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Experimental quantification of insect pollination on sunflower yield, reconciling plant and field scale estimates

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Abstract

Most crops grown in Europe, including sunflower (*Helianthus annulus L.*), benefit from insect pollination. However, valuing this benefit is not straightforward since estimates of the increase in sunflower yield vary from 18% to 100%. Most estimates have, moreover, been performed at plant scale, a scale that is not relevant for farmers who calculate at the field scale. In this four-year study, we quantified the contribution of insect pollination to sunflower yield at field and plant scales in working farm fields distributed along a gradient of pollinator diversity and abundance. Pollinators were found to increase field yield up to 40% (i.e. 0.7 t/ha) and by 31.3% at plant scale; the magnitude of effect on yield being therefore similar at both scales. The pollinators increased the yield by increasing the number of fertilized seeds per plant with no significant effect on the unit mass of the seeds although there was a trade-off between number of seeds and unit mass. Among pollinators, honeybees were the main taxon impacting sunflower yield. Sunflower

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plant density was a strong determinant of yield, with higher numbers attracting increased numbers of honeybees. Using pollinator and wind exclusion, we finally quantified the relative contributions of self-pollination (~40%), insect pollination (~35%) and wind pollination (~20%). Our results show, to the best of our knowledge, the first evidence of the key role of pollinators in sunflower production at field scale in real farming conditions, and underscore the need to maintain suitable conditions for pollinators in agricultural landscapes.

Keywords:

Agroecology, Pollination service, Honeybee, Wild pollinators, Biodiversity, Wind pollination, Sunflowers, Self-pollination

Introduction

Ecological intensification of agriculture has been promoted as a way of reducing chemical inputs by relying on pest control and pollination as ecosystem functions, rather than on agrochemicals (Bommarco, Kleijn, & Potts, 2013). Up to 70% of crops and 35% of agriculture production depend on insect pollination (Klein et al., 2007), which involve domesticated pollinators such as honeybees (*Apis mellifera*) and many wild bees (Garibaldi et al., 2013; Kleijn et al., 2015). However, recent declines of wild bees and honeybees and in the same time the increase of pollinator-dependent crop area may lead to pollination limitation at continental scale (Aizen & Harder, 2009). Despite strong evidence that pollinators are critical for agriculture production, insect pollination is still ignored in the selection of farming practices, farming systems and crops (Chen, Zhang, Liu, & Yu, 2017; Misganaw, Mengesha, & Awas, 2017). One reason may be a mismatch between the viewpoints of farmers, who measure yield at the field scale, and researchers who quantify the contribution of insect pollination at small field part (Garibaldi et al., 2016), plant scale (Bartomeus et al., 2014), or even smaller scales (Bos et al., 2007; Garibaldi et al., 2013). Such scales may not accurately account for the capacity of crop plants to compensate for a pollination deficit (Bos et al., 2007), nor for within- nor between field differences in yield (Kayad et al., 2016). An estimate of the contribution of pollinators will only be meaningful for farmers if the yield is measured at field scale along a pollinator gradient (Vaissière, Freitas, & Gemmill-Herren, 2011). Such studies are rare (Gaines-Day & Gratton, 2016; Lindström et al., 2016).

Sunflower (*Helianthus annulus L.*), the major oil seed crop in Europe (FAOSTAT, 2014), is highly dependent on pollinators whose contribution to yield is controversial and has been estimated from 18% up to 100% (i.e. doubling the yield). The dependence of the yield on pollinators has been found to be weak (Degrandi-Hoffman & Chambers, 2006; Tamburini, Lami, & Marini, 2017), medium (Aslan, Yavuksuz, & Asian, 2010) or strong (Carvalho et al., 2011; Garibaldi et al., 2016, Greenleaf & Kremen, 2006). The variability may be the result of the different methods that were used to estimate the pollinators' contribution, e.g. by using cages enclosing pollinators (Aslan et al., 2010), which may enforce plant-pollinator interactions seldom occurring naturally (Banda & Paxton, 1991), or by using experimental

fields, which may not be representative of farming practices (Degrandi-Hoffman & Chambers, 2006; Tamburini et al., 2017). Direct estimates of the contribution of insect pollination to sunflower yield in working farm fields are very rare (Carvalho et al., 2011, Garibaldi et al., 2016; Greenleaf & Kremen, 2006), and rarely consider the effect of plant density (Garibaldi et al., 2016, Carvalho et al., 2011; Greenleaf & Kremen, 2006). The variability may also be due to the pollinator metrics used: honeybee abundance (Greenleaf & Kremen, 2006; Pisanty, Klein, & Mandelik, 2013), wild pollinator diversity (Carvalho et al., 2011), wild bee abundance (Greenleaf & Kremen, 2006; Hevia et al., 2016) or total pollinator abundance (Garibaldi et al., 2016). Variability also arises from the method of estimating the pollinators' contribution: flower pollination success (Carvalho et al., 2011; Greenleaf & Kremen, 2006) or seed mass (Carvalho et al., 2011). These metrics do not take into account the trade-off between number of seeds and their unit mass (Tamburini et al., 2017). At the field scale, yield is the product of plant density, unit mass and number of seeds per flower head. The latter depends on pollination success, with the head diameter (Marinkovic, 1992) being a confounding variable since its average has been reported as decreasing with plant density (Ibrahim, 2012) while larger heads attract more pollinators (Pisanty et al., 2013). Finally, sunflower is also pollinated by self-pollination (Javed & Medhi, 1992) and wind pollination, though their contributions have never been differentiated (e.g. Degrandi-Hoffman & Chambers, 2006; Javed & Medhi, 1992).

Our study aimed to fill those gaps by assessing the effect of insect pollination on yield at plant and field scales in real farming conditions over four years using a single experimental design. Yield may depend on both pollinator abundance and diversity, which are very different between fields. In our study, fields were selected along gradients of landscapes with different densities of semi-natural habitats, meadows and organically farmed fields to ensure a wide range of pollinator abundances and diversities (Kennedy et al., 2013). In each field, we performed pollination exclusion experiments to determine the effectiveness of each type of pollination. We first assessed, at plant and field scales, the relationships between the various components of yield to test for potential trade-offs, especially between plant density, head diameter, number of seeds per head and seed unit mass. Then we investigated whether pollinators

(wild or domestic measured as abundance or diversity) increased yield at field and plant scales, accounting for the trade-offs identified. Finally, we evaluated the relative contributions of insect pollination, wind pollination and self-pollination on sunflower yields for a range of pollinator abundances and diversities.

Materials and methods

Study area and field selection

Pollinator exclusion experiments and farm surveys were conducted between 2013 and 2017 in the Long-Term Social Ecological Research site (LTSER) “Zone Atelier Plaine & Val de Sèvre”, a 450 km² study site located in the south of *Deux-Sèvres* district, central western France (Bretagnolle et al., 2018). Sunflower represents about 10% of the agricultural area. Experiments were conducted directly in working farm fields. Each year, we randomly selected 40 to 60 1 km² squares in the LTSER distributed along three gradients of landscape features: semi-natural habitats (hedges and forest fragments), meadows and organically farmed fields. All these landscape features have been shown to strongly influence pollinators (Kennedy et al., 2013). We used a moving window to select the squares (see Fahrig et al., 2011 for the procedure used) to minimize inter-gradient correlations. Within each square, one sunflower focus field was then selected when present. On average, the sunflower fields were 350 m apart (102 m to 1250 m). Field size ranged from 0.3 ha to 20.7 ha (mean 5.8 ha).

A first set of 97 sunflower fields (17, 5, 38 and 37 in 2013, 2014, 2015 and 2016 respectively) was used for an empirical assessment of pollinators on sunflower yield at the field scale. A second set of 67 fields (23, 27 and 17 from 2015, 2016 and 2017 respectively), of which half were also in the first set, was used for pollinator exclusion experiments. The two sets differed because not all farmers accepted either surveys or experiments in their fields in 2015 and 2016 (i.e. years where both surveys and experiments were carried out). No field was used in two different years. Experiments were carried out on 44 different hybrid restored varieties of sunflower, all of which were self-fertile. Most fields were cultivated using conventional practices (98%), the others were organically farmed.

Measurement of yield components

Information on farming practices (fertilizers, pesticides, sowing density, crop variety) and yield, for the 97 fields of the first set, were collected at the end of each cropping season by farm surveys. In 2016 and 2017, just prior to harvest, the number of sunflower plants was measured in 1 m² quadrats in the field border and at 15 m and 75 m from the field edge. Plant density at field scale was then estimated by averaging the number of plants over the three quadrats. Sunflower plants (see below) were collected five days before harvest. In the laboratory, head diameter (in mm) was measured twice and averaged for analyses and then heads were stored in individual bags and left into a heat chamber at 60 °C for 48 hours. Seeds were removed mechanically from the heads, and fertilized seeds were separated from empty seeds (arbitrary threshold of 9 mg) by seed density with a wind machine (Batteuse petites graines, ATID, France). Then fertilized seeds were counted twice with a seed counter (Contador 2, Pfeuffer, Germany). The repeatability between the two measurements was extremely high (less than 0.1% difference), so we used the average. Total seed mass (using only fertilized seeds) was measured (nearest 0.1 mg) and three individual fertilized seeds were randomly chosen and weighed to provide the individual unit mass (average of the three weights).

Pollination exclusion experiments

In 2015, twenty sunflower plants were selected per field in rows of four plants at five different positions (see Appendix A: Table 1). In 2016 and 2017, only 12 sunflowers were selected at three positions (see Appendix A: Table 1). The distance from the edge was studied because insect pollination in sunflower has been shown to decrease with distance from the edge (Hevia et al., 2016). The four plants within a row were chosen to be at the same growth stage (same overall height, same head flower diameter). Each plant within a given row was subject to a different treatment to estimate the contributions of large pollinators (LP), small pollinators (SP), wind pollination (W) and self-pollination (SF) at plant scale. We also used manual (hand) pollination (HP) in some rows, with or without insect pollinator exclusion, to estimate the effect of bagging sunflower heads (Wragg & Johnson, 2011).

Within each row, one plant was used as a control (see Appendix A: Table 1) with the flowers accessible to all pollination processes (LP+SP+W+SF). The head of a second plant was bagged with a coarse mesh (3 mm mesh size), preventing large insect pollination but allowing small pollinators (SP), wind-pollination (W) and self-pollination (SF), while a third plant was bagged with a fine mesh bag (0.6 mm mesh size; see Appendix A: Table 1), preventing all insect pollination (W+SF). The fourth plant on the row was treated differently depending on the year and plant position. Experimental design evolved throughout years to improve our ability to estimate pollinator contribution. Thus in 2015, it was bagged with osmolux, a tissue allowing only gas exchange thus excluding all types of pollination but self-pollination (SF). In 2016, only in the plant in middle position (15 m) was bagged with osmolux (Table 1): one of the other two was left open and hand-pollinated (HP) with pollen from a neighbouring plant, while the other was bagged with fine mesh and hand-pollinated. The bagged plant was either at the edge (0m) or the centre of the field (75m) depending on the field. In 2017, the fourth plants in the edge (0m) and centre rows (75m) were bagged with osmolux, while in the row 15 m from the edge, the fourth plants were left open and hand pollinated and a fifth plant was bagged with osmolux and hand pollinated (see Appendix A: Table 1). The plants were bagged at the very beginning of flowering and removed after the last flowers faded, to avoid the bag affecting seed formation. Sample size varied slightly because some sunflower plants died and some bags, especially osmolux, were found open or torn.

Pollinator sampling

We sampled the pollinators using two complementary methods. First, they were trapped in pan traps (Westphal et al., 2008). Traps were made of plastic bowls of 12 cm diameter and 10 cm deep filled with c. 600 ml of water with drops of soap and left in the field during 4 days per field. Pan traps were three different colours (yellow, blue or left white) to catch pollinators by colour preference (Westphal et al., 2008): The traps were mounted on wooden stakes, at vegetation height (Westphal et al., 2008) and installed during summer to cover the sunflower flowering period (~15th June to 22th August). Given that bees, in particular honeybees but also bumblebees and at least some wild bees (Zurbuchen et al., 2010), forage over large distances, we used other surrounding fields as well as the focal field to estimate the

pollinator community around the focal field, thus avoiding dilution, spillover or attraction effects (Holzschuh et al., 2016). Traps were set in fields with sunflowers, maize and meadows. The number of fields surveyed at a given buffer distance was quite variable (e.g. 1-10 fields at 2000 m from the focal field), with on average 28.8 pan traps in 4.0 fields. As pollinator abundance and diversity did not differ statistically between field edge and field core for 2013-2015 (see Appendix B: Fig. 1), the sampling effort was reduced to the field core with three pan traps placed twice during the season in 2016 and 2017.

We used sweep-nets to complement the pan trap catches, since pan traps may underestimate some pollinators, e.g. the honeybee (Westphal et al., 2008). In 2015, we swept two transects per field: one at the edge and one in the field core. In 2016 and 2017, we swept three transects: at the edge, 20 m from the edge and in the field core. All transects were 50 m in length and lasted 10 minutes measured with a chronometer to ensure approximately equal sampling effort. Honeybees were visually counted and other pollinators were caught by sweep net. We stopped the chronometer each time an insect (except for honeybee) was caught to remove it from the sweep-net, identify it visually or put it into a tube for later identification. We swept the transects between 8.30 am and 5.30 pm, when the air temperature was above 15°C and the weather sunny.

Professional entomologists identified all insects caught in the pan traps at genus level for wild bees and species level for hoverflies. For 2015-2017 only, we identified bees at species level. Abundance per guild was obtained by a hierarchical average procedure, starting with mean count per bowl colour and position in the field (core vs. edge), then averaging for each position in the field, and finally for each field. This was then averaged across all other fields within a radius of 2000m from the 25st of June (day 175 of year) to the 30th of August (day 240), covering the full sunflower flowering period each year. The diversity was represented either at genus or species level. A similar procedure was used for the sweep net captures. See Appendix B. for a description of the pollinator community.

Statistical analyses

We used linear models or linear mixed models (LM or LMM) for all analyses. As we expected sunflower yield to be influenced by farming practices, we first assessed the effects of these practices on

yield. Unexpectedly, none of the farming practices analysed here significantly affected yield (see Appendix D, Table 2), hence they were not included further in the analyses. We then analysed the relationships between yield (and various components of yield such as average head diameter), plant density (only available in 2016 and 2017) and sowing density. In these models, we accounted for the effects of year and distance to edge as fixed effects. Distance to edge was nested within the field ID, and the field ID was nested within year to account for the sampling design (the distance to edge varied between years and no field was sampled in different years). Next we investigated yield, with head diameter, plant density and year as explanatory variables.

We also examined the relationship between yield components at plant scale to check for potential trade-offs. Since the head diameter was expected to be the main driver of plant yield, we analysed relationships between head diameter and three plant yield components: the number of fertilized seeds, the seed mass and total seed mass per head. We used an asymptotic model to test whether the yield components saturated with head diameter. We used LMM since the field ID was included as a random effect as repeated measurements (i.e., different individual plants) were available for each field. Distance to edge and year were also included as fixed effects.

To analyse the effects of pollinators on yield at field scale, we built several LMs with different pollinator metrics, year and their interactions as explanatory variables. Pollinator metrics included pollinator diversity (bees and hoverflies at genus and species levels), abundance per guild (honeybees estimated by sweep nets, and wild bees, hoverflies and bumblebees estimated by pan traps) and total abundance. We used regression to quantify the effects of various pollinator metrics on yield, with the confidence interval obtained from the standard error of the model estimates, and compared the predicted yield at the lowest and highest values of each pollinator metric. The most relevant pollinator metric (honeybee abundance, see results) was then used to determine which sunflower plant yield component was most influenced by insect pollination. We built LMs with each sunflower yield component (number of fertilized seeds, seed unit mass and seed mass per m²) as successive dependent variables, and honeybee abundance, distance to edge, year and the interactions with honeybee abundance as explanatory variables.

Given the predominant effect of sunflower head diameter, it was added as an covariate in the model. To explore whether number of pollinators could saturate or decrease yield, we confronted a linear, a saturation and a humped model to analyse how sunflower yield changes with pollinator abundance. The asymptotic model, i.e. saturation hypothesis, was found to be the best model (see Appendix E: Table 3) and therefore kept for the analysis.

To quantify the contribution of the different types of pollination, we used the number of fertilised seeds per head as a measurement of yield per plant for each experimental treatment, since this parameter was the most affected by pollinator abundance. The use of bags as an exclusion treatment may potentially bias the estimated yield. We performed a preliminary analysis to check for such bias and corrected the number of fertilised seeds to take into account the effects of the bias. (see Appendix C). We then estimated the contribution of wind pollination as the difference between the number of fertilized seeds (corrected values) in the treatment which excluded all pollinators and the treatment allowing only self-pollination (SF). The contribution of pollination by small insects was estimated as the difference in the number of fertilized seeds between the large pollinator exclusion treatment and the treatment which excluded all pollinators, while the contribution of pollination by large insects was the difference in the number of fertilized seeds between the control and the large pollinator exclusion treatment. The overall pollinator contribution was the sum of the small and large pollinator contributions. In a few cases, the difference in the number of fertilised seeds between treatments was negative. Since a negative contribution cannot theoretically exist, negative values were arbitrarily set to 0 when the magnitude of the negative contribution exceeded 10% (see Appendix C). Setting these negative contributions did not affect the result (see Appendix C: Fig. 5). Differences in the contribution of the different types of pollination processes were tested using an LM and post-hoc test, with pollination type, year and their interaction as explanatory variables.

In all analyses, pollinator metrics, head diameter and plant density were used as predictor variables and were $\log(x+1)$ transformed. Honeybee abundance and seed mass per m² were $\log(x+1)$ transformed when used as response variable to meet normality and homoscedasticity assumptions. All

analyses were performed with R software (R Core team, 2015), using the “stat” package for linear models, multiple stepwise regression and post hoc tests and “lmerTest” for linear mixed models (Kuznetsova, Brockhoff, & Christensen, 2014). F and p-values of the LM (LMM) models are provided when we were interested in the relevance of the model for all variables. Otherwise, these values are provided only for the variable of interest.

Results

Components of sunflower yield at field and plant scales

Sunflower yield in farmers' fields was on average 2.08 tons.ha⁻¹ (range: 0.9-3.0, $n = 97$), and did not statistically differ between years (LM, $F_{3,93}=1.87$, $p = 0.14$). We observed a high variation of average head diameter between fields (17.7 cm, range 12.0-25.4), a smaller variation between years but not with distance from the edge of the field (Table 1). Average head diameter was not correlated with plant density (only available in 2016 and 2017) when accounting for year and distance to edge ($F_{6,116}=0.57$, $p = 0.45$). Sunflower plant density was also highly variable between fields (7.6 plants/m², range 3.3-10.7), slightly higher in 2017 than in 2016 (8.5 vs. 7 plants/m²) and increasing, though not significantly, from edge to core (Table 1). Plant density was unrelated to sowing density and other farming practices (see Appendix D: Table 3). Similar results were found for head diameter (see Appendix D: Table 3). Finally, field yield did not depend neither on plant density ($F_{1,20} = 2.49$, $p = 0.13$) in 2016 nor on head diameter ($F_{1,34}=0.072$, $P=0.48$) in 2015-16.

At the plant level, strong and positive relations were found between head diameter and number of fertilised seeds, seed mass, or seed mass per head (LMM, *all* $p < 0.001$, Figs 1A, B and C). The relationships were saturating for the number of fertilized seeds and head diameter and between seed mass per head and fertilized seeds (Fig. 1). At the plant level therefore, larger heads yielded higher total seed mass (Fig. 1C), but there was also a trade-off between the number of fertilized seeds and seed mass (Table 2, Fig. 1D) after accounting for head diameter.

Effect of pollinator abundance and diversity on sunflower yield at field and plant scales

Sunflower yield at field scale was significantly increased by honeybee abundance, for both pollinator sampling methods (Figs 2A and C; see Appendix E, Table 2 for statistical tests), with saturation for sweep net data. The yield increase between fields with the lowest honeybee abundance (0.5 honeybees/transect for sweep net) to fields with the highest abundance (116 honeybees/transect) was 41.1% (confidence interval: 30.5- 51.2), i.e. 0.72 t/ha yield increase. A similar value was obtained with pan trap data (41.5% increase, 30.4-52.6, Fig. 2C). Given that the abundance data were obtained within a predefined buffer zone and period (2000 m radius and 65 days), we checked the robustness of our results with varying spatial and temporal ranges: at 2000m, the relationships were independent of the time, but the relationships were slightly weaker with a smaller buffer zone (see Appendix E: Table 1). Among the other pollinators, only hoverflies increased sunflower yield, though this was due to 2013, a year where hoverflies were particularly abundant (Fig. 2E; see Appendix E: Table 2). Wild pollinator abundance and richness (either at genus or species levels), did not contribute to yield (Fig. 2B and D; see Appendix E: Table 2). According to our results, therefore, among bees, only honeybees affected sunflower yield at field level.

Next, we investigated which plant yield components were influenced by honeybee abundance. At the field scale, honeybee abundance was not correlated with average head diameter ($F_{1,65} = 1.19, p = 0.28$) for 2015-16-17, but honeybee abundance was correlated with plant density ($F_{1,42} = 4.52, p = 0.039$, Fig. 1E) for 2016-17. At plant scale, accounting for head diameter as a proxy of total number of flowers, honeybee abundance was significantly correlated with the total number of fertilized seeds per head ($F_{1,218}=32.45, p < 0.001$) with an increase of 31.3% between fields with 0.5 honeybees/transect and 116 honeybees/transect. This relationship was unaffected by distance of the plant from the edge (interaction between honeybee and plant position: $F_{1,218} = 0.2, p=0.65$). As expected from the trade-off between the number of seeds and the individual seed mass (Fig. 1D), seed mass was negatively correlated with honeybee abundance although not significantly ($F_{1,217} = 3.56, p = 0.06$). However, the positive correlation between honeybee abundance and total number of fertilized seeds was strong enough to result in a

positive correlation with seed mass per m² with a similar saturation to that observed for the yield at field scale ($F_{1,116} = 14.82$, $p < 0.001$, Fig. 1F). Overall therefore, honeybees increase yield by increasing the number of fertilised seeds per plant.

Pollination processes and their contribution to yield

The contributions of the various pollination processes were significantly different ($F_{4,312} = 58.36$, $p < 0.001$). Self-pollination (42.5%) and insect pollination (34.5%) were the main contributors to the number of fertilized seeds per head (Fig. 3A). Large pollinators (30.6%) such as honeybees and bumblebees had more effect than small pollinators (4% Fig. 3A). Only 23% of pollination was attributed to the wind (Fig. 3A). The contribution of insect pollinators varied between years (18% in 2015, 43% in 2016 and 2017), as did that of other pollination processes ($F_{2,312} = 4.85$, $p = 0.008$) except small pollinators (interaction year-pollination process, $F_{8,312} = 14.15$, $p < 0.001$). Honeybee abundance (sweep net data) varied between years (2.44, 22.7 and 29.8 honeybees/transect, $F_{2,64} = 43.32$, $p < 0.001$), and this variation significantly affected the insect contribution ($F_{1,64} = 35.4$, $p < 0.001$, Fig. 3B). The contribution of pollinators was marginally correlated with the yield estimated at field scale ($F_{1,35} = 3.5$, $p = 0.07$, Fig. 3C), confirming that the increase of pollinators contribution at plant scale resulted in an increase in yield at field scale.

Discussion

Our study revealed that honeybees were the main insect pollinators involved in sunflower yield: increasing honeybee abundance improved the relative contribution of insect pollination over other pollination mechanisms, and increased the number of fertilized seeds independently of head diameter. Barros et al. (2004) also found that yield increased with the number of seeds per m² rather than seed unit mass. Seed unit mass decreased non-significantly with increasing honeybee abundance, as a consequence of the trade-off between the mass and the number of fertilized seeds (see Barros et al., 2004; Tamburini et al., 2017). Such a trade-off is commonly found in plants (Jakobsson & Eriksson, 2000). Our results also showed that plant density was positively correlated with honeybee abundance, while head diameter was

not, suggesting that honeybees were more attracted by plant density than plant size. Moreover, higher honeybee abundance still increased seed mass per m² independently of the plant density. Increasing plant density was also shown to increase yield at field scale, in agreement with Barros et al. (2004). Plant density is thus a key feature that must be considered when studying sunflower yield and our results show that the contribution of pollinators cannot be directly estimated from pollination success or seed unit mass on their own.

Sunflower head diameter was found to be a strongly correlated with the number of fertilized seeds and seed mass (see also Marinkovic, 1992). Allowing for the effect of head diameter, we found that pollinators had a positive effect on the number of fertilised seeds, which was potentially due to the increase of pollination success with increasing pollinator abundance (Carvalho et al., 2011; Greenleaf & Kremen, 2006; Pisanty et al., 2013), though this was not directly estimated in our study. Honeybee abundance was not positively correlated with head diameter, contrary to Pisanty et al. (2013), who studied the difference of honeybee visits between small and large flowers within the same field. Surprisingly, we found that plant density was not negatively correlated with head diameter unlike Ibrahim (2012) who found a negative correlation for plant densities between 4.5 and 9 plants per m². Another study found that the number of flowers per head did not decrease with increasing density above about four plants per m² (Villalobos, Sadras, Soriano, & Fereres, 1994), well below the average value (7.6 plants per m²) we found in our study. Possibly, as there was no correlation between fertilizer dose and yield, soil resources were not limiting for sunflower development in our study. In addition, we found that fields with a high density of plants had a higher pollinator abundance, being probably more attractive due to an increase in resource availability, as found for other plants (Delmas, Fort, Escaravage, & Pornon, 2016).

The contribution of insect pollination was about the same level as self-pollination, confirming that modern hybrid sunflowers are highly self-fertile unlike wild sunflower species (Gandhi et al., 2005). Other studies found similar results of 26% to 70% (Degrandi-Hoffman & Chambers, 2006; Javed & Medhi, 1992). Since we estimated a relative contribution we cannot determine if the self-pollination rate is fixed or varies with pollinator abundance, which may increase the outcrossing rate (Wang, Yamasue,

Itoh, & Kusanagi, 1998). We also found that wind pollination contributed about 20%. Our study is the first to differentiate wind pollination from self-pollination. Wind pollination has not previously been demonstrated for sunflowers as they are particularly adapted to insect pollination (Wojtaszek & Maier, 2014). Wind tunnel experiments would be necessary to confirm our result (Cresswell & Osborne, 2004).

The increase in yield was up to 40%, close to the pollinators' contribution estimated at plant scale (34.5%), i.e. an additional gain of 0.7 t/ha, slightly depending on the method used to sample the pollinators (pan traps or sweep nets). This estimate is, to our knowledge, the first at field scale. It demonstrates the essential role of pollinators for farmers, though it is slightly lower than the increase in yield estimated from plant (Carvalho et al. 2011) or small-scale experiments (Garibaldi et al., 2016). In plant scale studies, the increases were 47% to 74% similar to Garibaldi et al. 2016, depending on the sunflower variety. However, in the study by Carvalho et al. (2011), wild bees had a positive effect. Other studies performed in farm fields have evaluated the pollinator contribution for male sterile lines (Greenleaf & Kremen, 2006) where pollination entirely depends on insects or wind. Finally we found that sunflower yield depended mainly on honeybee abundance, as already shown (Aslan et al., 2010; Degrandi-Hoffman & Chambers, 2006; Greenleaf & Kremen, 2006; Pisanty et al., 2013). Hoverflies were found to improve yield in only one out of the four years. Although such effect has not been found in other studies, hoverflies are known to pollinate other oil crops, such as oilseed rape (Jauker & Wolters, 2008). Wild bees were sometimes found to affect sunflower yield indirectly, by increasing the efficiency of honeybees in pollinating sunflower (Carvalho et al., 2011; Greenleaf & Kremen, 2006), but the direct effect of wild bees was found to be fairly low compared to that of honeybees (Greenleaf & Kremen, 2006; Pisanty et al., 2013).

In conclusion, although the importance of insect pollination for sunflower production has been already demonstrated using exclusion experiments (Aslan et al., 2010) or experimentally managed fields (Degrandi-Hoffman & Chambers, 2006; Tamburini et al., 2017), our study is the first to quantify how insect pollination effects at plant scale translate into yield at field scale under real farming conditions. In

addition, our results show that sunflower yield can be increased through the management of honeybee hives and plant density without resorting to agrochemicals.

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Appendix A-E. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXX.

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Table 1. Summary statistics of linear models investigating variation of plant density and head diameter for field, year and plant position. *P*-value significant ($p < 0.05$) are bold.

	Head diameter (N = 227)		Plant density (N = 123)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year	7.75	< 0.001	3.75	0.06
Plant position	2.85	0.01	2.99	0.09
Year \times Field ID	2.21	< 0.001	1.75	0.04
Field ID \times Plant position	0.81	0.82	0.68	0.88

Table 2. Summary statistics of linear mixed models investigating the trade-off between seed mass per plant and fertilized seeds, accounting for plant position and year. Field ID is used as random variable. *P*-value significant ($p < 0.05$) are bold.

	<i>F</i>	<i>P</i>
log(Head diameter+1)	203.57	< 0.001
Plant position	0.79	0.38
Year	4.27	0.02
log(Fertilized seeds+1)	20.46	< 0.001
log(Head diameter+1) \times Year	4.02	0.02
log(Fertilized seeds+1) \times Year	1.81	0.17

Fig. 1. Effect of head diameter on (A) fertilized seeds, (B) seed mass and (C) total seed mass per head. (D) Trade-off between seed mass (allowing for head diameter) and number of fertilized seeds. (E) Effect of plant density on honeybee abundance. (F) Effect of honeybee abundance on the total seed mass per m² (accounting for by head diameter). The black lines show the relationship averaged over the three years. 95% confidence bands are in grey. Lines for individual years are shown when year effect is significant ($p < 0.05$).

Fig. 2. Effect of different pollinators estimated by (A-B) sweep net or (C-E) pan trap method on sunflower yield. The black lines show the relationship averaged over the four years. 95% confidence bands are in grey.

Fig. 3. Mean (\pm 95% confidence interval) of contributions of different pollination processes (A). Bars with the same letter are not significantly different at 5% significance. (B) Effect of honeybee abundance (estimated by sweep net) on insect contribution (i.e. the sum of small and large pollinators contribution). (C) Effect of insect contribution on yield. The black line shows the relationship averaged over the three years. 95% confidence bands are in grey. The dashed line indicates a marginally significant relationship ($0.1 > p > 0.05$)

Fig.1.

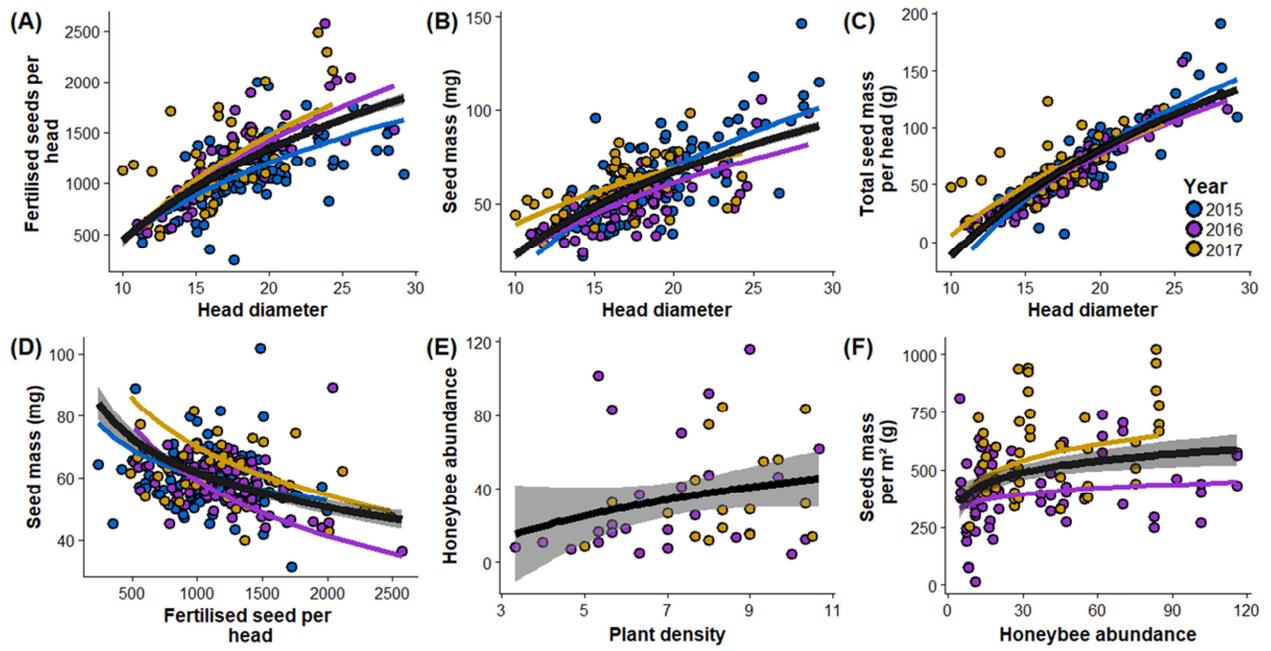


Fig.2.

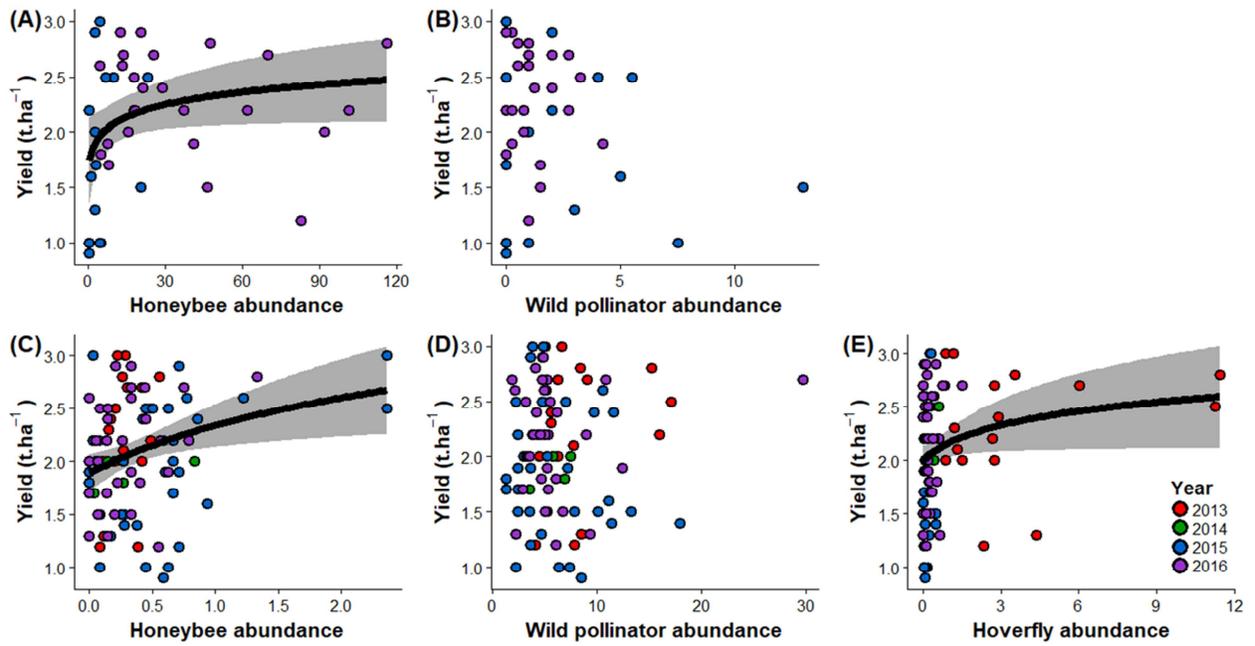


Fig.3.

