Towards ecological intensification:

The relative importance of wild pollinators as an agricultural input in seed production

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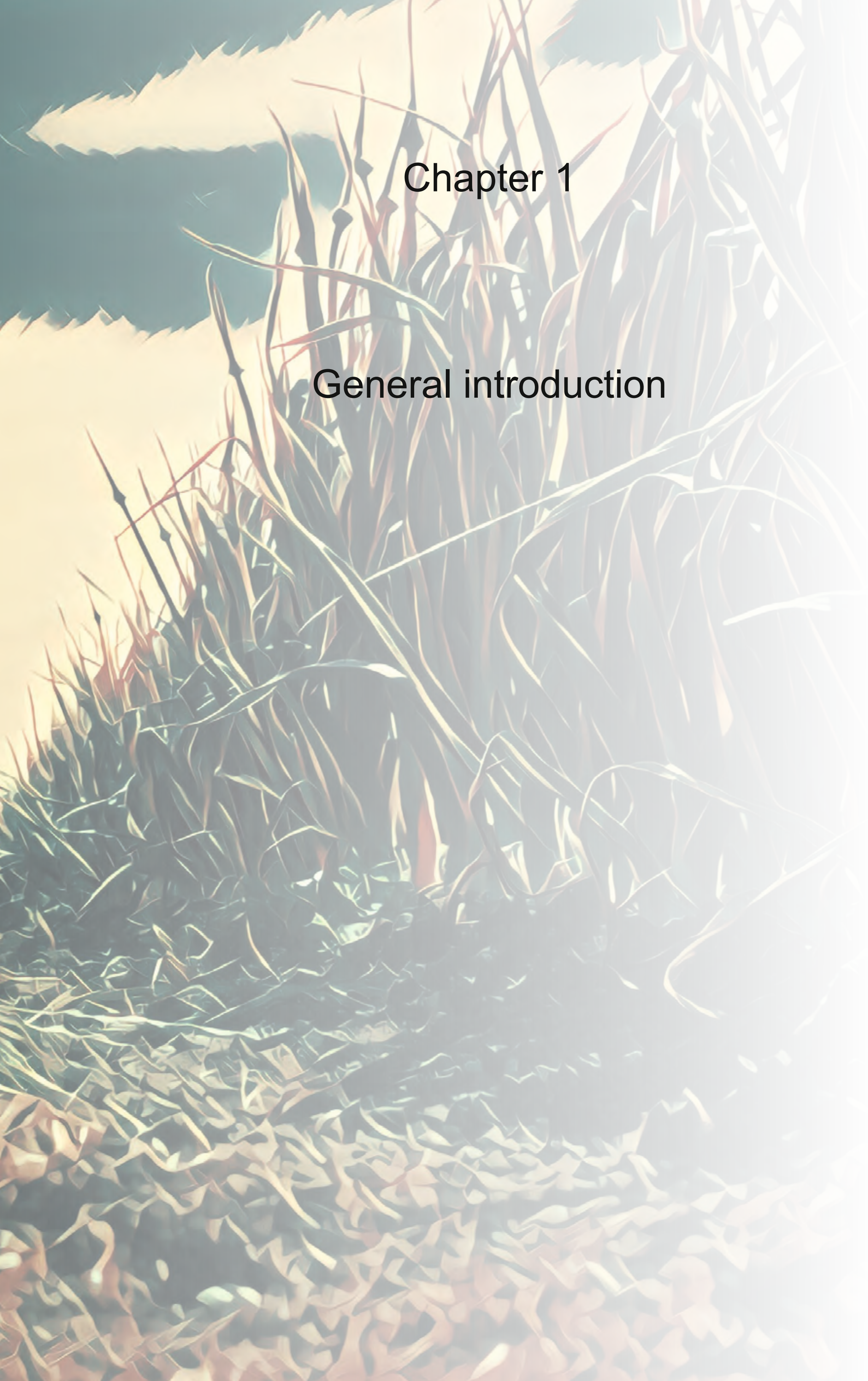
Chapter 1

General introduction



How to meet rising demands of food production

Agriculture is the most widespread form of human land-use in the world, with currently about 40% of Earth's ice-free land mass dedicated to agriculture (Foley et al. 2011). Because agriculture is such a dominant land-use, it is not surprising that agricultural practices have an influence on the natural ecosystems that surround agricultural lands (Foley et al. 2005). There are increasing worries that intensive agriculture does not make sustainable use of natural resources, and consequently, that intensive agriculture is detrimental to natural ecosystems (Foley et al. 2011, Tilman et al. 2011, Bommarco et al. 2013). To understand how intensive agriculture became to be unsustainable, we need to look at the history of agriculture. The origin of agricultural practices is still a topic of debate, but it is certain that agriculture has developed relatively slowly for long parts in the history of agriculture (Pringle 1998). There have been roughly three agricultural revolutions in Europe where agricultural development was relatively fast: the First Agricultural Revolution occurred around 10 000 BC when hunting and gathering was gradually replaced by settled agriculture (Pringle 1998). The Second Agricultural Revolution took place roughly in the period from the 17th to the 19th century, when small improvements in tools, infrastructure and the introduction and optimisation of the crop rotation increased the production relatively fast (Zanden 1991). The Third Agricultural Revolution is also known as the Green Revolution and started approximately mid-20th century, when technological advances allowed larger machines to work the land, artificial fertilisers (partially) replaced animal manure, and plant breeding increased productivity of crop plants (Tester and Langridge 2010). The effects of the Green Revolution are staggering: a 2.5-fold increase in cereal crop yields in only 50 years (Tilman et al. 2002). The Green Revolution is still taking place in large parts of the world and is characterised by agricultural expansion and agricultural intensification.

Agricultural development is in a positive feedback loop with population growth. With each step of agricultural development it can feed more mouths, but with increasing populations it is also required to find the next agricultural development to feed even more mouths. In that respect, the Green Revolution, or agricultural intensification, has been successful: there is still enough food to feed the rapidly growing world population (Rosegrant et al. 2001). The world population is expected to grow further to around 10 billion in the middle of the 21st century (Willett et al. 2019), and may then plateau (Lutz et al. 2001). It is therefore likely that the food demands to feed the growing world population will continue to increase for at least a couple of more decades (Godfray et al. 2010), but also to meet the increased consumption rate that comes with development (Tilman et al. 2011).

As the availability of agricultural land is limited, particularly in densely populated areas (Lambin and Meyfroidt 2011), modern agricultural practices are mostly aiming to increase crop yields per surface area (i.e. agricultural intensification). To date, some of the most successful measures taken to increase farm's crop yields are the increase of artificial inputs (i.e. fertilisers, irrigation and pesticides), development of improved crop varieties through plant breeding, farm specialisation, and more efficient land use through up-scaling of production systems and improved technology (Godfray et al. 2010). This up-scaling is facilitated by the so-called technological treadmill (Duffy 2009). With decreasing margins, it becomes profitable for growers to scale-up and farm more land. But because you farm more land in the same growing season, you need to have larger machines that can work the land more efficiently. Because of the increased costs of the new technology, the profit margins drop, and the treadmill continues. This development of agricultural intensification, facilitated by technological advances and artificial agricultural inputs has indeed made agriculture more efficient in terms of agricultural production per surface area.

Towards an ecologically sustainable agricultural system: ecological intensification

While technological advances allow for agricultural development, agriculture depends fundamentally on natural processes: the ecosystem services made freely available by the natural ecosystem. For example, crop plants depend on the nutrients that are available in the soil they grow on, and soil biota break down the dead plant material to make these nutrients available again to the plants (Bardgett and van der Putten 2014). One of the most tangible and visible ecosystem services is the pollination of crops that depend, at least partly, on animals that visit the crop flowers (IPBES 2016). Approximately one third of the world food production, but two-third of the crops, depends to some extent on animals for crop production (Klein et al. 2007). This service is therefore particularly valuable, and is estimated to contribute approximately 153 billion dollar of worldwide crop value annually (Gallai et al. 2009). The majority of pollinators are insects, and in particular bees and flies are important contributors (Garibaldi et al. 2013, Rader et al. 2016), making these groups essential for animal pollinated crop yields.

Paradoxically, agricultural intensification has severe negative effects on biodiversity and the associated ecosystem services on which agriculture so much depends (Tilman et al. 2011, Deguines et al. 2014, Wagg et al. 2014). In particular the combination of the eutrophication of semi-natural habitats (Clark and Tilman 2017) and the loss of semi-natural habitats (Hoekstra et al. 2004, Tscharntke et al. 2005) have caused large biodiversity declines in agricultural landscapes (Donald et al. 2001, Tscharntke et al. 2005, Potts et al. 2010). For example, with increasing levels of agricultural intensification, both the diversity and abundance of bees decline significantly (Le Féon et al. 2010). As a result, agricultural intensification practices to increase crop yield seem to be less effective with increasing dependency on insects for pollination (Deguines et al. 2014). This suggests that insect pollination is limiting crop yields of insect-dependent crops more than other agricultural management measures. This is becoming increasingly problematic, because the cover of insect-dependent crops is increasing worldwide (Aizen et al. 2008), mainly because we need those crops for a varied and healthy diet (Eilers et al. 2011). Additionally, a growth in demand of these crops is expected, because a shift towards a more plant-based diet is required for a sustainable food system (Springmann et al. 2016, Willett et al. 2019). To meet the demand for more pollination services, and to increase crop yields of insect-dependent crops (Breeze et al. 2014, IPBES 2016), the species delivering these pollination services need to be protected and promoted. This requires a shift towards a more sustainable way of farming where managing for ecosystem service delivery takes a more prominent role in day-to-day agricultural management.

A proposed solution lies in the concept of ecological intensification. The concept aims to make more sustainable use of ecosystem services in agricultural systems by conserving and restoring the biodiversity in agricultural landscapes (Bommarco et al. 2013). The general idea is that by restoring biodiversity levels, ecosystem service delivery (e.g. higher crop pollination levels) enhances and will increasingly contribute to crop yields (Kleijn et al. 2019). Depending on the system, the increased levels of ecosystem service delivery can then replace conventional agricultural inputs like fertiliser (‘ecological replacement’), or can even increase crop yields (‘ecological enhancement’). Simply put, to achieve higher levels of ecosystem services, agricultural management needs to be more wildlife friendly (i.e. reduce negative externalities of agriculture), and counter the landscape simplification by improving the quality of semi-natural habitats left, and where possible, increase the amount of natural elements in agricultural landscapes (Bommarco et al. 2013, Morandin and Kremen 2013, Kleijn et al. 2019). An important additional positive effect is that these measures will likely also benefit species that do not necessarily contribute to crop yields, but have high intrinsic value to us, people.

What works in crop pollination?

The number of crop pollination studies looking at the benefits of ecological intensification is growing rapidly, and there is a fair evidence base showing that management for ecosystem service delivery can potentially increase the abundance of crop pollinators (Kleijn et al. 2019). However, the agricultural sector is still reluctant to implement management for increased ecosystem service delivery (i.e. managing for more pollinators) into their day-to-day practices. Kleijn et al. (2019) have reviewed the knowledge gaps and reasons why in practice growers show little interest in managing for more pollinators. One of the main reasons is that studies looking at enhanced ecosystem service delivery often do not measure the effects the service has on crop yield, the main variable growers are interested in. Partly because of this, it is likely that growers still underestimate the contribution of pollinators to their crop yield.

One of the reasons why growers underestimate the contribution of pollinators could be that growers strongly rely on managed honeybees that may not always be effective in pollinating crops. Honeybees can be stocked in large quantities, and even though they are not always very efficient, the sheer numbers can make them effective at the field scale (Rader et al. 2009). Indeed, honeybees represented approximately half of the crop visitors in a large meta-analysis (Kleijn et al. 2015). Nevertheless, they contributed relatively little to fruit set of many crops when compared to the consistent positive contribution of wild pollinators (Garibaldi et al. 2013). The placement of honeybee hives can be costly and increases with increasing revenue of the crop (Rucker et al. 2012). However, the costs may be relatively small depending on other costs and revenue gains. For example, the costs of managed pollinators in apple and high-bush blueberry in the Netherlands are between 0.5-2.5% of the total costs (De Groot et al. 2015). This suggests that growers consider placement of honeybee hives as a relatively low-cost insurance against pollination failure. However, as honeybees often contribute little to crop yield, a more effective management strategy would be to invest in the pollinators species that contribute more to crop yield than managed honeybees.

A broad set of wild pollinator species contribute to crop pollination services (Garibaldi et al. 2013), but it is not yet clear whether the abundance of wild pollinators or the diversity of wild pollinators contributes more to crop pollination services. Several studies have shown that increasing species richness of the crop pollinator community relates to higher crop yields (Klein et al. 2003, Frund et al. 2013, Blitzer et al. 2016). This may be explained by the functional complementarity of different species (Hoehn et al. 2008), as different species can pollinate the crops on different times of the day (Rader et al. 2013), or may have different temperature envelopes (Frund et al. 2013, Kuhsel and Bluthgen 2015). Even closely related, and morphologically similar, pollinator species can exhibit different flower handling behaviour, contributing to species richness effects (Grab et al. 2019). On the other hand, other studies found that only a few species contribute to the majority of pollination services of crops (Kleijn et al. 2015, Winfree et al. 2015), suggesting that the abundance of a few species is more important for crop yield. However, the specific identity of these abundant crop pollinators, and how effective they may be, may differ between crops (Garratt et al. 2014, Rader et al. 2016). Managing for more pollinators of a subset of pollinator species (i.e. the most abundant ones), or to manage for a higher diversity of all crop pollinators requires a completely different approach. It is therefore essential to establish the relative contribution of these two components of the pollinator community to crop yield.

A downside of managing for more wild pollinators is that it is rather knowledge intensive and costly for growers (Kleijn et al. 2019). Taking pollinator-enhancing measures in agricultural landscapes in Europe often means that land has to be taken out of production and converted into flower rich semi-natural habitat like wildflower strips (Kleijn et al. 2019). The benefits of these wildflower strips therefore need to outweigh the costs of the lost agricultural land coverage, i.e. to cover the opportunity costs. Only few studies have actually performed such a cost-benefit analysis (Blaauw and Isaacs 2014, Pywell et al. 2015, Morandin et al. 2016). However, a first step in the process is to calculate what the relative contribution is of insect pollination on the revenue of a crop in real-world systems. With larger revenue of the crop and with increasing contribution of wild pollinators, it is increasingly likely that the costs of establishing wild flower strips are covered by the revenue gain.

Growers usually think it is more effective for crop yield to increase the conventional agricultural input levels of, for example, fertiliser and irrigation, than managing for increased insect pollination. However, whether this is the case depends on the relative contribution of these agricultural inputs to crop yield compared to insect pollination. Only recently, studies have looked into the relative contributions of, and possible interactions between, agricultural management and insect pollination (Garibaldi et al. 2018). However, most of them (but see Tamburini et al. (2017)) have used levels that do not relate well to the levels used by growers in their day-to-day practices (i.e. no fertiliser vs high fertiliser). While the use of these levels can give a good indication of the potential interactions between for example fertiliser inputs and insect pollination (Garibaldi et al. 2018), they are less relevant for growers. Conventional growers almost always apply fertiliser to their crop fields, so a no-fertiliser treatment level is not a convincing reference value for them. Instead, growers are more interested in whether they should, for example, apply 25% more or 25% less fertiliser than they currently do. Similarly, mass-flowering crops are in practice always visited by insect pollinators (Kleijn et al. 2015), making an insect pollinator-exclusion treatment of low relevance to growers. For growers it would be more relevant if studies are performed along a gradient of relatively few to relatively many insect pollinators, so that the results can be used to estimate how many insect pollinators are needed for high crop yields and whether crop yield increases with more insect pollinators. To convince the agricultural sector of the relative importance of insect pollination, and how pollination benefits interact with different agricultural input levels, we therefore need studies that use levels relevant to growers.

An important goal of the ecological intensification of insect pollinated crops is that by managing for more crop pollinators, for example by conserving or restoring semi-natural habitats, adverse effects of farming on biodiversity in the wider landscape are counteracted. While the general pattern is that a higher cover of semi-natural habitat in agricultural landscapes is usually beneficial for the crop pollinator community (Ricketts et al. 2008), surprisingly little is known about how the crop pollinator species pool is linked to the local pollinator species pool. Only a few pollinator species of the local species pool visit mass-flowering crops abundantly, and a larger group of pollinator species only occasionally visit crops (Senapathi et al. 2015). But the majority of the local pollinator community cannot be expected to visit crops, for example because they are cuckoo-species, or they are specialised on plants that are not cropped (Senapathi et al. 2015). These non-crop pollinators might be of little value for crop pollination services, but they do contribute to the stability of the ecosystem, for example through the pollination of wild plant species, or by stabilising pollinator population fluctuations (Alarcon et al. 2008, Thibaut and Connolly 2013). Furthermore, the wider pollinator biodiversity can be an intrinsic motivation of growers to protect biodiversity, regardless whether it contributes to crop pollination or not (Farmar-Bowers and Lane 2009). A relation between the wider pollinator community and crop pollinator community might then provide a powerful argument to convince growers to preserve semi-natural habitats, because conservation then not only benefits crop pollination services, but also the intrinsic values of pollinator diversity for which growers and non-growers may care.

Study system and outline of this thesis

The main aim of this thesis is to test the evidence base of the the importance of pollinator biodiversity in agricultural landscapes. We have used leek (*Allium porrum*) hybrid seed production (Box 1.1) as our study system. Hybrid seed production is a common practice in a wide range of vegetables and (oil)seed crops (George 2009). In this production system a male crop line (i.e. pollen producing) is crossed with the seed producing female crop line (i.e. no fertile pollen), and insect pollinators are essential to transfer the pollen from the male plant to the female plant (Brewster 2008). Insect pollinators are more important in hybrid seed production than in open-pollinated seed production (Box 1.1). In open-pollinated leek seed production about 30-40% of produced seeds are a result of self-fertilisation (De Clercq et al. 2003a), of which approximately half is likely caused by wind or passive self-fertilisation (Kumar et al. 1985). While leek is a relatively small seed crop in terms of agricultural production, pollination studies looking into leek seed production can be widely relevant to other crops. Pollinators contribute to the seed production and the plant breeding of approximately 30% of the crops in the world (Klein et al. 2007). Furthermore, numerous fresh-market crops are fully dependent on pollinators because these crops are obligate cross-pollinators, such as pumpkins (Pfister et al. 2017), and almond (Thomson and Goodell 2002). However, the results of pollination studies in leek seed production are likely to be less comparable to crops that depend less on insect pollinators. Leek hybrid seed production is a high-revenue and fully insect-dependent crop, which means that benefits of pollination are likely to be lower for low-revenue crops and for crops which depend only partly on insects for pollination (Klein et al. 2007). This project is a collaboration with BASF Vegetable Seeds, who commercially produces leek hybrid seeds together with local growers in France and Italy. The collaboration with a large seed company allows for performing large-scale studies that are agronomically realistic, and has the benefit that results can be directly disseminated to a large network of local growers.

Evaluating study methods is essential in keeping the scientific quality of research high (Elphick 2008). Estimating how frequent crop flowers are visited by pollinators (pollinator visitation rate) is a common method to link pollinators with the crop yield of single plants. However, there is no common ground on how long and how often plants should be observed to obtain reliable estimates of pollinator visitation rates, while also taking into account potential time-of-day and weather effects. To answer the question on how to efficiently obtain accurate estimates of pollinator visitation rate on crop plants we set out to observe pollinator visitation rate for three full days in two commercial fields (**chapter 2**). We used this data to study beyond which point longer observation times did not significantly improve the reliability of the estimate of the pollinator visitation rate, and how this observation duration is affected by the time of the day, and weather circumstances. In **chapter 3**, we applied this method in a correlative study in 36 commercial seed production fields in France and Italy along a gradient of expected pollinator abundance and richness to establish the relative contribution of pollinators and plant quality (as a proxy for agricultural management) to the crop yield and revenue of five crop lines. We furthermore compared how different functional pollinator groups contributed to crop yield, and whether abundance or species richness of pollinators was more important. Subsequently, to get insight in how agricultural inputs potentially interact with insect pollination we performed a full-factorial randomised field experiment, where we examined whether it is more beneficial to focus agricultural management on wild pollinators, on conventional agricultural inputs, or both (**chapter 4**). We used standardised and 50%-reduced levels of fertiliser, irrigation and pollination, and compared crop yields for three crop lines. Because most of the insect pollinators in the crop fields originate from the semi-natural habitats surrounding crop fields, we explore in **chapter 5** how the landscape complexity (i.e. semi-natural habitat cover) plays a role in shaping the local and crop pollinator species pool, and how this relationship is affected by crop flowering and with the level of association of pollinators with mass-flowering crops. By surveying the pollinators in both the semi-natural habitats and in the crop fields, we examined the relationship between the ecosystem service providing species and pollinator biodiversity in the wider landscape. The results of these studies are brought together in **chapter 6** where the implications and conclusions of this thesis are discussed and synthesised in the context of the recent developments in this field of research.

Box 1.1 Hybrid seed production

Not everyone is aware that the vegetables we buy at the market have to be grown from seeds, and consequently, that we also need vegetable seed producers. There are two common ways of producing vegetable seeds: open-pollinated seed production, and hybrid seed production (George 2009). With open-pollination seed production, fields contain flowering plants of a certain crop variety, all with similar characteristics, and the seeds produced will likely have the same characteristics. In hybrid seed production you specifically cross two genetically different parent lines (Wright 1980). The cross of these two parent lines will yield a F1-crop variety that has the desired characteristics of both parent lines.

One goal of hybrid seed production is that you increase uniformity of the plant characteristics in the crop variety. To ensure this, the parent lines are usually inbred (largely homozygotic) and individual plants of a parent line are genetic clones of each other. An additional benefit is that by specifically crossing two homozygotic lines, you can get the phenomenon of hybrid vigour, where the seeds produced by the cross of the parent lines are superior to their parents in terms of vigour. It is the task of plant breeders to select the parent lines in such a way that their cross inherits the desired characteristics, and ideally with the increased hybrid vigour. These benefits can be large, as hybrid-varieties can increase crop yields with 20-50% compared to open-pollinated varieties (Tester and Langridge 2010).

While in open-pollinated plants the seeds can be a result of self-pollination and outcrossing, in hybrid seed production you have obligatory outcrossing. The pollen of the male parent line needs to be transferred to the female parent line, and the female parent line then develops the F1-hybrid seeds. To ensure that the pollen actually come from the male parent line, the female parent line should be unable to produce fertile pollen, and is therefore often called a male-sterile line. Because of this, the reliance on pollinators for transferring the pollen is larger for hybrid seed production: in open-pollinated plants self-fertilising can occur through movement caused by wind, or by mere gravity, but this is much less likely in hybrid seed production. As an illustration, pollinators are essential in in hybrid sunflower seed production (Greenleaf and Kremen 2006), whereas for open-pollinated sunflower oil-seed production pollinators contribute for about 35% of the total crop yield (Perrot et al. 2019).

In this thesis, the importance of pollinators in hybrid seed production for several different female parent lines is studied. From a practical perspective these parent lines can be seen as sort of varieties, which is often taken into account in crop pollination studies. However, as the term variety is not synonymous to line, the correct term ‘line’ is used in this thesis.

Chapter 2

How to efficiently obtain accurate estimates of flower visitation rates by pollinators

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Abstract

Regional declines in insect pollinators have raised concerns about crop pollination. Many pollinator studies use visitation rate (pollinators/time) as a proxy for the quality of crop pollination. Visitation rate estimates are based on observation durations that vary significantly between studies. How observation duration relates to the accuracy of the visitation rate estimate is, however, unknown. We studied this relationship using six day-long observations (06:00h-19:00h) in leek-seed production fields (totalling 78 hours). We analysed beyond which point in time observing longer did not significantly improve the accuracy of the visitation rate estimate (minimum observation duration). We furthermore explored the relationship between the minimum observation duration and visitation rate, time of day and temperature. We found that the minimum observation duration (mean ± SD: 24 ± 11.9 minutes) was significantly related to visitation rate, where the observation time required to obtain accurate estimates decreased with increasing visitation rate. Minimum observation duration varied greatly between days and between fields but not within days. Within days, the visitation rates differed significantly only between the hour-intervals 06:00h-07:00h (lowest visitation rate) and 09:00h-11:00h (highest rate). Minimum observation duration decreased up to around 22˚C beyond which it remained fairly stable. Surprisingly, even after three day-long observations on the same plant we found new pollinator species visiting the flowers, suggesting that species-richness estimates based on plant observations alone probably underestimate true species richness. Because especially between-day variation in visitation rate on single plants can be large, reliable estimates of the pollinator visitation rate during the plant's flowering time require observations on multiple days. Standardising the number of pollinators rather than the time to observe (standardised pollinator timing approach: time to *n*-pollinator visits) may provide more consistent accurate assessments of visitation rate, especially for studies that use gradients in visitation rates to examine the contribution of pollinators to crop pollination.

Keywords

Minimum observation duration, visitation rate, pollination, crop systems, observation protocol, time of day, weather, species richness.

Introduction

Regional declines in insect pollinators have raised interest in pollination limitation of insect pollinated crops (Allen-Wardell et al. 1998, Potts et al. 2010). Seed or fruit set of an estimated 70% of the world crops benefits at least partially from pollinators (Klein et al. 2007). Because of that, an increasing body of literature has studied the relationship between crop yield and pollinators (Klein et al. 2007, Garibaldi et al. 2013, Garibaldi et al. 2016), how this is influenced by the effects of landscape structure (Ricketts et al. 2008) and what the relative contribution is of managed versus wild pollinators (Winfree et al. 2008, Garibaldi et al. 2013, Winfree et al. 2015). All these studies have in common that they link the number and diversity of pollinators visiting crop flowers per unit of time to some measure of crop yield. Such visitation rate estimates can be made at the scale of the wider landscape, agricultural fields, individual plants or even individual flowers. In the case where individual plants or flowers are harvested, the most accurate assessment of the contribution of pollinators to production comes from observations that directly link the number of pollinators visiting a plant to the fruit or seed set of that plant. Such an approach was used by 21 of the 41 studies in a crop pollination meta-analysis by Garibaldi et al. (2013).

The duration of pollinator observations on crop flowers varies greatly between studies and ranges from three minutes (e.g. Tamburini et al. (2016)) to 3.5 hours (e.g. Hoehn et al. (2008)). How this observation duration relates to the accuracy of visitation rate estimates is generally unknown. Observations of flower visitations by pollinators are usually made under more or less standardised environmental conditions to avoid results being influenced by inclement weather. Observations generally take place on sunny days without rain and temperature and wind speed thresholds are being used below which observations cannot be made (Kleijn et al. 2015). Nevertheless, weather conditions may vary greatly above these thresholds. Whether and how such variation influences the accuracy of visitation rate estimates is also unknown. Ideally, the observation duration should be as long as the shortest time period required for a visitation rate estimate that does not significantly deviate from the true visitation rate. Too short observations may lead to inaccurate estimates that are not representative for the observed plant, which in turn could lead to inaccurate conclusions on the effects of pollinators on crop yield. Too long observations would be inefficient and this time could better be invested in increasing sample size. What observation duration is most efficient for estimating pollinator visitation rates probably also depends on the visitation rate itself, as it is likely that a minimum number of encounters must exist for accurately estimating visitation rate (Burnham et al. 1980). But also the relationship between the visitation rate and observation duration is unknown.

To examine how the accuracy of visitation rate estimates is related to observation duration and whether this is influenced by weather conditions, we observed pollinators visiting leek plants in seed production fields in southern Italy. This crop is well suited for this kind of studies, as it is well visited by a wide variety of insect pollinators (Kleijn et al. 2015). The landscape of southern Italy is diverse which makes it possible to choose sites that differ in habitat suitability for pollinators and thus pollinator richness and abundance (Ricketts et al. 2008). We observed plants for full days to determine the true daily visitation rate. We then subdivided these days into intervals of different length (1 to 12 minutes) to determine at which observation duration the accuracy of the visitation rate estimate ceased to improve significantly (minimum observation duration). For each observation day, on both fields, we used the data to analyse the relationship between observation duration and estimated visitation rate. We then used this relationship to explore how time of day and weather conditions influence the minimum observation duration. Based on these results we discuss survey strategies that most efficiently produce reliable estimates of pollinator visitation rates.

Materials and methods

Study system

Commercial leek (*Allium porrum*) is mainly produced in Europe and comes in several hybrid varieties (Brewster 2008). Leek seeds are produced in hybrid seed production systems (Wright 1980). In these systems, a fully fertile inbred (male) line is crossed with a male sterile inbred (female) line to produce a high-yielding hybrid variety. Because the pollen of the male line have to be transferred to the female line and wind pollination plays no role (Brewster 2008), these systems fully rely on pollinators for pollination. Leek forms one primary umbel (flower head) and, depending on the line, one to three secondary umbels. Primary umbels can have up to 4000 flowers each of which can produce up to six seeds, like other *Allium* species (Brewster 2008, Simon and Jenderek 2010). The primary umbel contains open flowers for approximately three weeks, in which individual flowers open irregularly for a few days (Brewster 2008).

We selected two commercial leek-seed production fields in southern Italy that potentially attracted low or high amounts of pollinator individuals and species. The two fields were located about 40 km apart (field A & B) and were used to produce seeds of the same leek variety (i.e. the same male and female lines in both fields). Field A was located in a predominantly flat area, close to a small river, but otherwise surrounded by agricultural production fields, mainly wheat. Field B was located in hilly terrain. This area contained much more semi-natural habitat and was characterized by small-scale agriculture.

Observation protocol

In June 2015 we observed flower visitation by pollinators in both fields on three days from 6:00 hour until 19:00 hour (total observation time 78 hours). Sunrise in this area and time of year was around 5:30 hour and sunset at around 20:30 hour. This time period covered the full daily activity period of pollinators, as our observations showed that pollinator activity started only well after 6:00 hour and ceased before the end of observations. Within the fields, we selected a representative female plant before the start of flowering, approximately 20 meter from the edge of the field. We observed the same, individually marked, primary umbel over the three observation days and recorded each pollinator that touched the umbel. We identified the species in the field when possible, or caught and stored the pollinator to identify the species to the best possible taxonomic level otherwise. For each pollinator, we noted the landing time on the umbel to the minute.

We observed field A on 13, 16 and 22 June 2015 and field B on 19, 25 and 29 June 2015. In field A, the observed umbel was 80% flowering (20% of the flowers still closed), 90% flowering (10% setting seed) and 70% flowering (30% setting seed) on the respective observation days. In field B, the observed umbel was 80% flowering (20% of the flowers still closed), 100% flowering, and 70% flowering (30% setting seed), respectively.

We observed only on days without rain and with wind speeds below 8m/s (<5 Bft). During the observations, we recorded temperature (°C), relative humidity (%) and wind speed (m/s) every half hour with a handheld recorder (Digital Meter 50302).

Analyses

All data calculations and statistical analyses were done in R version 3.3.1 (R Core Team 2016). To analyse the relation between observation duration and the accuracy of the estimation of visitation rate we subdivided our day-long observations in time intervals of lengths ranging from 1 to 120 minutes. For each observation day, we then calculated the standard deviation (SD) of the visitation rates based on each time interval (i.e. 780 one-minute intervals, 390 two-minute intervals). SD is independent of sample size and this allows us to compare SDs from time intervals, with different sample sizes, with the SDs of the visitation rate of the day-long observations. The SD of the day-long observations was calculated as the mean SD from the time intervals from 80 to 120 minutes, as at those intervals the SD had always reached an asymptote. We then analysed at which observation duration (i.e. time interval) the SD of the estimated visitation rate no longer differed significantly from the SD of the actual visitation rate based on the day-long observation to determine the minimum observation duration. We tested for this by bootstrapping the 95% confidence interval (CI) for the SD of the estimated visitation rate for each observation interval, following Anderson and Santana-Garcon (2015) in the R-package ‘boot’ (Canty and Ripley 2015) with 10,000 bootstrap replicates. Subsequently, we identified the first observation duration for which the bootstrapped CI overlaps with the SD of the day-long visitation rate.

To examine whether minimum observation duration differs with more or less pollinators visiting the flowers per unit of time, we analysed how the calculated minimum observation duration was related to the day-long visitation rate using an ordinary least squares regression.

To analyse if visitation rate varies between different parts of the day, we analysed effects of time of day on hourly visitation rates. We regard time of day as a proxy for the complex interactions between the environment and pollinator activity patterns. We used a linear mixed effect model, with standardised hourly visitation rates as response variable, hour as independent fixed variable, and observation day nested within field as random variable to correct for nestedness of the data (function ‘lmer’ in R-package ‘lme4’ (Bates et al. 2015)). We performed pairwise comparisons between the hours using function ‘glht’ in R-package ‘multcomp’ (Hothorn et al. 2008). To allow comparison between observation days with different variation, we standardised visitation rates using Z-transformation by subtracting the mean day-long visitation rate and dividing by the SD of the day-long visitation rate.

To illustrate the implications of differences in time of day for the minimum observation duration, we used the relationship between visitation rate and minimum observation duration. We averaged observed hourly visitation rates for each of the six observation days and subsequently calculated the minimum observation duration for each hour. We then fitted a orthogonal polynomial regression to the second degree using ordinary least squares regression.

Similarly, we illustrate the implication of differences in weather for the minimum observation duration. As the weather variables were highly correlated with each other (Spearman’s *r*; see results), we only used temperature in this analysis, as this is the most easy variable to measure in the field. Firstly, we interpolated our temperature data linearly to 1 minute resolution using the function 'approx' in R. Secondly, we used the interpolated temperature data to calculate the observed visitation rate for each temperature unit (rounded to the nearest °C) and we used these visitation rates to estimate the minimum observation duration per temperature unit. Lastly, we fitted an orthogonal polynomial regression to the third degree using ordinary least squares regression.

Results

Observations

In field A, the total number of pollinators observed on a single umbel during an entire day was remarkably stable with 81, 87 and 77 visitors on the three observation days respectively (figure 2.1). In field B, the range was much larger with our single observed umbel receiving 166, 610 and 367 visitors on the three observation days respectively (figure 2.1). In both fields, we observed a steady increase in cumulative abundance throughout the day (figure 2.1).

We recorded in total 47 species in the two fields, with the most dominant species being *Apis mellifera* (993 individuals, 71.6% of total), *Lasioglossum malachurum* (150 individuals, 10.8% of total), *Bombus terrestris*-group (43 individuals, 3.1% of total) and *Andrena flavipes* (42 individuals, 3.0% of total). Cumulative species richness over the three days followed the same pattern in both fields. In field A we recorded 36 species, with 20 species on the first observation day, eight additional species on the second day and another eight additional species on the third day. In field B we found only 28 species despite the much larger number of visitors than on field A. Eighteen species were observed on the first day, five additional species on the second day and five more new species on the third observation day.

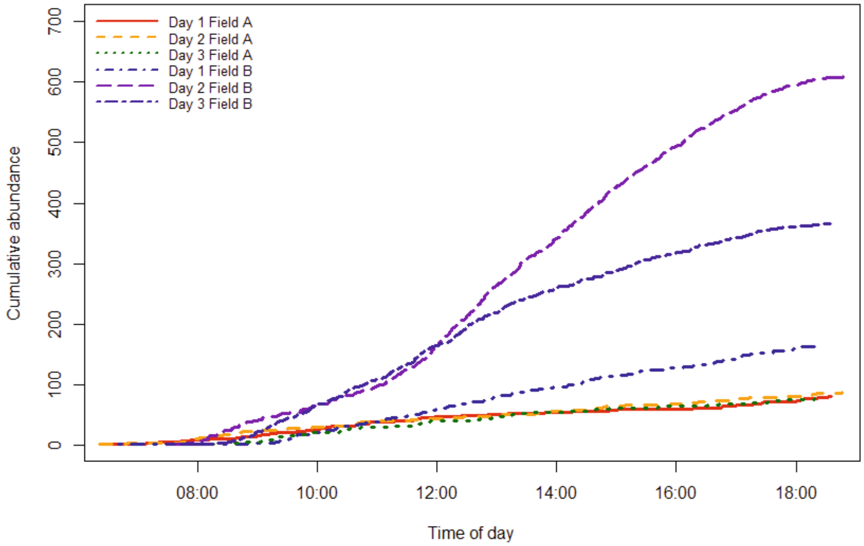

Figure 2.1 The cumulative abundance of pollinators visiting the focal plants (leek) in the two study sites on three observation days. Each visitor that landed on the umbel was recorded.

Minimum observation duration

On all observation days, increasing the observing duration resulted in a rapid decrease of the SD of the estimates of visitation rate towards an asymptote (figure 2.2). The minimum observation duration ranged from 7 to 36 minutes (mean 24.0 minutes ± 11.9 SD) between the observation days. In field A the minimum observation duration was 32, 36 and 28 minutes on the three different days on the same umbel, while in field B the variation was much larger with minimum observation durations of 29, 7 and 11 minutes on the three observation days. The minimum observation duration was negatively related to the number of pollinators visiting the umbels per minute. With a visitation rate of 0.6 pollinators per minute, 11.7 minutes were needed to accurately estimate visitation rate, but with a visitation rate of 0.2 pollinators per minute 30.3 minutes were needed (LM test $\beta = -40.602 \pm 7.479$ SE, Adj. $R^2 = 0.85$, $p < 0.01$, n = 6; figure 2.4A).

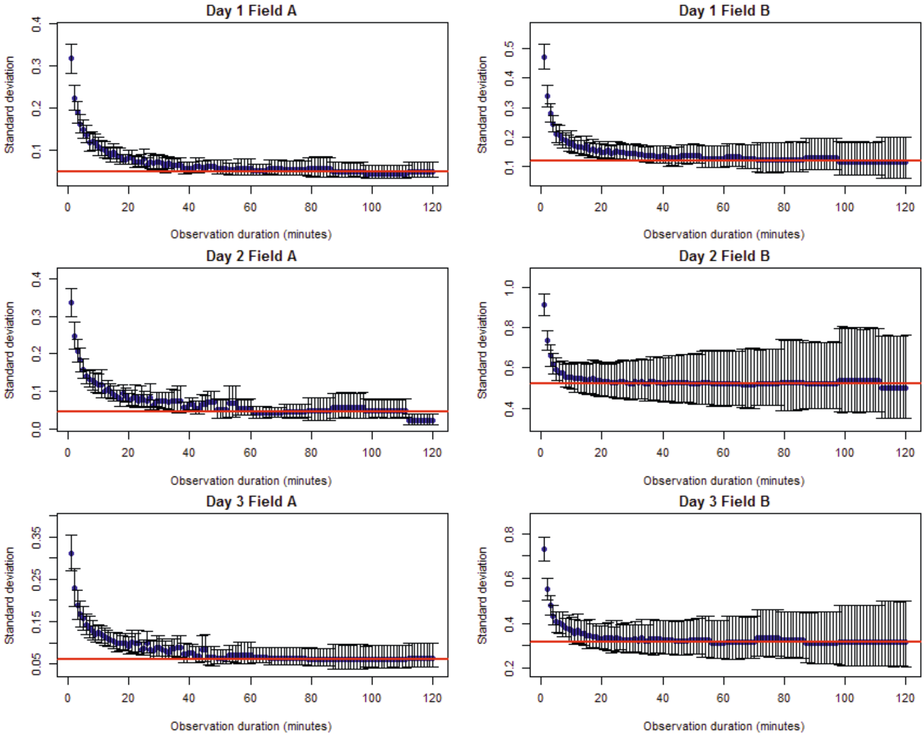

Figure 2.2 Decrease in standard deviation (SD) of visitation rate (pollinators/minute) with increasing observation durations. Points show bootstrapped SDs with 95% confidence interval. The mean SD was calculated for observation interval 80-120 minutes and is indicated by the solid red line.

Time of day

Visitation rates increased rapidly between 6 am and 9 am after which they decreased gradually during the remainder of the day (figure 2.3). The multi-comparison test revealed that visitation rates only differed significantly between the hourly observation intervals with the lowest (06:00h-07:00h) and highest visitation rates (09:00h-10:00h & 10:00h-11:00h; Tukey pairwise comparison, mean difference 9-6h = 2.20 ± 0.61 SE, z = 3.316, $p = 0.048$ & mean difference 10-6h = 2.20 ± 0.61 SE, z = 3.635, $p = 0.017$).

The relationship between minimum observation duration and time of day follows a clear U-shaped curve ($F_{2,10} = 83.24$, adj. $R^2 = 0.93$, $p < 0.001$; figure 2.4B), with an optimum (i.e. lowest minimum observation duration) at 12:00h with a minimum observation duration of approximately 18 minutes.

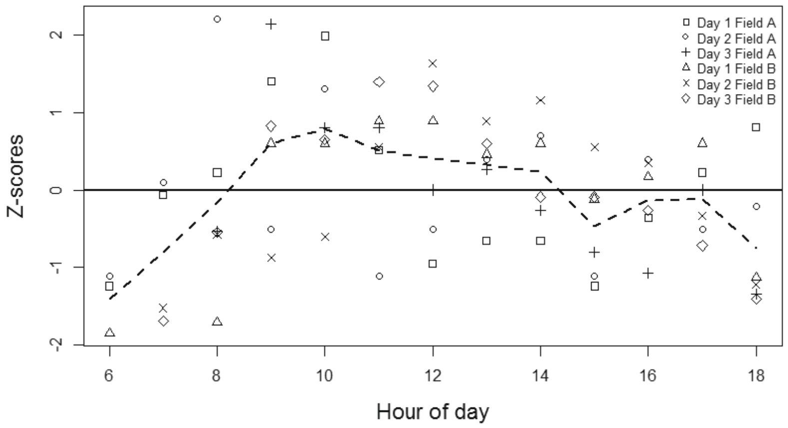

Figure 2.3 Standardised visitation rates (pollinators/minute) showing variation within observation days. Visitation rates were standardised using Z-transformation by subtracting the mean day-long visitation rate and subsequently dividing by the SD of the day-long visitation rate. Symbols with a Z-score of -1 indicate visitation rates 1 SD-unit below daily average visitation rate, and symbols with a Z-score of 1 indicate visitation rates 1 SD-unit above daily average visitation rate. The average Z-scores for all observation days combined is indicated by the dashed line.

Weather

Temperature, relative humidity and wind speed were strongly correlated. With an increasing temperature, relative humidity dropped (*r* = -0.853, *p* < 0.001) and wind speed increased (*r* = -0.337, *p* < 0.001), while with increasing relative humidity wind speeds were lower (*r* = -0.478, *p* < 0.001).

The relationship between temperature and the estimated minimum observation duration showed an optimum (i.e. lowest minimum observation duration) around 29 °C and minimum observation duration becomes steadily lower from 17 °C to 22 °C ($F_{3,20}$ = 18.37, Adj. R^2 = 0.69; *p* < 0.001). When the temperatures are above 29 °C, minimum observation duration increases again (figure 2.4C).

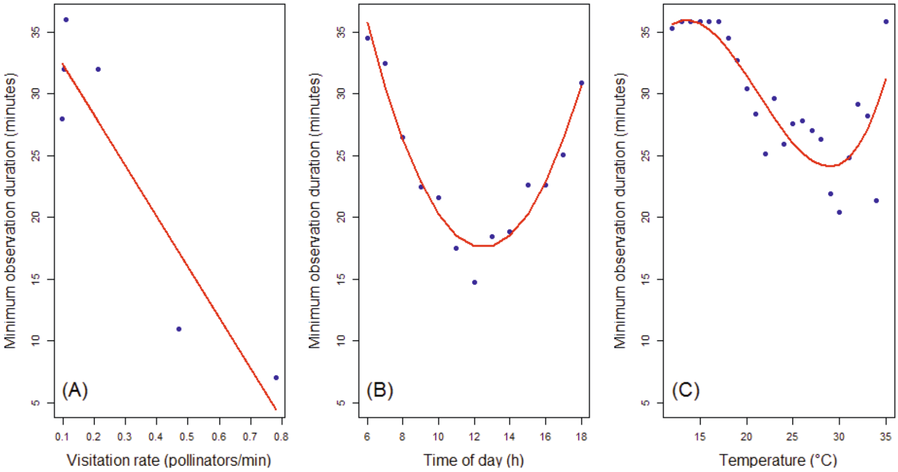

Figure 2.4 Relationships between (A) visitation rate (pollinators/minute), (B) time of day (h) and (C) temperature (˚C) with the minimum observation duration needed to accurately estimate visitation rate. The linear relationship between visitation rate and minimum observation duration (panel (A)) is used for calculating the minimum observation duration in panel (B) and (C). In all plots, predicted values are indicated by a solid red line.

Discussion

There is a rapidly increasing body of literature on pollination based on studies that survey the number of pollinators visiting individual plants or even flowers of crops. The durations of these plant observations vary greatly between studies and it is generally unknown how this affects the accuracy of the visitation rate estimates. Our study suggests that the minimum observation duration for efficiently and accurately estimating visitation rate may differ by a factor of about five between fields. Furthermore, even for one and the same umbel, fourfold differences in minimum observation duration were observed on different days. This variation was mainly due to differences in the number of pollinators visiting the flowers during our observations, as the minimum observation duration decreased significantly with increasing visitation rate. New species were visiting the observed umbel even at the end of the 39 hour long observation period which indicates that plant observations are not the best method for accurately estimating the pollinator species pool.

Even at the high visitation rate of 0.78 pollinators/minute we needed to observe the plant for 7 minutes to accurately assess visitation rate. Minimum observation durations of up to 36 minutes were found at visitation rates of 0.1-0.2 pollinators/minute which are commonly observed in other studies (e.g. (Ricketts 2004, Chacoff and Aizen 2006, Boreux et al. 2013a). Many studies make use of gradients in visitation rates to determine the effects of the contribution of pollinators to seed or fruit set of crops (Garibaldi et al. 2013). Because these studies make use of a fixed observation duration, our results suggest that estimates from plants with low visitation rates are structurally less accurate than estimates from plants with high visitation rates. This could affect the results of studies as it reduces the power of analyses to find statistically significant patterns. This problem can be avoided by determining the standardised observation duration based upon plants receiving the lowest visitation rates (i.e. least attractive plants, variety or crop). The disadvantage of this approach is that this would make the visitation rate estimates on plants with higher visitation rates (i.e. highly attractive plants, variety or crop) inefficiently long. A more elegant approach may be to scale the observation duration by the visitation rate, which can be done for example by measuring the amount of time it takes before a certain number of pollinators have visited the plant.

We found that visitation rates varied considerably between days within one field, but not in the other (figure 2.1). If we had focussed on only one day per field, but instead increased the number of fields, we might have under- or overestimated the average number of pollinators visiting umbels during the flowering period of leek in field B. If, for example, a pollination study focusses on a large gradient in visitation rates in a large geographic region, observing more fields (i.e. increasing sample size) may be of larger interest than improving accuracy within fields. However, our results suggests that studies that estimate visitation rate on plants based on single day observations might contain a lot of environmental noise which could significantly influence the outcomes (figure 2.1). This should be taken into account when designing studies.

Many studies make sure that pollinator observations are equally distributed over different parts of the day to account for pollinator activity peaks (Herrera 1990). In our study, only the period from 06:00h to 07:00h, which is well avoided in pollination studies (Kleijn et al. 2015), differed significantly in visitation rates from the most visited hours (09:00h to 11:00h). Within the period from 07:00h to 19:00h we found no significant differences in visitation rates. Additionally, when we look at the period from 09:00h to 17:00h, a generally accepted time frame within which pollinators can be surveyed (Kleijn et al. 2015), variation in the predicted minimum observation duration is small (figure 2.4B). Our results are based on full-day plant observations, allowing us to distinguish between within-day variation and between-day variation in visitation rates and the associated minimum observation durations. This suggests that variation in minimum observation duration is larger between days (figure 2.4A) than within days (figure 2.4B). If our results are representative for other study systems, this indicates that accounting for differences in pollinator activity across the day is less important than is generally assumed.

Many pollinator studies use the rule of thumb that pollinators can only be surveyed at temperatures of 15 ºC or higher (Kleijn et al. 2015). We found a sigmoid relationship between minimum observation duration and temperature, with a sharp decline in minimum observation duration between 17 °C and 22 °C and an optimum at around 29 °C (figure 2.4C). When temperatures rose further, minimum observation duration increased again. This relationship undoubtedly differs between plant-pollinator systems. For example, in warmer climates pollinator activity peaks will probably occur at higher temperatures than in colder climates and pollinator communities dominated by cold-tolerant bumble bees have lower activity peaks than pollinator communities dominated by solitary bees The number of days with weather conditions that are really suitable for surveying pollinators usually limits sample size of pollinator studies and raising the generally accepted 15 ºC temperature threshold below which no observations can be made might make it altogether impossible to perform well-replicated pollinator studies, especially at higher latitudes or altitudes. However, the influence of marginally suitable weather conditions could be incorporated better in study designs, for example, by making sure that days with better or worse conditions are evenly distributed over the experimental treatments or gradients.

Surprisingly, even after observing one and the same umbel for three full days, we found no saturation in the number of species visiting it. This means that regardless of the minimum observation duration, we would always have highly underestimated the total number of species visiting our plants during the receptive period of the flowers. Stigmata of onion flowers (*Allium cepa*, a close relative of leek) are receptive for two to five days (Moll 1954). If this is also the case for leek, even the last new pollinator species on the end of our third observation day could have increased seed set, for example through functional complementarity (Hoehn et al. 2008). But also for flowers that are only receptive for one day, pollinator species richness by plant observations alone would be underestimated. A better estimate of the total pollinator species pool in the system could be obtained with transect counts (Westphal et al. 2008). Transect counts can also be used to estimate visitation rate. However, because transect counts are done at a larger spatial scale than plant observations they less precisely describe the pollinators to which individually harvested plants have been exposed. Estimating both the total number of individuals and species to which a plant has been exposed during its flowering period can therefore probably be better done by a hybrid approach: estimating visitation rate by means of plant observations and species richness by standardised transect counts that cover more surface area, more flowers and therefore more readily detect less abundant species.

Evaluating the methods that are used to collect the data that are at the basis of scientific studies, even if they are generally accepted and widely used, is essential to uphold scientific quality in research (Elphick 2008). The lengths of the observation periods used to estimate the visitation rates that are at the core of the rapidly increasing number of pollination studies are largely based on general assumptions and rules of thumb. Our results suggest that more accurate and consistent estimates can be obtained by taking into account the effect visitation rate itself has on the reliability of its estimate. Standardising the number of pollinators rather than the time to observe may be both a more consistent and efficient approach. Determining the amount of time it takes to record a certain number of pollinators visits to the plant of interest ensures that observation duration is not too short in sites with low pollinator abundance and not too long in sites with high pollinator abundance. Accuracy of the estimates obviously increases with the number of pollinators that is used to time the visitation rate. In our study, timing the period until five pollinators visited the leek umbels gave on average estimates equal to those of the minimum observation duration. Such a standardised pollinator timing approach can easily be expressed in traditional units for visitation rate estimates (pollinators/time) allowing for easy comparisons with previous studies.

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Chapter 3

Insect pollination is at least as important for marketable crop yield as plant quality in a seed crop

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Abstract

The sustainability of agriculture can be improved by integrating management of ecosystem services, such as insect pollination, into farming practices. However, large-scale adoption of ecosystem services-based practices in agriculture is lacking, possibly because growers undervalue the benefits of ecosystem services compared to those of conventional management practices. Here we show that, under representative real-world conditions, pollination and plant quality made similar contributions to marketable seed yield of hybrid leek (*Allium porrum*). Relative to the median, a 25% improvement of plant quality and pollination increased crop value by an estimated \$18,007 and \$17,174 ha^{-1} respectively. Across five crop lines, bumblebees delivered most pollination services, while other wild pollinator groups made less frequent but nevertheless substantial contributions. Honeybees actively managed for pollination services did not make significant contributions. Our results show that wild pollinators are an undervalued agricultural input and managing for enhancing pollinators makes sense economically in high-revenue insect-pollinated cropping systems.

Keywords

crop pollination, crop yield, agricultural management, functional groups, species richness, visitation rate, agro-ecology, structural equation modeling.

Introduction

Agriculture depends critically on ecosystem services such as nutrient cycling, pest regulation and animal pollination. Paradoxically, these ecosystem services are increasingly being degraded by agricultural practices that aim to increase crop production (Tilman et al. 2011, Deguines et al. 2014, Wagg et al. 2014). For example, 70% of the world’s crops benefit from animal pollination (Klein et al. 2007), but at the same time agricultural intensification is one of the key drivers of the decline of wild pollinators in many parts of the world (Allen-Wardell et al. 1998, Kremen et al. 2002, Potts et al. 2010). Sustainable production of food, feed and fuel therefore requires solutions that integrate the management of ecosystem service-providing species into day-to-day agronomic management. This so-called ecological intensification of agriculture has been embraced by the scientific community and policy makers, in part because it potentially unifies crop production with biodiversity conservation in agricultural landscapes (Bommarco et al. 2013). However, the approach has seen little uptake amongst the main target group that should be implementing the practices, the agricultural sector (IPBES 2016). One of the reasons may be that they are unaware of the contribution of wild pollinators or underestimate its importance compared to conventional agricultural inputs (Munyuli 2011, Hanes et al. 2015). While the evidence base for the contribution of wild pollinators to crop production is substantial (Garibaldi et al. 2013, Kleijn et al. 2015, Garibaldi et al. 2016), little is known about how important that contribution is relative to that of typical agricultural management such as irrigation, or application of fertilizers and pesticides.

Recently, studies have started examining whether the contribution of insect pollination is influenced by agricultural management (Tamburini et al. 2016, van Gils et al. 2016, Garibaldi et al. 2018). However, it is difficult to infer real-world comparative effect sizes of pollinators and agricultural inputs from these studies because they used artificial settings (e.g. potted plants) or unrealistically large treatment contrasts (e.g. with and without pollinators; Garibaldi et al. (2018)). Whether managing for enhanced pollination makes sense agronomically and economically, depends essentially on whether a realistic increase can be obtained compared to the actual “business as usual” situation, rather than a situation without pollination. This can only be determined in farming systems along realistic gradients in insect pollination and agricultural management (Garibaldi et al. 2016). Furthermore, the relative contributions of wild pollinators and input-based management should be robust across crop varieties that are available to farmers. Crop varieties not only differ markedly in their response to traditional agricultural inputs such as fertilizers (Guarda et al. 2004) but also with respect to dependence on insect pollination (Klatt et al. 2014). An assessment of the agronomic potential of using pollination as an agricultural input should therefore ideally take into account the variation in responses of different varieties.

The contribution of pollination to agricultural production is at least partly determined by the composition of the pollinator community that is visiting the crop flowers. A higher species richness of crop visiting pollinators is often related to higher crop yields (Klein et al. 2003, Frund et al. 2013, Blitzer et al. 2016, Garibaldi et al. 2016), for example through functional complementarity (Hoehn et al. 2008), or larger spatial (Winfree et al. 2018) and temporal (Garibaldi et al. 2011a) stability of yield. Other studies suggest that crop pollination mainly depends on the services provided by just a handful of the most abundant species (Kleijn et al. 2015, Winfree et al. 2015). Furthermore, different insect pollinators may be more or less effective for particular crops (Rader et al. 2013, Rader et al. 2016). For example, long-tongued bumblebees (*Bombus* sp.) are the most important pollinators for field bean pollination (Garratt et al. 2014), and red mason bees (*Osmia rufa*) are more efficient pollinators than hoverflies in oilseed rape (Jauker et al. 2012). The diversity or visitation rate of specific pollinator groups may therefore be more strongly linked to crop yield than that of all pollinators combined.

To test if it is more productive for a farmer to manage for better plants or to manage for more pollinators and how this varies between crop varieties, we used five female genetic lines in each of 36 commercial hybrid leek (*Allium porrum*) seed production fields in France and Italy. The fields were located in representative agricultural landscapes to obtain a realistic level of variation in abundance and species richness of crop visiting insect pollinators. We used plant quality, measured as basal circumference, as a proxy for agricultural management since the crop plant integrates all interacting effects of management and environmental conditions and is therefore arguably the best indicator of successful management by farmers. We formed an *a priori* conceptual framework which we used in Structural Equation Modeling (SEM). To establish which functional group contributed most to pollination and how this compared to the relative contribution of plant quality to marketable seed yield, we ran multiple SEMs for each female line with visitation rate and species richness of different functional groups. The average effect sizes of variables across the best SEMs of the five lines then indicates the relative contribution of plant quality and pollination for yield of hybrid leek seed production in general, while a comparison of the best SEM per line shows how the relative contribution of plant quality and pollination, and which functional groups are contributing most to yield varies between crop lines.

Materials and Methods

Study system

We used commercial leek (*Allium porrum*) seed-production fields as our study system. High-yielding leek varieties are produced in hybrid seed production systems (Wright 1980) that cross inbred, fully fertile (male; produces pollen) lines with inbred, male sterile (female; no pollen) lines. Each inbred line is commercially selected for specific characteristics of the variety that results from the cross between the two inbred lines. Fields contain a single male and a single female line and pollen is predominantly transferred by insects, making insect pollinators essential for seed production (Brewster 2008). In our study regions, seeds are produced in southern Italy on small (0.5-2 ha) open fields, and in the Loire region in western France in semi-open tunnels (~0.1 ha). Honeybee colonies are placed in leek seed production fields in Italy, but not in France. In four French sites, bumblebee colonies (*Bombus terrestris*) were placed in the semi-open tunnels. Prior to each growing season, the seed company provides each grower with the same planting and cultivation protocol, but nevertheless considerable differences in management practices exist between fields due to for example differences in planting time or soil type.

Experimental setup

In autumn 2015, we selected 18 fields in Italy (study area ± 615 km^2) and 18 fields in France (study area ± 1,800 km^2) in landscapes located along a gradient of cover of potentially suitable pollinator habitat to obtain a representative level of variation in species richness and abundance. Pollinator habitat cover (mainly semi-natural grasslands, scrublands and woodlands; mean cover 22% ± 19.2 SD) was estimated using satellite images. Except for one pair of fields that was separated by approximately 850 meter, all fields were located at least one kilometer from other selected fields, which is beyond the foraging range of most bees (Greenleaf et al. 2007). In each of the 36 fields we planted five plants of five female production lines each in random order in a row or bed of the commercially grown female plants (i.e. 25 plants per field; female-lines coded B-F for this project). The plants were cultivated in the same way as the commercial plants.

Pollinator observations

We used plant observations to estimate pollinator visitation rate as a proxy for pollination (Fijen and Kleijn 2017, Garibaldi et al. 2018). We selected two representative plants per line in each field, which we observed throughout the flowering period of approximately three weeks in June-July 2016. We observed the primary flower head (umbel) of each plant for 20 minutes and recorded each pollinator that landed (bees and hoverflies; identified to the lowest taxonomic level possible). We repeated these observations at least three times (range: 3-5 times, mean: 3.3 times) throughout the flowering period with a minimum of four days between observations. Observations were carried out in dry circumstances, temperatures above 20 ˚C and wind speeds below 5 Beaufort (<8 m/s). To estimate pollinator species richness, we performed transect counts at the field level, since plant observations underestimate species richness (Westphal et al. 2008, Fijen and Kleijn 2017). On each plant observation day, counts were done in a single fixed transect per field totaling 150 m^2, divided in three continuous sub-transects of 50 m^2 (50 x 1 meter). In each sub-transect, we counted all pollinators (bees and hoverflies; identified to the lowest taxonomic level possible) on female plants in five minutes net observation time, excluding catching and handling time (i.e. 15 minutes per transect). Pollinators that could be identified on the wing were counted in the field. All other pollinators were caught and stored for later identification. We considered *Bombus terrestris* and *B. lucorum* as a single taxon c.f. Williams et al. (2012). Furthermore, we assumed that all individuals of *B. terrestris* came from the wild as the number of managed *B. terrestris* individuals was small compared to the average total number of individuals per field (up to 60 workers vs 1267 individuals).

Plant quality

Agricultural management consists of all measures taken and inputs used by growers to optimize plant size and vigor under the specific growing conditions of their farm (Sørensen et al. 1995). Plant size and vigor, in turn, are generally strongly related to seed production (Major 1980). We therefore measured several characteristics describing plant size and/or vigor: circumference of the pseudo stem at the base (basal circumference, rounded off to the nearest 5 mm), number of green leaves, height of the plant from the ground to the base of the umbel (height, to the nearest 1 cm), diameter of the flower stem 5 mm below the base of the umbel (flower stem diameter, to the nearest 0.01 mm) and the diameter of the umbel (umbel diameter, to the nearest 0.1 mm). Diameter was measured with a digital caliper. Height and circumference were measured with a tape measure. We measured each observed experimental plant and averaged measurements of each line in each field.

Marketable seed yield

The individually marked plants were harvested just before seed shedding. For each line in each field we pooled the two umbels and, after drying, threshed and cleaned by hand. All seeds were then counted with a seed counter (Contador, Pfeuffer GmbH). In one field, only one umbel of line F could be harvested and we doubled the seed count of that umbel for comparison. For unknown reasons, three plant pairs (two line B, one line C; all different fields) produced less than 1% of the average marketable seeds and these were excluded from the analysis. Seed quality was determined with a vigor test for each line in each field. In this test, three sets of 100 randomly selected seeds were sown in suboptimal circumstances and after 18 days, the vigor of the seedlings was assessed by experts in a NAL-authorized test (Naktuinbouw Authorized Laboratory). Vigor was categorized as (A) optimal, (B) suboptimal, (C) poor or (D) did not emerge. The average vigor scores (%) were calculated over the three sets. Usually, only seeds with vigor A or B are commercially sold. In practice, however, a too high proportion of vigor B plants is undesirable, depending on the quality standards of the company. For our study we assumed that all vigor A or B plants will be sold. Marketable seed yield was therefore calculated as the total number of seeds per line per field multiplied by the percentage seeds that were scored as vigor A or B.

Analysis – Structural Equation Modeling

Plant variables were generally correlated with one another. We therefore chose to include only basal circumference as a proxy for plant quality in subsequent analyses because this variable was most strongly correlated with the other measured variables (Supplementary table 3.3), and because it can more easily be used by the agricultural sector than the other variables, as other variables only become apparent relatively late in the growing season. Pollinator visitation rate was calculated as the average of all observations per female line per field. In addition to calculating visitation rates for all visitors combined, we also calculated separate visitation rates for honeybees (*Apis mellifera*) and the functional groups of bumblebees (*Bombus* sp.), solitary bees (mostly Halictidae and Andrenidae) and hoverflies (Syrphidae), resulting in five different visitation rates per line per field. For a measure of species richness effects, we first corrected for the difference in effort of finding new pollinator species between fields by using the chao1 estimator for calculating estimated species richness per field (Chao et al. 2009). We calculated estimated species richness of all pollinators, as well as for each of the functional groups separately (except for honeybees as they only comprise one species). In total, we estimated species richness for four groups (all species and three functional groups) per field, and five different visitation rates (all species, three functional groups and honeybees) resulting in 20 combinations of visitation rate and estimated species richness per female line. Preliminary analyses showed that the ranges of all measured variables largely overlapped between the two countries and the results were qualitatively similar when countries were analyzed separately. We therefore pooled the data for the final analysis. We standardized all variables before analysis to enable the comparison of the relative contribution of plant quality, pollinator visitation rate and pollinator richness on marketable seed yield.

We used Structural Equation Modeling (SEM; R-package 'lavaan' (Rosseel 2011, R Core Team 2018)) to estimate the relative importance of (different groups of) pollinators and plant quality. We first established a conceptual model of interactions with *a priori* hypotheses. We expected that three general factors were of importance in determining seed yield: Plant quality, visitation rate of (different groups of) pollinators and species richness of (different groups of) pollinators. For each of the 20 candidate models per line, we included direct effects of plant quality, visitation rate of a single group of pollinators, and estimated species richness of a single group of pollinators on marketable seed yield in our SEMs. We also included indirect effects of high quality plants attracting more individuals of pollinators. Furthermore, visitation rate and species richness are usually correlated, so we also tested this correlation in our model.

For all models, we computed bootstrapped standard errors and test statistics. We used the chi-square, and the Root Mean Square Error of Approximation (RMSEA) fit statistics to evaluate the model fit and discarded models that did not show an acceptable fit (Schermelleh-Engel et al. 2003). Because we were interested in which model explains the marketable seed yield best, we selected the best model based on the highest R^2 (Grace 2006, Weston and Gore 2006). We calculated the standardized effect sizes using the path coefficients for each factor (Grace 2006). To establish the relative importance of plant quality, visitation rate and species richness across lines, we averaged the standardized effect sizes of the best model per female line.

Analysis – Economic contribution

Standardized effect sizes poorly demonstrate the practical implications of scientific results. To illustrate the real-world impact of our findings we therefore estimated the economic contribution of changes in plant quality, pollinator visitation rate and pollinator richness. For this we required the unstandardized values of the factors to calculate the increase in marketable seed yield for each unit increase of the factor (e.g. one species extra increases marketable seed yield by *n*-seeds). We used the specific functional groups selected in the best model per line (figure 3.2 & table 3.1) and used the parameter estimates of a SEM based on the unstandardized values. We calculated the contribution to the value of the marketable seed yield for the whole range of the observed factors of the best models per line (Supplementary table 3.1 & 3.2). Leek seed prices from our collaborator or competitors are undisclosed information, and commercial seed prices were only available for the 28 worldwide leading crops and not for leek (Kynetec 2017). We therefore assumed leek seed prices were similar to those of related onion (*Allium cepa*) seeds. For our calculations, we used the average seed price (\$0.00144 seed^{-1}) of hybrid onion seed in 24 European countries from 2016 (Kynetec 2017). Leek seed production fields have on average a plant density of 110.000 plants per hectare, of which two-third are hybrid seed producing female plants (average density 7.3 female plants/m^2; c.f. Brewster (2008)). Using these values, we calculated the economic contribution of each factor per hectare (E, \$ ha^{-1}) for each line as:

$$E = \frac{p * i * v * d}{2}$$

Where p is the unstandardized parameter estimate (the slope predicting the number of seeds), i the increase in units from the low range to the high range (in cm, pollinators minute^{-1}, or species), v the market value (\$ seed^{-1}), and d the number of female plants per hectare (plants ha^{-1}). As we used two plants per line per field in our experiment, we divided the equation by two.

To estimate the effects of a management improvement that can be realistically achieved by growers we calculated for each factor what the increase in marketable seed yield would be if this factor would change from the median to the 75th percentile of the observed range. This resulted in an economic contribution for each line, and we averaged these contributions to draw general conclusions on the value of plant quality, pollinator visitation rate and pollinator richness on marketable seed yield, based on achievable within-range improvements.

Results

Between field variation in crop pollinators and plant quality in a real world farming system

In 394 hours of observing the umbels of our experimental plants, we counted a total of 1471 flower visiting pollinators. Even though the five female lines were planted within one meter of one another, the observed average visitation rate differed substantially between functional groups and lines, and ranged from 0.00 to 0.63 pollinators per minute (Supplementary table 3.1). The most frequent visitors were pollinator species that can be commonly found on crops throughout Europe (Kleijn et al. 2015): *Bombus terrestris (*25.4%), *Apis mellifera (*13.9%), *Andrena flavipes* (12.1%), *Lasioglossum malachurum* (7.0%) and *Bombus lapidarius* (6.9%). The field-level transect surveys confirmed the presence of considerable differences in the pollinator communities between the examined leek fields. Estimated species richness of all pollinators combined was high compared with other studies (Garibaldi et al. 2016) with 27.9 (SE: 1.3 SE) species per field but showed a wide range between fields from a minimum of only seven to a maximum of 113 species per field. Not all functional groups contributed equally to the species richness of the pollinator communities. The most species-rich group were the solitary bees, accounting for approximately half of the species (Supplementary table 3.2). Bumblebees made up 55% of the individuals at field level but were present with a mere 2.6 (SE: 0.2 SE) observed species per field. This functional group was dominated by the buff-tailed bumblebee *Bombus terrestris* and to a lesser extent the red-tailed bumblebee *B. lapidarius*. Together, these two species comprised 99% of all observed bumblebees in both the transects and the plant observations.

We used basal circumference as our proxy of plant quality as it was most strongly correlated with the other measured plant variables (see also Methods; Supplementary table 3.3). Plant quality varied considerably between fields and increased at least two-fold from lowest to highest quality plants in all lines (Supplementary table 3.1), showing that important differences in plant growth conditions existed despite the fact that all farmers received the same growing protocol.

The relative importance of pollination and plant quality on marketable seed yield

Across all five lines, marketable seed yield increased at least as strongly with pollination as with plant quality (figure 3.1). Effects of pollination and plant quality were mostly direct, with only one line showing a strong indirect effect of better quality plants attracting more pollinators, which in turn increased seed yield (line B; figure 3.2). The most consistent contributors to marketable seed yield were plant quality and bumblebee visitation rate with important contributions in four out of five lines (figure 3.2). Bumblebee visitation rate was included in more than half of the top-five models that explained most of the variation in seed yield in the five lines (table 3.1). In the fifth line, visitation rate of solitary bees explained yield variation best (Line E; figure 3.2). Visitation rate of all pollinators combined rarely featured in the models best explaining variation in marketable yield (table 3.1). Similarly, visitation rates of honeybees *Apis mellifera* hardly contributed to crop yield as indicated by their inclusion in only two of the top models explaining variation in marketable seed yield (table 3.1).

Species richness of hoverflies made an important contribution to marketable seed yield in two lines and species richness of all pollinators combined was important for seed yield of another line (figure 3.2). Species richness of hoverflies featured in almost half of the top-five models explaining marketable seed yield, and was included in the best model in four out of five lines (table 3.1).

Economic contribution

To illustrate the practical consequences of our findings we expressed the relationships described by the best models per line in economic terms. Pollinator visitation rate showed consistent positive relations with estimated crop value in all lines (figure 3.3). Plant quality and species richness were positively related to crop value in only four lines. An improvement in plant quality from the median to the 75th percentile of our observed range represented an estimated \$18,007 ha^{-1} increase in value. A similar improvement in pollinator visitation rates represented an estimated \$12,236 ha^{-1} increase in value, and an additional \$4,937 ha^{-1} for species richness of the functional group contributing most to marketable seed yield.

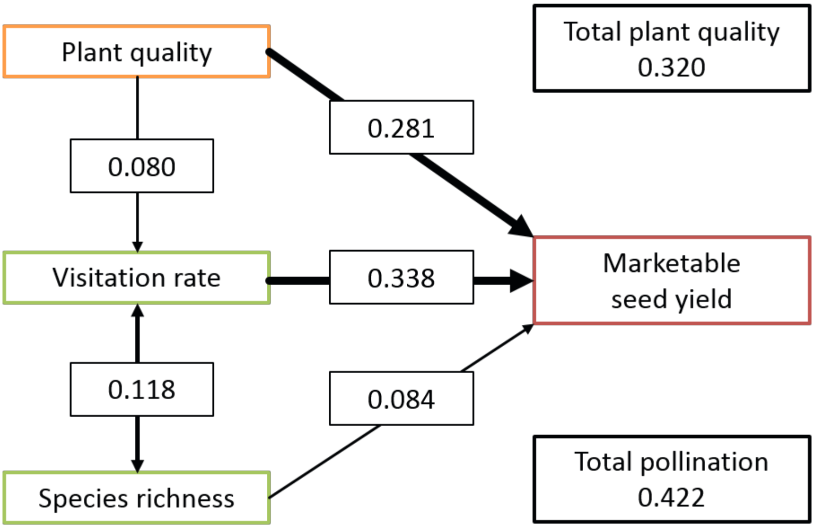

Figure 3.1 Averaged effect sizes of the selected SEMs per crop line. Standardized effect sizes in black rectangles indicate how strongly a factor is related to another. The total effect size of plant quality (upper right rectangle) consists of the direct effect of plant quality, and the indirect effect through visitation rate on marketable seed yield. The total effect size of pollination (lower right rectangle) consists of the direct effects of visitation rate and species richness. Plant quality is measured as basal circumference (cm). Effects of visitation rate (pollinators/minute) and species richness (estimated number of species per field) are contributed by different functional groups, depending on crop line. The double-headed arrow between visitation rate and species richness is a modelled correlation. For visualization purposes arrows with high effect sizes are made thicker. For simplicity, unexplained variance is not shown.

Table 3.1 The top five SEM models for each combination of pollinator group visitation rate and species richness per crop line. Models are ranked on the total explained variance (R^2), and the highest ranked model is in bold and illustrated in figure 3.2. All values are standardized effect sizes with 95% confidence intervals.

Line	Rank	Visitation rate	Species richness	R^2	Plant quality -> Marketable seed yield	Plant quality -> Visitation rate	Total effect plant quality	Visitation rate -> Marketable seed yield	Species richness -> Marketable seed yield	Visitation rate <-> Species richness
B	**1**	**Bumblebees**	**Hoverflies**	**0.320**	**0.090** **(-0.263 - 0.442)**	**0.426** **(0.188 - 0.665)**	**0.306** **(-0.062 - 0.673)**	**0.507** **(0.206 - 0.808)**	**-0.201** **(-0.537 - 0.135)**	**0.132** **(-0.177 - 0.382)**
	2	Bumblebees	Solitary bees	0.305	0.146 (-0.180 - 0.471)	0.422 (0.205 - 0.639)	0.347 (0.021 - 0.674)	0.477 (0.148 - 0.806)	-0.038 (-0.355 - 0.279)	0.127 (-0.161 - 0.414)
	3	Bumblebees	All pollinators	0.303	0.150 (-0.208 - 0.507)	0.410 (0.173 - 0.646)	0.344 (-0.005 - 0.692)	0.474 (0.141 - 0.807)	-0.041 (-0.517 - 0.435)	0.103 (-0.168 - 0.374)
	4	Hoverflies	Solitary bees	0.220	0.378 (0.052 - 0.703)	0.097 (-0.296 - 0.490)	0.347 (-0.003 - 0.698)	-0.316 (-0.689 - 0.057)	0.017 (-0.266 - 0.300)	0.002 (-0.150 - 0.154)
	5	Hoverflies	All pollinators	0.218	0.379 (0.053 - 0.704)	0.109 (-0.315 - 0.533)	0.344 (-0.015 - 0.703)	-0.319 (-0.675 - 0.037)	0.028 (-0.356 - 0.413)	0.078 (-0.053 - 0.210)
C	**1**	**Bumblebees**	**Hoverflies**	**0.223**	**0.233** **(-0.156 - 0.621)**	**-0.131** **(-0.510 - 0.248)**	**0.175** **(-0.244 - 0.593)**	**0.441** **(0.116 - 0.765)**	**0.048** **(-0.232 - 0.328)**	**-0.024** **(-0.237 - 0.188)**
	2	Bumblebees	Bumblebees	0.222	0.229 (-0.149 - 0.606)	-0.125 (-0.453 - 0.203)	0.176 (-0.230 - 0.581)	0.424 (0.084 - 0.764)	0.037 (-0.355 - 0.430)	0.404 (0.148 - 0.660)
	3	Bumblebees	All pollinators	0.221	0.230 (-0.145 - 0.605)	-0.130 (-0.477 - 0.218)	0.173 (-0.220 - 0.567)	0.439 (0.147 - 0.732)	-0.023 (-0.566 - 0.520)	-0.006 (-0.160 - 0.147)
	4	Bumblebees	Solitary bees	0.220	0.231 (-0.141 - 0.603)	-0.131 (-0.482 - 0.220)	0.173 (-0.222 - 0.568)	0.441 (0.143 - 0.739)	0.014 (-0.311 - 0.340)	-0.065 (-0.254 - 0.125)
	5	All pollinators	Bumblebees	0.105	0.203 (-0.188 - 0.594)	-0.139 (-0.444 - 0.165)	0.176 (-0.232 - 0.583)	0.194 (-0.149 - 0.537)	0.285 (-0.151 - 0.721)	-0.404 (-0.648 - -0.159)

D	**1**	**Bumblebees**	**All pollinators**	**0.105**	**0.042** **(-0.269 - 0.353)**	**-0.007** **(-0.305 - 0.291)**	**0.040** **(-0.284 - 0.363)**	**0.284** **(-0.222 - 0.791)**	**0.107** **(-0.334 - 0.548)**	**0.178** **(0.043 - 0.314)**
	2	Bumblebees	Solitary bees	0.104	0.043 (-0.274 - 0.360)	-0.003 (-0.290 - 0.284)	0.042 (-0.287 - 0.371)	0.285 (-0.212 - 0.782)	0.101 (-0.252 - 0.454)	0.180 (-0.023 - 0.382)
	3	Bumblebees	Bumblebees	0.100	0.039 (-0.283 - 0.360)	-0.072 (-0.321 - 0.178)	0.014 (-0.318 - 0.347)	0.340 (-0.212 - 0.891)	-0.094 (-0.491 - 0.303)	0.381 (0.141 - 0.621)
	4	Bumblebees	Hoverflies	0.094	0.030 (-0.280 - 0.340)	-0.028 (-0.337 - 0.282)	0.021 (-0.309 - 0.351)	0.301 (-0.204 - 0.806)	0.035 (-0.297 - 0.367)	0.064 (-0.149 - 0.277)
	5	All pollinators	All pollinators	0.049	0.016 (-0.283 - 0.316)	0.146 (-0.074 - 0.366)	0.040 (-0.282 - 0.361)	0.159 (-0.664 - 0.983)	0.116 (-0.358 - 0.590)	0.264 (0.104 - 0.424)
E	**1**	**Solitary bees**	**Hoverflies**	**0.635**	**0.605** **(0.373 - 0.837)**	**0.234** **(0.020 - 0.448)**	**0.670** **(0.462 - 0.877)**	**0.276** **(-0.020 - 0.571)**	**0.283** **(-0.015 - 0.581)**	**0.230** **(-0.082 - 0.542)**
	2	All pollinators	Hoverflies	0.584	0.628 (0.402 - 0.855)	0.284 (0.055 - 0.514)	0.670 (0.467 - 0.872)	0.145 (-0.196 - 0.486)	0.297 (-0.034 - 0.628)	0.343 (0.090 - 0.597)
	3	Bumblebees	Hoverflies	0.580	0.658 (0.443 - 0.873)	-0.098 (-0.265 - 0.068)	0.670 (0.457 - 0.882)	-0.115 (-0.348 - 0.117)	0.353 (0.082 - 0.624)	0.072 (-0.183 - 0.327)
	4	Honeybees	Hoverflies	0.575	0.637 (0.358 - 0.917)	0.327 (0.081 - 0.574)	0.670 (0.460 - 0.879)	0.098 (-0.422 - 0.618)	0.315 (0.010 - 0.619)	0.328 (0.081 - 0.575)
	5	Hoverflies	Hoverflies	0.571	0.701 (0.465 - 0.936)	0.416 (0.096 - 0.736)	0.670 (0.452 - 0.887)	-0.075 (-0.356 - 0.207)	0.371 (0.068 - 0.674)	0.388 (0.220 - 0.556)
F	**1**	**Bumblebees**	**Hoverflies**	**0.240**	**0.434** **(0.114 - 0.754)**	**-0.124** **(-0.397 - 0.150)**	**0.411** **(0.077 - 0.746)**	**0.183** **(-0.100 - 0.467)**	**0.182** **(-0.112 - 0.476)**	**0.074** **(-0.246 - 0.395)**
	2	Bumblebees	Bumblebees	0.239	0.445 (0.111 - 0.780)	-0.144 (-0.399 - 0.111)	0.407 (0.046 - 0.767)	0.269 (-0.034 - 0.571)	-0.201 (-0.493 - 0.091)	0.350 (0.110 - 0.590)
	3	All pollinators	Hoverflies	0.221	0.430 (0.101- 0.759)	-0.142 (-0.381 - 0.096)	0.411 (0.079 - 0.744)	0.131 (-0.368 - 0.630)	0.134 (-0.194 - 0.463)	0.470 (0.279 - 0.661)
	4	Honeybees	Hoverflies	0.216	0.420 (0.101 - 0.739)	0.092 (-0.563 - 0.379)	0.411 (0.076 -0.746)	-0.092 (-0.563 - 0.379)	0.211 (-0.102 - 0.525)	0.175 (-0.157 - 0.506)
	5	Solitary bees	Hoverflies	0.211	0.420 (0.090 - 0.750)	-0.123 (-0.46 - 0.101)	0.411 (0.081 - 0.742)	0.069 (-0.584 - 0.723)	0.166 (-0.174 - 0.507)	0.423 (0.227 - 0.618)

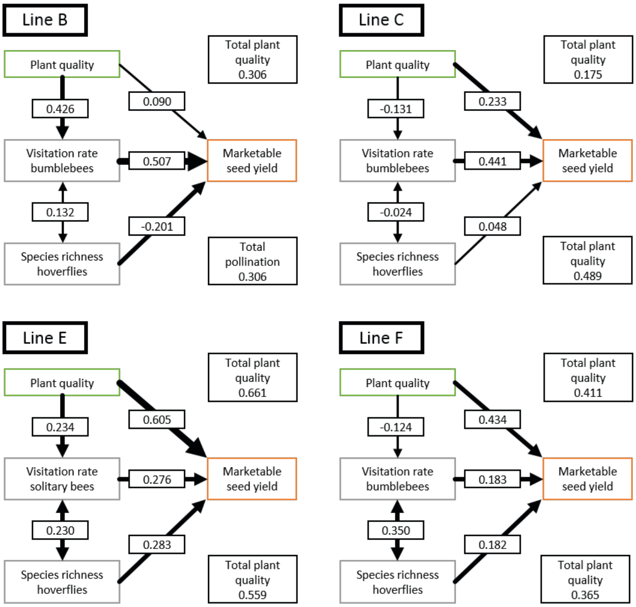

Figure 3.2 Selected SEM for each female line based on highest explained variation out of 20 candidate models. Plant quality is measured as basal circumference (cm). Effects of visitation rate (pollinators/minute) and species richness (estimated number of species) are contributed by different functional groups, depending on crop line. Effect sizes are standardized and indicate how strongly factors are related to another. The total effect size of pollination (lower right rectangle) consists of the direct effects of visitation rate and species richness. Plant quality is measured as basal circumference (cm). Effects of visitation rate (pollinators/minute) and species richness (estimated number of species per field) are contributed by different functional groups, depending on crop line. The double-headed arrow between visitation rate and species richness is a modelled correlation. For visualization purposes arrows with high effect sizes are made thicker. For simplicity, unexplained variance is not shown.

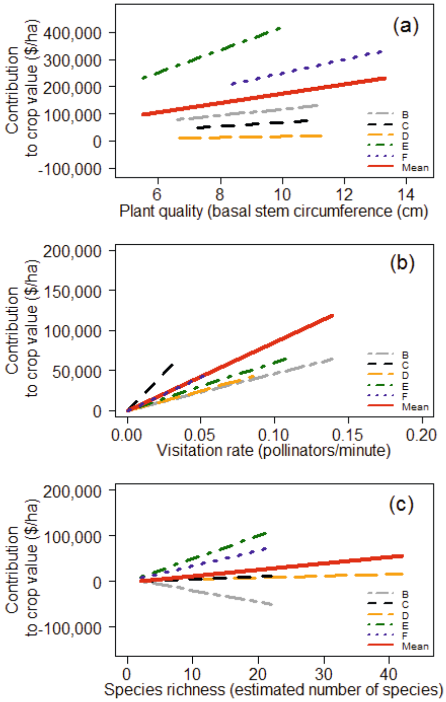

Figure 3.3 An illustration of the contribution of the measured factors to the estimated hybrid leek seed crop value. The contribution to the crop value along the observed 90% percentile of (**a**) plant quality (basal circumference (cm)), (**b**) visitation rate (pollinators/minute) and (**c**) species richness (estimated number of species), with average values of the other measured factors. The intercept for each line is set to zero. Economic value is calculated using the parameter estimates of the best SEM model and the observed 90% percentile measurements of each factor per line. The average slope of all female lines is superimposed with a solid red line.

Discussion

This study is the first endeavor comparing the relative contribution of pollination and regular agricultural management to crop yield, and shows that, although there were substantial differences between female lines, on average insect pollination was at least as important as plant quality in the examined cropping system. Better agricultural management, through its effects on plant quality, did not invariably result in higher yields as we surprisingly found no relationship between plant quality and yield in one of the five examined crop lines. In contrast, higher visitation rate of wild pollinators invariably resulted in higher marketable seed yield. Although our hybrid leek study system is fully dependent on insects to transfer pollen from the male to the female line, the important and systematic contribution of wild pollinators is still remarkable because our approach did not examine the effects of absence of pollinators (Garibaldi et al. 2018) but used the real-world variation in service-providing species. Our findings therefore suggest that wild pollinators are more consistently linked to marketable seed yield than plant quality. Since wild pollinators are rarely managed by growers this indicates that they are an undervalued agricultural input (IPBES 2016).

Interestingly, counter to findings from other studies (Klein et al. 2003, Garibaldi et al. 2013, Winfree et al. 2015, Garibaldi et al. 2016) visitation rate of all pollinators combined did not relate well to marketable crop yield, suggesting that not all flower visits add up to enhance pollination and crop yield. Individual functional groups of pollinators did relate strongly to marketable crop yield, however which group was most effective differed between female lines. Visitation rate of bumblebees seem to play a key role in hybrid leek pollination in four out of five lines (table 3.1). Bumblebees are known to be highly effective pollinators (Rader et al. 2009), and are amongst the most abundant pollinators in a wide variety of insect pollinated crops (Kleijn et al. 2015). Nevertheless, crop yield was best explained by visitation rate of solitary bees in the fifth line (Line E; figure 3.2). Because in each field the five lines were grown next to one-another, the most likely explanation for this result is that this line was more attractive to solitary bees than the other lines, possibly because specific floral traits, such as nectar accessibility, better matched the requirements of this functional group (Garibaldi et al. 2015). Indeed, solitary bee visitation rate of line E was almost twice as high as bumblebee visitation rate despite the fact that the opposite was the case in the neighboring line B (Supplementary table 3.1), and that at the field level almost 3.5 times more bumblebees were observed than solitary bees (Supplementary table 3.2). The pollinator groups that provide the key services may therefore not only differ between crops (Garratt et al. 2014), but also between lines of the same crop.

Species richness effects were less pronounced and less consistent than visitation rate effects but were nevertheless important in two of the examined crop lines. Here species richness of hoverflies contributed substantially to marketable seed yield, and furthermore appeared in almost half of the models best explaining variation in crop yield (table 3.1). Hoverflies are mainly active in the early morning (Herrera 1990) before most bees start visiting the crop and may therefore be the first pollinators to visit freshly opened flowers. This could have made them functionally complementary to the much more numerous wild bees (Frund et al. 2013). The negative relationship between hoverflies and seed yield in line B, not only for species richness but also for hoverfly visitation rate, suggests that hoverflies can also provide pollination disservices, but the exact mechanisms behind this remain unknown.

Our results suggest that for leek hybrid seed production two abundant bumblebees (*Bombus terrestris* and *B. lapidarius*) provide the majority of the pollination services, confirming previous observations that a small number of species provide the bulk of the services (Kleijn et al. 2015). This was not merely driven by abundance of species (Winfree et al. 2015) because the honeybee was the second most abundant flower visitor but hardly contributed to marketable seed yield. However, we found additional, sometimes important, contributions to crop yield of species richness of other functional groups of pollinators, and visitation rate of solitary bees was more closely related to crop yield of one line than the visitation rate of bumblebees (figure 3.2). Delivery of pollination services is therefore predominantly driven by abundance of key functional pollinator groups (Kleijn et al. 2015, Winfree et al. 2015), but, depending on the context, diversity and abundance of other pollinator groups may complement or largely replace the functional role of the dominant species. This indicates that a narrow focus of wild pollinator enhancing management that is just targeting a few species of dominant crop pollinators will effectively enhance pollination under most conditions. However, this strategy may not suffice to provide resilient pollination services under all circumstances (Hudewenz et al. 2014, Marini et al. 2015) or at larger time (Riedinger et al. 2015) or spatial (Winfree et al. 2018) scales, as it can fail to enhance the species that can step in or supplement service provision when the dominant species are performing suboptimal, thus acting as insurance to farmers (Yachi and Loreau 1999).

All but one line showed only direct effects of agricultural management and pollination on marketable seed yield, indicating that the contributions of conventional agricultural management and pollination to seed production were largely independent from each other (van Gils et al. 2016). This suggests that in theory insect pollination can replace external inputs such as fertilizer or pesticides to produce the same yield in a more sustainable way (Bommarco et al. 2013, Marini et al. 2015, Tamburini et al. 2017). However, a strategy that is probably more attractive to growers is to improve both insect pollination and agricultural management as this will result in the highest crop yields (Garibaldi et al. 2016). The main management strategy to enhance pollination in our study system is placement of honeybee hives (Rucker et al. 2012). In line with a growing body of evidence (Garibaldi et al. 2013), our study shows that managed honeybees cannot replace the pollination services provided by wild pollinators. Management aimed at increasing insect pollination should therefore be targeted at enhancing the abundance and diversity of the wild pollinator community.

Three types of measures can be taken that are increasingly difficult for farmers to integrate into their farming systems. First and foremost, to avoid further loss of wild pollinators, the existing semi-natural habitats in agricultural landscapes should be conserved as pollinators depend critically on them for nesting, shelter and food outside the crop flowering season (Westrich 1996, Ricketts et al. 2008, Garibaldi et al. 2011b, Dainese et al. 2017). Second, the quality of degraded semi-natural habitats in agricultural landscapes should be improved as this may be a cost-effective way to enhance wild pollinator communities (Morandin and Kremen 2013, M'Gonigle et al. 2015). Third, new wild pollinator habitats can be created by sowing diverse, native wildflower mixtures on field edges (Scheper et al. 2013, Blaauw and Isaacs 2014, Pywell et al. 2015, Sutter et al. 2017), which can even be targeted to particular groups of beneficial pollinators (Rundlof et al. 2014). Such practices are generally costly, which may prevent their adoption by single growers (Cong et al. 2014). However, our study shows that the economic benefits of increasing insect pollination in this hybrid-seed crop probably compensate for the costs of establishing wild flower strips (Blaauw and Isaacs 2014). This is likely the same for other insect pollinated hybrid seed production crops, which could be an economic incentive for seed companies to take the lead in pollinator-enhancing management in agricultural landscapes that are poor in semi-natural habitats. Here, seed companies could sponsor the establishment of high quality pollinator habitat near pollinator-dependent seed crops, thus lowering the risks for growers and making it more attractive to invest in wild pollinators (Blaauw and Isaacs 2014). This way the cultivation of high-revenue seed crops could instigate the development of a more pollinator-friendly agricultural landscape, which in turn could have important positive side-effects on farmland biodiversity, the productivity of low-revenue insect-dependent crops (Isaacs et al. 2017) and the aesthetic value of agricultural landscapes (Breeze et al. 2015).

Acknowledgements

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Supplementary information

Supplementary table 3.1 Descriptive statistics of the measured explanatory factors on the crop line level. Ranges, 25% percentile, median, 75% percentile and mean ± standard error of the observed plant quality and visitation rates used in the SEM models. Plant quality is measured as the basal circumference in centimeters. Visitation rate is expressed as the number of pollinators per minute.

	Plant quality (basal circumference (cm))					Visitation rate all pollinators (pollinators/minute)					Visitation rate honeybees (pollinators/minute)				
Line	Range	25% Percentile	Median	75% Percentile	Mean ± se	Range	25% Percentile	Median	75% Percentile	Mean ± se	Range	25% Percentile	Median	75% Percentile	Mean ± se
B	5.5-12.3	7.5	8.1	9.4	8.5 ± 0.3	0.000-0.6333	0.0438	0.0896	0.1396	0.1184 ± 0.0219	0.000-0.1667	0.0000	0.0000	0.0083	0.0127 ± 0.0056
C	3.2-11.8	8.5	9	10.3	9.1 ± 0.3	0.000-0.2063	0.0083	0.0313	0.0500	0.0415 ± 0.0082	0.000-0.0313	0.0000	0.0000	0.0062	0.0046 ± 0.0014
D	6.0-11.5	7.5	8.5	9.8	8.8 ± 0.2	0.000-0.5375	0.0234	0.0417	0.0917	0.0694 ± 0.0161	0.000-0.2375	0.0000	0.0000	0.0167	0.0139 ± 0.0067
E	5.1-10.8	6.2	7.3	8.4	7.5 ± 0.2	0.000-0.2500	0.0083	0.0250	0.0781	0.0539 ± 0.0110	0.000-0.0917	0.0000	0.0000	0.0015	0.0077 ± 0.0034
F	7.5-14.0	9.4	10.7	11.5	10.6 ± 0.3	0.000-0.3750	0.0156	0.0250	0.0798	0.0557 ± 0.0118	0.000-0.0417	0.0000	0.0000	0.0000	0.0038 ± 0.0015

	Visitation rate bumblebees (pollinators/minute)					Visitation rate solitary bees (pollinators/minute)					Visitation rate hoverflies (pollinators/minute)				
Line	Range	25% Percentile	Median	75% Percentile	Mean ± se	Range	25% Percentile	Median	75% Percentile	Mean ± se	Range	25% Percentile	Median	75% Percentile	Mean ± se
B	0.000-0.3000	0.0000	0.0156	0.0813	0.0454 ± 0.0110	0.000-0.3583	0.0000	0.0073	0.0250	0.0262 ± 0.0109	0.000-0.3000	0.0000	0.0125	0.0250	0.0262 ± 0.0092
C	0.000-0.0583	0.0000	0.0050	0.0083	0.0092 ± 0.0023	0.000-0.1313	0.0000	0.0000	0.0156	0.0135 ± 0.0048	0.000-0.0500	0.0000	0.0000	0.0113	0.0079 ± 0.0022
D	0.000-0.1813	0.0000	0.0073	0.0271	0.0232 ± 0.0061	0.000-0.2563	0.0000	0.0000	0.0188	0.0200 ± 0.0078	0.000-0.0750	0.0000	0.0000	0.0083	0.0094 ± 0.0028
E	0.000-0.1417	0.0000	0.0000	0.0167	0.0124 ± 0.0044	0.000-0.1571	0.0000	0.0000	0.0167	0.0220 ± 0.0070	0.000-0.0500	0.0000	0.0000	0.0083	0.0067 ± 0.0020
F	0.000-0.0833	0.0000	0.0000	0.0167	0.0128 ± 0.0036	0.000-0.2833	0.0000	0.0000	0.0224	0.0249 ± 0.0089	0.000-0.0583	0.0000	0.0000	0.0083	0.0062 ± 0.0021

Supplementary table 3.2 Descriptive statistics of the estimated species richness per field. Ranges, median and mean ± standard error of the estimated species richness per functional group. Note that species richness of the functional group honeybees was always one, as this species was always present. Species richness was estimated using Chao1 estimator based on the counted individuals in the transects.

Species richness	**Range**	**Median**	**Mean ± se**	**Individuals counted**
All pollinators	7-113	24.5	27.9 ± 1.3	26084
Honeybees	1-1	1	1.0 ± 0.0	4771
Bumblebees	1-5	3.0	2.6 ± 0.1	14438
Solitary bees	0-45	11.0	13.1 ± 0.7	4154
Hoverflies	2-24	8.0	9.1 ± 0.4	2721

Supplementary table 3.3 Correlation matrix of measured plant characteristics per crop line. The correlations between measured plant variables of the experimental plants (n=72 per female line). Basal circumference was chosen as the measure of plant quality as it had on average the highest average correlations with other variables, and because this measure is the most manageable measure for the agricultural sector. For example, the flower stem diameter, umbel width and height are only measurable during flowering, after which plant quality cannot be improved anymore. The number of leaves fluctuates during the season, as old leaves die off and new ones appear.

B	**Basal circumference**	**Flower stem diameter**	**Number of leaves**	**Umbel width**	**Height**	**Average correlation**
Basal circumference	X	0.84	0.51	0.59	0.52	0.62
Flower stem diameter		X	0.31	0.57	0.46	0.55
Number of leaves			X	0.33	0.47	0.41
Umbel width				X	0.40	0.47
Height					X	0.46

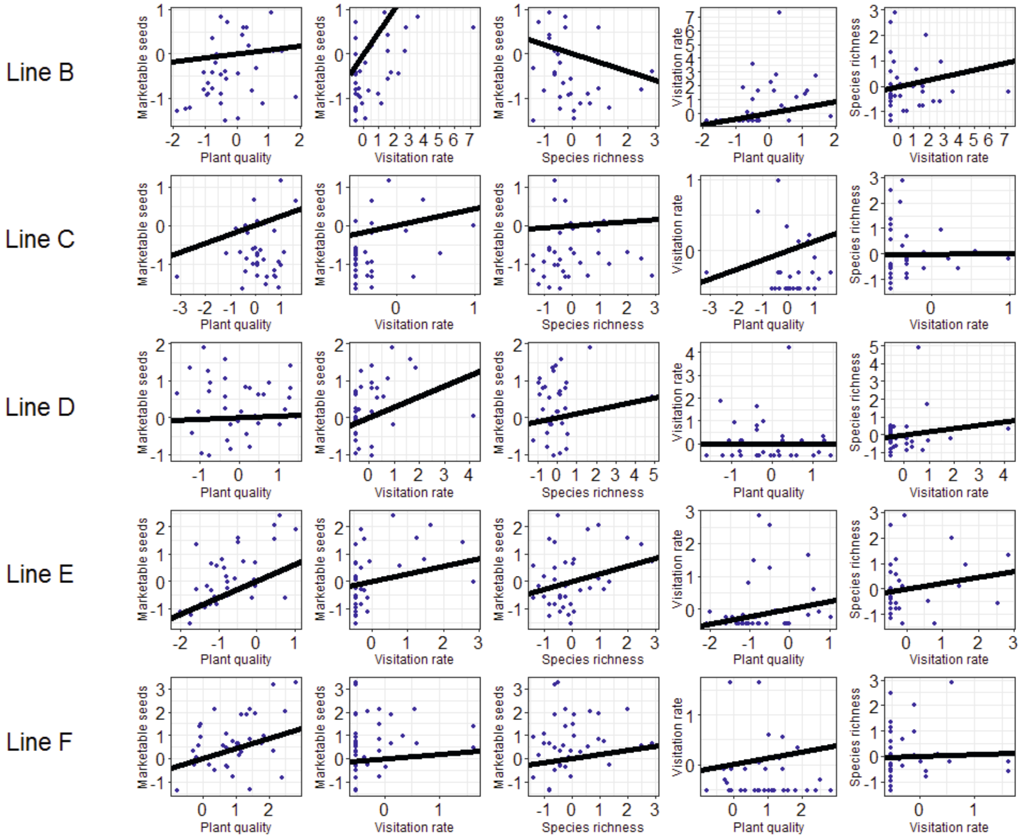
C	**Basal circumference**	**Flower stem diameter**	**Number of leaves**	**Umbel width**	**Height**	**Average correlation**
Basal circumference	X	0.88	0.21	0.71	0.37	0.54
Flower stem diameter		X	0.31	0.61	0.32	0.53
Number of leaves			X	0.04	0.39	0.24
Umbel width				X	0.23	0.40
Height					X	0.33

D	**Basal circumference**	**Flower stem diameter**	**Number of leaves**	**Umbel width**	**Height**	**Average correlation**
Basal circumference	X	0.71	0.28	0.66	0.10	0.44
Flower stem diameter		X	0.11	0.65	0.29	0.44
Number of leaves			X	0.24	0.08	0.18
Umbel width				X	0.10	0.41
Height					X	0.14

E	Basal circumference	Flower stem diameter	Number of leaves	Umbel width	Height	Average correlation
Basal circumference	X	0.79	0.47	0.76	0.13	0.54
Flower stem diameter		X	0.27	0.57	0.17	0.45
Number of leaves			X	0.29	0.26	0.32
Umbel width				X	0.10	0.43
Height					X	0.17

F	Basal circumference	Flower stem diameter	Number of leaves	Umbel width	Height	Average correlation
Basal circumference	X	0.60	0.35	0.67	0.12	0.44
Flower stem diameter		X	0.15	0.43	0.15	0.33
Number of leaves			X	0.12	0.39	0.25
Umbel width				X	0.27	0.37
Height					X	0.23

Supplementary figure 3.1: A visualisation of standardized correlations of the best models per female line (rows). The predicted slopes of the correlation of the best model SEMs (figure 3.2 in main article) are given with a black line (columns). Blue dots represent the raw standardized data points, and are not corrected for the other variables in the model. All data was standardized and values represent z-scores. For brevity we did not include which functional group of pollinators was contributing to visitation rate or species richness, for this see figure 3.2 and table 3.1 in main article.




Pollinators

Chapter 4

Insect pollination is the weakest link in the production of a hybrid seed crop

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Abstract

Ecological intensification of farming proposes that more effective use of ecosystem services can, in part, replace external inputs allowing farmers to maintain high crop yields while reducing adverse effects on the environment. However, uptake of ecological intensification among farmers is currently hampered by a lack of realistic studies on the agronomic benefits of enhancing ecosystem services vis-a-vis the benefits of conventional external inputs. Here, we use a full-factorial field experiment to test the relative and interactive effects of fertilisation, irrigation and pollination on crop yield of three parental crop lines of leek (*Allium porrum*) hybrid seed production. In a commercial leek seed production field, we assessed the agronomic performance of plants receiving conventional or 50% reduced external inputs and that were either continuously accessible to pollinators or only 50% of the time. For all crop lines, we found that reducing insect pollination had at least two times stronger effects on crop yield than similar reductions in fertilisation or irrigation. Surprisingly, reducing fertiliser inputs by half did not negatively affect crop yield (one line) or even increased crop yield (two lines), suggesting that in this system fertiliser is an over-applied agricultural input. Reducing irrigation did not affect crop yield in two lines but reduced crop yield in the third line. However, there were strong indications that this negative effect of reduced irrigation was due to reduced attractiveness for pollinators. Effects of fertilisation, irrigation and pollination on crop yield were additive, with the exception of pollination effects being influenced by fertilisation level in one of the lines. Under real-world conditions, reductions in wild insect pollination consistently reduced hybrid leek crop yield while reductions in external inputs did not. This suggests that in this cropping system insect pollination is the weakest link in the agricultural production process. Our findings help explain why the relation between agricultural intensification and yield growth disappears with the dependence of crops on insect pollination. For insect-depended crops, protection or promotion of wild pollinators in agricultural landscapes is essential for maintaining high yields.

Keywords

Ecosystem services, agricultural management, insect pollination, fertiliser, irrigation, interactions

Introduction

Intensive agriculture has adverse effects on biodiversity in agricultural landscapes (Donald et al. 2001, Tscharntke et al. 2005) and associated delivery of ecosystem services such as pest control (Karp et al. 2018) and insect pollination (Kremen et al. 2002, Kennedy et al. 2013). These negative environmental impacts have raised concerns about the sustainability of intensive agriculture in meeting rising demand for agricultural products (Godfray et al. 2010). Ecological intensification has been proposed as a more sustainable farming approach to maintain and/or enhance agricultural production while minimising negative environmental impacts. It encompasses the adoption of management practices to enhance biodiversity-based ecosystem service delivery to supplement or replace external inputs (Bommarco et al. 2013). However, effective uptake of the concept is limited so far (Kleijn et al. 2019), possibly because the agricultural sector does not perceive ecosystem service-providing organisms to be as important for crop productivity as regular agricultural inputs such as fertilisers or pesticides (IPBES 2016).

While there is a growing body of literature that shows that managing for biodiversity enhances the provision of key ecosystem services underlying agriculture (Kovacs-Hostyanszki et al. 2017), the evidence base may not yet be convincing enough for the agricultural sector to integrate biodiversity into farm management (Kleijn et al. 2019). One commonly raised argument is that biodiversity–based benefits do not outweigh the (opportunity) costs of the measures required to enhance biodiversity such as taking land out of production (Rundlöf et al. 2018, Sutter et al. 2018), at least in the first couple of years (Blaauw and Isaacs 2014, Pywell et al. 2015, Grab et al. 2018). Another commonly heard argument from growers is that they can improve yields via conventional agricultural inputs more easily than through managing for ecosystem services (Kleijn et al. 2019). However, whether managing for more inputs or enhanced ecosystem service delivery is more effective will depend on the contribution of the ecosystem services to crop yield relative to that of agricultural inputs (Fijen et al. 2018).

The most important external agricultural inputs that aim to increase crop yields are fertiliser and irrigation (Tilman et al. 2002, Tilman et al. 2011), while pesticides are mainly applied to reduce yield losses (Oerke 2005). Agricultural intensification has seen a steady rise in agricultural input levels in the last decades, corresponding with increasing yields (Tilman et al. 2002). However, for insect-dependent crops the increase in yields decreases with increasing insect dependency (Deguines et al. 2014), which suggests that pollination is currently often limiting yield of these crops. Globally, two-thirds of the crops depend at least partly on insect-pollination (Klein et al. 2007, Aizen et al. 2008), with wild pollinators generally contributing most to crop yield (Garibaldi et al. 2013, Garibaldi et al. 2016, Fijen et al. 2018). Recently, several studies have explored whether the relative benefits of insect pollination on crop yield depend on the levels of the agricultural inputs (Garibaldi et al. 2018). Many studies find that pollination and agricultural inputs have additive effects on crop yield, suggesting that both pollination and agricultural inputs need to be optimised to increase yield (van Gils et al. 2016, Garibaldi et al. 2018, Garratt et al. 2018).

However, most of these studies (but see Boreux et al. (2013b) & Tamburini et al. (2017)) have used all-or-nothing levels of, for example, insect pollination (no insect pollination vs open pollination) and fertiliser (no fertiliser vs fertiliser). Such extreme contrasts can provide useful information on the mechanisms regulating the contributions of pollination or fertilisation to crop yield, but they cannot reveal the contribution of pollination at different realistic input levels. For example, Tamburini et al. (2017) found that pollination benefits were optimal under intermediate fertilisation levels for crop yield of sunflower. Even for crops that fully depend on insect pollination (e.g. pumpkin (Hurd et al. 1971)) some input of fertilisation and irrigation is still necessary for high yields. Hence, results from all-or-nothing studies are hard to translate into day-to-day practices of farmers. To convince the agricultural sector of the relative importance of insect pollination compared to agricultural inputs, we need studies that use input levels resembling those in real-world systems.

Here we test the reliance of a conventionally managed insect-pollinated crop on pollinators and how this compares to, and possibly interacts with, application of fertilisation and irrigation. We used an experimental approach with a full-factorial, randomised block design in a commercial hybrid leek-seed production field in southern Italy and studied the response of three different crop lines. We compared conventionally managed plants receiving ambient pollinator visitation rates with plants receiving 50% reduced fertilisation and irrigation levels and whose flowers were accessible to pollinators half of the time. The results of this study can help to inform farmer management decisions on focussing on conventional agricultural inputs, insect pollination, or both, and how this varies between lines of the same crop.

Materials and methods

Study system

We used a commercial leek (*Allium porrum*) hybrid seed production field (one hectare) in southern Italy as our experimental field. Because of the hybrid seed production system, the seed producing female parent lines are fully dependent on insects to transport the pollen from the male parent line to the female parent line (Wright 1980, Brewster 2008). Leek is an attractive crop for a wide range of insect pollinators and may attract large numbers of pollinators, in particular bees and hoverflies (Fijen et al. 2018). We selected three different female parent lines in the experiment (referred to as line B, C and F), that varied in their average seed production based on an earlier study across commercial fields (Fijen et al. 2018). Leek plants were transplanted into the field in October 2016, and flowered around June 2017 for about 3-4 weeks. Leek forms a primary umbel and one to three secondary umbels, but for this study we focused on the primary umbel.

Experimental setup and treatments

We used a full-factorial randomised block design with five replicates. Each block contained eight plots in randomised order (two fertiliser x two irrigation x two pollination levels). Within each plot we placed six female plants of each female line and randomised the relative location of female lines to each other. Plants were planted in double rows, with 20 cm between the rows and 10 cm between the plants. To avoid that a treatment in one plot affected the neighbouring plots (for example during irrigation events), we placed 20 buffer plants between two subsequent plots (figure 4.1). The buffer plants were the male parent line of the commercial field, thereby also ensuring a sufficient and nearby pollen source. These plants also received the treatment of the closest plot of female plants. The two treatment levels were 100% (hereafter standard) and 50% (hereafter reduced) levels of conventional application rates of fertilisation, irrigation, and of ambient pollination levels.

We based the standard level of fertiliser on the conventional nurturing protocol for leek and adapted it to local growing conditions (Brewster 2008). The standard fertiliser treatment corresponded to a total of 200 kg/ha nitrogen (N), 120 kg/ha phosphorus (P), and 10 kg/ha potassium (K) in granular form, applied over several fertilising events during the growing season. For the reduced fertiliser level we reduced the NPK amount with 50% for each fertilising event.

In this crop system, irrigation is applied mostly in spring and summer, with the plants receiving approximately four hours of drip irrigation every three days (standard treatment). For the reduced irrigation treatment, we doubled the time between watering events (i.e. six days between events), and not the amount of water per event. We only applied the irrigation treatment from the start of flowering until harvest, as we expected this to be the period with potential water stress affecting seed production. It did not rain in the period from the start of flowering until harvest, so the irrigation was the only source of water in that period.

For pollination we used open-pollination (managed honey bees and wild pollinators) as the standard treatment, and for the reduced treatment we bagged the plants with small mesh bags made of bridal gown every other day during the flowering period. In this way we reduced the time that pollinators could visit the plants with 50%, and we expected that this would effectively reduce pollination success. Stigmatic receptivity of the closely related onion (*Allium cepa*) is approximately five days, but is highest three days after anthesis (Chang and Struckmeyer 1976), which suggests that pollination may depend on a plant being bagged that particular day. To remove any potential effect of bagging day, we treated all plants within a block similar (either all bag on, or all bag off), and randomised which block had the bags on or off at the start of the flowering period.

All other agricultural interventions like weeding, addition of micro-nutrients, or applications of pesticide were applied as in the commercial field. One plot (treatment standard fertiliser, reduced irrigation and reduced pollination) was lost due to the placement of an irrigation pipe, resulting in a total of 39 plots in the experiment.

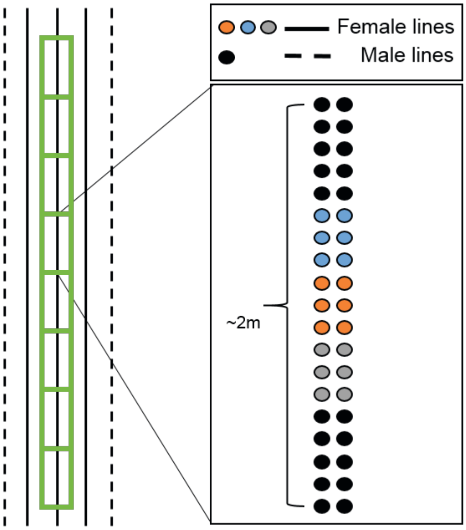


Figure 4.1 Schematic diagram of the experimental setup. The experiment was located in the middle bed of female plants (black solid line = bed of female plants, black dashed line = bed of male plants), with approximately one meter between beds. The green rectangle represents one block consisting of eight treatment plots. The inset shows a schematic setup of one plot, with six experimental plants per female line. Each dot represents a single female plant. The order of the female lines within a plot and the treatment per plot were randomized. The male plants represent a buffer zone between plots to ensure a high pollen load and to avoid effects of neighbouring treatments.

Plant, pollinator and yield measurements

Just before crop flowering we visually selected and marked two representative plants per line per plot as our experimental plants. To facilitate interpretation and explanation of yield effects, we measured several characteristics. Plant size was measured as the basal stem circumference (cm) of the experimental plants just after crop flowering, as this measure correlates well with other plant characteristics, and can be measured throughout the growing period (Fijen et al. 2018). We quantified nectar production as this may influence pollinator visitation rate and can be affected by the fertiliser and irrigation treatments (Gallagher and Campbell 2017). To this end, we bagged two flower heads per line per plot for 24 hours to allow nectar to build-up in the florets. After this period, we used 1 µl micro capillaries to measure for each plant the number of florets required to fill one micro capillary with nectar. We then calculated the average amount of nectar per floret per line per plot. Because the bagging of plants for nectar measurements would interfere with the pollination of the main experiment, we selected two additional plants per line per plot. Furthermore, because the bagging for nectar has the same effect as our reduced pollination treatment, we excluded the reduced pollination plots from nectar measurements. We measured nectar production of the plants in one block per day in the shortest time frame possible, and we randomised the order of plots. Nectar production of each plant was measured on three occasions.

To see if pollinator visitation rates differed between treatments we determined pollinator visitation rates for each experimental plant six times during the flowering period, with a minimum of three days between observations. Observations within blocks were done within the shortest time frame possible, and the order of observations in and between blocks was randomised. Plants which received a reduced pollinator treatment were effectively observed three times, as the other three times the bags excluded pollinator visitation and we assumed no visitation occurred (in total six observation rounds). We counted all bees and hoverflies that landed on the umbel during 30 minutes, or until five pollinators had visited the umbel (Fijen and Kleijn 2017). We then calculated visitation rate (pollinators/minute) for each line in each plot on each observation day, including the bagged days.

To measure seed yield we harvested the flower heads of the experimental plants just before seed shedding in August 2017, and we pooled the flower heads per line per plot. Flower heads were left to dry, and then threshed and cleaned. We counted the number of seeds using a seed counter (Contador, Pfeuffer GmbH). We subsequently assessed seed quality with a vigour test (see also Fijen et al. (2018)). In this test, three sets of 100 randomly selected seeds were sown in suboptimal circumstances and after 18 days, the vigour of the seedlings was assessed by experts in a NAL-authorized test (Naktuinbouw Authorized Laboratory). Vigour was categorized as (A) optimal, (B) suboptimal, (C) poor, or (D) did not emerge. The average vigour scores (%) were calculated over the three sets. For the marketable seed yield we calculated the total amount of good quality seeds (Vigour A + B * total number of seeds).

Analysis

We separated analyses per line because the sample size (n = 5 blocks) was relatively low, and four-way interactions would be difficult to analyse and interpret. Furthermore, to avoid pseudo replication, we averaged measurements and observations per line per plot. We performed all analyses using linear mixed effect models with block as random factor using the function ‘lmer’ in R-package lme4 with R-version 3.5.2 (Bates et al. 2015, R Core Team 2018).

We tested the effects of the treatments on plant size, nectar production, pollinator visitation rate and marketable seed yield in separate models. We constructed a full model with the treatments and their interactions, and assessed significance of treatment effects using backward model simplification based on likelihood ratio tests (Burnham and Anderson 2002). Because visitation rate could also be affected by plant size (e.g. visual cue) and nectar production (e.g. reward cue), we furthermore tested this in a separate model including plant size, nectar production and the two-way interaction, and block as random factor. We then simplified the model based on the same approach as above. Nectar production (average nectar per floret) and average visitation rate were log-transformed and log+1 transformed, respectively, to improve normality of residuals. As we did not measure nectar production in plots with 50% pollinator treatment, we excluded this treatment for that analysis. We excluded two extreme outlier in the analyses with visitation rate for line B and F (value >5 and >4 SD from mean, respectively).

Results

The fertilisation treatment had consistent effects on plant growth (figure 4.2A) with on average 7%, 11% and 9% smaller plants under reduced fertilisation than standard fertilisation in line B, C and F respectively (B: χ^2 (1) = 3.89, $P = 0.048$; C: χ^2 (1) = 17.30, $P < 0.001$; F: χ^2 (1) = 6.38, $P = 0.015$; figure 4.2A). Other treatments had no significant effect on plant size, nor were there any significant interaction effects ($P > 0.66$).

Treatment effects on nectar production differed slightly per line. Reducing fertiliser application lowered nectar production with 24% in both line C and F (C: χ^2 (1) = 6.41, $P = 0.011$; F: χ^2 (1) = 7.17, $P = 0.007$), while reducing irrigation lowered nectar production with 21% and 37% in line C and F (C: χ^2 (1) = 7.91, $P = 0.005$; F: χ^2 (1) = 16.30, $P < 0.001$) respectively. In line B, nectar production decreased with decreasing irrigation but the effect was stronger in the standard fertilisation treatment (i.e. significant interaction fertilisation x irrigation; χ^2 (1) = 6.18, $P = 0.013$; figure 4.2B).

The effects of treatments on visitation rate differed considerably between crop lines. The bagging treatment did not affect visitation rates in line B ($P = 0.70$; figure 4.2), even though flowers received zero visitors on the days they were bagged. Line C plants without bags had about three times as many visitors as plants with bags (figure 4.2C) and this effect was stronger for plants also receiving the standard fertilisation treatment (i.e. significant fertilisation × pollination interaction; χ^2 (1) = 4.65, $P = 0.03$). Both the reduced irrigation and the reduced pollination treatment decreased average visitation rate in line F (irrigation: χ^2 (1) = 4.76, $P = 0.03$; pollination: χ^2 (1) = 7.66, $P = 0.006$; figure 4.2C). Furthermore, we found that in line F, but not in the other lines, visitation rate was significantly lower with increasing plant size (χ^2 (1) = 4.86, $P = 0.03$), and that visitation rate increased with increasing nectar production (χ^2 (1) = 5.99, $P = 0.014$; figure 4.3).

The treatments affected marketable seed yield differently in each line, but the reduced pollination treatment had a significant negative effect in all of the three lines. For line B, we found a positive interactive effect of fertilisation and pollination (χ^2 (1) = 8.26, $P = 0.004$), indicating that pollination increased yield under standard, but not reduced fertilisation rate (figure 4.4). In line C we found that marketable seed yield was 60% higher under the reduced fertilisation rate (χ^2 (1) = 14.18, $P < 0.001$), and that the reduced pollination treatment had only 36% of the amount of marketable seeds as the standard pollination treatment (χ^2 (1) = 39.40, $P < 0.001$; figure 4.4). For line F we found that both the reduced treatments of irrigation (15% less; χ^2 (1) = 7.85, $P = 0.005$) and reduced pollination (27% less; χ^2 (1) = 21.38, $P < 0.001$) yielded significantly less marketable seeds (figure 4.4).

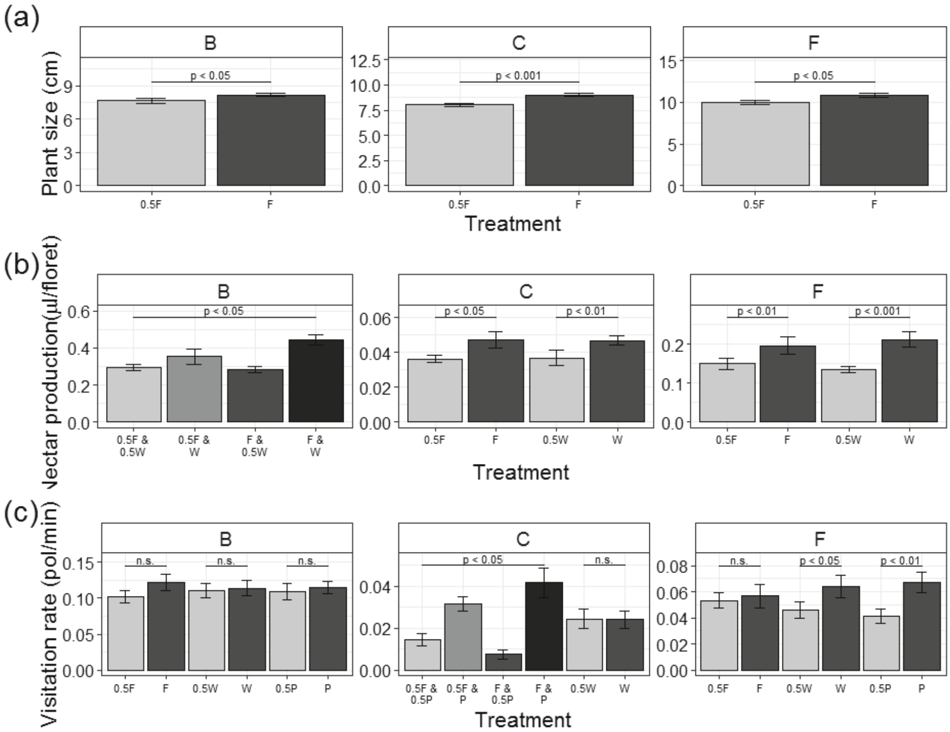


Figure 4.2 Treatment effects (F = fertilisation, W = irrigation, P = pollination) on plant characteristics and pollinators. Only treatment effects that are significant for at least one line are shown. (a) Plant size (only fertilisation), (b) nectar production (only fertilisation and irrigation treatments), and (c) pollinator visitation rate per line. Standard treatment levels are indicated in dark grey, reduced levels in lighter grey. Interaction or pairwise significance levels are indicated on top (n.s. = not significant). Bars show average values ± standard errors.

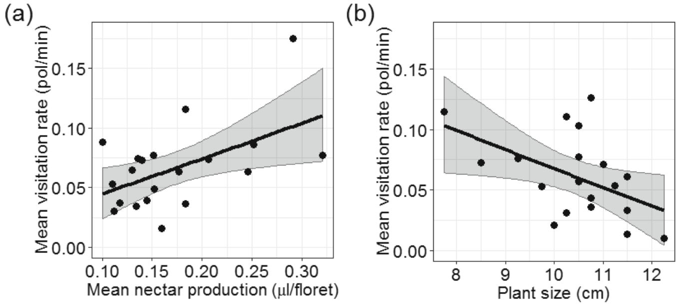


Figure 4.3 The relations between mean pollinator visitation rate and (a) mean nectar production and (b) plant size in line F. Relations were not significant for line B and C (see main text). Black points represent back-transformed partial residuals.

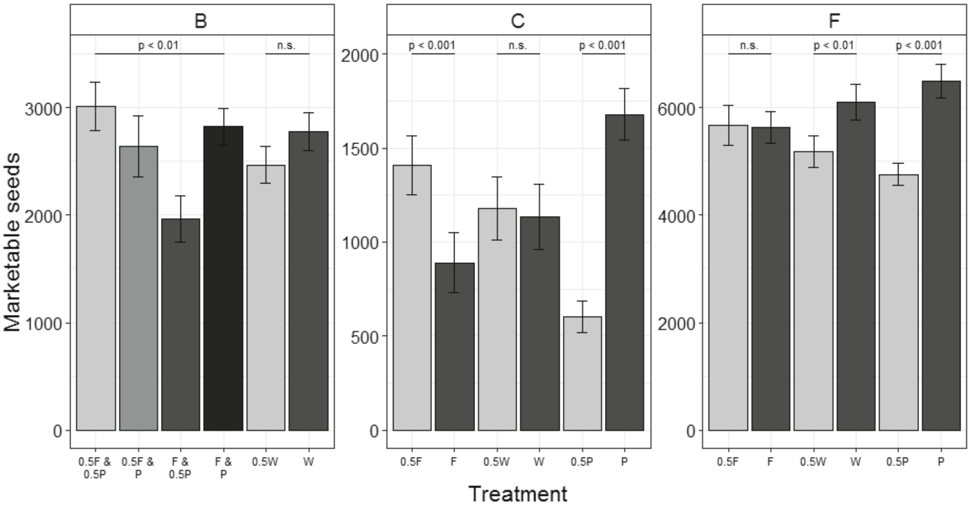


Figure 4.4 The effects of treatments (F = fertilisation, W = irrigation, P = pollination) on the total number of high-quality seeds per two female plants. Standard treatment levels are indicated in dark grey, reduced levels in lighter grey. Interaction or pairwise significance levels are indicated on top (n.s. = not significant). Bars show averages ± standard errors)

Discussion

Our results show that leek marketable seed yield is influenced more by a reduction in insect pollination than reductions in fertilisation and irrigation application rates, but the magnitude of effects differed between the crop lines. Surprisingly, although a 50% reduction in fertilisation reduced plant size in all three lines, it did not reduce seed yield in one of the lines and even increased it in the two other lines. The effects of reducing irrigation were less pronounced, but the results suggest that high irrigation rate may be beneficial for crop yield, and possibly act through the beneficial effects of irrigation on pollination. Treatment effects were mainly additive, but in one line the influence of pollinators became apparent only under standard fertiliser levels. These results indicate that NPK-fertilisers are over-applied, and that pollination is undervalued as an agricultural input in this crop system.

Of all treatments, manipulating pollination levels generally had the strongest effects on marketable seed yield, suggesting that in our study system variation in insect pollination influences crop yield more than variation in fertilisation or irrigation. The magnitude of effects differed between crop lines, however. The yield difference caused by the pollination treatment in lines C and F was around two times larger than the effect of the respective fertilisation and irrigation treatments. In line B the only significant effect on crop yield was caused by reduced pollination but only under the standard fertilisation treatment (i.e. significant interaction). That different lines displayed different yield responses to the pollination treatment is also reflected in the different effects of the pollination treatment on pollinator visitation rate of the crop lines. Plants of line B and F that were bagged every other day were visited by pollinators relatively more frequently on the days when they were not bagged compared to plants that were never bagged (i.e. visitation rate of bagged treatment was more than 50% of un-bagged treatment), thereby reducing the effective difference between pollinator treatments. These crop lines had relatively high nectar production rates, and this likely made the plants extra attractive on the days they were not bagged. We nevertheless found significant effects of the reduced pollination treatment on marketable seed yield in line B, possibly because florets were on average less receptive on un-bagged days in the reduced pollinator treatment (Chang and Struckmeyer 1976, Devi et al. 2015). In contrast, bagged plants of line C were relatively less attractive on the un-bagged days compared to the not-bagged plants, which was reflected in the strong effect of the pollinator treatment on marketable seed yield. Even though the crop lines responded differently to pollination reduction, our results suggest that in this cropping system insect pollination is the weakest link in the agricultural production process.

Unexpectedly, although fertiliser application had clear positive effects on plant size and nectar production, reducing fertiliser inputs by 50% did not affect crop yields, or even increased crop yield. From a farmer’s perspective, it may be understandable why such high levels of fertiliser are generally applied, as higher input levels resulted in larger plants, which can easily be observed in the field. Furthermore, over-application can act as a form of insurance against crop failure (Sheriff 2005). Nevertheless, crop yields were equally high or even higher with less fertiliser inputs, showing that increasing fertiliser levels in this high-input system is not the most appropriate way to maximise seed yield or reduce risk. A possible explanation for lower crop yields under conventional levels might be that the over-application of fertiliser lowered the concentration of other nutrients in the plant that are essential for seed production (Sørensen et al. 1995, Fageria 2001). For example, reduced boron concentrations may lead to reduced seed quantity or quality (Johnson and Wear 1967, Dordas 2006). The over-application of fertiliser may be more common and wide-spread than generally assumed, as it has also been found for the majority of global staple seed crops like wheat, rice and maize (Matson et al. 1998, Ju et al. 2009). This practice is not only pushing up costs of fertiliser application (Matson et al. 1998, Compton et al. 2011, Sutton et al. 2011), but also comes with undesirable high environmental costs (Foley et al. 2005, Kleijn et al. 2009, Vitousek et al. 2009).

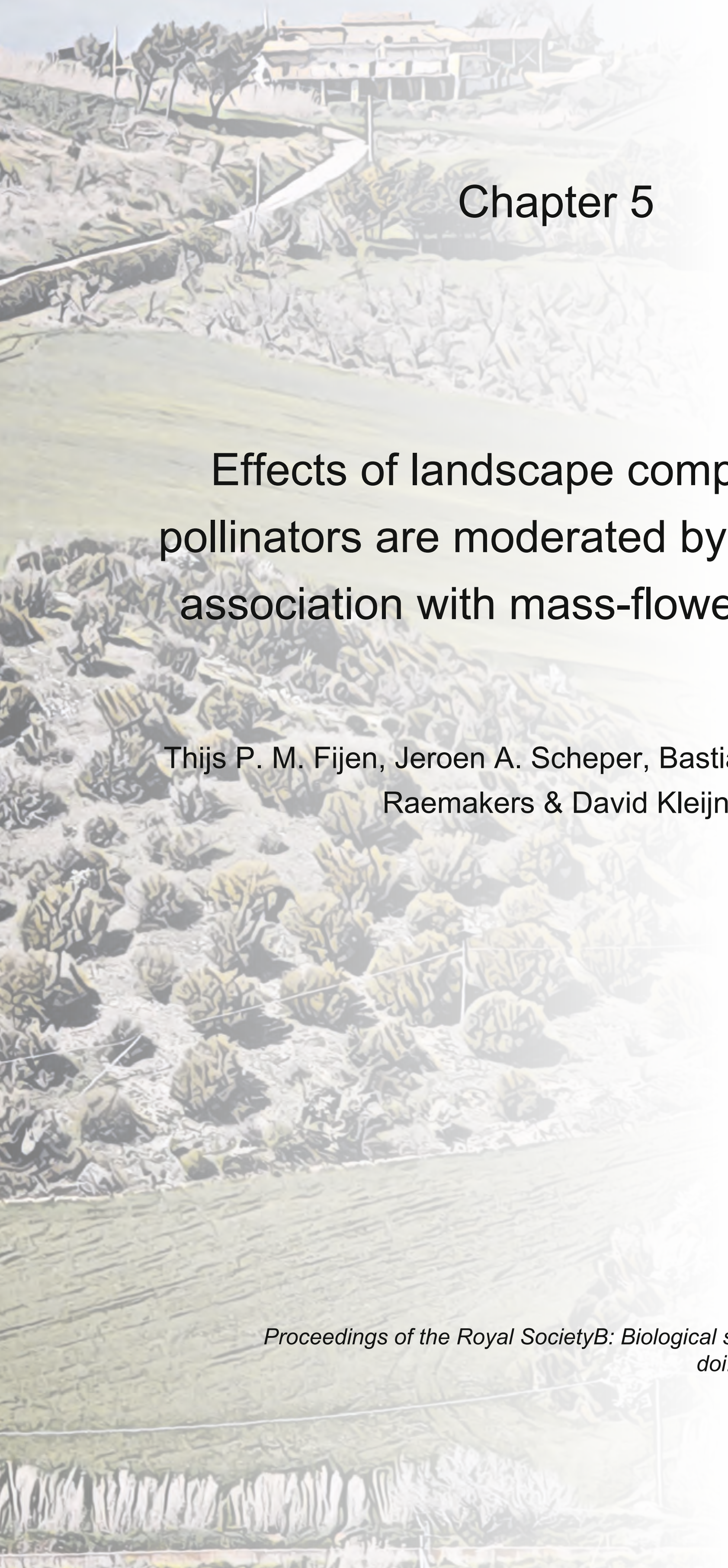
Reducing irrigation frequency during the crop flowering period generally had a negative effect on nectar production, but only in line F did it have a significant negative effect on crop yield. Concurrently, line F was also the only line where higher nectar production was positively related to pollinator visitation rate (figure 4.3), suggesting that benefits of irrigation for this line may largely be attributed to higher attractiveness of the plants for pollinators (Gallagher and Campbell 2017). The reduced nectar production in the two other lines did not significantly affect pollinator visitation rate, nor did it affect crop yield, possibly indicating that nectar quality might play a more prominent role in these lines. Although, across all lines, reduced irrigation had no clear effect on seed yield, it will probably become an increasingly important part of the day-to-day agricultural management under future climate change, with expected longer and more intense periods of droughts (Parry et al. 2004, Dai 2013). In addition to irrigating crops to promote plant establishment and growth, our results suggests that irrigation during the flowering period of insect dependent crops may act as a tool to increase or maintain high pollinator visitation rates, and thereby maintaining high pollen dispersal.

Our experiment was realistic and representative for the levels of agricultural management in this system. The plant sizes of all lines in the standard fertilisation treatment corresponded to the median plant size across 36 commercial fields in a previous study, while the visitation rate for the standard pollination treatment was around the average (above median; positively skewed) for these lines (Fijen et al. 2018). Furthermore, the reduced treatments decreased plant sizes and visitation rates (with the exception of line B) so that the levels approached the lowest 25% percentile of observations across fields. More than half of the crop fields in the previous study showed pollinator visitation rates well below the standard level in our study. Furthermore, honeybees are stocked in the same densities in each field. Whereas fertilisation and irrigation levels have seemed to reached, or overshot, the optimum input levels, there still lies great potential in enhancing pollinator populations. If wild pollinators can be effectively promoted, this may potentially close a large yield gap, even in this intensively managed agricultural system.

Although the patterns differed subtly between crop lines, our results show that effects of fertiliser application, irrigation and pollination on crop yield were largely additive in this system, making effects of different management strategies rather predictable. A reduction in insect pollination generally resulted in substantially lower crop yields. Contrastingly, a reduction in fertiliser inputs did not lower crop yields, and even increased crop yields in two crop lines. Our findings may explain why previous studies have found that the relation between agricultural intensification and crop yield growth decreases with increasing dependence of crops on insect pollination (Garibaldi et al. 2011a, Deguines et al. 2014). A further intensification by means of conventional agricultural inputs may therefore not be a very efficient approach for increasing crop yield of insect-dependent crops. Instead, putting more effort into promoting the abundance and diversity of wild pollinators is more likely to result in higher crop yields (Fijen et al. 2018). Whether doing this is actually cost-effective to a farmer will depend on the opportunity costs of pollinator-enhancing measures (Kleijn et al. 2019) and the expected yield increase per crop line. Our results show that, although it currently receives little or no attention in this system, pollination by wild insect is the agricultural input that has the highest potential to improve productivity.

Acknowledgements

We are grateful for the field assistance of Koen Verhoogt. BASF Vegetable Seeds kindly provided access to the field sites and assisted in the logistics of this study.



Chapter 5

Effects of landscape complexity on pollinators are moderated by pollinators' association with mass-flowering crops

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Abstract

Conserving and restoring semi-natural habitat, i.e. enhancing landscape complexity, is one of the main strategies to mitigate pollinator decline in agricultural landscapes. However, we still have limited understanding of how landscape complexity shapes pollinator communities in both crop and non-crop habitat, and whether pollinator responses to landscape complexity vary with their association with mass-flowering crops. Here, we surveyed pollinator communities on mass-flowering leek crops and in nearby semi-natural habitat in landscapes of varying complexity. Surveys were done before and during crop bloom and distinguished between pollinators that visit the crop frequently (dominant), occasionally (opportunistic) or not at all (non-crop). Forty-seven percent of the species in the wider landscape were also observed on leek flowers. Crop pollinator richness increased with local pollinator community size and increasing landscape complexity, but relationships were stronger for opportunistic than for dominant crop pollinators. Relationships between pollinator richness in semi-natural habitats and landscape complexity differed between groups with the most pronounced positive effects on non-crop pollinators. Our results indicate that while dominant crop pollinators are core components of crop pollinator communities in all agricultural landscapes, opportunistic crop pollinators largely determine species-richness responses and complex landscapes are local hotspots for both biodiversity conservation and potential ecosystem service-provision.

Keywords

Local pollinator species pool, crop pollinator species pool, landscape complexity, dominant crop pollinators, opportunistic crop pollinators, non-crop pollinators

Background

Biodiversity in agricultural landscapes is rapidly declining (Foley et al. 2005) along with the ecosystem services it provides (IPBES 2016). Loss of semi-natural habitat, or landscape simplification, is generally considered to be one of the main drivers underlying these declines (Ricketts et al. 2008). Insect pollinators of crops are amongst the best examined species groups and show a particularly consistent decline with reductions in the proportion of semi-natural habitat in the landscape (i.e. landscape complexity (Ricketts et al. 2008, Tscharntke et al. 2012)). Ecological intensification has been proposed as an approach to maintain high yield levels while at the same time promoting biodiversity in agricultural landscapes (Bommarco et al. 2013, Kleijn et al. 2019), in particular by conserving and restoring semi-natural habitats in agricultural landscapes (Morandin and Kremen 2013, Kleijn et al. 2019). However, we still lack the knowledge of how landscape complexity modifies the crop and non-crop pollinator communities in agricultural landscapes, and how pollinator responses differ depending on their association with mass-flowering crops. Elucidating these patterns may help us to design effective management strategies that unite the goals of enhancing and protecting crop pollination services with wider biodiversity conservation.

Pollinator species differ in the extent to which they use crops as a food source, which can be used to classify them into different functional groups. Crop flower visitation is generally dominated by a relatively small number of species that are particularly able to exploit mass-flowering crops and thereby contribute most to crop yield (dominant crop pollinators; (Kleijn et al. 2015)). Other pollinator species may only make opportunistically use of crop flowers, as they are only occasionally observed on crops and usually in small numbers (henceforth referred to as opportunistic crop pollinators). Recent studies have shown that these species can make a significant contribution to crop pollination that is additional to that of the dominant crop pollinators (Garibaldi et al. 2016, Fijen et al. 2018). However, the majority of all the species that occur in agricultural landscapes within flying distance of a crop may never be encountered on crop flowers because they for example do not collect pollen (cuckoo bees), or are specialised on other plant species (Senapathi et al. 2015). The proportion of the three different functional groups (dominant, opportunistic and non-crop pollinators) of pollinator species in the local species pool (i.e. the species in the semi-natural habitat) is unknown and may furthermore change with the size of the local species pool (figure 5.1). In turn, this is generally related to the proportion of semi-natural habitat in the landscape (figure 5.1) because agricultural fields rarely provide all the resources required by pollinators to complete their life cycle (Ricketts et al. 2008, Tscharntke et al. 2012).

It may be expected that the relationship with landscape complexity is stronger for non-crop pollinators than for species that can use crop resources because non-crop pollinators rely on semi-natural habitats for provision of all their resources, while crop visitors can obtain part of their floral resources outside semi-natural habitats. For similar reasons we may expect relationships with semi-natural habitat cover to be stronger for opportunistic crop pollinators than for dominant crop pollinators. With larger complexity of the landscape, not only the local species pool size may differ but also the relative contribution of the three groups of pollinators to that local species pool. Furthermore, these relationships are probably influenced by whether the crop is flowering or not. During crop flowering, part of the local species pool, i.e. the species that forage on crops, will be concentrated on the crop fields (Tscharntke et al. 2012), potentially freeing up floral resources for the pollinators remaining behind in the semi-natural habitats. Whether this strengthens or weakens relationships between landscape complexity and the species pools of the three groups of pollinators has yet to be determined.

Abundance, rather than diversity, of crop pollinators is the main contributor to crop pollination service delivery (Kleijn et al. 2015, Winfree et al. 2015). A final issue that therefore needs to be considered is whether the relationships between local pollinator species pool size and landscape complexity are indicative of the relationships between pollinator abundance and landscape complexity. This is not necessarily the case because in agricultural landscapes dominant crop pollinators species may make a small contribution to the local species pool but often make up the majority of all pollinators in the crop (Kleijn et al. 2015). In the crop, abundances of these dominant crop pollinators usually increase with increasing landscape complexity because with increasing cover of semi-natural habitat surrounding crop fields more individuals can move into these fields when they start to flower (Blitzer et al. 2012, Schellhorn et al. 2015, Holzschuh et al. 2016). We can only speculate how the migration of large numbers of pollinators out of the semi-natural habitats affects the abundances of the individuals that remain in the semi-natural habitats during crop flowering (Schellhorn et al. 2015).

Here we study whether pollinator communities on crop fields (crop pollinator species pool) are related to the pollinator communities in semi-natural habitats (local pollinator species pool) in landscapes of varying structural complexity, and whether this relation differs with the association of pollinator species with crops (figure 5.1). We surveyed pollinators in the landscape and in the mass-flowering, hybrid leek-seed production fields in 18 agricultural landscapes in Italy both prior and during crop flowering (see (Fijen et al. 2018) for the pollination effects on crop yield). We examined what proportion of the local pollinator species pool size contributes to crop pollination and examined whether the size of the local species pool was related to the number of species observed on crop flowers. We subsequently tested how the species pool size and abundance of pollinators are related to landscape complexity, and whether this was moderated by crop flowering, and by functional groups based on pollinators' association with crops. Our results provide important insights into whether and how landscape-scale management can simultaneously benefit biodiversity conservation and ecosystem service delivery objectives.

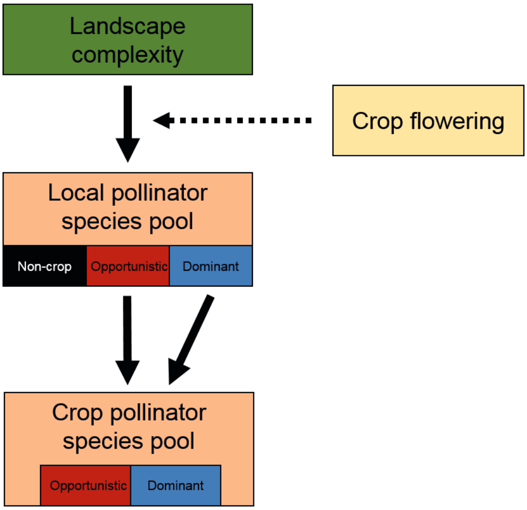

Figure 5.1 The local pollinator species pool (i.e. species pool in nearby semi-natural habitats) in agricultural landscapes is expected to be related to landscape complexity, but this relation may differ between functional groups of pollinators depending on their association with crops, and this relation may be moderated by crop flowering. The size of the local pollinator species pool is likely to be related to the crop pollinator species pool, but may differ for functional groups that differ in their association with crops.

Methods

Study system and landscape selection

Our study area is situated in southern Italy in a Mediterranean agricultural landscape spanning about 615 km^2. The main cultivated crop in this area is wheat, but several other crops are cultivated as well such as tomato, field bean, asparagus, and our focal crop leek (*Allium porrum*) for hybrid seed production. Flowering leek is an attractive crop for insect pollinators, and high abundances and a high diversity of species have been found on leek in earlier studies (Fijen and Kleijn 2017, Fijen et al. 2018). Depending on the altitude, leek flowers for approximately 4-6 weeks in June and July.

In autumn 2015 we selected 18 leek fields (0.5–2 ha) along a gradient of semi-natural habitat cover (%) to examine relationships between landscape complexity and species richness and abundance of pollinators in the landscape as well as in the crop (Fijen et al. 2018). Because altitude was generally positively correlated with the cover of semi-natural habitat, we took special care to select sites with lower altitude and high semi-natural cover and vice versa. After selection, landscape complexity ranged from 0.4% to 55.4% semi-natural habitat cover (mean = 20.30% ± 18.4 SD). The field sites were usually located 2000 metre or more from each other, so that there was no overlap in landscape cover. Because the average flight distance of most bees, the majority of leek pollinators (Fijen et al. 2018), is less than 1000 m (Zurbuchen et al. 2010), the field sites can be considered independent of each other.

Pollinator and flower surveys

In each landscape we surveyed pollinator abundance and species richness using standardised transect walks in the crop and in semi-natural habitats in the landscape surrounding the crop fields. Transects in the semi-natural habitat were located in flower rich herbaceous focal areas at on average 290 metre ± 145 SD (range: 77-537 metre) distance from the leek fields. Pollinators in the semi-natural habitat transects therefore had access to both the crop field and semi-natural habitats. On each observation day we walked one transect in semi-natural herbaceous habitat based on the most flower rich patches in the focal area, and one fixed transect in flowering leek fields. Transects in semi-natural habitats were visited five to seven times (mean 5.5 ± 0.8 se) of which 2-4 times before crop flowering (mean 2.6 ± 0.1 se) and 2-4 times during crop flowering (mean 2.9 ± 0.1 se). Crop transects were visited 3-5 times (mean 3.8 ± 0.7 se). We visited each transect with a minimum of five days between subsequent visits between

19 May 2016 to 10 July 2016 and only under weather conditions that are favourable for pollinator activity (Fijen and Kleijn 2017).

Transects consisted of 150m^2 of pollinator habitat, divided over three contiguous sub-transects of 50m^2 to spread sampling time evenly over the transect (Scheper et al. 2015, Holzschuh et al. 2016). Transect width was fixed to one metre in crop fields, but varied from 1-3 metre width in semi-natural habitat and length was adjusted accordingly. We observed each sub-transect for five minutes net collection time by slowly moving up and down alongside the transect boundary (15 minutes in total per transect), and recorded all bees and hoverflies that were clearly associated with flowers (i.e. excluded fly-bys) (Scheper et al. 2015, Holzschuh et al. 2016). Pollinators were identified on the wing whenever possible. When this was not possible pollinators were collected for later identification to the lowest taxonomic level possible. Directly after surveying the pollinators in each transect in semi-natural habitat, we visually estimated the flower cover (%) of each plant species for each sub-transect with increasing accuracy with decreasing flower cover (i.e. 10% cover with 1% accuracy, and 1% cover with 0.1% accuracy). If the flower cover of a species was estimated to be lower than 0.05% (250 cm^2 per sub-transect), we set the flower cover for that species at 0.025%. We summed the flower covers of each plant species to obtain total flower cover estimates (%).

Landscape complexity

We quantified landscape complexity as the cover (%) of semi-natural habitat such as woodland, semi-natural grassland, fallow arable fields, and road verges in a radius of 1000 metre around the centre of the leek fields. To estimate total cover of road side verges we first determined the total road length and then multiplied this with an assumed standard one metre width of road verge on each side of the road. The delineations and classifications were based on aerial imagery and ground-truthed by visual inspection of the fields and by using up-to-date RGB satellite imagery of 22 July 2016 (10m spatial resolution, source: Sentinel 2, processing level 1C). The main mass-flowering crops other than leek were field bean (mean 3.7% cover), tomato (1.8%) and asparagus (0.4%). Field bean flowered well before the sampling period of this study and in this study area is visited mainly by species that were no longer active during the current study (*Eucera* spp., *Anthophora plumipes*, *Xylocopa violacea* and *Bombus hortorum*; T. Fijen pers. obs. 2018). We considered the temporal overlap and/or range in cover of mass-flowering crops to be insufficient for producing meaningful results and therefore did not consider this factor in our analyses.

Analysis

Before analysis, we first assigned each encountered pollinator species to one of the functional groups dominant, opportunistic or non-crop pollinators. Kleijn et al. (2015) define dominant crop pollinators as bee species that comprise at least 5% of all crop pollinators in a single study. If we would use this criterion based on the data from our own survey, the maximum possible dominant crop pollinator species would be 20. Because the number of opportunistic pollinator species is not bound by such an upper limit this could inherently lead to opportunistic pollinators being more responsive to explanatory variables than dominant crop pollinators. We therefore classified pollinators as dominant crop pollinators if they were listed as dominant crop pollinators in any of the European crops by Kleijn et al. (2015). Unfortunately, such a database does not exist for hoverflies and we therefore chose to define hoverfly species as being dominant if they comprised at least 5% of all crop pollinators counted in our own crop fields. To check whether the use of different classification criteria affected the results we also ran two sets of analyses using alternative classifications: dominant crop pollinators based on our own survey data, or based on Kleijn et al. (2015) but excluding hoverflies. These different classifications resulted in the same overall patterns (supplementary information, figures S5.1-5.8) except for the densities of opportunistic crop pollinators in the semi-natural habitats before crop flowering when excluding hoverflies (supplementary information, figure S5.8, see results). All other pollinators found in our crop fields were classified as opportunistic crop pollinators. Species that were only encountered in semi-natural habitat were classified as non-crop pollinators. Honeybees (*Apis mellifera*) are common in the area, and because we are interested in the patterns of wild pollinators, we excluded honeybees from all analyses. However, analyses including honeybees as a dominant crop pollinator yielded qualitatively similar results (supplementary information, figure S5.9-5.12), and mean abundances of honeybees were not related to landscape complexity (supplementary information, figure S5.13). Because the sampling effort between field sites differed, we used bootstrapping to estimate the average cumulative number of species per functional group, standardised to the minimum number of transects walked (n = 2, 2, 4 and 3 for landscape before, during, and before and during crop flowering, and in the crop, respectively). For example, in a field site where we walked three transects in semi-natural habitat before crop flowering, we made 1000 random combinations of two observation dates, with replacement, and calculated average cumulative species richness for each combination. Pollinator abundance was averaged over all transects and was log-transformed before analyses to improve normality of residuals; flower cover and cover of semi-natural habitat were respectively log- and square root-transformed to reduce positive skew. We included the average flower cover in analyses in the semi-natural habitats, as this was highly variable between sites and may cause an attraction effect (Tscharntke et al. 2012), while this was not the case for the crop transects.

To test the relationship between the local pollinator species pool (i.e. the number of pollinator species in semi-natural habitat, before and during crop flowering) and the crop pollinator species pool, we first used linear regressions for richness and abundance. Subsequently, to test whether this relation was different for the dominant and opportunistic crop pollinator species pool (i.e. in the crop field) we used mixed effects models with site as random factor using the function 'lmer' in R-package *lme4*. Significance of effects was assessed using likelihood-ratio tests. Response variables were the number of pollinator species and abundance of pollinator species encountered in the crop fields. Explanatory variables were the total number of species encountered in the semi-natural habitat (before and during crop flowering), functional group and their interaction to test if the relationship between local and crop species pool differed between dominant and opportunistic crop pollinators.

To examine the relation between landscape complexity and pollinator species richness, we first performed separate linear regressions between landscape complexity and pollinator species richness, respectively measured in the semi-natural habitats and crop fields. To test how this relation was moderated by crop flowering and functional group, we used mixed effect models with site as random factor in separate analyses for pollinator richness in the crop and semi-natural habitat. For the response of species richness in the semi-natural habitat transects we included the three-way interaction of landscape complexity, functional group and crop flowering as fixed factors, and we included the average flower cover as this was highly variable between sites and may cause an attraction effect (Tscharntke et al. 2012), while this was not the case for the crop transects. Patterns of species richness in crop transects were analysed with a similar model except that only the effects of landscape complexity, functional group and their interaction were included as factors. In case of significant interactions, we further explored observed patterns by performing post-hoc analyses for each pollinator group separately.

Similar to analysis with species richness, we first tested whether overall abundance in the semi-natural habitat transects and in the crop transects was related to landscape complexity in simple linear regressions. To test the relation between landscape complexity and pollinator abundance for each functional group and whether this was moderated by crop flowering, we distinguished between the pollinator abundances before crop flowering and during crop flowering, resulting in a total of eight different abundances. As the abundances could differ two orders of magnitude between different locations or periods, we used separate linear regressions to test the relation of each of the eight abundances to semi-natural habitat cover.

Finally, to test if average pollinator abundances in nearby semi-natural habitats differed between before and during crop flowering periods, and to test if pollinator abundances differed between the semi-natural and crop transects, we compared the average abundances for each of the functional groups using mixed effect models with site as random factor. As explanatory variables we included period of sampling (before or during crop flowering), functional group and their interaction for the first model, and habitat type, functional group and their interaction for the second model. All statistical analyses were performed in R version 3.5.1 (R Core Team 2018).

Results

We counted 7578 pollinator individuals in leek transects and 4047 individuals in semi-natural habitat transects, comprising a total of 171 species of wild bees (n=8278 individuals, n=133 species) and hoverflies (n=3347 individuals, n=38 species). Eighteen species (10.5% of all species; mean 310 ± 482 SD individuals per species) were classified as dominant crop pollinators, with the five most dominant species being *Bombus lapidarius* (n=1480), *Andrena flavipes* (n=1448), *Bombus terrestris/lucorum* (n=1112), *Lasioglossum malachurum* (n=692) and *Syritta pipiens* (n=520). A total of 62 species (36.3%; mean 80 ± 237 SD individuals species^{-1}) were encountered only occasionally in crops and the remaining 91 species (53.2%; mean 12 ± 30 SD individuals species^{-1}) were only found in the semi-natural habitat transects. Eighteen species were only encountered in the crop fields (10.5%; mean 7 ± 11 SD individuals species^{-1}).

Local pollinator species pool size

The total crop pollinator species pool size was significantly positively related with the local species pool size in the semi-natural habitat transects ($F_{1,16} = 14.68$, $p = 0.001$), but this relation was stronger for the opportunistic crop pollinators than for the dominant crop pollinators (significant interaction effect local species pool × functional group: χ^2 (1) = 4.68, $p = 0.03$; figure 5.2A). In the most species-poor landscapes, the crop species pool was approximately as large as the local species pool, while in the species-rich landscapes the crop species pool comprised 71% of the local species pool size

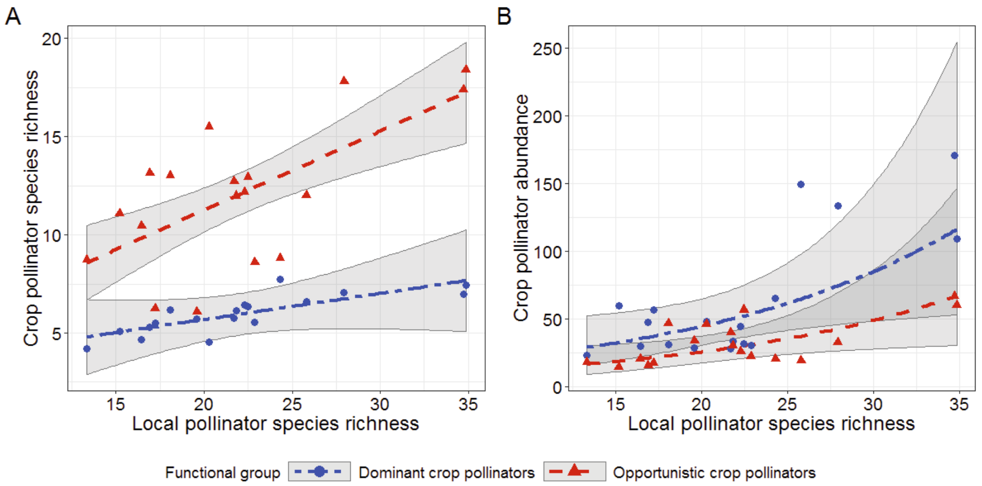

Figure 5.2 Relationships between the local pollinator species richness and the pollinator species richness (A) and abundance (B) in crops. Local pollinator species richness is based on transects in semi-natural habitat (both before and during crop flowering), while crop abundances and richness are based on transects in crop fields. Separate regressions are indicated for dominant crop species (blue circles) and opportunistic crop species (red triangles), and 95% confidence intervals are indicated with grey. Results are back-transformed partial residuals.

(figure 5.2A). With an increasing local species pool in the landscape, the abundance of pollinators in crop fields increased (χ^2 (1) = 5.24, p = 0.02; figure 5.2B) similarly for both dominant and opportunistic crop pollinators (i.e. no significant interaction effect local species pool × functional group: χ^2 (1) = 0.80, p = 0.37; figure 5.2B). Dominant crop pollinators were more abundant in crop fields than opportunistic crop pollinators (χ^2 (1) = 13.64, p = 0.003; figure 5.2B), and made up approximately 63% of all crop visitors across the entire gradient in local species pool.

Landscape complexity

The total size of the local species pool did not significantly increase with increasing landscape complexity ($F_{2,15}$ = 2.17, p = 0.11). There was only marginal support for a three-way interaction between functional group, period of sampling and landscape complexity (three-way interaction: χ^2 (2) = 5.30, p = 0.07; figure 5.3A), but both the two-way interactions between functional group and period (χ^2 (2) = 9.82, p = 0.007), as well as between functional group and landscape complexity (χ^2 (2) = 6.22, p = 0.04) were significant. There were no strong effects of landscape complexity before crop

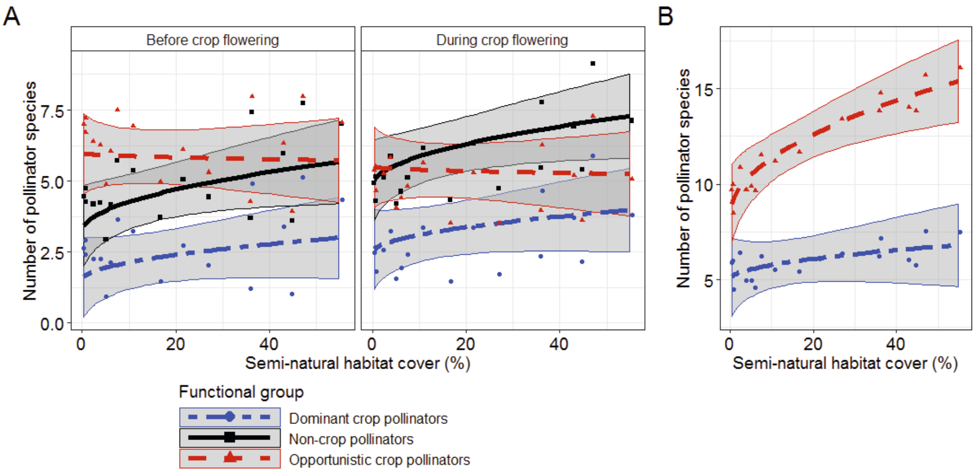

Figure 5.3 Relation between cover of semi-natural habitat (%) and pollinator species richness. Separate panels are given for (A) semi-natural habitat transects before and during crop flowering and (B) crop transects during crop flowering. Back-transformed regressions and predicted species richness are indicated for dominant crop pollinator species (blue circles), opportunistic crop pollinator species (red triangles), and non-crop pollinator species in the landscape (black squares). 95% confidence intervals are indicated with grey.

flowering, whereas during crop flowering, non-crop pollinators responded positively to semi-natural habitat cover ($F_{2,15}$ = 3.48, p = 0.03). Total pollinator species richness in the crop fields increased significantly with landscape complexity ($F_{1,16}$ = 8.93, p = 0.008). Both the species richness of dominant ($F_{1,16}$ = 8.93, p = 0.02) and opportunistic crop pollinators ($F_{1,16}$ = 8.93, p = 0.01) increased with increasing landscape complexity, and this effect was stronger for opportunistic crop pollinators (χ^2 (2) = 4.87, p = 0.03; figure 5.3B).

In the semi-natural habitats surrounding leek fields, the total average abundance of pollinators was not related to semi-natural habitat cover ($F_{2,15}$ = 4.85, p = 0.83), nor was one of the functional groups, both before and during leek flowering (p > 0.35, figure 5.4A-F). Abundances in the crop were generally related to semi-natural habitat cover ($F_{1,16}$ = 8.17, p = 0.01), but this was largely caused by the dominant crop pollinator abundance ($F_{1,16}$ = 14.74, β = 0.12, p = 0.001; figure 5.4G), as the abundance of opportunistic crop pollinators was not related to semi-natural habitat cover ($F_{1,16}$ = 0.77, β = 0.03, p = 0.39; figure 5.4H).

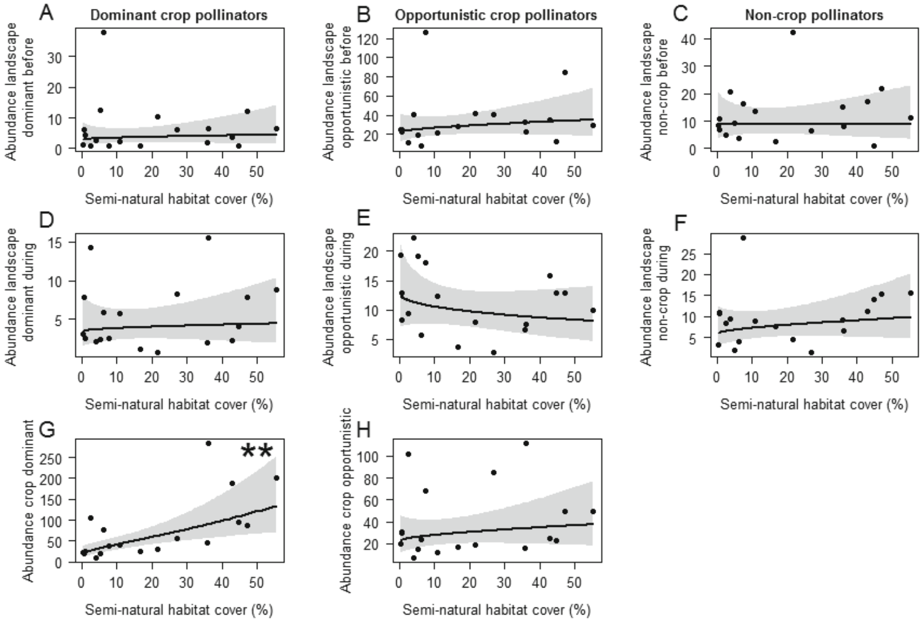

Figure 5.4 Relation of pollinator abundances with semi-natural habitat cover (%). Abundances were separated in (A-C) semi-natural habitat transects before crop flowering, (D-F) semi-natural habitat transects during crop flowering and (G & H) crop transects. Panel A, D and G reflect dominant crop pollinator abundances, B, E and H opportunistic crop pollinator abundances and C & F non-crop pollinator abundances. Results are back-transformed partial residuals corrected for flower cover. Panel A-F & H show no significant relation, while G is significant ($p<0.01$, indicated with **). 95% confidence intervals are indicated with grey.

Crop flowering

Crop flowering did not alter the abundances of dominant crop pollinators in the landscape (mean log-difference = 0.04 ± 0.12 se, $z = 0.30$, $p = 0.99$) or abundances of non-crop pollinators in the landscape (mean log-difference = 0.06 ± 0.12 se, $z = 0.454$, $p = 0.99$). However, the opportunistic crop pollinators showed a 60% decline in abundances in the landscape when the nearby crop was flowering (mean log-difference = 0.45 ± 0.12 se, $z = 3.596$, $p = 0.004$; figure 5.5A). This pattern may be largely explained by hoverflies because analyses without hoverflies showed no difference in abundance of opportunistic pollinators before and during crop flowering (electronic supplementary information, figure S5.8). Abundances of dominant crop pollinators were about 10 times higher in the crop than in the semi-natural habitat in the surrounding landscape (mean log-difference = 0.98 ± 0.08 se, $z = 11.577$, p <

0.001; figure 5.5B). This was mainly caused by surprisingly low abundances of dominant crop pollinators in the landscape even before crop flowering. In semi-natural habitats surrounding leek fields, dominant crop pollinator abundances were almost four times lower than abundances of opportunistic crop pollinators (mean log-difference = 0.58 ± 0.08 se, z = 6.834, $p < 0.001$) and 1.8 times lower than abundances of non-crop pollinators (mean log-difference = 0.26 ± 0.08 se, z = 3.121, $p < 0.02$). Abundances of non-crop pollinators in the landscape were around half of the abundances of opportunistic crop pollinators (mean log-difference = 0.32 ± 0.08 se, z = 3.713, p = 0.001). Perhaps surprisingly, abundances of opportunistic crop pollinators in the crop were comparable to those in the landscape (mean log-difference = 0.17 ± 0.08 se, z = 2.005, p = 0.26).

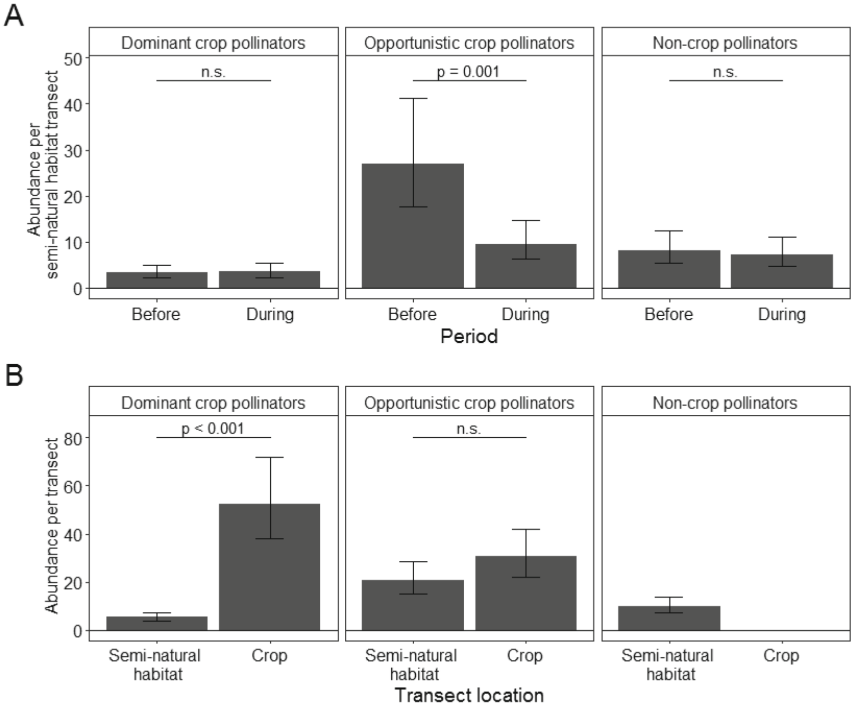

Figure 5.5 (A) Back-transformed mean abundances of dominant, opportunistic and non-crop pollinators in the landscape, before and during crop flowering. (B) Back-transformed mean abundances of dominant and opportunistic in the landscape (before and during crop flowering together) and in the crop, and mean abundances of non-crop pollinators in the semi-natural habitat for comparison. Error-bars are 95% confidence interval. Pairwise significance values are indicated on top (n.s. = not significant).

Discussion

Ecological theory predicts that the species richness of pollinators on crops is determined by the size of the local pollinator species pool which, in turn, depends mainly on the quantity of resources that is available in semi-natural habitats (Mandelik et al. 2012, Tscharntke et al. 2012, Schellhorn et al. 2015). However, the empirical evidence to date is scarce. Here we provide partial support for these relationships by showing that the abundance and species richness of pollinators in crops is directly and positively related to the size of the local species pool in the surrounding landscape. This relation differs, however, between functional groups, with dominant crop pollinators showing weaker relations than opportunistic crop pollinators (figure 5.2). Surprisingly, significant relations with landscape complexity, a proxy for overall resource availability, were restricted to species richness and abundance of pollinators on crop flowers, and to non-crop pollinators in semi-natural habitats during crop flowering. Landscape complexity did not explain species richness or abundance of crop pollinators in transects in the wider landscape at any time, and dominant crop pollinators were virtually absent in the semi-natural habitats surrounding crop fields, even before bloom of the crop.

Our results indicate that it is important to distinguish between different functional groups when considering crop pollination or pollinator conservation. The species richness of opportunistic crop pollinators was much more strongly related to the local species pool than that of dominant crop pollinators, which could explain why in species-poor landscapes dominant crop pollinators make up a much larger proportion of the crop-visiting pollinator communities than in species-rich landscapes (Kleijn et al. 2015). This makes dominant crop pollinator species a relatively constant component of crop pollinator communities (Carvalheiro et al. 2013), and variation in the overall species richness of pollinators on crops seems to be primarily determined by the larger number of less common species that use crop flowers opportunistically. Interestingly, the higher species richness of opportunistic species compensated for their lower abundance per species, as the relative abundance on crop flowers of the two functional groups remained fairly constant along the gradient of local species pool size (figure 5.2B). The species that were not encountered on crops were even less abundant than the opportunistic crop pollinators in the semi-natural habitat, but as a group, the non-crop pollinators did make up more than half of the total number of observed pollinator species across all study sites. Although non-crop pollinators were also observed in species-poor landscapes (figure 5.3A&B) the majority of the non-crop pollinator species was probably restricted to species-rich landscapes where 30% of the local pollinator species pool was never observed on the crop, a pattern which is in line with other studies (M'Gonigle et al. 2015, Harrison et al. 2017). In contrast to the crop pollinators, hardly any of the species that relied solely on semi-natural habitats were observed in large numbers. Potential reasons for this could be that non-crop pollinators may have restricted or specialised pollen diet requirements (Wood et al. 2018b), or that in semi-natural habitats resource availability was too low and scattered to maintain species with larger populations for the duration of their activity period.

Surprisingly, species-richness and abundance of pollinators in the wider landscape was generally not related to landscape complexity. At first glance this would indicate that the hypothesised positive relationship between landscape complexity and local species pool size is not supported. However, we think the lack of response in both abundance and richness was caused by the pollinators spreading out evenly over the available pollinator habitat up to a certain carrying capacity (Tscharntke et al. 2012). Other studies examining pollinators inside semi-natural habitat likewise fail to find relationships with landscape complexity (Steffan-Dewenter 2003, Kleijn and van Langevelde 2006). Estimating pollinator population sizes requires taking into account the total area of pollinator habitat as well as the density per unit area (Kleijn et al. 2018). Complex landscapes by definition contain larger surface areas of pollinator habitat. Equal pollinator densities in simple and complex landscapes then translate into larger population sizes in complex landscapes. Species richness does not show linear relationships with surface area (Steffan-Dewenter 2003), but the surveyed transects were relatively small and did not exhaustively represent all available semi-natural habitat types in the landscapes. The same process may largely explain why also here we failed to observe relationships with landscape complexity. The only exception were the non-crop pollinators, whose species richness in semi-natural habitats was positively related to landscape complexity during crop flowering. This can be explained by crop flowering temporally alleviating competition between the non-crop pollinator species and the crop pollinators in the wider landscape (Holzschuh et al. 2011, Henry and Rodet 2018) which may have had more pronounced effects on resource availability in complex than in simple landscapes. Other studies have found honey bees to influence wild bee densities through competition for floral resources (Henry and Rodet 2018). Although, resource competition between managed and wild bees probably also occurred in this study it is unlikely to explain the observed relationships between wild bees and landscape complexity because honey bees densities, both in the crop and in semi-natural habitats, were constant across the landscape complexity gradient.

In contrast to effects in the semi-natural habitats, we did find clear positive relationships of landscape complexity on species richness and abundance of pollinators on crop flowers. Mass-flowering crops like leek generally concentrate pollinators that are within flight range of the field (Holzschuh et al. 2016) as indicated by the general higher abundance and richness of, in particular, the dominant crop pollinators in crop fields than in the semi-natural habitat. When the surrounding landscape contains a lot of pollinator habitat, more pollinators may be attracted on the crop field than when these landscapes contain little of such habitats (Ricketts et al. 2008). Crop fields thus magnify relations between pollinators and the proportion of semi-natural habitats in the landscape, and because opportunistic crop pollinators are more reliant on semi-natural habitats than dominant crop pollinators (Carre et al. 2009), the relationship with opportunistic pollinator species richness may have been more pronounced than the relationship with dominant crop pollinators. In summary, these findings support that complex landscapes have larger pollinator species pools of all three functional groups even though this is not always reflected in higher species richness or abundance per transect in semi-natural habitats. Moreover, it shows that the crop pollinator species pool in mass-flowering crops is mostly limited by the available pollinator habitat cover in the landscape surrounding the crop fields.

Opportunistic crop pollinators were on average as abundant in the semi-natural habitat as in the flowering crop, but before crop flowering their abundances in the landscape were much higher. This likely reflects that opportunistic crop pollinators generally use crop fields as a useful additional food source when it starts flowering, but the reduced and still relatively high abundance of opportunistic crop pollinators in semi-natural habitats suggests that they require additional floral resources from the semi-natural habitats as well (Blitzer et al. 2012, Schellhorn et al. 2015). Unexpectedly, we found no evidence for such spill-over effects for dominant crop pollinators, despite that most dominant crop pollinators were eusocial or multivoltine bees and must have large populations in the area. In fact, we hardly found dominant crop pollinators in the landscape at all, even though the abundances of dominant crop pollinators in the crop were about five times higher than those of opportunistic crop pollinators. This suggests that most dominant crop pollinators foraged in habitats that were not part of our sampling design. It is possible that these species were foraging in other mass-flowering crops that were flowering before the leek crop. Dominant crop pollinators may consist of species that preferentially exploit mass-flowering crops, as it may provide fitness advantages to collect large amounts of resources in a short period of time. In natural systems this mechanism may have evolved in response to abundant mass-flowering wild species such as some canopy trees (Kamper et al. 2016), or certain species of Brassicaceae and Rosaceae (Scheper et al. 2014), of which many crops in Europe have derived (e.g. *Brassica napus* and *Rubus fruticosus* (Carre et al. 2009, Scheper et al. 2014)). In agricultural landscapes these dominant crop pollinators may simply hop from one mass-flowering crop to another mass-flowering crop for their food sources (Riedinger et al. 2014). Although this would make these crop hoppers less dependent on semi-natural habitats for food availability, they nevertheless depend on these habitats for nesting (Williams and Kremen 2007) or food sources when there is no mass-flowering crop flowering (Mandelik et al. 2012, Carvalheiro et al. 2013), which probably underlies the relationship between dominant pollinators on crops and landscape complexity. Insights in the whereabouts of this key group of crop pollinators before flowering of the focal crop can help better understand the contribution of wild pollinators to crop pollination, and to identify effective pollinator-supporting strategies.

Recent studies suggest that pollinator abundance and species richness have significant complementary effects on crop pollination (Garibaldi et al. 2016, Fijen et al. 2018, Winfree et al. 2018). While pollinator abundance is strongly determined by dominant crop pollinators, species richness is more strongly determined by opportunistic crop pollinators. Our study shows that the relationship with landscape complexity, and therefore dependence on semi-natural habitats, differs between these different functional groups of pollinators, with opportunistic crop pollinators being more dependent on semi-natural habitats than dominant crop pollinators. Dependence of non-crop pollinators on semi-natural habitats was even higher than of opportunistic crop pollinators. However, all three groups seem to increase with increasing complexity of the landscape, resulting in the largest local pollinator species pool in the wider landscape as well as the largest pollinator species pool on the mass-flowering crop in the most complex landscapes. Since in this cropping system higher pollinator abundance and species richness is directly related to higher marketable seed yield (Fijen et al. 2018), this suggests that complex landscapes are local hotspots for biodiversity conservation as well as ecosystem service provision. In addition to the intrinsic value of biodiversity and aesthetics, this may provide an important argument for the preservation of semi-natural habitats in times of land-use change.

Acknowledgements

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Supplementary information

Supplementary information belonging to: Fijen, T.P.M., Scheper, J.A., Boekelo, B., Raemakers, I., Kleijn, D. 2019 Effects of landscape complexity on pollinators are moderated by pollinators' association with mass-flowering crops. Proceedings of the Royal Society B: Biological Sciences doi: 10.1098/rspb.2019.0387

In this supplementary information we present results of analyses where we use slightly different definitions of dominant crop pollinators. First, species are classified as being dominant crop pollinators if they comprised of at least 5% of all crop pollinators in our study (figures S5.1-5.4). Second, we show results of analyses where we have used the definition as used by Kleijn et al. (2015, *Nature communications* 6-7414), and as in the main manuscript, but where we excluded hoverflies and honeybees (figures S5.5-5.8). Third, we classified dominant crop pollinators as per Kleijn et al. (2015, *Nature communications* 6-7414) but included wild bees, hoverflies and honeybees (figures S5.9-5.12). We furthermore show that honeybee abundances were not significantly related to landscape complexity (figure S5.13).

Dominant crop pollinators defined as all species comprising at least 5% of all individuals on crop flowers in this study

The total crop pollinator species pool size was significantly positively related with the local species pool size in the semi-natural habitat transects ($F_{1,16} = 12.90$, $p = 0.002$), but this relation was stronger for the opportunistic crop pollinators than for the dominant crop pollinators (significant interaction effect local species pool × functional group: $\chi^2 (1) = 9.41$, $p = 0.002$; figure S5.1A). With an increasing local species pool in the landscape, the abundance of pollinators in crop fields increased ($\chi^2 (1) = 7.22$, $p = 0.007$; figure S5.1B) similarly for both dominant and opportunistic crop pollinators (i.e. no significant interaction effect local species pool × functional group: $\chi^2 (1) = 0.21$, $p = 0.64$; figure S5.1B). Dominant crop pollinators were generally more abundant in crop fields than opportunistic crop pollinators ($\chi^2 (1) = 32.43$, $p < 0.001$; figure S5.1B).

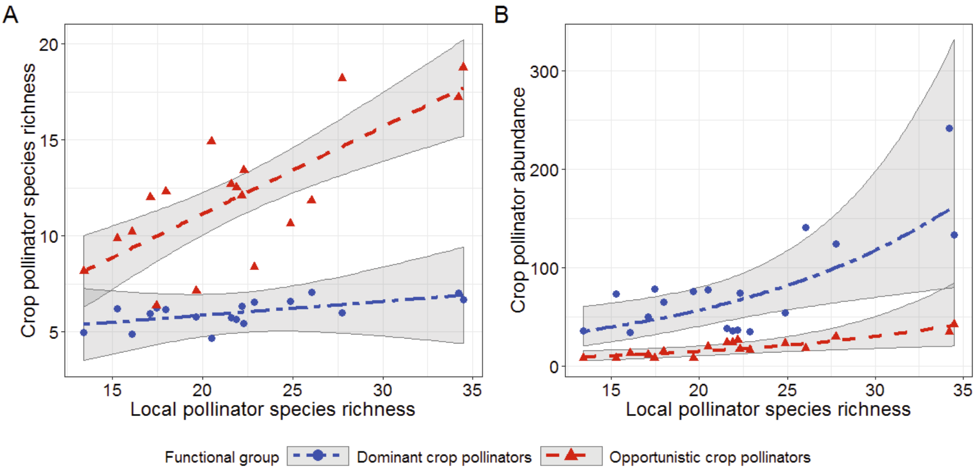

Figure S5.1 Relationships between the local pollinator species richness and the pollinator species richness (A) and abundance (B) in crops. Here, we defined dominant crop pollinators as all species comprising at least 5% of all visits to crop flowers in our study. Local pollinator species richness is based on transects in semi-natural habitat (both before and during crop flowering), while crop abundances and richness are based on transects in crop fields. Separate regressions are indicated for dominant crop species (blue circles) and opportunistic crop species (red triangles) and 95% confidence intervals are indicated with grey. Results are back-transformed partial residuals.

The total size of the local species pool did not significantly increase with increasing landscape complexity ($F_{2,15}$ = 2.17, p = 0.11). The three-way interaction between functional group, period of sampling and landscape complexity was significant (three-way interaction: χ^2 (2) = 6.94, p = 0.03; figure S5.2A). There were no strong effects of landscape complexity before crop flowering, whereas during crop flowering, non-crop pollinators responded positive to semi-natural habitat cover ($F_{2,15}$ = 2.98, p = 0.045). Total pollinator species richness in the crop fields increased significantly with landscape complexity ($F_{1,16}$ = 8.93, p = 0.008). The species richness of dominant ($F_{1,16}$ = 31.57, p < 0.001) and opportunistic crop pollinators ($F_{1,16}$ = 6.10, p = 0.03) increased similarly with increasing landscape complexity (i.e. no significant interaction functional group x landscape complexity (χ^2 (1) = 3.37, p = 0.07; figure S5.2B).

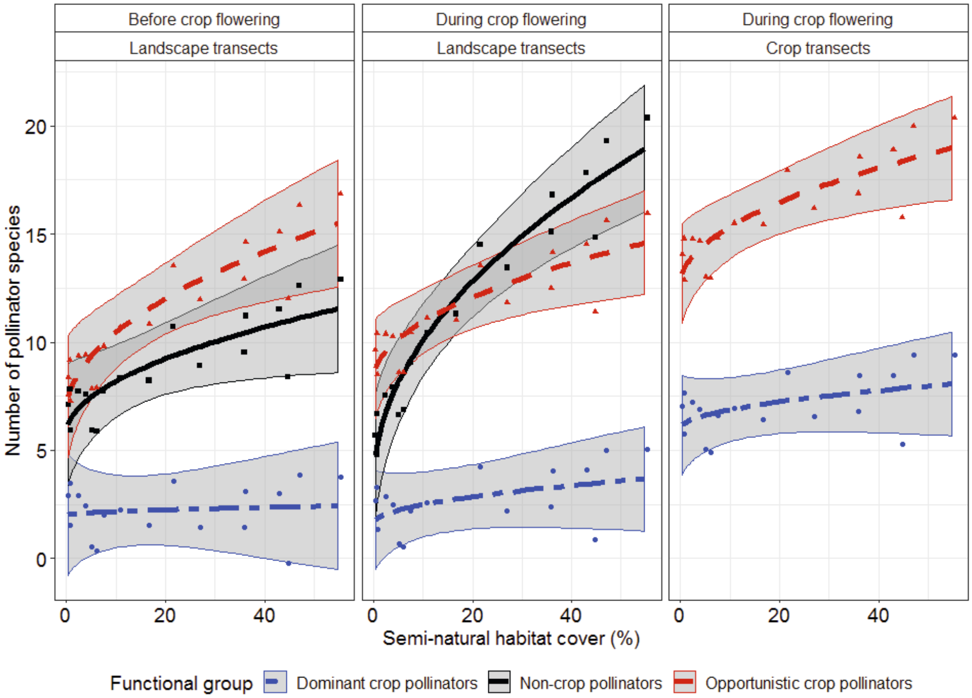

Figure S5.2 Relation between cover of semi-natural habitat (%) and pollinator species richness. Here, we defined dominant crop pollinators as all species comprising at least5% of all visits to crop flowers in our study. Separate panels are given for semi-natural habitat transects before, and during crop flowering, and crop transects during crop flowering. Back-transformed regressions and predicted species richness are indicated for dominant crop pollinator species (blue circles), opportunistic crop pollinator species (red triangles), and non-crop pollinator species in the landscape (black squares). 95% confidence intervals are indicated with grey.

In the semi-natural habitats surrounding leek fields, the total average abundance of pollinators was not related to semi-natural habitat cover ($F_{2,15}$ = 0.77, p = 0.88), nor was one of the functional groups, both before and during leek flowering (p > 0.38, figure S5.3A-F). Abundances in the crop were generally related to semi-natural habitat cover ($F_{1,16}$ = 5.35, p = 0.03), but this was stronger for the dominant crop pollinator abundance ($F_{1,16}$ = 8.50, β = 0.10, p = 0.01; figure S5.3G), as the abundance of opportunistic crop pollinators was only marginally related to semi-natural habitat cover ($F_{1,16}$ = 4.27, β = 0.06, p = 0.06; figure S5.3H).

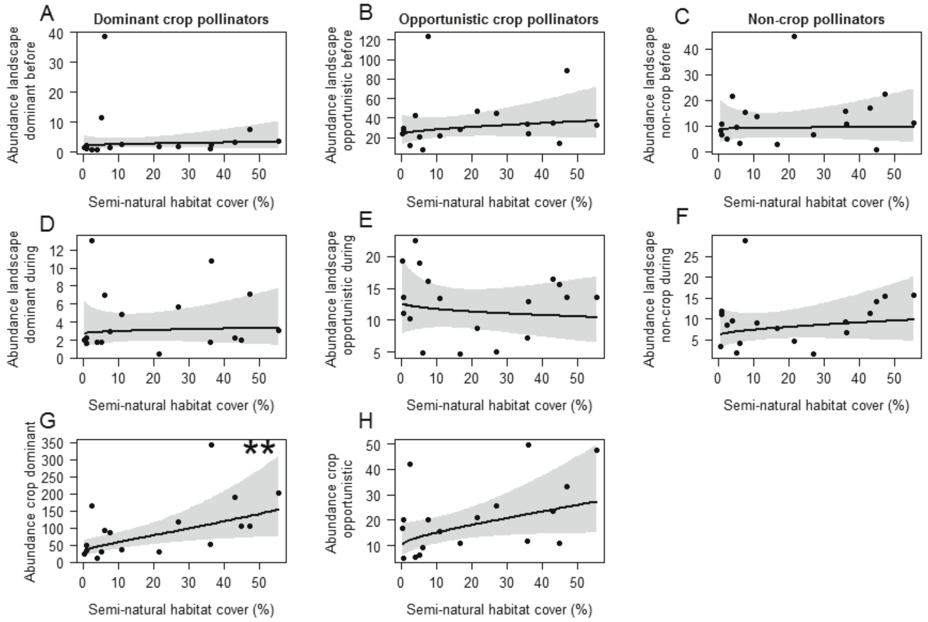

Figure S5.3 Relation of pollinator abundances with semi-natural habitat cover (%). Here, we defined dominant crop pollinators as all species comprising at least5% of all visits to crop flowers in our study. Abundances were separated in (A-C) semi-natural habitat transects before crop flowering, (D-F) semi-natural habitat transects during crop flowering and (G & H) crop transects. Panel A, D and G reflect dominant crop pollinator abundances, B, E and H opportunistic crop pollinator abundances and C & F non-crop pollinator abundances. Results are back-transformed partial residuals corrected for flower cover. Panel A-F show no significant relation, while G is significant (p<0.01, indicated with **) and panel H marginally significant (p = 0.06). 95% confidence intervals are indicated with grey.

Crop flowering did not alter the abundances of dominant crop pollinators in the landscape or abundances of non-crop pollinators in the landscape. However, the opportunistic crop pollinators showed a strong decline in abundances in the landscape when the nearby crop was flowering (figure S5.4A). Abundances of dominant crop pollinators were much higher in the crop than in the semi-natural habitat in the surrounding landscape. Abundances of opportunistic crop pollinators in the crop were comparable to those in the landscape (figure S5.4B).

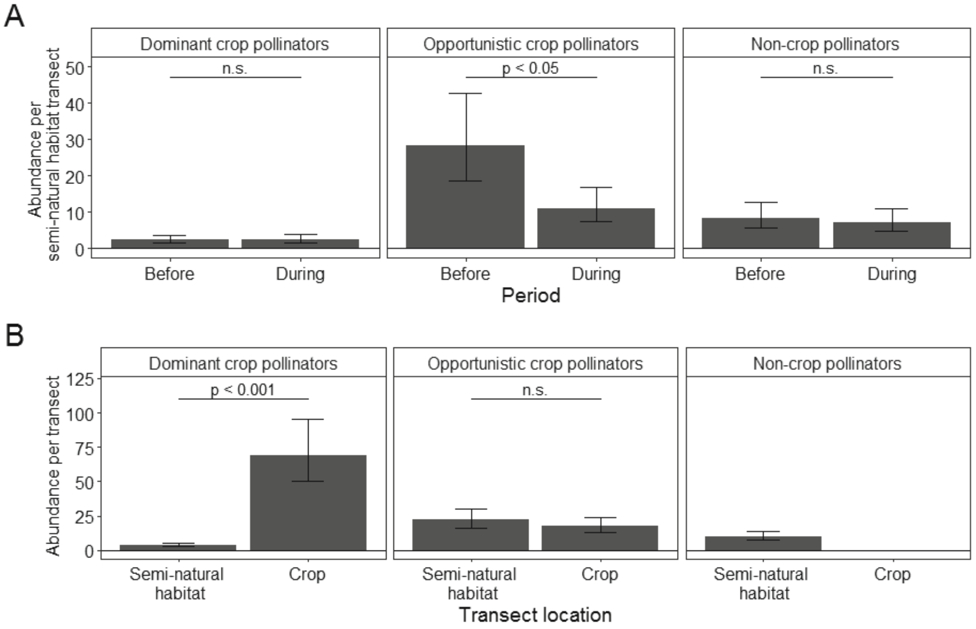

Figure S5.4 (A) Back-transformed mean abundances of dominant, opportunistic and non-crop pollinators in the landscape, before and during crop flowering. (B) Back-transformed mean abundances of dominant and opportunistic in the landscape (before and during crop flowering together) and in the crop, and mean abundances of non-crop pollinators in the semi-natural habitat for comparison. Here, we defined dominant crop pollinators as all species comprising at least5% of all visits to crop flowers in our study. Error-bars are 95% confidence interval. Pairwise significance values are indicated on top (n.s. = not significant).

Definition of dominance as in main article – excluding hoverflies and honeybees

The total crop pollinator species pool size was significantly positively related with the local species pool size in the semi-natural habitat transects ($F_{1,16} = 20.56$, $p < 0.001$), but this relation was stronger for the opportunistic crop pollinators than for the dominant crop pollinators (significant interaction effect local species pool × functional group: $\chi^2 (1) = 4.10$, $p = 0.04$; figure S5.5A). With an increasing local species pool in the landscape, the total abundance of pollinators in crop fields increased ($\chi^2 (1) = 5.24$, $p = 0.05$; figure S5.5B). Furthermore, dominant and opportunistic crop pollinators increased similarly with increasing local species pool size (i.e. no significant interaction effect local species pool × functional group: $\chi^2 (1) = 2.20$, $p = 0.14$; figure S5.5B). Dominant crop pollinators were generally more abundant in crop fields than opportunistic crop pollinators ($\chi^2 (1) = 9.97$, $p = 0.002$; figure S5.5B).

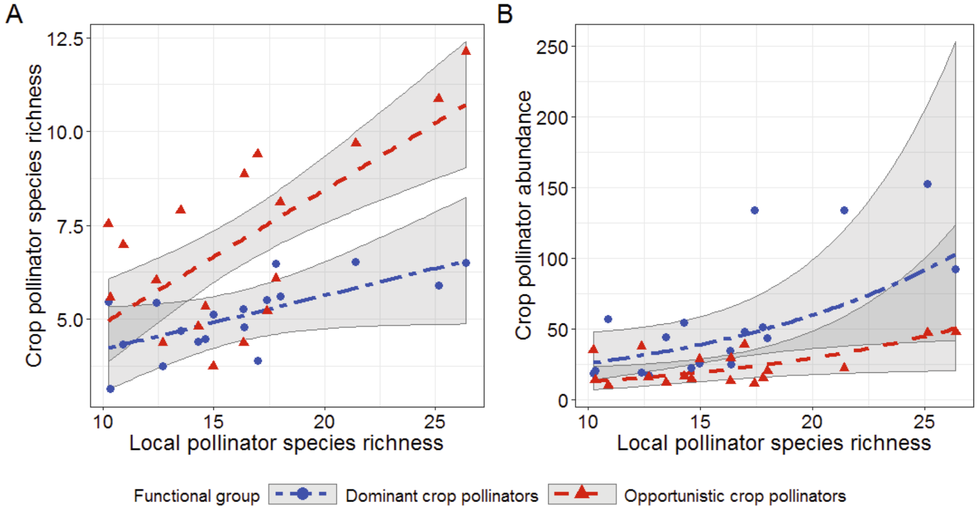

Figure S5.5 Relationships between the local pollinator species richness and the pollinator species richness (A) and abundance (B) in crops, excluding hoverflies and honeybees. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications* 6-7414). Local pollinator species richness is based on transects in semi-natural habitat (both before and during crop flowering), while crop abundances and richness are based on transects in crop fields. Separate regressions are indicated for dominant crop species (blue circles) and opportunistic crop species (red triangles) and 95% confidence intervals are indicated with grey. Results are back-transformed partial residuals.

The total size of the local species pool did not significantly increase with increasing landscape complexity ($F_{2,15}$ = 2.19, p = 0.08). There was no support for a three-way interaction between functional group, period of sampling and landscape complexity (three-way interaction: χ^2 (2) = 0.73, p = 0.70; figure S5.6A), but both the two-way interactions between functional group and period (χ^2 (2) = 6.99, p = 0.03), as well as between functional group and landscape complexity (χ^2 (2) = 9.71, p = 0.008) were significant. There were no strong effects of landscape complexity before crop flowering, whereas during crop flowering, non-crop pollinators responded marginally positive to semi-natural habitat cover ($F_{2,15}$ = 2.46, p = 0.07). Total pollinator species richness in the crop fields increased significantly with landscape complexity ($F_{1,16}$ = 8.93, p = 0.008). The species richness of dominant ($F_{1,16}$ = 4.41, p = 0.05) and opportunistic crop pollinators ($F_{1,16}$ = 5.65, p = 0.03) increased similarly with increasing landscape complexity (i.e. no significant interaction functional group x landscape complexity (χ^2 (1) = 2.59, p = 0.10; figure S5.6B).

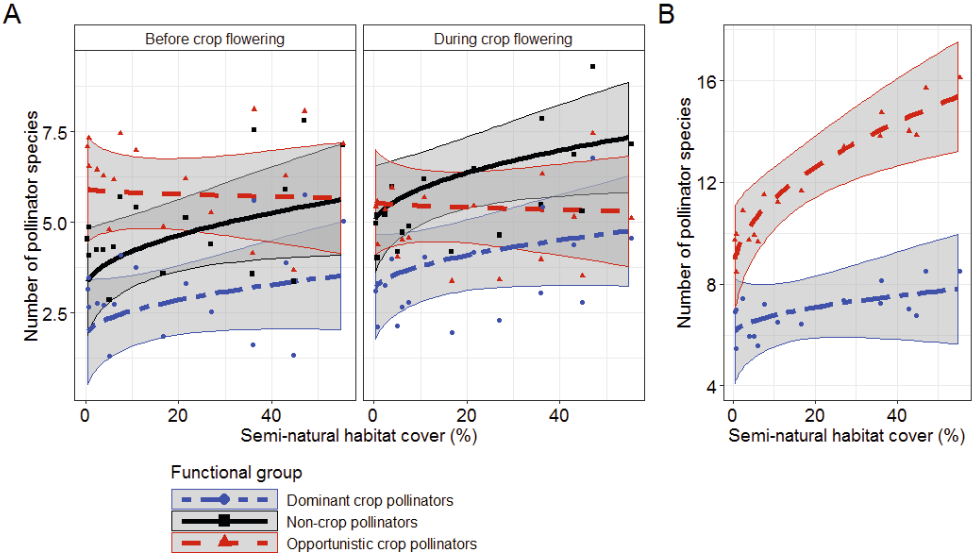

Figure S5.6 Relation between cover of semi-natural habitat (%) and pollinator species richness, excluding hoverflies and honeybees. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications* 6-7414). Separate panels are given for (A) semi-natural habitat transects before and during crop flowering and (B) crop transects during crop flowering. Back-transformed regressions and predicted species richness are indicated for dominant crop pollinator species (blue circles), opportunistic crop pollinator species (red triangles), and non-crop pollinator species in the landscape (black squares). 95% confidence intervals are indicated with grey.

In the semi-natural habitats surrounding leek fields, the total average abundance of pollinators, excluding hoverflies and honeybees, was not related to semi-natural habitat cover ($F_{2,15} = 0.89$, $p = 0.84$), nor was one of the functional groups, both before and during leek flowering ($p > 0.43$, figure S5.7A-F). Abundances in the crop were generally related to semi-natural habitat cover ($F_{1,16} = 6.37$, $p = 0.02$), but this was largely caused by the dominant crop pollinator abundance ($F_{1,16} = 11.40$, $\beta = 0.12$, $p = 0.004$; figure S5.7G), as the abundance of opportunistic crop pollinators was not related to semi-natural habitat cover ($F_{1,16} = 0.77$, $\beta = 0.03$, $p = 0.50$; figure S5.7H).

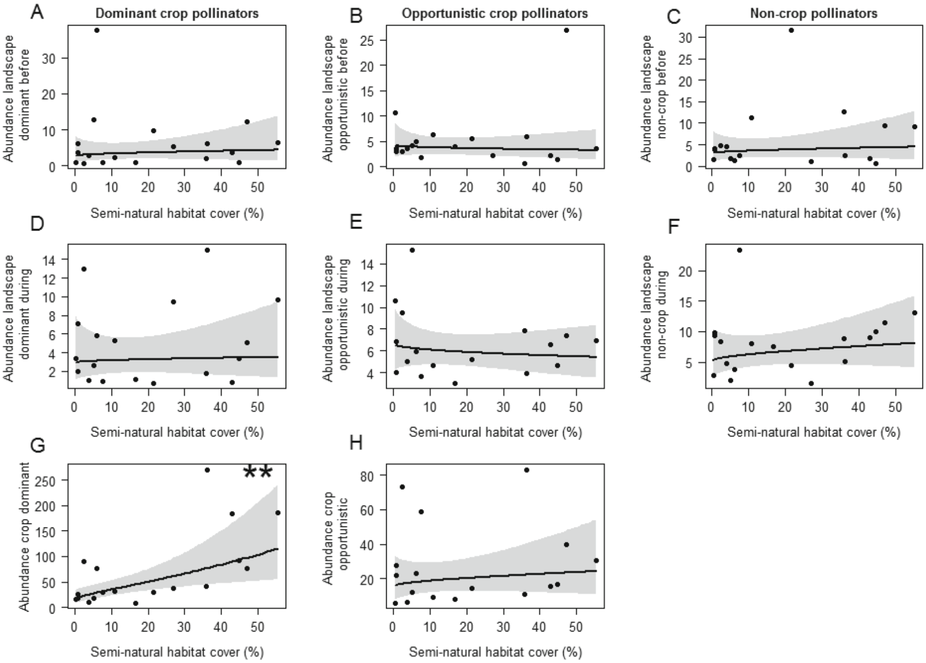

Figure S5.7 Relation of pollinator abundances, excluding hoverflies and honeybees, with semi-natural habitat cover (%). Classification of dominant crop pollinators follows Kleijn et al. (2015, N*ature communications* 6-7414). Abundances were separated in (A-C) semi-natural habitat transects before crop flowering, (D-F) semi-natural habitat transects during crop flowering and (G & H) crop transects. Panel A, D and G reflect dominant crop pollinator abundances, B, E and H opportunistic crop pollinator abundances and C & F non-crop pollinator abundances. Results are back-transformed partial residuals corrected for flower cover. Panel A-F & H show no significant relation, while G is significant (p<0.01, indicated with **). 95% confidence intervals are indicated with grey.

Crop flowering did not alter the abundances of dominant, opportunistic or non-crop pollinators in the landscape (figure S5.8A). Abundances of dominant and opportunistic crop pollinators were much higher in the crop than in the semi-natural habitat in the surrounding landscape (figure S5.8B).

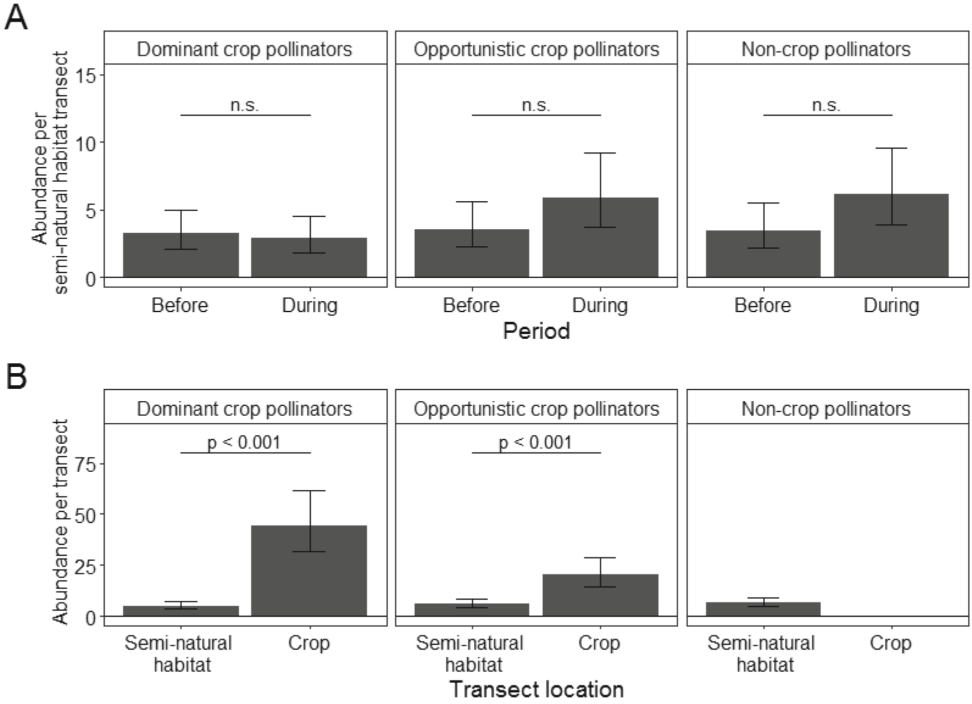

Figure S5.8 (A) Back-transformed mean abundances of dominant, opportunistic and non-crop pollinators in the landscape, before and during crop flowering, excluding hoverflies and honeybees. (B) Back-transformed mean abundances of dominant and opportunistic in the landscape (before and during crop flowering together) and in the crop, excluding hoverflies and honeybees. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications* 6-7414). Mean abundances of non-crop pollinators in the semi-natural habitat are shown for comparison. Error-bars are 95% confidence interval. Pairwise significance values are indicated on top (n.s. = not significant).

Definition of dominance as in main article – including hoverflies and honeybees

Honeybee (*Apis mellifera*) hives are placed in the leek fields during crop flowering and are ubiquitous in the study area. Because we were interested in the patterns of wild pollinators, we have excluded honeybees from all analyses in the main article. Here we present results of analyses that include honeybees to show that the results are qualitatively the same. We furthermore show that honeybee abundances were not significantly related to landscape complexity (figure S5.13).

The total crop pollinator species pool size was significantly positively related with the local species pool size in the semi-natural habitat transects ($F_{1,16} = 15.06$, $p = 0.001$), but this relation was stronger for the opportunistic crop pollinators than for the dominant crop pollinators (significant interaction effect local species pool × functional group: χ^2 (1) = 4.87, $p = 0.03$; figure S5.9A). With an increasing local species pool in the landscape, the total abundance of pollinators in crop fields increased only marginally (χ^2 (1) = 5.24, $p = 0.09$; figure S5.9B). But when accounting for the functional groups, an increasing local species pool size increased pollinator abundance (χ^2 (1) = 3.97, $p = 0.046$) similarly for both dominant and opportunistic crop pollinators (i.e. no significant interaction effect local species pool × functional group: χ^2 (1) = 0.04, $p = 0.85$; figure S5.9B). Dominant crop pollinators were generally more abundant in crop fields than opportunistic crop pollinators (χ^2 (1) = 22.09, $p < 0.001$; figure S5.9B).

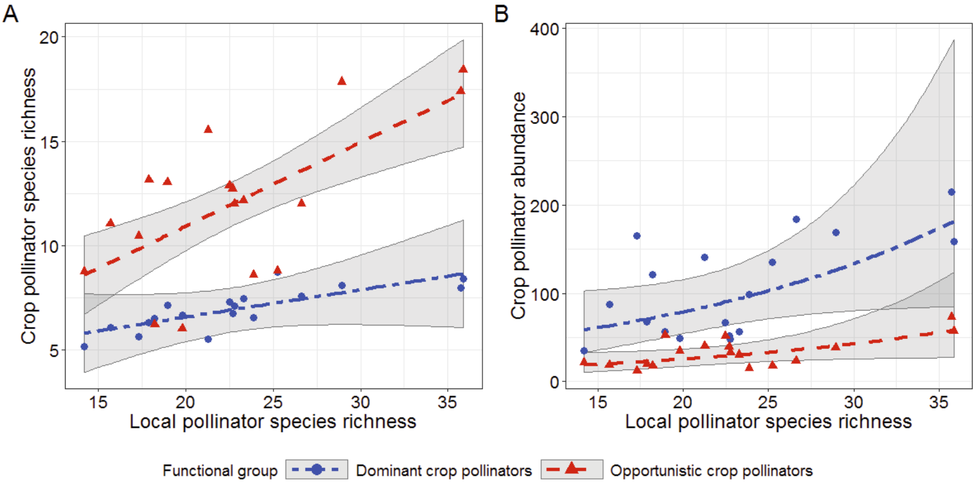

Figure S5.9 Relationships between the local pollinator species richness and the pollinator species richness (A) and abundance (B) in crops, including honeybees. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications 6-7414*). Local pollinator species richness is based on transects in semi-natural habitat (both before and during crop flowering), while crop abundances and richness are based on transects in crop fields. Separate regressions are indicated for dominant crop species (blue circles) and opportunistic crop species (red triangles) and 95% confidence intervals are indicated with grey. Results are back-transformed partial residuals.

The total size of the local species pool did only marginally increase with increasing landscape complexity ($F_{2,15}$ = 2.84, p = 0.06). The three-way interaction between functional group, period of sampling and landscape complexity was only marginally significant (three-way interaction: χ^2 (2) = 4.93, p = 0.08; figure S5.10A). The two-way interaction between functional group and period (χ^2 (2) = 9.94, p = 0.007), as well as between functional group and landscape complexity (χ^2 (2) = 6.23, p = 0.044) were significant. There were no strong effects of landscape complexity before crop flowering, whereas during crop flowering, non-crop pollinators were positively related to semi-natural habitat cover ($F_{2,15}$ = 3.48, p = 0.03). Total pollinator species richness in the crop fields increased significantly with landscape complexity ($F_{1,16}$ = 8.93, p = 0.008), and this was stronger for the opportunistic crop pollinators than the dominant crop pollinators (i.e. significant interaction functional group x landscape complexity (χ^2 (1) = 4.87, p = 0.03; figure S5.10B).

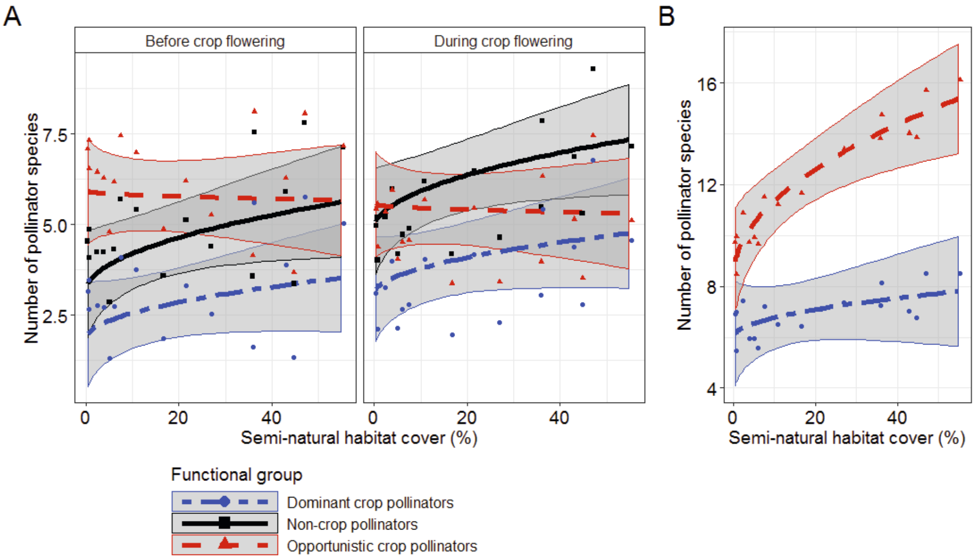

Figure S5.10 Relation between cover of semi-natural habitat (%) and pollinator species richness, including honeybees. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications* 6-7414). Separate panels are given for (A) semi-natural habitat transects before and during crop flowering and (B) crop transects during crop flowering. Back-transformed regressions and predicted species richness are indicated for dominant crop pollinator species (blue circles), opportunistic crop pollinator species (red triangles), and non-crop pollinator species in the landscape (black squares). 95% confidence intervals are indicated with grey.

In the semi-natural habitats surrounding leek fields, the total average abundance of pollinators was not related to semi-natural habitat cover ($F_{2,15}$ = 3.78, p = 0.47), nor was one of the functional groups, both before and during leek flowering (p > 0.35, figure S5.11A-F). Abundances in the crop were marginally related to semi-natural habitat cover ($F_{1,16}$ = 3.86, p = 0.07), but this was mainly due to the dominant crop pollinator abundance ($F_{1,16}$ = 4.74, β = 0.08, p = 0.045; figure S5.3G), as the abundance of opportunistic crop pollinators was not related to semi-natural habitat cover ($F_{1,16}$ = 0.77, β = 0.03, p = 0.39; figure S5.11H).

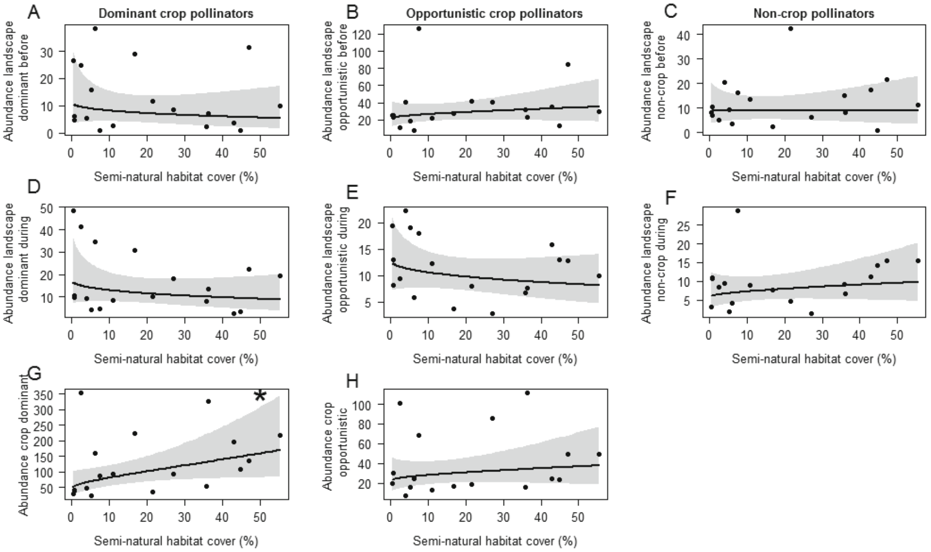

Figure S5.11 Relation of pollinator abundances, including honeybees, with semi-natural habitat cover (%). Abundances were separated in (A-C) semi-natural habitat transects before crop flowering, (D-F) semi-natural habitat transects during crop flowering and (G & H) crop transects. Panel A, D and G reflect dominant crop pollinator abundances, B, E and H opportunistic crop pollinator abundances and C & F non-crop pollinator abundances. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications* 6-7414). Results are back-transformed partial residuals corrected for flower cover. Panel A-F & H show no significant relation, while G is significant (p<0.05, indicated with *). 95% confidence intervals are indicated with grey.

Crop flowering did not alter the abundances of dominant crop pollinators in the landscape or abundances of non-crop pollinators in the landscape. However, the opportunistic crop pollinators showed a strong decline in abundances in the landscape when the nearby crop was flowering (figure S5.4A). Abundances of dominant crop pollinators were much higher in the crop than in the semi-natural habitat in the surrounding landscape. Abundances of opportunistic crop pollinators in the crop were comparable to those in the landscape (figure S5.4B).

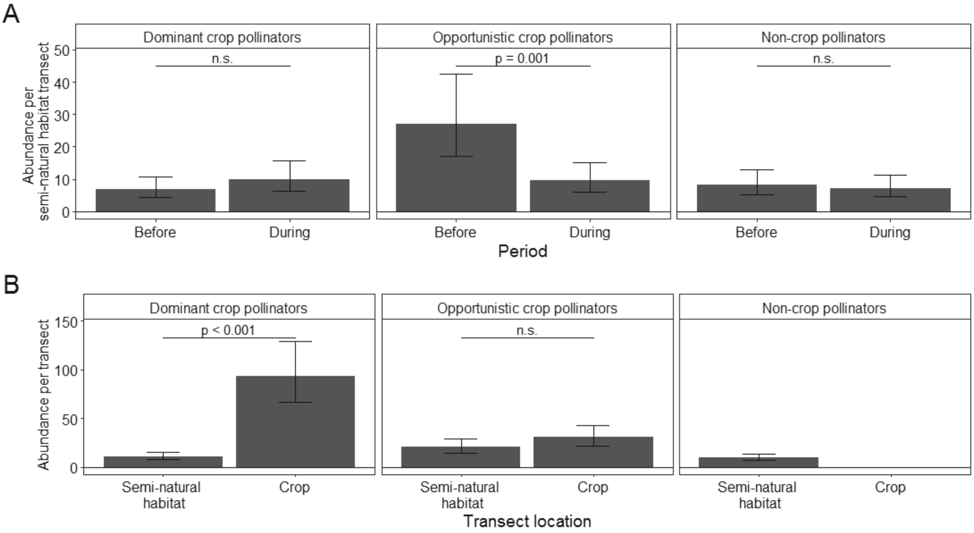

Figure S5.12 (A) Back-transformed mean abundances of dominant, opportunistic and non-crop pollinators in the landscape, before and during crop flowering, including honeybees. (B) Back-transformed mean abundances of dominant and opportunistic in the landscape (before and during crop flowering together) and in the crop, and mean abundances of non-crop pollinators in the semi-natural habitat for comparison. Classification of dominant crop pollinators follows Kleijn et al. (2015, *Nature communications* 6-7414). Error-bars are 95% confidence interval. Pairwise significance values are indicated on top (n.s. = not significant).

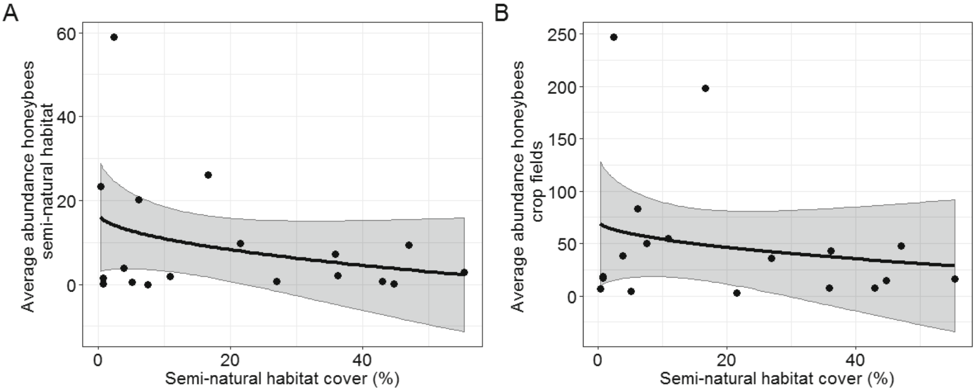

Figure S5.13 (A) Back-transformed mean abundances of honeybees in the landscape (simple linear regression: $F_{1,16}$ = 1.75, p = 0.20), and (B) in the crop fields (simple linear regression: $F_{1,16}$ = 0.70, p = 0.41) along the measured gradient of semi-natural habitat cover (%). 95% confidence intervals are indicated with grey.

Chapter 6

General discussion

Introduction

In this thesis we have aimed to enhance the evidence base of the concept of ecological intensification. Ecological intensification proposes that by taking better care of the species that deliver the ecosystem services, high crop yields can be maintained while at the same time biodiversity in agricultural landscapes can be protected (Bommarco et al. 2013). There is increasing support for the hypothesis that by enhancing biodiversity levels, ecosystem service delivery to crops can be enhanced (Kleijn et al. 2019). However, several barriers exist that need to be crossed before it is likely that the agricultural sector can be convinced of the effectiveness of ecological intensification. A key barrier is that studies showing the benefits of ecological intensification are not measured at scales that are relevant to the agricultural sector. In this thesis we have aimed to provide convincing and evidence-based arguments for ecological intensification in real-world systems by studying the relative importance of wild pollinators compared to the conventional agricultural management in commercial leek hybrid seed production (Box 1.1). First, to establish how pollinator visitation rate on leek flowers varies between days and with varying weather conditions, we have observed pollinator visitation rates for three full-days on single leek plants in two crop fields (**chapter 2**). We have used this data to explore how we can efficiently obtain reliable estimates of pollinator visitation rate on leek flowers, a common method in crop pollination studies. In **chapter 3**, we have established the relative contribution of insect pollination and agricultural management to crop yield along a gradient of expected pollinator abundance and richness in 36 agricultural crop fields in France and Italy. The next step was to test how changes in the agricultural management in a single crop field affect crop yield and how this effect interacts with varying insect pollination levels (**chapter 4**). Lastly, because the majority of insect pollinators originate from the semi-natural habitat surrounding the crop fields, we have looked at the relationship of semi-natural habitats with the abundance and species richness of the crop pollinator community (**chapter 5**). By combining the results of these studies, we show that under the real-world variation of environmental and agronomic conditions wild insect pollination is an undervalued agricultural input, and that improving wild insect pollination has potential to sustainably improve crop yields while at the same time maintaining high biodiversity levels in agricultural landscapes.

Reliable proxies for plant breeding and day-to-day management

In this thesis we have performed studies in such a way that measures were relevant for growers and relatively easy to measure. This also means that we have used proxies for, for example, pollination services and agricultural management. By definition, these proxies are an approximation of reality, and may therefore introduce some noise to the data. Nevertheless, we are confident that measures of these proxies are reliable (Garibaldi et al. 2018). Here, we discuss how some of these proxies may be of use in day-to-day agricultural management and in plant breeding.

Sampling methods for measuring pollinator abundance, densities or species richness are only snapshots of reality (Russo et al. 2015), but we show that they turn out to be good proxies for the pollination services if planned and executed properly. The total number of pollinator species that visited a single plant on two crop fields after three full days of observation was 28 and 36, respectively (**chapter 2**). We estimated on the basis of three to five 150m^2-transect counts that the crop fields were visited on average by approximately 28 pollinator species (**chapter 3**). While we cannot directly link the transect counts to the whole-day observations, these same order of magnitudes suggest that the estimated species richness based on transect counts may be representative for the number of species visiting single plants during the whole flowering period. Combining plant observations with transect counts likely allows for efficiently obtaining accurate estimates of pollinator visitation rates of single plants, as well as obtaining good estimates of the pollinator species richness in the crop fields. Simplified versions of these transect counts could give growers a better idea on how well-visited the crop is by pollinators. While, non-experts have been shown to be able to identify bumblebee species as accurately as experts in a comparative study (Austen et al. 2016), growers are usually non-experts in identifying insect pollinators, although bumblebees are probably the positive exception (Carvell et al. 2016). However, with a little help it is likely to be possible for growers to count all the bumblebees, honeybees, other bees and hoverflies as functional groups. The time-effort for doing transect walks is relatively small, and if performed with a standard protocol, it could at least give an impression on how the abundance may compare with other days or years, or with other fields. This can allow growers to identify pollination problems early on, and to adjust agricultural management accordingly.

One of the potential day-to-day applications of these transect walks could be to irrigate more often if insect abundance is relatively low. We found that higher frequency of irrigation events increased nectar production in all three investigated crop lines, and in one crop line this also significantly increased pollinator visitation rate and crop yield (**chapter 4**). Nectar in *Allium*-crops is relatively sugar rich, and *Allium*-crops are therefore quite attractive for pollinators (Pamminger et al. 2019). Measuring nectar production and quality is relatively labour intensive and may be difficult to measure and, consequently, of little added value to growers.

However, nectar production may be useful as a proxy for pollinator visitation rate during plant breeding. Nectar production is usually correlated to pollinator visitation rate, and strongly confounded by the genetic crop line (**chapter 4**; Silva and Dean (2000)). In the first place, this may be one of the reasons why some crop lines were better visited by pollinators than others (**chapter 4**). In the second place, it suggests that by taking nectar production into account during plant breeding, new crop lines (or varieties) can be selected that are extra attractive for pollinators (Prasifka et al. 2018).

In **chapter 3** we assumed that agricultural management is aimed at raising high-quality leek plants, and that the plant quality can best be expressed in measuring the basal stem circumference (i.e. plant size). This variable can be measured throughout the growing season and is strongly correlated to plant biomass (De Clercq et al. 2003b). We found that plant size was positively correlated to marketable seed yield, suggesting that plants with larger basal stem circumference do indeed produce more marketable seeds. However, when we halved fertiliser inputs in **chapter 4**, we found that these plants had a 10% smaller stem circumference than plants with standard fertiliser inputs, but this did not affect, or even increased the number of marketable seeds. All else being equal, this suggests that factors other than fertiliser play a more important role in the positive relationship between plant size and marketable seed yield found across fields in **chapter 3**. There are a several other agricultural management factors that can influence plant size, such as micronutrient availability, pest pressure, planting dates, soil type, and weather conditions (Brewster 2008). However, most of them are relatively invisible or difficult to manage, whereas artificial fertiliser input is relatively straight forward: apply more artificial fertilisers and the plants grow faster. Because artificial fertiliser is easy to apply, introducing a target plant size as an agricultural management goal might then results in the over-application of fertiliser, with counter-effective results on crop yield. Which agricultural management factors are more important in shaping crop yield needs further study, but it is essential that effects of agricultural management should always be translated into crop yield for an objective assessment of effectiveness. In conclusion, the proxies measured for pollination and agricultural management in this thesis generally gave a good indication of crop yield.

Wild insect pollination is relatively more important than conventional agricultural management

Growing a crop from planting to harvest can be described as managing a chain of events where everything should be optimal. In this thesis, we found that insect pollination in the flowering phase explained more than half of the crop yield, suggesting that under current production conditions insect pollination is the most important factor in leek hybrid seed production. Across the real-world variation in agronomic conditions of 36 commercial leek seed production fields, we found that the variation in insect pollination had on average stronger effects on marketable seed yield than the agricultural management aimed at increasing plant size (**chapter 3**). Furthermore, in a more controlled experiment on a single crop field we showed that a reduction in insect pollination had twice as strong an effect on marketable seed yield than a reduction in common agricultural management inputs such as fertiliser and water (**chapter 4**). Leek plants for seed production grow for approximately one full year, of which it flowers only 4-6 weeks, and it is therefore surprising that this relatively short period determines so much of the crop yield. This could suggest that conventional agricultural management is already relatively optimised for crop yield (e.g. planting date), so that the small variation in agricultural management (e.g. timing of fertilising) has only little effect on crop yield. An additional reason could be that the relative contribution of insect pollination to crop yield has been underestimated, and, consequently, has received less attention in conventional agricultural management. All in all, managing for enhanced wild insect pollination seems to show largest potential in increasing yields in high-input systems of insect-pollinated crops.

We found that wild pollinators contributed most to the marketable seed yield of leek (**chapter 3**), and the contribution could for a large part be mostly to the abundance, and to a lesser extent the species richness of wild crop pollinators. Species communities usually consist of a few common species making up most of the abundance, and many less abundant species adding little to abundance but more to species richness (Scheffer et al. 2017). Furthermore, abundance and species richness are often correlated in real world systems (Larsen et al. 2005), making it difficult to infer whether abundance or species richness drives pollination services. By looking at abundance and species richness of functional groups of pollinators, we could side-step the common correlation between abundance and species richness. We found that in particular bumblebees delivered most services, but this was complemented by the abundance of solitary bees and the species richness of hoverflies. It is not until recently that studies have suggested that both abundance and species richness of pollinators are important for high crop pollination levels, for example when looking at crop pollination over a large spatial area (i.e. state or country; Winfree et al. (2018)), or to make sure that there are large enough pollinator populations to pollinate large crop fields (Garibaldi et al. 2016). A recent (unpublished) meta-analysis, including the data collected in **chapter 3**, showed that for 89 crop systems around the world, changes in crop pollinator richness mediates changes in pollinator abundance and consequently affects crop pollination services (Dainese et al. 2019). This suggests that a species rich crop pollinator community supports the total pollination services delivered, although the majority of services is delivered by a few abundant species (Kleijn et al. 2015).

Furthermore, the abundance of a crop pollinator species is not always an indication of how much they contribute to crop yield, because the identity of the crop pollinator is also important. Like in many other crops (Garibaldi et al. 2013, Kleijn et al. 2015), honeybees were common visitors to leek plants (**chapter 2 & 3**), but they did not relate well with marketable seed yield (**chapter 3**). A possible explanation for the lack of a relation is that honeybee's foraging behaviour differs between foraging for nectar or pollen (Kumar et al. 1985, Pankiw and Page 2000), which has large effects on the number of pollen deposited (Cane and Schiffhauer 2001) and subsequently their effectiveness (Javorek et al. 2002, Garibaldi et al. 2013). Honeybees are generally foraging for nectar on *Allium*-flowers (Brewster 2008), which likely reduces their pollination effectiveness on *Allium*-crops. But even if honeybees deposit large amounts of pollen when visiting crop flowers (Pfister et al. 2017), it is sometimes found that in the end honeybees do not increase crop yields (Garibaldi et al. 2013, Pfister et al. 2018). The mechanism behind this surprising pattern is unknown so far, but it is likely that the pollen carried by honeybees are of relatively poor quality, for example due to little movement between male and female flowers (Free 1993, Brittain et al. 2013). These between plant movements are particularly important in hybrid seed production because of the separate male and female parent plants (**chapter 1**, box 1.1). It is therefore not beneficial to ensure a high total abundance of crop pollinators in general, but rather to ensure high total abundance of efficient crop pollinator species that effectively pollinate crops (Garratt et al. 2014, Marzinzig et al. 2018).

Although leek hybrid seed production is a specific production system with limited coverage world-wide, our pollination studies in leek are nevertheless relevant to other crop systems for several reasons. More than half of the insect-dependent crops has similar levels of insect-dependency as leek, either during seed production, plant breeding or for the fresh-market (Klein et al. 2007). Furthermore, hybrid seed production is an increasingly common seed production system for vegetables and oil-seed crops (George 2009). However, leek hybrid seed production is also a special system because it is an exceptionally attractive crop for insect pollinators and high numbers of pollinator species can be encountered on leek flowers (**chapter 3 & 5**; (Brewster 2008)). The wild relative of leek (*Allium ampeloprasum*) is a native plant in the Mediterranean area, and it is likely that much of the pollinator community is adapted to using the flowers, and there is even an *Allium*-specialist, the onion yellow-face *Hylaeus punctulatissimus* (Muller et al. 2006). Leek may attract fewer pollinator species in non-native areas, because the local pollinator species are less likely to be adapted to members of this plant genus. For example, sunflower in Europe, or apple in South-Africa attract less pollinators than the same crop in its native range (Kleijn et al. 2015). Nevertheless, high crop yields may be obtained for these crops as well, if an effective pollinator is present. For example, the common leek-pollinator species buff-tailed bumblebee (*Bombus terrestris*-group c.f. (Williams et al. 2012)) can also be a common dominant crop pollinator in places where it is introduced, such as on highbush blueberry in Chile (Vieli et al. 2016), or on onion in New Zealand (Howlett et al. 2009). This also suggests that the contribution of pollinators to crop yield in other crops or geographical areas may potentially be more or less similar to the patterns found in leek. However, the exact contribution will depend on how much these crops depend on pollinators for crop yield, and whether effective pollinator species are abundantly present or not.

Dealing with insect pollination in agricultural management

We found that conventional agricultural inputs like fertiliser and irrigation cannot replace insect pollination in insect-dependent crops. Benefits of insect pollination were largely independent of the agricultural management aimed at raising plant size (**chapter 3**), and benefits of pollination, fertiliser and irrigation were predominantly additive (**chapter 4**). Only since about a decade ago, studies started to look into the potential interactions between agricultural management and ecosystem service delivery to crops (Garibaldi et al. 2018). These interactions play a pivotal role in the concept of ecological intensification, which predicts that ecosystem services can replace conventional agricultural inputs while maintaining crop yields (‘ecological replacement’), or that by enhancing ecosystem service delivery (‘ecological enhancement') benefits may be increased (Bommarco et al. 2013, Kleijn et al. 2019). Ecological replacement is generally assumed to take place if the benefits of the ecosystem service are larger with lower inputs (i.e. negative interaction between service delivery and inputs), while ecological enhancement can take place when there is no interaction (i.e. additive effects) or a positive interaction between service delivery and inputs (Bommarco et al. 2013, Kleijn et al. 2019). Insights in these potential interactions can therefore guide management advice on how to make optimal use of ecosystem service delivery. We found in only one crop line (line B) that insect pollination benefits depends on the agricultural management aimed at plant size (i.e. fertiliser; **chapter 3 & 4**), but for the other four crop lines we only found independent, additive, effects of agricultural management and insect pollination. The latter seems to be supported by a recent overview suggesting that agricultural management and insect pollination complement each other in shaping crop yield (Garibaldi et al. 2018). In practice this means that effects are rather predictable (i.e. more pollination means higher crop yield), and that both agricultural management and insect pollination need to be optimised independently of each other in order to reach highest yields.

Surprisingly, higher artificial fertiliser inputs did not result in higher crop yields, showing that more is not always better. In **chapter 3** we found that almost half of the variation in marketable seed yield could be explained by plant size, indicating that larger plants are likely to have larger crop yield. An easy measure to increase leek plant size is increasing fertiliser input (Sørensen et al. 1995, Brewster 2008). However, in **chapter 4** we found that even though the plants were larger in a standard fertiliser treatment, crop yields were at least equally high when standard fertiliser input levels were halved. At the same time, reducing insect pollination levels showed at least two-fold larger effects on crop yield. This suggests that in cropping systems that already receive high input levels, growers overestimate the relative contribution of fertiliser on crop yield, and likely underestimate the relative contribution of insect pollination on crop yield. While this obviously does not mean that artificial fertiliser use should be abandoned all-together, over-application of fertiliser needs to be avoided. Our results indicate high crop yields can be maintained under significantly reduced fertiliser application rates and that higher yields can be achieved by effectively increasing wild pollinator levels.

Whether growers should fully, or partly, abandon placement of honeybees in crops which do not clearly benefit from honeybees is a complicated discussion. Growers sometimes place honeybee hives as a form of insurance (Breeze et al. 2014, de Groot et al. 2016), as honeybees are the easiest way to ensure large numbers of pollinators, for example when the time window of crop pollination is short due to inclement weather. Surprisingly, however, the same argument has been raised by commercial blueberry growers who perceive wild pollinators as an insurance in case they cannot rent honeybee hives (Hanes et al. 2015). Together, this suggests that a crop pollinator community comprising of both managed and wild pollinators is the most secure option for growers (Isaacs et al. 2017), although caution should be given to the potential competition between honeybees and wild pollinators (Blitzer et al. 2012, Lindstrom et al. 2016b, Magrach et al. 2017, Henry and Rodet 2018). Furthermore, grower's knowledge on which pollinators contribute to their crop seems to play an important role in the decision to place honeybee hives or not. Commercial blueberry growers who rented managed pollinators believed that wild pollinators contributed only relatively little to crop yield, largely because they were uncertain of the contribution of wild pollinators to crop yield (Hanes et al. 2015). Removing this uncertainty by showing growers which pollinators effectively pollinate their crops will likely result in a reduction in the use of honeybee hives in crops that do not necessarily benefit from honeybee pollination.

The choice of crop variety is important in agricultural management, for example to suit a crop to the local growing conditions. However, estimates of pollination benefits for crop yield usually only take one crop variety into account (Melathopoulos et al. 2015). Indications from the studies present (Hudewenz et al. 2014, Klatt et al. 2014, Lindstrom et al. 2016a, Perrot et al. 2019), and our studies (**chapter 3 & 4**), show that pollination benefits on crop yield can vary substantially between varieties. However, the rapid and continuous development of new varieties does not allow to extensively study the relative contribution of insect pollination on crop yield for each new variety. So when a new variety enters the market, it may be in practice unknown how this variety responds to insect pollination. By observing five different hybrid seed crop-lines, we now have a fairly good understanding on how wild insect pollination affects hybrid seed production of leek in general (**chapter 3**), and it is likely that these results are indicative for non-studied lines of this crop as well. If seed producers of insect-dependent crop varieties follow the same approach, this also means that they should be able to provide evidence-based advise to growers on which pollinators contribute most to crop yield of most varieties.

Complex landscapes for crop pollination services and pollinator biodiversity

We found that with increasing cover of semi-natural habitat in agricultural landscapes (i.e. landscape complexity) both crop pollination service provision and overall pollinator biodiversity increases (**chapter 5**). The main contribution of crop pollination comes from the abundance of a few dominant species, but this is complemented by a diverse set of pollinators that usually occur in smaller numbers (**chapter 3**). Both of these components of crop pollination increased substantially with increasing landscape complexity, and also with size of the local pollinator community. However, about half of the encountered local pollinator species were not observed on flowering leek fields (**chapter 5**). Even though some of these species may contribute to pollination services of other crops (Winfree et al. 2018), or their contribution may differ from year-to-year (Alarcon et al. 2008), it is clear that a large part of the local pollinator species pool does not contribute to crop pollination (Kleijn et al. 2015, Senapathi et al. 2015). As a result, the provision of crop pollination services is not a good argument to protect these non-contributing species (Kleijn et al. 2015). However, because both the contributing and non-contributing pollinator species increase with increasing landscape complexity in agricultural landscapes, increasing landscape complexity for the purpose of increasing crop pollination services can simultaneously benefit the conservation of non-contributing pollinator species.

The handful of dominant crop pollinator species that delivered most of the crop pollination services were hardly present in the herbaceous semi-natural habitat (**chapter 5**), suggesting that these species were foraging in habitats we did not survey, such as in other flowering crops or in the woody vegetation. Two common European bumblebee species made up 55% of all counted individuals in the crop fields (**chapter 3**): red-tailed bumblebee (*Bombus lapidarius*) and the buff-tailed bumblebee-group (*Bombus terrestris/lucorum* c.f. Williams et al. (2012)). These species are commonly found on a variety of European crops (Kleijn et al. 2015), and it seems that these species have specialised on mass-flowering plant species, such as crops. This may have allowed them to be able to persist in agricultural landscapes (Redhead et al. 2018). If these species have specialised in hopping from one crop to another, then this also provides opportunities for targeted enhancement of the services they provide. For example, mass-flowering crops can follow up on each other, thereby ensuring high pollen and nectar availability throughout the flying period (Schellhorn et al. 2015, Hovestadt et al. 2019), and potentially boosting population sizes (Westphal et al. 2003, Riedinger et al. 2014) and reproductive success (Rundlof et al. 2014). However, at present we do not know for certain where these crop hoppers reside throughout their flight period, making it difficult to recommend effective management strategies.

While we have not looked at the benefits of increasing landscape complexity in agricultural landscapes, for example by planting wildflower strips or hedgerows, these measures are likely to be effective in enhancing crop pollination services (Kleijn et al. 2019). An often overlooked problem is that taking these measures for pollinators are costly because, at least in Europe, it usually means that productive land has to be taken out of production (Kleijn et al. 2019). The benefits of the measures therefore have to weigh up against these costs, and very few studies have actually tested this (Blaauw and Isaacs 2014, Morandin et al. 2016). While we did not performed pollinator-enhancing measures, we did measure the estimated benefits of wild insect pollination for crop revenue (**chapter 3**) and found that increasing wild crop pollinator levels with 25% relative to the median can increase crop revenue with about \$17 000 ha^{-1}. Because of the high revenue of leek hybrid seed production, measures to increase wild pollinators are therefore likely to be profitable in this crop, but for many low-revenue crops it may be less cost-effective. The main aim of wild pollinator-enhancing measures such as wildflower strips is to provide extra food resources and nesting sites in order to increase population size. However, there are strong indications that growth of pollinator populations shows a time lag of a few years after pollinator-enhancing measures have been established (Blaauw and Isaacs 2014, Scheper et al. 2015, Grab et al. 2018), likely because the pollinator generation time is often longer than one year. Because of this time-lag, it might be possible that annually sown wildflower strips next to annual or bi-annual crops have only a limited benefit on both pollinator populations and insect-pollinated crops. Instead, more effective pollination-enhancing measures on the long term are probably permanent flower-rich linear landscape features (Morandin and Kremen 2013, Kremen and M'Gonigle 2015, Dainese et al. 2017). Many of pollinator species that provide crop-pollination services collect surprising amounts of pollen from woody plants (Wood et al. 2018a, Wood et al. 2018b, Hass et al. 2019), with up to 80% of the pollen collected from woody plants in spring (Kamper et al. 2016). It may therefore be not surprising that restoration of woody elements has been shown to enhance crop pollination services (Morandin et al. 2016, Dainese et al. 2017), but the effects of restoration of woody elements on crop yield have not yet been studied. To convince the agricultural sector of the effectiveness of semi-natural habitat restoration, the lack of studies looking into the effects of habitat restoration on crop yield is one of the most important challenges to date that needs to be tackled.

Future directions and concluding remarks

In this thesis we have shown that wild pollinators contribute relatively more to crop yield than conventional agricultural management in a high-input agricultural production system. We show that mostly abundance of a few bumblebee species contributes most to crop yield, but that species richness can complement in providing crop yield. While we now have a fairly good idea on how insect pollination contributes to crop yield of leek, we still lack information to guide effective crop pollinator-enhancing management. It seems likely that conservation and restoration of semi-natural habitats will contribute to enhanced crop pollination service delivery, but to date we do not know where the main contributors, the dominant crop pollinators, reside before leek flowering. Future studies can provide insight in whether these pollinators have specialised in hopping from crop to crop, or that they also make use of natural resources where we have not measured, such as in canopy trees. Additionally, we found that relatively complex, and pollinator species rich agricultural landscapes provide higher levels of crop pollination services. Ecological theory predicts that with increasing pollinator species richness, the crop pollination service delivery will fluctuate less from year to year (Gunderson 2000). While this has been shown in relatively small-scale studies in plant diversity experiments (Tilman et al. 2006, Ruijven and Berendse 2007, Isbell et al. 2011), to date there are no studies that explicitly test whether pollinator species richness can increase stability of crop pollination service delivery and how this affects crop yield. Lastly, it is essential that studies evaluating pollinator-enhancing management measure the effects on crop yield and/or crop revenue on the farm level (Blaauw and Isaacs 2014, Kleijn et al. 2019). These insights can provide convincing arguments on how to ensure global food security by making sustainable use of natural resources.

An underlying motive of this thesis is to convince the agricultural sector to move towards a more ecologically responsible farming system, where *‘growers take better care of their landscape’.* The perception of what is taking care of a landscape has, however, distinctly different meanings for a grower and an ecologist (Gobster et al. 2007). For example, growers may perceive clean (i.e. no weeds) and straight uniform crops as a well-tended agricultural landscape, while ecologists may prefer to see small scale fields with abundant flowers, hedges and trees. The changing landscape caused by agricultural intensification (i.e. landscape simplification) can evoke strong emotions in conservationists, described in Dutch as ‘landschapspijn’ (literally: landscape pain). It is the almost physical pain people feel when they see that the landscape has been changed beyond repair, for example because they see hedgerows removed or an old oak cut down. Part of these changes are driven by what growers think is a well-tended landscape (e.g. removing that old dying hedgerow), and partly by the current farming system where the focus is on the maximisation of agricultural production (Tscharntke et al. 2012). In the end, we need agriculture for our food security, and we also need, and value, biodiversity in agricultural landscapes (Kremen and Merenlender 2018), so it is important that growers and ecologists work closely together to improve the system for agriculturally productive and aesthetically pleasing landscapes for everyone.

In this thesis we have shown that the conservation of pollinator biodiversity in agricultural landscapes is important for agricultural production of insect-pollinated crops. Moreover, conservation measures aimed at increasing pollinator biodiversity are likely to benefit other biodiversity and their services as well. For example, similar relationships between semi-natural habitat cover in agricultural landscapes and biodiversity have been found for several species groups such as birds (Flynn et al. 2009), butterflies and moths (Ekroos et al. 2010), mammals (Flynn et al. 2009) and plants (Liira et al. 2008). These species groups are in one way or another connected to each other, so that the decline of a single group will have effects on associated species groups. For example, a decline in flowering plant species has been associated with a decline in insect pollinators (Biesmeijer et al. 2006, Scheper et al. 2014). Similarly, wintering farmland birds depend for a substantial part on seeds of plants that benefit from insect pollination (Henderson et al. 2004, Albrecht et al. 2007), suggesting that effects of one group (pollinators) can affect another group (birds) even though there are no obvious direct links. Conversely, conservation or restoration measures targeted to one group can cascade through the whole ecosystem (Seddon et al. 2014). This can make conservation measures targeted at crop pollination services relevant to growers of non-insect pollinated crops as well, because they can simultaneously increase crop pollination services and pest control services, and reduce soil erosion in non-insect pollinated crops (Schulte et al. 2017). Addressing these links between multiple ecosystem services is essential to transform the whole agricultural system towards a more sustainable system (Kremen and Merenlender 2018, Kleijn et al. 2019), because it has to become mainstream that biodiversity matters for everyone.

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Summary

Agriculture depends fundamentally on ecosystem services such as nutrient cycling and animal pollination. However, the species delivering these services are under increasing pressure due to the conventional agricultural practices that aim to increase agricultural production. For example, while about 70% of the world's crops depend at least partly on animals for crop pollination, agricultural intensification has been identified as one of the main drivers of declining wild pollinators that deliver these services. To counter these negative effects on biodiversity, ecological intensification has been proposed, which aims to maintain, or even increase, agricultural production by making more use of the ecosystem services. To achieve this, managing for enhanced ecosystem service delivery should become part of the day-to-day agricultural management. Because managing for ecosystem services is more wildlife friendly than conventional agricultural management, local biodiversity may benefit as well. There is an increasing evidence base showing that managing for enhanced ecosystem service delivery has the potential to increase crop pollination services. However, the agricultural sector is still reluctant to implement management for increased crop pollination services. A possible reason for this could be that the agricultural sector underestimates the relative contribution of wild pollinators to crop yield compared to conventional agricultural inputs such as fertiliser and irrigation. This thesis looks at the relative importance of wild pollinators to crop yield of commercial leek (*Allium porrum*) hybrid seed production in real-world systems. By using realistic levels of insect pollination and agricultural inputs that are relevant to growers, we contribute to the evidence base of ecological intensification by showing that high crop yields can be maintained while at the same time biodiversity can be protected in agricultural landscapes.

Evaluating data collection methods is essential for performing high-quality studies. A common method to link insect pollinators to the yield of single crop plants is to observe how frequently crop flowers are visited by insect pollinators (pollinator visitation rate). However, there is no common ground among researchers on how long plants should be observed for a reliable estimate of pollinator visitation rate, and whether this estimate differs with weather conditions and time of day. In **chapter 2** we set out to observe single leek plants for three full-days on two crop fields, and determined beyond which point a longer observation time did not improve accuracy of the pollinator visitation rate estimate on leek flower heads. We found that the observation time required to obtain accurate estimates (minimum observation duration; mean ± SD: 24 ± 11.9 min) decreased with increasing visitation rate. Visitation rates differed markedly between the two sites, and between the three observation days at a single site, but hardly within observation days. Weather circumstances and time of the day had little effect on the minimum observation duration. Surprisingly, even at the end of the third day-long observation (i.e. after 39 hours observing a single plant) we found new pollinator species visiting the flowers. This suggests that plant observations usually underestimate pollinator species richness. Because most variation in visitation rate was between days and between sites, crop pollination studies estimating pollinator visitation rate should ideally visit crop plants on several days. A combination of plant observations for estimating pollinator visitation rate on several days, and transect counts on several days for estimating pollinator species richness are likely to provide the most reliable estimates of crop pollination services on single crop plants.

We have applied these methods in **chapter 3** in a correlative study across 36 commercial leek hybrid seed production fields in France and Italy. We used the natural variation in abundance and species richness of insect pollinators, and agricultural management to test the relative contributions of these variables to marketable seed yield of five crop lines. We found that under real-world conditions insect pollination was at least as important for marketable seed yield as the agricultural management aimed at raising high quality plants. Relative to the median, a 25% improvement of plant quality and pollination increased crop value by an estimated \$18,007 and \$17,174 ha^{-1} respectively. The relative importance of plant quality and insect pollination on crop yield differed between crop lines. Most notably, the abundance of bumblebees was most positively correlated to pollination services in most lines, but this was complemented by the abundance and species richness of other wild pollinators for some crop lines. We found that honeybees, managed for crop pollination services, did not contribute to marketable seed yield of any crop line. These results suggest that wild insect pollinators are an undervalued agricultural input and that managing for more wild pollinators is likely to be profitable in high-revenue crops like leek hybrid seed production.

To gain insight in whether agricultural management interacts with insect pollination, we complemented the correlative study with a full-factorial field experiment in **chapter 4**. We compared crop yield of three crop lines under standard and 50%-reduced levels of fertilisation, irrigation and insect pollination. We found that on average the effects of reduced insect pollination on crop yield were at least twice as large as the effects of reduced fertilisation and irrigation. Effects were mostly additive, suggesting that effects are rather predictable. However, crop yields of all crop lines were at least as high, or even higher, under the 50%-reduced fertilisation treatment, suggesting that artificial fertiliser is over-applied. We found indications that effects of irrigation on crop yield could work through increased nectar production, and subsequently, higher attractiveness for pollinators. These results suggest that insect pollination is the weakest link in agricultural production of insect-dependent crops in high-input systems, and that protection or promotion of wild pollinators in agricultural landscapes is essential for maintaining high yields.

Increasing wild pollinator populations in agricultural landscapes is likely to be achieved by enhancing landscape complexity (i.e. semi-natural habitat cover). Ecological theory predicts that complex landscapes are more pollinator species rich than simple landscapes, and therefore are also more crop pollinator species rich, but empirical evidence is lacking. We explored in **chapter 5** how the semi-natural habitat cover (i.e. landscape complexity) plays a role in shaping the local and crop pollinator species pool, depending on the pollinators' association with mass-flowering crops, and whether or not the crop is flowering. We surveyed pollinator communities in semi-natural habitats and in crop fields before and during crop flowering, and made a distinction between pollinators that visit crops frequently (dominant), occasionally (opportunistic) or not at all (non-crop). Non-crop pollinator species richness in the semi-natural habitats responded most positively to landscape complexity. Surprisingly, dominant crop pollinators were almost absent from the semi-natural habitats, even though they comprised about 80% of the crop pollinator community and abundance in the crop increased with landscape complexity. Species richness of the crop pollinators was positively related to local pollinator species richness and with landscape complexity, and effects were stronger for the opportunistic crop pollinators than for the dominant crop pollinators. About half of the local pollinator species pool, mostly opportunistic crop pollinators, were also encountered on leek flowers. Additionally, abundance of opportunistic crop pollinators in the semi-natural habitat dropped at the onset of crop flowering, suggesting that for this group of species mass-flowering crops provide resources additional to the natural resources. Our results indicate that increasing landscape complexity has the potential to provide high levels of both biodiversity and crop pollination services.

The results of these studies show that wild insect pollination is the most important factor in leek hybrid seed production in the current high-input system. Insights in the whereabouts of the dominant crop pollinators can guide crop pollinator-enhancing management targeted on the most contributing species. While we have not looked at the effects of such pollinator-enhancing management in agricultural landscapes, these practices are likely to be effective in enhancing crop pollination services, as well as for promoting non-contributing species in agricultural landscapes. Increasing pollinator biodiversity in agricultural landscapes may also show to provide relatively more stable crop pollination services over time, but to date we lack the empirical evidence. Agriculture is essential in providing food security, and protecting the biodiversity in agricultural landscapes is important for ecosystem services and intrinsic values. It is therefore pivotal that ecologists work closely together with the agricultural sector to achieve agriculturally productive landscapes where biodiversity conservation is mainstream.

Samenvatting

De landbouw is fundamenteel afhankelijk van ecosysteemdiensten zoals voor de omzetting van dood plantmateriaal in beschikbare voedingstoffen en de bestuiving van planten. Echter, de soorten die deze diensten verlenen staan onder toenemende druk door het gangbare agrarische beheer dat is gericht op het verhogen van de productie. Bijvoorbeeld, hoewel ongeveer 70% van de gewassen in de wereld (gedeeltelijk) afhankelijk zijn van insecten voor gewasbestuiving, is intensivering van de landbouw een van de belangrijkste oorzaken voor de achteruitgang van de wilde soorten die deze gewassen bestuiven. Om deze negatieve effecten op biodiversiteit tegen te gaan, is ecologische intensivering van de landbouw voorgesteld. Ecologische intensivering heeft als doel om de productiviteit van gewassen even hoog te houden ten opzichte van de gangbare landbouw, of zelfs te verhogen, door slimmer gebruik te maken van ecosysteemdiensten in plaats van het gebruiken van kunstmatige middelen zoals kunstmest en gewasbeschermingsmiddelen. Om dit te bereiken, moet het beheer gericht op het verbeteren van ecosysteemdiensten onderdeel worden van het gangbare, dagelijkse beheer van boeren. Omdat beheer gericht op het verbeteren van ecosysteemdiensten beter is voor de natuurlijke omgeving, kan dit beheer daarnaast ook de lokale biodiversiteit versterken. Een groeiend aantal wetenschappelijke studies laat zien dat beheer gericht op het verbeteren van ecosysteemdiensten de gewasbestuiving mogelijk kan vergroten. Echter, de landbouwsector is nog terughoudend in het uitvoeren van het beheer gericht op gewasbestuiving door insecten. Een mogelijke reden hiervoor is dat de landbouwsector de toegevoegde waarde van insectbestuiving op hun gewasopbrengst onderschat, met name ten opzichte van gangbare middelen als bemesting en gewasbeschermingsmiddelen. Dit proefschrift kijkt naar de relatieve bijdrage van wilde bestuivers aan de opbrengst van commerciële prei-hybridezaadproductie (*Allium porrum*) onder gangbare teeltomstandigheden. Door realistische niveaus van insectbestuiving, bemesting en irrigatie te gebruiken, dragen we bij aan de kennis over ecologische intensivering. We doen dit door te laten zien dat hoge gewasopbrengsten behouden kunnen blijven terwijl de lokale biodiversiteit beschermd kan worden in agrarische landschappen.

Het evalueren van dataverzamelingsmethoden is essentieel om de kwaliteit van wetenschappelijke studies hoog te houden. Een algemene methode om insectbestuiving aan de opbrengst van een enkele gewasplant te relateren, is om te observeren hoe vaak bloemen van de plant bezocht worden door insecten (bestuiver-bezoekfrequentie). Echter, er bestaat geen overeenstemming tussen onderzoekers over hoe lang planten geobserveerd moeten worden voordat de schatting van bestuiver-bezoekfrequentie betrouwbaar is, en hoe deze schatting afhangt van weercondities en de tijd van de dag. In **hoofdstuk 2** hebben we een enkele preiplant geobserveerd gedurende drie volle dagen en op twee productievelden. Aan de hand van deze observaties hebben we bepaald na welke observatieduur het langer observeren de schatting van bestuiver-bezoekfrequentie niet meer nauwkeuriger maakte. We vonden dat de observatieduur die nodig was om een nauwkeurige schatting te krijgen (minimale observatieduur; gemiddelde ± SD: 24 ± 11.9 minuten) afnam met een hogere bezoekfrequentie van bestuivers. De bezoekfrequentie van bestuivers verschilde opvallend veel tussen de twee productievelden en tussen de drie observatiedagen op een enkel productieveld, maar nauwelijks binnen een observatiedag. Weersomstandigheden en tijd van de dag hadden weinig effect op de minimale observatieduur. Opvallend genoeg vonden we aan het eind van de derde volledige dag-observatie (dus na 39 uur observatie van een enkele plant) nog nieuwe soorten bestuivers op onze preiplant. Dit suggereert dat plantobservaties normaal gesproken de soortenrijkdom van bezoekers onderschatten. Omdat de meeste variatie in bezoekfrequentie tussen dagen en tussen de productievelden was, zouden gewasbestuivingsstudies die bezoekfrequentie meten, idealiter de planten op meerdere dagen moeten observeren. Een combinatie van plantobservaties op meerdere dagen voor een schatting van bezoekfrequentie, en transecten op meerdere dagen voor een schatting van bestuiversoortenrijkdom zorgen dan waarschijnlijk voor de meest betrouwbare schattingen van gewasbestuivingsdiensten op een enkele gewasplant.

We hebben deze methoden toegepast in **hoofdstuk 3** in een correlatieve studie in 36 commerciële prei-hybridezaadproductievelden in Frankrijk en Italië. We hebben de natuurlijke variatie in talrijkheid en soortenrijkdom van insectbestuivers en agrarisch beheer gebruikt, om te testen wat de relatieve bijdrage van deze factoren zijn op de marktwaardige zaadopbrengst van vijf gewaslijnen. We vonden dat onder gangbare omstandigheden insectbestuiving op zijn minst zo belangrijk was voor de marktwaardige zaadopbrengst als het agrarische beheer gericht op het verkrijgen van een hoge plantkwaliteit. Een 25%-verbetering van plantkwaliteit en bestuiving ten opzichte van de mediaanverhoogde de gewaswaarde respectievelijk met $18,007 en $17,174 per hectare. De relatieve bijdrage van plantkwaliteit en insectbestuiving op de marktwaardige zaadopbrengst verschilde tussen de gewaslijnen. De gewasopbrengst van de meeste lijnen was het meest positief gecorreleerd aan de talrijkheid van hommels , maar dit werd aangevuld door de talrijkheid en de soortenrijkdom van andere wilde bestuivers in sommige gewaslijnen. We vonden dat honingbijen, die geplaatst worden voor gewasbestuiving, voor alle gewaslijnen nauwelijks bijdroegen aan de marktwaardige zaadopbrengst. Deze resultaten suggereren dat wilde bestuivers een ondergewaardeerde agrarische productiefactor zijn en dat beheer gericht op het verhogen van wilde bestuiversaantallen waarschijnlijk voordelig is voor gewassen met een hoge economische waarde, zoals prei-hybridezaadproductie.

Om een beeld te krijgen van de wisselwerking tussen agrarisch beheer en insectbestuiving, hebben we de correlatieve studie aangevuld met een veldexperiment in **hoofdstuk 4**. We vergeleken gewasopbrengst van drie gewaslijnen onder standaard en 50%-gereduceerde niveaus van bemesting, irrigatie en bestuiving. We vonden dat de effecten van gereduceerde bestuiving gemiddeld genomen twee keer zo groot waren als de effecten van gereduceerde bemesting en irrigatie. Effecten waren voornamelijk complementair, wat suggereert dat de effecten redelijk voorspelbaar zijn. Opvallend genoeg waren de gewasopbrengsten van alle lijnen even hoog, of zelfs hoger, bij de planten die 50% minder bemesting ontvingen, wat suggereert dat bemesting overmatig wordt toegepast in dit systeem. We vonden aanwijzingen dat de effecten van irrigatie hoofdzakelijk voor een hogere nectarproductie zorgt, wat vervolgens de aantrekkelijkheid voor insectbestuivers verhoogt. Deze resultaten impliceren dat insectbestuiving de zwakste schakel is in de productie van insectafhankelijke gewassen in intensieve landbouwsystemen, en dat de bescherming en stimulering van wilde bestuivers in agrarische landschappen essentieel is voor het behoud van hoge gewasopbrengsten.

Het vergroten van wilde bestuiverpopulaties in agrarische landschappen kan waarschijnlijk worden bereikt door de landschapscomplexiteit te verhogen (i.e. hoeveelheid half-natuurlijk habitat). Volgens ecologische theorie verwachten we dat complexe landschappen soortenrijker zijn dan simpele landschappen, en daardoor ook dat complexe landschappen rijker zijn in gewasbestuiversoorten, maar empirisch bewijs hiervoor ontbreekt. In **hoofdstuk 5** hebben we uitgezocht hoe het oppervlakte half-natuurlijk habitat (i.e. landschapscomplexiteit) een rol speelt in het vormen van de lokale bestuivers- en de gewasbestuiversgemeenschap, afhankelijk van hoe vaak bestuivers op massaal-bloeiende gewassen af komen, en of het gewas al dan niet aan het bloeien is. We hebben de bestuiversgemeenschappen gemonitord in de half-natuurlijke gebieden en in de gewassen zowel vlak voor, als gedurende de gewasbloei. We hebben daarbij een onderscheid gemaakt in bestuivers die de gewassen vaak (dominante gewasbestuivers), soms (opportunistische gewasbestuivers), of niet (niet-gewasbestuivers) bezoeken. Soortenrijkdom van de niet-gewasbestuivers in de half-natuurlijke gebieden reageerden het meest positief op landschapscomplexiteit. Opvallend genoeg waren dominante gewasbestuivers vrijwel afwezig in de half-natuurlijke gebieden, ook al bestond 80% van de totale gewasbestuivergemeenschap uit deze soorten en namen de aantallen in het gewas toe met toenemende landschapscomplexiteit. Gewasbestuiversoortenrijkdom was positief gerelateerd aan de lokale bestuiversoortenrijkdom, en eveneens met landschapscomplexiteit. Deze effecten waren sterker voor de opportunistische gewasbestuivers dan voor de dominante gewasbestuivers. Ongeveer de helft van de lokale bestuiversgemeenschap, hoofdzakelijk opportunistische gewasbestuivers, werd aangetroffen op preibloemen. De talrijkheid van opportunistische gewasbestuivers in de half-natuurlijke gebieden nam substantieel af toen het gewas begon met bloeien, wat suggereert dat voor deze groep bestuivers massaal-bloeiende gewassen een aanvulling zijn op het natuurlijke voedselaanbod. Onze resultaten suggereren dat het vergroten van de landschapscomplexiteit positief kan zijn voor een grote biodiversiteit en voor een goede gewasbestuiving.

De resultaten van deze studies laten zien dat bestuiving door wilde insecten de meest belangrijke factor is voor prei-hybridezaadproductie in het huidige hoge-inputsysteem. Inzichten in waar de dominante gewasbestuivers zich in het landschap bevinden kan helpen om het beheer gericht op de soorten die relatief het meest bijdragen aan gewasbestuiving te verbeteren. Hoewel we niet hebben gekeken naar effecten van maatregelen die bestuivingsecosysteemdiensten moeten bevorderen (bijvoorbeeld bloemstroken), lijkt het waarschijnlijk dat deze maatregelen effectief kunnen zijn in het verbeteren van gewasbestuiving, alsmede in de bescherming van niet-gewasbestuivende soorten in agrarische landschappen. Het verhogen van de bestuiversoortenrijkdom in agrarische landschappen kan er mogelijk ook voor zorgen dat de gewasbestuiving stabieler verloopt over de tijd heen, maar hiervoor missen we momenteel bewijs. Landbouw is essentieel voor onze voedselvoorziening, en het beschermen van biodiversiteit in agrarische landschappen is belangrijk voor ecosysteemdiensten en voor de intrinsieke waarde die biodiversiteit levert en heeft. Het is daarom belangrijk dat ecologen nauw samenwerken met de agrarische sector om agrarische landschappen te creëren die productief zijn, en waar bescherming van biodiversiteit de normaalste zaak van de wereld is.

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Perhaps the most-read part of a thesis, is the word of thanks to all the people that have helped and supported me, knowingly and unknowingly, during the completion of this thesis, or even well before I even knew I was going to do a PhD. I cannot express my gratitude well enough, but I will give it a shot.

First and foremost, Charlotte. Thank you for being so supportive during all these years. Sorry that I had to leave so often, and so long, for fieldwork in Italy and France, particularly next to all the birding weekends (and some birding holidays). I am super happy that I'm allowed to call you my wife (even though I'm still not used to that word). Let's see what the future holds!

Of course, thank you David, as a supervisor and colleague. I remember that it was quite strange to present myself during the job interview in front of you, as you already knew all the projects I had worked on in my MSc because you had supervised them. I also remember that you joined the 'birders' in an informal conversation at the Netherlands Annual Ecology Meeting, when we were discussing biodiversity loss (of birds, of course!) due to intensive agriculture. You told me about a potential new project working on pollinators. I knew nothing about pollinators, and might have come over as not interested... In all honesty, I applied for the vacancy mostly because I could work with a supervisor I knew and with I could work together. That part has not changed, but thanks to you I now also really want to continue to work on the topic. Thanks also for stepping into my office (or me in yours), and convincing me to do just that small extra bit of work to make the work just that tiny bit better. Also thank you for allowing me to disagree, and for the more informal talks during coffee breaks, travels in the car or plane and during dinners. Up to another three years (at least?) of working together on, and talking about, exciting topics!

Then my co-promotors, Jasper and Jeroen! Jeroen, as my roommate you were always available for all nonsense, content or statistical questions. More than once you commented on a draft paper and noticed an overlooked mistake, that because we now knew it, had to be changed. This caused a few days of stress -hoping the results don't change too much- but luckily they rarely did, and now I knew the statistics were better than before. Thanks for your critical looks, and your humour during the informal social events! Jasper, your practical experience and analytical thinking have helped me a lot during the setting up of experiments and framing of papers. Also your views on the ‘game of science’, and how different people play the game are food for thought. Speaking of games: thank you for organising the PEN-football-pools!

I’m so fortunate to have worked, and continue to work with such a nice and fun group at PEN (Plant Ecology and Nature Conservation). Jan, Frans en Hennie, jullie hebben misschien geen flauw benul hoe fijn we het vinden dat jullie ons altijd op komen halen voor gezamenlijke koffie-, lunch- en theepauzes. Ik probeer er zo min mogelijk over te slaan, want zelfs al moet er écht wat gebeuren, dat kwartiertje pauze is een soort van reset. Dank daarvoor! Petra en Gerda, super dat jullie altijd dingen voor ons kunnen regelen :). De overige stafleden: Elmar, Joop, Juul, Liesje, Maaike, Michiel, Monique en Philippine. Dank voor het ondersteunen, voor het meedenken en de leuke avondjes! And of course the PhD and Postdoc-team: Aaron, Eline, Chen, Davide, George, Hamza, Job, Lisette, Maarten, Marinka, Miguel, Natalie, Peng, Pengyao, Robert, Rúna, Sina, Wei, and Zulin. You make the group dynamic, fun and exciting!

Much of the practical work was supported by a whole team of MSc-students and assistants. Please know that without your help, this would have not been possible. Bastiaen, Cassandra, Cristina, Dennis, Dianne, Koen, Nicole, Timo and Win: thank you! Not only for the practical work, but also for challenging me and feeding me with your ideas.

The leek-production team of BASF (formerly Bayer Vegetable Seeds – Crop Science) has been incredibly helpful and supportive of this project. Our half-yearly meetings were always lively and full of questions. Thank you, Martje, for coordinating this project so well. Ronald, thanks in particular for letting me join on your inspection trips to the fields, very insightful! Toon and Philip, thanks for thinking along from the eyes of plant breeders. Johan, endless numbers of curious questions and identifying potential problems, excellent! Agnes, you came into the project only later, but your support was essential and I am so grateful that you also liked the project. Paul and Peter, thanks for assessing all these leek seedlings on their vitality, and for your help in the BASF-lab. In our experiments we have made use of numerous production fields in France and Italy. This was of course not possible without the support of the growers, but a large part can be attributed to the managing support of Mikael and Jean-Cristophe in France, and to Alberto and Antonio in Italy. Antonio, mille mille gracie! In all four years we have had a field season in Italy, and it was a joy for me to be there (expressed in the kilos I gained..). You took me into the fields, warned me for dangerous roads, learned me Italian words and where to get the good food. Your enthusiasm for the bees, and your dedication to the project really have made this project a success. Take care! Also thanks to the support of the team in Italy, in particular Sabato and Salvatore.

I've spent a couple of years in the PhD-council of the graduate school of PE&RC, and the group of people that I should thank is too large to mention here. So, in short: PPC thanks for the great time, nice events and the constructive work we have done for the PhD-program.

Een groot voordeel van Wageningen is dat er veel mensen 'blijven hangen' en die trekken dan weer leuke mensen aan. Hieronder ook de 'Geheime club van 500', oorspronkelijk omdat we gewoon maar 500 meter van elkaar af woonden. De spelletjesavonden op dinsdagen waren legendarisch, ook al speel ik nu echt nooit meer Kolonisten van Catan. Jammer genoeg wonen we nu weer verder van elkaar en zien we elkaar (deels) minder. Laten we het vooral weer oppakken!

De Birding Basterds, wat een stelletje zijn we toch ook. Wat geniet ik enorm van de manier waarop wij vogelen. We gaan er volle bak voor en iets anders behoort niet tot de opties. Ik weet oprecht niet of al die weekendjes vogelen met jullie nou productief ('ik moet het nú afmaken') of contra-productief ('ik moet eigenlijk nu vogelen') waren voor mijn promotieonderzoek, maar ik weet wel dat ik niet zonder kan. Het heeft me vooral geleerd om goed te plannen. In het specifiek wil ik 'D18' bedanken: Jorrit, Marijn, Pieter, Rutger en Tim. Dat we nog jaren in de blokhut mogen blijven zitten, inclusief alle bende en verhalen die er bij horen.

Pap en mam, Marleen, Tim, Luuk en Florian, en Hanneke, Joris en Juna, zelf nadenken en werken voor wat je graag wilt doen, dat zit er bij ons ingebakken. Voor mij is dat onderzoek doen en in de natuur bezig zijn. Dat hebben jullie mede mogelijk gemaakt, en ik heb geluk met jullie als familie. Liebe Walthers, vielen Dank, dass ihr mich in eurer Familie aufgenommen habt.

Er blijven nog een aantal mensen over die me hebben geholpen met het onderzoek, heel praktisch of juist door vroeg in mijn leven mijn nieuwsgierigheid voor natuur te voeden. Allereerst Ivo, zonder jou hadden we alleen maar met morphospecies van alle bijen kunnen werken in Italië, en dat jij die duizenden (!) bijen met (veel) plezier hebt gedetermineerd, daar heb ik heel veel respect voor! Vast geheel onbewust, maar wel erg toevallig: Pieter van Breugel was mijn scheikundeleraar en mentor op de middelbare school. Nu, jaren later, raken onze werkvelden zich, want Pieter is een bijenspecialist, met name van de wilde bijen die veel te weinig aandacht krijgen. Ergens is die enthousiasme voor bijen toch een keer overgesprongen. Zo zijn er vroeger toch veel mensen geweest die het enthousiasme gevoed hebben. Een kleine (onvolledige) greep: ome Huub en Wim voor de wandelingen en het hokken, Toy en de Jeugdvogelwacht Uden, en de JNM voor alle natuurkampen. Die tochten door de natuur zijn fantastisch geweest en daar ben ik ontzettend dankbaar voor.

About the author

Thijs Peter Mathias Fijen was born on 30 October 1990 in Veghel, The Netherlands. His enthusiasm for nature was sparked already on the primary school ‘t Ven in Veghel, where the local school nature guides took the kids out on small excursions into the woods and the herb-gardens. Now it became clear: where other boys usually wanted to be fireman or the like, Thijs wanted to be a nature warden (boswachter). Soon after he started to focus on birds, and through the magazine Vrije Vogels, he came into contact with the youth organisation Jeugdbond voor Natuur- en Milieustudie (JNM). In his early teenage years he spent the weekends playing soccer, but later he realised he just wanted to watch birds. In these years he spent most weekends watching birds, but also learning a lot about all other living creatures. Thijs completed his VWO-education in 2008 at the Zwijsen College, Veghel. At the age of 17, Thijs moved to Wageningen to start the bachelor study ‘Bos- en Natuurbeheer’ (Forest and Nature Conservation), and later the master Forest and Nature Conservation at Wageningen University (WUR). He graduated the bachelor in 2011, and the masters in 2013.

From 2002 until 2015, Thijs was a member of the JNM and has joined (and organised) about 100 camps, spanning from a weekend in the Netherlands to two weeks in foreign countries. Gradually, more and more species groups came into the attention of Thijs, and his knowledge of species also helped to feed the curiosity in what is happening in nature. Next to his studies, Thijs worked part-time at the postal office, but later also more or less full-time at Regelink Ecologie & Landschap, where he monitored protected birds in municipalities and spent many nights surveying bats all over the Netherlands.

Birds are a keyword in Thijs’ life. Not only does he spend most weekends watching birds, twitching (go and see reported rare birds) or searching for rare birds, he also makes sound recordings of birds since 2010. In particular he likes to identify, or search for identification features in, bird calls of species with similar calls. Because of his passion for birds, it may come as no surprise that he did both his master thesis and internship in birds. In his master thesis, Thijs looked at the time budget and diet of Crab Plovers (*Dromas ardeola*) in the Sultanate of Oman in collaboration with Dr. Roeland Bom and Dr. Jan van Gils at the Royal Netherlands Institute for Sea Research (NIOZ). For his master internship, Thijs gathered funding and a project team together with Dr. Johannes Kamp (Westfälische Wilhelms Universität, Münster, Germany), and Dr. Ruslan Uruzaliev (Association for the Conservation of Biodiversity of Kazakhstan (ACBK)) to work on the breeding ecology of Black Larks (*Melanocorypha yeltoniensis*).

Black Larks have the peculiar behaviour of collecting animal dung to ‘pave’ the entrance of their nest, and Thijs’ mission was to find out why. Both of these projects were supervised by Prof. Dr. David Kleijn (at that time Research Ecology Group, now Plant Ecology and Nature Conservation Group, WUR). Other research projects in his studies were focussed on moths. In his bachelor thesis, Thijs studied whether it was possible to identify moth communities based on moth records per habitat type at the Butterfly Conservation Netherlands (Vlinderstichting) together with Dr. Frank van Langevelde (Resource Ecology Group, WUR) and Dr. Dick Groenendijk (Vlinderstichting). Later in his masters, moths were again in the picture in a study on the effect of artificial night lighting on drinking frequency of moths, supervised by Dr. Koert van Geffen (Plant Ecology and Nature Conservation Group, WUR) and Dr. Frank van Langevelde.

After his masters, Thijs started a PhD project on Crab Plovers and their prey again at the Royal Netherlands Institute for Sea Research (NIOZ) in 2014. However, due to financial difficulties, the project ended soon after. At that point, a PhD-vacancy with his MSc supervisor David Kleijn working on the relative importance of wild pollinators for hybrid seed production got his attention. Thijs was appointed as PhD-candidate at the chair group Plant Ecology and Nature Conservation at WUR in 2015, and the result of this PhD-project lies in front of you.

Thijs continues to work with birds in his spare time: searching for rare birds, writing identification articles, validating rarity reports in the Dutch Birding Rarity Committee, and as a member of the editorial board of the magazine Dutch Birding. For his professional career, Thijs continues to work on crop pollinators, pollinator trends, and motivations to protect pollinator biodiversity in a three-year postdoc project at Plant Ecology and Nature Conservation, WUR, Wageningen.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Complementarity and synergism among ecosystem services supporting crop yield

Post-graduate courses (5.5 ECTS)

- GIS in practice; PE&RC (2016)
- Introduction to zero-inflated models in R; (2018)
- Bugs at your service; PE&RC (2019)

Laboratory training and working visits (2.4 ECTS)

- Mechanistic understanding of the effects of green infrastructure at different spatial scales on crop ecosystem services; Estación Biológica de Doñana (2017)
- Which combination of traits makes a dominant crop pollinator dominant; Estación Biológica de Doñana (2019)

Invited review of (unpublished) journal manuscript (4 ECTS)

- Journal of Insect Conservation (2016)
- Journal of Applied Ecology (2 x 2018)
- PLOS One (2019)

Deficiency, refresh, brush-up courses (0.3 ECTS)

- Workshop identifying bees; European Invertebrate Study (EIS) (2015)

Competence strengthening / skills courses (2.1 ECTS)

- Scientific writing; Wageningen Into Languages (2016)

- PhD Workshop carousel; WGS (2016)

PE&RC Annual meetings, seminars and the PE&RC weekend (4.3 ECTS)
- PE&RC Day (2015-2018)
- PPC (2015-2018)
- Mediterranean documentary evening (2018)

Discussion groups / local seminars / other scientific meetings (5.5 ECTS)
- Insect pollination & sustainable agriculture special interest group meeting; Reading, UK (2014)
- WEES Seminar (2015, 2018)
- WEES Workshop (2016, 2018)
- Bayer Bee-Care & Bayer; oral presentation (2017)
- PSI Discussion group (2017)
- TKI Network event; poster presentation (2018)
- Dutch seed symposium; oral presentation (2018)

International symposia, workshops and conferences (9.3 ECTS)
- NAEM; oral presentation (2017)
- Student conference on conservation science; oral presentation; Cambridge, UK (2017)
- NAEM; poster and oral presentation (2018)
- European congress for conservation science; poster and oral presentation; Jyväskylä, Finland (2018)

Lecturing / supervision of practicals / tutorials (10.8 ECTS)
- Ecology 1; excursions (2015, 2016, 2017, 2018)
- Introductie omgevingswetenschappen (2016)
- Landscape geography (2016, 2017)
- Ecology 2 (2016, 2018)
- Ecological aspects of bio-interactions (2017)
- Restoration ecology (2017, 2018, 2019)
- Ecology of communities, ecosystems and landscapes; excursion (2017, 2019)
- Academic consultancy training; scientific advisor (2018)
- Bos- en natuurbeheer beheermaatregelen en –planning (2018, 2019)
- Ecology 1; tutorials (2018, 2019)

- Research master cluster (2018, 2019)
- Capita selecta: nature in a crowded country (2018, 2019)
- Seminar week HAS guest lecture (2019)

Supervision of MSc students

- Bastiaen Boekelo: the effect of landscape composition on pollinator communities in and around mass-flowering leek fields in Italy
- Timo Boom: pollinator abundance and species richness are both important for hybrid leek (*Allium porrum*) seed production in Italian agricultural landscapes
- Christina Pappa: exploiting the wildflower strips opportunities as a mitigation measure for bumblebee loss in the Netherlands
- Cassandra Vogel: effect of agricultural management in leek-seed production on pollinator visitation rate, species richness, and proportional visitation by functional groups
- Koen Verhoogt: effect of agricultural management on nectar production and pollinator visitation rate in leek-seed production
- Dennis Heupink: the potential of early mass-flowering crops as a pollinator boost for a late mass-flowering crop
- Winfried Vertommen: unravelling the spatio-temporal distribution of wild pollinators in and around crop fields. Is onion an effective boosting crop for leek?

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