The role of pollinators and plant diversity in the pollination services – Dutch apple orchards as a test case

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SUMMARY

Pollinator-dependent products are a very important part of human diets, global food production hence being highly affected by animal pollination. The ongoing biodiversity losses and degradation of the ecosystem services by human activity enhances food insecurity. Further research on the relationships between ecosystem functions and species diversity are needed to develop sustainable and more effective farm management plans. For example, it is still unclear if the presence of low-abundant wild plants (weeds) within the orchards has a positive or negative effect for crop flower visitation. Moreover, despite the increasing number of studies done on the mechanisms causing positive impacts of pollinator diversity (rather than abundance) on pollination services (e.g. foraging complementarity and/or differential responses to climatic conditions), there are still uncertainties. This study aims to analyze the importance of plant and insect diversity for the pollination service, identify potential pollinators of the apple crops and of the weeds and evaluate their performance under different weather conditions. Flower (weed and apple flowers) abundance and visitation surveys were done in 10 apple farms within the Netherlands, from April-June 2013 in three different periods: before, during and after the apple peak of flowering. Although a clear effect of co-flowering weed abundance for the apple visitation was not found, there are results suggesting that it could increment the pollinator richness and consequently the apple pollination. The results show that Diptera specimens visited apple flowers even when sensitive temperature pollinators such as honey bees could not be active, which suggest a complementarity stabilizer mechanism for the pollination service. Therefore, the presence of weeds also attracts apple visitors, without showing a competition or facilitation effect but enhancing the diversity of pollinators. This is important because the results also suggest that different species of apple visitors foraged on different time periods, also providing stabilization for the pollination service. This study concludes that insect and plant diversity could play an important role for pollination.

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1- INTRODUCTION AND OBJECTIVES

1.1- Pollination as an ecosystem function

Ecosystem services such as nutrient cycling, soil formation, and pollination are crucial to environmental stability (Cardinale *et al.,* 2012*;* Garibaldi *et al.,* 2013; Winfree, & Kremen, 2009). The service of pollination is crucial for wild plant reproduction (Ollerton *et al.,* 2011), food production (Klein *et al*., 2007), and human nutrition (Eilers *et al.,* 2011). More than 70% of the leading global food crops, accounting for 35% of the global food production, are affected by pollination of flower-visiting animals (Klein *et al;* 2007).

Research about pollinator efficiency is currently a relevant research topic. It has been suggested that pollination by managed honey bees supplemented pollination by wild insects rather than substituting it for (Garibaldi *et al*., 2013). There are several examples of other crop species for which non-Apis pollinator species are more effective than Apis for crop pollination (e.g. almond, see Bosch, & Blas, 1994a; coffee, see Klein *et al*., 2003; and blueberry, see Javorek *et al*., 2002; Klein *et al.,* 2007). Most ecosystem services are being degraded by human activity (Millennium Ecosystem Assessment, 2005), and the study of the relationships between human disturbance, biodiversity loss and the loss of ecosystem functioning or services is becoming important (Hooper *et al.* 2005; Kremen, 2005).

Although modern farming practices have enabled overall higher crop productivity (Aizen *et al.,* 2008; 2009), pollinating insects have decreased dramatically in Western Europe, North America and Asia since the 1950s (Potts *et al.,* 2010*)* often due to the isolation from natural habitat (Klein *et al.,* 2003; Garibaldi *et al*., 2011) and declines in pollinator dependent crop yields have been observed (Garibaldi *et al*., 2011). These declines combined with the increasing demand for animal-pollinated crops in human diets (Garibaldi *et al.,* 2009) can accelerate conversion of natural areas to cropland.

1.2- Importance of pollinator diversity

Understanding the relationship between ecosystem functions and species diversity, will help to predict the consequences of species losses for ecosystem functioning (Hunt, & Wall, 2002; Solan *et al.,* 2004; Cardinale *et al.,* 2012). Biological diversity could enhance ecosystem service provision by increasing the mean level of services provided, and/or by providing more consistent (stable) services over space and time against disturbance by a variety of stabilizing mechanisms (Winfree, & Kremen, 2009). It has been suggested that complementarity plays an important role in pollination service: greater pollinator diversity could provide greater complementarity leading to an increase in pollination service and it could be important within environmental changes, because different species maybe don't respond equally (Brittain *et al.,* 2013) and climatic conditions strongly affect the foraging activity of pollinator insects (Kevan, & Baker, 1983; Corbet, 1990). Therefore, diversity has been studied to be an important provider of mechanisms such as foraging complementariety of insects groups (Boyle, & Philogene, 1985; Boyle, 1987; Jacob-Remacle, 1989; Batra, 1994) or diferential responses to climatic conditions (Willmer *et al.,* 1994; Vicens, & Bosch, 2000; Tuell, & Isaacs, 2010). Pollination success in coffee is positively correlated with pollinator functional group richness (Klein *et al.,* 2008) and pollinator functional diversity explained more of the variance in the seed set of pumpkin than species richness (Hoehn *et al*., 2008). However, little is known about the role of complementarity in ecosystem functions mediated by pollinators. There are only a few studies about how spatial complementarity of pollinator communities interacts with environmental change (Brittain *et al.,* 2013) and furthermore*,* few studies have investigated whether stabilizing mechanisms occur in real landscapes affected by human disturbance such as crops (Winfree, & Kremen, 2009).

1.3- Apple crop pollination

Apple *(Malus domestica)* production is dependent on insect pollinators (Free, 1964; Delaplane, & Mayer, 2000) and is one of the most important crops in the Netherlands. The majority of apple cultivars are self-incompatible and depend almost entirely on insects (especially bees) for cross-pollination (Free, 1964; McGregor, 1976; Gardner, & Ascher, 2006). Several species of *Andrena, Bombus, Halictus, Lasioglossum, Osmia,* as well as *Colletes inaequalis* are known to collect pollen from apple flowers (Atwood, 1933; Brittain, 1933, 1935; Phillips, 1933; Boyle, & Philogene, 1983; Gardner, & Ascher, 2006).

In apple orchards *O.cornuta* and muscoid flies (Family Muscidae) were observed foraging under light rain when honey bees were not active and *O.cornuta* was the only pollinator species observed foraging in the orchards under high wind speeds (Vicens, & Bosch, 2000). Such complementarity could be an extremely important mechanism for ensuring stable crop production, and more in countries northern countries such as The Netherlands.

1.4- Interaction between apple flowers and weeds

The effects of the presence of weeds within crops for apple pollination are still not clear. Previous studies conclude that co-flowering plants can either compete for (Campbell,& Matlon, 1985) or facilitate pollination (Lopezaraiza-Mikel *et al*., 2007). However, weeds are commonly seen as a cause of crop productivity loss, due to fear of competition for soil resources or for flower pollinators (Weiss 1983; Carvalheiro *et al;* 2010), so they are usually removed from extensive cultivation fields (Weiss, 1983). Farmers are advised about the importance of maintaining and restoring pollinator communities, which normally involves the creation of areas rich in plant diversity (ecological compensation areas), but they are still uninformed and unsure to apply those measures because they are afraid of competition for resources between wild plants within the crop (also said weeds) and the crop *(*Weiss, 1983*)*.

Some studies have suggested that allowing the natural occurrence of wild plants within crop fields enhances crop productivity (Carvalheiro *et al;* 2011; 2012). Further studies are necessary to better understand the relationship between the composition of wild plant communities and the interactions and benefits to crop flower visitation. Such relationships are likely to vary across crop species and regions of the world (Kohler et *al*., 2007), depending on the nesting requirements and foraging ability of the resident flower visitors (Lonsdorf *et al.,* 2009) and also between landscape characteristics.

1.5- Objectives of the study

The objectives of this study are:

1- To evaluate the importance of plant and insect diversity for the pollination service within Dutch apple farms.

Based on the literature reviewed above (e.g. Carvalheiro *et al*., 2011, 2012), no negative effects (competition) are expected with a higher abundance of weeds. Contrary, a positive consequence is expected (such as a higher insect diversity or higher apple visits).

2- To identify potential pollinators of the apple crops and of the weeds and to evaluate their differences and their performance under different weather conditions

Different insects groups are expected to be found with different weather conditions. We expect to find an important number of honey bees if the weather conditions are optimal, because of the presence of honeybees hives in the majority of the orchards. However, *Apis* bees or other temperature sensitive insects such as butterflies are not expected to be found in low temperatures (less than 20 degrees). In the non-optimal temperatures for pollination (under 15 degrees) other bee groups (such as *Osmia* species or Bumblebees), Diptera specimens and ants, are expected to be found.

These analyses were done before, during and after the apple flowering.

2- MATERIALS AND METHODS

2.1- Study area and timing

This study was carried out in 10 apple farms (1 field per farm) spread throughout the Netherlands, from April to June of 2013. The farms were distributed randomly in three different provinces of the Netherlands: Flevoland, Gelderland and Zeeland, the minimum distance between farms being 3.5 km (Figure 1). Six of the farms selected were under conventional management and the other four were organically managed, all of them with similar orchard characteristics (cultivar, age of trees and planting pattern). The main difference between the treatments in the conventional managed farms and the organic managed farms is that conventional use synthetic pesticides (herbicides and insecticides), whereas the organic managed farms substitute the herbicides for mechanical control and only can use specific insecticides permitted by the European law (such as sulphur lime) or fungicides.

Figure1. Map of the Netherlands showing the farms location (red points)

Flower visitation surveys were done in three different periods: before apple flowering (end of April 2013), during peak of flowering (half/end of May 2013) and after apple flowering (beginning of June 2012), with the aim of capturing the temporal variation of insect visitation patterns. Normally, the peak flowering season of Dutch apples is at the end of April and beginning of May (*farmers personal communication*) but the weather conditions of the study year (a lot of rain and very low temperatures) delayed it more than two weeks.

As apple flowers and weed flowers do not open very early at the morning (Vaissière *et al.,* 2011; personal observation), within each period, each farm has been surveyed twice during the day: once in the morning (M, 0900-1400h) and once in the afternoon (A, 1500-1700h). Therefore, each survey information on the time of the day (M/A), the time period (1/2/3) and the farm (1-10) were annotated. Surveys were done by walking slowly along two transects of 30 x1 m (a total 60 x 1 m transect) located always in the middle of one Elstar apple field of each farm, making sure the weed abundance present on the transect was representative of the weed abundance in the whole field.

2.2- Cultivar selection

The cultivar selected was Elstar, which is one of the most economically important cultivar of the Netherlands The majority of apple varieties are self-incompatible and depend almost entirely on insects to be pollinated (especially bees) for cross-pollination (Free, 1964., McGregor 1976., Gardner, & Ascher, 2006). However, it seems that from all the apple cultivars, Elstar apple is not the most pollinator dependent (farmers personal comunication).

2.3- Data collection

To assess the abundance of flowers within the farm field (weed and apple flowers), we first walked each transect to identify all present flowering plant species, and counted number of floral units. One floral unit corresponds to 1 cm^2 of flowers, the minimum size needed to allow one medium-sized visitor to forage without preventing visitation in the adjacent floral unit (Carvalheiro *et al*., 2011). For the apple, one floral unit corresponded to one flower, but for compound species such as some weeds (e.g. *Taraxacum officinale*) one flower unit contains several flowers. Whenever identification was not possible, wild plant species were collected for later identification. As in during peak of flowering the apple flowers are very abundant, the total number of apple flowers in the transect was estimated by counting the number of open flowers of three randomly selected trees from each transect and the average of flower per tree was then multiplied by the total number of trees counted in the transect.

After plant abundance surveys, we walked again each transect to assess flower visitation to all plant species. We observed each section of the transect for 30 seconds (so that each flower unit is observed for the same time), during which all insects that contacted with the reproductive structures of flowers were recorded as well as the identity of the plant species visited. Whenever possible, visitors were collected for later identification, using nets and killing tubes with ethyl acetate for the insects. If the flower visitor escaped, we assumed it did not return to the transect within the observation period and registered broad information on visitor identity (e.g. honeybee, other bee, fly). All specimens collected were pinned and bees and flies were separated to species or morphospecies level by professional taxonomists (see acknowledgements). Coleoptera and ants were broadly separated to morphospecies by LR and some that could not be identified properly (damaged or uncertain) were considered "non-identified". As rare interactions might easily be missed by the survey method, species richness surveys were complemented by including any new interactions detected outside the transect survey (extra specimens collected) as rare interactions (frequency of occurrence = 0.01; as in Carvalheiro *et al.,* 2010)

To evaluate the effect of weather conditions, cloud cover, temperature and humidity information were recorded in the beginning of each survey with a portable weather station.

2.4- Data analysis

2.4.1- Flower diversity and abundance (apple and weeds) in each farm

To evaluate the differences of flower abundance and richness between farm fields, linear mixed effect models (GLMM), were run with the program R (R Development Core Team, 2011) using lme4 package (R package version 0.999375-42) (Bates *et al.,* 2011). The models related weed richness, weed abundance, *T. officinale* abundance, *B. perennis abundance* and apple flower abundance with the farm treatment (organic or conventional) and the period (before, during and after the peak of apple flowering). As data was not normally distributed, it was analysed using a Poisson error distribution and factor farm (site) as a random effect. Factor period was also included as a random effect in the models which period was not the explanatory variable of the model.

2.4.2- Apple and weed pollinator abundance, richness and visitation

To evaluate the effect of weeds, climate, treatment, insect richness and plant richness on apple visitation rate (number of insect visits/flower) data was analysed using general linear mixed effects models (GLMM). As number of apple flowers observed varied during the samplings, visitor abundance (overall and per each group of principal visitors: honey bees, wild bees, ants and other flying visitors) was standardized for each survey by dividing the number of apple flowers visits by the number of the apple flowers presents in the transect sampled, and then multiplying the rate per the average number of apple flowers of all the farms during the peak of flowering: standardized visitor abundance. As data was not normally distributed, it was analysed using a Poisson error distribution. For these analyses only the surveys with apple flower abundance higher than 30 flowers and with a temperature higher than 15 degrees and without rain were included.

We then evaluated how apple visitation was affected by the abundance of the two most important weed species *T. officinale* and *B. perennis* (which together made up 87.9% of the overall wild flower abundance), wild flower richness, temperature and treatment use (organic or conventional) also checking for any interaction between these variables (pairwise interactions). Site (farm) and period were included as random effects. The most important explanatory variables for apple visitation were determined via a model selection procedure using the AIC (Akaike Information Criterion), a measure of the relative quality of a statistical model which selects the most balanced model in terms of complexity and amount of information explained (model with the lowest AIC).

Another linear mixed effect models testing the effect of pollinator richness on "standardized visitor abundance" was run using also Poisson distribution. Moreover, the effect of wild flower abundance on total pollinator richness (apple and weeds) was tested (adding farm and period as random effects).

Finally, to verify the model, some complementary analyses were done with *T.officinale*, in order to understand the role of weed abundance on visitation and to test if those effects should be considered. If the negative effect of abundance of *T. officinale* on apple visitation was due to competition for flower visitors between the two plants, we would expect an increase of visitation to *T. officinale* with the higher abundance of it. To test that, we created the same models as before but changing the "Standardized apple visitor abundance" for "Standardized *T. officinale* visitor abundance".

2.4.3- Effect of climate on visitation patterns

To evaluate the effect of climate on apple and *T. officinale* visitation the relative number of visits of each group of insects was calculated for each survey. GLMM were used to check the effect of temperature (which was correlated with humidity and cloud cover), with farm and period as random effects. Linear equations were extracted from the model in order to predict the effect of temperature on the visitation and understand the role and differences of each insect group on the apple and weed pollination. The response variable (relative abundance) was log-transformed in order to normalize residuals. The range of temperature we sampled was from 11 to 19 Celsius degrees. Finally, to test if the effect of temperature was significantly different between insect visitor groups, a simple linear model was created where the interaction between temperature and insect group factor was tested.

3- RESULTS

3.1- Importance of plant and insect diversity for the pollination service

3.1.1- Determination of flower diversity and abundance of weeds and apple flowers in each farm.

Wild flowers (weeds) abundance and richness varied between farms, ranging from 0 to 1382 flower units per survey and richness between 0 and 5 species. Farm treatment affected significantly the wild flower abundance, organic managed farms having a significant higher value of weeds abundance than the conventional managed farms (*Z-value=-2.421, p-value=0.01*, Table 2). However, wild flower richness was not significantly affected by farm treatment, although organic farms tended to have more *species* (Table 2).

The richness of wild flowers found in the different farms was very low (a maximum of 5 species of plants found, Table 1), and there were only two species that were really present in the farms regularly: *Taraxacum officinale* and *Bellis perennis.* Abundance of *T. officinale* has a tendency to increase in organic farms, although the p-value is non-significant *(p-values <0.1).* No significant tendencies were observed with *B. perennis* (Table 2).

Table 1. Species of weeds found in the samplings and their averaged and rounded abundance per transect (30x1m) in total (Total) and per farm treatment (Org=organic, Conv=conventional) in each period (P1- before peak of apple flowering, P2- during peak of apple flowering, P3 – after peak of apple flowering).

	Abundance P1			Abundance P2			Abundance P3		
Weed species	**Org**	**Conv**	**Total**	**Org**	**Conv**	**Total**	**Org**	**Conv**	**Total**
Taraxacum officinale	215	42	111	912	117	434	12	13	12
Bellis perennis	44	276	183	98	50	68	217	424	341
Ranunculus repens	0	0	0	19	0	1	82	3	35
Anthriscus sylvestris	0	0	0	5	0	2	0	0	0
Veronica arvensis	4	1	2	0	1	0	1	0	0

Apple flower abundance were supposed to be different in each period (before, during and after the peak of apple flowering). All the surveys of the "before" period were with an apple flower abundance of 0 unless 2 farms, where some flowers were already open (7, 54 AF/transect). During the peak of flowering (period 2), the values of apple flower abundance obtained in each survey were between the minimum value of 6460 AF/transect to the maximum value of 19600 AF/Transect. Finally, the last period (after peak of apple flowering) the minimum number of unit flowers obtained was

17AF/Transect and the maximum 903 flowers. As it was expected the factor period affects significantly positively the apple flower abundance (*p-value<0.01*), being the slope of "period 2" higher than the "period 3", and the slope of "period 3" more important that the slope of "period 1". (Table 2)

Table 2. Linear mixed effect model results for the analysis of response variable wild flower richness (WFR), wild flower abundance (WFA), apple flower abundance (AFA), *T. officinale* abundance and *B. perennis* abundance, related with treatment (Organic-Org vs Conventional-Conv) and/or period (P1- before peak of apple flowering, P2- during peak of apple flowering, P3 – after peak of apple flowering).

Response variable	Estimate	Effect	Period	Test	P-value
WFA	-1.2663	Org>Conv		*Z= -2.421*	0.01
WFA			P1<P2>P3	Chi=1377.1	<2.2e-16
WFR	-0.2927	Org>Conv		Z= -1.365	0.172
AFA			P1<P2>P3	Chi=456670	<2.2e-16
***T. officinale* abundance**	-2.381	Org>Conv		Z=-1.667	0.095
***B. perennis* abundance**	0.147	Org<Conv		Z=0.185	0.853

3.1.2- Comparison of pollinator visits between apple flowers and weeds

Apple flower visitation

Despite the high number of apple flowers observed during the peak of flowering, the apple flower visitation was low during it (a maximum of 42 visits per transect). During the peak 334 visits were recorded in the two surveys per sampling (morning and afternoon). No apple visits were detected before the peak of apple flowering, as almost no apple flowers were open. After the peak of apple flowering, where some apple flowers were still open, the maximum of visits to apple recorded per survey and farm were 13 and the total visits recorded in all farms during that period were 58. During the peak, 51% of the visits were from honeybees and a 37% from other flying visitors (Figure 2), whereas after the peak the composition of insects changed, a 65% of the visitors were other flying visitors (Diptera specimens and Coleoptera) and honeybees become only a 14% of the visitors, almost the same percentage than the other bees (12%). Ants were the less important group of apple visitors and become more important after the peak of flowering than during it (9% and 5%) (Figure 2)

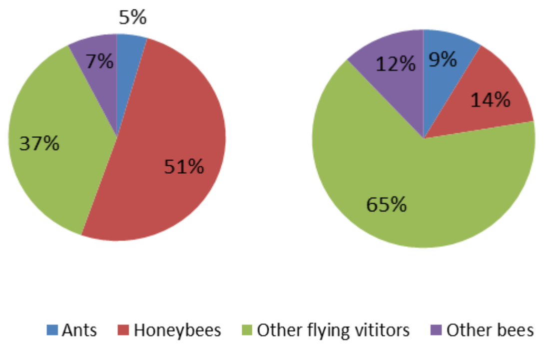


Figure 2. Percentage of apple visitors recorded during (left graph) and after (right graph) the peak of apple flowering , separated to morphospecies level: Ants, honeybees, other flying visitors (Diptera and Coleoptera) and other bees.

There was a positive significative correlation *(p-value<0.05)* between the standardized visitor abundance and the total richness of pollinators visiting (apple and weed flowers) during a sampling (Table 3). The model run to see if that total richness of pollinators was related with the wild flower abundance also show a significant positive tendency *(p-value <0.5)* (Table 3).

Table 3. Linear mixed effect model results for the analysis of "standardized visitor abundance" (SVA) explained by" total pollination richness" (PRT) and linear mixed effect model results for the analysis of " total pollination richness" (PRT) explained by "wild flower abundance" (WFA).

Response variable	Explanatory variable	Z-value	P-value
SVA	PRT	9.49e-13	1.51e-10
PRT	WFA	8.219	<2e-16

Wild flower visitation

The weeds received a total of 202 visits in all the samplings. *T. offcinale* was the weed which more visits received during all the study (141 visits). More visits to wild flowers were detected before the peak of apple flowering (72 visits) than in the peak of flowering period (39 visits recorded) and after the peak of apple flowering (30 visits recorded). In all the periods the "other flying visitors" group was the most important in terms of absolutes visits, 67% of them in the before apple flowering, the 33% during the apple flowering peak and the 80% after it. Ants were especially representative of the visits during the before apple flowering period (80% of the visits), but also during and after the peak of apple flowering (25% and 10%). Honeybees visited few times *T. officinale,* they represent the 3% of the visits in the first sampling period, the 8% in the second and the 10% in the third sampling period. Other bees group (wild bees or bumblebees), only visited *T. officinale* before the apple flowering period, representing only a 4% of the visits (Figure 3).

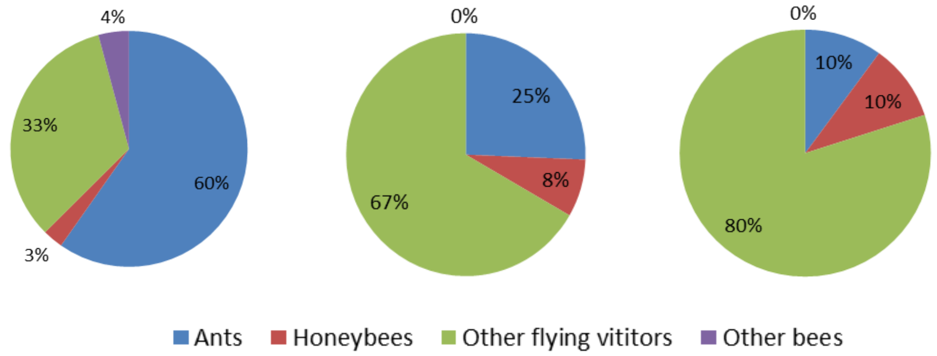


Figure 3. Percentage of wild flowers visitors recorded before (left graph), during (middle graph) and after (right graph) the peak of apple flowering separated to morphospecies level: Ants, honeybees, other flying visitors (Dipera and Coleoptera) and other bees.

Interactions between apple flowers and weeds

No clear effect of the presence of weeds in the visitation of apple flowers was detected in this study (Table 4).

Table 4. Linear mixed effect models developed to explain "Apple visitation standardized" response, and different explanatory variables affecting it: *T. officinale* abundance, Treatment (Treat), Temperature (Temp), Wild flower richness (WFR), Wild flower abundance (WFA) and *B. perennis* abundance. P- values are presented for each term and interactions included in each of the six best models (see d.f and AIC)

MODELS	***T. officinale* abundance**	**Treat**	**WFR**	**WFA**	**Temp**	***B.perennis* abundance**	**Temp*Treat**	**WFR*Treat**	**d.f**	**AIC**
Model 1	<0	ns	<0	-	<0.05	<0	<0	ns	9	359
Model 2	<0	ns	<0	-	<0.01	-	<0	<0	8	395
Model 3	<0	ns	<0	-	-	-	<0	<0	7	399
Model4	<0	-	-	-	<0.05	<0	-	-	5	437
Model 5	<0	ns	-	<0.05	-	-	<0	-	6	448
Model 6	<0	-	<0	-	-	-	-	-	4	474

**ns=non signifficant*

As the table shows (Table 4), "Model 1" was the best model (lowest AIC value). It suggest that the abundance of *T. officinale* has a significant negative effect on the apple flower visitation *(z-value <0.01*), while *B. perennis* abundance had a positive effect *(z-value <0.01).* Moreover, the model suggest that wild flower richness has a positive effect for the apple flower visitation *(z-value <0.01)*, but only in organic farms because, the interaction of "Wild flower richness" with "Treatment" is significant *(z value <0.01*), richness having a negative effect to the apple visitation. Finally, the model suggest that "Temperature" has also a negative effect to the apple visitation *(z value<0.05)*.

Moreover, the detailed analyses on *T .officinale* visitation done to test potential competition effect, show that the visitation rate to *T. officinale* also decreased with an increase of *T. officinale* abundance. (Figure 4)

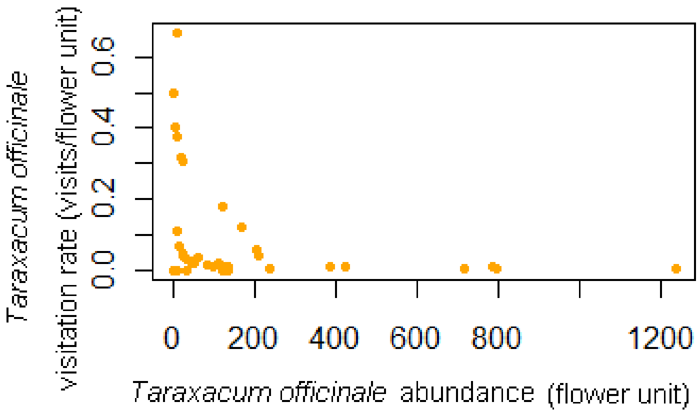


Figure 4. Changes in *Taraxacum officinale* visitation rate (visits/*T.officinale* flower) with the increase of *Taraxacum officinale* abundance (flower units)

3.2- Identification of potential pollinators of the apple crops and of the weeds and evaluation of their performance under different weather conditions

3.2.1- Identification of potential pollinators of the apple crops and of the weeds

Recorded visitors (Data used for the visitation analysis)

This data was identified to morphospieces level during the samplings. From the 594 specimens used for the data analysis 394 were found visiting apple flowers and 200 weed flowers. A 33% of visits recorded were from honeybees, a 5% from other bees (bumblebees or wild bees), a 10% were ants and finally, the majority of visits (52%) were of other flying visitors (Diptera, Coleoptera, and non-identified) (Figure 5).

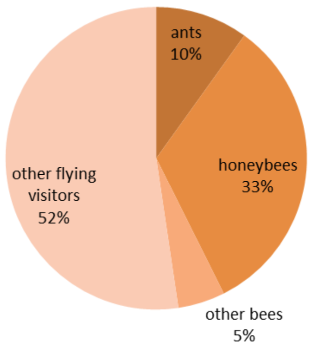
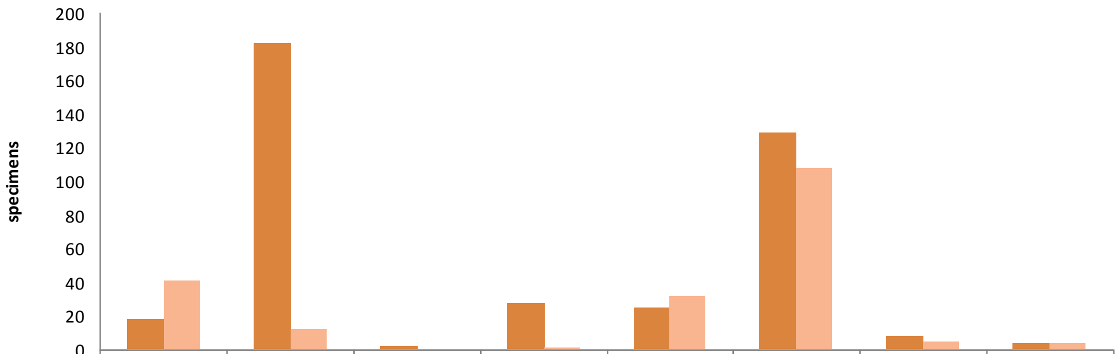


Figure 5. Percentage of total recorded visitors during all the visitations samplings separated to morphospecie level: Ants, honeybees, other flying visitors (Diptera and Coleoptera) and other bees.

Figure 6, shows which of those visits were to apple flowers and which ones were to the weeds present in the transect with more detail of species groups. Honeybees were the major recorded visitors in apples (182 visits) whereas only 12 visits of honeybees were recorded in the weeds. Similar patterns occurred with the bumblebees and the wild bees, which were almost only found visiting apple flowers. On the other hand, the visits of Diptera species were very high in both type of flowers (154 visits to apple and 139 visits to weeds flowers). From those Diptera spieces, hoverlflies were found regularly (total of 25 visits recorded to apple flowers and 31 to weeds flowers). However, visits of ants were important in the weeds (41 visits) and not as important in the apple (18 recorded visits). Finally, Coleoptera spieces and other non-identified insects were few recorded in both flower types (Figure 6).



	ants	honeybees	wild bees	bumblebees	hoverflies	other flies	coleoptera	non-identified
APPLE	18	182	2	27	25	129	8	3
WEEDS	41	12	0	1	31	108	4	3

Figure 6. Total number of recorded visitors during all the visitations samplings for apple flowers and weeds, separated to detailed morphospecie level: Ants, honeybees, other flying visitors, Coleoptera, wild bees, bumbles bees, hoverflies and non-identified specimens.

Collected visitors (including “extra” specimens)

For richness evaluations we also considered specimens collected outside of the survey transects. Considering such “extra” visitors a total of 631 specimens were recorded and from those 304 specimens were collected (48.2%) From them, 97 were bees, 145 Diptera species, 50 ants, 9 Coleoptera species and finally 3 categorized as “non-identified visitors” as they could not be properly identified.

In the case of Diptera specimens, 89 were collected visiting apple flowers and 56 visiting wild flowers. The groups found in each type of flower had different frequencies and presences. The diversity of Diptera families (13 families) found visiting apple flowers was bigger than the found for wild flowers (9 families) (Table 5). Specimens from Syrphidae family (hoverflies) were the most collected visiting wild flowers (34.09%) followed by specimens from Anthomyiidae family (22.73%) and Empididae family (22.73%), whereas for the apple flowers the most found Diptera family was Bibionidae (25.58%), followed by Anthomyiidae (23.26%) and Syrphidae (17.44%). Empididae family specimens were few found visiting apple flowers (9.30%), (Table 5).

Table 5. Results of the identification of Diptera visitors (Family level) and bee visitors (Specie level) collected: total abundance and relative abundance (%) (accounting the extra specimens only for the bees), considering separately visits to apple flowers and to weeds.

Family	**Absolute abundance**					**Relative abundance**	
	Apple	Weeds	*Taraxacum officinale*	*Bellis perennis*	*Raunculus repens*	Apple	Weeds
Syrphidae	15	15	6	8	1	17,44	34,09
Bibionidae	22	0				25,58	0,00
Tipulidae	4	1	1			4,65	2,27
Empididae	8	10	4	6		9,30	22,73
Sarcophagidae	6	1		1		6,98	2,27
Calliphoridae	4	4	1	3		4,65	9,09
Hybotidae	1	1			1	1,16	2,27
Chalcidoidea	1	2	1	1		1,16	4,55
Sciaridae	1					1,16	0,00
Anthomyiidae	20	10	2	7	1	23,26	22,73
Lauxaniidae	1					1,16	0,00
Sciomyzidae	1					1,16	0,00
Non-identified	2					2,33	0,00
TOTAL	**86**	**44**	**15**	**26**	**3**	**100,00**	**100,00**
Species	**Absolute abundance**					**Relative abundance**	
	apple	weeds				apple	weeds
Bombus terrestris-group	5	0				6,25	0,00
Bombus pascuorum	0,01	0				0,01	0,00
Bombus lapidarius	0	0				0,00	0,00
Andrena haemorrhoa	2,01	0				2,51	0,00
Andrena chrysosceles	0	3,01				0,00	50,00
Apis mellifera	73,01	3,01				91,23	50,00
TOTAL	**80,03**	**6,02**				**100**	**100**

* the extra specimens of bees are considered with an abundante of 0.01. The only weed visited by bees was *T. officinale*

In the case of bees, 3 different genus and 6 different species were found (considering also the extras). The majority of specimens collected were honeybees *(A. mellifera),* found basically visiting apple flowers (91.23%). 3 different species from *Bombus* genus were found, only visiting apple or just flying around (extra data): 6 specimens from *Bombus terrestris*-group, 3 *Bombus* and 1 *Bombus lapidarius* . Finally, also some wild bees were found: 6 specimens of *Andrena chrysosceles* were collected only visiting weeds (*T.officinale*) and 3 specimens of *Andrena haemorrhoa* where collected while visiting apple flowers. (Figure 7, Table 5)

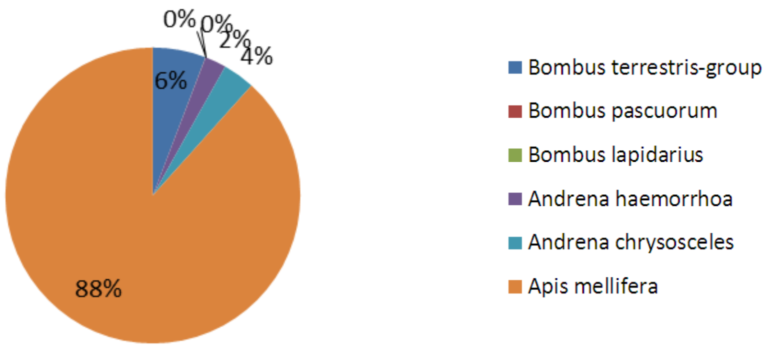


Figure 7. Percentage of total collected bee visitors separated to specie level (extra specimens collected accounted with an abundance of 0.01)

The other group of insects were identified by LR to morphospieces level: ants and Coleoptera species, which correspond exactly with the "recorded data".

3.2.2- Evaluation of pollinator performance in different weather conditions for apple flowers and for the weeds

The three environmental variables measured in the field where: temperature (ºC), relative humidity (%) and cloud cover (%). These three variables were correlated between them; being temperature negatively correlated with the increase of humidity *(p-value<0.05)* and cloud cover *(p-value<0.05)* (Figure 8). Considering all the samplings done (also considering some extra ones non used for the visitation analysis), the average of temperature in each period was of 18.6ºC in the first sampling period (period 1), 19.2 ºC during the peak of apple flowering (period 2), and in the after apple flowering sampling (period 3) of 22.5 ºC.

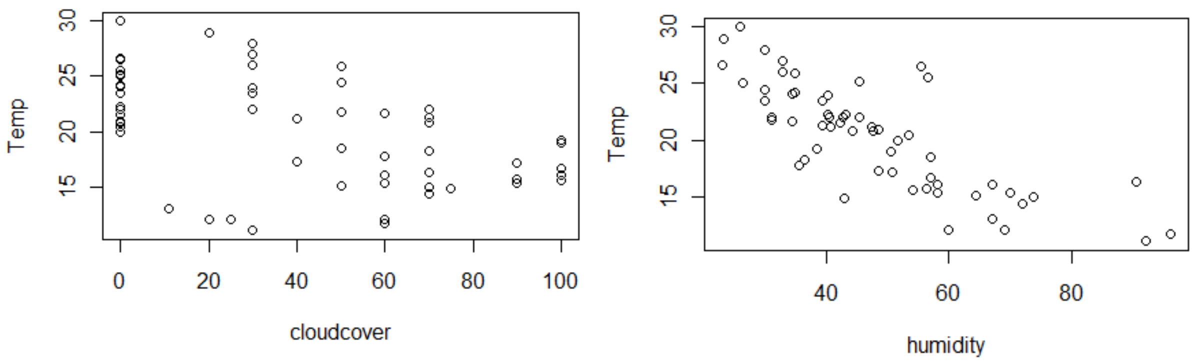


Figure 8. Graphs showing the significant negative correlation between temperature (Temp) and cloud cover (Left graph) and temperature (Temp) and humidity (Right graph) of all the surveys.

Temperature had a different effect on different apple visitor groups (interaction temperature*group being significant) while *T. officinale* visitor groups consistently increased with temperature (Table 6).

Table 6. Linear model results of the effect of temperature (Temp) and insect group (I.group) to the relative abundance of apple visitors (first table) and relative abundance of *Taraxacum officinale* visitors (second table). The interactions between the variables (Temp*I.group) were also considered in the model.

Apple	**F-value**	**P-value**
Temp	0	1
Insect group	28.5289	2.89E-12
Temp*I.group	5.0382	0.00317

T. officinale	**F-value**	**P-value**
Temp	4.3834	0.0383
Insect group	4.3417	0.0061
Temp*I.group	0.5652	0.639

Indeed, in Figure 9 we can observe in the case of apple visitation, how "Other flying visitors" group remains important in all the temperature range, but especially when it is low (under 10ºC) and honeybees are not active. When the temperature increases (above 25ºC) the relative abundance of honeybees increase (from a 20% to a 40%), while the "other flying visitors" decrease (From 60% to 40%), so it seems that one group compensates the other. Moreover, ants seem to be also important when temperatures remains low, their abundance become 0% around the 23 degrees. On the other hand, other bees (bumblebees and wild bees) were not abundant in any range of temperature, having a relative abundance of almost 0% along all the temperatures.

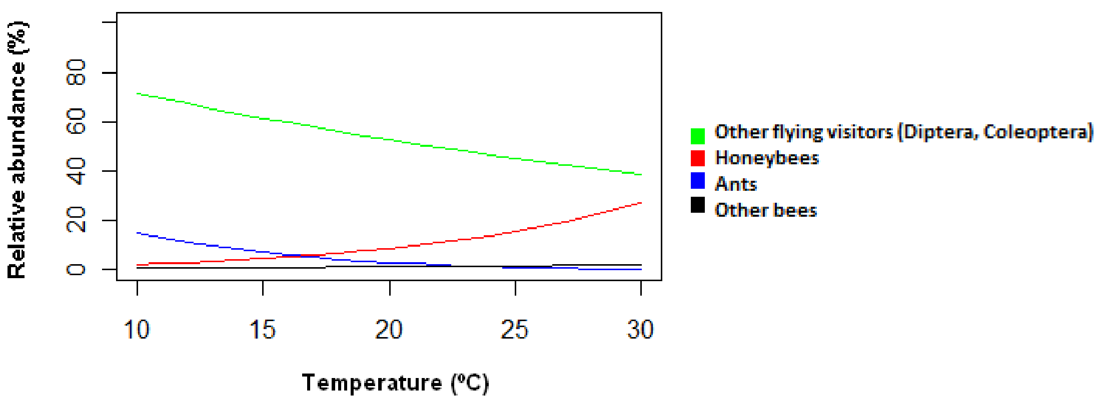


Figure 9. Graph showing the linear equations specific from each insect group of apple visitors, extracted from a linear mixed effect model, which relates temperature with the relative abundance of visitor groups (%) of apple flowers: Honeybees, ants, other flying visitors and other bees.

The same graph was created with *T. officinale* visitation instead of apple flowers. We saw that the patterns changed. The “other flying visitors” continue being the most important visitors in all the temperatures, but instead of decreasing when the temperatures are high, their relative abundance also increases with it (Figure 10). Honeybees and “other bees” group, have a very low relative abundance in all temperatures, which suggest that those are not the potential pollinators of *T. officinale* when apple flowers are present. The only visitors that have shown the same pattern in both flower types are ants, whose relative abundance is highest in low temperatures and it decrease when temperatures increases. The difference of ants visiting *T.officinale* is that their relative abundance is never of a 0%, they were found visiting *T.officinale* in all the sampling temperatures.

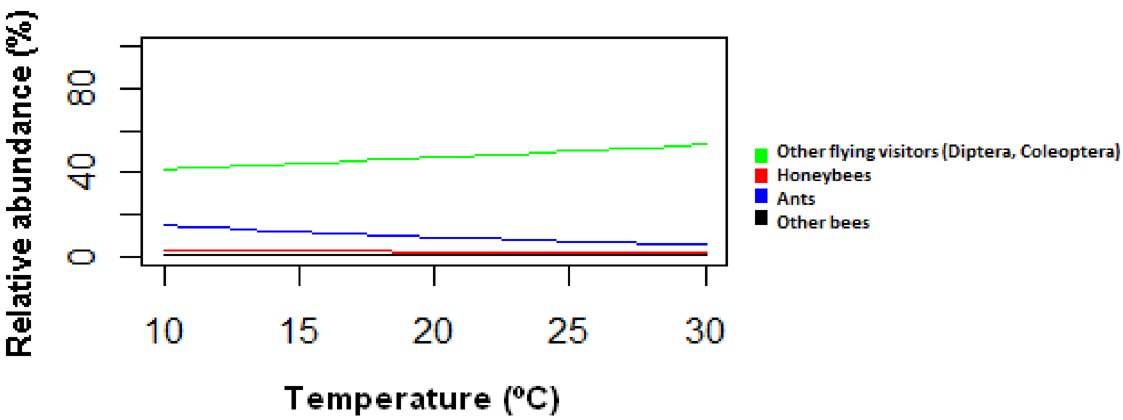


Figure 10. Graph showing the linear equations specific from each insect group of *T. officinale* visitors, extracted from a linear mixed effect model, which relates temperature with the relative abundance of visitor groups (%) of *T. officinale*: Honeybees, ants, other flying visitors and other bees.

4- DISCUSSION

4.1- Importance of plant and insect diversity for the pollination service in Dutch apple farms

In agreement with previous studies (Kremen *et al.,* 2002; Carvalheiro *et al.,* 2010; Carvalheiro *et al.,* 2012; Klein, 2009; Winfree, & Kremen, 2009) our results suggest that insect and plant diversity are important for the pollination service. Even though no clear direct correlation between apple visits and wild flower abundance was found (Table 4), there we show that pollinator richness increases the apple visitation rate (visits/flower*survey) and that wild flower abundance affects positively the pollinator richness (Table 3). Therefore, higher wild flower abundance may contribute to a higher pollinator richness foraging in the crops, which could increase the apple visitation and the success in pollination *(*see also Richards, 2001; Ghazoul, 2005). Firstly, the lack of shared bee species (the most important group of visitor for apple) between apples and weeds suggests that apple visitors are not the same as weeds visitors, so there is no evidence of competition in terms of visitors groups. Diptera group was an important group of apple visitors, and also visited weeds. However, the relative abundance of Diptera families found in each type of flower was not exactly the same.

Anthomyiidae and Syrphidae familes were important visitors of both type of flowers. However, Bibionidae was only important as a visitor of apple flowers and Empididae was more important as a visitor of weeds. (Table 5).

Despite finding few non-*Apies* bees, some solitary bees were found visiting both weeds and apple flowers (Table 5). Although there are studies concluding that *Andrena haemorrhoa* is one of the solitary bees which more visit *T. officinale* (Free 1967), we only found this species visiting apple rarely (only 2 visits) , and the only solitary bee we found foraging on weeds was *Andrena chrysosceles*, which visited also apple flowers during the surveys. Moreover, bumblebees were never found visiting a weed flower, even they were present in the transect.

T. officinale was one of the weeds which have created more controversy in the literature about their effects for crop flowers visitation and it is the one that worries the farmers the most (personal farmers communication). However, the fact that visits to wild flowers were more frequent before the peak of apple flowering suggests a facilitative effect between weeds and apple, as weeds are providing resources for apple pollinators during a period of flower scarcity which may attract insects in the orchards before the flowering. Therefore, no evidence was found of competition effect for pollination between apple flowers and weeds. Bad weather conditions during the peak of flowering should be considered as a limiting factor of our study in terms of total number of visits received, and the little number of farms sampled which made hard create simple models showing clear tendencies. It would be interesting to study further in one hand, if instead of a generalized effect, there is a threshold of weed abundance in which the consequences for the crop flowers visitation become negative and on the other hand, to study the positive effect of the visitors attracted to the orchard by wild flowers before the presence of crop flowers, and their role in the pollination during the following periods. The fact that that organic managed farms have significant higher weed abundance than conventional farms (Table 2), suggest a negative effect of the conventional management. The fact that no differences in weed richness were found is likely because total number of species was extremely low, we only found 5 different plant species in our transects (Table 1).

To summarize, although this study does not find a positive effect of co-flowering wild plants for apple flowers visitation as found in previous studies (Carvalheiro *et al.,* 2011), the results suggest an indirect positive effect due to increased flower resources before the peak of apple flowering.

4.2- Identification of potential pollinators of the apple crops and of the weeds and evaluation of their performance under different weather conditions

As previous studies showed (Vicens, & Bosch, 2000) Diptera species were found to be important visitors of apple flowers under weather conditions on which other species groups are not capable to forage. However, contrary to other studies (Vicens, & Bosch, 2000) no Muscoidae flies were found. Our results support the fact that climatic conditions strongly affect the foraging activity of pollinators (Kevan, & Baker, 1983; Corbet, 1990). Other flying visitors group (mostly Diptera specimens) was constant during all the range of temperatures, and they were especially important in terms of relative abundance when temperature is low (under 15ºC) or not very high (under 20ºC), a common scenery in countries as the Netherlands (Figure 9). Among Diptera, hoverflies have played an important role (Table 5) and recent studies showed that they can be efficient pollinators of crops such as oilseed rape (Jauker, & Woulters, 2008). Furthermore, our results show that honeybees become important in terms of relative abundance above 25ºC (approximately 40% of relative abundance). Surprisingly, ants changed a lot their relative abundance with temperature and within periods, they seem to be only important in low temperatures conditions (Figure 9).

Previous studies conclude that pollinator species differ in their thermal biology (Herrera, 1997; Bishop, & Armbruster, 1999) which could mitigate the impact of climate change on pollinator service stability. Therefore, our results suggest the existence of some compensatory mechanisms that could ensure the pollination in non-optimal conditions, by differential responses to weather conditions. Linking that with the results of visitation of apple flowers and weeds, we can also predict a foraging complementarity effect which could also be understood as a stability mechanism of pollination. The findings of this study support previous evidences about biological diversity could enhance ecosystem services by providing more stability by stabilizing mechanisms such as complementarity (Winfree, & Kremen, 2009).

Future studies should study deeply which is the abundance of more specific groups of insects with temperature (which is related with cloud cover and humidity), and also with other climate variables, which could be also important such as wind speed. As far as we are concerned few studies about stabilizing mechanisms have been carried out in landscapes disturbed by human activity like apple crops.

Our results, are rellevant in a context of diversity loss and fast cropland expansion which compromise the sustainable development (Carvalheiro *et al.,* 2012), suggesting that the presence of weed flowers, which is higher in organic farms, could enhance the pollinator diversity, which is at the same time crucial for increase the efficiency of pollination service. Pollinator-dependent food crop production, have become increasingly important in human diets (Aizen *et al.,* 2008) and our results could be useful for future management farming techniques, not expensive and which could help to improve the farming efficiency at the same time than contributing to cope with the loss of diversity.

4.3- Limitations of the study

This study has been limited firstly for the small number of farms selected and low number of surveys done. Moreover, we have to consider that the amount of weeds in each farm was not a constant value and that is explained because of the mowing time between the rows varied among far. Future studies involving experimental set-up to enhance weed diversity and abundance could help better understand the potential benefits of these plants to apple production. Moreover, number of managed pollinator hives and information about pesticides application are variables to take in account to get more reliable results. Finally, the findings could change depending on the studied crop or even the cultivar.

This study will be extended if possible with the data of the final year production of each farm (apple production 2013), with the aim to understand the relationship between it and farm management and characteristics and also to analyse the relationship of visitation results with the pollination and apple final production, which is the economical important variable.

5- CONCLUSIONS

Few studies about stabilizing mechanisms have been carried out in landscapes disturbed by human activity like apple crops. This study shows that insect and plant diversity plays an important role as a stability ecosystem pollinator service provider. While a higher abundance of co-flowering wild plants did not enhance significantly apple pollination, a higher abundance of weeds before apple flowering season guaranteed flower resources for crop pollinators during times with fewer resources. Such flower resources were particularly important for flies, a group that was able to forage under climatic conditions that were not favourable for bees. Indeed, one the most interesting findings of our study is the importance of Diptera specimens such as hoverflies (Syrphidae family) as a pollinator service stabilizers, as they were present in all the weather conditions, and during all the periods and are considered efficient pollinators. The findings of this study contribute to understand the compensatory mechanisms that help ensure the pollination in non-optimal conditions, giving an explanation for the positive effect of plant and insect richness on crop productivity.

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