscape-scale floral resource mapping is a highly time and resource-intensive task. An important question is therefore whether more easily obtained classical habitat maps may be sufficient to predict certain groups of bees, and to what extent more refined "functional habitat maps" can improve predictions (Vanreusel et al. 2007; Lausch et al. 2015).

In this study, we quantified landscape-scale spatio-temporal floral resource availability and bee communities across 20 agricultural landscapes in Switzerland. In particular, we addressed the following research questions:

(1) What is the contribution of different habitat types to floral resources for bees in agro-ecosystems during different times of the year? (2) Are abundance and species richness of bees driven by landscape-scale abundance and diversity of floral resources? (3) What is the relative importance of floral resources provided by different semi-natural habitat types and crops for solitary bees, social bees, rare bees and important crop-pollinating bees? Does relative importance shift during the season? (4) Do floral resource maps better predict richness and abundance of bees than classical habitat maps, and are predictions improved by accounting for seasonal availability of resources?

Materials and methods

Study design and estimation of flower abundance and diversity

A total of 20 landscape sectors of 500 m radius each (hereafter 'landscapes') were randomly selected in the north-eastern Swiss Plateau (overview in Supplementary methods; Fig. S1). Landscapes were characterized by mixed farming with dominant arable crops intermixed with grasslands, horticulture (intensive fruit production), and semi-natural habitats. Habitats were classified into four types; crops (including arable areas and horticulture), grasslands (managed intensively as well as extensively), semi-open habitats (i.e., hedgerows, and extensively managed traditional orchards and single trees) as well as forest edges. The latter three were classified as semi-natural habitat. To assess flower availability in the 20 landscapes, habitat types were classified into detailed categories according to their floral composition (Fig. 1). In each landscape, habitat types were manually mapped based on aerial photographs, which were ground-truthed in the field and subsequently digitalized with a geographical information system (ArcGIS version 10.6, ESRIs).

Flower abundance and flower species diversity (Simpson index; Simpson, 1949) of entomophilous flowering plants (excluding wind-pollinated plants; according to the BiolFlor plant trait database; Klotz et al, 2002) was estimated for each habitat type as following: Flower abundance in habitat types ($F_{habitat}$) was the sum of the seasonal flower abundance provided by each flowering plant species occurring in this habitat ($F_{species}$). $F_{species}$ was a product of the species-specific flower density

(i.e., the number of flowers per m^3, e.g., in tree crowns; $D_{species}$), flower size (volume; $S_{species}$), the potentially flower bearing volume occupied by the species ($V_{species}$) and the flowering duration ($T_{species}$)

$$F_{species} = D_{species} \times S_{species} \times V_{species} \times T_{species}$$

$$F_{habitat} = \sum_{i=species} F_{species}$$

The resulting habitat maps are a product of detailed field surveys that allow insights on floral resources provided by each species for a specific landscape, habitat type and date during the three months of bee sampling. In the supplementary methods a detailed description of the floral mapping method and estimation of flower abundance and diversity are provided, S1 Data (electronic supplementary) gives detailed information on habitat categorisation and landscape parameters.

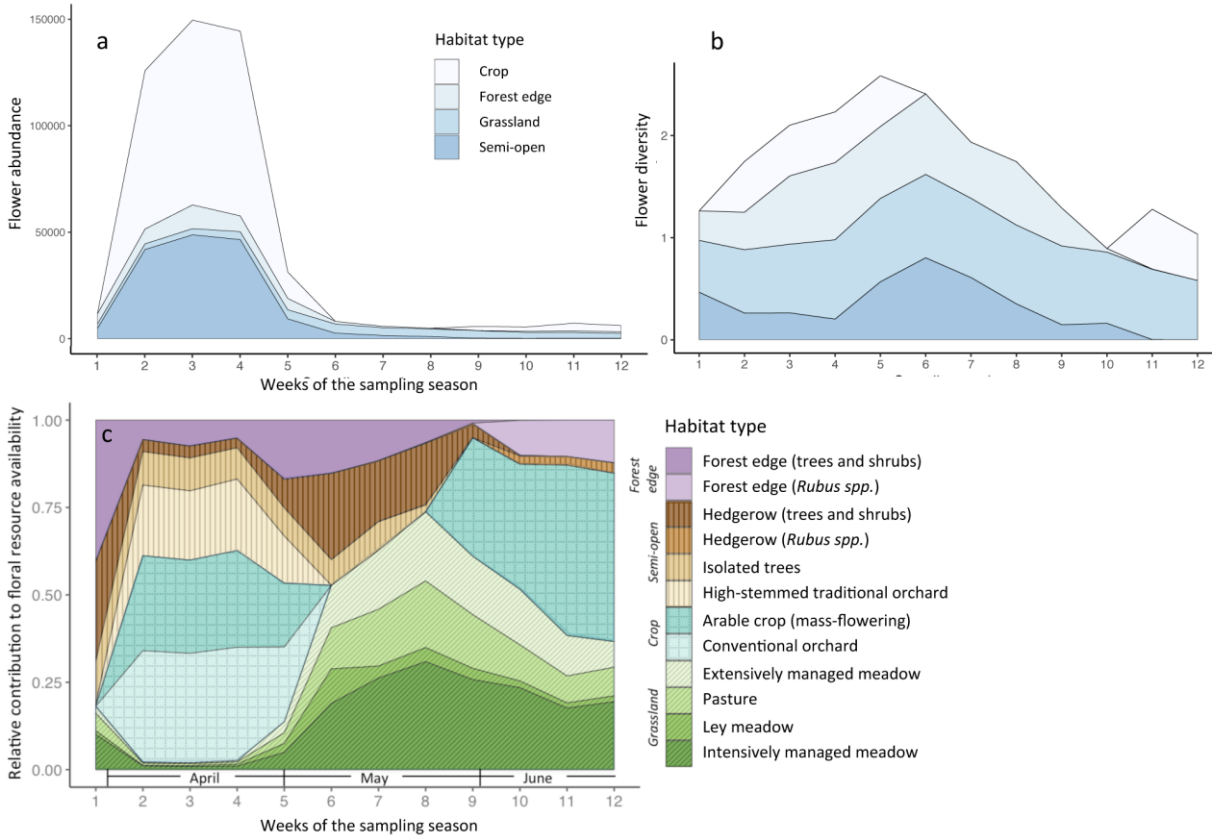

Fig. 1. Temporal shifts in the contribution of major habitat types (crops, grassland, forest edges and semi-open habitat (i.e., hedgerows and single trees) over the season (12 sampling weeks from April to end of June) in terms of a) floral resource abundance b) flower diversity (Simpson diversity) and c) relative contribution to floral resource availability over time (see S1 Data for background data).

Sampling of bees

In each landscape, bees were sampled with four traps constructed as combination of a passive window intersection trap together with a pan trap ("combi traps"; Duelli et al. 1999). Combi traps have been demonstrated to be highly effective for quantifying bees and other flying insects in agricultural landscapes (Duelli et al. 1999). These combi traps consisted of two plexi-glass windows (50cm x 42cm) arranged cross-wise over a large yellow funnel-shaped pan trap (42.5 cm upper diameter). The

pan trap (12 litre volume) was filled with water and a drop of soap to reduce surface tension and enhance trapping success. Traps were set up along grassy field margin and grassland edges at least 150 m apart but as close to the centre as possible. Bees were sampled from early April until late June 2018 and traps emptied weekly. Samples of the four traps were pooled per landscape for analysis. Bees were stored in 70 % ethanol until pinning and identification by experts. We excluded managed *Apis mellifera* L., the Western honeybee and the two solitary bee species *Osmia cornuta* and *O. bicornis* from further analyses because they are often managed in high numbers for the pollination of fruit orchards in the study region. Conservation status of species was taken from Amiet (1994), important crop pollinators and social bees and solitary bees were categorised according to Kleijn et al. (2015) and Scheuchl & Willner (2016).

Statistical analysis

Linear model analyses were used to test the effects of landscape explanatory variables (i.e., floral resource and habitat descriptors) on bee response variables (pooled bee samples of the four traps per landscape). Explanatory variables were centred and scaled prior to analysis to be able to directly compare parameter estimates of different models. In all models, bee abundance was log-transformed to meet linear model assumptions. Results of these analyses were qualitatively identical to those of generalised linear models using quasi-Poisson error distribution. To be able to use adjusted-R^2 values for direct comparison of the goodnees-of-fit of linear (but not generalized linear) models differing in the number of explanatory variables, we present the results of the linear model analyses.

To test the effects of landscape-scale flower abundance and diversity on the abundance and species richness of bees (research question 2) separate linear models for each bee descriptor (abundance, species richness) and group of bees (rare bees, solitary bees, social bees and important crop pollinators) were run. To explore variation explained by floral abundance and diversity of the four major habitat types (forest edge, semi-open habitat, grassland and crops) for different groups of bees (research question 3), separate linear models for each descriptor (bee abundance, species richness) and group of bees (rare bees, solitary bees, social bees and important crop pollinators) were run. To avoid potential co-linearity issues due to high correlations of floral abundance and diversity of some habitat types VIFvalues ≥ 3; Zuur, Ieno, & Smith, 2007) separate models with flower abundance of the four habitat types or their floral diversity were run.

To explore variation explained by early and late floral abundance and diversity of the four major habitat types for bees appearing early or late in the season, models with early or late flower abundance or diversity in each of these habitat as explanatory variables and early or late species richness or abundance of bees were run. Effects of early floral resource contributions of the four major habitat types were tested for early as well as for late bee abundance and species richness. Effects of late floral resources were only tested on late bees.

To test whether floral resource maps accounting for seasonal availability of floral resources better predict richness and abundance of bees than classical habitat maps (question 4) the amount of explained variation (R^2) and goodness-fit (adjusted R^2) the models described above with early or late floral resource contributions (floral abundance or diversity) of the four major habitat types were compared to the models with simple landscape proportions covered by the four habitat types (without

accounting for their seasonal floral resource contributions early or late in the season) for the response variables bee species richness and bee abundance (log-transformed).

All statistical analyses were performed using the software R (version 3.4.1); R Core Team, 2017).

Results

Contribution of different habitat types to floral resources from April to June

The relative importance of different habitat types in terms of floral abundance and diversity varied strongly over the season (Fig. 1). Flower abundance was highest early in the season (April), with highest relative contributions of semi-open habitat and crops. Flowering trees and shrubs belonging to the genera *Prunus, Pyrus* and *Malus* (Rosaceae) in forest edges and hedgerows, in traditional orchards, as single trees and in intensive orchards made large contributions to flower abundance (Fig 1). The relative contribution of grasslands to flower abundance increased towards mid-season, reaching almost 75% by the end of May. Flower diversity peaked around mid-season, mainly due to semi-open habitat (Fig. 1). Floral diversity of forest edges and semi-open habitat declined strongly towards the end of the sampling season while grasslands provided high floral diversity throughout the season. Crops exhibited generally low floral diversity (Fig. 1).

Floral resources driving abundance and species richness of bees

Over the entire sampling period 4742 wild bees have been sampled, comprising 108 species. The genera most commonly collected were *Andrena* (47.4%), *Lasioglossum* (29.3%), *Bombus* (12.4%), *Colletes* (5.8%) and *Halictus* (3.0%). Solitary bees made up 58.1% of the bees sampled, followed by social bees (36.0%) and 5.8% parasitic bees (S2 Data). A total of 45.5 % were classified as important crop pollinating wild bees, and 6.3% as rare bees.

Total bee abundance and species richness increased with landscape-level flower abundance, but not diversity (Table 1; Fig. 2). Total bee abundance and species richness increased significantly with flower abundance contributed by forest edge (Table 2). Furthermore, bee abundance increased with crop flower abundance. Bee abundance and species richness also increased with flower diversity of forest edges and grasslands (Table1).

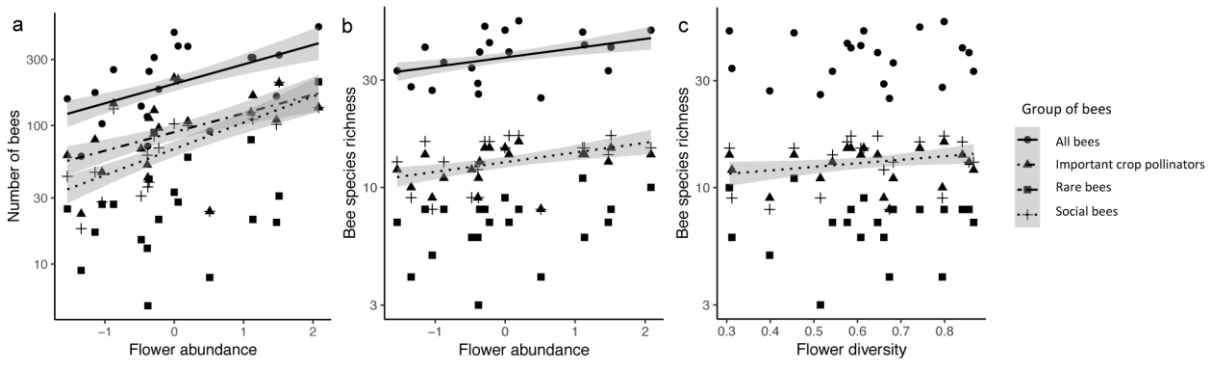

Fig. 2. Relationships between a) landscape-scale flower abundance and bee abundance, b) landscape-scale flower abundance and bee species richness and c) landscape-scale floral diversity (Simpson index) on bee species richness of different groups of bees. Grey areas indicate 95 % confidence intervals along lines from linear regression models.

Table 1. Summary of linear model analysis testing the effects of floral resources (floral abundance or diversity) of major habitat types on abundance (log-transformed) and species richness of different groups of bees (social bees, solitary bees, rare bees and important crop pollinating bees). Parameter estimates (slope) were retained from linear regression models with scaled data. Significant effects ($P \leq 0.05$) are indicated in bold. d.f. = degree of freedom; SE = standard error. (See also Fig. 2)

Response variable	df	R2	Adjusted R2	AIC	Flower predictor	Parameter estimate	se	p-value
Total bee species richness	17	0.224	0.132	58.67	**Total flower abundance**	**0.54**	**0.24**	**0.042**
					Total flower diversity	0.31	0.24	0.229
Total bee abundance	17	0.324	0.244	55.90	**Total flower abundance**	**0.65**	**0.23**	**0.011**
					Total flower diversity	0.25	0.23	0.291
Rare bee species richness	17	0.162	0.064	60.19	*Total flower abundance*	*0.46*	*0.25*	*0.087*
					Total flower diversity	0.22	0.25	0.393
Rare bee abundance	17	0.193	0.098	59.45	*Total flower abundance*	*0.47*	*0.25*	*0.078*
					Total flower diversity	0.07	0.25	0.792
Crop pollinator species richness	17	0.205	0.112	59.14	*Total flower abundance*	*0.51*	*0.25*	*0.053*
					Total flower diversity	0.30	0.25	0.237
Crop pollinator abundance	17	0.247	0.159	58.06	**Total flower abundance**	**0.56**	**0.24**	**0.033**
					Total flower diversity	0.18	0.24	0.470
Social bee species richness	17	0.377	0.304	54.26	**Total flower abundance**	**0.65**	**0.22**	**0.009**
					Total flower diversity	**0.55**	**0.22**	**0.023**
Social bee abundance	17	0.407	0.337	53.29	**Total flower abundance**	**0.71**	**0.21**	**0.004**
					Total flower diversity	0.20	0.21	0.375
Solitary bee species richness	17	0.092	-0.014	61.80	Total flower abundance	0.34	0.26	0.220
					Total flower diversity	0.09	0.26	0.750
Solitary bee abundance	17	0.160	0.062	60.24	*Total flower abundance*	*0.46*	*0.25*	*0.089*
					Total flower diversity	0.21	0.25	0.412

Temporal shifts in early and late season floral resources driving bee abundance and richness

More bee individuals and species were sampled in the first half of the sampling period (beginning of April to mid-May; 72.8%) than in the second half (mid-May to end of June; 27.2%). Forest edge flower abundance and diversity was positively related with the abundance and species richness of bees active early but also later in the season (Fig. 3), a pattern driven by social bees with activity periods covering early and late periods (see also supplementary results Tables S3 and S4). Early grassland diversity was positively related with early and late bee abundance and species richness and late grassland diversity was positively related with late bee abundance and species richness. Early crop flower abundance but not early semi-open habitat had a positive effect on early bee abundance as well as late bee abundance and species richness (Fig. 3).

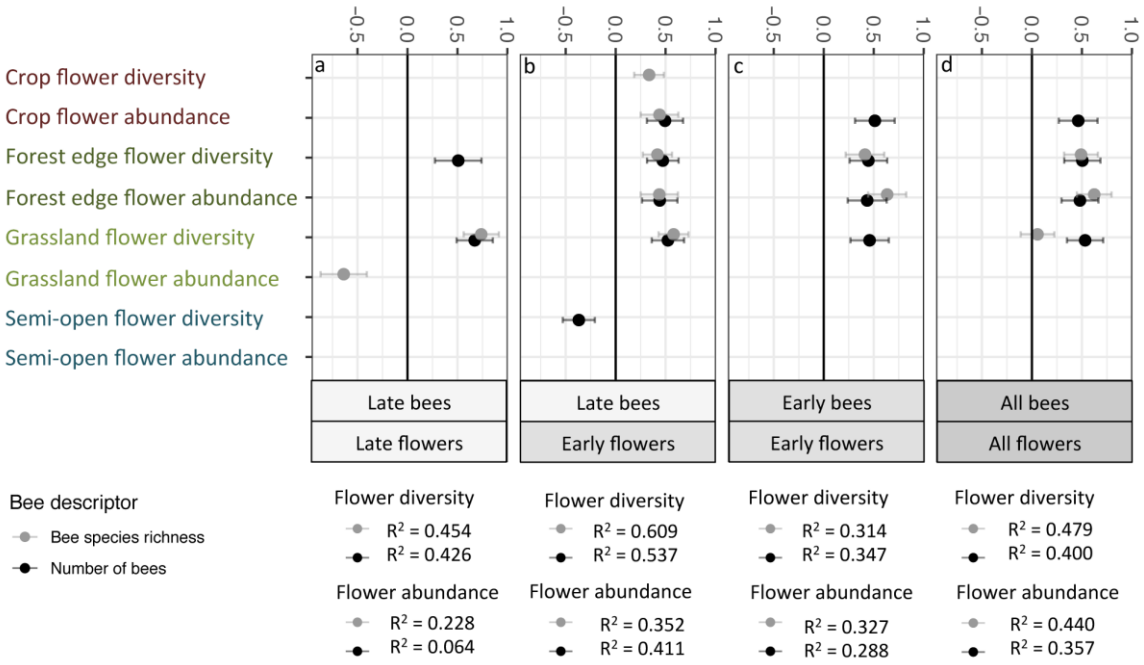

Fig. 3. Parameter estimates (slope) of significant relationships of habitat types for temporal subsets of bees (abundance (log-transformed) and species richness) and flowers (abundance and diversity) from linear regression models with scaled variables. Early season covers the first six weeks of the sampling season beginning in April and late season covers the last six sampling weeks, ending mid-June. R^2 describes adjusted-R^2 values indicating the goodnees-of-fit for linear models. See Table S2 in the Supplementary material for a summary of the results.

Relative importance of floral resources contributed by different habitat types for different groups of bees

Social bees, rare bees and important crop pollinators, but not solitary bees increased with landscape-scale flower abundance (Fig. 2, Table 1). Only social bees increased with landscape-scale flower diversity. The relative importance of floral abundance and diversity of specific habitat types differed for different groups of bees: forest edge flower abundance and diversity had a positive effect on species richness of all bees, except forest edge flower diversity in rare bees (Fig. 4). Solitary and rare bees abundance and species richness related positively to forest edge flower abundance and abundance and species richness of solitary bee and crop pollinators correlated positively with forest edge flower diversity (Fig. 4). Floral diversity, but not abundance in grasslands was positively related to the abundance and species richness of social bees, solitary bees and important crop pollinators, and species richness of rare bees (Fig. 4). Flower abundance of crops was positively related to abundance of social and rare bees (Fig. 4). No significant relationship between floral abundance or diversity of semi-open habitat was found for any of the studied bee groups, except a negative relationship between flower diversity of semi-open habitat and social bee abundance (Fig. 4). In social bees and important crop pollinating bees flower diversity explained 17.7% to 39.4% more variation than flower abundance (Fig. 4; see also Supplementary S1; Table 3a,b), while in solitary bees and rare bees explained variation between flower abundance and diversity was comparable.

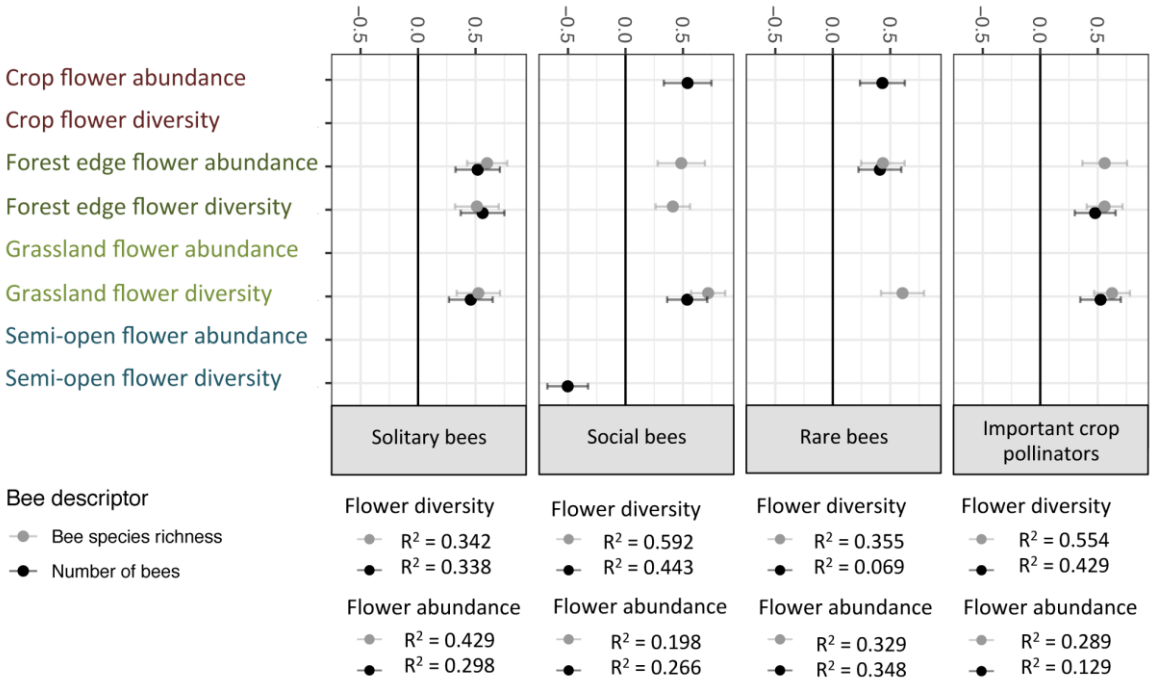

Fig. 4. Parameter estimates (slope) of significant relationships of linear model analysis testing the effects of floral resources (floral abundance or diversity) of major habitat types on abundance (log-transformed) and species richness of different groups of bees (social bees, solitary bees, rare bees and important crop pollinating bees). Estimates were retained from linear regression models with scaled data. R^2 describes adjusted-R^2 values indicating the goodnees-of-fit for linear models. See also Supplementary material Table S4.

Do floral resource maps predict bees better than classical habitat maps?

Floral resource maps performed equally well or better than classical habitat maps, with varying importance of different habitat types and temporal subsets (Table 2). A clear improvement over classical habitat maps was achieved when investigating temporal subsets in floral resource maps (54% and 61% of variation explained for wild bee abundance and richness, respectively; Fig. 3).

Discussion

Spatio-temporal distribution of floral resources in agricultural landscapes

The present study is among the first providing a landscape-scale assessment of the spatio-temporal availability of floral resources across major habitat types in agricultural landscapes. Our findings reveal a strong decline in overall landscape-level floral resource abundance during the main activity period of most bee species from early April to late June in the agricultural study region. Especially mass-flowering high-stem fruit trees of traditional orchards and arable crops (74% of early flower abundance), and to a lesser extent forest edges and hedgerows (12%) contributed to a more than ten-fold higher overall floral abundance early in in the season (April) compared to later time periods. Thus, habitat types supporting flowering trees and shrubs, such as hedgerows and single trees, including high-stem fruit trees of traditional orchard meadows, forest edges and intensive orchards, contributed substantially to the high floral resource availability in early spring (more than 70%), but also mass-flowering arable crops such as oilseed rape. However, there was a strong decline in the contribution of woody plants to floral resource availability later in the season and a striking shift towards herbaceous plants contributing to landscape-level floral resource availability in summer (52% mainly provided by

flowering plants of grasslands). In fact, floral resource abundance per area (i.e., floral density; Fig. S5) of herbaceous semi-natural habitat types, mainly grasslands, did not strongly increase during the season, but rather their relative contribution to landscape-level flower abundance increased as a result of the pronounced decline in floral resources from woody plants. These findings indicate, that similar observed shifts from woody to herbaceous pollen use by highly generalist bee pollinators, such as *Bombus terrestris* and *Osmia biocornis* (Bertrand et al. 2019) reflect an opportunistic tracking of the most abundantly available floral resources from different vegetation and habitat types across the agricultural landscape and season. Thus, even for such highly polylectic bee pollinators, multiple habitat types of both woody and herbaceous vegetation are required to ensure floral resource availability throughout the season (Cole et al. 2017; Bartual et al. 2019). Interestingly, landscape-level flower diversity showed a much less pronounced temporal dynamic, although it was clearly higher in spring (April/May) than in summer. Semi-natural habitats, and in particular grasslands and forest edges, contributed most to landscape-level floral diversity (Baude et al., 2016; Dicks et al., 2015; Lonsdorf et al., 2009). The high flower diversity of grasslands was mainly driven by meadows managed extensively according to the prescriptions of the Swiss agri-environment scheme (e.g., no fertilizer input; postponed first cut in mid-June). They had a 31% higher floral diversity compared to intensively managed grasslands ($t = 3.02$, $df = 37.4$, $P = 0.005$). Thus, appropriate management of grasslands, but also of woody semi-natural habitats (Staley et al. 2012), is key to achieve high ecological quality in terms of floral diversity (Albrecht et al. 2007b; Kennedy et al. 2013; Cole et al. 2020).

Floral resources driving wild bees in agricultural landscapes

Despite the very high amounts of floral resources (but relatively low diversity) provided by semi-open habitat during a relatively short time early in the season, mainly through massive floral resources contributed by mass-flowering trees in traditional orchards or as single trees, they failed to show positive relationships with any of the studied group of bees. In contrast, floral resources, especially floral diversity, provided by forest edges and grasslands had the most consistent positive effects on all four studied groups of bees, while floral resources provided by crops were positively associated only with particular groups, mainly social bees and interestingly, rare bees. Targeting management to conserve and restore flower-rich forest edges and grasslands should therefore offer great potential to simultaneously promote rare bee species of high conservation concern as well as wild bees important for crop pollination and thus create win-win situations for biodiversity conservation and ecological intensification (Albrecht et al. 2007a; Ekroos et al. 2014, 2020; Senapathi et al. 2015; Sutter et al. 2017). High floral diversity ensures a high level of spatio-temporal heterogeneity and complementarity of available resources for a range of bee taxa and may be associated with disproportionally high availability of key plant species offering floral resources of particular importance for different target groups of pollinators (e.g., Sutter et al., 2017). Also temporal complementarity through the combined contribution of forest edges early in the season and grasslands later in the season may have contributed to the observed positive effects on bees, in particular bees with long activity periods (Schellhorn et al. 2015). Moreover, our findings highlight the importance of floral resources early in the

Table 2. Summary of linear model analysis of the effect of habitat proportion and floral resources (floral abundance or diversity) of the four major habitats on wild bee abundance (log-transformed) and richness. Parameter estimates (slope) were retained from linear regression models with scaled data. Significant effects ($P \leq 0.05$) are indicated in bold (d.f. = degree of freedom; SE = standard error). SNH: Semi-natural habitat

Predictor variable	Response variable	d.f.	R^2	Adjusted R^2	AIC	Habitat type	Estimate	SE	*P*-value
Coarse habitat proportion	Total bee species richness	17	0.087	0.037	59.90	SNH (semi-open, forest edge, grassland)	-0.29	0.22	0.206
	Total bee abundance	17	0.114	0.066	59.29	SNH (semi-open, forest edge, grassland)	-0.34	0.22	0.144
Specific habitat proportion	Total bee species richness	15	0.541	0.419	52.16	Semi-open	0.12	0.21	0.571
						Forest edge	**0.73**	**0.21**	**0.003**
						Crop	0.31	0.28	0.293
						Grassland	-0.04	0.27	0.890
	Total bee abundance	15	0.457	0.313	55.51	Semi-open	0.21	0.23	0.379
						Forest edge	**0.60**	**0.22**	**0.018**
						Crop	0.35	0.31	0.270
						Grassland	-0.13	0.30	0.679
Total flower abundance	Total bee species richness	15	0.558	0.440	51.41	Semi-open	0.16	0.18	0.394
						Forest edge	**0.62**	**0.17**	**0.003**
						Crop	0.31	0.18	0.108
						Grassland	-0.30	0.20	0.133
	Total bee abundance	15	0.492	0.357	54.17	Semi-open	0.23	0.20	0.255
						Forest edge	**0.48**	**0.19**	**0.020**
						Crop	**0.47**	**0.19**	**0.030**
						Grassland	-0.21	0.20	0.321
Total flower diversity	Total bee species richness	15	0.590	0.479	49.95	Semi-open	-0.06	0.17	0.716
						Forest edge	**0.49**	**0.17**	**0.011**
						Crop	0.12	0.17	0.512
						Grassland	**0.06**	**0.17**	**0.001**
	Total bee abundance	15	0.527	0.400	52.77	Semi-open	-0.22	0.18	0.243
						Forest edge	**0.50**	**0.18**	**0.014**
						Crop	-0.07	0.19	0.705
						Grassland	**0.53**	**0.18**	**0.010**

season, not only for early active bees, but also for bees still active later in the season, such as bumblebees (e.g. Westphal et al. 2003; but see Rundlöf et al. 2014). Availability of early floral resources can be key for colony growth in the critical early phase of colony development (Westphal et al. 2003, 2009; Williams et al. 2012), and potentially reproductive success and population growth (Westphal et al. 2009; Williams et al. 2012)). Our findings suggest that such positive effects of early floral resources are not restricted to bumblebees, but also affect a large proportion of other social wild bees and solitary bees with long activity periods (Supplementary material; Table S2; S2 Data). Yet, our results also highlight the importance of continuous floral resource availability and diversity at the landscape scale throughout the season for these bees, such as social bees, which were among the studied groups of bees benefitting most from high landscape scale floral diversity and abundance throughout the season (Table 2; Steffan-Dewenter et al. 2002; Williams et al. 2012; Rundlöf et al. 2014), with the generalists among them, such as many important crop pollinators, being able to also use resource pulses provided by mass flowering crops (Westphal et al. 2003; Rundlöf et al. 2014; Spiesman et al. 2017).

Landscape-scale assessments on the role of spatio-temporal floral resources driving bee communities across a high number of landscapes, almost inevitably comes with some limitations. For example, we

are aware that the potential foraging range of the most mobile bees included in this study, such as bumblebees, is considered larger than the studied 500 m radius landscapes (Goulson et al. 2002). However, the actual foraging range of most bees studied here is considered much smaller (Greenleaf et al. 2007; Zurbuchen et al. 2010), and even for bumblebees the average realized foraging ranges is generally only few hundred meters (Osborne et al., 2008; Walther-Hellwig & Frankl, 2000). Although we acknowledge that further assessments on even larger scales could have provided additional insights, the studied scale is appropriate for our assessments, especially when considering the small-scaled mosaic type mixed farming system typical for Swiss and many other Central European agricultural landscapes. Furthermore, it was not possible to adequately quantify floral resource availability also in the more interior parts of forest lots, and therefore their role for floral resource availability for bees could not be assessed there.

Can functional floral resource maps predict bees better than classical habitat maps?

Our results highlight not only pronounced spatial heterogeneity of floral resource availability across major habitat and vegetation types in agricultural landscapes, but further indicate strong variation of floral resource abundance and diversity within these habitat types, as illustrated by the significant variation in floral diversity of grasslands influenced by their management, as well as strong temporal variation within and across habitat types. Consequently, considering the positive relationships of floral resources and bees, functional floral resource maps accounting for such marked spatio-temporal variation of resources across habitats predicted bees generally much better than classical habitat maps. In fact, simple categorisation of the landscape by the amount of semi-natural habitat entirely failed to predict wild bee pollinators. This strongly supports propositions to utilize functional resource maps as a tool to refine predictions of biodiversity and associated ecosystem services at the landscape scale (Lonsdorf et al. 2009), as well as to improve the effectiveness their management, e.g. by identifying management priorities to achieve improved spatio-temporal availability of the basic resource needs of the target organisms in agricultural landscapes (e.g. Dennis et al. 2006; Moore et al. 2010; Schellhorn et al. 2015).

Conclusions and implications for management and policy

The findings of our study imply the need of a landscape perspective for the conservation and restoration of bee pollinators and their pollination services through enhancements of floral resource availability in agroecosystems. The pronounced seasonal shift of floral resource contribution from different woody vegetation including single trees, forest edges or hedgerows, as well as arable crops towards grasslands and other herbaceous vegetation later in the season highlight the crucial role of habitat and habitat diversity at the landscape scale. These results also reveal the particularly high potential of flowering trees and mass-flowering crops to transiently boost floral resource quantities, while flowering species rich forest edges and grasslands play a key role for ensuring a high and continuous floral diversity in agricultural landscapes. Our results show that management extensification in grasslands can strongly enhance the provisioning of floral resource diversity and thus the potential of grasslands to sustain bee pollinators. Indeed, floral resource diversity offered by forest edges and grasslands could be identified as key drivers for different conservation target groups

of bee pollinators, including rare bee species of particular conservation concern, as well as important crop pollinators. Hence, targeting management on these habitats has a high potential for win-win situations and synergies between landscape management for rare bee species conservation and for crop pollinators and their pollination services. Finally, we conclude that functional floral resource maps at the landscape scale, especially when temporally and spatially sufficiently resolved, can more adequately predict bee pollinator abundance and species richness in agricultural landscapes compared to classical habitat maps. They can represent a valuable tool contributing to more targeted and effective pollinator conservation and restoration in agricultural landscapes.

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Supplementary material

Electronic supplementary

S1 Data. Landscape classification and composition

S2 Data. Bee functional groups

Supplementary methods

General approach

Bee abundance and species richness was assessed on 20 landscape sectors of 500m radius (Fig. S1). Landscapes were characterised by classical habitat maps including four habitat types grasslands (intensively and extensively managed), crops (including arable land and intensive orchards), semi-open habitat (with hedgerows, single trees and traditional orchards) and forest edge. Predictive performance of classical habitat maps was compared to functional habitat maps, that held information on either flower abundance or flower diversity within each of the four habitat types. Landscapes were selected to form a gradient in habitat proportion of the four habitat types that did not exceed variation inflation factors (VIF; Fox (2018) of more than 3 (Zuur et al. 2007)). For each vegetation type, we developed a specific protocol to quantify floral resources in the field. Specific protocols were necessary to account for the differences in the floristic composition, phenology and three-dimensional structure of the various vegetation types. For instance, along forest edges and hedgerows (but not within forests), we comprehensively mapped crown volumes of all shrub and tree species present and assessed floral resources through species specific floral traits. In grasslands, on the other hand, floral resources were quantified on 10 m^2 sampling plots specific for meadow types and landscapes. Sampling of meadows was repeated to account for the marked phenological differences in flowering time among grassland plants. This appendix describes how flower abundance and diversity was assessed in the four habitat types in each landscape. Key to all calculations is the formula described in the following section.

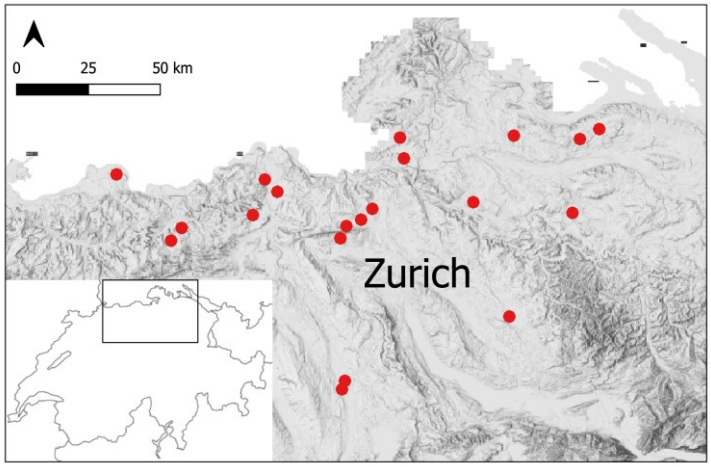

Fig. S1. Geographical distribution of landscape sectors in the north-eastern Swiss Plateau (Map type: swissALTI3D relief shading, source: Federal office of topography, swisstopo)

Calculation of flower abundance

To estimate flower abundance as adequate as possible, the four habitat types described above were split into further sub-units based on vegetation composition or mappable approach. We call these vegetation types and refer to S1 Data for a detailed list (e.g. extensive grasslands, hedgerows) and their assignment to conventional habitat types. Fine-grained information about the flower abundance in each vegetation type allowed us to compute flower abundance in the four habitat types: sum up the flower abundances of vegetation types that make up a habitat type, see equation for $F_{habitat}$ below. Flower abundance in a vegetation type ($F_{veg.type}$) was the sum of the flower abundance of all insect pollinated flowering species present in a vegetation type and landscape. The flower abundance of a species ($F_{species}$) was estimated from the product of the flower size (flower volume) of a single flower of that species (S_{flower}), the average number of flowers of the species per m^3-volume in the flowering part of the plant, e.g. the tree crown (flower density $D_{species}$), and the volume (in m^3) occupied by the flowering parts of the plant species in a habitat and landscape (e.g. flowering crown volume of a shrub or tree; $V_{species}$). To account for variation in the duration of flowering periods of different species affecting their contribution to $F_{species}$ this product was multiplied by its flowering period (e.g., the estimated average number of days the species was flowering during this time period; $T_{species}$).

$$F_{species} = S_{species} \times D_{species} \times V_{species} \times T_{species}$$

$$F_{veg.type} = \sum_{species} F_{species}$$

$$F_{habitat} = \sum_{veg.type} F_{veg.type}$$

Flower mapping of crops, grasslands, semi-open and forest edges need different mapping approaches to retain comparable precision, due to different vegetation characteristics. Therefore, D, S, V and T were assessed differently depending on species and habitat types. The following sections describe in detail how this was done.

Species' flower volume ($S_{species}$)

Inflorescences and single flowers greatly vary in size and volume, which is likely to be related to the amount of offered floral resources. We therefore explored the relationships of flower area (projection area from the top) and flower volume (approximated as cylinders using inflorescence diameter as cylinder width and corolla depth as cylinder length; Fig. S2) with floral nectar availability of 72 plant species frequently flowering in the study region for which provided nectar amounts are reported (using the extensive database provided by Baude et al. (2016). We also explored the relationship of these flower traits with pollen volumes provided by flowers of 27 flowering plant species (Hicks et al. 2016). Flower diameter and corolla depth were obtained from a floral trait database compiled for most flowering plant species of the study region (Frey et al., *in prep.*). For most floral types flower volumes were taken from individual flowers, except for Asteraceae (Fig. S2b) and male catkin flowers (Fig. S2c), since recognising open flowers was difficult. For the few species lacking information in the trait database, values were obtained from own measurements of flowers in the study region, or average

values of other species of the same genus represented in the trait database were used. If values considerably varied among species of the same genus, the values of the most similar species of the same genus was used (according to Info Flora; Juillerat et al. 2017). Flower volume showed close and significant positive linear relationships with both amount of nectar and pollen (Fig. S3).

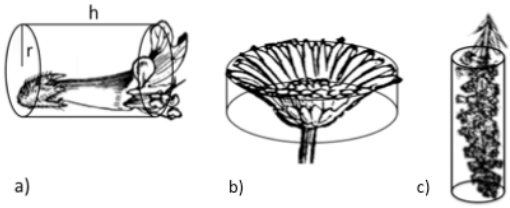

Fig. S2. Flower volume approximation. Flowers and inflorescences were approximated as cylinders with h = cylinder height = corolla depth and r = radius = 0.5* flower diameter or 0.5*inflorescence diameter (adapted from Fitch et al. 1924).

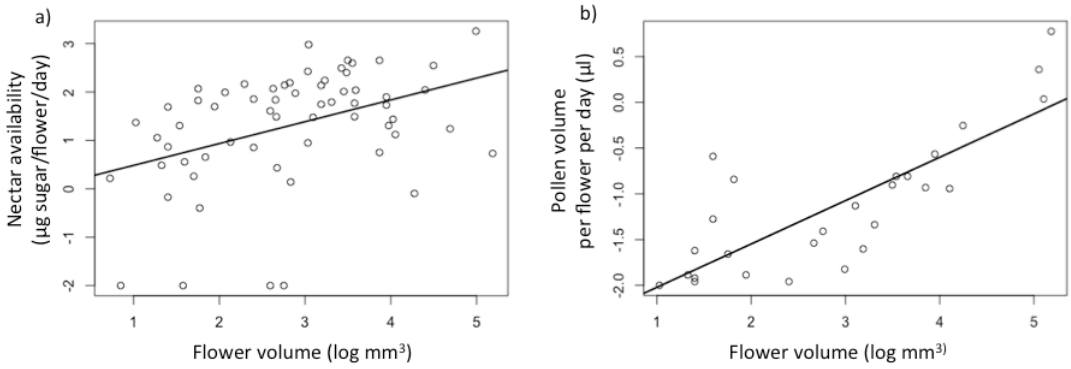

Fig. S3. Results from linear regression models between resource availability and flower volume. For a) nectar availability and log flower size (P = 0.001, Baude et al. (2016) as well as b) pollen availability and log flower size (b), P < 0.001, Hicks et al. (2016).

Flower density ($D_{species}$)

Flower density ($D_{species}$) describes the number of open flowers in the flowering parts of the species during its flowering period. Flower density was assessed differently for woody plants, arable crops and grasslands. Flower density in woody species (trees and shrubs of forest edges, hedgerows, orchards and single trees; see S1 Data) was assessed by counting the number of flowers in flowering parts of trees and shrubs within 20 cubes of 1 m^3 (two cubes each in 10 representatives per species) during the species' flowering period.

Flower density in crops were counted in 10 cubes of 1 x 1 x 1 m size in two fields per crop type during the peak flowering period.

Flower density assessments in grasslands were more complicated. Grasslands vary in flower composition depending on management, season and factors like soil types and exposition, which leads to differences between landscapes. For this reason, grasslands were classified into four management types; Permanent grasslands, extensive grasslands, ley meadows, and grazing lands (see S1 Data for classification criteria). Flower densities of grassland species were assessed per grassland type and landscape over the entire bee sampling period (beginning of April to end of June) in roughly three week intervals, leading to a total of five sampling rounds. Flower densities per species

in grasslands were counted within 10 x 1 m^3 cubes in 2 representatives (if present) per meadow type (20 cubes) per landscape (20) per sampling round (5) leading to 6060m^3 counted.

Mapping of woody plants to estimate $V_{species}$ of flowering tree and shrub species

To quantify flower bearing plant volumes, approaches optimised for the different habitat types were applied. For crops und meadows this was done by simply transferring square meters to cubic meters, since height of flower horizons do not exceed one meter. To estimate the floral resource contribution of tree and shrub species along forest edges and hedgerows in a landscape, the volume of flowering parts of all tree and shrub species potentially visited by bees for floral resource use (S1 Data) were estimated along the entire length of all forest edges and hedgerows in each landscape (c.a.38 km). To this end, forest edges and hedgerows were split into segments of two-meters (covering the entire width of the woody vegetation of hedgerows, and a depth of ten meters into the forest along forest edges). Within each of these segments, the presence of all woody species was recorded. Furthermore, the volumes of the flowering parts of each species in the upper crown layer, the middle crown layer, and the shrub layer was estimated for each segment by estimating crown height within the segment (Fig. S4). Volumes of flower parts in hedgerows and of the middle crown layer and the shrub layer of forest edges were directly estimated in the field. Since estimates on high trees are difficult and become un-precise, a GIS approach using a digital vegetation height map was applied to assess the height of the upper crown layer to estimate flower part volumes of this layer: woody elements were digitized in ArcGIS version 10.6. (ESRI) and a vegetation height map with a 1 m resolution available for Switzerland (Ginzler 2018) was placed over the orthophoto. To avoid underestimation of vegetation height along forest edges with relatively sparse tree cover average maximum tree height per segment was used. The height of the upper crown layer was calculated by subtracting the height minus the height of the upper part of the middle crown layer recorded in the field. Ground-truthing confirmed that this approach yielded reasonably precise and robust estimations of tree heights and estimates of flowering crown volumes of the upper crown layer.

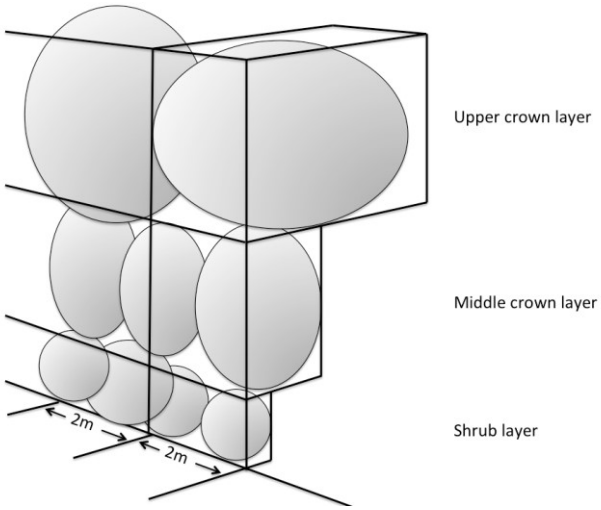

Fig. S4. Schematic illustration of volumes of flowering parts of trees and shrubs estimated for different layers along forest edges.

Isolated trees identified on the orthophoto were assigned to species in the field and the outline digitised as polygon in ArcGIS. Volumes of flowering tree were approximated based on estimated

crown projection area and crown height. For fruit trees in orchard plantations crown volumes were calculated by multiplying orchard area with estimated ratio of tree coverage, and a standard approximation of 5 m crown height for traditional orchards and 2 m crown height for intensive orchards (Anbautechnik Bioobst, FiBL).

Estimation of flowering period ($T_{species}$)

For flowering trees, shrubs and crops average flowering period was estimated based on field observations. Although flowering periods vary between species, flowering periods observed were generally similar with an average duration of approximately 21 days. We therefore used this approximation of average flowering period and the observed peak of each species' flowering period to estimate the start and end date of flowering in the study region. For grasslands it was possible to determine the flowering period based on continuous floral assessments during the season: a species' flowering period was defined as the period from the first to the last day it was recorded flowering in a sample plot. Very rare flowering herbaceous species that occurred in less than 1% of all sampling plots (22 species) were excluded from further analyses. Due to the rare occurrence they did not allow to retain reliable flowering durations. Average floral density per species was calculated from squares counted within the flowering season of this species.

Flower diversity

Flower diversity defined by the Simpson index ((Simpson 1949); implemented in the R vegan package 2.5-2; Oksanen et al. (2018)) was calculated from species specific flower abundance of each habitat type (grassland, crop, semi-open or forest edge) and landscape.

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Supplementary Results

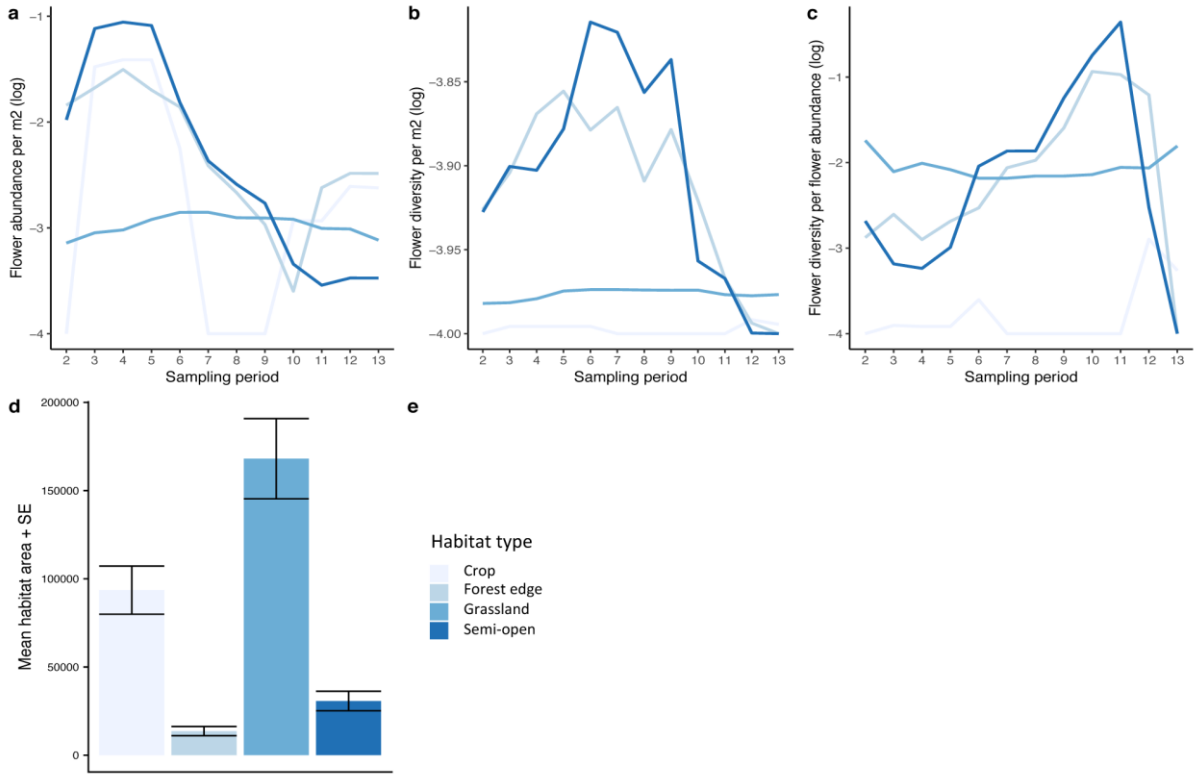

Fig. S5. Temporal distribution of (a) average log-transformed flower abundance and (b) flower diversity per m^2 and (c) the ratio between average flower diversity and average flower abundance of each habitat type during the season, and (d) mean area [m^2] of each habitat type per landscape.

Table S1. Total number of mapped entomophilous flowering plant species of each major habitat type early (beginning of April to mid-May) and late (mid-May to end of June) in the season. See Supplementary methods for detailed description of flower mapping.

Habitat type	Time period	Floral species richness
Crop	Early	2
Forest edge	Early	22
Grassland	Early	51
Semi-open habitat	Early	23
Crop	Late	4
Forest edge	Late	11
Grassland	Late	54
Semi-open habitat	Late	12

Table S2. Parameter estimates (slope) of linear regression models of the effect of floral resources (floral abundance or diversity; scaled) of the four major habitat types early or late in the season on wild bee abundance (log-transformed) and richness. Significant effects ($P \leq 0.05$) are indicated in bold (d.f. = degree of freedom; SE = standard error). See also Fig. 3.

Floral	Response	d.f.	R^2	Adjusted	AIC	Habitat type	Estimate	SE	*P*-value
Early flower abundance	Early bee species richness	15	0.469	0.327	55.09	Semi-open	-0.01	0.20	0.988
						Forest edge	**0.63**	**0.19**	**0.004**
						Crop	0.33	0.19	0.107
						Grassland	-0.04	0.20	0.863
	Early bee abundance	15	0.438	0.288	56.21	Semi-open	0.16	0.21	0.468
						Forest edge	**0.43**	**0.20**	**0.042**
						Crop	**0.51**	**0.20**	**0.021**
						Grassland	-0.16	0.21	0.458
Early flower diversity	Early bee species richness	15	0.458	0.314	55.47	Semi-open	0.10	0.20	0.611
						Forest edge	**0.41**	**0.19**	**0.050**
						Crop	*0.37*	*0.20*	*0.079*
						Grassland	0.31	0.20	0.138
	Early bee abundance	15	0.484	0.347	54.50	Semi-open	-0.08	0.19	0.680
						Forest edge	**0.45**	**0.19**	**0.032**
						Crop	0.24	0.19	0.229
						Grassland	**0.46**	**0.19**	**0.031**
Late flower abundance	Late bee species richness	15	0.391	0.228	57.83	Semi-open	0.17	0.21	0.434
						Forest edge	0.15	0.21	0.499
						Crop	-0.37	0.24	0.142
						Grassland	**-0.64**	**0.23**	**0.015**
	Late bee abundance	15	0.261	0.064	61.68	Semi-open	0.15	0.24	0.531
						Forest edge	0.14	0.24	0.572
						Crop	-0.31	0.26	0.253
						Grassland	*-0.50*	*0.25*	*0.067*
Late flower diversity	Late bee species richness	15	0.569	0.454	50.89	Semi-open	-0.13	0.19	0.511
						Forest edge	*0.47*	*0.23*	*0.057*
						Crop	0.29	0.23	0.224
						Grassland	**0.74**	**0.18**	**0.001**
	Late bee abundance	15	0.547	0.426	51.90	Semi-open	-0.20	0.19	0.313
						Forest edge	**0.51**	**0.23**	**0.045**
						Crop	0.32	0.23	0.192
						Grassland	**0.67**	**0.18**	**0.002**
Early flower abundance	Late bee species richness	15	0.489	0.352	54.32	Semi-open	0.29	0.20	0.160
						Forest edge	**0.44**	**0.19**	**0.033**
						Crop	**0.44**	**0.19**	**0.035**
						Grassland	-0.33	0.20	0.114
	Late bee abundance	15	0.535	0.411	52.43	*Semi-open*	*0.39*	*0.19*	*0.057*
						Forest edge	**0.44**	**0.18**	**0.025**
						Crop	**0.49**	**0.18**	**0.015**
						Grassland	-0.27	0.19	0.183
Early flower diversity	Late bee species richness	15	0.691	0.609	44.25	Semi-open	-0.21	0.15	0.184
						Forest edge	**0.42**	**0.15**	**0.012**
						Crop	**0.34**	**0.15**	**0.040**
						Grassland	**0.58**	**0.15**	**0.001**
	Late bee abundance	15	0.634	0.537	47.61	**Semi-open**	**-0.37**	**0.16**	**0.037**
						Forest edge	**0.47**	**0.16**	**0.009**
						Crop	0.20	0.16	0.227
						Grassland	**0.52**	**0.16**	**0.006**

Table S3. Summary of linear model analysis testing the effects of floral resources (floral abundance or diversity) of major habitat types on abundance (log-transformed) and species richness of different groups of bees (social bees, solitary bees, rare bees and important crop pollinating bees). Parameter estimates (slope) were retained from linear regression models with scaled data. Significant effects ($P \leq 0.05$) are indicated in bold. d.f. = degree of freedom; SE = standard error. See also Fig. 4.

Response variable	Floral resource predictor	d.f.	R^2	Adjusted R^2	AIC	Habitat type	Estimate	SE	*P*-value
Social bee species richness	Flower abundance	15	0.367	0.198	58.59	Semi-open	0.30	0.22	0.196
						Forest edge	**0.49**	**0.21**	**0.033**
						Crop	0.25	0.22	0.269
						Grassland	-0.19	0.23	0.411
	Flower diversity	15	0.678	0.592	45.10	Semi-open	-0.26	0.15	0.108
						Forest edge	**0.41**	**0.15**	**0.015**
						Crop	-0.08	0.15	0.630
						Grassland	**0.72**	**0.15**	**<0.001**
Social bee abundance	Flower abundance	15	0.420	0.266	56.82	Semi-open	0.35	0.21	0.112
						Forest edge	0.27	0.20	0.189
						Crop	**0.54**	**0.21**	**0.020**
						Grassland	-0.08	0.22	0.732
	Flower diversity	15	0.560	0.443	51.30	**Semi-open**	**-0.50**	**0.18**	**0.012**
						Forest edge	*0.31*	*0.18*	*0.094*
						Crop	-0.13	0.18	0.471
						Grassland	**0.54**	**0.17**	**0.007**
Solitary bee species richness	Flower abundance	15	0.549	0.429	51.80	Semi-open	-0.06	0.16	0.763
						Forest edge	**0.60**	**0.17**	**0.004**
						Crop	0.31	0.18	0.112
						Grassland	-0.28	0.19	0.165
	Flower diversity	15	0.481	0.342	54.64	Semi-open	0.08	0.19	0.668
						Forest edge	**0.51**	**0.19**	**0.017**
						Crop	0.16	0.20	0.430
						Grassland	**0.52**	**0.19**	**0.014**
Solitary bee abundance	Flower abundance	15	0.446	0.298	55.93	Semi-open	0.08	0.21	0.688
						Forest edge	**0.52**	**0.19**	**0.017**
						Crop	0.32	0.20	0.132
						Grassland	-0.28	0.21	0.201
	Flower diversity	15	0.477	0.338	54.76	Semi-open	0.02	0.19	0.930
						Forest edge	**0.56**	**0.19**	**0.010**
						Crop	0.02	0.20	0.929
						Grassland	**0.46**	**0.19**	**0.029**

Rare bee species richness	Flower abundance	15	0.470	0.329	55.03	Semi-open	0.02	0.20	0.905
						Forest edge	**0.44**	**0.19**	**0.036**
						Crop	*0.37*	*0.20*	*0.083*
						Grassland	-0.35	0.21	0.112
	Flower diversity	15	0.491	0.355	54.23	Semi-open	0.08	0.19	0.683
						Forest edge	0.29	0.19	0.145
						Crop	-0.13	0.20	0.512
						Grassland	**0.61**	**0.19**	**0.006**
Rare bee abundance	Flower abundance	15	0.486	0.348	54.44	Semi-open	0.11	0.20	0.591
						Forest edge	**0.41**	**0.17**	**0.044**
						Crop	**0.43**	**0.20**	**0.043**
						Grassland	-0.34	0.21	0.114
	Flower diversity	15	0.265	0.069	61.57	Semi-open	0.04	0.23	0.867
						Forest edge	0.16	0.23	0.486
						Crop	-0.10	0.23	0.690
						Grassland	*0.47*	*0.23*	*0.054*
Crop pollinator species richness	Flower abundance	15	0.439	0.289	56.18	Semi-open	0.24	0.21	0.258
						Forest edge	**0.56**	**0.19**	**0.011**
						Crop	0.25	0.20	0.246
						Grassland	-0.24	0.21	0.274
	Flower diversity	15	0.648	0.554	46.84	Semi-open	-0.24	0.16	0.156
						Forest edge	**0.56**	**0.16**	**0.003**
						Crop	0.09	0.16	0.588
						Grassland	**0.63**	**0.16**	**0.001**
Crop pollinator abundance	Flower abundance	15	0.313	0.129	60.24	Semi-open	0.29	0.23	0.227
						Forest edge	0.31	0.22	0.169
						Crop	0.39	0.23	0.102
						Grassland	-0.17	0.24	0.480
	Flower diversity	15	0.549	0.429	51.80	*Semi-open*	*-0.37*	*0.18*	*0.055*
						Forest edge	**0.48**	**0.18**	**0.016**
						Crop	-0.01	0.18	0.942
						Grassland	**0.53**	**0.18**	**0.009**

Table S4. Summary of linear model analysis testing the effects of early floral abundance or diversity on late-active social bees other than bumblebees. Parameter estimates (slopes) were retained from linear regression models with scaled data. Significant effects ($P \leq 0.05$) are indicated in bold. d.f. = degree of freedom; SE = standard error. These patterns were not present in solitary bees.

Response variable	d.f.	R^2	Adjusted R^2	AIC	Floral resource predictor	Estimate	SE	*P*-value
Late social species richness	17	0.225	0.133	61.31	*Early flower abundance*	*0.46*	*0.22*	*0.053*
					Early floral diversity	0.28	0.22	0.217
Late social bee abundance	17	0.4475	0.382	54.54	**Early flower abundance**	**0.69**	**0.19**	**0.002**
					Early floral diversity	0.13	0.19	0.488

Chapter 3

Aphid predators reduce pest aphids and are better predicted by classical habitat maps than floral resource maps

Lolita Ammann, Aliette Bosem-Baillod, Philipp W. Eckerter, Martin H. Entling, Matthias Albrecht and Felix Herzog

Abstract

Context

Resource maps have been proposed as an alternative to classical habitat maps for prediction of beneficial insects. Predatory insects such as hoverflies or ladybirds contribute to the natural control of agricultural pests, but also use plant pollen or nectar as supplementary food resources.

Objectives

We aimed at predicting the abundance of crop pest predating insects and the pest control service they provide with the best possible mapping approach, using both detailed flower resource mapping and classical habitat maps.

Methods

We selected 19 landscapes of 500 m radius along a gradient of varying landscape composition and mapped them with both approaches. In the centres of the landscapes, aphid predators – hoverflies (Diptera: Syrphidae), ladybeetles (Coleoptera: Coccinellidae) and lacewings (Neuroptera: Chrysopidae) – were surveyed in experimentally established faba bean phytometers (*Vicia faba* L. Var. Sutton Dwarf) and their control of introduced black bean aphids (*Aphis fabae* Scop.) was recorded.

Results

Landscapes with higher proportions of forest edge as derived from classical habitat maps supported higher abundance of aphid predators, which in turn enhanced aphid pest control on faba bean. Floral resource maps failed to predict predator abundance or aphid control services.

Conclusions

Classical habitat maps allowed to link landscape composition with predator abundance and pest control. Floral resource maps probably failed prediction because predators require shelter and alternative prey in addition to flower availability. Semi-natural forest edges seem to support the populations of the predators investigated, and the service they provide.

Introduction

As natural enemies of crop pests, pollinators and decomposers, insects provide important ecosystem services to agriculture (Losey and Vaughan 2006). Public awareness of declining insect numbers and risks associated with pesticide applications increase the pressure on agriculture to find more sustainable management practices. The presence of predatory insects at the right moment and in sufficient quantity in agricultural fields can help to avoid insecticide applications against crop pests (Thies and Tscharntke 1999; Losey and Vaughan 2006; Tschumi et al. 2015). Thus, how to maintain and promote populations of natural enemies of crop pests in agroecosystems that spill into agricultural fields is of great interest. The conservation and restoration of areas of natural- and semi-natural habitats (SNH) even in intensively used farmland is often (e.g. Martin et al. 2019; Rusch et al. 2016; Sutter et al. 2018; Tscharntke et al. 2012), but not always enhancing populations of predatory insects and the pest control services they provide (Karp et al. 2018; Tscharntke et al. 2016). A better understanding of which landscape and habitat features are critical for an effective conservation of predatory insects is therefore urgently needed. Often, interactions between few species or guilds can decide whether provision is working or not (Evans 2008; Tscharntke et al. 2016). For example, SNH can not only host natural enemies, but also antagonists of natural enemies (Martin et al. 2013) or preferred hosts for pest species (Heimpel et al. 2010). SNH comprise a large set of different habitat types such as forest lots, hedgerows or grasslands (Herzog et al. 2017) that can differ significantly in their potential to sustain natural enemies (Schirmel et al. 2018; Bartual et al. 2019), providing food, shelter and overwintering sites (Holland et al. 2016). A better understanding which features of such habitats drive predator numbers and thus the potential to contribute to natural pest control services would represent a big step towards more effective and efficient conservation biocontrol.

Many insect pest predators in agricultural landscapes rely on floral food resources to complete their life cycle (e.g. hoverflies, lacewings and parasitoids) or to overcome times of scarce prey supply (Landis et al. 2000; Symondson et al. 2002; Wäckers and Van Rijn 2012; Lu et al. 2014). For example, larval growth in ladybirds is clearly enhanced by supplementary pollen resources and wild flower strips tailored to floral resource needs of predators efficiently enhances pest control in crops (Jonsson et al. 2015; Tschumi et al. 2015, 2016). Unlike wild flower strips, SNH such as forest edges are often located in some distance to the field and it is less clear, how their floral resources promote pest control in crops. To date, we lack knowledge on the response of predators to landscape scale floral resource availability based on flower availability in major habitat types including crops. By mapping and quantifying the (spatio-temporal) availability of floral resource characteristics, we expected to gain important insights into predators' requirements to the landscape. Such refined "functional habitat maps" have been proposed to improve the prediction of species and functional groups (Vanreusel and Van Dyck 2007; Lausch et al. 2015) – although generating such maps is significantly more laborious than "classical" habitat mapping. Knowing which floral resources predators require, and in which habitat types they prevail, will allow for specific recommendations on habitat and agricultural landscape design, provided that their population increase actually translates in improved pest control.

We asked the following research questions:

1) Do floral resource maps predict aphid predator abundance better than classical habitat maps and is there a habitat type of particular importance?
2) Do crop pest predators increase with the amount of SNH in the landscape?
3) Are black bean aphid populations reduced by predator numbers on faba bean?

Methods

Study design and experimental setup

Nineteen agricultural landscape sectors of 500 m radius (hereafter landscapes) were selected in northern Switzerland, near Zürich (See Supplementary material Fig. S1 for spatial distribution of landscapes). Landscapes covered a gradient of varying shares of forest edges, semi-open habitats (hedgerows, tree rows and single trees), grasslands (permanent intensively managed grasslands, permanent extensively managed grasslands and pastures) and crops (mass-flowering crops, intensive orchards and ley meadows). Habitat maps of the four habitat types were established using aerial images that were verified and supplemented based on field observations (Fig. 1a, 1b) and amalgamated in ArcGIS (ESRI) with a minimal mapping unit of 1 sqm. Forest edge, semi-open habitats and grasslands were grouped as semi-natural habitat.

Floral resource maps were established according the same four habitat categories as in classical habitat maps. Floral resources were assessed between beginning of April and mid-May 2017, the time period most relevant for the control of aphid pests in cereals, oilseed rape and fruit production of the study region (Stähler Pflanzenschutz, Switzerland). Flower availability at the landscape level was calculated from the sum of all flowering species recorded in the four major habitat types mentioned above. Flower availability was assessed for each landscape and habitat type separately at local scale, with daily resolution over the sampling season for each flowering species, except grasses. Flower availability of a species ($F_{species}$, day * m^3) was assessed by evaluating its potentially flower bearing volume in a landscape and habitat type ($V_{species}$ e.g. of tree crowns or the flowering horizon in crops), which was multiplied by its species specific flower density within $V_{species}$ ($D_{species}$), as well as flower size ($S_{species}$, volume taken by individual flowers) and flowering duration ($T_{species}$). Flower diversity was calculated using the Simpson index (Simpson 1949; implemented in the R vegan package 2.5-2 (Oksanen et al. 2018)), based on flower availability per habitat type. Mapping resulted in two types of floral resource maps: flower availability and flower diversity (Fig. 1c, 1d). See Supplementary material for a detailed description the mapping procedures.

Predator and aphid survey

In the center of each of the 19 landscapes, at the edge of a winter wheat field, a patch of ten faba bean (*Vicia faba* L. Var. Sutton Dwarf) phytometer plants was established. Faba bean plants had been raised in an insect-prove greenhouse. At the start of bean flowering, 48h before translocation to the field, plants were infested with approximately 20 black bean aphids (juvenile *Aphis fabae* Scop., purchased from Katz Biotech AG) following Eckerter et al. (*subm.*). Aphids were transferred on a single *V. faba* leaf, which was pinned below the uppermost crown of small leaves (i. e. at the

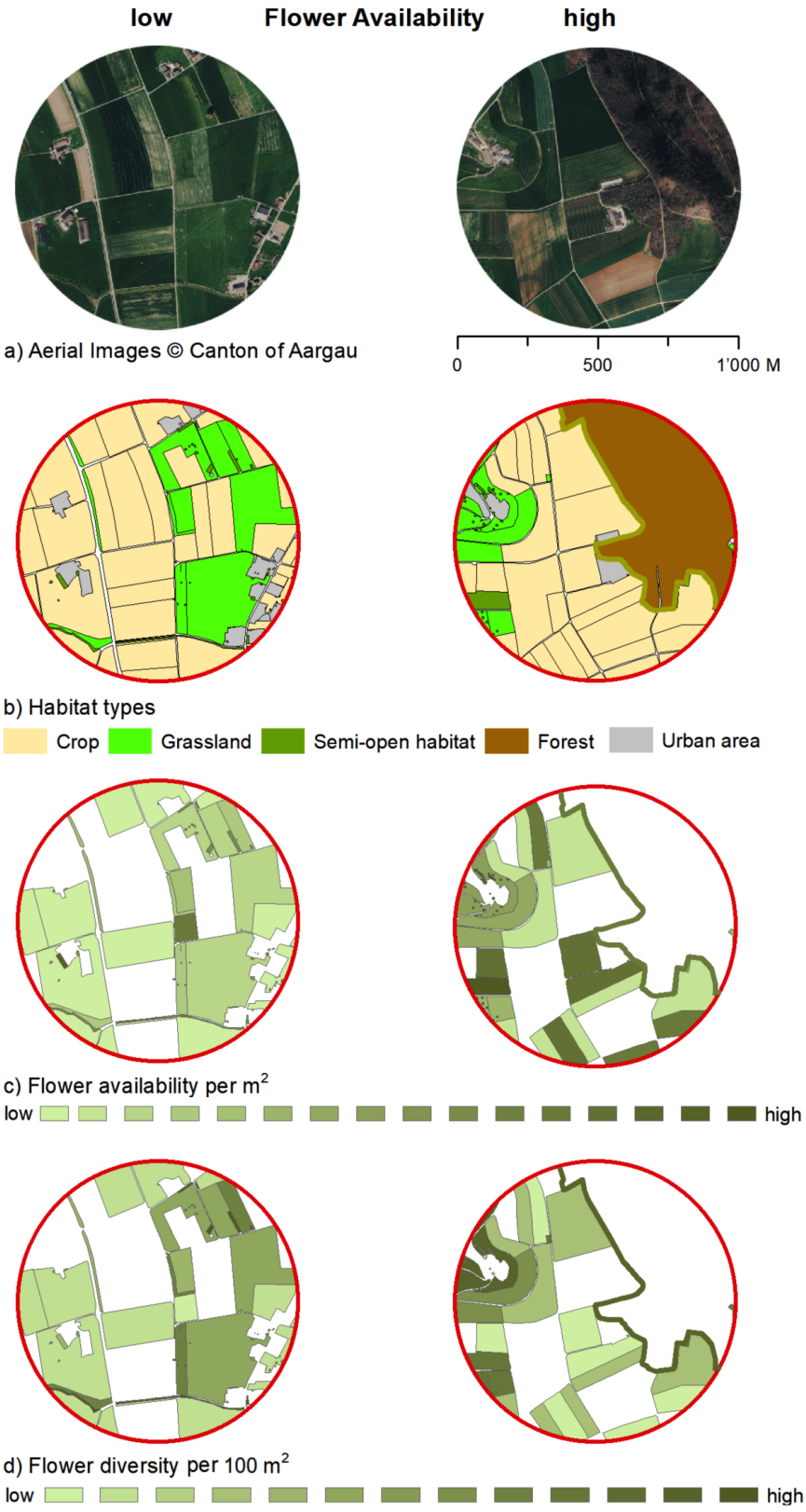

Fig. 1. Two landscapes with high and low flower availability. Plot pair b) shows habitat categories for classical habitat maps. Plot pair c) shows average flower availability (m^3 times flowering duration) per m^2 for habitat sub-categories of the depicted landscapes (low = 0.0002, high = 1.3251). Plot pair d) shows average flower diversity (Simpson index) per $100m^2$ for habitat sub-categories of the depicted landscapes (low = 0.0003, high = 0.0097).

youngest plant part) close to the stem. All black bean aphids were counted again immediately after translocation to the field (used as initial "starting population" number in the analyses). The numbers of

black bean aphids and their predators (coccinellids, chrysopids and syrphids present as eggs, larvae, or adults on phytometer plants) were recorded after two days (approximately 48h), four days (approx. 96 hours) and 14 days after exposure. The few aphids that migrated from the environment into faba beans (e.g. *Megoura viciae* Buckton) were not taken into account for analysis, since numbers would rather relate to landscape scale aphid pools than predation on the faba beans. Furthermore, mummies of parasitoids were excluded as they were present quite abundantly, but not identifiable with sufficient certainty after less than two weeks of development (often inflated appearance, but no change in colour yet). See supplementary material Fig. S2 for a graph of the experimental layout.

Statistical analysis

Aphis fabae population growth (hereafter equivalent with aphid control) was defined as the change in total *A. fabae* numbers per landscape from the first counting round immediately after exposure until to the last counting round after 14 days, with pooled aphid numbers across individual phytometer plants. Numbers of ladybeetles, hoverflies and lacewings per landscape were pooled across life-history stages, sampling rounds and individual phytometer plants per landscape. Relations between predators and aphid control, as well as their relation to landscape parameters were assessed with linear regression models. For each response variable (predators, aphid control) and each map type (classical habitat maps and functional resource maps based on flower availability and flower diversity) a separate model was computed. Each model included the four habitat categories as predictors (grasslands + semi-open + forest edges + crops). To test effects from SNH, habitat areas of semi-open habitat, grasslands and forest edges were pooled and tested against predators and aphid control in separate models. Landscape level floral availability and diversity were derived from pooling over habitat types and tested separately, as for SNH (see Table 1). The best map was identified based on model performance via Akaike information criteria corrected for small sample sizes (AICc). To meet linear model assumptions, predator numbers were log-transformed. Potential co-linearity between explanatory variables was checked based on variation inflation factors (VIFs; car package version 3.0-2; Fox, 2018), making sure a threshold of three was not reached (Zuur et al. 2007). All analysis were performed using R version 3.4.1 (Team 2017). Means ± 1 standard error are reported throughout.

Results

Crops covered on average around 30% of the landscape, providing more than 50% of floral resources available in the landscape (56% provided by *Brassica napus*), but only 12% of floral diversity (Fig. 2). Grasslands and forest edges provided the highest amounts of flower diversity (33% and 31% respectively) but contributed relatively little to total flower availability (2%, of which 47% were *Cerastium spp.*, and 14%, of which 20% were *Prunus spp.*, respectively). Unlike grasslands (12% landscape cover), forest edges covered a very small proportion of the landscape (<5%), similarly to semi-open habitat, which provided almost 30% of total flower availability (traditional orchards) but was less diverse than forest edges (22% of total landscape-level diversity). The two woody SNHs (forest edges and semi-open habitat) provided by far the highest diversity as well as the highest flower availability relative to the area covered (Fig. 3).

Table 1. Results of linear regression models for effects of landscape variables on predator numbers (log) and aphid control on faba bean. Significant *p*-values are indicated in bold ($p < 0.05$). See material and methods section for detailed information on models and parameters. SNH: Semi-natural habitat (grassland, forest edge, semi-open).

Response	Fixed effect	df	AICc	Habitat	*F*-value	*p*-value
Predators	Total flower abundance	17	52.40	Landscape-level	0.070	0.794
	Total flower diversity	17	34.24	Landscape-level	0.489	0.494
	Habitat area	17	33.84	SNH	0.858	0.367
	Habitat area	**14**	33.57	Crop	2.300	0.152
				Grassland	0.038	0.848
				Forest edge	**9.649**	**0.008**
				Semi-open	0.847	0.373
	Flower abundance	14	40.91	Crop	0.424	0.526
				Grassland	0.104	0.752
				Forest edge	1.855	0.195
				Semi-open	2.420	0.142
	Flower diversity	14	40.93	Crop	1.409	0.255
				Grassland	0.887	0.362
				Forest edge	2.995	0.106
				Semi-open	2.012	0.178
Aphid control	Total flower abundance	17	331.94	Landscape-level	0.055	0.817
	Total flower diversity	17	330.78	Landscape-level	1.135	0.302
	Habitat area	17	329.51	SNH	2.383	0.141
	Habitat area	14	339.13	Crop	0.639	0.438
				Grassland	1.097	0.313
				Forest edge	0.910	0.356
				Semi-open	0.021	0.888
	Flower abundance	14	339.34	Crop	0.009	0.926
				Grassland	2.448	0.140
				Forest edge	0.356	0.560
				Semi-open	0.021	0.886
	Flower diversity	14	342.59	Crop	0.107	0.749
				Grassland	0.005	0.945
				Forest edge	0.093	0.766
				Semi-open	0.281	0.604
	Predators	**17**	**29.70**	**-**	**5.211**	**0.036**

A total of 129 predators were sampled on the bean phytometer plants, of which 63% were Coccinellids, 28% Syrphids and 9% Chrysopids. SNH area covered more than 10% of the total landscape area, but did not significantly explain predator numbers or aphid control, neither did landscape-scale flower availability or diversity. However, when separating habitat categories into finer components (forest edges, crop, grasslands, semi-open habitat), predator numbers increased with the proportion of forest edge (Table 1, Fig. 2). No other habitat type could explain predators significantly, neither from functional resource maps, nor from classical habitat maps. Thus, functional resource maps did not improve prediction over classical habitat maps (AICc was best in classical habitat maps; Table 1).

The average number of black bean aphids on field bean phytometer plants increased from 283.2 (± 26.3) after translocation of invested plants to 1183.8 (± 289.8) two weeks later. Aphid control was positively related to predator numbers (Table 1, Fig. 3) but did not relate to any landscape descriptor that predators were tested for (Table 1).

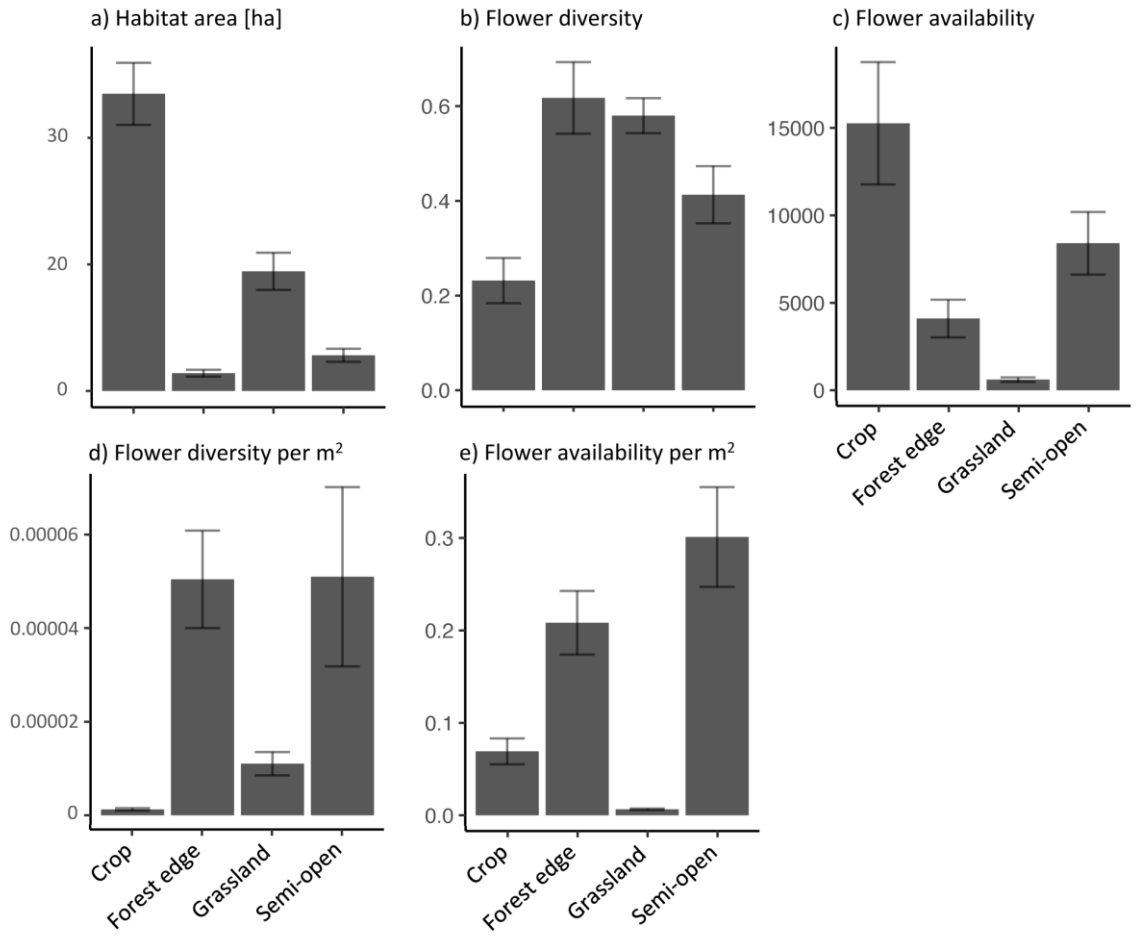

Fig. 2. Distribution of a) average habitat area, b) average flower diversity (Simpson's index), c) average flower availability (flower volume * flowering days), d) average flower diversity per habitat area (Simpson's index) and e) average flower availability per habitat area over landscapes for the four habitat types (+/- standard error). See Appendix for detailed information on calculation of flower abundance and diversity.

Discussion

Numbers of the studied aphid predators, i.e., hoverflies, lady beetles and lacewings on faba beans could be explained with classical habitat maps, but not with floral resource maps, although they consume floral resources at least in certain life-history stages or to supplement their animal diet. In the context of biodiversity conservation, e.g. (Dennis et al. 2006; Moore et al. 2010; Turlure et al. 2019) had argued, that resource maps ("functional habitat") would predict the occurrence of target organisms better than classical habitat maps based on land use or vegetation types. We had therefore hypothesized that floral resource maps would predict predating insects and their effectiveness better than classical habitat maps and had mapped the availability (Schirmel et al. 2018) and diversity of flower resources in the landscapes investigated. This laborious mapping allowed detailed evaluations of flower resources that are known to be vital for the three insect groups investigated. More than 50 % of landscape-level floral resources were provided by crops, of which the large majority came from oilseed rape and fruit trees, which both have relatively short flowering periods. Grasslands providing 25 times less flowers still exhibited the second highest flower diversity after forest edges, in particular extensively managed meadows. That the large variation in floral resource availability between

landscapes as well as habitat types did not mirror in predators abundance and performance was therefore surprising.

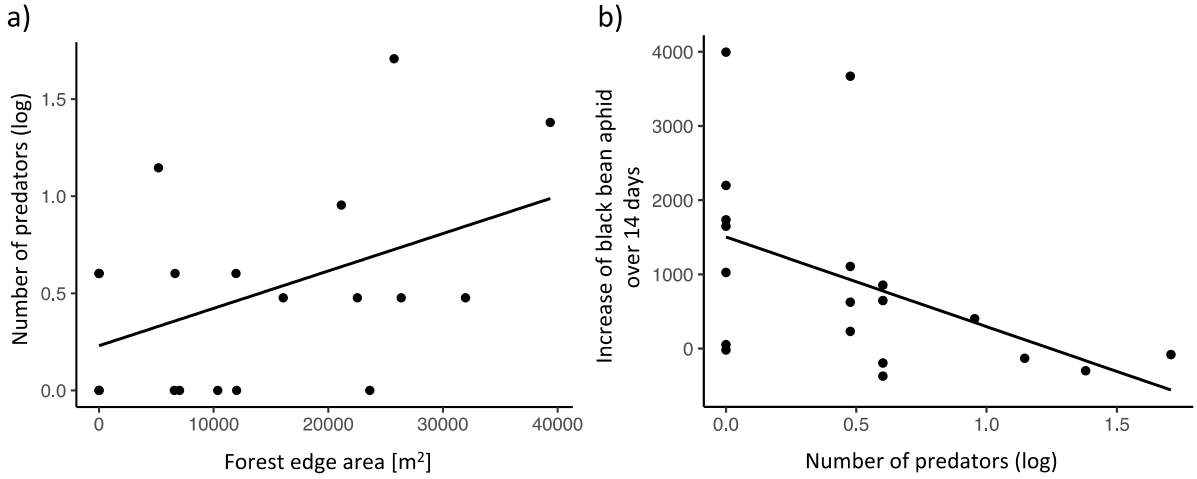

Fig. 3. Number of predators on faba beans (ladybirds, lacewings and hoverflies on 10 phytometer plants per landscape) in relation with a) amount of forest edge habitat in the landscape and b) aphid control (restriction of black bean aphid population growth over 14 days on faba bean; see Table 1 for parameters).

Natural enemies and pest control have been shown to be related to landscape-level environmental traits in the past (Bianchi et al. 2006; Chaplin-Kramer et al. 2011; Veres et al. 2013; Rusch et al. 2016), although with some inconsistencies in their response to non-crop habitat (Karp et al. 2018). Those inconsistencies may be partly related to structural differences between SNH types. Our results show, how variable different types of SHN are at least in their floral provisions to the landscape. Floral resource maps failing to predict aphid predators on faba bean suggests, that other drivers are more important in the studied agroecosystems, such as alternative prey availability, overwintering habitat and shelter (Landis et al. 2000; Burgio et al. 2006; Schirmel et al. 2018). Findings in this and previous studies indicate that forest edges may be particularly important in providing such resources. Although forest habitats can promote pests in some cases (e.g. Kheirodin 2020) they have mostly been positively associated with predator numbers and pest control (Nicholls et al. 2001; Alhmedi et al. 2009; Gardiner et al. 2009; Mitchell et al. 2014). Here, increasing proportion of forest edge habitat was related to higher predator abundance, which in turn led to increased aphid control (Fig. 3). Forest edges are a prominent habitat for many natural enemies (Ingrao et al. 2017; Schirmel et al. 2018; Bartual et al. 2019) and have been identified as important sources of prey and shelter (reviewed by Holland et al. 2016). For example stinging nettles, found prevalently along forest edges in the studied landscapes, are hosts of some of the most important alternative prey for ladybirds (Chapter 4; Ammann et al. 2020) and were found to host ladybirds as well as hoverflies prior to crop colonisation (Alhmedi et al. 2009).

However, Holland et al. (2016) found resources in grassy habitats to be at least equally important, which contrasts with our findings. Grasslands, similar to crops, differ in their management and the associated degree of chemical and mechanical disturbance experienced by predators (Giller 1997), a factor not investigated in this study. An additional reason for the lack of prediction by crops

could be temporal patchiness of crop food resource availability (Schellhorn et al. 2015; Baude et al. 2016).

Whether floral resources are important limiting factors and therefore improve prediction of insects compared to classical habitat maps seems to depend on factors such as the agricultural system investigated or the group of insects and effects assessed. A related study found comparable relations for pollination services, which were better predicted with classical habitat maps than floral resource maps (Eckerter et al. *subm*.). Recent findings by Bartual et al. (2019) similarly failed to show floral effects on predators on a landscape-scale, indicating that resource complementation for predators, despite effective at small scale, needs to address resources other than flowers for promotion of predators on a landscape-scale. In the same field study, wild bees did relate positively to floral resources (Bartual et al. 2019). The fact that the abundance of wild bees, unlike natural enemies, is better predicted with floral resource maps compared to classical habitat maps (Chapter 2; Ammann et al. *in prep.*) may emphasize the distinct differences in resource requirements of different functional groups to the landscape.

We believe this to be the first time that flower resources were evaluated with this degree of detail at landscape level, and related to natural enemies of crops and to the actual mechanism of pest control. Resource mapping is a tedious process. To minimize errors and inaccuracies, data collection in the field was whenever possible restricted to counting, measuring and presence-absence characterisation of landscape parameters, avoiding observer bias through estimates. However, flower availability as well as flower diversity values are derived from numerous generalizations, such as flower size or flower density, which may be subject to some deviations. It is therefore possible, that some flowering species were somewhat over- or underestimated in their contribution to the landscapes. Still, since the same parameters were applied over all landscapes, we believe that this does not impair comparisons between landscapes. Another reason for the missing link between resource maps and the occurrence of aphid predators may be that they are not very specialized in the use of plant resources, but seem the be rather opportunistic in pollen consumption (Bertrand et al. 2019).

Conclusions

We draw three main conclusions. First, floral resource maps performed poorly at predicting the studied flower-visiting aphid predators. This was an unexpected result, because we hoped that more detailed resource maps would perform better. Still, this finding actually supports the further use of state of the art habitat mapping in landscape ecological investigations in relation to pest control, which is much less time consuming than the detailed mapping of resources. Second, classical habitat maps allowed to explain the occurrence of predators of crop pests. Still, broad dichotomous classifications of habitat types into SNH and crop habitat, sometimes termed “landscape structure” and “matrix”, is not sufficient. Instead, different types of SNH (and possibly crops, depending on the purpose of the investigation) must be differentiated. Third, not only floral resources (pollen, nectar) are needed to promote predators of crop pests and the service they provide, but other services (shelter, alternative prey, etc.) available at e.g. forest edges must also be factored in.

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Supplementary material on landscape mapping

General approach

Nineteen landscape sectors of 500 radius were selected along a gradient of varying shares of forest edges, semi-open habitats (hedgerows, tree rows and single trees), grasslands (permanent intensively managed grasslands, permanent extensively managed grasslands and pastures) and crops (mass-flowering crops, intensive orchards and ley meadows) (Fig. S1). Landscapes were selected to form a gradient in habitat proportion of the four habitat types that did not exceed variation inflation factors (VIF; Fox 2018) of more than 3 (Zuur et al. 2007).

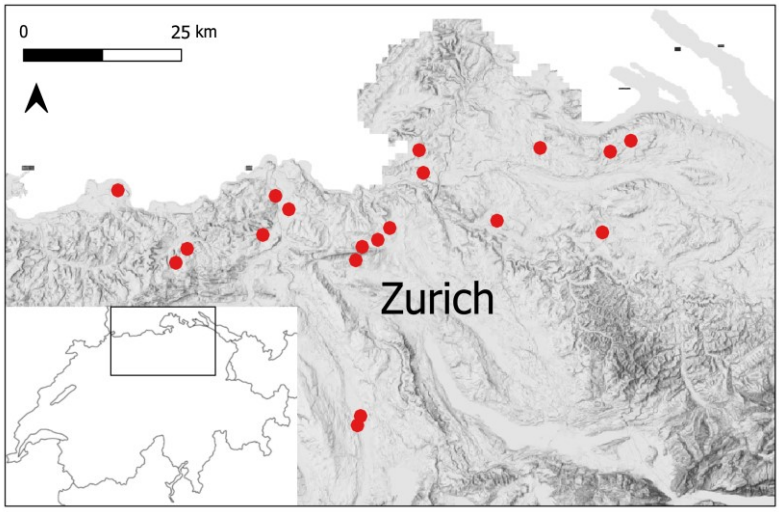

Fig. S1. Geographical distribution of landscapes in the north-eastern Swiss Plateau (Map type: swissALTI3D relief shading, source: Federal office of topography, swisstopo)

Predictive performance of classical habitat maps was compared to functional resource maps that held information on flower availability and on flower diversity within each of the four habitat types. Floral resources were assessed in the field between the beginning of April and mid-May 2017. Flower availability assessments were done according to floral composition and structure of different habitat types. For example, along all linear woody elements, the flower bearing crown volume of woody species was mapped comprehensively. Floral availability was then calculated from crown volumes and species specific floral traits (flower size, flower density, flower duration) retained from individual representatives of the species. In meadows, flower densities in different types of meadows were mapped within 10 representative square meters in each landscape, several times throughout the season, to account for temporal variation in floral composition. This appendix describes in detail, how flower availability and diversity were assessed in the four habitat types.

Calculation of flower availability

To estimate flower availability the four habitat types form above were split into further sub-units based on vegetation composition (e.g. intensively and extensively managed meadows) or mappable approach (e.g. single trees and forest edges), called vegetation types here. Flower availability in the four habitat types was summarized from the flower availability found in individual vegetation types. Flower availability in a vegetation type ($F_{veg.type}$) was the sum of the flower availability of all flowering

species present in a vegetation type that were accessible for predators, except grasses (see section on floral resource accessibility). The flower availability of a species ($F_{species}$) was estimated as the product of the flower size (flower volume) of a single flower of that species (S_{flower}), the average number of flowers of the species per m^3-volume in the flowering part of the plant, e.g. the tree crown (flower density $D_{species}$), and the volume (in m^3) occupied by the flowering parts of the plant species in a habitat and landscape (e.g. flowering crown volume of a shrub or tree; $V_{species}$). To account for variation in the duration of flowering periods of different species affecting their contribution to $F_{species}$ this product was multiplied by its flowering period (e.g., the estimated average number of days the species was flowering during this time period; $T_{species}$).

$$F_{\text{species}} = S_{\text{species}} \times D_{\text{species}} \times V_{\text{species}} \times T_{\text{species}}$$

$$F_{\text{veg.type}} = \sum_{species} F_{\text{species}}$$

$$F_{\text{habitat}} = \sum_{veg.type} F_{\text{veg.type}}$$

To account for the differences in three dimensional structure and species composition, $D_{species}$, $S_{species}$, $V_{species}$ and $T_{species}$ were assessed differently depending on species and vegetation type. For all assessments, estimations in the field were avoided, by restricting data collection to measurements, counts and presence-absence characterisation of flower and landscape parameters to avoid observer bias. The following sections describe in detail how this was done.

Species' flower volume ($S_{species}$)

Numbers of flowers will not necessarily translate directly into floral resource availability to insects. Depending on size and floral traits, the amount and accessibility of nectar and pollen varies. We therefore explored the relationships of flower area (projection area from the top) and flower volume (approximated as cylinders using flower diameter as cylinder width and corolla depth as cylinder length) with floral nectar availability of 72 plant species frequently flowering in the study region using the extensive database provided by Baude et al. (2016). We also explored the relationship of these flower traits with pollen volumes provided for flowers of 27 flowering plant species (Hicks et al. 2016). Flower diameter and corolla depth were obtained from a floral trait database compiled for most flowering plant species of the study region Frey et al. (*in prep*). For most floral types, flower volumes were taken from individual flowers, except for Asteraceae (inflorescence diameter used as cylinder width) and male catkin flowers, since recognising open flowers was difficult. For species lacking information in the trait database, values were obtained from own measurements of flowers in the study region, or average values of other species of the same genus represented in the trait database used. If values varied strongly among species of the same genus, the value of the most similar species with a similar geographical distribution was used (according to Info Flora; Juillerat et al. 2017). Flower volume (log-transformed) showed close and significant positive linear relationships in regression models with nectar (df = 1, t = 3.42, P = 0.001) and pollen availability (df = 1, t = 12.04, P < 0.001).

Floral resource accessibility

Many predators lack the long tongues of bees that would allow them to access most flowers. Therefore, they usually rely on relatively simple floral shapes, with open access to pollen and shallow corolla tubes for access of nectar (Colley and Luna 2000; Fiedler and Landis 2007; Haaland et al. 2011). Flowers with no open access to pollen or nectar were excluded from analysis. Nectar access was categorised as open, if pollinator behaviour recorded by Frey et al. (*in prep*) was classified as primitive, nectar tube length was shorter than 1 mm and van Rijn and Wäckers 2016 did not note else based on experimental data. Pollen access was categorised as open if Frey et al. (*in prep*) classified the flower associated pollinator behaviour either as primitive or for crawling in. Pollen access was categorised as not possible if pollen resources were marked as hidden or buzzing pollinators were needed. Since most flowers had open pollen access, results did not deviate from analysis without this pre-selection.

Flower density ($D_{species}$)

Flower density describes the number of open flowers in the flowering parts of the species during its flowering period. Flower density was assessed differently for woody plants, arable crops and grasslands. Flower density in woody species (trees and shrubs of forest edges, hedgerows, orchards and single trees) was assessed by counting the number of flowers in flowering parts of trees and shrubs within 20 cubes of 1 m^3 (two cubes each in 10 representatives per species) during the species' flowering period.

Flower density in crops (including lay meadows) was based on counts in 10 cubes of 1 x 1 x 1 m size in two fields per crop type during the peak flowering period.

Flower density assessments in grasslands were more complicated. Grasslands vary in flower composition depending on management, season and factors like soil types and exposition, which leads to differences between landscapes. For this reason, grasslands were classified into three management types: Permanent grasslands, extensive grasslands and pastures. Flower densities of grassland species were assessed per grassland type and landscape from beginning of April until mid-May, in roughly three week intervals. Flower densities per species in grasslands were counted in 10 x 1 m^3 cubes in 2 representatives (if present) per meadow type (20 cubes) per landscape (19) per sampling round (3).

Mapping of woody plants to estimate $V_{species}$ of flowering tree and shrub species

To quantify potentially flower bearing plant volumes in the landscape, approaches optimised for the different habitat types were applied. For crops und meadows this was done by transferring square meters retained from areal maps into cubic meters, since none of them have flower horizons higher than one meter. To estimate the floral resource contribution of tree and shrub species along forest edges and hedgerows in a landscape, the volume of flowering parts of all tree and shrub species potentially visited by insects for floral resource use were estimated along the entire length of all forest edges and hedgerows in each landscape (ca. 38 km). To this end, forest edges and hedgerows were split into segments of two-meters (covering the entire width of the woody vegetation of hedgerows, and a depth of ten meters into the forest along forest edges). Within each segment, the presence of all

woody species was recorded and the volumes of the flowering parts of each species in the upper crown layer, the middle crown layer, and the shrub layer were estimated. Volumes of flower parts in hedgerows and of the middle crown layer and the shrub layer of forest edges were directly estimated in the field. Since estimates on high trees are difficult and become un-precise, a GIS approach using a digital vegetation height map was applied to assess the height of the upper crown layer to estimate flower part volumes of this layer: woody elements were digitized in ArcGIS version 10.6. (ESRI) and a vegetation height map with a 1 m resolution available for Switzerland (Ginzler 2018) was placed over the orthophoto. To avoid underestimation of vegetation height along forest edges with relatively sparse tree cover, average maximum tree height per segment was used. The height of the upper crown layer was calculated by subtracting the height minus the height of the upper part of the middle crown layer recorded in the field. Ground-truthing confirmed that this approach yielded reasonably precise and robust estimations of tree heights and estimates of flowering crown volumes of the upper crown layer.
Isolated trees identified on the orthophoto were assigned to species in the field and the outline digitised as polygon in ArcGIS. Volumes of flowering trees were approximated based on estimated crown projection area and crown height. Crown volumes of fruit trees in intensive orchards were calculated by multiplying orchard area with estimated ratio of tree coverage and a standard approximation of 2 m crown height. Crown volumes of fruit trees in high-stem traditional fruit orchards were calculated the same way but with a standard approximation of 5 m crown height (Anbautechnik Bioobst, FiBL).

Estimation of flowering period ($T_{species}$)

For flowering trees, shrubs and crops average flowering duration was set to 21 days based on observations in the field. Flowering season started 10 days prior to the recorded flowering peak and ended 10 days after the flowering peak. For grasslands it was possible to determine the flowering period based on continuous floral assessments during the season: a species' flowering period was defined as the period from the first to the last day it was recorded flowering in a sample plot. Very rare flowering meadow species that occurred in less than 1 % of all sampling plots (22 species) were excluded from further analyses. Due to the rare occurrence they did not allow to retain reliable flowering duration.

Flower diversity

Flower diversity defined by the Simson index (Simpson 1949; implemented in the R vegan package 2.5-2 (Oksanen et al. 2018)) was calculated from species specific flower availability of each habitat type (grassland, crop, semi-open or forest edge) and landscape.

Experimental setup for survey of natural enemies and aphids

In the center of each landscape, at the edge of a winter wheat field, a patch of ten faba bean (*Vicia faba* L. Var. Sutton Dwarf) phytometer plants was established.

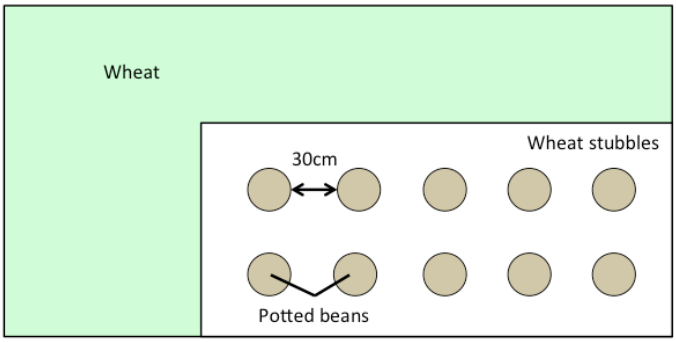

Fig. S2. Faba bean setup in wheat fields.

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Chapter 4

Insights into aphid prey consumption by ladybirds: Optimising field sampling methods and primer design for High Throughput Sequencing

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Abstract

Elucidating the diets of insect predators is important in basic and applied ecology, such as for improving the effectiveness of conservation biological control measures to promote natural enemies of crop pests. Here, we investigated the aphid diet of two common aphid predators in Central European agroecosystems, the native *Coccinella septempunctata* (Linnaeus) and the invasive *Harmonia axyridis* (Pallas; Coleoptera: Coccinellidae) by means of high throughput sequencing (HTS). For acquiring insights into diets of mobile flying insects at landscape scale minimizing trapping bias is important, which imposes methodological challenges for HTS. We therefore assessed the suitability of three field sampling methods (sticky traps, pan traps and hand-collection) as well as new aphid primers for identifying aphid prey consumption by coccinellids through HTS. The new aphid primers facilitate identification to species level in 75% of the European aphid genera investigated. Aphid primer specificity was high *in silico* and *in vitro* but low in environmental samples with the methods used, although this could be improved in future studies. For insect trapping we conclude that sticky traps are a suitable method in terms of minimizing sampling bias, contamination risk and trapping success, but compromise on DNA-recovery rate. The aphid diets of both field-captured ladybird species were dominated by *Microlophium carnosum*, the common nettle aphid. Another common prey was *Sitobion avenae* (cereal aphid), which got more often detected in *C. septempunctata* compared to *H. axyridis*. Around one third of the recovered aphid taxa were common crop pests. We conclude that sampling methodologies need constant revision but that our improved aphid primers offer currently one of the best solutions for broad screenings of coccinellid predation on aphids.

Introduction

Insects, including crop pollinators and predators of crop pests, provide important ecosystem services to agriculture. Advancing our understanding of the dietary resource needs of service-providing insects is critical to effectively promote them by agricultural landscape management (Tschumi et al. 2015; Gurr et al. 2017; Sutter et al. 2017). Various methods have been used to investigate diets of insects, each with distinct advantages and disadvantages (Birkhofer et al. 2017). High-throughput sequencing (HTS) has been increasingly adopted as a standard method for dietary analysis of both prey consumed by predators and plants consumed by herbivores, given its high accuracy (Pompanon et al. 2012) and its capacity to detect a broad range of consumed species simultaneously (Pearson et al. 2018; Eitzinger et al. 2019). However, several methodological constraints remain for HTS-based dietary analyses, in particular with respect to the analysis of prey diets of insect predators. First, insects are small animals, which yield minute amounts of gut content, making it difficult to distinguish between contamination and actual prey consumed. Second, especially for this system, close taxonomic proximity between predators and prey makes it challenging to specifically amplify prey DNA, which is especially important since the entire animal is used, rather than just faecal samples. Furthermore, collecting large numbers of individual insect predators, from which prey DNA can be isolated and contamination avoided, is difficult. For example, widely-used approaches such as pitfall trapping, vacuum sampling or sweep-netting may ensure collection of insects in sufficient numbers and in satisfactory condition for DNA analysis (Triltsch 1997; Berkvens et al. 2010; Thomas et al. 2013; Piñol et al. 2014), but these sampling methods can introduce cross-contamination through interaction of insects in the sampling containers (Greenstone et al. 2011; King et al. 2012; Athey et al. 2017). Moreover, the resource-use patterns found in studies using such sampling methods are often prone to an “observer bias”, i.e. they may be dependent on the choice of sampling location. For example, if predators are hand-collected directly from easily accessible plants, samples may be biased towards prey associated with the sampled host plants and the very local habitat, rather than adequately representing dietary use or preferences of mobile insects in their entire foraging range. Sampling methods using traps that capture moving insects beyond the immediate trapping location, such as interception traps that capture insects during flight (Chapman and Kinghorn 1955; Duelli et al. 1999), or traps attracting insects over relatively large distances via colour, scent or light (Duelli et al. 1999), could be more suitable for these reasons. However, also the use of trap-sampling methods presents challenges: for example, low insect sampling effectiveness during short trapping periods, or the risk of low DNA recovery rates due to DNA degradation if trapping periods are longer and thus restricted potential for DNA analysis (Harper et al. 2006; Gagnon et al. 2011). Furthermore, in amplicon-based HTS analyses of diet, the choice of PCR primers is critical (Piñol et al. 2014). To increase amplification probability of target DNA, a primer pair should amplify as broad a range of potentially consumed food taxa as possible, whilst ideally not amplifying the consumer species itself (Deagle et al. 2006). Moreover, the amplicons generated should allow distinction of consumed taxa at an appropriate taxonomic resolution. For such studies, the primers need to target a gene with primer sites conserved between target species, while amplicons need to be sufficiently short to survive digestion but sufficiently long as to provide the required taxonomic information.

A group-specific aphid primer pair published by Harper et al. (2005) promised amplification of a wide range of aphid species. However, it was not clear how well-suited it was for HTS, nor how well it might amplify, and distinguish between, different aphid species, and to what extent it also would amplify ladybirds and other arthropod taxa. Amplification performance of primers on environmental DNA can differ from *in silico* results and *in vitro* amplification of mock communities. For example, both of the latter methods showed the Clarke primers to be useful for DNA metabarcoding of insects (Clarke et al. 2014; Elbrecht et al. 2016). In a study by Alberdi et al. (2018), however, the same primers failed to amplify prey DNA from environmental samples, due to extensive amplification of non-target DNA. It is therefore important to test primer performance on real samples collected from the landscape. We focused on *Coccinella septumpunctata* (Linnaeus) and *Harmonia axyridis* (Pallas), two ladybird species (Coleoptera: Coccinellidae) known as key aphid predators in temperate agricultural landscapes (Symondson et al. 2002; Straub and Snyder 2006). While *C. septempunctata* is native to Europe, the Asian *H. axyridis* was introduced into European agricultural systems in the 1990s (Adriaens et al. 2008). The role of *H. axyridis* as a natural enemy of crop pests motivated its introduction into many agroecosystems as a non-native biocontrol agent, from where it quickly spread and out-competed local ladybird populations (Evans 2004; Brown et al. 2008). Both ladybird species are amongst the most abundant ladybirds in the studied German and Swiss agricultural regions (Klausnitzer 2002; Eschen et al. 2007). Their high functional importance as natural enemies of aphids has led to several prey choice and digestion studies under artificial conditions (Ware and Majerus 2007; Alhmedi et al. 2008). However, far less is known about aphid prey use of the two ladybirds in real agricultural landscapes. Yet, such knowledge is critical for targeted promotion of the two species as crop aphids' natural enemies, as well as to inform management decisions with respect to the conflicting role of the invasive *H. axyridis* as pest control agent on one hand and predator or competitor with native insect species on the other hand. We therefore investigated the aphid prey of *C. septempunctata* and *H. axyridis*, compared the advantages and disadvantages of different trap and hand-collection based sampling approaches in terms of sampling effectiveness and aphid DNA detectability in ladybird guts. We modified the aphid-specific primer pair designed by Harper et al. (2005) with respect to the applicability of HTS for investigating aphid prey use of functionally important ladybird species at the landscape scale. Specifically, we compared: (I) primer specificity between the existing and modified primer pairs as well as resolution of aphid identification; (II) the number of sampled ladybirds and their suitability for DNA analysis between sampling methods, and (III) aphid prey diets of field-sampled *C. septempunctata* and *H. axyridis*.

Methods

In silico and *in vitro* primer specificity

The Harper et al. (2005) general aphid primer pair amplifies a region of 308 bp of the mitochondrial cytochrome *c* oxidase I subunit (COI) gene. To assess the suitability of the primer pair for this study, a sequence library was produced by downloading and clustering COI sequences of Coleoptera, Coccinellidae, Hemiptera, Hymenoptera, Aphididae, Neuroptera and Araneae from GenBank (Benson et al. 2014) via PrimerMiner v.0.18 (Elbrecht et al. 2017). Of these, Coccinellidae and Aphididae are directly relevant to the present study, whilst the other taxa were included to assess any broader

potential of the modified primers for other studies. Sequences were aligned in Geneious Prime 2019.1.1. ((Kearse et al. 2012) via MAFFT 1.3.7. (Katoh et al. 2002)) and primer binding sites visually assessed on a subset of thirty species of aphids and coccinellids, represented by at least five sequences each. Subsequently, several modifications were made to the primer sequences to increase exclusion of ladybird DNA from amplification (Table 1), thus maximising recovery of prey reads (Deagle et al. 2006). PrimerMiner v.0.18 (Elbrecht et al. 2017) was used to visualize differences in the alignment of both the modified primers and those designed by Harper et al. (2005) to the target binding sites over the whole library (S1 Fig). The improvement of in silico primer target specificity was visualized and compared using PrimerMiner v.0.18 (Elbrecht et al. 2017) with the default table for mismatch scoring and a penalty score of >120.

The primers were further tested in vitro with DNA extracted from several ladybird, aphid and alternative predator specimens to approximately match those groups tested in silico, with particular focus on aphid diversity. These included ladybirds C. septumpunctata and H. axyridis, aphids Aphis fabae, Myzus cerasi, Brachycaudus lychnidis, Sitobion avenae, Aphis rumicis and Microlophium carnosum, and alternative predators Chrysoperla carnea, Loricera pilicornis, Pardosa pullata, Syrphidae sp. and Ichneumonidae sp. Extraction of DNA used Qiagen DNeasy Blood & Tissue Kits (Qiagen, Manchester, UK) following manufacturer instructions, but with an extended lysis time of 12 h for better penetration of chitinous insect tissue. Both primer pairs were tested in 5 µl reaction volumes comprised of 1 µl DNA, 2.5 µl PCR Multiplex Kit (Qiagen) and forward and reverse primers at 2 ng µl-1. All PCRs were carried out following: 95 °C for 15 min, then 35 cycles of 94 °C for 30 s, 51 °C for 30 s and 72 °C for 90 s, and a final extension of 72 °C for 10 min. PCR products were visualised via gel electrophoresis in 2 % agarose gels illuminated with UV light, the DNA stained with SYBR®Safe (Thermo Fisher Scientific, Paisley, UK).

Table 1. Primers designed by Harper et al. (2005) compared with those modified for this study. Details were calculated using ThermoFisher's Primer Analyzer. The primers designed by Harper et al. (2005) were reported in an unconventional manner which has been corrected in this table to allow comparison with the new modifications.

Primer	Sequence (5'-3')	Direction	Source	Tm [°C]	GC content	Molecular weight [g mol^{-1}]
Aph344F	GGAACAGGWACAGGATGAAC	F	Harper et al. (2005)	60.2	50%	6228.6
Aph149R	AATCAAAATAAATGTTGATA	R	Harper et al. (2005)	49.5	15%	6156.2
Aph344.MF	GGAACAGGWACAGGATGAACWA	F	This study	62.6	45.5%	6850.6
Aph149.MR	AATCARAATARATGTTGATA	R	This study	49.2	20%	6172.1

Taxon resolution of amplicon region

While primer specificity assessments need reference databases with broad taxon coverage, investigation of taxonomic resolution of a given amplicon mainly relies on correctly identified sequences. Especially in aphids, where morphological identification is sometimes impossible (Heie 1986), it is difficult to obtain sequences from accurately identified specimens. Nevertheless, reference

databases should be as comprehensive as possible to provide sufficient insight into both intra- and inter-specific variability. For this, the best currently available dataset for European aphids was used ((Clamens et al. 2014); It has been deposited on GenBank (Benson et al. 2014) (KF638720 to KF639739) and PhylAphidB@se website, http://aphiddb.supagro.inra.fr), which covers the full 658bp Folmer barcoding region (Folmer et al. 1994) of COI. Aphid species expected in the study region, based on vegetation and aphid-host plant relations, were added to the library from GenBank. Library sequences not identified to species level and covering less than 296 base pairs of the amplicon region were excluded. The library produced contains 1160 sequences comprising 999 sequences from the aforementioned aphid database (Clamens et al. 2014) and 161 additional sequences from GenBank (S1 Table), totalling 282 species across 95 genera. Sequences were aligned in ClustalX (Larkin et al. 2007), manually checked in BioEdit (Hall 1999) and trimmed in MEGA5 (Tamura et al. 2011). To assess aphid taxon assignability, a Blastn algorithm in Blast+ (Camacho et al. 2009) with a clustering threshold of 90% was performed on the aforementioned library. After visually screening the matches the threshold was increased to 98.36% allowing a maximum of matches at species level while excluding deviating matches as often as possible. If matching sequences originated from the same species exclusively, a taxon was considered identifiable to species level. If several species matched, it was considered identifiable to genus level, since no incorrect matches occurred for this similarity threshold at higher taxonomic levels. This library was subsequently used as a reference database for aphid species identification of our field samples. The sequence similarity threshold informed on clustering thresholds necessary for centroid generation during bioinformatics processing of field samples (99%). This similarity threshold is rather high and leads to a high number of OTUs in ladybird taxa, which would allow taxon assignment with lower similarity thresholds.

Study regions and ladybird sampling

Fieldwork was conducted in 2016 in agricultural landscapes of northern Switzerland (50 km radius around Zurich) and southern Germany (20 km radius around Landau, Pfalz). A total of 23 independent agricultural landscape sectors of 500 m radius (hereafter landscapes) were chosen with different land use compositions. In each of the landscapes, five (Switzerland, 12 landscapes) or three (Germany, 11 landscapes) sampling points were randomly selected and equipped with two types of traps (sticky trap and combi trap, see below), adding up to a total of 186 traps. To minimize the risk of sampling non-target species of high conservation concern, traps were not set up in or near nature conservation areas. Trapping was in accordance with national legislation. We obtained permits for trapping in Germany from the “Struktur- und Genehmigungsdirektion Süd”, AZ 42/553-254 486/16. In Switzerland no permits were necessary, since no trapping was done in protected areas. Sampling points were located at least 200 m apart from each other. Ladybirds were sampled at each sampling point every two weeks from April to July, yielding eight sampling rounds (S2 Table). Combi traps are a combination of pan traps and intersection window traps, having two plexi-glass windows arranged cross-wise over a yellow funnel of 42.5 cm upper diameter (Obrist and Duelli 2010; S2 Fig). At the bottom of the funnel a whirl-pack® bag (Sigma-Aldrich) was attached, filled with 95% ethanol, ensuring that captured ladybirds were preserved immediately after trapping. Each sticky trap consisted of two wooden plates (891 cm x 210 cm) painted with three lengthwise strips of UV-reflecting colour (yellow,

blue, white; Sparvar UV reflecting colour of Spray-Color GmbH) for maximum attractiveness (S2 Fig). Transparent acetate foils (Folex Foils Laserptinter BG-64 from OfficeWorld Switzerland) were attached to the plates and sprayed with insect glue (Soveurode spray glue from Witasek, Austria). The foils and the bags were mounted at two week intervals and collected after four sampling days. This is a comparably long period for samples on sticky traps intended for genetic use, but it allows collection of sufficient numbers of individuals with a reasonable sampling effort (Stephens and Losey 2004). In the 11 German landscapes, in addition to these two trap-sampling methods, habitats in the immediate surrounding of the sampling points were hand-sampled: ladybirds were collected with sweep nets from the vegetation of major habitat types present. All sampled *C. septempunctata* and *H. axyridis* were visually identified, collected into separate tubes filled with 95% ethanol and stored at -18°C until further processing.

Laboratory procedures

To reduce PCR inhibitors in the ladybird bodies and to minimize the risk for potential contamination, elytra, wings, legs and heads of ladybirds were removed before DNA extraction. Isolation of ladybird guts was not possible due to disruption of internal tissue through storing in 95% ethanol. Extractions were performed with the QIAGEN® Frozen Plant Tissue (DNeasy 96) kit on a total of 619 ladybirds following homogenisation with a QIAGEN® TissueLyser II bead mill (Qiagen, Manchester, UK). On each extraction plate (96 samples) three to six negative controls were included. The tubes assigned for negative control were treated precisely as any other sample starting from DNA extraction throughout all laboratory steps until visualisation of the PCR product. For aphid DNA amplification, the modified primers detailed above were used (Table 1). Molecular identifier tags (MID-tags) were attached to both primer pairs so that individual ladybirds could be identified after pooling during bioinformatic processing. The PCR reaction volume of 6.5µl consisted of 3.125µl Multiplex mix (Quiagen) and 0.125µl primer solution per primer, yielding a concentration of 10pmol/µl primer plus 2.125µl water and 1µl DNA per reaction tube. All PCRs took place in a GeneAmp9700 PCR system performing the following cycles: 95°C for 15min, 40 x (94°C for 30s, 51°C for 90s, 72°C for 90s) and a terminal phase of 72°C for 10min. PCR cycling conditions were optimized using PCR temperature gradients followed by examining the intensity of the PCR product after gel electrophoresis. Gel electrophoresis was run in a 2% agarose gel in Tris-acetate-buffer (TAE) running for 40min at 140 Volt, stained with 0.5 mg ml^{-1} SYBR®Safe (Thermo Fisher Scientific, Paisley, UK) to identify successful PCR amplification and to monitor possible contamination of negative controls included in the samples. All samples yielding a positive PCR product were quantified by Qubit measurements (ThermoFisher Scientific, Waltham, MA, UK) and pooled equimolarly into two pools to ensure sufficient read depth for sequencing. The pools were purified with SPRIselect (© 2012 Beckman Coulter, Inc.; left side selection with a ratio of 0.8 for both pools) to remove primer dimer and then further processed with the NEXTflex® Rapid DNA-Seq Kit from BiooScientific for library building. HTS was performed with an Illumina MiSeq Sequencer at the Genomics Research Hub at Cardiff University School of Biosciences using a MiSeq Reagent Kit v3 from Illumina (600 cycles with 2 x 300 bp). Raw MiSeq data for all samples described in the manuscript have been uploaded to NCBI Sequence Read Archive under SRA Accession number PRJNA563315. Information on bioinformatics procedure can be found in the

bioinformatics section below and in the supplementary material as well as detailed individual-level taxonomic data in the file S1 Data.

Bioinformatics

Paired-end Illumina reads were filtered for quality using Trimmomatic v0.32 (Bolger et al. 2014). The command ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 was used to remove adapters. Leading and trailing low quality bases were removed if their quality score was below 3. A minimum length of 250 bp and a minimum average base quality score of 20 over a sliding window of four bases were specified. Filtered reads were then aligned using FLASH v1.2.11 (Magoč and Salzberg 2011). The trim.seqs command was used in Mothur v1.37.1 (Schloss et al. 2009) to assign reads to their respective sample identifications based on MID tag sequence combinations (with S5_P1Oligos.txt and S5_P2Oligos.txt for the respective pools, located in the supplementary material), and allowing for one mismatch, prior to MID tag and primer removal. Subsequently, reads were demultiplexed into one file per sample using bespoke perl scripts (Supplementary material; Demultiplexing). Chimeric sequences alongside those appearing fewer than 10 times in a single sample were removed using the unoise2 and minuniquesize commands in Usearch v9.2.64 (Edgar 2010). This threshold of 10 was later adjusted to 13 for pool1 and 97 for pool2 as a method to mitigate for tag-jumping, contamination or sequencing errors following Dunn et al. 2018; Supplementary material; Mitigating tag-jumping). Usearch v9.2.64 was also used to cluster similar sequences into centroids using an identity threshold of 99% utilising the cluster_fast algorithm. The header line for each centroid was then annotated with the sample identification before concatenating all centroids into a single file ready for taxonomic assignment. The Blastn algorithm in Blast+ (Camacho et al. 2009) was used for taxonomic assignment against the library described above for analysis of taxonomic resolution. Blastn parameters were identical to the ones used for identification of taxon resolution in the region amplified i.e. a minimum read length of 296 bp and a minimum sequence similarity of 98.36%. For centroids that did not match to the library, a Blastn search was performed on GenBank.

Statistical analysis

Differences in sampling effectiveness (i.e. the number of captured ladybird individual per trap and sampling interval) of the two trap types applied (sticky traps and combi traps) were analysed by running generalised linear mixed models (GLMM) with Poisson error distributions using R package lme4 v1.1-17 (Bates et al. 2011). Models included trap type, ladybird species (*C. septempunctata* and *H. axyridis*) and their interaction as fixed factors as well as country, landscape and sampling point as nested random factors with 4 sampling intervals as random slope. Sampling intervals comprised two pooled sampling rounds of a four day duration each, with sampling effort standardised between the two trap types. DNA recovery rate (presence-absence data; i.e. the number of ladybird individuals in which aphid DNA was detected (presence) or not detected (absence) using a certain sampling method at a sampling point during a sampling interval) was compared between hand-sampled ladybirds and trap-sampled ladybirds. Samples from the two trap types (sticky traps and combi traps) were pooled in this model since no significant differences in recovery rate were detected (not shown). A GLMM with binomial error distribution and the same random structure as described above was run. In both models

log-likelihood ratio tests were used for statistical inference (Zuur et al. 2009). To explore differences in prey species used by *C. septempunctata* and *H. axyridis*, multivariate differences in detected consumed aphid species composition were assessed using the adonis function implemented in the R package vegan (2.5-2) (Oksanen 2007). The adonis function is applied on distance measures derived from a matrix, which in this case contains proportions of detected aphid species per landscape per sampling round. The matrix was Hellinger-transformed to deal with the relative data type and the high zero-ratio (Legendre and Legendre 1998) before Euclidean distances were calculated. The adonis function included ladybird species as factor using sampling round as stratum on the 999 permutations performed, so differences in aphid species composition would not interfere with differences between sampling rounds. Visualisation of the data was performed with non-metric multidimensional scaling (NMDS) of Hellinger-transformed Euclidean distances with k=2, using the metaMDS function. All statistical analysis were performed in R version 3.4.1 (Pinheiro et al. 2017).

Results

In silico and *in vitro* primer specificity

Alignments displayed clear mismatches between the primer sequences designed for this study and ladybird sequences (S1 Fig). *In silico* evaluation of the primers designed by Harper et al. (2005) suggested successful amplification of 90.61% of aphids and 29.55% of coccinellids. The primers modified for this study, however, successfully amplified 91.78% of aphids and 0% of coccinellids. The modified primer pair achieved increased amplification for Hemiptera generally and, other than a relatively low percentage of Hymenoptera, did not amplify any of the other predatory groups evaluated (Fig 1). These results were ratified in the *in vitro* tests (S4 Fig), with the Harper et al. (2005) primers achieving broad amplification success with only the Ichneumonid wasps not amplifying, although some of the ladybirds and alternative predators were amplified faintly. The modified primers, however, amplified all aphids (one slightly fainter) but none of the ladybirds or alternative predators.

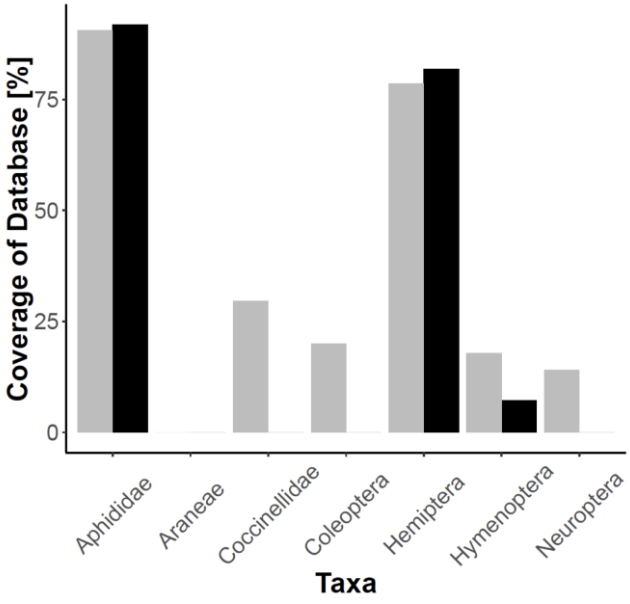

Fig. 1. Percentage coverage by the two primer pairs for different taxa. The new modified primers designed in this study (black) and those of Harper et al. (2005) (grey).

Taxon resolution of amplicon region

All 1,160 aphid sequences investigated from GenBank were assignable to genus level with a sequence similarity of 98.36% or better. Of the 32 genera investigated, 24 allowed taxon resolution to species level, covering a total of 69 species. In eight genera, taxonomic resolution to species level was not possible (*Aphis*, *Betulaphis*, *Brachycaudus*, *Dysaphis*, *Macrosiphonella*, *Macrosiphum*, *Uroleucon*, *Wahlgreniella*). Intra-specific similarity was 99.81% ± 0.05 for all species represented by two sequences or more. Within-genus variability was calculated for sequences that could only be identified to genus level and which were represented by more than two taxa per genus. Their average sequence similarity was 99.74 ± 0.23%.

OTUs retrieved from field samples

Initial read numbers following HTS were 5,668,854 and 2,772,817 from the first and second sequencing runs, respectively, resulting in an average of 6,348 and 8,531 reads per sample in each pool. After removing adaptors and low quality reads with Trimmomatic v0.32, 1,639,236 and 1,300,972 reads remained. Following alignment with FLASH v1.2.11, 1,622,258 and 1,279,716 reads were retained. Finally, 1,258,164 and 515,858 sequences remained after pairing aligned sequences with their respective MID tags in Mothur v1.37.1. Of the 141 OTUs (molecular operational taxonomic units) retrieved from analysed ladybirds, 43 could be assigned to aphid DNA sequences in the library and 89 were assigned to ladybirds (S1 Data). Eight OTUs did not match any sequence in the library and therefore a Blastn search was performed on GenBank. One further OTU could be assigned to the aphid *Laingia psammae* uniquely matching with more than 99% occurring in one ladybird individual. Resulting read numbers added up to 83,815 reads for aphids and 848,353 reads for ladybirds (see also OTU rarefaction curve Fig S3). Given the 0% amplification of ladybirds in the *in silico* and *in vitro* tests, the ladybird read proportion found in field samples is rather high. A total of 21 aphid genera were found in ladybirds. Four taxa (*Aphis*, *Brachycaudus*, *Macrosiphum*, *Wahlgreniella*) could only be assigned to genus level. A total of 20 aphid species were distributed over the 17 other genera retrieved from ladybird guts. *Microlophium carnosum* and *Aphis* spp. were the most common taxa identified. They exhibited both the highest read numbers (45,492 and 15,057, respectively) and the highest frequency in ladybird guts (found in 51.1% and 22.6 % of ladybirds positive for aphids, respectively) (S3 Table, S1 Data).

Comparison of field sampling methods

A total of 1,040 *C. septempunctata* and *H. axyridis* were sampled with the two trap-sampling methods (S2 Data). With 720 (average per trap = 0.53 ±0.04) individuals in total, sticky traps yielded significantly more ladybirds than combi traps (320 individuals, average per trap = 0.25 ±0.02). Significantly more *H. axyridis* (854) than *C. septempunctata* (186) were captured. According to an interactive effect of trap type and ladybird species, the representation of *H. axyridis* was stronger in sticky traps (88.6% of individuals) than in combi traps (67.5% of individuals; Fig 2, Table 2). Hand-collections in Germany yielded more ladybirds than trap sampling, yielding 237 *C. septempunctata* and 359 *H. axyridis* individuals.

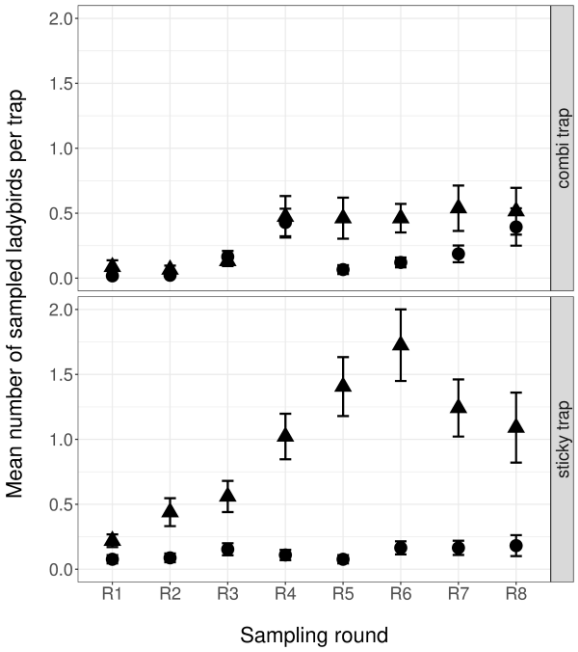

Fig. 2. Mean (± SE) numbers of sampled ladybirds with the two trap types (combi traps and sticky traps). Circles denote *C. septempunctata* and triangles denote *H. axyridis*. Sampling rounds indicate two-week sampling intervals from April to July. See methods section for detailed description of trap types and sampling design.

Aphid DNA recovery

Genetic analyses were performed on a subset of 619 ladybirds (213 *C. septempunctata* and 406 *H. axyridis*), the remaining samples were used for palynological analyses published elsewhere (Bertrand et al. 2019). Of those analysed here, 330 were hand-sampled and 289 were sampled with traps. Aphid DNA was detected in 186 ladybirds. Of the hand-sampled ladybirds, 167 were positive for aphids consisting of 82 *C. septempunctata* and 85 *H. axyridis*. A total of 19 ladybirds, from which aphid DNA was recovered, were trap-sampled, consisting of 12 *H. axyridis* and 7 *C. septempunctata*. DNA recovery rate in hand-sampled ladybirds was almost eight times higher (50.6%) than in those sampled with traps (6.6%). In addition, aphid DNA recovery was higher in *H. axyridis* (41.7%) than in *C. septempunctata* (23.9%) (Table 2, S1 Data).

Table 2. Statistical evaluation of trapping success and DNA recovery rates. Statistical inference using log-likelihood ratio tests for generalized linear mixed-effect models to test for differences in (a) ladybird trapping effectiveness of the two trap types ("trap type"; combi trap vs. sticky trap) for the two ladybird species (*C. septempunctata* and *H. axyridis*), and (b) aphid DNA recovery rates from ladybird guts for hand-sampled vs. trap-sampled ladybirds (combi traps and sticky traps combined; "sampling type") for the two ladybird species. See Methods section for detailed description of sampling design and methods, and statistical analyses.

Response	Fixed effects	*df*	X^2	*p-value*
a) Ladybird trapping effectiveness	Trap type x ladybird species	1	62.7	< 0.001
	Ladybird species	1	464.9	< 0.001
	Trap type	1	155.8	< 0.001
b) Aphid DNA recovery rate from ladybird guts	Trap type x ladybird species	1	0.7	0.413
	Ladybird species	1	6.5	0.011
	Sampling method	1	36.8	< 0.001

Ladybird diet

The diet of hand-sampled *C. septempunctata* was more variable than that of *H. axyridis* (multivariate dispersion: F = 12.03, P = 0.005). Despite some overlap in the species composition of aphids consumed by the two ladybird species the analysis revealed significant differences in aphid species compositions consumed by *C. septempunctata* and *H. axyridis* (Fig 3, F = 4.67, P = 0.020). *Microlophium carnosum* (stinging nettle aphid found in 88 ladybirds) and *Aphis* spp. (found in 25 ladybirds) were the most common taxa consumed by both ladybird species. However, *M. carnosum* was more often consumed by *H. axyridis* (72.9%) than by *C. septempunctata* (36.6%, Fig 4, S1 Data). For *C. septempunctata, Aphis* spp. and *S. avenae* (cereal aphid) comprised a greater fraction in the diet (28.0% and 13.4%) compared to *H. axyridis* (16.5 % and 2.4%, Fig 4). All other aphid taxa were only found in a few ladybird individuals. Numbers of trap-sampled ladybirds positive for aphids were too low (a total of 19 individuals) for statistical comparison of ladybird prey. While hand-sampled ladybirds were positive for 18 aphid taxa, the 19 trap-sampled ladybirds were positive for 12 taxa, of which six were found in trap-sampled ladybirds exclusively (Fig 4). Thus, the most commonly consumed aphid taxa by hand-sampled ladybirds could also be found in trap-sampled individuals.

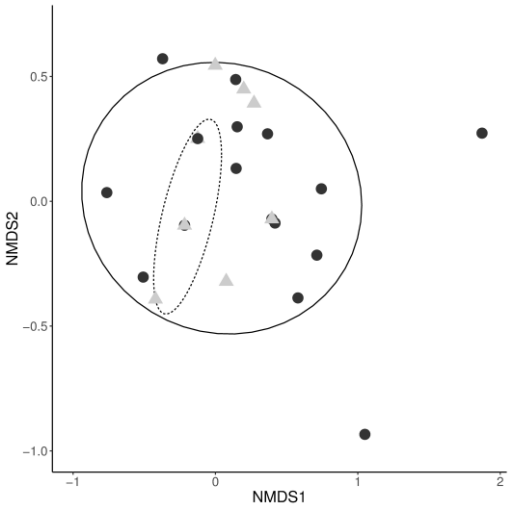

Fig. 3. NMDS ordination graph for the two hand-sampled ladybird species. comparing aphid prey species composition. Full line with points are *C. septempunctata*, grey triangles with the dashed line are *H. axyridis* (stress = 0.06), circles indicate a 95% confidence interval.

Discussion

Our results allow insights into the aphid diet of two of the functionally most important ladybird species of Central European agricultural landscapes, *C. septempunctata* and *H. axyridis,* as well as the methodological possibilities and challenges of HTS as a means for investigation of dietary use of insects in real landscapes. Evaluation of the modified primer pair showed promising results *in silico* regarding both coverage and specificity, further ratified *in vitro* with the modified primers showing far greater specificity for aphids. A wide range of aphid taxa were also amplified from field-sampled ladybirds, although with a loss in specificity. The increased predator amplification for gut content samples could suggest that the identifying tags used in this study increased predator amplification, which could be avoided by attachment of tags with the sequencing adapters rather than before the

PCR stage, although this could be associated with different biases. The amplicon region proved suitable for aphid identification to species level in most taxa, allowing insights into dietary use of hand-sampled ladybirds. Trap-sampling was mainly subject to low DNA recovery rates, yielding too few beetles positive for aphids for robust statistical analysis of prey composition, despite reasonable trapping numbers in sticky traps. *M. carnosum* and *Aphis* spp. were identified as the most frequently consumed prey in hand-sampled *C. septempunctata* and *H. axyridis*, and were also common in trap-sampled individuals.

Primer suitability

In silico and *in vitro* evaluation of both the primers designed by Harper et al. (2005) and the novel modifications from this study demonstrated improved specificity achieved by the modified primers. The modified primers achieved slightly larger coverage of aphids with greatly reduced amplification of coccinellids. The lack of amplification of many common agricultural predator groups such as spiders and ground beetles also suggests that the modified primers may be more broadly applicable to the HTS-based investigation of aphid predation by other species. The proportion of 94% predator reads recovered in our study is certainly at the high end of predator read proportions found in invertebrate predator studies (Piñol et al. 2014). Nevertheless, *in silico* and also *in vitro,* tests provide an insight

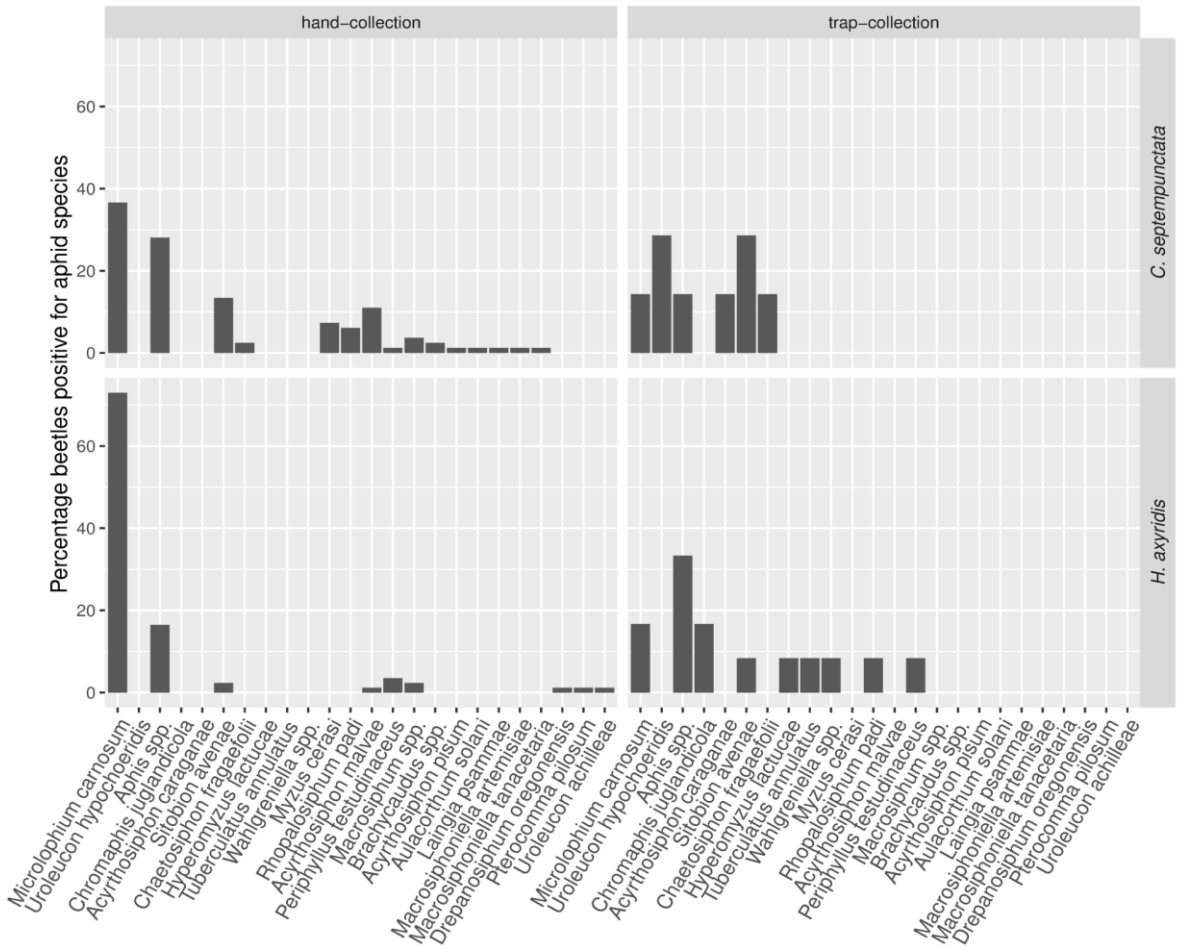

Fig. 4. Aphid prey species consumed by the two studied ladybird species. Total number of hand-sampled ladybirds positively tested for aphids was 167 compared to 19 trap-sampled ladybirds (see S1 Data for more detail).

into the potential bias against specific taxa by primers during the PCR process. That the modified primers had a 0% success rate with coccinellids *in silico* indicates a strong bias against them in PCR, likely resulting in a greater proportion of prey DNA reads post-sequencing than would be achieved with the primers designed by Harper et al. (2005) regardless of predator amplification.

The number of ladybird OTUs retrieved was relatively high. In many cases, several OTUs were attributed to the same species within the same sample. This does not, however, indicate that different individuals were present, with large variation in sequences observed in other metabarcoding studies of invertebrate diet (Lafage et al. 2019). Such variation could be due to sequencing errors, for example, but equally could arise from additional copies of the COI gene, such as nuclear mitochondrial pseudogenes, which are difficult to distinguish from mitochondrial DNA (Moulton et al. 2010). The assumption that these sequences represent the same individual is consequently justified, especially given that the focus of this study is on the predation of aphids, and the ladybird identifications were morphologically confirmed prior to sequencing. Instances in which ladybird DNA, other than that of the predator respective to each sample, was identified may indicate intraguild predation. Ladybirds are known to engage in intraguild predation, particularly consumption of other ladybird eggs and larvae (Gardiner et al. 2011); whilst this is unarguably of agricultural significance, it was beyond the remit of this study, but highlights the possibility of future investigations of this aspect of ladybird biocontrol dynamics. Equally, co-occurrence of different ladybird samples could indicate cross-contamination of ladybirds during trapping. Attempts were, however, made to mitigate risk of this by removal of external wing cases and other non-essential body parts, indicating a greater likelihood of the aforementioned intraguild predation. When amplifying DNA of the predator in dietary metabarcoding, the high volume of predator reads generated could also increase the rate of tag-jumping and misassignment between samples, which may result in predator reads appearing in other samples. Whilst this would be problematic for broader dietary studies, the focus of this study on aphid predation circumvents the issue, although it is certainly worth considering for future studies pertaining to intraguild predation.

Taxonomic identification for this amplicon region was possible down to species level in 75% of European aphid genera based on the library used. This is good considering the relatively short amplicon length of 308 bp and the often cryptic taxonomy of aphids (Ortiz-Rivas and Martínez-Torres 2010), though resolution may be weaker on a global scale. The taxa for which resolution was lower also proved difficult to identify to species level both morphologically and genetically, even when using the entire Folmer COI barcoding region (Clamens et al. 2014). It is questionable whether any sequence fragment in a size suitable for gut content analysis could facilitate better taxonomic resolution within COI. Other aphid specific primer pairs have been reported for the ribosomal 18S and mitochondrial COII barcoding regions (Chen et al. 2000; Staudacher et al. 2016), but both markers did not provide enough reference data on NCBI or BOLD to even identify all species found in ladybird guts analysed here. Currently, there seems to be a trade-off for broad taxonomic assessments between ribosomal and mitochondrial barcoding regions. It is not yet clear which region provides better taxonomic resolution (Clarke et al. 2014), but currently 16S primer sets seem to give better taxonomic coverage, amplifying taxa more evenly, while far more sequence information is available for COI

(Clarke et al. 2014; Elbrecht et al. 2016). An extensive reference database for sequence identification is a prerequisite for any study aiming at obtaining a realistic insight into the dietary habits of its study organisms. Especially on a global scale, barcode availability for any aphid amplicon region is still low. The last count by Lee et al. (2017) of the approximately 5000 species of aphids described in the world revealed that only 10% had a barcode on one of the commonly used platforms (BOLD or NCBI), which imposes major limitations to HTS.

Trapping methods and DNA recovery

Sticky traps were more effective in trapping ladybirds than combi traps in the present study. However, DNA recovery was similarly limited for both trap types, yielding very few individuals testing positive for aphid DNA in the ladybirds' guts. Hand-sampling seems more effective in this respect, with a DNA recovery rate almost eight times higher than in trap-sampled ladybirds. Even though sticky traps did not yield sufficient data to statistically evaluate aphid prey composition, they do provide valuable information, putting results of hand-sampled ladybirds into perspective. The dominant species found in hand-sampled ladybirds were found in trap-sampled ladybirds as well. Of the 12 taxa identified in trap-sampled ladybirds, six were not found in hand-sampled ladybirds, however. Remarkably, two of these species feed on high stemmed trees exclusively, which are difficult to access when hand-sampling: *Chromaphis juglandicola* on *Juglans regia* (Walnut) and *Tuberculatus annulatus* on *Quercus* spp. (Oak). In contrast, none of the aphid species detected in hand-sampled ladybirds are specific to high stemmed trees. Another noticeable result concerns the number of aphid taxa recovered by trap-sampling and hand-sampling. The ratio of aphid taxa found in ladybirds relative to the number of ladybirds testing positive for aphids was six times higher in trap-sampled ladybirds (0.63) than in hand-sampled ladybirds (0.11). This indicates that diet information derived from hand sampling is biased towards low numbers of species. Given the eight times higher aphid DNA detection rate in hand-sampled individuals, the use of traps might seem to be a high price to pay for a less biased, broader insight into predator diets. However, DNA recovery rates from trapped ladybirds have the potential to be improved (e.g. with shorter trap activity periods), while minimized sampling bias is crucial to inform on ladybird diet at the landscape scale. Taking these findings together, they suggest that sticky trap-sampling likely gives more representative insights into ladybird diet by considerably reducing sampling bias towards the sampled local vegetation. A further advantage of sticky trap-sampling is the reduction in potential cross-contamination, reported to be problematic in methods allowing interactions of trapped insects in the sampling container, such as in combi traps, sweep-netting, beating or vacuum sampling (Greenstone et al. 2011; King et al. 2012; Athey et al. 2017). Thus, there are some limitations for this method mainly imposed by trade-offs between sampling effectiveness, DNA recovery rate and sampling effort. DNA detectability half-lives are influenced by many factors but were generally found to be less than a day for aphids in arthropod predator guts (Chen et al. 2000; Harper et al. 2005). The detection rates on field samples yielded by our primers were unknown but sufficient trapping rates were a prerequisite for any aphid DNA detection and were ensured by elongating trapping periods to four days (Stephens and Losey 2004). In future we recommend daily sample collection from sticky traps with more sampling rounds and/or traps to ensure sufficient sample size.

This comes at a cost of higher sampling effort, but appears necessary for a representative picture of predator diet.

Ladybird diet

Of the 18 aphid taxa identified in the 167 hand-sampled ladybirds' guts, six were prey taxa shared between *C. septempunctata* and *H. axyridis*. *M. carnosum* was consumed by almost twice as many *H. axyridis* as *C. septempunctata*; nevertheless, it was the main prey found in both ladybirds. Similarly, the percentage of recovered pest taxa was comparable between ladybird species (33.4% and 46.7%, respectively), though, unlike *H. axyridis*, *C. septempunctata* consumed clearly more *S. avenae,* which aligns with the findings of Honěk and Martinková 2005, who highlighted the association between *C. septempunctata* and cereal crops. Most other taxa were only detected in a relatively low number of ladybird individuals in either ladybird species, with limited overlap between *C. septempunctata* and *H. axyridis*. Accordingly, prey composition differed significantly between the two ladybird species. Several reasons are discussed for the rapid increase in dominance by the invasive *H. axyridis* in European ladybird communities, mainly intraguild predation, apparently common in *H. axyridis* (Pell et al. 2008), and food resource competition (Roy et al. 2006; Honek et al. 2016). Shared use of frequently consumed aphid preys, which are specialised on a specific host plant species, as shown in our study, certainly increases the potential for resource competition between the two studied ladybird species. This makes *C. septempunctata* vulnerable to competition and intraguild predation by *H. axyridis* and may be a reason why *H. axyridis* so strongly dominates local ladybird populations as recorded in this and other studies (Alhmedi et al. 2009; Honek et al. 2016). Despite these results according with previous findings, a potential observer bias present in the hand-collected ladybird samples should be considered, given that an effect by choice of the local sampling vegetation cannot be excluded here. For this reason, future improvement of trap-based sampling methods is important.

Conclusions and implications

This study highlights some important methodological challenges, but also presents potential solutions towards improved sampling, when using HTS for investigations of prey use by insect predators at the landscape scale. Our modified primers gave us insights into aphid prey use by ladybirds, amplifying a wide range of aphid taxa that could be identified to species level. The primer set used for this study still has restrictions in both specificity and taxon resolution, but, as long as insufficient reference data are available for primers situated in more suitable barcoding regions, we think that this primer is probably among the best current solutions for broad taxonomic screenings for aphids in ladybird guts.

For acquiring insights into diets of mobile flying insects at scales beyond local vegetation (e.g. at the landscape scale), we recommend sticky traps for future investigations. Our findings indicate a more complete spectrum of prey taxa retrieved, including taxa from a broader range of habitats likely used by prey that are not usually accessible via hand-sampling. Our findings regarding the species composition of the consumed aphids by the invasive *H. axyridis* compared to the native *C. septempunctata* indicate significant dissimilarities in prey communities, but also several shared aphid prey, including host-specific species. The latter thus provides some support with real agricultural landscape data on resource competition between the invasive and the native species, as has been

suggested by previous studies. The dominance of nettle aphids in both ladybird species underlines the role of nettle as an important source habitat of beneficial insects in agricultural landscapes.

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Supplementary material

Electronic supplementaries

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0235054

S1 Table. Library for aphid taxon resolution assessments.

S1 Data. Analysed ladybirds with aphid OTU and read information.

S2 Data. Trapped ladybirds.

S5 P1SampleList1. File for deplexing pool1.

S5 P1SampleList2. File for deplexing pool1.

S5 P2SampleList1. File for deplexing pool2.

S5 P2SampleList2. File for deplexing pool2.

S5 P1Oligos. File for deplexing pool1.

S5 P2Oligos. File for deplexing pool2.

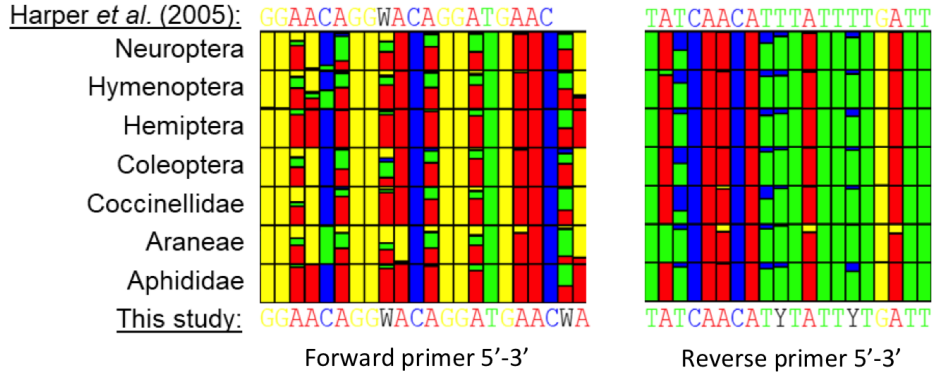
Supplementary results

S2 Table. Sampling rounds and corresponding dates.

Round	Time interval	Dates
R1	Int 1	01/04/16 - 14/04/16
R2	Int 1	15/04/16 - 27/04/16
R3	Int 2	28/04/16 - 08/05/16
R4	Int 2	09/05/16 - 19/05/16
R5	Int 3	20/05/16 - 30/05/16
R6	Int 3	31/05/16 - 13/06/16
R7	Int 4	14/06/16 - 26/06/16
R8	Int 4	27/06/16 - 12/07/16

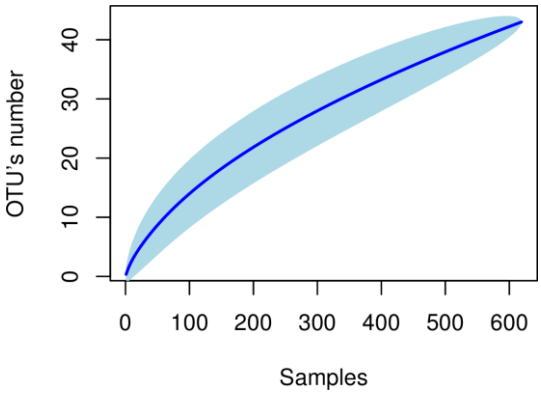
S3 Table. Aphid OTU and species information retrieved from ladybird samples.

Aphid taxa	Aphid reads	OTU per taxon	Occurrence in *C. septempuctata*	Occurrence in *H. axyridis*
Acyrthosiphon caraganae	17	1	1	0
Acyrthosiphon malvae	1968	2	9	1
Acyrthosiphon pisum	22	1	1	0
Aphis spp.	15057	10	24	18
Aulacorthum solani	17	1	1	0
Brachycaudus spp.	1471	2	2	0
Chaetosiphon fragaefolii	429	1	3	0
Chromaphis juglandicola	770	1	0	2
Drepanosiphum oregonensis	556	1	0	1
Hyperomyzus lactucae	59	1	0	1
Laingia psammae	258	1	1	0
Macrosiphoniella artemisiae	37	1	1	0
Macrosiphoniella tanacetaria	14	1	1	0
Macrosiphum spp.	1603	3	3	2
Microlophium carnosum	45492	2	31	64
Myzus cerasi	2023	2	6	0
Periphyllus testudinaceus	4548	3	1	4
Pterocomma pilosum	205	1	0	1
Rhopalosiphum padi	383	1	5	1
Sitobion avenae	4089	3	13	3
Tuberculatus annulatus	259	1	0	1
Uroleucon achilleae	123	1	0	1
Uroleucon hypochoeridis	4363	1	2	0
Wahlgreniella spp.	52	1	0	1
Total	83815	43	105	101

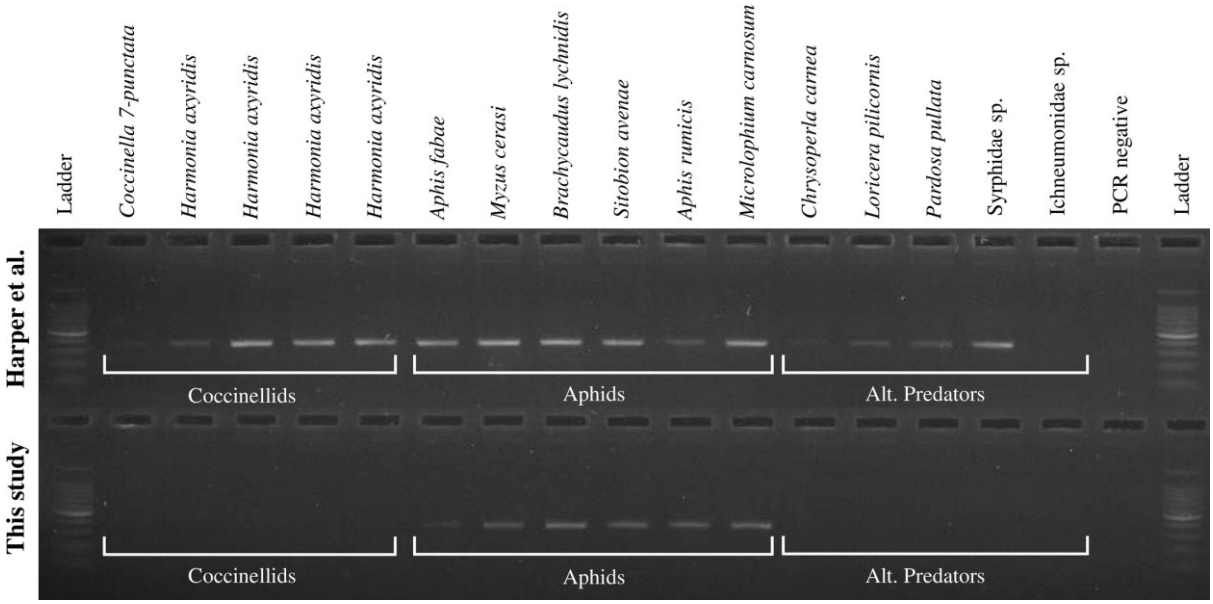


S1 Fig. Visualisation of primer binding sites. Primers modified for this study and those used by Harper et al. (2005) aligned with mass-alignments of taxa downloaded via PrimerMiner. The forward primer is on the left, the reverse primer on the right, both sequence alignments are oriented in 5' – 3' direction.

S2 Fig. Sampling point with the two trap types. The combi trap (yellow, 42.5 cm upper diameter) has a whirl-pack® bag (Sigma-Aldrich) attached on the bottom, filled with 95% ethanol and a roof on top to prevent dilution through rain. For the sticky trap (891cm x 210cm), foils (Folex Foils Laserptinter BG-64 from OfficeWorld Switzerland) are attached with clothes pegs and/or tape to the coloured board (yellow, blue, white; Sparvar UV reflecting colour of Spray-Color GmbH) and were then sprayed with glue (Soveurode spray glue from Witasek, Austria).



S3 Fig. OTU rarefaction curve over ladybird samples. Curvature index: $y = 0.5826x^{0.675}$ on 619 samples.



S4 Fig. *In vitro* comparison of primers. The modified primers of this study show increased specifity towards target aphid DNA, missing to amplify ladybird DNA.

Details on bioinformatics procedures

This supplementary document contains information for demultiplexing samples, including the necessary skripts and detailed procedures for dealing with tag-jumping.

Demultiplexing

This section includes two sets of perl scripts to demultiplex the data following the previous processing steps in Mothur v1.37.1 (41). Both perl scripts are edited for use with Pool 1. The four associated text files required to complete these processing steps on Pool 1 and Pool 2 are located in the supplementary material called “S5_P1SampleList1.txt” and “S5_P1SampleList2.txt” for Pool 1 and “S5_P2SampleList1.txt” and “S5_P2SampleList2.txt” for Pool2.

Script 1 “deplexstep1.pl” :

```
#!/usr/bin/perl
unless ($#ARGV == 0)
{
print "Usage: deplexstep1.pl S5_P1SampleList1.txt";
die;
}

open (INLIST, "<$ARGV[0]") || die;

# replace 'XXX' with your input and output directories
$indir = " XXX ";
$outdir = “XXX ";

# Loops through the list of your samples (in 'S5_P1SampleList1.txt ') and performs the commands for each one
while (<INLIST>) {
$lib = $_;
chomp($lib);

# A shortcut to read or write a file for each of your samples, each file having the same extension
$readids1 = $lib . "_ids.txt";
$fa1 = $lib . ".fasta";

# split fasta read IDs into files grouped by sample ID. Replace 'XX' with the name of the '.groups' file (output from mothur)
system("grep -w $lib $indir/XX.groups  | awk '{print \$1}' > $outdir/$readids1");
}
exit;
```

Script 2 “deplexstep2.pl” :

```
#!/usr/bin/perl
unless ($#ARGV == 0)
{print "Usage: deplexstep2.pl S5_P1SampleList2.txt";
die;}

open (INLIST, "<$ARGV[0]") || die;

# replace 'XXX' with your input and output directories
$indir = " XXX ";
$outdir = “XXX ";

# Loops through the list of your samples (' S5_P1SampleList2.txt ') and performs the commands for each one
while (<INLIST>) {
$lib = $_;
```

```
chomp($lib);

# A shortcut to read or write a file for each of your samples, each file having the same extension
$fa1 = $lib . ".fasta";

$readidsa = $lib . "a_ids.txt";
$readidsb = $lib . "b_ids.txt";
$readids2 = $lib . "_ab_ids.txt";

# combine the list of sequence names for 'a' and 'b' matches
system("cat $outdir/$readidsa $outdir/$readidsb >> $outdir/$readids2");

# split the trimmed fasta file into reads specific to each sample. Replace 'XX' with the name of your
trimmed fasta file (output from mothur)
my $command1 = 'perl -ne'."'".'if(/^>(\S+)/){$c=$i{$1}}$c?print:chomp;$i{$_}=1 if'." @ARGV'"."
$outdir/$readids2 $indir/XX.fasta > $outdir/$fa1";

system ($command1);
}
exit;
```

Mitigating tag-jumping

In this supplementary section we outlined the steps taken to mitigate the combined effects of tag-jumping (after Dunn *et al.* 2018). As detailed in the methods section of the manuscript, using Usearch v9.2.64 we initially removed those sequences that appeared fewer than 10 times in a sample. The bioinformatics pipeline was then run to the end, giving a preliminary output table on which decisions over cut off levels were made. Since only samples testing positive for a PCR product after gel electrophoresis were pooled, samples yielding no PCR band should not occur in the dataset. We therefore screened the preliminary output table for occurrence of any samples positive for Aphid DNA that were not pooled. The highest sum of reads found in such samples was then applied as a new threshold and the bioinformatics pipeline was re-run from the Usearch step onwards, using the newly identified thresholds in the minuniquesize command. Thus, all samples including less than this number of reads were excluded from any further analysis. For pool 1 the read threshold was set to 12 giving 8.06 % of false positives and in pool 2 to 96 giving 7.00% false positives (false positive percentages are percentage of unpooled samples showing positive for aphid DNA).

Reference:

Dunn JC, Stockdale JE, Moorhouse- Gann RJ, et al (2018) The decline of the Turtle Dove: Dietary associations with body condition and competition with other columbids analysed using high-throughput sequencing. Mol Ecol 27:3386–3407

Chapter 5

Synthesis and outlook

Lolita Ammann

Optimising landscapes for pollinators and natural enemies of crop pests

Understanding interactions of specific ecosystem service (ES) providing groups and the resources they use in a given landscape is an important step forward towards ecological intensification (Bommarco et al. 2013). However, the biggest challenge probably comes along with creating landscapes that simultaneously provide different functional groups to the right crops without mutual impairment (Bommarco et al. 2013). Our results show, how variable the floral resources can be, that SNHs and crops provide and to what extent pollinators and natural enemies of pest aphids differ in their habitat preferences and food resource needs. All three chapters point to the importance of forest edge habitat for promoting pollinators and natural enemies. Both functional groups were more abundant in landscapes with higher shares of forest edge habitat. For rare bees and important crop pollinators, floral resource abundance and composition offered by forest edges was important throughout the season. For the investigated predators, despite partly relying on floral resources to complete their life cycle (Landis et al. 2000; Symondson et al. 2002; Wäckers and Van Rijn 2012; Lu et al. 2014), other resources offered by forest edges seem to be even more important (Bartual et al. 2019). Gut content analysis of ladybirds detected large portions of individuals consuming stinging nettle aphid (*Microlophium carnosum*), a species often occurring along forest edges in the studied region (Chapter 4; Ammann et al., 2020a). Hence, forest edges may have the potential to offer important alternative prey to aphidophagous ladybirds, but likely also to other natural enemies of crop pests (reviewed by Holland et al. 2016). Furthermore, the increased predator numbers on faba bean phytometers relating to forest edge habitat did translate into improved aphid control, indicating the potential of forest edges for natural pest control (Chapter 3; Ammann et al. 2020b).

The floral resource maps showed, how important the spatio-temporal variation of flower availability can be in different SNH such as grassland, forest edges, semi-open habitat, but also crops. Crops and semi-open habitat provided the majority of floral resources, creating a huge resource pulse in April with the flowering of oilseed rape and fruit trees. This resource pulse did relate much less to bees than forest edges and grasslands, although their contributions to total flower abundance was much less (Chapter 2; Ammann et al. 2020c). Grasslands provided by far the smallest portion of floral resources in the landscape, yet supporting a steady supply of high floral diversity, similar to forest edges. Grasslands and forest edges had a clear positive effect on all groups of bees, showing the important role of SNHs can take in complementing large crop resource pulses through offering diverse and abundant alternative supply of flowers throughout the season (Scheper et al. 2014; Baude et al. 2016; Sutter et al. 2017). Our results reveal the potential of forest edges and grasslands to synergistically support conservation objectives, such as biodiversity conservation, protection of rare bees and promotion of crop pollinators and pest predators and the functions they provide.

Given the large differences in amount and composition of floral resources in different habitat types and the distinct effect of habitats on bees and predators, it is not surprising, that SNH without further characterisation fail predicting the abundance and diversity of the two functional groups. Despite research finding generally positive effects of SNH on pollinators and pest predators, inconsistencies were also observed (e.g. Tscharntke et al. 2012; Rusch et al. 2016; Sutter et al.

2018a; Martin et al. 2019), indicating that we need to better understand underlying mechanisms. Recent work has emphasized the importance of more detailed characterisation of landscapes, including more precise identification of habitat types (Bartual et al. 2019; Kheirodin 2020) and more appropriate characterisation of habitat traits (Bartual et al. 2019; Cole et al. 2020; Kheirodin 2020). The extremely detailed habitat maps established for the landscapes that were investigated, meet those requirements. The improved prediction of bees through floral resource maps, as compared to classical habitat maps, confirm that “functional habitat maps” are more adequate. However in some situations, there might be factors other than flowers, which may be more important for prediction of pollinators and pest enemies. Findings from a similar field study indicate that pollination services are better predicted by classical habitat maps (Eckerter et al. *subm*). As for pollination services, in this thesis prediction of the abundance of pest predators was not improved by floral resource maps. Predators seem to be limited by other resources than flowers offered by forest edges, possibly shelter or alternative prey (Holland et al. 2016; Schirmel et al. 2018; Sutter et al. 2018b). Unlike bees, natural enemies’ food resources are not directly linked to vegetation but interrupted by a further trophic link. This is why I investigated the prey found in the guts of coccinellids, using high throughput sequencing. Although it is a powerful tool for broad scale screenings of taxa (Weber and Lundgren 2009; Pompanon et al. 2012), limitations such as the lack of sufficient reference data, suitable sampling methods and the right primers for amplification still exist (King et al. 2008; Baker et al. 2014; Lee et al. 2017). Our findings help to address those limitations gaining insights into the prey use of lady birds on a landscape-scale, leading to recommendations about sampling strategy and a novel aphid primer.

I do believe, that the deeper we dig into ecological processes and relations, the more specific these relations become in respect to different agro-ecological systems. Each system is a picture of its own, built from the puzzle pieces of its inhabitants and their interplay. I have the impression that we currently try to complete a picture with pieces from different puzzles. Focusing on understanding few systems in their entirety could probably lead research forward more quickly, allowing to adapt working models to similar systems, once completed.

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Ammann L, Bosem-Baillod A, Eckerter PW, Entling MH, Albrecht M, Herzog F (XXXXb) Aphid predators reduce pest aphids and are better predicted by classical habitat maps than floral resource maps. Landsc ecol

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Appendix

A: Status and author contributions of publication chapters

B: Author affiliations

C: Curriculum vitae

D: Declaration according to §8 of the Promotionsordnung des Fachbereichs 7: Natur- und Umweltwissenschaften der Universität Koblenz-Landau, Campus Landau vom 14.06.2013

Appendix A

Status and author contributions of publication chapters

Chapter 2 – in preparation
L.A., M.A., A.B., F.H., and M.E. designed the study; L.A. and A.B. collected the data and lead the field work; D.F. collected and compiled the flower trait data; L.A. performed the analysis and L.A. and M.A. wrote the manuscript.

Chapter 3 - submitted
L.A., M.A., A.B., F.H., P.E and M.E. designed the study; L.A. and A.B. collected the data and lead the field work; L.A. and M.A. performed the analysis and L.A. wrote the manuscript.

Chapter 4 - accepted
L.A., M.A., C.B., F.H., L.M. and M.E. designed the study; L.A., C.B., and L.M. collected data and lead the field work. N.P. provided aphid data. R.M., J.C., A.E, M.A. and L.A. processed and analysed the data. L.A. wrote the manuscript.

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Appendix C: Curriculum vitae

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11/2012 – 07/2014	M.Sc in Systematics and Evolution; "*Linking patterns of plant diversity and water use efficiency along two elevational gradients in La Réunion"*
09/2009 – 08/2012	B.Sc in Biology
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04/2015 – 10/2015	Internship at experimental unit of agriculture, Strickhof Lindau, Switzerland
06/2014 – 09/2014	Internship at WSL (Swiss federal institute for Forest, Snow and Landscape research)
11/2014	Habitat restoration in Sinclair Wetlands, Dunedin, New Zealand
12/2012 – 01/2013	Internship at the Department of Systematics and Evolutionary Botany, Zürich, Switzerland

Publications

Published (peer-reviewed)

Bertrand C., Eckerter P., Amman L., Entling M.H., Gobet E., Herzog F., Mestre L., Tinner W., Albrecht M. (2019) Seasonal shifts and complementary use of pollen sources by two bees, a lacewing and a ladybeetle species in European agricultural landscapes. Journal of Applied Ecology 56(11), 2431 – 2442 DOI: 10.1111/1365-2664.13483

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Sylvester S. P., Quandt D., Ammann L., & Kessler, M. (2016). The world's smallest Campanulaceae: Lysipomia mitsyae sp. nov. *Taxon*, *65*(2), 305-314.

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Ammann L., Moorhouse-Gann R.J., Cuff J., Bertrand C., Mestre L., Pérez Hidalgo N., Ellison A., Herzog F., Entling M.H., Albrecht M., Symondson W.O.C. (2020) Insights into aphid prey consumption by ladybirds: Optimising field sampling methods and primer design for High Throughput Sequencing. PLoS One

Submitted
Ammann L., Bosem-Baillod A., Eckerter P.W., Entling M.H., Albrecht M., Herzog F. Aphid predators reduce pest aphids and are better predicted by classical habitat maps than floral resource maps. Landscape ecology

In preparation
Ammann L., Bosem-Baillod A., Herzog F., Frey D., Entling M.H., Albrecht A. Spatio-temporal floral resource availability across different habitats drives wild bee communities in agricultural landscapes. Journal of Applied Ecology

Frey D., Amman L., Albrecht M., Moretti M. Functional and structural blossom and flower traits of animal pollinated plants of urban gardens. Data in brief

Conference presentations

Ammann L., Bosem-Baillod A., Eckerter P.W.,. Entling M.H., Albrecht M., Herzog F. Classical habitat maps or food resource maps – which one predicts predator abundance in agricultural fields better? Milan, International Association of Landscape Ecology IALE, 1-5 July 2019.

Ammann L., Moorhouse-Gann R.J., Cuff J., Bertrand C., Mestre L., Pérez Hidalgo N., Ellison A., Herzog F., Entling M.H., Albrecht M., Symondson W.O.C. Analysis of spatio-temporal food resource exploitation by aphid predators may help to promote pest control services. Vienna, Annual meeting GfÖ, 10-14 September 2018.

Poster

Ammann L., Bertrand C., Mestre L., Herzog F., Eckerter P.W., Entling M.H., Albrecht M., Symondson W.O.C. Assessing food resource requirements of key crop pollinators and predators of agricultural pests. Annual meeting BES, 11-14 December 2016

Appendix D

Declaration according to §8 of the Promotionsordnung des Fachbereichs 7: Natur- und Umweltwissenschaften der Universität Koblenz-Landau, Campus Landau vom 14.06.2013

Erklärung des Doktoranden darüber,

dass er die eingereichte Dissertation selbstständig verfasst hat und alle von ihm für die Arbeit benutzten Hilfsmittel und Quellen in der Arbeit angegeben sowie die Anteile etwaig beteiligter Mitarbeiterinnen oder Mitarbeiter sowie anderer Autorinnen oder Autoren klar gekennzeichnet sind;

dass er nicht die entgeltliche Hilfe von Vermittlungs- oder Beratungsdiensten (Promotionsberater oder andere Personen) in Anspruch genommen hat;

dass er die Dissertation nicht in gleicher oder ähnlicher Form als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung im In- oder Ausland eingereicht hat;

ob er die gleiche oder eine andere Abhandlung in einem anderen Fachbereich oder einer anderen wissenschaftlichen Hochschule als Dissertation eingereicht hat, ggf. mit welchem Erfolg; - nicht zutreffend

dass ihm bewusst ist, dass ein Verstoß gegen einen der vorgenannten Punkte den Entzug des Doktortitels bedeuten und ggf. auch weitere rechtliche Konsequenzen haben kann;

Zürich, 30.06.2020

Lolita Ammann