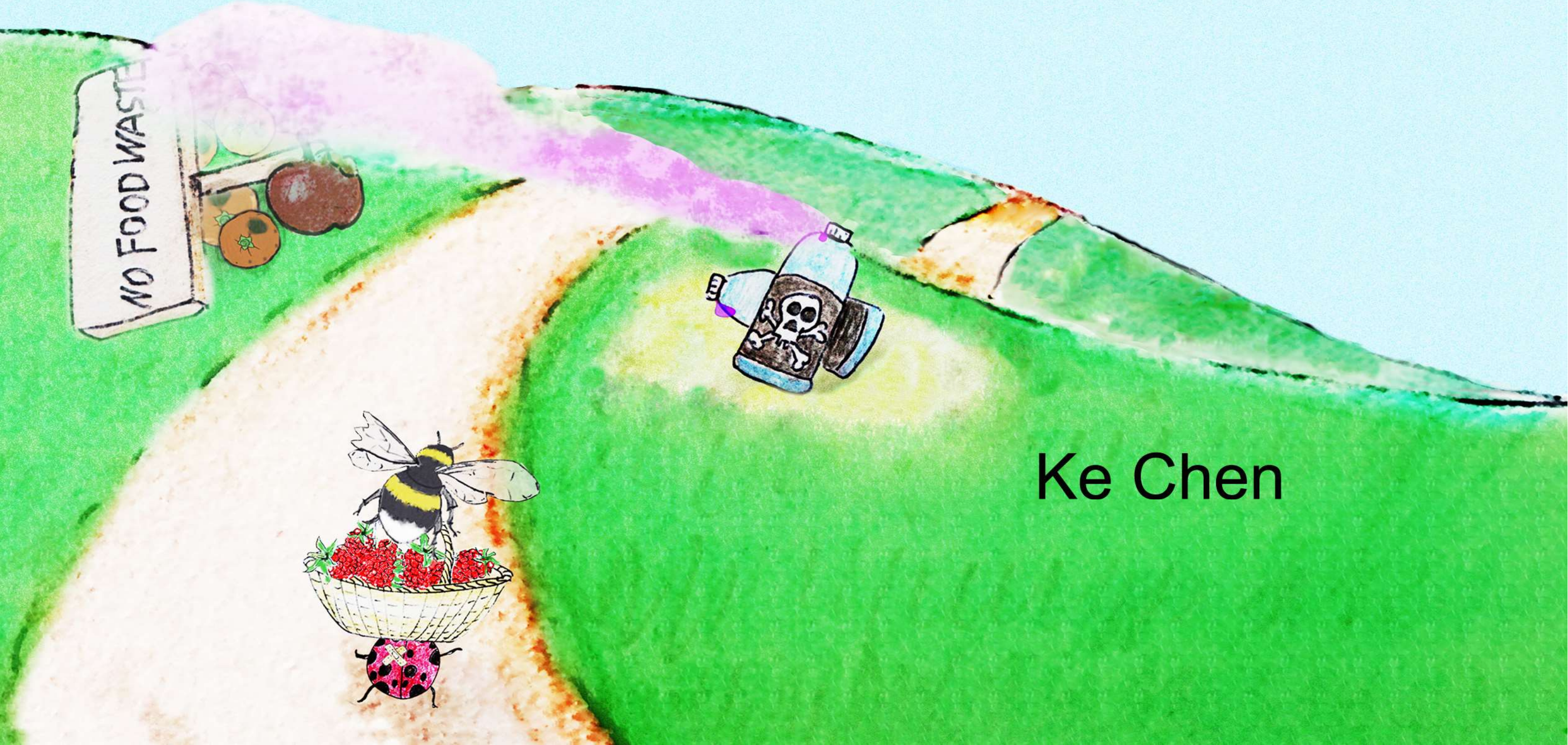
Ecosystem service benefits in a perennial fruit crop:

How do insect pollinators, arbuscular mycorrhizal fungi and soil organic matter shape raspberry production?



Ke Chen

Propositions

1. Understanding the outcomes of managing ecosystem services under different agricultural contexts is more important than exploring the underlying mechanisms.
   (this thesis)

2. Interacting effects of multiple ecosystem services are not useful for agricultural practice.
   (this thesis)

3. Reducing food waste is a more rational strategy for ensuring food security than boosting agricultural output.

4. Societal impact is a more meaningful criterion for scientific success than academic citation scores.

5. The relationship between technological development and human wellbeing is unimodal rather than linear.

6. Efforts to eradicate hunger must take priority over efforts to protect the environment.

Propositions belonging to the thesis, entitled
Ecosystem service benefits in a perennial fruit crop: How do insect pollinators, arbuscular mycorrhizal fungi and soil organic matter shape raspberry production?
Ke Chen
Wageningen, 19 April 2022

Ecosystem service benefits in a perennial fruit crop:
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Ke Chen

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Ke Chen

Ecosystem service benefits in a perennial fruit crop: How do insect pollinators, arbuscular mycorrhizal fungi and soil organic matter shape raspberry production?

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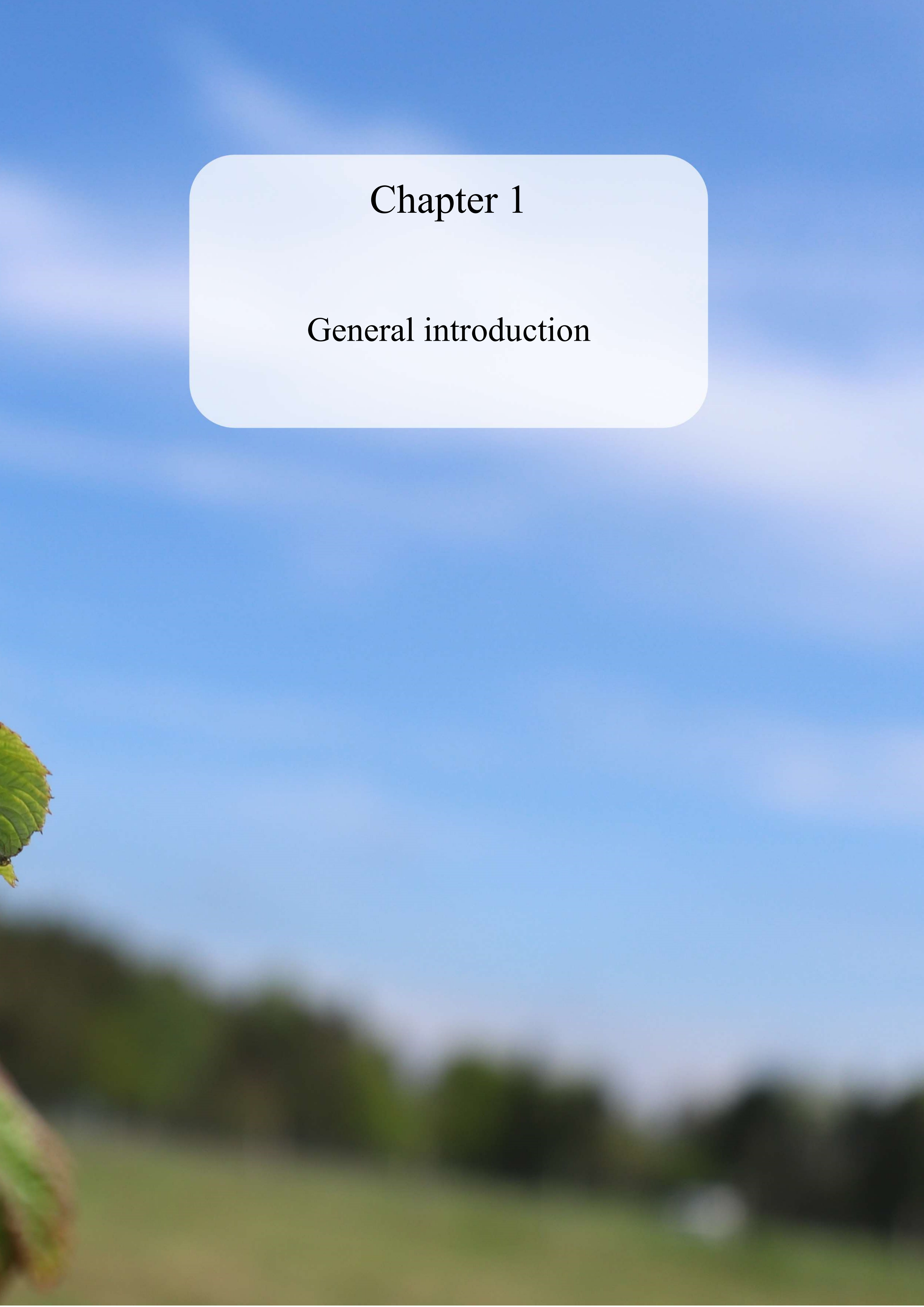


Chapter 1

General introduction

Future agriculture has to reduce environmental impacts and increase yield

The global population has expanded ca. 2.5-fold from 3.1 billion in 1961 to 7.6 billion in 2018, while global cereal yield increased 3-fold and per capita cereal increased 1.3-fold during the same period (FAO 2018). This achievement can largely be attributed to the adoption of practices developed during the *"Green revolution"* (Khush 1999), such as the use of high-yielding crop varieties (Khush 2001; Tilman *et al.* 2001) and the expansion of mechanized monocultures (Altieri 2009). In addition, these practices have been accompanied by intensive use of inputs such as artificial fertilizers, pesticides and water (Stewart *et al.* 2005). For example, between 1961 and 2018, nitrogen fertilizer consumption per hectare of cropland increased about ninefold (FAO 2018). The downside of these developments has been that the intensive farming practices have caused severe environmental problems (Rodriguez *et al.* 2004) such as soil degradation, water pollution (Carpenter *et al.* 1998; Novotny 1999), and biodiversity loss (Benton *et al.* 2003; Geiger *et al.* 2010; Dudley & Alexander 2017). Agriculture has become a major contributor to global climate change, accounting for up to 30% of all anthropogenic greenhouse gas emissions, in particular contributing to 84% of total nitrous oxide emissions (Smith *et al.* 2008; Kang & Banga 2013). Therefore, if the current agricultural intensification continues or strengthens, agro-ecosystems and human wellbeing could suffer considerable and irreversible damages (Tilman *et al.* 2001).

In addition to reducing the environmental impacts, agriculture is facing an unprecedented challenge of further increasing productivity for food security (Godfray *et al.* 2010; Gerland *et al.* 2014). By 2050, the global population is expected to reach 9.6 billion. This is 39% more people than in 2010 but with an estimated 60% higher food demand, due to the economic development and the associated increased consumption rates (Godfray *et al.* 2010; Gerland *et al.* 2014; van Dijk *et al.* 2021). Increased production is also essential to fight hunger in underdeveloped regions (World Health Organization 2019). Despite the fact that the global average yields have improved dramatically in recent decades, by 2018 about 11% of people globally still did not have access to sufficient food and almost 20% of people in Africa were suffering undernourishment (World Health Organization 2019). The pressure on increasing agricultural productivity cannot be released by converting more natural ecosystems into farmland. Agriculture currently already occupies about 40% of Earth's ice-free terrestrial surface, and the rest is mostly land unsuitable for agriculture, e.g., deserts, tundra and mountains (Young 1999; Foley *et al.* 2011). Furthermore, since the last century, the rate of increasing agricultural productivity has been declining despite the ever-increasing external inputs (Ray *et al.* 2013). For example, after decades of significant increase, the rate of increase in cereal yields has been plateauing in some of the world's most important cereal-producing countries (Cassman *et al.* 2010; Grassini *et al.* 2013). This suggests that feeding the growing world population may need a different approach than conventional agricultural practices.

The twofold goal for future agriculture, i.e., safeguarding food security and protecting the environment, can only be achieved by bringing together the expertise of multiple disciplines (Foley *et al.* 2011; Fraser *et al.* 2016). Breeding technologies, for example, can contribute to new crop varieties with elevated yield potential and increased tolerance to the changing environmental conditions (Tester & Langridge 2010; Zaidi *et al.* 2019). From the perspective of socioeconomics, initiatives and measures to decrease food waste have considerable potential to reduce food demand, as about one-third to half of the global food is estimated to be lost during post-harvest processes, such as transport, storage and retail (Lundqvist *et al.* 2008; Foley *et al.* 2011; Tscharntke *et al.* 2012). Modifying conventional farming practices to make them more robust and productive, for example by adoption of intercropping and more diverse crop rotations, can also contribute to increased food safety (Berzsenyi *et al.* 2000; Zhang & Li 2003). In addition to these options, ecological intensification has been proposed as a promising paradigm that can replace conventional agricultural intensification (Bommarco *et al.*

2013; Tittonell 2014; Kleijn *et al.* 2019). Ecological intensification holds great potential to either increase yield formation sustainably or to reduce artificial inputs while sustaining high yield (Bommarco *et al.* 2013).

Definition of ecological intensification

Ecological intensification is based on the intensive and smart use of ecosystems services in the agricultural production process (Bommarco *et al.* 2013; Tittonell 2014). Ecosystems services are broadly defined as the benefits that humans obtain from ecosystems (Millennium Ecosystem Assessment 2005), and they can be grouped into four categories: provisioning services, cultural services, regulating services and supporting services (Figure 1.1a). In any given location, agricultural production (provisioning services) depends on supporting services (e.g., nutrient cycling services provided by beneficial soil fungi; Figure 1.1a) and regulating services (e.g., crop pollination provided by bees; Figure 1.1a) (Zhang *et al.* 2007; Bommarco *et al.* 2013). The contribution of ecosystem services to crop production is usually determined by one or several of the most limiting underpinning services (Bommarco *et al.* 2013; Motzke *et al.* 2015; Fijen *et al.* 2020). I illustrate such relationships in Figure 1.1b, where yield level is mainly determined by pest regulation, the assumed most limiting service. A common approach to increase yield in conventional agriculture is to apply artificial inputs to raise any limiting services, such as applying fertilizer to remove nutrient limitation or applying pesticides to reduce pest damage (Figure 1.1b). In this framework, increasing a non-limiting service is not very efficient. For example, the yield of watermelon (*Citrullus lanatus*) under common farming practices was not enhanced by increasing fertilization or irrigation, which were already applied in sufficient quantity, but was enhanced by increasing the insect pollination services (Sawe *et al.* 2020).

Ecological intensification can aim to promote yield sustainably through elevating the most limiting ecosystem services (i.e. ecological enhancement; c.f. Bommarco *et al.* (2013)). For instance, higher yields can be achieved by increasing the limiting ecosystem service through biodiversity-based practices (e.g., increasing pest regulation through the creation of beetle banks in the case of Figure 1.1b), without increasing artificial pesticides inputs (MacLeod *et al.* 2004). Alternatively, ecological intensification can also aim to minimize the environmental impacts while maintaining a certain yield level by using ecosystem services to (partially) replace anthropogenic inputs (i.e. ecological replacement; c.f. Bommarco *et al.* (2013)). For example, pesticides and artificial fertilizers can be (partially) replaced by enhancing natural enemies or inoculating soils with microorganisms that increase nutrient uptake of crop plants (Figure 1. 1b). In this way, the negative environmental consequences of artificial inputs could be reduced without compromising crop yield. Ecological enhancement and replacement are not mutually exclusive and can be applied in combination to increase yield with reduced artificial inputs, resulting in win-win situations for agricultural production and the environment (Bommarco *et al.* 2013; Tamburini *et al.* 2017; Kleijn *et al.* 2019).

The implementation of ecological intensification rests on diverse management practices that can be applied to enhance biodiversity and the associated delivery of ecosystem services. Examples of such management practices include the creation of wildflower strips, hedge management, application of organic amendments and inoculation of beneficial soil microorganisms (Duru *et al.* 2015; Kleijn *et al.* 2019; Tamburini *et al.* 2020). Currently, such management practices are rarely used by farmers, although both scientists and policymakers have increasingly advocated ecological intensification as an environmentally friendly way towards food security (Garibaldi *et al.* 2019; Kleijn *et al.* 2019; Kremen 2020). A potential barrier hindering the adoption of ecological intensification is the remaining knowledge gap between theory and practice, since the transition towards ecological intensification is a knowledge-intensive process (Caron *et al.* 2014; Kleijn *et al.* 2019).

The effects of multiple ecosystem services on production may interact

One set of key knowledge gaps is whether and how the effects of different ecosystem services on crop production interact (Kleijn *et al.* 2019). Ecosystem services in agricultural ecosystems are not delivered in isolation. Multiple services may influence each other's effects on crop production in complicated ways (Bennett *et al.* 2009; Garibaldi *et al.* 2018). The effects of multiple ecosystem services on crop production may interact both negatively and positively (Power 2010; Garibaldi *et al.* 2018; Tamburini *et al.* 2019). A positive interaction between the effects of ecosystem services occurs when the cumulative benefits of several ecosystem services on crop yield outweigh the sum of their individual benefits (Figure 1.1c). For example, in a recent study on oilseed rape (*Brassica napus* L.), the combined effects of pollination and florivorous pest control interacted positively, and the underlying mechanism was that pest control increased pollinator visitation rate by enhancing flower lifetime and consequently increased yield disproportionally (Sutter & Albrecht 2016). A negative interaction, on the other hand, indicates that the cumulative benefits of several ecosystem services are less than the sum of their individual benefits (Figure 1.1c). For instance, Strauss and Murch (2004) found that effects of pollination and simulated herbivorous pest control negatively interacted on the production of cantaloupe (*Cucumis melocrops*), and the mechanism was that, under sufficient insect pollination, plants compensated for the herbivore damage. The effects of ecosystem services can also be independent, in which case there is no interaction between them (Figure 1.1c). Furthermore, the combined effects of ecosystem services on production may be influenced by agricultural management practices, such as artificial fertilizer inputs (Tamburini *et al.* 2017) and irrigation (Klein *et al.* 2015). Depending on the conditions, the nature of such interactions may even differ in a single crop. The effects of pollination on oilseed rape, for example, can interact with nitrogen inputs positively (Garratt *et al.* 2018b), negatively (Marini *et al.* 2015) or not at all (van Gils *et al.* 2016).

Studies testing the benefits of a single ecosystem service on crop production are common (e.g., (Reeves 1997; Blaauw & Isaacs 2014; Baum *et al.* 2015; Stein *et al.* 2017)). Interactions between the effects of multiple ecosystem services are less studied (Garibaldi *et al.* 2018; Tamburini *et al.* 2019), and even less is known whether and how such interactions are influenced by agricultural management practices. A comprehensive understanding of the relationships of multiple ecosystem services and agricultural management practices on crop production may be essential for designing management strategies that result in ecological intensification (Bennett *et al.* 2009; Barber & Soper Gorden 2014). A key question is whether farmers can manage ecosystem services in such a way that it will predictably result in synergies (positive interactions) while avoiding the unwanted trade-offs (negative interactions) (Gaba *et al.* 2014; Sutter & Albrecht 2016).

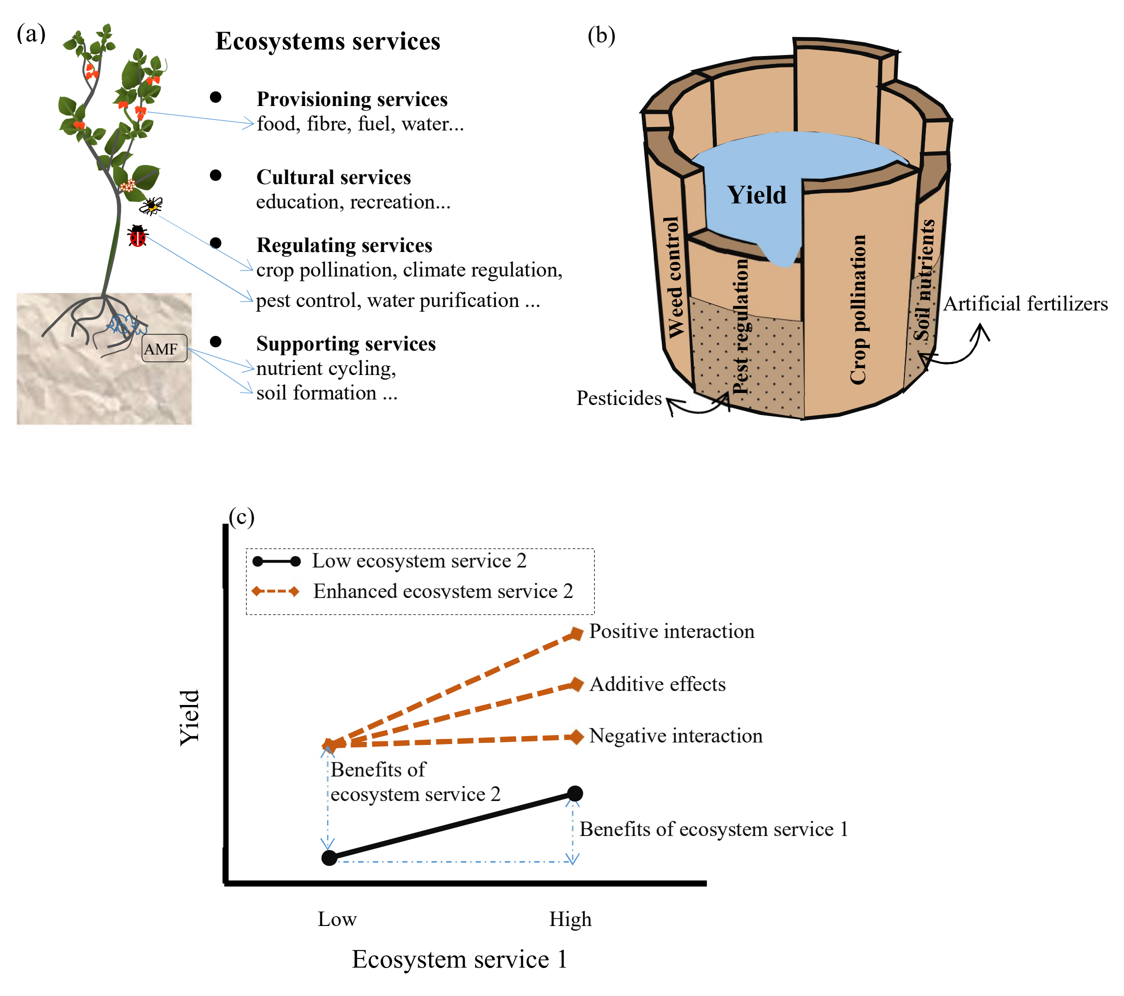


Figure 1.1 A graphical illustration of important concepts that are being addressed in this thesis. (a) The four categories of ecosystem services (adapted from Millennium Ecosystem Assessment (2005)), and several examples of the ecosystem service providers, e.g., arbuscular mycorrhizal fungi (AMF). (b) A conceptual illustration to illustrate how multiple ecosystem services contribute to yield. The dotted area shows that the services are replaced by anthropogenic inputs (e.g., artificial fertilizers and pesticides) (modified from Bommarco *et al.* (2013)). (c) Interactive effects of two ecosystem services on crop production (adapted from Garibaldi *et al.* (2018)).

Study system and outline of this thesis

In this thesis, I mainly focus on exploring the interactive effects of insect pollinators, soil organic matter (SOM) and arbuscular mycorrhizal fungi (AMF) on crop production under different fertilizer levels. All three factors can be important components of ecological intensification since they provide or regulate pivotal ecosystem services for agricultural production (Bowles *et al.* 2017; Garratt *et al.* 2018b; Gemmill-Herren *et al.* 2021). Their effects on crop production have been well studied in isolation (Box 1.1), but little is known about whether their effects interactively shape crop production and whether their interactions are influenced by fertilizer inputs. I selected raspberry (*Rubus idaeus* L.) as the study system. Raspberry is an important perennial fruit crop with a global gross production value of $1.5 billion (FAO 2018). Over the last few decades, the cultivation of commercial raspberry has widely expanded across many temperate and subtropical regions of the world (Rao & Snyder 2010; Burton-Freeman *et al.* 2016; Giuffrè *et al.* 2019). Its popularity is primarily driven by the growing consumer interest in its health benefits as the source of antioxidant phytochemicals and its appealing flavour (Rao & Snyder 2010; Burton-Freeman *et al.* 2016; Giuffrè *et al.* 2019). The commercial varieties of raspberry are self-compatible, while high-quality fruit production nevertheless benefits from visitation by insect pollinators (Daubeny & Kempler 2003).

Modern commercial varieties have been bred to reach their maximal yield with high dependence on artificial inputs, which might impede the benefits of ecosystem services (Tamburini *et al.* 2019). Therefore, I thought that testing the effects of AMF and SOM on wild relatives of crops would be informative to fully understand their importance for fruit formation. I performed an investigation to test the relationships between SOM content and AMF colonization rate and fruit production of wild raspberries (**chapter 2**). Specifically, I harvested berries from 15 sites of wild raspberry populations in the forests surrounding Wageningen. I collected soil samples for SOM content measurement and root samples to determine AMF colonization rate. Because wild raspberries are self-incompatible and their reproduction relies entirely on insect pollination services (Roach 1985), I could not perform pollinator exclusion treatments to test the benefits of pollination services.

In the next chapters of my thesis, I tried to further unravel how AMF inoculation, SOM content and insect pollinators affect the quantity and quality of commercial raspberry production under different artificial fertilizer inputs. Considering that simultaneously testing interactive effects of four factors would be logically challenging and results of such complex study designs can be difficult to interpret (particularly the three-way and four-way interactions), I performed three separate pot-field experiments and each involved different combinations of two of the three examined ecosystem services (AMF inoculation, SOM content and pollinators).

In **chapter 3**, I tested the combined effects of pollinators, SOM content and fertilizer inputs on raspberry production. I used a randomized complete block design with plants grown in low and high SOM content soils, with and without access to insect pollination, and receiving four levels of fertilizer applications. I tested how these factors interactively influenced raspberry quality (single berry weight and soluble solids content) and quantity (fruit number and yield). In addition, I conducted a pollinator survey on plants of open pollination treatments and investigated the effects of SOM content and fertilizer treatments on flower visitation rates.

In **chapter 4**, I tested the combined effects of pollinators, AMF inoculation and fertilizer inputs on raspberry production. A randomized complete block design was adopted with three crossed factors: AMF inoculation (AMF inoculated vs AMF non-inoculated), pollination treatment (open pollination vs pollinator excluded) and four levels of fertilizer inputs. I analysed how the treatments in conjunction affected the yield parameters (flower number, fruit set, fruit number, single berry weight and yield), and I explored the pathways explaining the relationships. I surveyed the pollinators visiting the flowers and analysed how AMF inoculation and fertilizer inputs influenced this process.

In **chapter 5**, I explored the interactive effects of AMF inoculation, SOM content and fertilizer inputs on raspberry production, following the protocol used in the previous two chapters for consistency. I analysed the interactive effects of the treatments on flower number, fruit set, fruit number, single berry weight and yield.

I integrated and discussed the findings of chapters 2-5 in **chapter 6** in the wider context. I synthesized the (interacting) relations of the multiple ecosystem services and fertilizer inputs on raspberry production and fruit quality. In addition, I proposed some possible mechanisms for explaining the results. I also discussed the individual effects of the three studied ecosystem properties and evaluated their potential for achieving ecological intensification. Finally, I suggested some directions for future studies in this field of research.

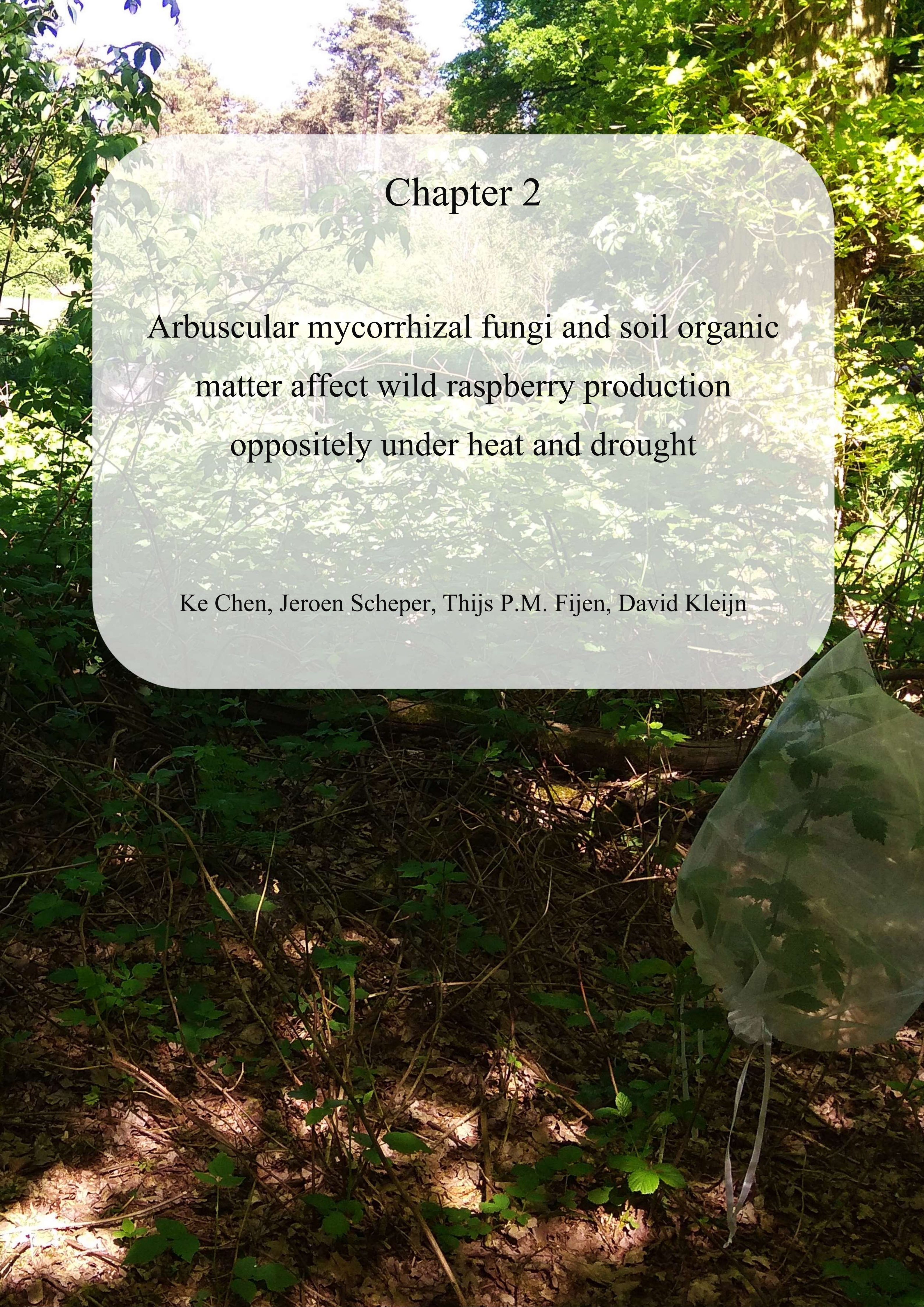
Box 1.1 Pollinators, soil organic matter and arbuscular mycorrhizal fungi

Pollinators (including bees and other insects, birds and mammals) provide pivotal pollination services for sustaining the yield of ca. 75 % of leading crops worldwide, accounting for 35% of the global food production (Klein *et al.* 2007). More importantly, these pollinator-dependent crops are a key source of various micronutrients; for example, they supply around 90% of vitamin C and 98% of lycopene to the human diet (Eilers *et al.* 2011). Experimental evidence suggests that increasing pollination services can promote production and contribute to food security (Blaauw & Isaacs 2014; Motzke *et al.* 2015; Garratt *et al.* 2018a; Fijen *et al.* 2020). Furthermore, adequate pollination services are often essential to achieve crop market quality, e.g. by ensuring a minimum required fruit size or shelf life (Garratt *et al.* 2014; Klatt *et al.* 2014; Fijen *et al.* 2018). Pollination effects on crop production in agro-ecosystems may interact with other natural processes and environmental conditions, such as nutrient availability and pest control (Garibaldi *et al.* 2018; Tamburini *et al.* 2019). If we want to be able to fully utilise the benefits of pollination services it is important to study the interactions between pollination and other factors (Tamburini *et al.* 2019).

Soil organic matter (SOM) is broadly defined as all the biologically derived organic material in the soil and it is a fundamental indicator for soil quality (Baldock & Skjemstad 2000). SOM relates to almost all soil properties, including soil structural stability and water-holding capacity (physical properties), cation exchange capacity and pH (chemical properties). Additionally, the mineralization of SOM provides nutrients to the plants and energy to the soil microbial communities (biological properties) (Krull *et al.* 2004; Liu *et al.* 2006). SOM regulates the flow of various ecosystem services, e.g., soil formation, nutrient cycling and water retention (Dominati *et al.* 2010; Bommarco *et al.* 2013), and therefore it is often used as a proxy for soil services (Bommarco *et al.* 2013; Williams & Hedlund 2014). SOM content generally relates positively to crop production and growth (Pan *et al.* 2009; Oldfield *et al.* 2019; Lal 2020). For example, Garratt *et al.* (2018b) found that greater SOM content was associated with a 10% yield increase for wheat in a large-scale field trial. However, the relationship between SOM content and yield can also be neutral or unimodal (Bauer & Black 1994; van Gils *et al.* 2016; Oldfield *et al.* 2020). One possible explanation for the varying relationships might be that the effects of SOM on production can interact with the concomitant factors, including management interventions, such as fertilizer and irrigation (Gagic *et al.* 2017; Oldfield *et al.* 2020), as well as ecosystem services, such as pest control (Garratt *et al.* 2018b). Current studies focusing on the interactions between SOM and the concomitant factors are relatively rare, and more studies are needed to explore the interactions.

Arbuscular mycorrhizal fungi (AMF) of the phylum *Glomeromycota* are widespread soil microorganisms in terrestrial ecosystems, consisting of ca. 350-1000 fungal species (Öpik *et al.* 2014; Davison *et al.* 2015). AMF can colonize roots of about 72% of all vascular terrestrial plants, including the majority of cultivated crops (Plenchette *et al.* 2005; Brundrett & Tedersoo 2018). AMF develop an extensive hyphae network through growing intercellular hyphae inside roots of the host and extraradical hyphae within the soil to reach further beyond the root zone (Jeffries *et al.* 2003; Plenchette *et al.* 2005; Rajtor & Piotrowska-Seget 2016). Therefore, AMF considerably help the host plants exploit poorly mobile ions, notably inorganic phosphate (up to 90% of P in the host plants can be provided by AMF) and nitrogen (van der Heijden *et al.* 2015). Besides providing assistance with resource uptake, AMF can provide other supporting ecosystem services, e.g., improving soil structure and soil aggregation (Rillig & Mummey 2006), as well as enhancing host plant tolerance to abiotic and biotic stresses, such as heat, salinity, drought, diseases and pathogens (Bethlenfalvay & Barea 1994; Gollotte *et al.* 2008b; Begum *et al.* 2019). In exchange, AMF may consume ca. 4% - 20% of photosynthetic carbon of the host plants for metabolic needs (Smith & Read 2010; Rajtor & Piotrowska-Seget 2016).

AMF inoculations have been reported to enhance the productivity of various crops, and AMF are being used as biofertilizers in some agriculture systems (Baum *et al.* 2015; Srivastava *et al.* 2015; Berruti *et al.* 2016; Zhang *et al.* 2019). However, studies are beginning to show that the effects of AMF could range from mutualistic to parasitic, depending on a variety of agricultural management practices, environmental variables and other ecosystem service providers, and thus further studies are needed to understand the effects of AMF in real-world systems (Bryla & Duniway 1997; Barber *et al.* 2013; Ziane *et al.* 2017; Wang *et al.* 2018).



Chapter 2

Arbuscular mycorrhizal fungi and soil organic matter affect wild raspberry production oppositely under heat and drought

Ke Chen, Jeroen Scheper, Thijs P.M. Fijen, David Kleijn

Abstract

Ecosystem services are of key importance for agricultural production. Replacing or enhancing agricultural inputs with these services will likely contribute to a more sustainable global food production, while minimizing harm to the environment. Arbuscular mycorrhizal fungi (AMF) and soil organic matter (SOM) can be important for crop growth and production as they may enhance crop nutrient uptake and increase drought tolerance. Natural populations of crop species are exposed to natural selection and can therefore be assumed to be growing at optimal levels of these environmental factors which can be useful as a benchmark for crop production. Using data from fifteen sites in the Netherlands, we examined the relationships between wild raspberry (*Rubus idaeus* L.) fruit production and AMF colonization and SOM content. Surprisingly, we found that single berry weight and branch yield of wild raspberry were negatively related to AMF colonization. Single berry weight increased with SOM content. Relative to the mean, a 25% increase in AMF colonization or SOM content resulted in an estimated 18.7% decrease and 33.6% increase in single berry weight respectively. The negative effect of AMF on yield was probably due to the unusual hot and dry weather during the study period. We did not find indications of interacting effects of AMF colonization and SOM content on fruit production, making the effects of managing these variables rather predictable. While further studies are needed to examine the role of AMF under more normal conditions, our study suggests that management of SOM content can make a significant functional contribution to raspberry crop production under low input conditions.

Keywords

Arbuscular mycorrhizal fungi, drought, ecosystem services, heat, soil organic matter, wild raspberry

Introduction

Agriculture has to become more productive to feed the growing world population (Godfray *et al.* 2010). Modern agriculture sustains high yields by heavily relying on external inputs of agrochemicals, irrigation and fossil fuels (Novotny 1999). However, the contribution of intense agricultural inputs to agricultural productivity has almost plateaued (Cassman *et al.* 2010). Modern agriculture, on the other hand, is criticised for its serious environmental costs, such as climate change due to greenhouse gas emissions (Solomon *et al.* 2009), degradation of soil quality (Lal 1993), biodiversity loss (Conrad *et al.* 2006; Tsiafouli *et al.* 2015; Sánchez-Bayo & Wyckhuys 2019), water pollution and eutrophication (Novotny 1999). Ecological intensification has been proposed as a potential solution for this challenge (Bommarco *et al.* 2013). It entails the promotion of biodiversity-based regulating and supporting ecosystem services, such as nutrient cycling, pollination and pest control, to replace or reduce anthropogenic inputs while sustaining or even increasing crop yield. The concept is supported by results of a range of studies that find that utilising natural ecosystem services can contribute significantly to agriculture yield and quality (Candido *et al.* 2015; Motzke *et al.* 2015; Fijen *et al.* 2018) although a number of key knowledge gaps still need to be addressed before farmers can be expected to embrace it (Kleijn *et al.* 2019).

A group of organisms that provide important supporting ecosystem services to crops are arbuscular mycorrhizal fungi (AMF). AMF in the phylum *Glomeromycota* are obligate symbionts, which can intracellularly colonize roots of about 72% of all vascular terrestrial plants (Brundrett & Tedersoo 2018). AMF generally benefit their host's growth and reproduction by enhancing the absorption of mineral nutrients and water (Smith & Read 2010), by increasing plant resistance against biotic stresses like fungal pathogens (Gollotte *et al.* 2008b; Smith & Read 2010) as well as by raising plant tolerance for abiotic stress factors like salinity and heavy metal toxicity (Bethlenfalvay

& Barea 1994). Additionally, AMF can help improve soil structure and soil aggregation, thus contributing to soil quality (Rillig & Mummey 2006).

Soil organic matter (SOM) is not an ecosystem service providing species group itself but moderates the activity of important organism groups such as fungi or bacteria that are more difficult to measure directly (Bommarco *et al.* 2013). It is a broad term to describe all dead biologically derived matter in soil and is often used as a proxy for soil fertility (Magdoff & Weil 2004). SOM is generally positively related to soil quality as it stabilizes soil aggregates (Williams & Hedlund 2014), improves water-holding capacity (Diaz-Zorita *et al.* 1999; Bradford 2014) and reduces soil erosion (Schmidt *et al.* 2011). Additionally, SOM provides gradual-release nutrients, especially organic nitrogen (Bradford 2014). Therefore, SOM content is often positively related to plant growth and seed or fruit production (Pan *et al.* 2009; Oldfield *et al.* 2019; Lal 2020). For example, in a large-scale winter wheat field trial covering 84 fields in five European countries, Garratt *et al.* (2018b) found that a 11% increase in SOM content was associated with a 10% yield increase.

Agricultural crops, especially modern varieties, have been bred under high-input conditions which may obscure the benefits of ecosystem services (Tamburini *et al.* 2019). It could therefore be informative to study the importance of AMF or SOM on wild plants of crops of which still natural populations occur. These populations are still entirely dependent on diverse ecosystem services for survival and reproduction. They are exposed to natural selection and can therefore be assumed to be growing at optimal levels of service provision which can be useful as a benchmark for crop production. Additionally, wild relatives of crop plants are important genetic resources, which could be used for breeding new varieties that utilize ecosystem services more effectively. The majority of the studies on the importance of AMF or SOM have been conducted on cultivated species under experimental conditions (Taylor & Harrier 2000; Shi *et al.* 2016; van Gils *et al.* 2016; Garratt *et al.* 2018b) and much less is known about how AMF or SOM affect the growth and reproduction of plants in the wild (but see

(Streitwolf-Engel *et al.* 1997; Sharma *et al.* 2009)). Furthermore, while interactive effects of AMF and SOM on cultivated plants have been demonstrated (Rillig 2004; Gryndler *et al.* 2009), it is largely unknown whether AMF is more important for wild plants growing in sites with low compared to high SOM content.

Here, we examine the relationship between AMF colonization and SOM content, and their potential interaction, on fruit production of natural raspberry (*Rubus idaeus*. L.). Natural populations of the species are widespread in temperate forests (Roach 1985) and plants are able to form symbiotic relationships with AMF (Taylor & Harrier 2000). In 2018, we selected fifteen natural raspberry populations, with at least ten raspberry plants in each site. In each population, we took soil and root samples to quantify SOM content and AMF colonization rate, and linked these to the weight and branch yield of raspberry fruits that were produced. We measured the base diameter of the studied plants and the canopy openness of each site to correct for effects of inherent differences between plants and sites. Because the summer of 2018 was an exceptionally hot and dry growing season, our study unintentionally tested the relationships of AMF and SOM on the fruit yield of wild raspberries under drought and heat stress.

Materials and methods

Study design

In spring 2018, we selected fifteen study sites of wild raspberry populations in an area of about 34.5 km^2 (N51° 58'29" - 52°00'06", E5°39'31" - 5°49'24"; Supplementary figure 2.1), in the centre of the Netherlands. Sites were located at least 400 meters from each other (range: 400-2450 m, mean±SD= 776±494.2 m). Ten sites were in the forest, four in the forest edge and one in the edge of a farm field but under trees (Supplementary figure 2.1). All sites were located on sandy soils. In each site, we selected three raspberry plants of similar size that were standing 2-4 meters from each other. Of each plant, we selected the largest branch as study unit and marked it with a plastic label. To avoid fruit predation, we enclosed the chosen branches with netting bags made of bridal gown

(50×65cm in size, mesh size in 0.1 mm, white and semi-transparent) just before fruit maturity and until the end of the harvest. We visited all sites twice a week for three weeks in total to collect the fruits when they turned to bright red and the polidrupe could be detached easily from the receptacle. Every fruit from the labelled branch was weighed to determine single berry weight, and the berry weight from the same branch was summed up to determine the branch yield.

To quantify SOM content, we took four 30 cm deep soil cores near each study plant with a semi-cylindrical chamber gouge auger (⌀=2 cm). The four samples were pooled and homogenized and SOM content was measured following the loss on ignition method (Salehi *et al.* 2011). Samples were dried at 105°C overnight and weighed, then ignited at 550 °C for three hours, and after which samples were cooled and weighed. SOM content (%) was calculated by the following equation (Salehi *et al.* 2011):

$$\mathrm{SOM}\ (\%) = \left[\frac{\text{dry weight } (105°\mathrm{C}) - \text{ weight after Ignition } (550°\mathrm{C})}{\text{dry weight } (105°\mathrm{C})}\right] \times 100$$

AMF colonization rate was measured by sampling fine roots from each studied plant. After the fruit harvest, in August 2018, roots smaller than 1 mm in diameter were collected in the rooting zone (5-30 cm depth). Root samples were cut to the length of 1-2 cm and cleared with 10% (w/v) KOH at 90 °C for 1 h in water bath. Roots were neutralized by 5% HCl for 1 minute and then stained with 0.05% trypan blue in lactophenol at 90 °C for 5 minutes (Phillips & Hayman 1970). AMF colonization rate was subsequently measured following the grid line intersect method (Giovannetti & Mosse 1980). We spread stained roots evenly on a 9 cm diameter petri dish with 0.5 × 0.5 inch squares grid lines, and checked at each line intersection whether the roots were infected or not, using a dissecting microscope (Novex Microscope K-Range). Root segments were re-spread several times until 150 intersections were scanned (Giovannetti & Mosse 1980; Sun & Tang 2012). AMF colonization rate was calculated as the percentage of the colonised intersections of the total observed intersections.

To be able to correct for potential raspberry plant size effects on yield components, we measured the diameter of each studied plant at the base of the main stem (about 1 cm above ground) using a Digital Vernier Caliper. To check if light availability plays a role in fruit production, we measured the canopy openness in each site. Firstly, we took hemispherical pictures with a Nikon E4500 camera equipped with a fish-eye lens at 180° in August of 2018, at the height of the studied branch at each site. The percentage of canopy openness was subsequently determined using the Gap Light Analyser (Version 2.0) software (Frazer *et al.* 1999).

Because 2018 was exceptionally hot and dry, we explored whether and how the weather conditions in the year of study would differ from the average weather conditions. We collected daily precipitation and daily maximum temperature data from January 1st 2008 to December 31st 2018 from the Royal Netherlands Meteorological Institute (KNMI 2018). We used data of the Deelen weather station, which is the nearest weather station to the selected sites at about 10-20 km.

Statistical analysis

Response and explanatory variables were averaged over the three plants within each site prior analyses to avoid pseudoreplication. In site 1 and 4 (Supplementary table 2.1) only one intact branch was available, and in site 3 and 5 (Supplementary table 2.1) only two branches were available as bags had been damaged or removed from the other branches.

We used linear regression analyses to examine the relationships between SOM and AMF on one hand and single berry weight and branch yield on the other. Although there was considerable variation in canopy openness (%) among sites (range: 13.8%-70.0%, mean = 27.3%±1.43%, mean±SD), preliminary analyses revealed that differences in light availability were not correlated with single berry weight (estimate= 0.180, $t_{1,12}$= 0.366, p= 0.721) or branch yield (estimate= 13.715, $t_{1,12}$= 1.068, p= 0.306). Similarly, we found no significant relationships between base diameter and single berry weight (estimate= -0.044, $t_{1,12}$= -1.600, p= 0.136) or branch yield (estimate= -0.582,

$t_{1,12}$= -0.809, p= 0.434). Canopy openness and base diameter were therefore excluded from further analyses. We performed separate linear regression analyses with single berry weight and branch yield as response variables, and AMF colonization rate (%), SOM content (%) and their interaction as explanatory variables. Models were simplified by backward elimination of non-significant predictors through comparing the full model and reduced model using the likelihood ratio test. Normality and homoscedasticity of model residuals were confirmed by visual inspection of diagnostic plots. The collinearity for the simplified models was checked by estimating the variance inflation factors (VIF), and we found VIFs for all variables were below 3, which indicated that no strong collinearity was in the models.

For the weather data, we calculated monthly weather parameters of precipitation (the sum of daily precipitation per month) and temperature (the average daily-maximum temperature per month). One-sample t-tests were then used to compare the monthly weather conditions of 2018 to those of the previous decade (2008-2017).

All analyses were carried out in R (R Core Team 2015). Plots for the regression visualization of best fitted models illustrating the relationships between explanatory variables and response variables were made using the ‘visreg’ package (Breheny & Burchett 2013) in R.

Results

Weather conditions

The examined wild raspberry plants were exposed to severe dry and hot weather conditions during their main growth and production period. Compared to the same period during the previous decade, wild raspberry plants in the sites experienced significant dryer conditions in June ($t_{1,9}$=4.39, P=0.002; Figure 2.1a) and July ($t_{1,9}$=8.11, p<0.001; Figure 2.1a), with only 17.7% and 4.8% precipitation of the mean precipitation of the past ten years respectively. The monthly temperature of May, June and July of

2018 was 4.5°C , 1.6°C , and 5.0°C higher than the average monthly temperatures of 2008-2017 ($t_{1,9}=-6.91$, $p<0.001$; $t_{1,9}=-3.96$, $p=0.003$; $t_{1,9}=-8.93$, $p<0.001$ respectively; Figure 2.1b). The optimum temperature range for most of the raspberry cultivars is 16 to 24 °C (Gotame *et al.* 2013), while the raspberry plants in our study experienced nine days with temperatures above 30°C from May to July 2018, with a maximum of 37°C on 26th July.

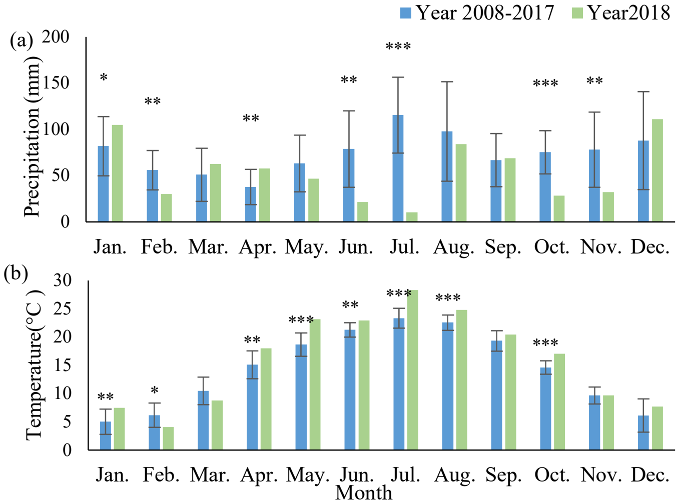


Figure 2.1 Historical precipitation and temperature recorded in Deelen weather station, the Netherlands. a) average monthly precipitation (mean±SD) from 2008 to 2017 and monthly precipitation of 2018, b) the average monthly temperature (mean±SD) from 2008 to 2017 and monthly temperature of 2018. Asterisks indicate levels of significance (*P<0.05, **P<0.01, ***P<0.001) for difference between weather conditions of 2018 and the mean of the previous 10 years using one-sample t-test.

Relationships between SOM and AMF and fruit production

Single berry weight varied more than four-fold from the harvested smallest to the largest fruits (0.19 vs 0.74 g per fruit, Supplementary table 2.1). Average branch yield ranged from 0 to 25.2 g per site, as in four sites (site 7-10) fruits were either dried out or no fruits were produced at all (Supplementary table 2.1). AMF colonization rate of the wild raspberry roots varied from 1.54% to 23.6% (Supplementary table 2.1), and SOM content of the selected sites ranged widely (3.18%-13.1%, Supplementary table 2.1).

Single berry weight significantly decreased with AMF colonization rate (Table 2.1), with 25% increase in AMF colonization rate comparing to the mean resulting in *ca.* 0.06 g (18.7%) decrease in raspberry single berry weight (Table 2.1; Figure 2.2). Single berry weight significantly increased with SOM content (Table 2.1). A 25% increase in SOM content comparing to the mean resulted in a 33.6% increase in single berry weight (Figure 2.2). The model with these two predictors explained 34.5% of the variance for single berry weight (df=12, P= 0.031, R^2= 0.34). The patterns for the branch yield were broadly similar (Table 2.1). Branch yield decreased with AMF colonization rate and showed a strong positive trend with SOM content (Table 2.1; Figure 2.2), which a 25% increase in AMF colonization or SOM content comparing to the mean resulted in an estimated 39.7% decrease and 45.8% increase respectively. In total 27.3% of the variance of the branch yield was explained by the model (df=12, P= 0.059, R^2= 0.27). Furthermore, we found no evidence that the relationships between yield components and AMF colonization rate were different at high and low SOM content (AMF*SOM interaction not significant).

Table 2.1 Results of the models. Significance is in bold with a significance level P<0.05 and followed by asterisks.

Response variables	Explanatory variables	d.f	Estimate	Std. Error	t value	Pr(>|t|)
Single berry weight	AMF	1,12	-2.945	1.260	-2.336	**0.038***
	SOM	1,12	6.255	2.144	2.917	**0.013***
	AMF: SOM	1,11	23.059	43.160	0.534	0.604
Branch yield	AMF	1,12	-86.232	33.597	-2.567	**0.025***
	SOM	1,12	117.196	57.158	2.050	0.063
	AMF: SOM	1,11	1150.678	1112.458	1.034	0.323

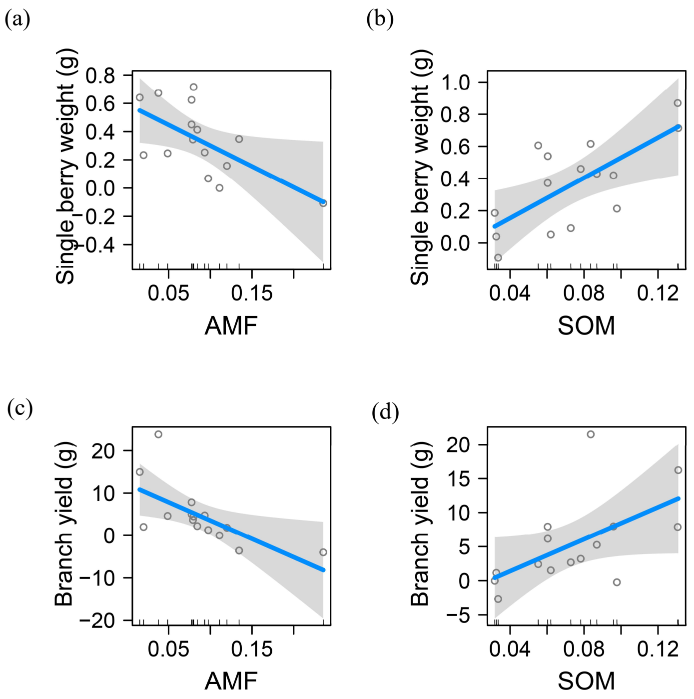


Figure 2.2 Partial residual plots of the regression visualization of the simplified models for representing relationships between explanatory variables (AMF, SOM) and response variables (single berry weight and branch yield), with the 95% confidence interval shaded in grey. (a) estimated relationship between AMF and single berry weight, (b) estimated relationship between SOM and single berry weight, (c) estimated relationship between AMF and branch yield and (d) estimated relationship between SOM and branch yield. Plotted points in the figure represent partial residuals.

Discussion

We found negative relationships between AMF colonization and single berry weight and branch yield of wild raspberry. Although AMF are mainly regarded as mutualistic symbionts (Rillig *et al.* 2001; Jeffries *et al.* 2003; Smith & Smith 2012; Shi *et al.* 2016), other studies have found similar negative relationships with plant performance (Klironomos 2003; Reynolds *et al.* 2005; Jacott *et al.* 2017). Our investigation also showed that SOM has positive relationships on the wild raspberry production, both the single berry weight and branch yield. We didn't find any interactions between the relationships of AMF and SOM on single berry weight or branch yield of wild raspberry.

Possible explanations for the negative relationships between fruit production and AMF colonization rate are the drought and high temperatures during the study period, which are atypical for the study area (Figure 2.1). The adverse environmental conditions may have increased the plant's costs of associating with AMF much more than it did the benefits. Bryla and Duniway (1997) found that under severe drought, AMF have negative relationships on leaf health of wheat (*Triticum aestivum* L.). Similarly, Martin and Stutz (2004) found AMF-inoculated pepper (*Capsicum annuum* L.) plants have lower P levels than non-AMF inoculated ones at high temperatures. The dry and hot weather might therefore have changed the relationship between AMF and wild raspberry plants from positive to negative. This could also explain why approximately a quarter of the raspberry plants failed to produce any fruits at all even though they all flowered and initiated fruit set. An alternative, or additional, explanation is that different species of colonizing AMF can have contrasting relationships with the host plant. The majority of AMF species have positive relationships with host plant fitness but negative cases have been reported. For example, Martin and Stutz (2004) investigated the effects of diverse mycorrhizal treatments, two AMF isolates and their mixture, on the shoot weight of the host pepper plants and found the effects of different isolates vary from negative to positive. We did not identify the AMF species that colonized the raspberry plants and cannot exclude the possibility that these were predominantly antagonistic species. Elucidating the exact mechanisms that led to the observed pattern requires further study, however, an important take-home-message is that under stressful conditions the costs of associating with AMF may outweigh the benefits for plants.

SOM management can play an important role in modern farming enterprises, especially in the face of climate change. In our study, higher SOM content related to significantly higher single berry weight and tended to be positively related to branch yield of wild raspberry plants. The positive relationship between SOM content and fruit production could be due to the benefits of multiple ecosystem services from SOM such as nutrient provisioning and soil structure improvement. However, given the dry conditions during the fruit maturation stage we think that especially the positive effects of SOM on water holding capacity of the soils have played a positive role (Diaz-Zorita *et al.* 1999; Bradford 2014). This suggests that raspberry growers that manage for high SOM soils become less vulnerable to periods of drought and less dependent on access to irrigation water.

What ultimately will matter to farmers are the net effects of these variables across the entire growing season or even across multiple growing seasons (Kleijn *et al.* 2019). If in the long term, the benefits outweigh the costs, it will be a worthwhile variable for farmers to manage AMF. So far, longer-term studies examining the effects of AMF and SOM are missing. AMF colonization rates of our wild raspberry plants (1.54%-23.6%) were relatively low compared to rates observed by Taylor and Harrier (2000) who inoculated their raspberry (with AMF colonization rates from 20%-65%). Which rates more closely resemble the situation in commercial raspberry plantations is unclear but it is clear from our study that even relatively low colonization rates can have significant effects. The application of AMF as a management practice is further complicated by the widespread practice of fungicide use in conventional raspberry production (Gollotte *et al.* 2008a), even though AMF colonization can potentially reduce the susceptibility of crops to fungal diseases (Gollotte *et al.* 2008b; Smith & Read 2010). Managing SOM content offers better prospects. Our results suggest that, compared to natural populations, conventional raspberry production occurs at relatively low SOM contents. Open field raspberry production is recommended to be done in soils with SOM content ranging from 3%-4% (Zdorovtsov *et al.* 2011). Our wild raspberry populations were found growing in soils that ranged in SOM content from 3.18%-13.1%. Within this range we observed a linearly increasing relationship with fruit production. This suggests that maintaining, or even better, enhancing SOM content should be an important aspect of the agronomic management of raspberries. This is particularly important in countries with a high proportion of open field cultivation and with relatively low-input management practices such as China (Dai *et al.* 2008) where raspberry production is rapidly increasing (Yang & Gao 2005).

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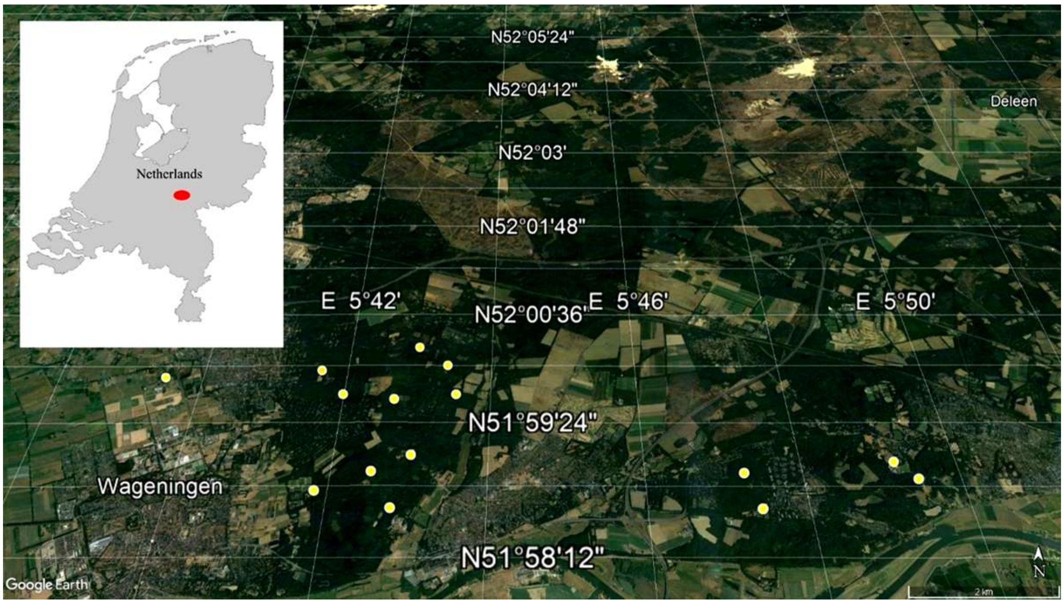
Supplementary materials



Supplementary figure 2.1 Map of the study region. The wild raspberry sites (yellow dots) involved in the experiment were located near Wageningen (red oval area), the Netherlands. The map was created with Google Earth Pro 7.3.

Supplementary table 2.1 Results of the investigation (mean±SD)

Site	AMF colonization rate (%)	SOM content (%)	Base diameter (mm)	Canopy openness (%)	Fresh berry weight (g)	Branch yield (g)
1[a]	7.76±1.23	3.18±0.02	8.82±1.13	41.62±0.53	0.19	0.19
2	8.00±3.33	5.53±0.15	5.95±0.80	13.83±1.14	0.61±0.07	2.42±1.56
3[b]	23.56±7.48	13.10±3.63	7.05±1.45	23.12±0.37	0.26±0.03	2.82±2.06
4[c]	3.78	8.36	7.68	22.59	0.74±0.06	25.16
5[b]	13.46±4.73	13.07±2.29	5.98±0.47	18.07±0.78	0.71±0.11	3.19±2.82
6	12.00±3.33	9.60±3.02	8.57±0.11	40.59±0.26	0.30±0.03	4.53±0.71
7	9.33±6.00	3.26±0.20	9.37±0.70	27.13±4.27	0	0
8	4.89±2.12	3.36±0.71	14.52±4.33	20.67±0.23	0	0
9	11.11±3.49	7.29±0.19	8.57±0.97	20.89±1.51	0	0
10	9.76±5.25	6.21±0.64	6.57±0.60	14.71±1.33	0	0
11	7.76±0.57	6.04±1.45	6.23±0.95	27.47±4.42	0.55±0.06	6.37±2.66
12	7.92±1.29	8.70±1.14	8.51±0.14	23.96±3.50	0.43±0.05	5.33±1.66
13	8.44±3.32	7.83±1.20	13.20±1.44	21.14±0.15	0.45±0.05	2.83±0.70
14	2.00±2.00	9.78±2.32	7.18±0.29	23.98±1.02	0.39±0.06	4.93±1.45
15	1.54±0.97	6.04±0.30	7.24±1.05	68.98±0.46	0.56±0.03	13.50±2.68

[a] Site only has one branch left intact and produce one berry

[b] Sites only have two intact branches left

[c] Site only has one intact branch left, labels missed

Chapter 3

Insect pollination and soil organic matter improve raspberry production independently of the effects of fertilizers

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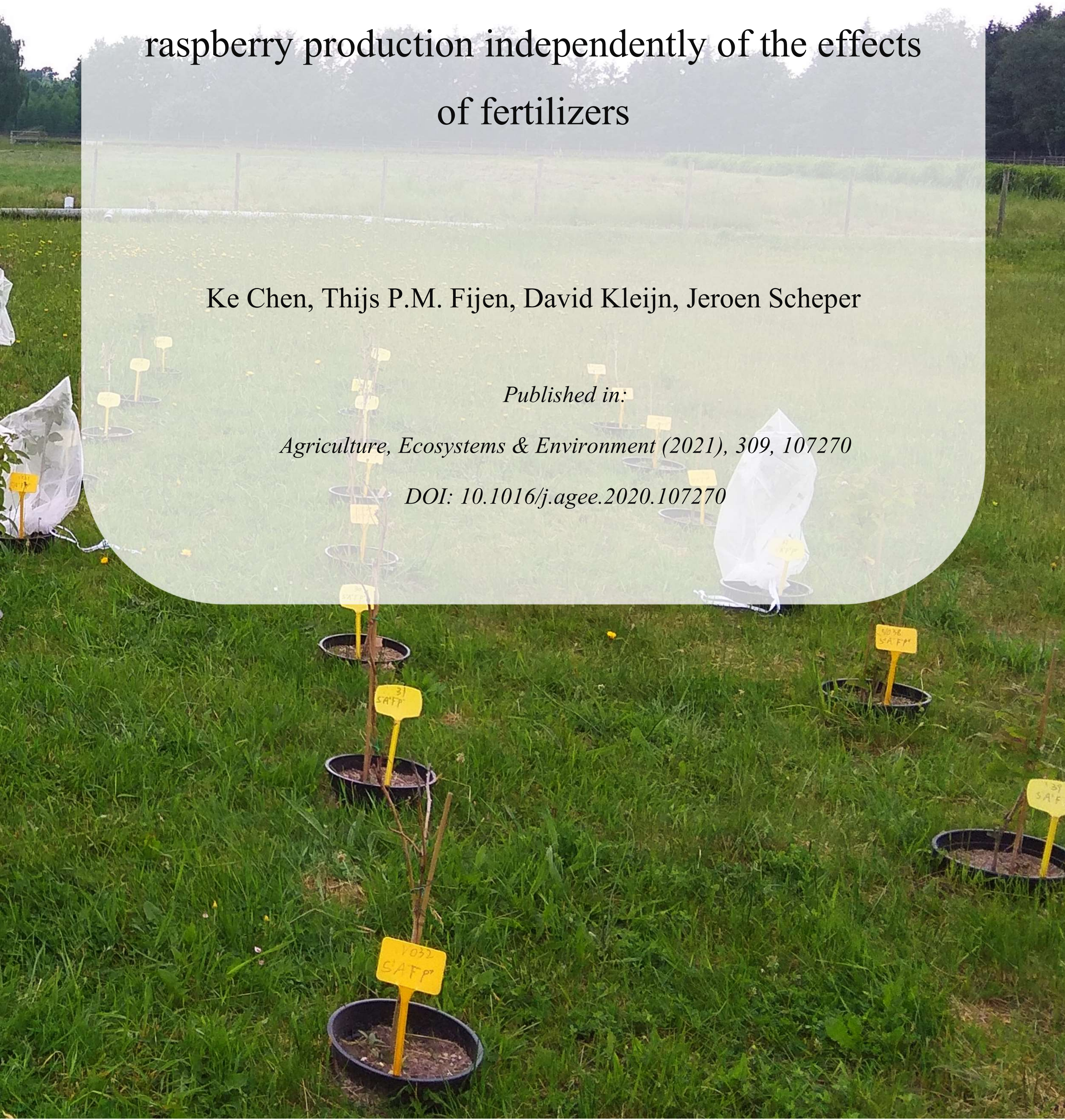

Abstract

Intensive agriculture faces the challenge of contributing to feeding the increasing global population while minimizing its adverse effects on the environment. Ecological intensification can help achieve this as it proposes to supplement artificial inputs with ecosystem services such as pollination, nutrient cycling and water retention. The mixed results of previous studies with respect to the potential of using ecosystem services for ecological intensification suggests more data is needed from a wider range of contexts to explore the potential of this approach in practice. We conducted an experiment which studied the effects of all combinations of insect pollination (open pollination vs pollinators excluded), soil organic matter (SOM) content (1.66% vs 3.73%) and four levels of fertilizer applications, on the quantity and quality of raspberry (*Rubus idaeus* L.) production. We were particularly interested in interacting effects on crop yield between the ecosystem services and fertilizer application. Insect pollination significantly increased single berry weight (11%) and raspberry yield (33%). SOM content enhanced visitation rate of pollinators and increased the single berry weight by 20.5%, but SOM did not contribute significantly to fruit number or yield. SOM contributed to the soluble solids content of the fruits; however, this effect interacted with pollination and fertilizer inputs in a non-linear way. Fertilizer application positively contributed to single berry weight, fruit number and thus overall yield but did not influence in any way the effects of pollination and SOM on raspberry production. Our results provide evidence that ecosystem services contribute to fruit production and can potentially be used to (partly) replace artificial fertilizer inputs while maintaining productivity but our results also suggest that yield maximization requires enhancing both ecosystem services and fertilizer application.

Keywords

Ecological intensification, ecosystem services, fertilizer, pollination, raspberry, soil organic matter (SOM)

Introduction

Intensive agriculture sustains high yields by heavily relying on inputs of agrochemicals, resources and energy (Matson *et al.* 1997). Although the intensification of agriculture has increased global food production (Cassman *et al.* 2010), it has come at high environmental costs, such as pollution of ground and surface waters (Novotny 1999), greenhouse gas emission (Robertson *et al.* 2000) and biodiversity loss (Karp *et al.* 2012; Tsiafouli *et al.* 2015), giving rise to concern about the sustainability of this paradigm. With the global human population continuously growing, increasing food production without incurring adverse effects on the environment is one of the main challenges that agriculture is currently facing. Ecological intensification has been proposed to address these challenges. It relies on managing biodiversity to enhance ecosystem service delivery which can then complement artificial inputs to increase yield (i.e. ecological enhancement), or can partially replace artificial inputs while sustaining productivity (i.e. ecological replacement; c.f. Bommarco *et al.* (2013)). Essential ecosystem services supporting agricultural production are pollination and soil services, such as nutrient cycling, water retention and infiltration (Power 2010). So far, empirical studies are mixed with respect to the potential of using ecosystem services for ecological replacement or enhancement (Tamburini *et al.* 2019). Studies carried out in a wider range of contexts (e.g. crop, soil type, climate) are needed to explore the potential of this approach in practice and under real-world conditions (Kleijn *et al.* 2019).

Animal-mediated pollination enhances the yield of ca. 75% of global leading crops and accounts for 35% of the global food production (Klein *et al.* 2007). Animal pollination furthermore benefits human wellbeing by improving crop quality (Klatt *et al.* 2014). Pollination effects on crops are often moderated by environmental conditions that may influence the resource allocation strategy and/or fruit development process (Bos *et al.* 2007), such as nutrient availability (Tamburini *et al.* 2017), water (Klein *et al.* 2015) and pest control (Melathopoulos *et al.* 2014). However, we are still a long way from predicting how environmental conditions influence the contribution made to crop production by pollinators. In a review, Tamburini *et al.* (2019) found that pollination benefits to crops can increase, decrease or be unrelated to nutrient availability or can even show a unimodal relationship with maximum benefits at intermediate nutrient availability levels. Even for the influence on pollination of the same factor in the same crop, different studies may find contrasting results (Tamburini *et al.* 2019). The benefits of pollination on oilseed rape, for example, can be affected by nitrogen inputs positively (Garratt *et al.* 2018b), negatively (Marini *et al.* 2015) or not at all (van Gils *et al.* 2016).

Soil organic matter (SOM) links closely to several important provisioning and regulating soil ecosystem services (Dominati *et al.* 2010; Bommarco *et al.* 2013). Therefore, SOM is often used as a proxy for soil services (Magdoff & Weil 2004; Garratt *et al.* 2018b), and hereafter we refer SOM as an ecosystem service. SOM contributes to plant growth and productivity, through its effects on soil properties (Williams & Hedlund 2014), such as soil structural stability (Krull *et al.* 2004) and water-holding capacity (Díaz-Zorita *et al.* 1999); through providing various macro- and micronutrients by mineralization (Fageria 2012); or through promoting diverse soil microbial communities by providing them nutrients and energy (Degens *et al.* 2000; Drenovsky *et al.* 2004). Nevertheless, the effects of increasing SOM content on crop production are variable, that some studies showing positive effects (Garratt *et al.* 2018b) but others showing no effects (Hijbeek *et al.* 2017). This is possibly due to the effects of SOM on crop production being influenced by environmental variables and management interventions (Oldfield *et al.* 2020). Fertilizer inputs, in particular, can interact with the effects of SOM, as Gagic *et al.* (2017) found that the relationship between yield and SOM content was much more pronounced in unfertilized than in fertilized crops. Nevertheless, other studies found independent effects of SOM and fertilizers (Oldfield *et al.* 2020). Whether managing SOM can partially replace fertilizer application to contribute to the sustainability of farming systems is therefore still an open question.

Different ecosystem services can also influence one another. For example, Lundin *et al.* (2013) found that the benefits of enhancing red clover pollination and pest control simultaneously outweighed the sum of yield gains obtained when increasing each service separately. Bartomeus *et al.* (2015) found just the opposite; that pollination effects on oilseed rape yield increased with increasing pest pressure. As far as we know, whether the interactions between two different ecosystem services are influenced by fertilizer availability has never been tested before. To assess the potential of replacing external agricultural inputs with the management of ecosystem services, it is essential to know not only whether the effects on crop yield of different ecosystem services, such as pollination and SOM content interact, but also how this interaction is affected by fertilizer application. Figure 3.1 illustrates such a three-way interaction along the lines of findings by Tamburini *et al.* (2017, 2019). At low fertilizer levels, effects of SOM and pollination on crop yield can be positive and interacting with pollination benefits being stronger at high SOM content because the nutrients provided by SOM can partly alleviate any macronutrient limitation that is restricting seed or fruit set (Figure 3.1a). At intermediate fertilizer levels, pollination and SOM content can both be positively related to crop yield but no longer influence one another (Figure 3.1b). At high fertilizer levels, pollination still contributes to crop production, but the abundance of nutrients provided by artificial fertilizers have made the nutrient contribution of SOM to crop growth redundant (Gagic *et al.* 2017) and the benefits from pollination and SOM do not interact (Figure 3.1c). The hypothetical patterns in Figure 3.1 suggest that the influence of two ecosystem services on one another may depend on the absolute level of fertilizer input. Testing how fertilizer application influences the interaction between the effects of two ecosystem services therefore requires experiments involving a wide range of fertilizer application levels and examining responses at three or more levels of fertilizer application because of potentially non-linear relationships between nutrient availability and ecosystem service benefits (Tamburini *et al.* 2017).

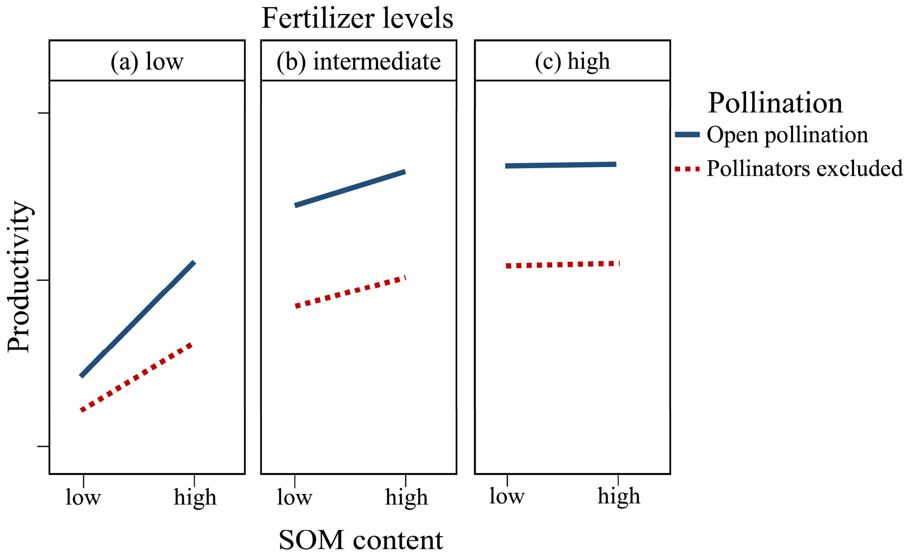

Figure 3.1 Conceptual diagram illustrating potential interactive effects of soil organic matter (SOM) and pollination on crop productivity under low, intermediate and high fertilizer input levels.

Here, we experimentally tested the combined effects of pollination, SOM and fertilizer inputs on fruit production of raspberry (*Rubus idaeus* L.), which is an increasingly important fruit crop that has not been studied yet within the context of ecological intensification. Although most raspberry cultivars are considered self-compatible (Keep 1968), they nevertheless benefit from insect pollination for high-quality fruit production (Colbert & De Oliveira 1990; Sáez *et al.* 2018). In this study, we used potted raspberry plants and exposed them to different levels of pollination (open pollination vs pollinator excluded) and SOM content (1.66% vs 3.73%) in combination with four levels of fertilizer application rates to examine potential non-linear fruit production response patterns. We measured fruit quantity as well as quality, as these both determine raspberry production value (Parker *et al.* 1991; Mauromicale *et al.* 2011). We specifically asked whether (i) SOM content and fertilizer application rates interactively affect pollinator visitation on raspberry; (ii) whether and how pollination, SOM content and fertilizer applications interactively affect fruit quantity, and (iii) whether and how these factors interactively affect fruit quality.

Materials and methods

Experimental material and site

Raspberry is an economically important perennial fruit crop with a global gross production value of $1.9 billion in 2016 (FAO 2016). Raspberry production has strongly increased over the last decades, partly due to growing consumer interest in its health benefits (Burton-Freeman *et al.* 2016; Giuffrè *et al.* 2019). Commercial raspberry seedlings of cultivar *'Tulameen'* were used in this study. *'Tulameen'* is a self-compatible cultivar (Daubeny & Anderson 1991), and it is one of the most popular raspberry cultivars worldwide in diverse climatic conditions (Aprea *et al.* 2009). We purchased raspberry seedlings from a local fruit tree supplier, with an average height of ca. 60 cm.

The experiment was carried out from September 2018 to August 2019. The site is at an experimental farm (51° 59'47 "N, 5° 39'36 "E) of Wageningen University and Research, Wageningen, the Netherlands. It is in the temperate climate, with a mean annual precipitation of 868.71±116.65 (mean±SD) mm and monthly mean temperature ranging from 3.02±1.98 °C (January) to 18.21±1.75 °C (July) (average data from the year 1999 to 2018 (KNMI 2018)). An apiary was located within 500 m of the experimental site, and an abundant and diverse wild pollinator community was observed during a pilot experiment at the experimental site in spring and summer 2018.

Experimental setup

We designed a field trial using potted plants to measure combined effects of animal pollination, SOM and fertilizer inputs on raspberry production. We adopted a complete randomized block design, applying the following three crossed factors: (i) pollinators excluded vs (open) insect pollination, (ii) low SOM content vs high SOM content, and (iii) four levels of fertilizer application. The 16 treatment combinations were repeated seven times (i.e. 7 blocks, 112 experimental plants in total). Plants were randomly placed in a block arrangement in the field with one meter between pots within and between rows. Pots were buried into the ground, with the top ca. 3-5 cm above the ground and all plants received equal and ample irrigation.

Before transplanting, we carefully washed away any soil adhering to the roots to ensure that plants were exposed to the experimental SOM content treatments. Every washed seedling was transplanted into a 10-litter plastic pot (diameter 28 cm), filled with the same amount of high SOM (SOM content: 3.73%, available N: 112.35 mg/kg, available P: 1.09 mg/kg, available K: 40.73 mg/kg) or low SOM (SOM content: 1.66%, available N: 39.27 mg/kg, available P: 0.60 mg/kg, available K: 14.85 mg/kg) soils. The two SOM treatment soils were obtained by mixing two sandy soils that differed in SOM content but were similar in soil texture. One was normal sandy agricultural soil, with SOM content of 4.58%; another was river sand, of which SOM content was 0.63%. The two soils were mixed thoroughly in ratios of 85%:15% and 35%:65% respectively, to obtain the desired experimental soil types. The resulting final SOM content of the two treatments was determined by means of the loss on ignition method (Salehi *et al.* 2011).

The pollinator exclusion treatment was conducted by covering the whole plant with a mesh bag just before flowering. The white semi-transparent bags were 50×65cm in size, with the mesh size of 0.1 mm. The mesh bags excluded all insect visitors while allowing pollination by wind. Plants of open pollination treatments were kept open in the field with free access to pollinators. After blooming, all plants were covered with the same mesh bags to avoid fruit loss from predation until the end of the harvest (Blaauw & Isaacs 2014).

Four contrasting levels of fertilizer were applied to plants. The levels varied from 0, 33, 66 and 99 $kg{\cdot}ha^{-1}$ of N per year. A locally commonly used solid fertilizer (CropSolutions Co., Perth, UK) was used in the experiment. It contained the following nutrient concentrations: 10.80% N, 13.44% K, 5.89% P, and 7.20% S. Fertilizer treatments were split into three applications: first one at about two weeks after the transplant, the second one at bud break and the last one seven weeks after the second application. We chose the dosages of fertilizer application to represent low, medium and optimum fertilizer levels, based on a study reviewing fertilizer application rates on raspberries (Strik 2005).

Pollinator observations

From May 20th to June 17th (the blooming period), we used the focal point observation method to determine pollinator visitation rate (Fijen & Kleijn 2017). We conducted 10-minute pollinator censuses for each plant of the open pollination treatment, randomly repeated ten times at different times of the day (morning, noon and afternoon). Only flower visitors were recorded that contacted anthers or stigmas of flowers from the observed plant. Bees were identified to species level in the field, and other pollinators were identified to order. Each pollinator visiting the observed plant was counted once regardless of how many flowers it visited. Observations were only performed during sunny or slightly cloudy days with low wind velocity (Sáez *et al.* 2012) and with temperatures above 12 ° C.

Yield measurements

We collected ripe berries every two days for eight weeks, when fruits turned to bright red and the polidrupe can be detached easily from the receptacle (Sáez *et al.* 2014). We measured parameters about quantity and quality immediately in the lab. Single berry weight of every berry was weighed, and the total number of produced fruits was determined for each plant. Total yield was calculated by summing the single berry weights from the same plant. For the first ten berries from each plant, we measured their soluble solids content (SSC) using an Atago Hand Refractometer (Atago Co., Tokyo, Japan). SSC indicates the proportion (%) of dissolved solids, which consists mainly of sugars (65%) and is often used as a proxy for sugar content and quality of fresh fruits (Martínez-Romero *et al.* 2006; Beckles 2012). For ease of communication, in the text, we henceforth use sugar content when discussing the results of the soluble solids content.

Data analysis

Until harvest, 82 out of 112 raspberry plants survived. The death of 30 plants was most probably attributed to root damage caused by the process of removing adhering soil before transplant. The number of surviving plants differed per treatment combination, ranging from 2-7 (Supplementary table 3.1), making our design unbalanced. We therefore adopted linear mixed-effects models to analyse our data. Models were built in R (R Core Team 2015) using the function lme() in the nlme package, using the maximum likelihood estimation method (Pinheiro *et al.* 2019).

Data were averaged per plant prior to analyses to avoid pseudoreplication. We fitted separate models with single berry weight, fruit number, total yield and soluble solids content as response variables and block as a random factor. Pollination, SOM content, fertilizer application rate and their interactions were fixed factors and we also included a quadratic term for fertilizer application rate to test for non-linear effects. For all models, the significance of fixed effects was determined by backward model simplification using likelihood ratio tests. Normality and homoscedasticity of model residuals were checked by visual inspection of diagnostic plots. To test the effects of treatments on flower-visitor abundance, we built a model using SOM content, fertilizer application rate, a quadratic term for fertilizer application rate and their interactions as fixed factors, with block as a random factor. Flower visitor abundance was square-root transformed to improve the normality and homoscedasticity of residuals. We checked for collinearity in all minimum adequate models by estimating variance inflation factors (VIF), and no strong collinearity existed in any of the models (VIFs<3).

Results

We observed 774 pollinators, of six different taxa, visiting the flowers of the experimental raspberry plants. *Bombus pratorum* was by far the most dominant species, making up 56% of total visits. Other visits were made by *Bombus lapidarius* (29%), *B.*

pascuorum (7%), *B. terrestris* congl. (5%), *Apis mellifera* (2 %) and *B. sylvestris* (0.1 %).

Table 3.1 Effects of soil organic matter content (SOM; high vs low), pollination (open-pollinated vs pollinators excluded) and fertilizer application rate (0, 33, 66, 99 kg N·ha^{-1}·year^{-1}) on pollinator visitation rate (open-pollinated plants only, n=43) and raspberry fruit production variables (n=82). All analyses were performed using linear mixed-effects models. $\chi 2$ and P values were calculated through likelihood ratio tests by comparing the full model with the reduced model. Bold values represent significant effects (P<0.05).

	Visitation rate (sqrt)		Fruit number		Single weight		Yield		Soluble solids	
	$\chi^2_{(1)}$	P	$\chi^2_{(1)}$	P	$\chi^2_{(1)}$	P	$\chi^2_{(1)}$	P	$\chi^2_{(1)}$	P
SOM	4.54	**0.033**	0.14	0.709	10.163	**0.001**	2.81	0.094	4.64	**0.031**
Pollination			3.46	0.063	4.725	**0.030**	5.20	**0.023**	0.19	0.660
Fertilizer	3.45	0.063	13.90	**<0.001**	9.336	**0.002**	17.57	**<0.001**	2.90	0.089
Fertilizer^2	0.26	0.613	3.77	0.052	0.011	0.915	2.47	0.116	0.23	0.635
SOM:fertilizer	0.20	0.656	1.35	0.246	0.410	0.522	0.63	0.426	1.88	0.171
SOM:fertilizer^2	0.46	0.496	0.04	0.851	0.565	0.452	0.04	0.841	1.81	0.179
SOM:pollination			<0.01	0.992	1.110	0.292	0.42	0.519	0.00	0.952
Pollination:fertilizer			0.36	0.548	0.021	0.886	0.42	0.516	0.11	0.746
Pollination:fertilizer^2			3.04	0.081	2.383	0.123	3.82	0.051	0.32	0.573
SOM:fertilizer:pollination			1.73	0.189	1.166	0.280	2.64	0.104	0.01	0.917
SOM:fertilizer^2:pollination			0.13	0.723	0.033	0.855	0.24	0.623	6.99	**0.008**

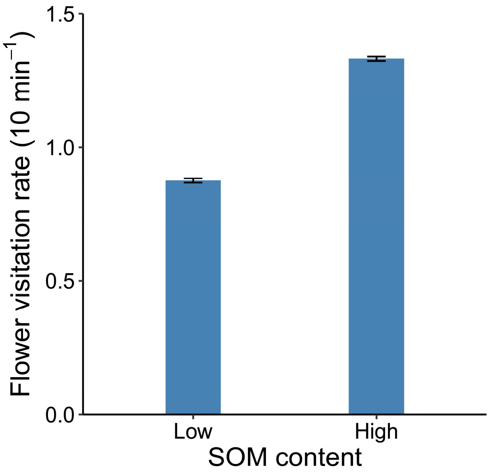

Figure 3.2 Effects of soil organic matter (SOM) content on flower visitation rate (number of visits per 10 min) of raspberry. Results represent the predicted back-transformed values from the minimum adequate model. Error bars show ± 1 S.E.

Raspberry flower visitation by pollinators was only significantly influenced by SOM content, with plants in high SOM content soils attracting about 40% more visitors than low SOM content soils (Table 3.1; Figure 3.2). Fruit number increased significantly and linearly with increasing fertilizer inputs and not with any other factors, although there was some support for a curvilinear relationship with fertilizer application and a trend for higher fruit number of open-pollinated plants (Table 3.1). Single berry weight was significantly affected by all three manipulated factors: SOM, pollination and fertilizer inputs (Table 3.1; Figure 3.3). Open-pollinated plants had 11.4% higher single berry weight than netted plants. Raspberry plants in high SOM content soil produced 20.5% higher single berry weight fruits than plants in low SOM content soil. Increasing fertilizer inputs from 0 to 99 kg N·ha^{-1}·year^{-1} increased single berry weight from 1.7 g to 2.2 g. Raspberry yield, essentially the product of fruit number and size, was only significantly affected by pollination and fertilizer inputs (Table 3.1; Figure 3.4). Open-pollinated plants had a 33% higher total plant yield than plants from which pollinators had been excluded. Plant yield linearly increased from 24.6 g in plants grown in non-fertilized soils to 55.7 g in plants grown in soils receiving fertilizer of 99 kg N·ha^{-1}·year^{-1}. Additionally, we found a near-significant interaction between insect pollination and fertilizer application (Table 3.1; Supplementary figure 3.1). Interestingly, while there was no evidence for interactions between the effects of experimental variables on pollinators or plant biomass variables, we found a significant three-way interaction on sugar content as indicated by soluble solids content (SSC) (Table 3.1; Figure 3.5). The sugar content of berries from which pollinators had been excluded, showed a concave relationship with fertilizer application rate on low SOM content soils but a convex relationship on high SOM content soils (Figure 3.5a). As a result, the sugar content was higher in high than in low SOM content soils at intermediate fertilizer application rates, but not at low or high fertilizer application rates. The sugar content of the open-pollinated berries did not differ systematically between plants growing in high or low SOM content soils and fertilizer levels, as indicated by overlapping confidence intervals. The mean sugar content of berries from plants growing in high SOM content soils was almost invariably higher than the sugar content of berries from plants growing in low SOM content soils which explains the significant main effect of SOM content (Table 3.1).

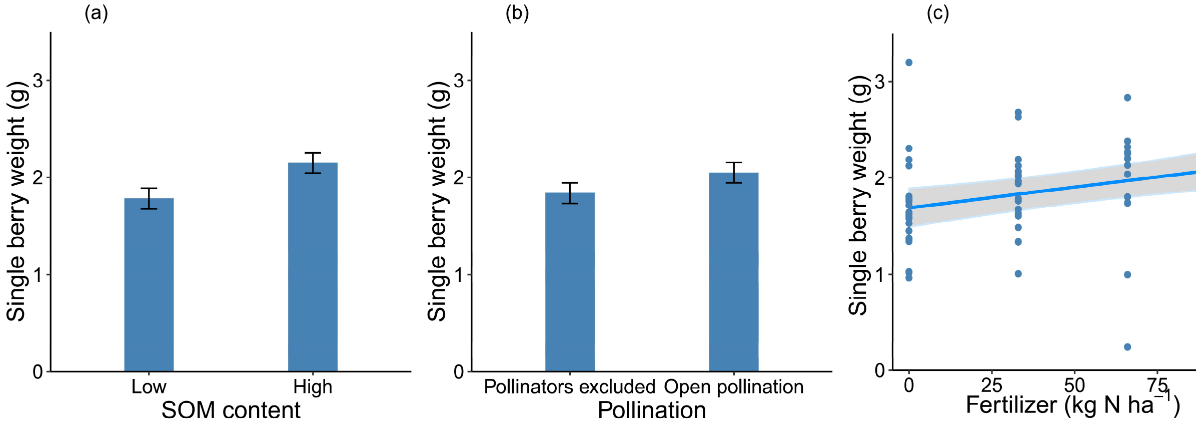

Figure 3.3 Effects of a) soil organic matter (SOM) content, b) pollination and c) fertilizer application rate on per plant single berry weight. Graphs show the predicted effects based on the minimum adequate model; error bars show ± 1 S.E; the grey shading shows the 95% confidence interval. Plotted points in (c) represent partial residuals.

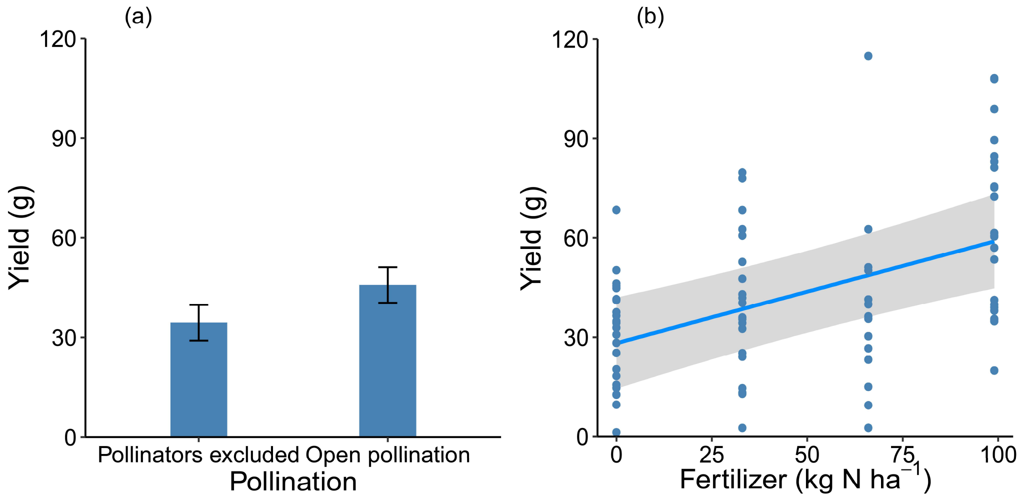

Figure 3.4 Effects of a) pollination and b) fertilizer application rate on per plant yield. Graphs show the predicted effects based on the minimum adequate model; error bars show ± 1 S.E; the grey shading shows the 95% confidence interval. Plotted points in (b) represent partial residuals.

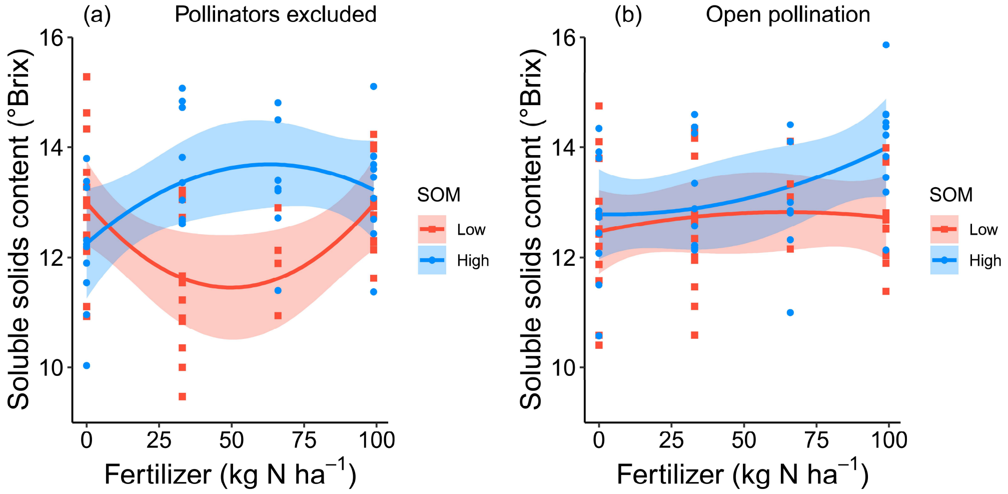

Figure 3.5 Interactive effects of soil organic matter (SOM) content and fertilizer application rate on per plant soluble solids content under a) pollinators excluded and b) open pollination. Graphs show the predicted effects based on the minimum adequate model; the blue/red shading shows the 95% confidence interval. Plotted points represent partial residuals.

Discussion

We found positive effects of insect pollination and artificial fertilizer inputs on raspberry single berry weight and yield. SOM content enhanced the visitation rate of pollinators and seemed to be particularly important for qualitative aspects of raspberry production: single berry weight and sugar content (as indicated by SSC), although effects on this last variable interacted with pollination and fertilizer inputs. We found no evidence for any other interacting effects of our two investigated proxies for ecosystem service delivery, pollinator visitation rate and SOM content, with our proxy for agricultural management intensity, fertilizer application rate. This suggests that these ecosystem services affect crop yield independently from farm management.

Insect visitation rate was positively affected by SOM content but not by fertilizer application. We are not aware of any other study that shows that SOM content can influence a plant's attractiveness to flower visitors, but a recent study on field bean did show that organic manure application increased bumblebee flower visitation compared to inorganic fertilizer application (Banaszak-Cibicka *et al.* 2019). Possibly the diverse mixture of micro- and macro-nutrients, released through SOM mineralization, enhances the quality of floral traits, such as nectar sugar content and flower longevity, which are important factors determining a flower's attractiveness to pollinators (Poveda *et al.* 2005; Fageria 2012). This could then explain why fertilizer applications did not influence pollinator visitation rates since the applied artificial fertilizers only contained the main nutrients N, P, K and S. If the link between SOM content, micro-nutrients and pollinator visitation rate is confirmed by further studies this could be of interest to farmers with crops that are pollination-limited. In our study, an estimated 132 and 185 pollinators visited the flowers of the plants in low and high SOM soils respectively, assuming a ten hours' daily visitation period and a 2.5-day life span for raspberry flowers (Sáez *et al.* 2014). Previous studies showed that each raspberry flower only needs ca. 1.3-10 insect visits to fulfil adequate pollination (Chagnon *et al.* 1991; Sáez *et* *al.* 2014; Andrikopoulos & Cane 2018). This suggests that all open-pollinated flowers were more than adequately pollinated.

Pollination contributed to raspberry yield through a significant positive effect on fruit size and we additionally found a trend of pollination contributing to fruit set (fruit number, P=0.063). This is in line with findings from previous studies (Cane 2005; Lye *et al.* 2011). The mechanism in raspberry most likely entails an increase in the number of fertilized ovules caused by enhanced transport of pollen to the stigmas (Colbert & De Oliveira 1990), which results in the development of a higher proportion of drupelets and thus higher berry weight (Sáez *et al.* 2014). Insect pollination resulted in a 33% higher yield compared to wind-pollinated raspberry plants. This is a bit lower than expected based on Klein *et al.* (2007) who classified raspberry as greatly dependent on insect pollination with an average yield decrease of 65% without pollinators. However, the contribution of insect pollination to yield differs considerably between varieties of the same crop (Klatt *et al.* 2014; Fijen *et al.* 2018). The cultivar *'Tulameen'* that we used in our study is a self-compatible cultivar (Daubeny & Anderson 1991), of which production ought to be less dependent on insect pollination than self-incompatible cultivars. This could explain the relatively modest effects of insect pollination. In addition, the near-significant interaction (P=0.051) between insect pollination and fertilizer application indicates that without pollination the contribution of fertilizer on yield would be positive only with high fertilizer inputs (Supplementary figure 3.1). We failed to find any clear effects of pollination on sugar content (Table 3.1; Figure 3.5), which is similar to some previous studies (Walters 2005; Hogendoorn *et al.* 2010).

High SOM content contributed significantly to single berry weight, but SOM content did not significantly enhance total yield per plant, although we did observe a trend (P<0.1). Our results are therefore intermediate between studies finding no effects of SOM content on crop yield (van Gils *et al.* 2016; Hijbeek *et al.* 2017) and studies finding positive effects of SOM content on crop yield (Quiroga *et al.* 2006; Pan *et al.* 2009; Wei *et al.* 2016). In contrast to studies by Gagic *et al.* (2017) and Hijbeek *et al.*

(2017), we did not find the benefits of SOM on yield or on single berry weight diminishing with fertilizer application rates (no significant interaction between effects of SOM content and fertilizer application rate). Because our artificial fertilizers only provided N, P, K and S this could suggest that the main role of SOM did not lie in providing these macro-nutrients but rather in a combination of providing extra micro-nutrients (Drenovsky *et al.* 2004), greater soil structural stability, water-holding capacity or more favorable redox conditions for root growth (Gleason *et al.* 2003; Oldfield *et al.* 2020). This could possibly also explain the variable effects of SOM on crop yield in other studies. Under rainy conditions or in soils rich in micro-nutrients and with good water holding capacity, the contribution of SOM to crop growth would be much less pronounced than in soils poor in micro-nutrients and with poor water holding capacity. Furthermore, our study was done using a perennial crop species, which complicates comparisons with other studies that were mostly done on annual crops (Quiroga *et al.* 2006; Pan *et al.* 2009; van Gils *et al.* 2016; Oldfield *et al.* 2020). Perennial species may be less responsive to short-term local conditions than annual species because they often have access to larger resources stored in roots and stems from previous seasons or may use resources for survival or vegetative growth rather than seed or fruit set (Ehrlén & Van Groenendael 2001; Langley *et al.* 2002).

The only evidence for fertilizer application influencing the interaction between effects of two ecosystem services was observed for sugar content although the patterns were not clear-cut (e.g. along lines depicted in Figure 3.1) and a bit hard to explain. Berry sugar content seemed to increase (non-linearly) across the whole range of fertilizer application rates at high SOM content while such a pattern was absent at low SOM content. The fact that mean berry sugar content of plants growing at high SOM content was higher than that of low SOM content at virtually all combinations of the other treatments and the significant main effect suggests that SOM content is particularly important for berry sugar content, although it seems to depend on fertilizer use and pollination level. Mauromicale *et al.* (2011) previously found that increasing SOM content by organic supplementation increased SSC and other fruit qualities of tomato. Sugar content is a major physiological property for fruit quality, which would significantly influence consumer choice, thus improving the marketable value (Parker *et al.* 1991; Mauromicale *et al.* 2011).

Our pot experiment explored the potential of managing pollination and SOM content as a possible way to supplement or partially replace artificial fertilizer application with the objective of making raspberry production more sustainable. For the main yield variables, we found only additive linear relationships with the examined production variables (i.e. Figure 3.1b). Such relationships mean that external fertilizer inputs can indeed be partially replaced by ecosystem services. For example, by providing ample pollination, fertilizer inputs can be reduced by 39 kg N fertilizer per hectare per year and still have the same total yield of plants without pollination, while the near-significant trend (P=0.09) between SOM and total yield suggests that by enhancing SOM content from 1.66% to 3.73%, 30 kg N fertilizer per hectare per year can be replaced without yield loss. Moreover, plants without pollination would need around 53 kg N fertilizer per hectare more to get the same fruit size as the plants with ample pollination; and approximately 85 kg N fertilizer per hectare extra would be needed for plants in low SOM soils to produce the same size fruit as the plants in high SOM soils. Decreasing fertilizer inputs would be beneficial for the environment (Vitousek *et al.* 1997; Dirzo & Raven 2003). However, it is questionable whether this approach makes sense economically for farmers because our findings indicate that yield can be maximized by enhancing both ecosystem services and fertilizer application (i.e. ecological enhancement; Bommarco et al. 2013). Only when external inputs are costly and their price exceeds the costs of managing ecological processes will it make economic sense to partially replace them with the management of pollination or SOM content. When this is not the case, uptake of practices to partly replace the use of external fertilizer inputs, such as the creation of wildflower strips and adding organic amendments to build up SOM, will probably rely on governmental subsidies or tax support. Alternatively, in developing countries, where artificial fertilizer may be expensive, farmers can use these insights to enhance agricultural productivity by making use of natural resources.

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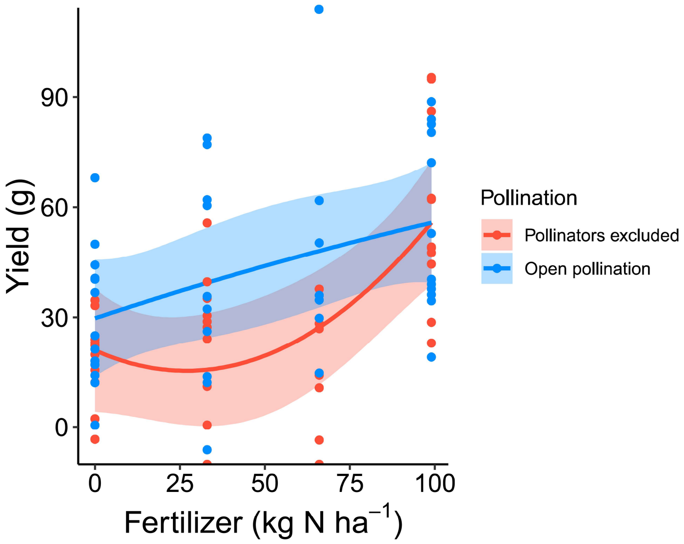
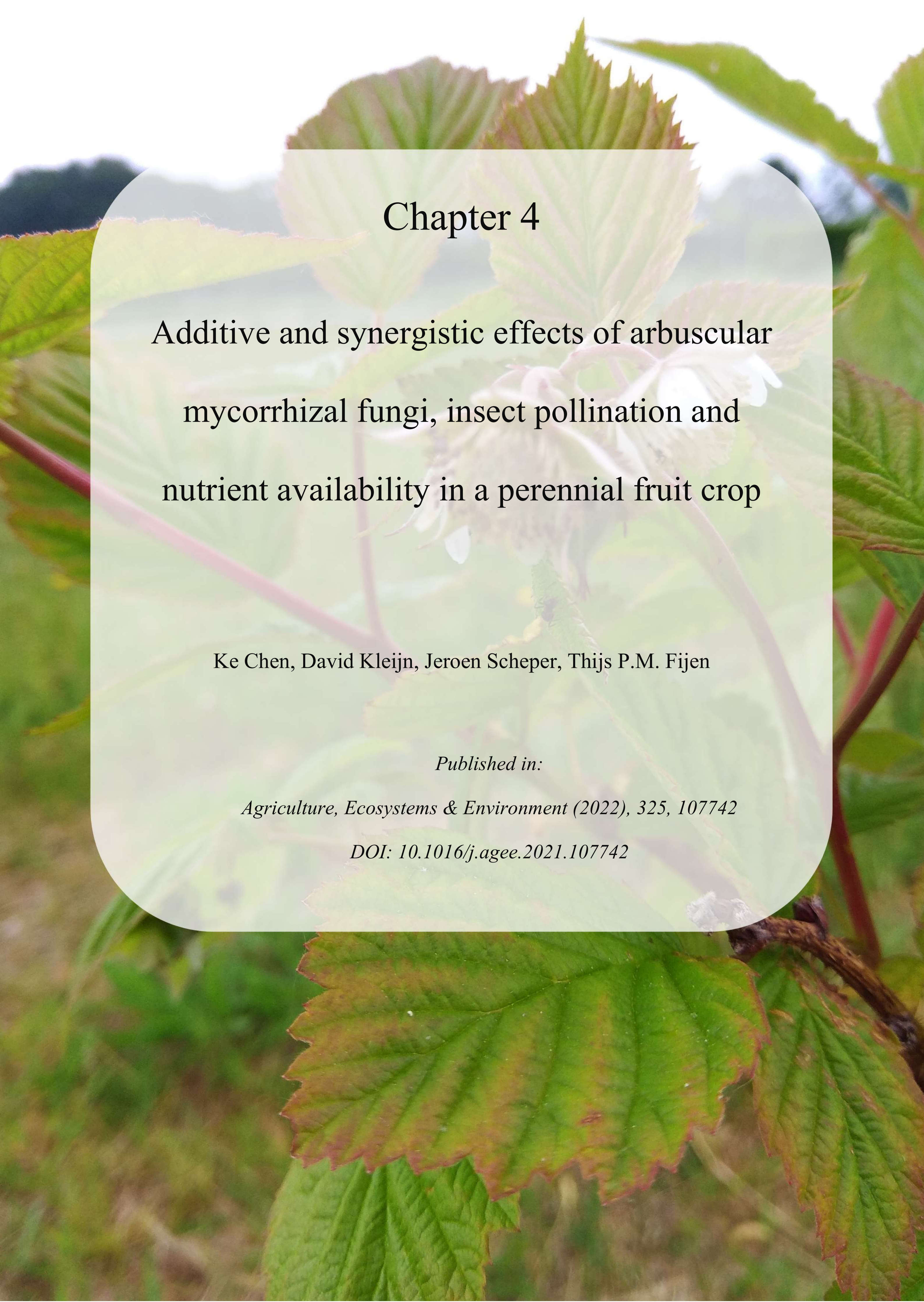
Supplementary materials

Supplementary table 3.1 The number of replicated raspberry plants per treatment combination.

Pollination	Fertilizer (kg·ha^{-1} of N per year)	SOM content	No. plants
open pollination	0	low	7
open pollination	0	high	6
open pollination	33	low	7
open pollination	33	high	4
open pollination	66	low	3
open pollination	66	high	4
open pollination	99	low	7
open pollination	99	high	5
pollinators excluded	0	low	7
pollinators excluded	0	high	4
pollinators excluded	33	low	5
pollinators excluded	33	high	5
pollinators excluded	66	low	2
pollinators excluded	66	high	5
pollinators excluded	99	low	6
pollinators excluded	99	high	5



Supplementary figure 3.1 Interacting effects of pollination and fertilizer application rates on per plant yield (near significant interaction, p=0.051). The graph shows the predicted effects of the model; the shadings show the 95% confidence interval. Plotted points represent partial residuals.



Chapter 4

Additive and synergistic effects of arbuscular mycorrhizal fungi, insect pollination and nutrient availability in a perennial fruit crop

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Abstract

Managing ecosystem services might reduce the dependence of modern agriculture on external inputs and increase the sustainability of agricultural production. Insect pollinators and arbuscular mycorrhizal fungi (AMF) provide vital ecosystem services for crop production, but it has not been tested whether their effects on crop yield interact and how their effects are influenced by nutrient availability. Here we manipulated insect pollination, AMF inoculation and fertilizer application (four levels) in a randomized complete block design with potted raspberry (*Rubus idaeus* L.) to assess the interacting effects of these variables on crop yield. AMF inoculation increased the per-plant flower number by 33% and the fruit number by 35%, independently from insect pollination and fertilizer application. Single berry weight furthermore increased more strongly with fertilizer application rates in AMF inoculated plants than in non-inoculated plants. As a consequence, AMF inoculation boosted raspberry yield by 43% compared to non-inoculated plants. AMF inoculation increased pollinator visitation rate per plant under intermediate fertilizer levels, suggesting additional indirect effects of AMF inoculation on yield. Fruit yield of pollinated plants increased more strongly with fertilizer application rate than the yield of plants from which pollinators had been excluded. At maximum nutrient availability, the combined benefits of both ecosystem services resulted in a 135% higher yield than that of fertilizer-only treatments. Our results suggest that the benefits of ecosystem services on yield can be additive or synergistic to the effects of conventional management practices. Intensive, high-input farming systems that do not consider the potential adverse effects of management on ecosystem service providing species might risk becoming limited by delivery of ecosystem services. Proactively managing ecosystem services, on the other hand, has the potential to increase crop yield at the same level of external inputs.

Keywords

Arbuscular mycorrhizal fungi, ecological intensification, fertilizer, interaction, insect pollination

Introduction

Agriculture depends on a wide array of ecosystem services (Costanza *et al.* 1997; Klein *et al.* 2007), but agricultural inputs like fertilizer have adverse effects on the species providing those services and on the wider environment (Bakhshandeh *et al.* 2017). Ecological intensification has been put forward as a promising way to make agriculture more sustainable and reduce negative impacts on the environment (Bommarco *et al.* 2013; Kleijn *et al.* 2019). This approach proposes to manage for biodiversity to complement or (partially) replace external inputs with production-supporting ecosystem services. Although ecological intensification is increasingly being advocated by scientists and policymakers as an environmentally friendly way towards food security (Pywell *et al.* 2015; IPBES 2016), it is rarely adopted by farmers (Kleijn *et al.* 2019). Farmers manage complex agro-ecosystems, with the interplay of several agronomic and environmental factors shaping crop yield. Evidence that a single ecosystem service has a positive effect on crop yield might not be convincing enough for farmers to change their day-to-day practices (Dainese *et al.* 2019; Kleijn *et al.* 2019). Ecological intensification might be more appealing to farmers when multiple ecosystem services together can synergistically enhance crop yield. This requires insight into the effects of multiple ecosystem services on crop yield simultaneously, whether and how these services interact and how their benefits are influenced by conventional agricultural practices. However, we are only just starting to understand how multiple ecosystem services might interact (Garibaldi *et al.* 2018; Tamburini *et al.* 2019), and we know even less how these interactions are being influenced by agricultural management. Here we contribute to addressing this knowledge gap by examining the interacting effects of aboveground insect pollination and belowground arbuscular mycorrhizal fungi (AMF) inoculation on crop yield of raspberry (*Rubus idaeus* L.) and how this is affected by different fertilizer application levels.

AMF are able to form symbiotic associations with about 72% of all vascular terrestrial plants (Smith & Read 2010; Brundrett & Tedersoo 2018), including the majority of field crops (Plenchette *et al.* 2005). AMF provide a range of services to plants, such as facilitating mineral nutrient uptake (mainly phosphorus and nitrogen), enhancing disease resistance and stress tolerance, and improving soil structure (Smith & Read 2010; Chen *et al.* 2018). AMF colonization of crop plants can significantly increase crop yield (Zhang *et al.* 2019). However, current agricultural practices, such as high fertilizer inputs and tillage, are likely to inhibit AMF growth, and root colonization might currently be suboptimal in many agricultural systems (Bhadalung *et al.* 2005; Jansa *et al.* 2006; Verbruggen *et al.* 2013). Farmers might actively manage for increased AMF colonization through reduced tillage (Bowles *et al.* 2017), or by inoculating the soil or seedlings, but whether this is effective for crop yield is less studied (Tamburini *et al.* 2020). Interestingly, AMF might also have indirect effects on crop production as the presence of AMF in plant roots can moderate the behaviour of other service-providing species groups. For example, Gange and Smith (2005) found that plants with AMF can significantly increase pollinator visitation frequency, which indicates that AMF and pollinator service delivery might interactively shape crop yield (Wolfe *et al.* 2005; Saini *et al.* 2019). However, AMF might also provide disservices to the host plant's growth and development, for example by reducing phosphorus uptake (Smith *et al.* 2004). Whether the net balance of AMF inoculation is positive for raspberry crop yield, and how this varies under different levels of fertilizer application is unknown.

Pollinators are important ecosystem service providers as they enhance yields in two-thirds of the leading global crops, and contribute to 35% of the global food production (Klein *et al.* 2007). Pollination might alter a number of interrelated qualitative and quantitative yield parameters such as fruit/seed set and size (Bommarco *et al.* 2012; Klatt *et al.* 2014; Fijen *et al.* 2018). However, the positive effect of pollination on a particular yield parameter does not automatically result in a higher total crop yield. For example, in sunflower (*Helianthus annuus* L.) increasing insect pollination can contribute to higher seed set but with smaller seeds resulting in the same overall yield (Tamburini *et al.* 2017), probably because yield is constrained by other factors, such as nutrient availability (Garibaldi *et al.* 2018). Particularly for high-revenue fruit crops like raspberry (Daubeny & Kempler 2003), both yield quantity and quality are important for farmers. To make more reliable predictions of the benefits of ecological intensification for agriculture, it is therefore important to gain insight into how effects of insect pollination shape crop yield through these intercorrelated yield parameters, and how this is affected by other ecosystem services such as those provided by AMF, or management practices such as fertilizer application.

Here, we experimentally manipulated insect pollination, AMF inoculation and nutrient availability on raspberry crop plants in a randomized complete block design to explore whether and how AMF inoculation, insect pollination and fertilizer application interactively affect crop yield, which to our knowledge has not been studied before. The main objectives of this study were (i) to test the effects of AMF inoculation and fertilizer application rates on pollinator visitation, (ii) to examine the effects of pollination and AMF inoculation on five yield quality and quantity parameters and how their effects are influenced by fertilizer application, and (iii) to explore the pathways explaining the relationships among the variables. The insights obtained in our study might help advance our understanding of whether and how we can integrate different ecosystem services into farming practices to make agriculture more sustainable.

Materials and methods

Study system

We used raspberry as our study crop, which is an increasingly important fruit crop with a global production value of $1.5 billion in 2018 (FAO 2018). We used the cultivar '*Tulameen*', which is among the most popular raspberry cultivars worldwide due to its high marketable quality, mainly the appearance and flavour (Aprea *et al.* 2009). It is a self-compatible cultivar, but high-quality fruit production nevertheless benefits from visitation by insect pollinators (Daubeny & Kempler 2003; Chen *et al.* 2021). The study was carried out on an experimental field of Wageningen University & Research in Wageningen, the Netherlands (51° 59' 47" N, 5° 39' 36" E; 780 mm mean annual precipitation, 9.4 °C mean annual temperature).

Experimental design

In August 2019, we purchased raspberry plants with a height of ca. 60 cm from a local fruit tree supplier. To ensure that all plants were exposed to the same soil conditions, we carefully washed away any soil adhering to the roots of raspberry plants prior to transplanting. Each plant was then planted into a 10-litre plastic pot (upper diameter 28 cm, holes in the bottom for drainage but covered with root cloth to minimize root growth out of the pot), and filled with un-sterilized former agricultural soil (SOM content: 1.95%, available N: 14.0 mg/kg, available P: 0.6 mg/kg, available K: 19.4 mg/kg). Soils were not sterilized to reflect real-world conditions in agricultural fields where plants can be colonized by AMF already present in the agricultural soil.

As our AMF treatment, we added either alive inoculum (inoculated) or sterilized inoculum (non-inoculated). We used the commercially available *Rhizophagus intraradices* inoculum (MYKOS® Xtreme Gardening, Canada). To sterilize the inoculum for our non-inoculated treatment, we autoclaved it at 121 °C for two hours (Changey *et al.* 2019). During transplantation, we gave each plant two tablespoons of inoculum or sterilized inoculum spread evenly on the roots.

The fertilizer treatments comprised four levels: 0, 33, 66 and 99 kg ha^{-1} of N per year. The fertilizer levels were selected to include the range from no to optimum N inputs, as the recommended annual fertilizer N application rates for raspberry range from 45 to 85 kg/ha (Strik 2005). The annual dose was divided into three applications: the first application at two weeks after transplanting (October 30, 2019), the second application at the bud break (March 16, 2020) and the last application just before flower opening (April 24, 2020). We selected a local commonly used fertilizer for the experiment, containing 10.80% N, 13.44% K, 5.89% P, and 7.20% S (CropSolutions Co., Perth, UK).

Our experimental site is known to host pollinators, mainly wild bumblebees and managed honey bees, in sufficient densities to result in an optimal fruit set of raspberry plants (Chen *et al.* 2021). To examine the effect of insect pollination, we excluded pollinators from half of the plants and used open-pollinated plants as positive controls. We covered every plant of the pollinator exclusion treatments with a white semi-transparent mesh bag (mesh size 0.1 mm) before the onset of flowering and kept plants covered throughout the flowering period. The mesh bags allowed wind pollination but excluded all insect visitors. To avoid predation of the developing fruits, we covered all plants after flowering with the mesh bags until harvest.

We used a randomized complete block design with AMF inoculation (two levels), pollination (two levels) and fertilizer (four levels) fully crossed to measure their individual and interacting effects on raspberry productivity. This resulted in 16 treatment combinations, which were randomly assigned to individual raspberry plants and replicated in five blocks, bringing the total to 80 experimental plants. Potted plants were spaced one meter apart both within and between rows and dug into the soil to protect the roots from extreme temperatures. All plants received equal and ample irrigation, and weeds were regularly removed by hand.

Measurements

For each plant of the open pollination treatment, we conducted ten-minute pollinator censuses from May 12 - 27th 2020 to see if the AMF inoculation and fertilizer treatments affected the pollinator visitation rate. We randomly observed plants ten times on different days (morning or afternoon), and only during sunny or slightly cloudy days and with low wind velocity, following the focal point observation method (Fijen & Kleijn 2017). We only recorded flower visitors that contacted anthers or stigmas of flowers per plant. All flower visitors were identified on the wing, with bees identified to species level and other pollinators to order level. From June 15 onward, we harvested ripe berries every other day and weighed the fresh weight of each berry in the lab. Additionally, we counted the wilted and aborted flowers of each plant.

To assess whether AMF inoculation results in higher final AMF colonisation rate compared to non-inoculated plants, we collected fine roots (<1 mm in diameter) after the harvest of all the plants. Firstly, two grams of roots from each plant were cleared and stained following a protocol adjusted from Koske and Gemma (1989). AMF colonization rate was subsequently measured following the gridline intersect method (Giovannetti & Mosse 1980). AMF colonization rate was calculated as the percentage of the colonised intersections of the total observed intersections.

Data analysis

Four plants died over winter prior to fruit production, resulting in a dataset for 76 plants (Supplementary table 4.1). Prior to analyses, single berry weight was averaged per plant to avoid pseudoreplication. Total flower number per plant was calculated as the sum of the total fruit number and the total number of flowers that did not develop into fruits (e.g. wilted or aborted flowers). Per-plant fruit set was calculated by dividing the fruit number by the total flower number and expressed as a percentage.

We fitted linear mixed-effects models to quantify the relations between the experimental treatments and response variables. We fitted separate full models for each of the response variables: flower number, fruit number, fruit set (%), single berry weight (g/fruit) and total yield (g/plant), and included "block" as a random factor in all models. Independent variables included pollination, AMF inoculation, fertilizer application rate and all their possible interactions (i.e. all two- and three-way interactions). We also included a quadratic term for fertilizer application rate to test for non-linear relations between fertilizer levels and raspberry production (Tamburini *et al.* 2017). The full models were simplified by removing non-significant predictors (backward elimination) using likelihood ratio tests with removal thresholds of $p > 0.05$, until the resulting minimum adequate model consisted only of variables that contributed significantly to the outcome (Zuur *et al.* 2009). Finally, we tested the effects of AMF inoculation and fertilizer treatments on the average pollinator visitation rate (visitors/10 min), including the quadratic term for fertilizer application rate, and their interactions, and "block" as a random factor. For this analysis we only used the open pollination treatment plants. The models were built using the function lme() in the nlme package with the maximum likelihood estimation method (Pinheiro *et al.* 2019). Statistical assumptions of normality and homoscedasticity of model residuals were inspected visually through diagnostic plots using the function plotresid() in the RVAideMemoire package. All analyses were performed in R (R Core Team 2020).

Results

Total visits and flower visitation rate

Altogether, 682 individual pollinators were observed, divided over seven taxa: *Apis mellifera* (471 individuals), *Bombus terrestris* congl. (132 individuals, including *B. terrestris* and *B. lucorum* due to overlapping morphology, cf. Williams *et al.* (2012)), *B. pascuorum* (55 individuals), *B. lapidarius* (13 individuals), *B. pratorum* (7 individuals), hoverfly (3 individuals) and *B. sylvestris* (1 individual). AMF inoculation and fertilizer application interactively influenced pollinator visitation rate per plant (Table 4.1). Pollinator visitation rate increased with fertilizer levels, and was higher for plants that had been inoculated with AMF than for non-inoculated plants at intermediate fertilizer application rates, but not at low or high fertilizer application rates (Table 4.1; Figure 4.1). Besides, the pollinator visitation rate was strongly correlated with the number of flowers per plant (Supplementary figure 4.1).

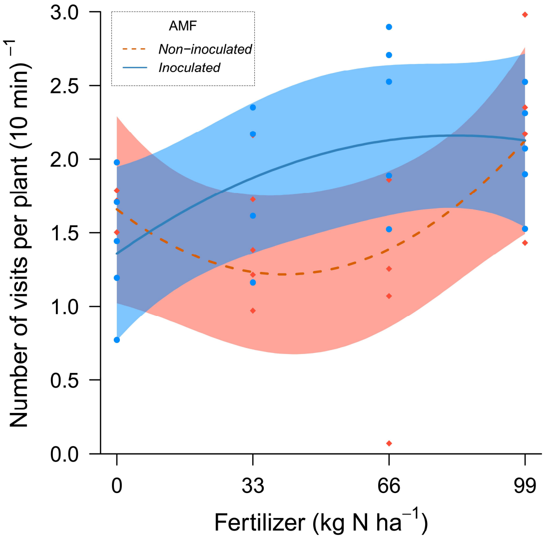

Figure 4.1 Interactive effects of AMF inoculation and fertilizer application rates on pollinator visitation rate (number of visits per 10 min) of raspberry. The lines are predicted by the minimum adequate model; shadings show the 95% confidence interval, and points represent partial residuals.

Table 4.1 Effects of arbuscular mycorrhizal fungi (AMF; inoculated vs non-inoculated) and fertilizer application rates (0, 33, 66, 99 kg N·ha^{-1}·year^{-1}) on flower visitation rate (open-pollinated plants only, n=37). Only the estimates from the minimum adequate model were included. All analyses were performed using a linear mixed-effects model. Bold values represent significant effects (P<0.05).

	Flower visitation rate		
	Estimate	$\chi^2_{(1)}$	P
AMF	-0.299	2.096	0.148
Fertilizer	-0.022	5.394	**0.020**
Fertilizer^2	0.000	0.396	0.529
AMF:fertilizer	0.041	0.284	0.594
AMF:fertilizer^2	0.000	5.234	**0.022**

Table 4.2 Effects of arbuscular mycorrhizal fungi (AMF; inoculated vs non-inoculated), pollination (open-pollinated vs pollinators excluded) and fertilizer application rates (0, 33, 66, 99 kg $N\cdot ha^{-1}\cdot year^{-1}$) on raspberry fruit production variables (n=76). Only the estimates from the minimum adequate model were included. All analyses were performed using linear mixed-effects models. Bold values represent significant effects ($P<0.05$).

	Flower number			**Fruit set**			**Fruit number**			**Single berry weight**			**Yield**		
	Estimate	$\chi^2_{(1)}$	P	Estimate	$\chi^2_{(1)}$	P	Estimate	$\chi^2_{(1)}$	P	Estimate	$\chi^2_{(1)}$	P	Estimate	$\chi^2_{(1)}$	P
AMF	12.880	8.074	**0.004**		0.007	0.933	8.553	5.436	**0.02**	-0.158	0.277	0.599	14.485	7.712	**0.005**
Pollination		0.022	0.881	-2.530	9.093	**0.003**	-0.291	6.916	**0.009**		2.083	0.149	-2.207	10.165	**0.001**
Fertilizer	0.310	19.934	**<0.001**	-0.108	0.944	0.331	0.116	14.059	**<0.001**	0.001	8.725	**0.003**	0.228	23.003	**<0.001**
Fertilizer^2		1.807	0.179		2.277	0.131		2.6	0.107		0.885	0.347		1.186	0.276
AMF:fertilizer		0.29	0.59		0.309	0.578		0.002	0.966	0.004	4.146	**0.042**		1.17	0.279
AMF:fertilizer^2		3.565	0.059		0.577	0.448		0.607	0.436		1.164	0.281		0.324	0.569
AMF:pollination		0.071	0.79		0.375	0.54		0.04	0.841		0.14	0.708		0.552	0.458
Pollination:fertilizer		0.054	0.817	0.349	8.517	**0.004**	0.207	4.699	**0.03**		0.39	0.532	0.400	8.705	**0.003**
Pollination:fertilizer^2		0.686	0.407		0.616	0.432		0.116	0.734		0.79	0.374		0.229	0.632
AMF:fertilizer:pollination		0.35	0.554		3.412	0.065		0.577	0.447		0.025	0.874		0.231	0.631
AMF:fertilizer^2:pollination		0.174	0.677		1.218	0.27		0.026	0.873		3.228	0.072		0.339	0.56

Flower number, fruit set and fruit number

The number of flowers per plant increased independently by both factors that (potentially) influence the nutrient acquisition, i.e. fertilizer inputs and AMF inoculation. Fertilizer inputs linearly increased flower number (Table 4.2), with plants receiving 99 kg N·ha^{-1} producing 105% (57.7 g) more flowers than the unfertilized plants (28.1 g; Figure 4.2a). Compared to the non-inoculated plants, AMF inoculation increased flower number by 33% (from 36.6 to 48.7 g; Table 4.2; Figure 4.2b). There was a near-significant interaction (P=0.059) between the effect of AMF inoculation and the quadratic term of fertilizer application rate, with AMF inoculated plants receiving intermediate fertilizer application rates producing the most flowers (Table 4.2; Supplementary figure 4.2).

Fruit set was mainly altered by an interaction between the insect pollination and the fertilizer treatment, where pollination benefits were most pronounced at the higher fertilizer application rates (significant pollination × fertilizer interaction; Table 4.2). From the lowest to the highest level, fertilizer application increased the fruit set of open-pollinated plants by 37% and had little effect on fruit set in bagged plants (Figure 4.2c). Fruit set was not affected by AMF inoculation (Figure 4.2d).

Pollination and fertilizer application rate interactively affected fruit number with open-pollinated plants receiving 99 kg N·ha^{-1} producing 162% (51.7 g) more fruits than unfertilized plants (19.7 g). This increase was only 53% when pollinators were excluded (Table 4.2; Figure 4.2e). Additionally, AMF inoculation independently increased fruit number by 35% (from 25.5 to 34.3 g; Figure 4.2f).

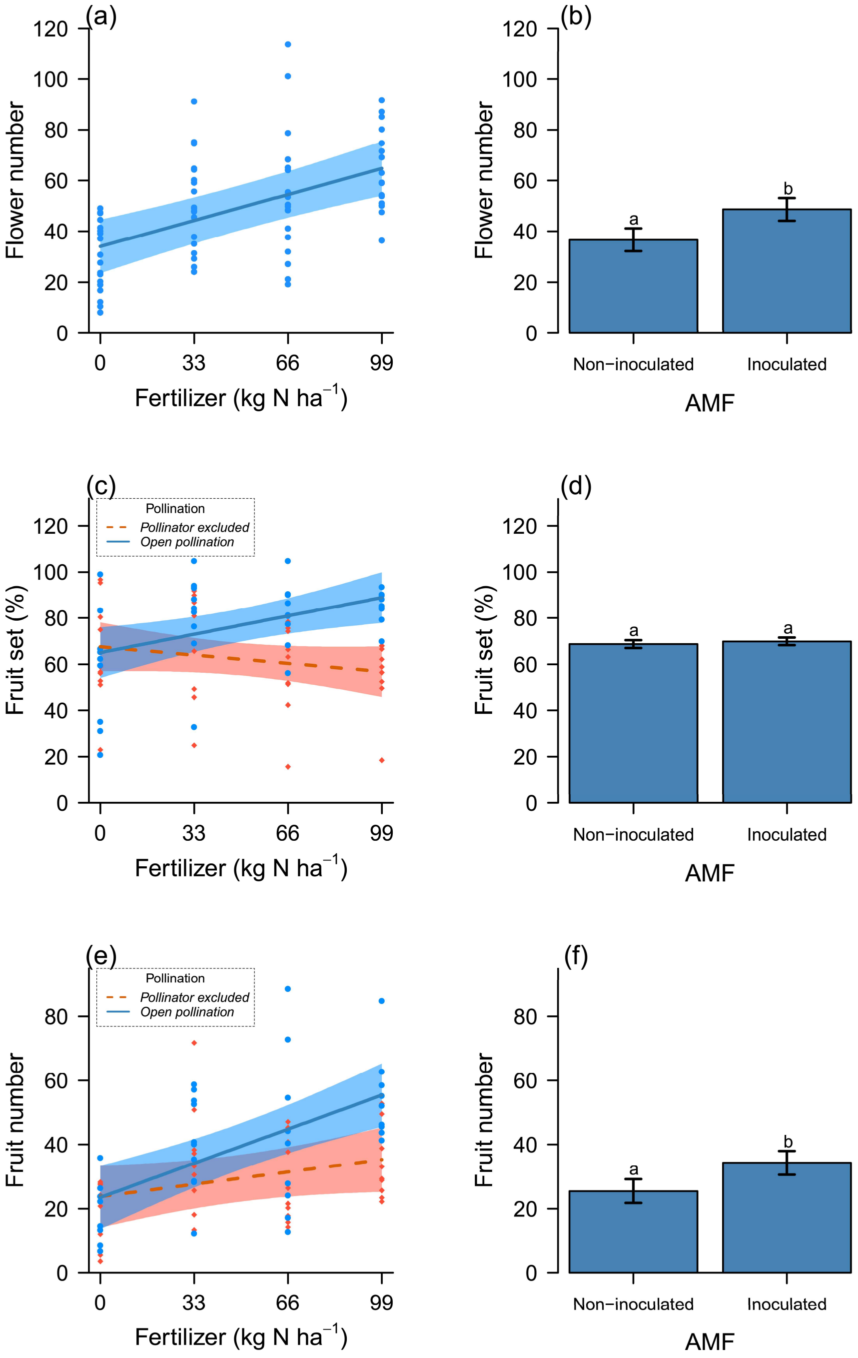

Figure 4.2 Effects of AMF inoculation, pollination and fertilizer application rates on flower number (a and b), fruit set (c and d), and fruit number (e and f) per plant. Graphs show predicted values of the minimum adequate models; panel (d) shows non-significant estimated mean fruit set for AMF treatments as calculated in a model including AMF treatment (p=0.93) and the minimum adequate model parameters, and is shown for completeness. Different letters in barographs indicate significant differences among AMF treatments. Shadings show the 95% confidence interval, and points represent partial residuals; error bars show ± 1 SE.

Single berry weight and yield

Increasing fertilizer application rates influenced single berry weight interactively with AMF inoculation treatments, with a much more pronounced positive response in AMF inoculated plants compared to the non-inoculated plants (Table 4.2; Figure 4.3). Pollination treatments did not influence single berry weight (Table 4.2).

The total yield is essentially the product of per-plant fruit number and single berry weight. However, total yield largely reflected effects of treatments on total fruit number, albeit stronger, while the significant interaction of AMF inoculation and fertilizer application on single berry weight was not reflected in the pattern for total yield (Table 4.2; Figure 4.4). Total yield was positively related to fertilizer application rate, but these effects were much more pronounced in open-pollinated plants than in plants from which pollinators had been excluded; plants with insect pollination produced 90% more yield than bagged plants under our highest fertilizer input level (83.8 g and 44.2 g separately; Figure 4.4a). On top of that, the yield of AMF inoculated plants significantly increased by 43% compared to the non-inoculated plants (from 35.1 to 50.2 g; Figure 4.4b). Under the highest fertilizer input, raspberry plants with open pollination and AMF inoculation produced the highest yield, on average 90.4 g berries, which was 135% more than the yield of plants receiving only the fertilizer application (38.5 g).

AMF colonization rate

We found no significant difference between AMF colonization rate of the AMF inoculation or fertiliser treatments at the end of the experiment (59.6%). This was likely because all plants had been well colonized by either inoculated AMF or indigenous AMF in the soils after 12 months' growth (Ortas 2012; An-Dong *et al.* 2013).

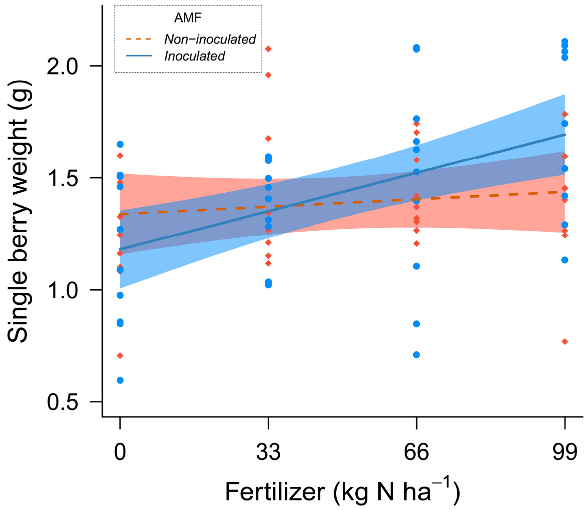

Figure 4.3 Interactive effects of AMF inoculation and fertilizer application rates on average single berry weight (g) per plant. The lines are predicted by the minimum adequate model; shadings show the 95% confidence interval, and points represent partial residuals.

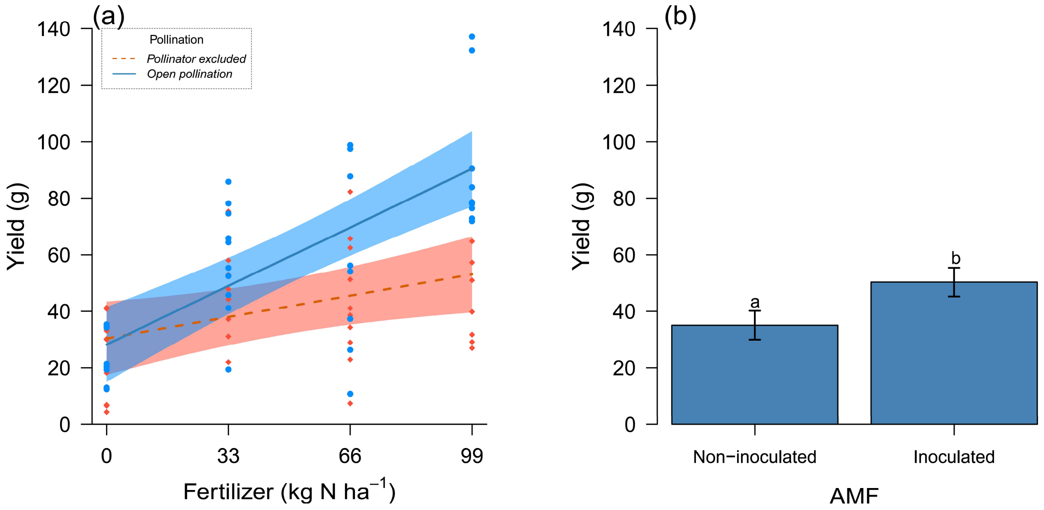

Figure 4.4 Effects of a) fertilizer application rates and pollination, b) AMF inoculation on yield per plant. Graphs show predicted values of the minimum adequate model (both); shadings show the 95% confidence interval, and points represent partial residuals (a); error bars show ± 1 SE (b). Different letters in the barograph indicate significant differences among AMF treatments.

Discussion

Our results indicate positive effects of AMF inoculation on raspberry yield that were independent of the effects of pollination and fertilizer application, and positive synergistic effects of pollination and fertilizer inputs on yield. AMF inoculation enhanced the fruit-producing potential of plants by increasing the number of flowers per plant, independent from fertilizer. Insect pollination subsequently increased the likelihood that these flowers developed into fruits but only when plants received enough fertilizers. This probably suggests that poorly fertilized plants have insufficient resources for maximum fruit set. Interestingly, at intermediate fertilizer levels, AMF inoculation also enhanced pollinator visitation rates suggesting intricate indirect effects of one ecosystem service on another. Our findings imply that the simultaneous management of below- and aboveground ecosystem services can substantially increase the yield-enhancing effects of fertilizer application and represent a compelling example of ecological enhancement (i.e. enhancing yield by increasing ecosystem service delivery without increasing agricultural inputs) *sensu* Bommarco *et al.* (2013).

AMF inoculation contributing to raspberry yield directly and indirectly

AMF inoculation contributed to raspberry yield mainly through enhancing the number of flowers and by allowing plants to develop larger fruits, as AMF inoculation did not affect fruit set. The 35% increase in fruit numbers of plants inoculated with AMF was very similar to the 33% increase in flower numbers of AMF inoculated plants, suggesting that AMF inoculation did not have a direct effect on fruit number but mostly on flower number. The effect on flower number might be due to the ability of AMF to increase plant nutrient concentrations (especially P and K) and to raise hormone levels stimulating bud-formation which have both been observed to lead to the development of larger numbers of flowers (Long *et al.* 2010). The positive effect of AMF inoculation on fruit size has been found in strawberry as well (Bona *et al.* 2015), but in our case the benefits were only expressed under ample fertilizer inputs. Possibly, at low fertilizer application rates, soil nutrient availability was the main limiting factor, while at higher fertilizer application rates, plant nutrient uptake capacity became a more limiting factor which AMF are known to improve (Wang *et al.* 2018). Surprisingly, when no fertilizer was applied, AMF-inoculated plants developed slightly smaller fruits than the plants that had not been inoculated, which could be the result of the competition for N with the host (Wang *et al.* 2018; Ingraffia *et al.* 2020). The interaction between AMF inoculation and fertilizer application did not carry over into the final yield. Raspberry plants are readily colonized by AMF (Taylor & Harrier 2000) and regardless of treatment, all plants had formed associations with AMF to some degree by the end of the study. Our results, therefore, provide a conservative estimate of the potential contribution of AMF to raspberry crops.

Interestingly, our results indicate that AMF inoculation can also indirectly contribute to raspberry production through increasing pollinator visitation rate and thus pollination. Pollination has been shown to be an important factor limiting raspberry production, even in self-compatible cultivars like the one used in the present study (Chen *et al.* 2021). In our study, AMF inoculation and fertilizer inputs interactively shaped pollinator visitation rate per plant, and the pattern resembled their near-significant interaction on flower number ($p = 0.059$; Supplementary figure 4.2), which is an important plant trait to affect attractiveness to pollinators (Gange & Smith 2005). Therefore, it seems likely that the effects of AMF inoculation on pollinator visitation rate operated through their influence on flower number. However, we cannot rule out the possibility that AMF inoculation also influenced pollinator visitation rate through altering the composition of nectar and pollen (Somme *et al.* 2015; Bennett & Meek 2020).

Synergistic effects of insect pollination and fertilizer on raspberry production

Insect pollination and fertilizer inputs showed synergistic effects on raspberry yield. The possible pathway to explain the interacting effects starts with the positive effect of fertilizer on flower number, which simultaneously increased both the number of flowers that can potentially be pollinated and developed into fruits, as well as increased the attractiveness of the plant to pollinators (Conner & Rush 1996). Increased pollinator visitation rate in turn improved fruit set of the plants in the open pollination because increased insect pollination generally enhances the transfer of pollen for ovule fertilization (Sáez *et al.* 2020). Interestingly, the benefits of insect pollination and fertilizer inputs seem to be depending on each other, as in the absence of the one, the benefits of the other diminish. For example, in the absence of fertilizer inputs, pollination benefits on fruit set are negligible, suggesting that nutrient availability limited the potential benefits of insect pollination to develop additional fruits (Garratt *et al.* 2018a). Similarly, in the absence of insect pollination, solely increasing fertilizer inputs did not increase fruit set at all. This suggests that raspberry is probably limited by multiple 'resources' at the same time (Garibaldi *et al.* 2018), and that both need to be optimized to reach the highest raspberry crop yield. It also indicates that in our study system, ecosystem service benefits critically depend on the right management of external inputs and thus cannot easily replace them.

Because insect pollination did not influence single berry weight, the pollination-induced effects on fruit set carried over into similar effects on fruit number (Figure 4.2e) and eventually the yield (Figure 4.4a). In a previous study using the same experimental system we did find positive effects of insect pollination on raspberry fruit size but not on fruit number (Chen *et al.* 2021). Plants have multiple ways to invest their most limiting resources (compensation mechanism; (Garratt *et al.* 2018a)), which suggests that if one ecosystem service partially removes one limitation (e.g. nutrient-constrained flower development) this might impose new limitations to a subsequent process (e.g. nutrient-constrained drupelet development of raspberry fruits). However, it is noteworthy that regardless of the exact pathway, insect pollination resulted in substantially increased total raspberry crop yield in both studies.

The potential of capitalizing on ecosystem services in farming systems

Our results highlight the importance of maintaining ecosystem service providing species in agro-ecosystems. Not only did we find that without pollination and AMF inoculation raspberry yield would be substantially reduced, but yield effects of fertilizer were much less pronounced in the absence of ecosystem services. Agricultural production methods that do not consider potential adverse effects on ecosystem service providing species might risk shifting the system to one that is limited by delivery of ecosystem services rather than by management intensity (Deguines *et al.* 2014; Fijen *et al.* 2020). This is not a trivial issue as, for example, AMF colonization might be adversely affected by the application of some types of pesticides (Hernández-Dorrego & Parés 2010; Hage-Ahmed *et al.* 2019). A farmer trying to control a disease using fungicides might succeed in minimizing disease damage only to lose the benefits provided by AMF. Our results furthermore suggest that proactively managing for ecosystem services can even increase crop production independently of conventional management practices such as fertilizer application, or can enhance the yield increases due to such practices as here with pollination. Such an approach could address the increasing demands for safe and healthy food that is typically associated with crop production methods that rely on natural processes rather than external inputs (Yiridoe *et al.* 2005). Here we found additive and synergistic benefits among insect pollination, AMF inoculation and fertilizer inputs. Given that other species groups can have additional yield impacts through, for example, biological pest control or nutrient cycling, the ultimate benefits to agricultural production of capitalizing more on natural processes could be substantially higher.

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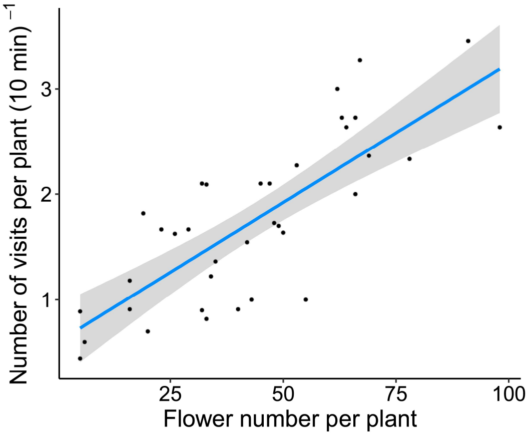
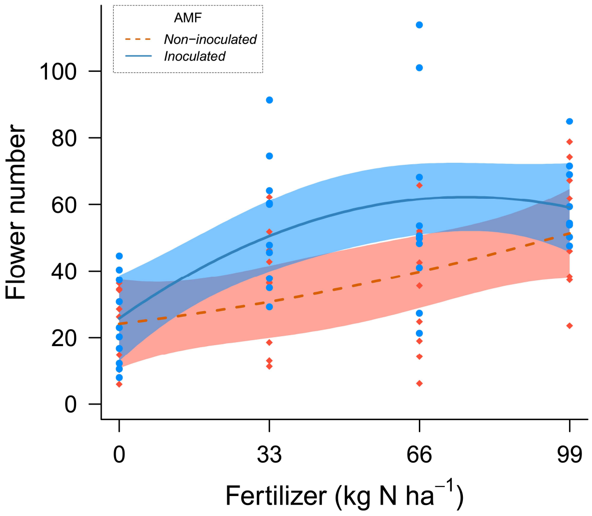
Supplementary materials

Supplementary table 4.1 The number of replicated raspberry plants survived in each treatment combination.

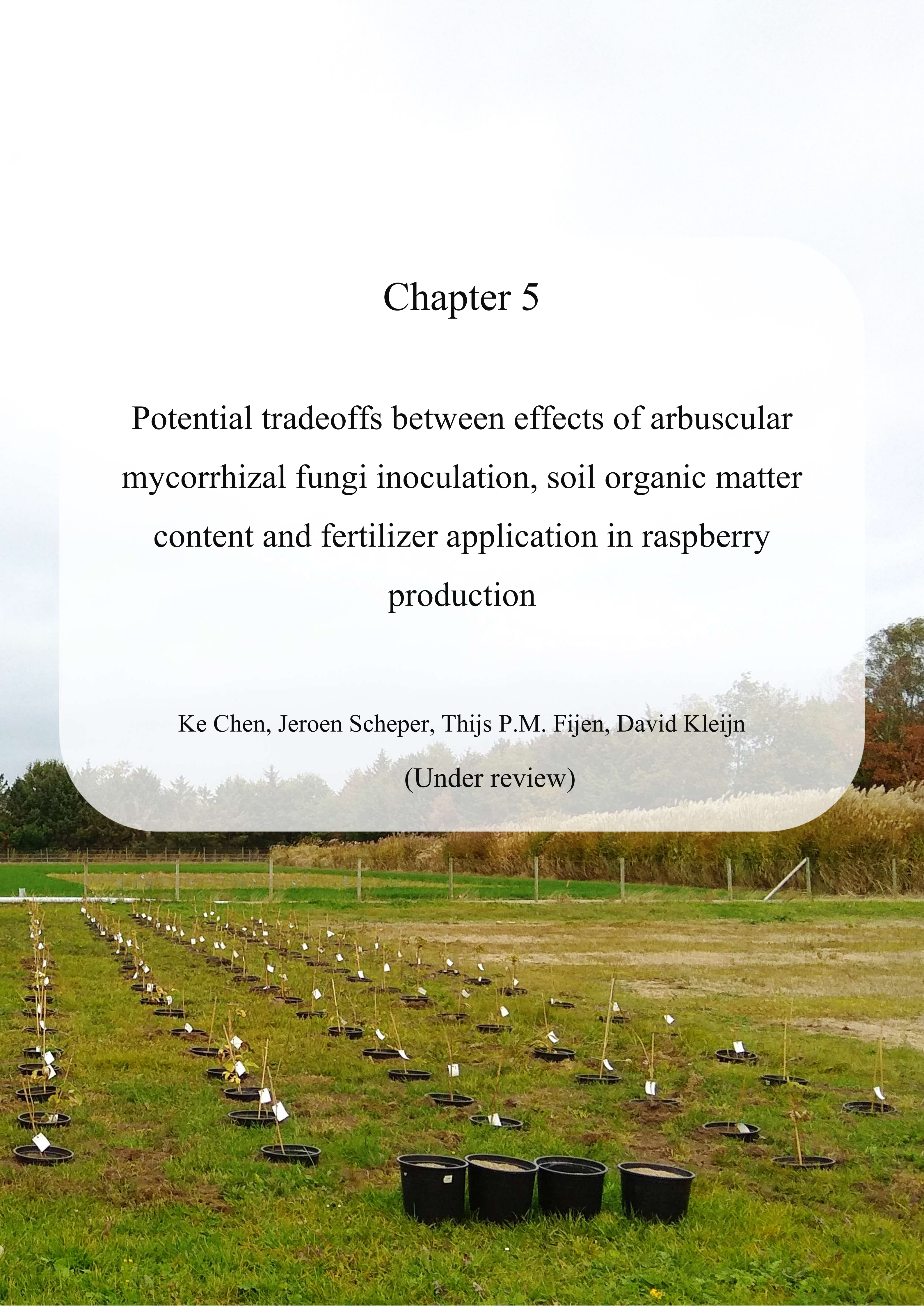
Pollination	Fertilizer (kg·ha^{-1} of N per year)	AMF	No. plants survived
Pollinators excluded	0	Non-inoculated	5
Pollinators excluded	0	Inoculated	5
Pollinators excluded	33	Non-inoculated	5
Pollinators excluded	33	Inoculated	5
Pollinators excluded	66	Non-inoculated	5
Pollinators excluded	66	Inoculated	5
Pollinators excluded	99	Non-inoculated	5
Pollinators excluded	99	Inoculated	4
Open pollination	0	Non-inoculated	4
Open pollination	0	Inoculated	5
Open pollination	33	Non-inoculated	5
Open pollination	33	Inoculated	5
Open pollination	66	Non-inoculated	4
Open pollination	66	Inoculated	5
Open pollination	99	Non-inoculated	4
Open pollination	99	Inoculated	5



Supplementary figure 4.1 The relation between flower number and flower visitation rate (number of visits per 10 min) per plant, with the shading showing the 95% confidence interval. The graph bases on a simple linear regression model and the equation is $y = 0.60 + 0.03x$ ($r^2 = 0.61$, $p < 0.001$)



Supplementary figure 4.2 Interactive effects of AMF inoculation and fertilizer application rates on flower number per plant (near significant interaction, p=0.059). The lines are predicted by the minimum adequate model; shadings show the 95% confidence interval, and points represent partial residuals.



Chapter 5

Potential tradeoffs between effects of arbuscular mycorrhizal fungi inoculation, soil organic matter content and fertilizer application in raspberry production

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(Under review)

Abstract

Ecological intensification has been proposed as an alternative paradigm for intensive agriculture to boost yield sustainably through utilizing ecosystem services. A prerequisite to achieving this is to understand the relations between multiple ecosystem services and production, while taking growth conditions such as nutrient availability into consideration. Here, we conducted a pot-field experiment to study the interactive effects of soil organic matter (SOM) content and arbuscular mycorrhizal fungi (AMF) inoculation on the production of raspberry (*Rubus idaeus* L.) under four levels of fertilizer application. Raspberry flower number, fruit number and yield only significantly increased with fertilizer inputs but were not impacted by SOM content or AMF inoculation. Fruit set and single berry weight were influenced by both SOM content and AMF inoculation, in complex three-way interactions with fertilizer application. Fruit set of AMF inoculated plants increased with fertilizer inputs in low SOM soils, but decreased with fertilizer inputs under high SOM soils, with the highest fruit set occurring at no fertilizer inputs. In low SOM soils, the relation between single berry weight and fertilizer application was more pronounced in inoculated plants than in non-inoculated plants, while in high SOM soils the relative benefits of AMF inoculation on single berry weight decreased with increasing fertilizer inputs. We attribute the lack of effects of AMF inoculation and SOM content on flower number, fruit number and yield mainly to potential tradeoffs between the experimental variables that all influence resource uptake by plant root systems. Our results suggest that potentially beneficial effects of AMF and SOM can be offset by each other, probably driven by the dynamic relations between AMF and the host plants. The findings reveal fundamental implications for managing AMF inoculation and SOM management simultaneously in real-world agricultural systems.

Keywords

Arbuscular mycorrhizal fungi, ecological intensification, fertilizer, interactive effects, soil organic matter

Introduction

Conventional agricultural intensification cannot meet the twofold challenge facing agriculture: increasing yield to feed the growing world population while minimizing negative externalities on the environment (Tilman *et al.* 2002; Godfray *et al.* 2010). It is increasingly difficult to further promote productivity through mainstream intensive farming practices (Cassman *et al.* 2010; Grassini *et al.* 2013), because the production is increasingly limited by critical natural ecosystem services, such as insect pollination (Lebuhn *et al.* 2013; Deguines *et al.* 2014) and soil formation (Pimentel & Burgess 2013). Additionally, these intensive farming practices have caused severe environmental problems, such as soil and water pollution (Carpenter *et al.* 1998; Rodríguez-Eugenio *et al.* 2018) and biodiversity loss (Potts *et al.* 2010; Ponge *et al.* 2013), which are threatening human-wellbeing (Cassman *et al.* 2010). Ecological intensification has been proposed as a promising alternative for conventional intensive agriculture. It is based on managing multiple ecosystem services to complement and/or replace artificial inputs to maintain or enhance productivity while reducing negative environmental impacts (Bommarco *et al.* 2013; Kleijn *et al.* 2019). Ecological intensification has been advocated as an environmentally friendly way towards food security (Pywell *et al.* 2015; IPBES 2016) and an increasing number of studies provide proof of concept for this paradigm (Tittonell 2014; Tamburini *et al.* 2016; Garratt *et al.* 2018b). There are still knowledge gaps between theory and practice, however, which limit the adoption of ecological intensification by the agricultural sector (Kleijn *et al.* 2019). For example, when multiple ecosystem services are managed in conjunction, their effects on production could interact synergistically, negatively or not at all (Garibaldi *et al.* 2018; Tamburini *et al.* 2019). Understanding whether and how different ecosystem services interact in shaping crop production is of importance to maximize the benefits of ecological intensification and promote its adoption (Kleijn *et al.* 2019).

Soil organic matter (SOM) and arbuscular mycorrhizal fungi (AMF) are two natural factors that provide or influence vital ecosystem services in cropping systems (Bommarco *et al.* 2013; Garratt *et al.* 2018b; Zhang *et al.* 2019). SOM is often used as a proxy for soil services, as it is able to mediate the flow of soil ecosystem services (Bommarco *et al.* 2013; Williams & Hedlund 2014), and it strongly affects almost all soil properties (Krull *et al.* 2004). Examples include soil structural stability and water-holding capacity (physical properties), cation exchange capacity and pH regulation (chemical properties), and nutrient supply for microbial communities (biological properties) (Krull *et al.* 2004). SOM content, therefore, often relates positively to crop production (Garratt *et al.* 2018b; Oldfield *et al.* 2020).

AMF are widespread soil microorganisms from the phylum *Glomeromycota*, and they can form symbiotic associations with the majority of the cultivated crops (Tawaraya 2003; Rillig *et al.* 2019). AMF develop an extensive hyphal network through proliferating their hyphae inside plant roots (intracellular hyphae) as well as within the soil (extraradical hyphae), thus acting as a bridge between plant and soil (Jeffries *et al.* 2003; Smith & Read 2010; Rajtor & Piotrowska-Seget 2016). AMF mainly help their host plants exploiting poorly mobile ions (notably inorganic phosphate) that are beyond the root zone, in exchange for photosynthetic products from the host for metabolic needs (Smith & Read 2010). Besides assisting with resource uptake, AMF colonization can also benefit the hosts by enhancing their tolerance to abiotic and biotic stresses, such as drought, salinity, diseases and pathogens (Begum *et al.* 2019; Diagne *et al.* 2020). Indirectly, AMF can benefit the hosts via improving soil structure and soil aggregation (Rillig & Mummey 2006). Inoculation of AMF has been found to promote crop yield (Baum *et al.* 2015; Srivastava *et al.* 2015; Zhang *et al.* 2019), especially where the indigenous AMF communities have been degraded by agricultural practices (Jin *et al.* 2013; Manoharan *et al.* 2017).

A wealth of studies have shown that AMF and SOM can influence each other (Joner & Jakobsen 1995; Zhou *et al.* 2020). AMF are able to positively influence SOM content directly, through producing glomalin-related soil proteins (Wright & Upadhyaya 1996; Rillig 2004), which are significant components of SOM (Jones *et al.* 2004; Zhang *et al.* 2017). Additionally, AMF has been found to affect the decomposition of SOM negatively (Zhou *et al.* 2020) or positively (Paterson *et al.* 2016). On the other hand, various organic compounds released from the decomposition of SOM have been shown to influence AMF growth and activity, either positively (Gryndler *et al.* 2009) or negatively (Ravnskov *et al.* 1999). However, as far as we know, so far no studies ever clearly tested whether and how their effects on crop production interact. Furthermore, agricultural practices, in particular artificial fertilizer application, can influence the effects of both AMF (Bakhshandeh *et al.* 2017) and SOM (Oldfield *et al.* 2020) as it also influences nutrient availability of crop plants. It is therefore essential to take fertilizer inputs into consideration when test the interacting effects of AMF and SOM on crop production. Here, we examined (1) the combined effects of AMF inoculation and SOM content on the production of raspberry (*Rubus idaeus* L.) and (2) how they are affected by fertilizer application.

Materials and methods

Study system

Raspberry was used as the study crop, which is an important perennial fruit crop, with growing consumer interest due to its health benefits and flavours (Burton-Freeman *et al.* 2016; Giuffrè *et al.* 2019). We selected the commercial cultivar *'Tulameen',* as it is among the most popular raspberry cultivars in a range of climatic conditions (Aprea *et al.* 2009) and is locally available. The study was conducted in an experimental field of Wageningen University & Research in the Netherlands, from August 2019 to September 2020.

Experimental setup

We adopted a randomized complete block design, combining the following three crossed factors: (i) low SOM content vs high SOM content, (ii) AMF inoculated vs non-inoculated and (iii) four levels of fertilizer application. The SOM treatments were obtained by mixing different proportions of two types of sandy soils which had different SOM content (0.3% vs 4.6%) resulting in either 1.95% SOM content soils ('low SOM' treatment; available N: 14.0 mg/kg, available P: 0.6 mg/kg, available K: 19.4 mg/kg) or 3.96% SOM content soils ('high SOM' treatment; available N: 43.1 mg/kg, available P: 0.6 mg/kg, available K: 26.6 mg/kg). As for AMF treatments, we used *Rhizophagus intraradices* inoculum (MYKOS® Xtreme Gardening, Canada). Half of the original inoculum was autoclaved at 121 °C for two hours as sterilized inoculum for non-inoculated treatments (Changey *et al.* 2019). The four levels of fertilizer treatments represented the equivalent 0, 33, 66 and 99 kg ha^{-1} of N per year, ranging from no to optimum N inputs (Strik 2005). The fertilizer used was a compound fertilizer (CropSolutions Co., Perth, UK), containing 10.80% N, 13.44% K and 5.89% P.

We purchased 160 raspberry cuttings from a local supplier, with an average height of ca. 60 cm. To avoid the influence from the original peaty substrate, we carefully washed away the soil adhering to the roots in early August 2019. We added the recommended dose of AMF inoculum or an equal volume of sterilized inoculum evenly to the washed roots of the plants. The plants were then transplanted to a 10-litre plastic pot (upper diameter 28 cm, holes in the bottom for drainage but covered with cloth to minimize root growth out of the pot) and filled with low or high SOM soils according to the experimental design. However, higher than expected mortality occurred, possibly due to the cuttings being damaged during the roots washing process combined with the late summer heat. Only 56 plants survived out of the 160 plants, and 48 of them were of good health and thus were selected for further experimentation in three blocks. To carry out the experiment with sufficient replication, we additionally purchased another 160 raspberry cuttings in early October 2019. Because 60 cm cuttings were no longer available, we used plants with an average height of ca. 25 cm. Strictly following the earlier described protocol and using the same materials, the new batch of cuttings were washed, inoculated and transplanted into the low or high SOM soils. In this round, 110 out of the 160 new cuttings survived. These 110 plants were arranged into seven blocks. In total, the experiment therefore started out with 158 potted raspberry plants.

Plants were placed randomly in the field in a block arrangement, with one meter within and between rows. Pots were dug into the soil to protect the roots from extremely high or low temperatures. The fertilizer treatments were applied by splitting the annual dose (0, 33, 66 and 99 kg ha^{-1} of N) into three applications: the first one in the autumn, the second one at bud break in early spring of the following year and the last one at early flowering. All plants received equal and ample irrigation (depending on the weather conditions), and weeds in the pots were regularly removed. Prior to berry ripening, all plants were bagged with mesh bags to avoid predation by animals. We harvested and weighed the ripe berries when they had just turned bright red. We summed up the berry weight from the same plant to get the total yield and fruit number. Additionally, we carefully counted the wilted or aborted flowers that failed to develop into fruits, which in combination with the fruit number allowed us to estimate the flower number.

Data analysis

Until harvest, 41 plants from the first batch survived and developed fruits; all 110 plants from the second batch survived, but only 25 of them developed fruits. Since we mainly focus on the effects of treatments on production, only the plants that produced fruits were involved in the data analysis (sample size n=66). We ran separate linear mixed-effects models using the function lme() of the nlme package in R (R Core Team 2020) to study the interacting effects of SOM, AMF and fertilizer on flower number, fruit number, single berry weight (g/fruit) and total yield (g/plant), and included “block” as a random factor. We included the origins of the plants as a covariable in all models, to account for differences between plants from the first and the second batch. Because the fruit set followed a binomial distribution, we used the function glmmTMB() to run the same models assuming a binomial distribution (Brooks *et al.* 2017). Response variables were averaged per plant to avoid pseudoreplication and were transformed if necessary to meet the normality and homoscedasticity assumptions of the models.

Full models were simplified by removing non-significant predictors (backward elimination) using likelihood ratio tests with removal thresholds of $p > 0.05$, until the resulting minimum adequate model consisted only of variables that contributed significantly to the outcome (Zuur *et al.* 2009; Heinze & Dunkler 2017).

Results

The number of flowers per plant was only influenced by fertilizer inputs (Table 5.1). Plants receiving 99 kg N·ha^{-1} produced 32% more flowers than plants without any fertilizer inputs (Figure 5.1a). Similarly, fruit number and total yield per plant were only affected by fertilizer inputs (Table 5.1). The fruit number of the plants grown with the highest fertilizer inputs was 69% higher than that of plants receiving no fertilizer (Figure 5.1b). Increasing fertilizer inputs from 0 to 99 kg N·ha^{-1} increased yield from 25.7 g to 63.5 g (Figure 5.1c). SOM content or AMF inoculation did not affect these yield

Table 5.1 Effects of arbuscular mycorrhizal fungi (AMF; inoculated vs non-inoculated), soil organic matter (high vs low SOM content) and fertilizer application rates (0, 33, 66, 99 kg N·ha^{-1}·year^{-1}) on raspberry fruit production variables (n=66). Bold values represent significant effects ($P<0.05$).

	Flower number (sqrt transformed)		**Fruit set**		**Fruit number (ln transformed)**		**Single berry weight**		**Yield (ln transformed)**	
	$\chi^2_{(1)}$	**P**	$\chi^2_{(1)}$	**P**	$\chi^2_{(1)}$	**P**	$\chi^2_{(1)}$	**P**	$\chi^2_{(1)}$	**P**
AMF	0.185	0.667	1.614	0.204	0.232	0.630	2.070	0.150	0.005	0.943
SOM	0.601	0.438	22.136	**0.000**	0.936	0.333	1.304	0.254	0.137	0.711
Fertilizer	5.107	**0.024**	23.883	**0.000**	6.433	**0.011**	10.593	**0.001**	14.914	**0.000**
Origin	29.620	**0.000**	17.136	**0.000**	27.739	**0.000**	13.807	**0.000**	28.936	**0.000**
AMF:fertilizer	0.014	0.907	13.303	**0.000**	0.671	0.413	0.670	0.413	0.241	0.624
AMF:SOM	0.033	0.855	1.054	0.305	0.292	0.589	3.356	0.067	0.246	0.620
SOM:fertilizer	1.132	0.287	0.715	0.398	0.008	0.928	0.073	0.787	0.019	0.891
AMF:SOM:fertilizer	0.768	0.381	16.053	**0.000**	0.047	0.829	4.722	**0.030**	1.438	0.230

parameters, nor did they influence the effect of fertilizer (no significant interactions; Table 5.1).

A three-way interaction was found between the effects of AMF inoculation, SOM content and fertilizer inputs on fruit set (Table 5.1). In low SOM soils, the fruit set increased with increasing fertilizer inputs, for both inoculated and non-inoculated plants (Figure 5.2a). In high SOM soils, the fruit set of non-inoculated plants showed a positive relationship with fertilizer inputs, while the fruit set of inoculated plants was highest in unfertilized soils and decreased with increasing fertilizer inputs (Figure 5.2b).

There was also a three-way interaction between the three experimental variables on the single berry weight per plant (Table 5.1). In low SOM soils, the relationship with fertilizer application rate was much more pronounced for AMF inoculated plants than for non-inoculated plants (Figure 5.3a). In high SOM soils, single berry weight was consistently higher in AMF inoculated plants than in non-inoculated plants, although the difference seemed to decrease with increasing fertilizer inputs (Figure 5.3b).

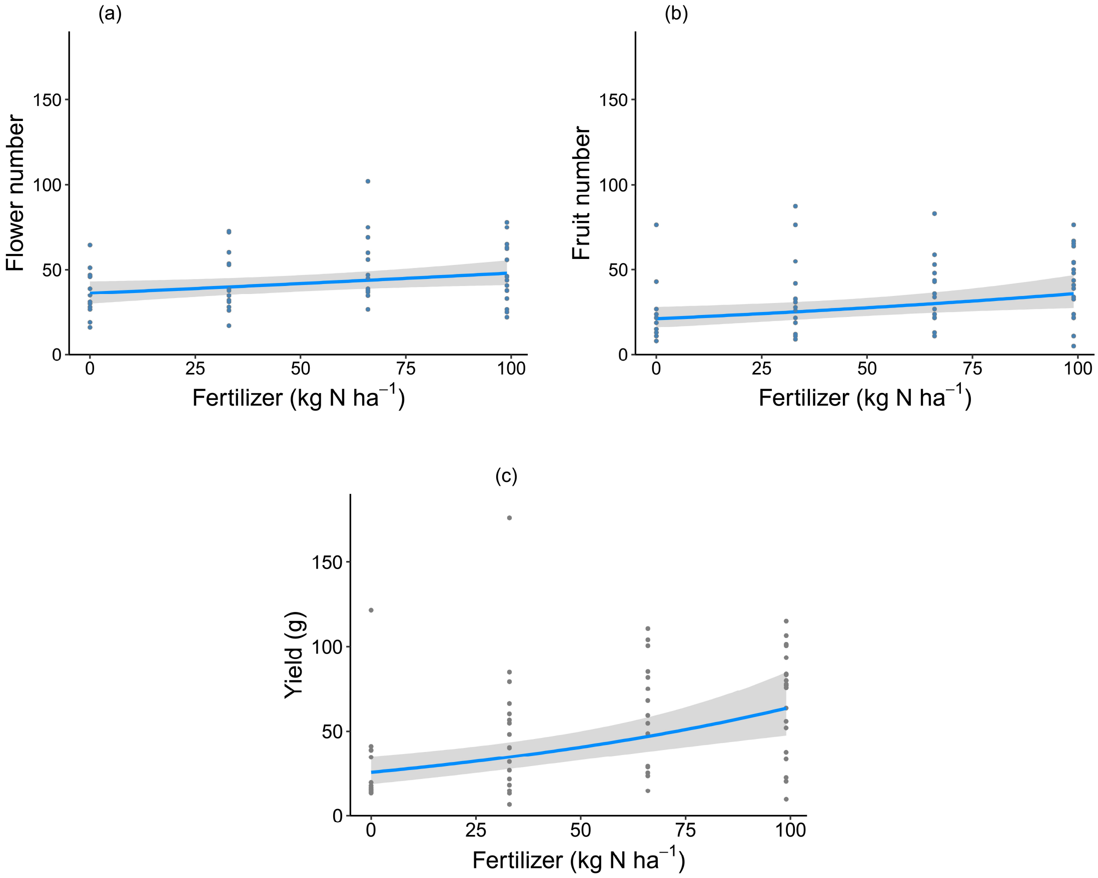

Figure 5.1 Effects of AMF inoculation, SOM and fertilizer application rates on flower number (a), fruit number (b) and yield (c) per plant. Graphs show predicted values of the minimum adequate models. Shadings show the 95% confidence interval, and points represent partial residuals.

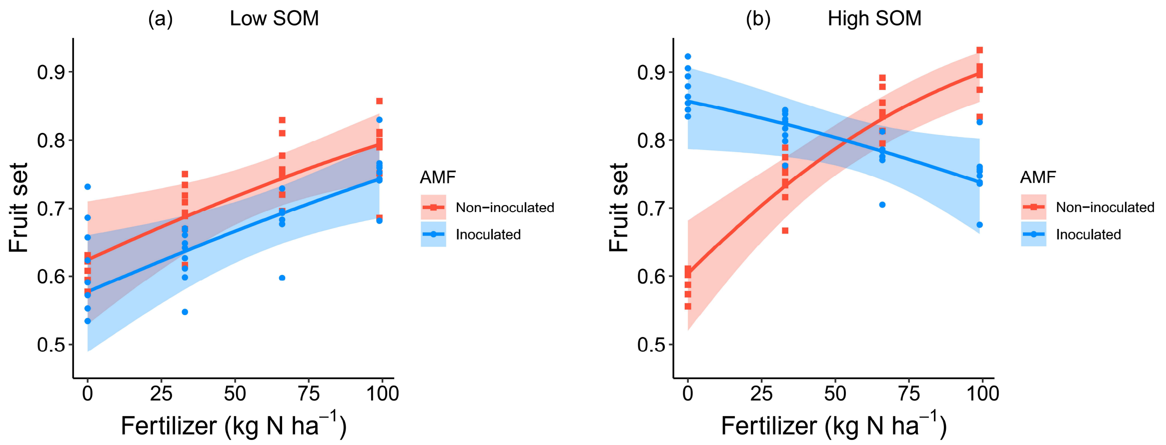

Figure 5.2 Interactive effects of AMF inoculation, SOM and fertilizer application rates on fruit set per plant. The lines are predicted by the minimum adequate model; shadings show the 95% confidence interval, and points represent partial residuals.

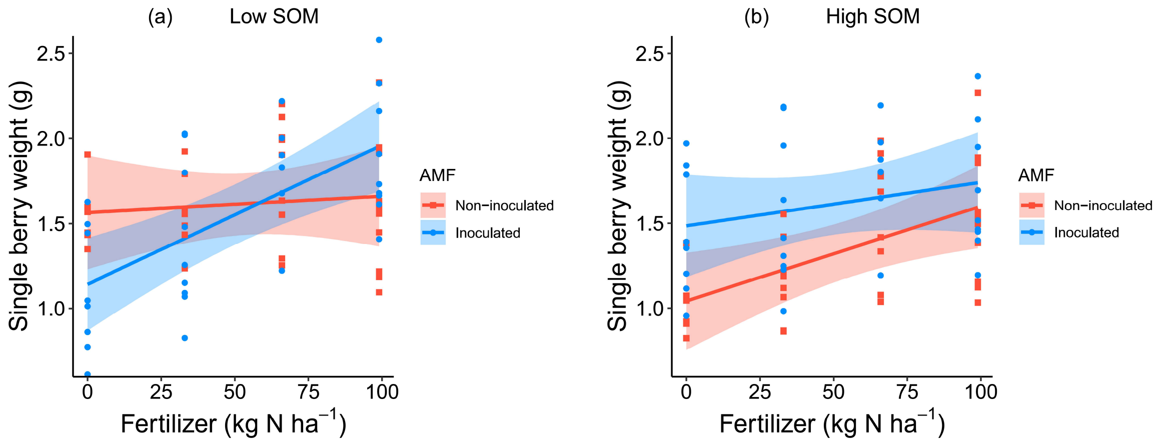

Figure 5.3 Interactive effects of AMF inoculation, SOM and fertilizer application rates on average single berry weight (g) per plant. The lines are predicted by the minimum adequate model; shadings show the 95% confidence interval, and points represent partial residuals.

Discussion

In this study, we found that the numbers of flowers and fruit, as well as the most important parameter from the perspective of farmers, yield per plant, were only driven by fertilizer inputs and were not significantly impacted by AMF inoculation or SOM content. The positive relation between fertilizer application and fruit set in AMF inoculated plants in low SOM soils, changed into a negative relation in high SOM soils.

Similarly, at low SOM the relation between fertilizer application and single berry weight was more pronounced in inoculated plants than in non-inoculated plants, but in high SOM soils it was the other way around. This suggests that the effects of AMF and SOM on these yield parameters cancel each other out and as a result did not contribute to the final yield.

At first glance, the lack of effects of AMF inoculation and SOM content on yield and flower number and fruit number, may seem at odds with results of earlier studies done using this same study system. For example, Chen *et al.* (2022) found significant positive effects of AMF inoculation on raspberry flower number, fruit number and yield. Furthermore, in chapter 2 of this thesis we found that SOM content was positively related to the yield of wild raspberry. However, the first study was only done at low SOM content levels (1.95%; the same as the current low SOM content treatment), while the second study was exclusively done in high SOM content soils (mean 7.4%, range 3.2-13.1%), and neither of these studies simultaneously manipulated both SOM content and AMF inoculation. Potential tradeoffs between the effects of the two factors on raspberry yield would therefore not become apparent in these studies. This is further supported by the fact that Figure 5.3a is almost an exact copy of Figure 4.3 in chapter 4. Both these graphs show the effects of fertilizer and AMF on single berry weight under the same low SOM content levels. Furthermore, in previous experiments we showed that part of the effects of AMF and SOM could be explained by their positive influence on flower visitation rate by pollinators (Chen *et al.* 2021; Chen *et al.* 2022). Because pollinators were not considered in this study, this may have left unexplained any potential indirect effects of AMF inoculation and SOM content on flower visitation rate and consequently the final yield.

In low SOM soils, AMF-inoculated plants produced smaller raspberries than the non-inoculated plants under low fertilizer inputs, while beneficial effects of AMF inoculation on berry weight only became apparent at adequate fertilizer inputs. One possible explanation for this is that under nutrient deficiency AMF have to compete for the limiting nitrogen for their hyphae development against the host plants, reducing the resources that host plants can allocate to fruit development (Reynolds *et al.* 2005; Wang *et al.* 2018). In high SOM soils and under low fertilizer inputs, AMF inoculation increased both single berry weight and fruit set compared to those of the non-inoculated treatments (Figure 5.2b; Figure 5.3b), likely because AMF could help acquire nutrients from soil organic matter to compensate for the effects of artificial inputs (Hodge & Fitter 2010). However, the benefits of AMF inoculation tended to decrease (Figure 5.3b) or even change into parasitic effects (Figure 5.2b) with increasing fertilizer application rate, a pattern found in previous studies as well (Smith *et al.* 2009; Hoeksema *et al.* 2010; Jin *et al.* 2017). The demonstrated tradeoff between effects of AMF inoculation and SOM content at different fertilizer application rates on berry weight and fruit set might explain why we didn’t observe any effect of these factors on flower number, fruit number and yield. The negative interaction could be explained by the cost-benefit relation between AMF and the host plants (Smith *et al.* 2009; Hoeksema *et al.* 2010; Jin *et al.* 2017). Host plants share up to 20% of total photosynthetic carbon with AMF, as the cost to maintain the symbiotic associations (Willis *et al.* 2013), while receiving mineral nutrients and other resources absorbed by AMF as the benefit (Smith & Read 2010). The cost-benefit relations vary from positive to negative, depending on the environmental context and the identity of AMF and the host plants (Cavagnaro *et al.* 2021). Under high fertilizer inputs and high SOM soils, the host plants might obtain adequate nutrients via their own root systems (Wu *et al.* 2005; El Kinany *et al.* 2019), which decreases the dependence on the assistance of AMF over nutrients acquisition. However, if the associated cost do not decrease, or less strongly, this may result in a net negative benefit which may explain the decreasing benefits of AMF for single berry weight and fruit set with increasing fertilizer levels in the present study. In addition, the decreasing benefits of AMF inoculation might also be explained by the direct suppressing effects of the host plants on AMF growth. When plants obtain sufficient nutrients and water via their own root system in high nutrients soils (high SOM and fertilizer in this study), they may suppress AMF development (Grman 2012).

Consequently, the suppressed AMF contributed less to production and this might indirectly constrain the benefits delivery of SOM since AMF can enhance the decomposition of SOM (Hodge *et al.* 2001; Gui *et al.* 2017).

Although our study is based on only one study in one crop species, it is the first one to explore the interactive effects of AMF and SOM under a range of fertilizer application rates. Our results provide an indication that the benefits of AMF and SOM on crop yield offset each other. This finding contributes to the understanding of the dynamic effects of AMF inoculation on crop production. For example, Yamawaki *et al.* (2013) found significant positive effects of AMF inoculation on turmeric (*Curcuma longa* L.) production under greenhouse conditions but no effects were found under field conditions, and they attributed the differing outcomes to the influence of indigenous AMF. However, the lack of beneficial effects of AMF inoculation under field conditions could also be caused by tradeoffs due to the interactive effects between AMF, SOM and fertilizer, according to our findings. Therefore, our findings may have important implications for applying AMF as biofertilizers in practical cropping systems, which has been increasingly proposed as a key solution for accomplishing sustainable agriculture (Igiehon & Babalola 2017; Basu *et al.* 2018). For example, when SOM content is high, inoculating AMF might not be such a good idea as when SOM content is low, unless with reduced fertilizer inputs. This study starts the exploration of the combined effects of AMF and SOM on raspberry production under several fertilizer inputs, and further research over a wider range of contexts (e.g. crop, soil type, climate, irrigation and fungicides) is needed to identify their interactive effects under real-world conditions.

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Chapter 6

General discussion

Introduction

Ecological intensification has been proposed as an alternative paradigm for current intensive farming systems, and aims to augment yield without extra environmental costs or aims to minimize negative impacts on the environment while sustaining crop yield (Bommarco *et al.* 2013; Kleijn *et al.* 2019). The implementation of ecological intensification relies on making intensive and smart use of ecosystem services, through managing diverse organisms that provide or regulate these services (Bommarco *et al.* 2013; Tittonell 2014; Motzke *et al.* 2015; Fijen *et al.* 2020). An increasing number of studies provide proof of concept for this paradigm (Petit *et al.* 2015; Pywell *et al.* 2015; Tamburini *et al.* 2016; Garratt *et al.* 2018b), but there are still considerable knowledge gaps that limit the adoption of ecological intensification by the agricultural sector (Kleijn *et al.* 2019). A good example of this is that the effects of multiple ecosystem services on crop production might interact with each other either negatively or positively (Garibaldi *et al.* 2018). So far, studies have studied the interactive effects of only a few combinations of ecosystem services (Garibaldi *et al.* 2018). Insect pollinators, soil organic matter (SOM) and arbuscular mycorrhizal fungi (AMF) can provide or influence key ecosystem services for agricultural production, but it is unknown whether and how their effects on crop production interact. In this thesis, I aimed to explore their combined effects and test whether their effects are influenced by fertilizer inputs. Firstly, I performed an investigation to study the relationships between SOM content and AMF colonization rate on berry production of wild raspberry populations (**chapter 2**), which could provide a benchmark for the importance of AMF and SOM on the production of the commercial varieties. In the next chapters, I performed pot-field experiments to test the interactions of the examined ecosystem properties and fertilizer inputs on raspberry (*Rubus idaeus* L.) production. I chose not to perform a single experiment involving all four experimental variables because the results of three- and four-way interactions are notoriously difficult to interpret. Therefore, I studied all combinations of two ecosystem services and fertilizer application in three separate experiments. In **chapter 3**, I tested the combined effects of insect pollinators, SOM content and fertilizer inputs on single berry weight, fruit number and yield of raspberry, following a randomized complete block design. Additionally, I surveyed the flower visitation rates, and analysed how these were related to SOM content and fertilizer inputs. Following the same experiment design, I tested the effects of insect pollinators, AMF inoculation and fertilizer inputs on the yield formation of raspberry in **chapter 4**. Response variables included flower number, fruit set, fruit number, single berry weight and yield. I explored the relationships between AMF inoculation and fertilizer inputs on flower visitation rates. Additionally, the AMF colonization rate of plants from different treatments were measured. In **chapter 5**, I explored the interactive effects of AMF inoculation, SOM content and fertilizer inputs on flower number, fruit set, fruit number, single berry weight and yield of raspberry, following the protocol of the previous two chapters for consistency. In the current chapter, I synthesize and discuss the results of the previous chapters.

Combined effects of the examined ecosystem properties on raspberry production

The results of this thesis suggest that the effects of AMF, pollinators and SOM on raspberry yield formation were predominantly additive. This is in line with the limited number of other studies that examine interactive effects among multiple ecosystem services, usually on annual crops (reviewed in (Bennett *et al.* 2009; Garibaldi *et al.* 2018; Tamburini *et al.* 2019)). For example, about 60% of the studies evaluating the interactive effects of regulating ecosystem service combinations (e.g., pollination and pest control) found additive effects (Garibaldi *et al.* 2018). My findings suggest that the outcome of managing these variables for bolstering yield can be rather predictable because the outcome is simply the sum of the benefits of the individual ecosystem services. Therefore, farmers might improve their crop production by managing ecosystem services simultaneously (e.g., insect pollination and SOM content according to **chapter**

3, insect pollination and AMF inoculation according to **chapter 4**) to fully benefit from these services.

The results of **chapter 5** seem to complicate such clear management prescriptions. Also in that chapter, no significant interaction was found between the effects of AMF inoculation and SOM content on raspberry yield production, which at first glance indicated their effects were additive. However, based on the results of **chapter 3 & 4,** in **chapter 5** positive effects on raspberry yield production were expected from AMF inoculation and SOM content. The lack of such effects of AMF inoculation and SOM content on yield, therefore, might indicate interactions exist. This was further supported by their offsetting interactive effects on fruit set and single berry weight, which both are important yield-related variables (**chapter 5**). The net benefits of AMF inoculation on these two yield parameters decreased with increasing fertilizer inputs at high SOM content soils (**chapter 5**). The reason for not detecting any significant interaction of AMF inoculation and SOM content on yield might be because the potential interaction has been masked by the profound effects of fertilizer inputs. Overall, results in **chapter 5** indicate that effects of AMF inoculation and SOM content on yield might interact, which deserves future research on this topic.

Fruit quality was found to be interactively affected by the experimental variables. In **chapter 3**, insect pollination, SOM and fertilizer inputs interactively influenced soluble solids content in a non-linear way. In **chapter 5**, SOM, AMF and fertilizer inputs interactively affected single berry weight, with patterns that implied trade-offs between their effects (Figure 5.2). Both single berry weight and soluble solids content are key physiological properties for fruit quality and significantly influence consumer choice and marketable value (Parker *et al.* 1991; Mauromicale *et al.* 2011). These findings indicate that practices to manage insect pollination, SOM content and AMF inoculation have the potential to increase fruit quality. However, it is difficult to formulate clear recommendations from this thesis for managing these ecological properties to improve fruit quality since the three-way interactions were complex. This thesis sheds some light on the combined effects of ecosystem services on fruit quality, but further studies are needed to see if more clear-cut patterns can be found which could translate into unambiguous management advice.

Increases in raspberry yield by insect pollination depends on fertilizer inputs

The results of the experimental studies showed beneficial effects of insect pollination on raspberry yield that were influenced by fertilizer inputs. In **chapter 4**, the yield of pollinated raspberry plants increased more strongly with fertilizer application rate than the yield of plants from which pollinators had been excluded. A possible explanation for the synergistic effects starts with the positive effect of fertilizer on flower number, which increased the number of flowers that can potentially developed into fruits after pollination. Insect pollination generally enhances the transfer of pollen for ovule fertilization (Sáez et al. 2020), but the development of the fertilized ovules into fruits requires significant amounts of nutrients (Bos *et al.* 2007; Tamburini *et al.* 2019). In the absence of fertilizer inputs, pollination benefits on fruit set were negligible (**chapter 4**), suggesting that nutrient availability limited the potential benefits of insect pollination to develop additional fruits (Garratt *et al.* 2018a).

In **chapter 3,** insect pollination and fertilizer inputs independently influenced raspberry yield, indicating that their effects are additive, in agreement with some previous studies (Tamburini *et al.* 2019). In addition, their effects on yield near-significantly interact in a non-linear pattern (P=0.051; Supplementary figure 3.1), so that the net benefits of insect pollination (i.e. the differences between open pollinated and bagged plants) were highest at the intermediate fertilizer inputs level. The resource limitation mechanism mentioned in the previous paragraph might also explain the low benefits of pollination under low fertilizer input (Bos *et al.* 2007; Tamburini *et al.* 2019). On the other hand, the benefits of pollination for crop production decreased at high fertilizer inputs. This phenomenon can be explained by the fact that the plants in the pollinator excluded treatments may allocate the same amount of resources to a smaller number of fruits to produce heavier fruits, thus compensating for the lack of pollination (Marini *et al.* 2015).

The combined effects of insect pollination and fertilizer inputs on raspberry production found in **chapter 3** and **chapter 4** were different. Previous research also found different results of the combined effects of insect pollination and fertilizer inputs on crop production, which varied from negative, additive, unimodal to positive with the underlying mechanisms still being largely unclear (Garibaldi *et al.* 2018; Tamburini *et al.* 2019). In this thesis, the different results might be driven by the different factors that were simultaneously manipulated, SOM in **chapter 3** and AMF in **chapter 4**. Both SOM and AMF can influence soil nutrient availability in subtly different ways (Krull *et al.* 2004; van der Heijden *et al.* 2015); therefore, they might also influence the effects of fertilizer inputs in different ways. In other studies, the use of different crop species might also contribute to the inconsistent results, for example, because different crops respond differently to fertilizer inputs (Greenwood *et al.* 1980; Weih *et al.* 2011). In general, my studies found consistent beneficial effects of insect pollination on raspberry production, although the benefits of pollination service were moderated by fertilizer inputs. The results highlight the importance of ensuring sufficient insect pollination services remain available to maximize crop yield for safeguarding food security.

The beneficial effects of AMF are shaped by plant growth conditions

AMF are obligate symbionts, and they depend on photosynthates delivered by the host plants (Smith & Read 2010). In exchange, AMF provide or regulate various ecosystem services such as the uptake of resources (Smith & Read 2010) and thus they are usually considered to be beneficial for agricultural production (Smith & Read 2010). However, in **chapter 2**, single berry weight and branch yield of wild raspberry were negatively related to AMF colonization rate, probably due to the severe dry and hot weather conditions. Although previous studies mainly found positive relations between AMF and drought or heat (Mathur *et al.* 2018; Chareesri *et al.* 2020), some other studies also found negative effects of AMF on crop production under such conditions (Bryla & Duniway 1997; Martin & Stutz 2004). One possible explanation for the negative relations could be a shift in cost-benefit relations under the stressed conditions, where the benefits provided by AMF decreased and the host plant's cost of associating with AMF might outweigh the advantages (Bryla & Duniway 1997; Martin & Stutz 2004). An alternative or additional explanation could be that the effects of AMF are species-specific; that some AMF species contribute more to host plants than other species (Taylor & Harrier 2000; Martin & Stutz 2004). I did not identify the AMF species in **chapter 2** and cannot exclude the possibility that these were predominantly antagonistic species. In the following experimental studies I therefore used a commercial inoculum composed of a single species *Rhizophagus intraradices* for AMF treatments in **chapter 4&5**. This AMF species has been proven as a beneficial one for many crop plants, including raspberry (Taylor & Harrier 2000; Fracasso *et al.* 2020; Stoffel *et al.* 2020). Nevertheless, because these studies were carried out under real-world conditions, even the experimental plants could have been colonized by wild AMF species which could have different relations with raspberry.

In **chapter 4**, AMF inoculation significantly increased flower number, fruit number and yield. This indicates that the inoculation of AMF has the potential to contribute substantially to raspberry production in this study system. In **chapter 5,** however, following the same experimental setup as **chapter 4** but testing the interactive effects of AMF and SOM, AMF inoculation did not influence any yield relevant parameters. This might be because the effects of AMF on these yield parameters had been offset under high nutrient conditions (high SOM content and fertilizer inputs). This trade-off hypothesis is supported by the negative interacting effects on single berry weight and fruit set (**chapter 5**). Two mechanisms can be used to explain the negative interaction: the cost-benefit relation theory and suppressing effects of the host plants on AMF growth. Under conditions with adequate resources, such as the high fertilizer inputs and high SOM soils in **chapter 5**, host plants may be able to take up sufficient nutrients via their own root systems instead of depending on additional absorption by AMF (Wu *et al.* 2005; El Kinany *et al.* 2019), but AMF nevertheless requires photosynthetic products from the host for metabolic needs (Smith & Read 2010). This may have resulted in trade-offs between experimental variables on the single berry weight and fruit set. Another possible mechanism for explaining the interactions can be the direct suppressing effects of the host plants on AMF growth. In high nutrients soils, host plants might suppress AMF development by reducing carbon allocation (Grman 2012). The two possible mechanisms are somehow exclusive, and future studies are needed to identify the underlying mechanisms.

Next to the previous mentioned variables, the efficiency of AMF inoculation is also largely shaped by the native AMF communities (Pellegrino *et al.* 2011; Köhl *et al.* 2016). In **chapter 4**, I found no significant difference between AMF colonization rate of the AMF inoculation or fertiliser treatments at the end of the experiment. The findings indicated that all plants in the experiments had been well colonized by either inoculated AMF or indigenous AMF in the soils, about 12 months after the inoculation. The beneficial effects of AMF inoculation might therefore be due to the fact that AMF inoculated plants have a head start with AMF colonization in the early stage of the plant development. For example, this could explain why flower number was higher in AMF inoculated plants than non-inoculated plants in **chapter 4**. Even though the AMF colonization rate was the same between treatments, the species composition of AMF could be different, and thus their effects on yield might be different (Marro *et al.* 2020). This thesis did not identify the AMF species composition and therefore cannot contribute to the understanding of the relationships between inoculated AMF species and indigenous AMF species, which are currently less studied (Pellegrino *et al.* 2011; Köhl *et al.* 2016). Furthermore, the effects of AMF inoculation on crop production can be complicated by the widespread practice of pesticides use in conventional raspberry production (Gollotte *et al.* 2008a; Łozowicka *et al.* 2012), even though AMF colonization can potentially increase the resistance of crops to fungal diseases (Gollotte et al. 2008b; Smith & Read 2010). Giovannetti *et al.* (2006) found that pesticides (including fungicides, insecticides and herbicides), even at much lower concentrations than those indicated for use, negatively influenced the spore germination and mycelial growth of the studied AMF species. In this thesis, however, I did not apply or study the effects of any pesticides. Therefore, it remains unknown whether and how pesticides influence the combined effects of AMF with concomitant factors, such as SOM and fertilizer inputs. Overall, before making significant investments in AMF inoculation as a component of ecological intensification, more studies are needed to elucidate the interactions between inoculated AMF and the surrounding conditions.

The effects of SOM on raspberry production

SOM is an important ecosystem property that influences a series of ecosystem services that underpin crop production, including nutrient availability and water-holding capacity (Krull et al. 2004; Liu et al. 2006). However, the effects of SOM content on crop yield vary. Some studies find positive effects (Pan *et al.* 2009; Oldfield *et al.* 2020) while others find no effects of SOM content (van Gils et al. 2016; Hijbeek et al. 2017). In **chapter 2**, SOM content showed positive relationships with the single berry weight and branch yield of wild raspberry. Considering the atypical drought stress during the investigation, the positive effects might largely be due to the water holding capacity services regulated by SOM. In the field experiments, I did not find significant effects of SOM on yield formation in either **chapter 3** or **chapter 5**. This finding is consistent with the results of van Gils *et al.* (2016), who used an experimental design that was similar to my own, with potted plants that were irrigated with equal and sufficient water. The adequate irrigation could offset the functioning of SOM on water-holding capacity and thus weaken the delivery of benefits of SOM on the final yield. Furthermore, my study used a perennial crop species, complicating comparisons with other studies that were mostly done on annual crops (Pan *et al.* 2009; van Gils *et al.* 2016; Oldfield *et al.* 2020). Perennial species may allocate resources for vegetative growth rather than reproduction (Ehrlén & Van Groenendael 2001; Langley *et al.* 2002). Therefore, the reproduction of perennial species may be less responsive to relatively short-term changes in local conditions (SOM treatments in this case only lasting 12 months) than annual species.

It seems that this thesis cannot provide convincing evidence for enhancing SOM content in agricultural soils as an efficient intervention for achieving ecological intensification. Nevertheless, I did find beneficial effects of SOM on flower visitation rate and single berry weight in **chapter 3**, probably due to the micronutrients released through SOM mineralization and other benefits provided by SOM (Poveda *et al.* 2005; Fageria 2012). Besides, SOM content interactively influenced soluble solids content (**chapter 3)**, fruit set and single berry weight (**chapter 5**), with other experimental variables. In summary, these findings suggest that the effects of SOM might contribute to raspberry production, but they are less pronounced and more subtle than the effects of insect pollination, AMF inoculation or artificial fertilizer.

Concluding remarks and future directions

This thesis found no interacting effects of AMF, pollinators and SOM on raspberry yield formation. However, I found some evidence that the effects on some yield-related variables and fruit quality of ecosystem services interacted with fertilizer application. This thesis also provided information for evaluating the individual effects of these ecosystem services on raspberry production. Insect pollination should be the most important component for achieving ecological intensification among the three tested ecosystem properties, since insect pollination showed consistent positive effects on raspberry production. Insect pollination contributed to increasing raspberry yield by 33% compared to the non-pollinated plants (**chapter 3**), and the increment can reach 90% at the highest fertilizer input level (**chapter 4**). AMF inoculation could also be a potential option for ecological intensification, while it might be less important than insect pollination. In **chapter 4**, AMF inoculation increased yield by 43% compared to the non-inoculated treatments, in line with some previous studies (Sadhana 2014; Igiehon & Babalola 2017). However, the beneficial effects of AMF inoculation could be varying and largely influenced by several factors, e.g., SOM and fertilizer inputs (**chapter 4**), climatic stresses (**chapter 2**) and other variables (Köhl *et al.* 2016; Hage-Ahmed *et al.* 2019). Therefore, it requires further studies to identify the relationships between the effectiveness of AMF inoculation and the surrounding conditions. SOM might be the least promising component for ecological intensification. In the experimental chapters, SOM content did not show positive effects on yield formation under conditions with ample water (**chapter 3&5**), while SOM content related positively to wild raspberry production under summer drought stress **(chapter 2)**. Besides, SOM content influenced some fruit quality traits (**chapter 3&5**). Therefore, SOM might potentially sustain or enhance crop yield in real-world conditions where plants are exposed to water stress (Kane *et al.* 2021), but the results in this thesis do not provide direct support for advocating SOM as an essential practice for ecological intensification. This thesis confirms the importance of fertilizer inputs for maximising production and shows that the benefits of fertilizer inputs can be additive or even synergistic to the benefits of the examined ecosystem services on raspberry yield (**chapter 3&4**). Interestingly, if anything the results of my thesis suggest that the benefits of ecosystem services might be less profound under low fertilizer inputs (e.g. the interactions presented in Figure 3.1a, Figure 4.1, Figure 4.2c&d, and Figure 4.4a). Possibly, at low fertilizer inputs, nutrient limitation reduces the benefits of ecosystem services on agricultural production. Therefore, decreasing fertilizer inputs for the sake of the environment, one of the possible goals of ecological intensification, might not be in the best interests of farmers. On the other hand, reducing the application of other artificial inputs that are harmful to the ecosystem service providing species, e.g., insecticides and fungicides, may not only contribute to the environment but may also contribute to crop yield by enhancing ecosystem services such as insect pollination, AMF colonization and biological pest control.

More practical field studies over a wider range of contexts (e.g. different crops, soil types, climate, irrigation and fungicides) are needed to test the combined effects of ecosystem services, as studies in this thesis are based on only one crop species and in one region. Additionally, longer-term field trials are necessary to evaluate the effects of ecosystem services, since those from AMF and SOM, for example, are apt to become more substantial over longer time periods, especially for perennial crops (Augé 2004). Studies are also needed to explore the underlying mechanisms. In this thesis, I mainly focused on the effects of the multiple ecosystem services, while the exact mechanisms that can explain the trends and patterns have yet to be elucidated. For example, it remains unknown what has incurred the trade-offs between AMF inoculation and SOM content on raspberry yield. Furthermore, it is essential to evaluate the costs and benefits for building such a new paradigm of ecological intensification (Kleijn *et al.* 2019). For farmers, the net economic benefits of adopting biodiversity-enhancing practices would be a major motivation to change their day-to-day practices. This thesis has explored some of the benefits of managing ecosystem services for agricultural production but hasn't addressed the costs of doing so. This represents an interesting and important topic for future research.

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Summary

Summary

Future agriculture faces a twofold challenge: increasing yields to safeguard food security while simultaneously reducing its environmental impact. Ecological intensification has been proposed as a solution to this challenge. It entails the integration of ecosystem service management into farming practices to enhance or maintain yields while minimizing artificial inputs and environmental externalities. An increasing number of empirical studies have shown beneficial effects of ecosystem services on crop production, but the majority of these studies have focused on the effects of only a single ecosystem service in isolation. In contrast, in real-world agricultural systems, crop yield is shaped by several ecosystem services simultaneously. These different ecosystem services may interact (positively or negatively) and their interactions may furthermore depend on agricultural management practices. To assess the potential of ecological intensification in promoting yields and reducing external inputs, it is essential to understand the combined effects of different ecosystem services on agricultural production. The aim of this thesis was to examine whether and to what extent different ecosystem services interactively shape crop yield, and whether and how this is affected by agricultural management. Focusing on raspberry (*Rubus idaeus* L.), a globally important perennial fruit crop, I examined the potential interactive effects of the ecosystem services provided by insect pollinators, arbuscular mycorrhizal fungi (AMF) and soil organic matter (SOM) on the quantity and quality of raspberry production, and whether these effects were influenced by artificial fertilizer inputs.

Natural relatives of commercial raspberry are widespread in temperate forests. These natural relatives depend entirely on several ecosystem services for survival and reproduction. Therefore, investigating the effects of ecosystem services on natural raspberry production would be informative to fully understand their importance, and could act as a benchmark for commercial raspberry production. In **chapter 2,** I set out to test the relationships between SOM content and AMF colonization rate on natural

raspberry production. I found that under the examined natural conditions, effects of AMF colonization rate and SOM content did not interact but instead independently affected wild raspberry production. AMF colonization rate was negatively related to berry weight and yield, possibly because of the unusually hot and dry weather during the study period, which may have resulted in unbalanced cost-benefit relationships between AMF and the host plants. Conversely, SOM content was positively related to single berry weight and branch yield of wild raspberry, possibly due to the positive effects of SOM on the water holding capacity of the soils. These results suggest that under low input conditions, maintaining or enhancing SOM content can make a significant functional contribution to raspberry production.

In **chapter 3,** I performed a pot-field experiment to unravel the combined effects of pollinators, SOM content and fertilizer inputs on raspberry production. The experiment followed a randomized complete block design, with the experimental plants being exposed to two levels of SOM content (low vs high), two levels of insect pollination (open pollination vs pollinators excluded) and four levels of fertilizer applications. Insect pollination and fertilizer application independently increased single berry weight and yield. SOM content increased single berry weight, and also affected soluble solids content of fruits. The latter effect depended on the effects of insect pollination and fertilizer inputs though (i.e. three-way interaction), with positive effects of SOM being most pronounced at intermediate fertilizer levels and when pollinators were excluded. Total yield was not affected by SOM content. Although SOM content enhanced the visitation rate of pollinators, no interaction was found between the effects of insect pollination and SOM content on production. The positive effects of pollination and SOM content on the quality and/or quantity of raspberry production suggest that these ecosystem services can be key components in the implementation of ecological intensification in this cropping system.

In the next chapter, I explored the combined effects of insect pollinators, AMF inoculation and fertilizer inputs on raspberry production (**chapter 4**). A randomized block design was adopted with three crossed factors: insect pollination (open pollination vs pollinators excluded), AMF inoculation (AMF inoculated vs non-inoculated) and four levels of fertilizer applications. I found synergistic interactions between insect pollination and fertilizer inputs on raspberry fruit set and fruit number, and consequently on fruit yield. AMF inoculation significantly increased flower number, fruit number and yield, independently from insect pollination and fertilizer application. AMF inoculation furthermore had indirect effects on insect pollination through increasing pollinator visitation rate under intermediate fertilizer levels. Single berry weight was influenced interactively by AMF inoculation and fertilizer application, with a much more pronounced positive response of AMF inoculation under high fertilizer inputs. Results in this chapter indicate that the effects of insect pollination and AMF inoculation on raspberry production can be synergistic or additive to the effects of conventional management practices (i.e. fertilizer application).

In **chapter 5,** following the same experimental design as the previous two chapters, I conducted an experiment to explore the interactive effects of AMF inoculation, SOM content and fertilizer inputs on raspberry production. I found that only fertilizer inputs significantly increased flower number, fruit number and yield. However, AMF inoculation, SOM content and fertilizer inputs interactively influenced fruit set and single berry weight (three-way interactions). In low SOM soils, fruit set increased with fertilizer inputs for both AMF inoculated and non-inoculated plants, but in high SOM soils, the fruit set of AMF inoculated plants decreased with fertilizer inputs. In low SOM soils, the single berry weight of AMF inoculated plants increased more pronouncedly than the non-inoculated plants when the fertilizer inputs were high. In high SOM soils, the net benefits of AMF inoculation on single berry weight decreased with increasing fertilizer inputs. These results indicate that the beneficial effects of AMF and SOM can potentially be offset by each other, particularly under high fertilizer input levels.

The results of these studies show that, although some yield relevant parameters depended on complex three-way or non-linear two-way interactions between ecosystem services (provided by insect pollinators, AMF and SOM) and fertilizer application, effects of ecosystem services on raspberry yield are mainly additive, and generally complement the effects of agricultural inputs. Results of this thesis also demonstrated the individual benefits of the examined ecosystem services. Comprehensively, insect pollination should be the most important ecosystem service for raspberry production to be maintained in this system, followed by AMF inoculation and then the SOM content. Additionally, this thesis highlighted the importance of fertilizer inputs in maximizing production, as in the low inputs of fertilizer, the benefits of ecosystem services disappear. A general conclusion would be that insect pollination, AMF inoculation and SOM content have potential to be managed as components of ecological intensification, and fertilizer inputs are essential to sustain their benefits. However, before implementing management to enhance these ecosystem services in real-world farming systems, more studies are needed to test their effects on other crops in the broader context, to explore the underlying mechanisms as well as to evaluate the net economic benefits.

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About the Author

Ke Chen was born on May 28, 1991, Leshan, Sichuan province, China. In the year 2010, he left his village to start the undergraduate study at Southwest University in Chongqing city. He majored in landscape gardening and enjoyed many interesting courses and activities.

After graduation, he continued his master's studies at Southwest University from 2014 to 2017, supervised by Prof. Xiaohong Yang. He was involved in several research projects during this phase of study, including testing the beneficial effects of arbuscular mycorrhizal fungi (AMF) on mulberry growth, identifying the sex of Ginkgo biloba by using molecular and morphological methods, and evaluating the adaptability of four woody plants against winter flooding and summer drought.

Since 2017, he moved to the Netherlands to pursue his PhD in Wageningen University under the supervision of David Kleijn (promoter), Jeroen Scheper (daily supervisor) and Thijs P.M. Fijen (daily supervisor). He worked on testing the interacting effects of pollinators, arbuscular mycorrhizal fungi (AMF), soil organic matter (SOM) and fertilizer inputs on raspberry production through conducting field experiments and forest investigation.

Publications

Chen K., Fijen T. P., Kleijn D. & Scheper J. (2021). Insect pollination and soil organic matter improve raspberry production independently of the effects of fertilizers. Agriculture, Ecosystems & Environment, 309, 107270.

Chen, K., Kleijn, D., Scheper, J. & Fijen, T. P.. (2022). Additive and synergistic effects of arbuscular mycorrhizal fungi, insect pollination and nutrient availability in a perennial fruit crop. Agriculture, Ecosystems & Environment, 325, 107742.

Chen K., Scheper, J., Thijs P.M. Fijen & Kleijn D.. Potential tradeoffs between effects of arbuscular mycorrhizal fungi inoculation, soil organic matter content and fertilizer application in raspberry production. (Under review).

Chen K., Scheper, J., Thijs P.M. Fijen & Kleijn D.. Arbuscular mycorrhizal fungi and soil organic matter affect wild raspberry production oppositely under heat and drought. (In progress).

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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)

- How arbuscular mycorrhizal fungi (AMF), soil organic matter (SOM) and pollination interactively affect crop production

Writing of Project proposal (4.5 ECTS)

- Interactive effects of soil quality and pollination on soft fruit production

Post-graduate courses (5.9 ECTS)

- Bugs at your service: fundamentals and application of arthropod-mediated ecosystem services; PE&RC/SENSE (2019)
- Advances in intercropping: principles and implementation; PE&RC (2021)
- Design of experiments; WIAS/PE&RC (2017)
- Linear models; PE&RC/SENSE (2018)
- Generalized linear models; PE&RC/WIMEK (2019)
- Mixed linear models; PE&RC/WIMEK (2019)

Deficiency, refresh, brush-up courses (2.1 ECTS)

- Basic statistics; PE&RC/SENSE (2017)
- Introduction to R for statistical analysis; PE&RC/SENSE (2018)

Laboratory training and working visits (1.5 ECTS)

- Soil biology lab skills course for assessing soil functions; Soil Biology Group of Wageningen University (2021)

Competence strengthening / skills courses (3.5 ECTS)

- Speaking skills; Wageningen in'to Languages (2017)
- Reviewing a scientific paper; WGS (2019)
- Scientific writing; Wageningen in'to Languages (2019)
- Careers after PhD; online seminar; WGS (2021)

Scientific integrity/ethics in science activities (0.6 ECTS)

- Scientific integrity; WGS (2018)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.7 ECTS)

- PE&RC First years weekend (2017)
- PE&RC Day (2017, 2018, 2019)
- PE&RC Midterm weekend (2019)
- PE&RC Last years weekend; online (2020)

Discussion groups / local seminars or scientific meetings (5.7 ECTS)

- R Users meeting (2017)
- Wageningen Evolution & Ecology Seminar (WEES) (2017-2018)
- Workshop on serious gaming for participatory research (2018)
- Plant-Soil feedbacks symposium-linkages between root traits and soil biota (2019)
- Current themes in ecology biodiversity in crisis perspectives on how to bend the curve (2019)
- What artificial intelligence and robotics could and couldn't offer for rethinking agricultural systems (2021)
- Re-design of (agro)-ecosystems (2021)

International symposia, workshops and conferences (4.8 ECTS)

- Netherlands annual ecology meeting; the Netherlands (2020)
- Scandinavian association for pollination ecology conference; online (2020)
- Netherlands annual ecology meeting; online (2021)

BSc/MSc thesis supervision (4 ECTS)

- How do pollination, arbuscular mycorrhizal fungi (AMF) and fertilizer interactively affect raspberry production
- Optimizing the potential of raspberry production Understanding the interacting effects of soil organic matter, fertilizer, and insect pollination on raspberry production

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