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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.

1. 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.,

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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., 2018), 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 and Weil, 2004; Garratt et al., 2018), and hereafter we refer SOM as an ecosystem service. SOM contributes to plant growth and productivity, through its effects on soil properties (Williams and 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., 2018) 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. Fig. 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 (Fig. 1a). At intermediate fertilizer levels, pollination and SOM content can both be positively related to crop yield but no longer influence one another (Fig. 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 (Fig. 1c). The hypothetical patterns in Fig. 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).

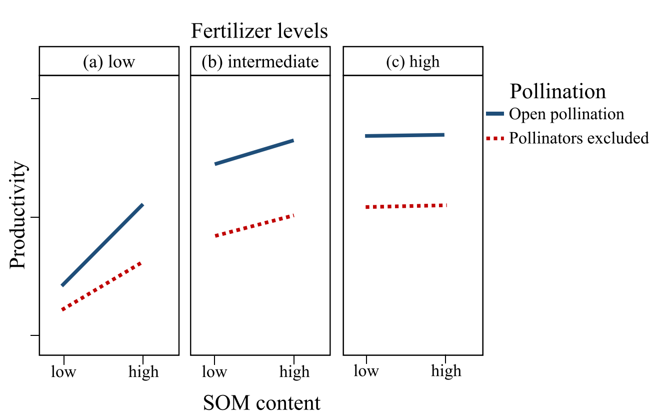

Fig. 1. Conceptual diagram illustrating potential interactive effects of soil organic matter (SOM) and pollination on crop productivity under low, intermediate and high fertilizer input levels.

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 and 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 exclusion) 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.

2. Materials and methods

2.1. 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 and 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 1999–2018 (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.

2.2. 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 × 65 cm 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 and Isaacs, 2014).

Four contrasting levels of fertilizer were applied to plants. The levels varied from 0, 33, 66 and 99 kg 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).

2.3. Pollinator observations

From May 20th to June 17th (the blooming period), we used the focal point observation method to determine pollinator visitation rate (Fijen and 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.

2.4. 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 fruit 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.

2.5. 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 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, significance of fixed effects was determined by backward model simplification using likelihood ratio tests. For the final results, we reached minimum adequate models through selecting only the parameters whose effects were significantly different from zero ($P < 0.05$). 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).

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 %).

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 1, Fig. 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 1). Single berry weight was significantly affected by all three manipulated factors: SOM, pollination and fertilizer inputs (Table 1, Fig. 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

Table 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. All χ2 and P values were calculated through likelihood ratio tests by comparing the full model with the reduced model. For final results, only minimum adequate models including only the significant effects were used. 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**

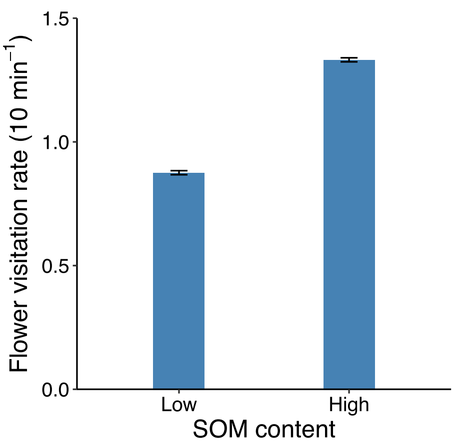

Fig. 2. Effects of soil organic matter (SOM) content on flower visitation rate (number of visits per 10 min) of raspberry. Results represent the predicted back-transformed values from the minimum adequate model. Error bars show ± 1 S.E.

fertilizer inputs (Table 1, Fig. 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 1, Supplementary Fig. 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 1, Fig. 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 (Fig. 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. 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 1).

4. Discussion

We found positive effects of insect pollination and artificial fertilizer inputs on raspberry single berry weight and yield. SOM content enhanced 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

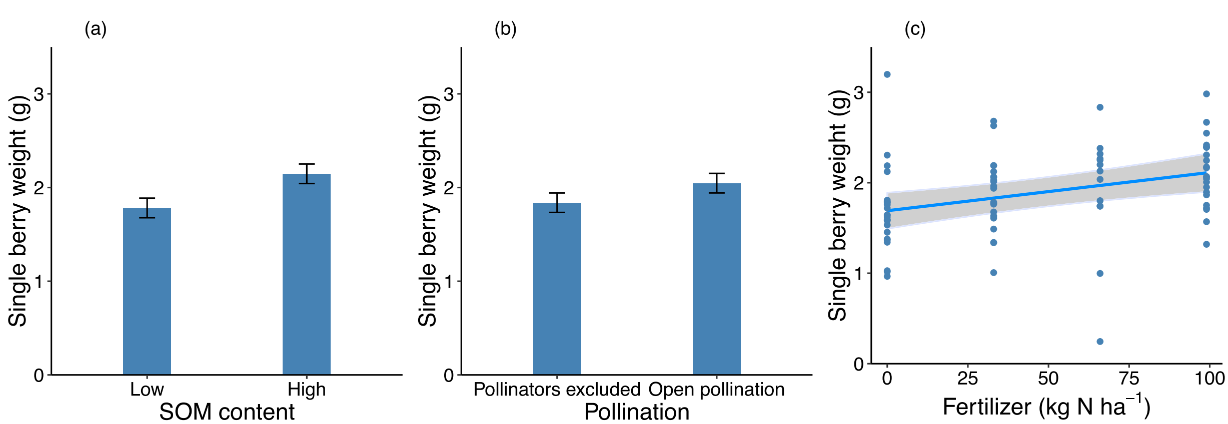

Fig. 3. Effects of a) soil organic matter (SOM) content, b) pollination and c) fertilizer application rate on per plant single berry weight. Graphs show the predicted effects based on the minimum adequate model; error bars show ± 1 S.E; the grey shading shows the 95 % confidence interval. Plotted points in (c) represent partial residuals.

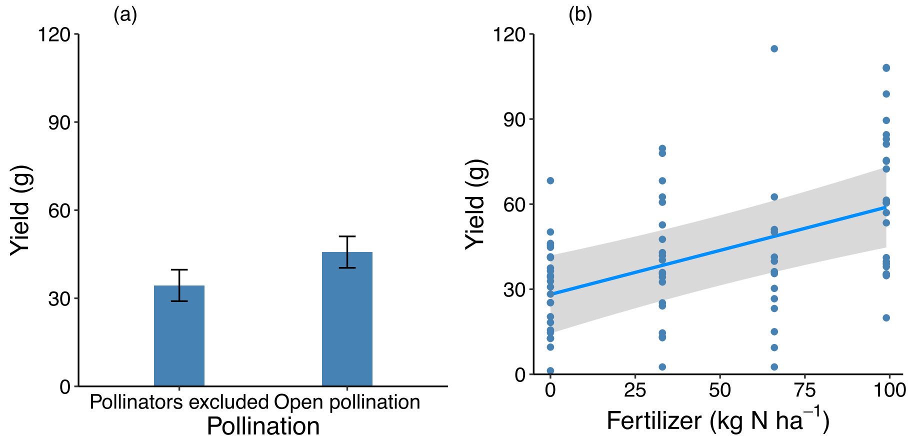

Fig. 4. Effects of a) pollination and b) fertilizer application rate on per plant yield. Graphs show the predicted effects based on the minimum adequate model; error bars show ± 1 S.E; the grey shading shows the 95 % confidence interval. Plotted points in (b) represent partial residuals.

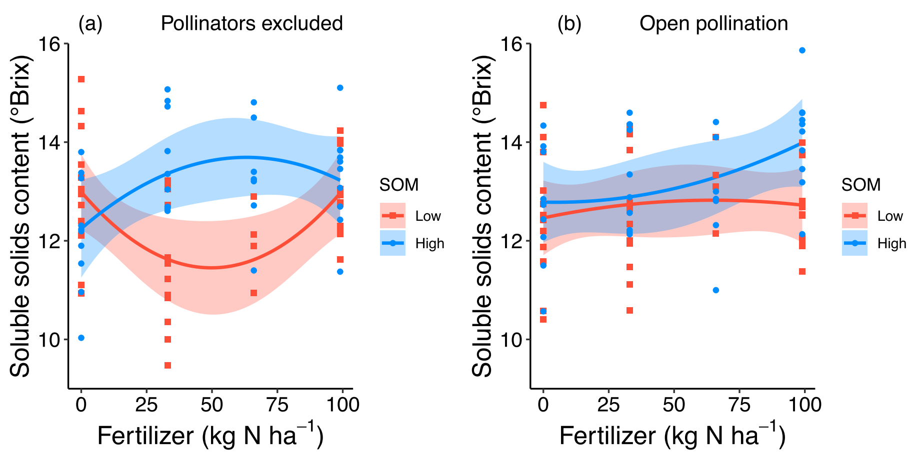

Fig. 5. Interactive effects of soil organic matter (SOM) content and fertilizer application rate on per plant soluble solids content under a) pollinators excluded and b) open pollination. Graphs show the predicted effects based on the minimum adequate model; the blue/red shadings show the 95 % confidence interval. Plotted points represent partial residuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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 and 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 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 and 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 and 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 Fig. 1). We failed to find any clear effects of pollination on sugar content (Table 1, Fig. 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 and 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 Fig. 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 improve 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. By growing plants in pots in an open field, we were able to standardize the soil properties in the treatments while allowing for natural pollination levels and pollination behaviour. We think our experiment is representative for full-field conditions because we have used agronomically realistic treatment levels but acknowledge that effect sizes may differ in real-world situations as they are then also influenced by other agronomic management practices and local environmental conditions. For the main yield variables, we found only additive linear relationships with the examined production variables (i.e. Fig. 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 and 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.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.107270.

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