



Contrasting impacts of pesticides on butterflies and bumblebees in private gardens in France



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ABSTRACT

Private gardens are an important food source and refuge for animals in urban areas because they represent a large part of the green space. It has been shown that garden management regime (water use, floral composition) may impact the species they shelter. However, due to access restrictions, lack of regulations and the difficulty of data collection on private property, the impact of management practices and in particular pesticide use has seldom been assessed in private gardens. Using data collected in the framework of a nationwide participatory monitoring scheme in France, we assess here, for the first time, the effect of private garden management on two important groups of flower-visiting insects, i.e. butterflies and bumblebees, at a large scale. We show that the correlation between butterfly and bumblebee abundance and use of insecticides and herbicides is negative, whereas the use of Bordeaux mixture (fungicide approved for organic use), fungicides and anti-slugs is positively correlated with butterfly and bumblebee abundance. We hypothesize that herbicides have an indirect negative impact on insects by limiting the amount of available resources, and that the Bordeaux mixture, fungicides and slug repellants have an indirect positive impact on these insects by fostering healthier plants, probably offering higher level of resources to pollinators. Moreover, we show that the impact of pesticides varies according to the landscape, the negative effect of insecticides being more important in highly urbanized areas. Overall, our results show that gardener practices can have a positive impact on flower-visiting insects, even in a highly anthropized, urban landscape.

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1. Introduction

Private gardens represent an important part of green spaces in cities, e.g. 23% in Sheffield (UK), (Gaston et al., 2005), or 36% in Dunedin, New-Zealand (Mathieu et al., 2007). Representing nature oases in cities, green spaces are known to positively influence human health and wellbeing (Fuller et al., 2007; Gross and Lane, 2007; Gaston et al., 2007). Furthermore, it has been suggested that private gardens might mitigate the impact of urbanization on biodiversity (Goddard et al., 2010). Even if each garden taken individually is too small to be of biological importance, gardens taken as a whole can be an important component of urban floristic diversity (Thompson et al., 2003; Smith et al., 2006b; Loram et al., 2008; Stewart et al., 2009) and provide important sources of food and shelter for birds (Cannon et al., 2005; Davies et al., 2009), wild bees (Fetridge et al., 2008; Samnegård et al., 2011) and amphibians

(Gaston et al., 2005). Private gardens can also provide landscape connectivity for plants and animals (Rudd et al., 2002; Sperling and Lortie, 2010; Vergnes et al., 2012, 2013). However, they may also have a negative impact on the environment: for instance, Dehnen-Schmutz et al. (2007) and Marco et al. (2010) have shown that ornamental plants cultivated in private gardens could be an important vector of plant invasions. Assessing the role of private gardens in maintaining urban biodiversity still requires an understanding of the factors driving the biodiversity hosted within these private areas.

Landscape and local scale factors may impact urban biodiversity. Pardee and Philpott (2014) showed that presence of native plants in gardens but also landscape characteristics, such as amount of semi-natural area in the landscape, influence urban bee diversity. Similar results were shown for British moths (Bates et al., 2013). Furthermore, Bergerot et al. (2011) showed that the level of urbanization in the landscape surrounding private gardens was a strong driver of the diversity and composition of butterfly communities in gardens, with lower species richness and lower occurrence of feeding specialists in strongly urbanized sites. On

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the other hand, [Smith et al. \(2006a\)](#), found that the extent of green space around gardens only occasionally explained the abundance of 22 invertebrate groups, and that most variables correlated with abundance occurred at the scale of the garden itself. These seemingly contradictory results might arise from temporal and spatial variability, or from a lack of power of the analysis performed. Untangling the local and landscape effects on insect diversity in gardens might require larger datasets encompassing various garden types and levels of urbanization.

Another difficulty of studying private gardens is that they are unregulated habitats with various water and chemical use intensity and vegetation structure. Moreover, these characteristics are generally unknown, depending on each gardener's own decisions ([Mathieu et al., 2007](#)). Although the effect of management practices on private gardens has been little studied, it has been shown that increased pesticide use on residential yards may negatively impact the environment ([Robbins et al., 2001](#)). Direct effects on species abundance in private gardens have seldom been studied, but available results suggest it could be important, especially because their use in gardens is unregulated and the amount private gardeners use may be significant. [Smith et al. \(2006a\)](#) included pesticide use in their study of invertebrates in urban gardens at a city scale, but this factor was pooled in a global management intensity index including several variables, such as weeding, pruning, watering or bird feeding. Such an aggregated index of management intensity makes it difficult to identify the components that most affect biodiversity. More specifically, [Byrne and Bruns \(2004\)](#) and [Cheng et al. \(2008\)](#) have revealed the negative impact of pesticides on non-target soil microfauna, whereas [Politi Bertoncini et al. \(2012\)](#) have shown it on floristic composition, and [Stewart et al. \(2009\)](#) found a negative correlation between lawn management intensity, including use of phytochemicals, and the presence of various plant species in urban lawns. There are a few citizen-science studies that have investigated bumblebees or Lepidoptera in private gardens (e.g. [Lye et al., 2012](#); [Bates et al., 2013](#)); however, to our knowledge, the impact of pesticide use on biodiversity, and especially flower-visiting insects, has never been studied in private gardens at a large scale and in different landscape contexts.

Restricted access to private gardens and the difficulty of data collection on biodiversity and management in this habitat probably accounts for the paucity of research on this topic. When collecting data in private gardens, citizen science is an efficient tool because garden owners can directly provide the data ([Cooper et al., 2007](#)). Based on a nationwide citizen survey on private gardens in France, we assess here the relative impact of local scale factors (i.e. garden structure and management) and landscape composition (i.e. proportion of urban area) on two groups of pollinating insects, butterflies and bumblebees. We specifically measured the impact of pesticides on these insects, depending on the type of pesticide (e.g. herbicide, molluscicide, insecticide), and quantified this impact relatively to other factors, such as garden characteristics and urbanization level. We hypothesized that gardening practices would have a larger impact on insect abundance in densely urbanized districts than in more rural districts.

2. Material and methods

2.1. Insect data

Data came from two citizen monitoring schemes: the French garden butterfly observatory and the French bumblebee observatory (<http://vigienature.mnhn.fr/>). For these nationwide programs, citizens identify and count butterflies and bumblebees in their garden between March and October, following a simple protocol and a

closed list of 28 common species or species groups of butterflies (see [Appendix A](#) for full species list and mean abundances) and 11 bumblebee morphospecies (i.e. recognizable taxonomic units based on external morphology, which may not correspond to species – see [Appendix B](#) for full morphospecies list and mean abundances). No constraint on the frequency of observation is imposed, and volunteers record online each month the maximum number of individuals of each species/morphospecies seen simultaneously in the garden during the previous month. To reduce heterogeneity in the dataset due to non-independence between individual detection probability for species seen in groups, all monthly abundances that were above 10 (0.4% of all data) were leveled to a maximum value of 10 ([Julliard et al., 2006](#)). Visit frequency per month in each garden was recorded. We used data collected from 2009 to 2011 in 3722 gardens for the butterfly monitoring and 1119 gardens for the bumblebee monitoring. About 95% of gardens monitored for bumblebees were also monitored for butterflies. Due to the impossibility of assigning a species to a morphospecies with certainty for bumblebees, we only used total bumblebee abundance in analyses. For butterflies, we also only used abundance in analyses, because diversity and abundance were strongly correlated ($\rho = 0.9$).

2.2. Garden data

Volunteers recorded variables on garden structure and management. Garden structure was described as (1) garden area, (2) an index of nectar resources, calculated as the number of types of flowering plants in the garden, among a closed list of 12 species/plant types (i.e. *Buddleja*, *Centaurea* sp., *Valeriana* sp., *Pelargonium* sp., lavenders, crucifers, nettles, bramble, ivy, clovers, aromatic plants, fruit trees); this list was built with plants non-specialists can easily identify and that are common in gardens, and that offer resources (food or shelter) to butterflies, (3) an index of garden naturalness, this was calculated as follows: in the garden description, the observer states whether the garden has fallow, nettles, ivy and/or brambles (these three plants being usually considered as weeds by gardeners), dead trees and stems. Each of these items was scored one if present, zero if absent, and the naturalness index was calculated as the sum of these scores.

The use (or not) of herbicide, insecticide, fungicide, Bordeaux mixture (fungicide based on copper sulfate and approved for organic use), anti-slug and fertilizer defined garden management as reported by observers. They had to characterize their use of the different chemical types as “often”, “seldom” or “never”: however, only ca. 1% reported a regular (“often”) use of pesticides. For this reason, we used only two classes, “use” or “no use” of each of the chemicals.

2.3. Landscape data

Data were recorded in gardens located in ca. 3000 different districts (“communes”) out of 36,570 in France ([Fig. 1](#)). The mean area of a district in France is 15 km². We characterized the landscape of each district using CORINE land cover map (CLC project co-ordinated by the European Environmental Agency) dated 2006. CLC is established from satellite images with a resolution of 1:100,000 and includes 44 land cover classes grouped into five main (level-one) categories: urban areas, agricultural areas, forests and semi-natural areas, wetlands and water bodies. For each garden we quantified the proportion of urban areas in the district.

2.4. Data analysis

Independence between garden structure, management and landscape variables was tested with Pearson's correlations. For

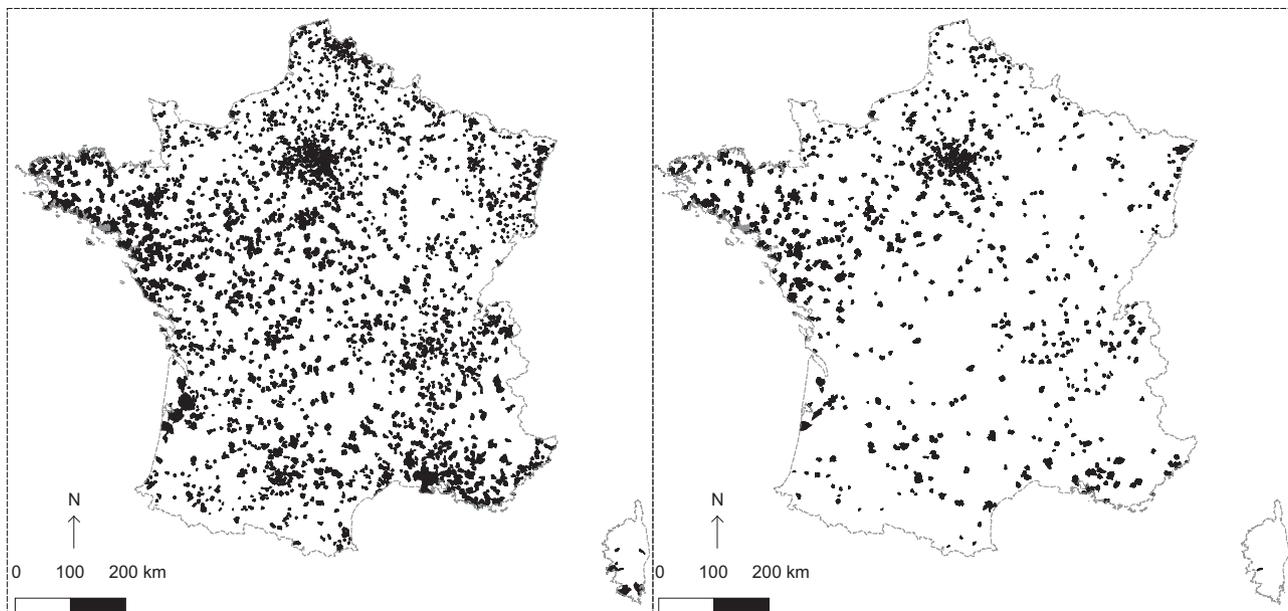


Fig. 1. Location of monitored gardens in France for butterflies (left) and bumblebees (right). Districts with at least one garden monitored in one year appear black.

these and subsequent analyses, insect abundance was log-transformed ($\log(x + 1)$) and landscape variables were square-root transformed.

For butterfly and bumblebee global abundance (all species/morphospecies within each taxa group were pooled), a linear mixed model was computed with month nested in region nested in year as random variables, to control for spatial and temporal pseudoreplications (Pinheiro and Bates, 2000). Garden structure (area, nectar resources), management (garden naturalness, use of products), sampling effort (garden visit frequency) and landscape (proportion of urban area in the district) were included as fixed factors. Interactions between proportion of urban area in the district and (1) garden area, (2) nectar resources, (3) garden naturalness and (4) the use of products (i.e. insecticide, herbicide, fungicide, Bordeaux mixture, slug repellent, fertilizer) were also calculated. All explanatory variables except use of products were standardized by retrieving means and dividing by standard deviations to compare directly the magnitude of model variables responses. We compared the Akaike information criterion (AIC values) of the full model with a null model including random effects only, to test if the selected variables really improve the understanding of abundance variation. The model was run with the R software (R 2.13.2; R Development Core Team, 2013) using the nlme package (Lindstrom and Bates, 1990).

3. Results

355,326 butterfly individuals of 28 species or group of species (see Appendix A) and 52,631 bumblebee individuals of 11 morphospecies (see Appendix B) were recorded in all gardens. Their abundances showed the same correlation trends along explanatory variables (Appendix C). As all these correlations were weak ($\rho \leq 0.5$), we considered them as independent.

The slope of the relationship between butterfly and bumblebee abundances, and significant variables of the linear mixed models were examined (Table 1). AIC values of full models were considerably smaller than those of null models (Full model AIC values: 14,157 and 66,142; null model AIC values: 15,216 and 73,953, respectively for bumblebees and butterflies) suggesting that explanatory variables used in the full model improved the understanding of the main drivers of abundance.

As predicted, the number of observer visits in the garden significantly increased the number of individuals observed.

3.1. Landscape level

The proportion of urban areas in the garden district was associated with a decrease in abundance of butterflies (slope estimate = -0.15 ; $p < 0.001$) and bumblebees (slope estimate = -0.06 ; $p = 0.02$).

3.2. Garden structure

Garden area was the variable with the largest effect on butterfly abundance (slope estimates = 0.18 with $p < 0.001$; Table 1). It also had a large effect on bumblebee abundance (slope estimate = 0.14 ; $p < 0.001$). The effect of garden area on pollinator abundance was larger when the garden was located in highly urbanized districts and this held particularly true for bumblebees. The variety of nectar resources in the garden was also an important driver of pollinator abundance (slope estimates = 0.1 and 0.08 , respectively, for butterflies and bumblebees; $p < 0.001$). This effect was greater for bumblebee abundance when the garden was located in a less urbanized district. Garden naturalness was associated with an increase in butterfly abundance (slope estimate = 0.06 for butterflies) but not of bumblebees. This effect of garden naturalness was enhanced in gardens situated in less urbanized districts.

3.3. Garden management

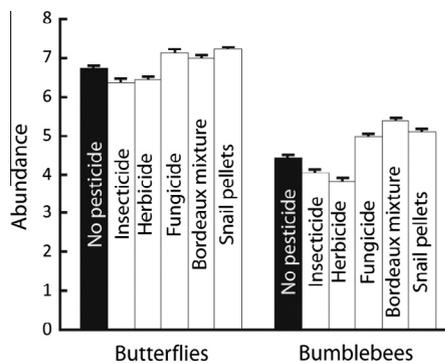
The use of insecticides was negatively associated with butterfly and bumblebee abundances (slope estimate = -0.07 for bumblebees and -0.05 for butterfly; $p < 0.001$). The use of herbicides showed the same negative associations on butterfly (slope estimate = -0.04 ; $p < 0.001$; Fig. 2) and bumblebee abundances (slope estimate = -0.12 ; $p < 0.001$). However, the use of fungicides, Bordeaux mixture and snail pellets were positively associated with butterfly (slope estimate = 0.05 ; 0.03 and 0.06 , respectively; $p < 0.001$) and bumblebee abundances (slope estimate = 0.09 , 0.16 , 0.12 , respectively; $p < 0.01$).

The negative effect of insecticides on butterflies and bumblebees was more important in highly urbanized districts. The positive

Table 1Summary table of model output (degrees of freedom (numDF), Slope estimates (and standard-errors) of fixed variables, *t*-values and *p* value).

Variables	Pollinator abundance							
	Butterflies				Bumblebees			
	numDF	Slope estimates (standard-error)	<i>t</i> -value	<i>p</i> -value	numDF	Slope estimates (standard-error)	<i>t</i> -value	<i>p</i> -value
(Intercept)	31,252	2.04 (0.06)	33.1	***	5773	1.70 (0.07)	25.3	***
Urban area in the district (UrbDist)	31,252	-0.15 (0.01)	-16.1	***	5773	-0.06 (0.02)	-2.3	*
Garden area (Area)	31,252	0.18 (0.005)	38.2	***	5773	0.14 (0.01)	10.7	***
Nectar resources (DivNect)	31,252	0.10 (0.004)	22.3	***	5773	0.08 (0.01)	7.3	***
Garden naturalness (DivNat)	31,252	0.06 (0.004)	14.1	***	5773	0.02 (0.01)	1.6	0.12
Garden visit frequency	31,252	0.14 (0.004)	36.4	***	5773	0.13 (0.01)	13.9	***
UrbDist*Area	31,252	0.02 (0.004)	5.1	***	5773	0.12 (0.01)	9.6	***
UrbDist*DivNect	31,252	-0.01 (0.004)	-1.7	0.09	5773	-0.03 (0.01)	-2.6	**
UrbDist*DivNat	31,252	-0.01 (0.005)	-2.2	*	5773	-0.002 (0.01)	-0.2	0.88
Insecticide use (INSE)	31,252	-0.05 (0.01)	-5.0	***	5773	-0.07 (0.02)	-3.3	***
Herbicide use (HERB)	31,252	-0.04 (0.01)	-4.2	***	5773	-0.12 (0.02)	-5.3	***
Fungicide use (FUNG)	31,252	0.05 (0.01)	5.6	***	5773	0.09 (0.02)	4.1	***
Bordeaux mixture use (BORD)	31,252	0.03 (0.01)	3.9	***	5773	0.16 (0.02)	7.4	***
Snail pellets use (SLUG)	31,252	0.06 (0.01)	7.1	***	5773	0.12 (0.02)	5.4	**
Fertilizer use (FERT)	31,252	-0.01 (0.01)	-1.3	0.18	5773	0.01 (0.03)	0.3	0.78
UrbDist*INSE	31,252	-0.02 (0.01)	-2.7	**	5773	-0.04 (0.02)	-2.8	**
UrbDist*HERB	31,252	-0.01 (0.01)	-1.2	0.22	5773	-0.07 (0.02)	-3.1	**
UrbDist*FUNG	31,252	-0.05 (0.01)	-5.8	***	5773	-0.08 (0.02)	-3.9	***
UrbDist*BORD	31,252	0.03 (0.01)	4.0	***	5773	0.01 (0.02)	1.6	0.11
UrbDist*SLUG	31,252	0.04 (0.01)	4.2	***	5773	-0.0002 (0.02)	0.4	0.71
UrbDist*FERT	31,252	0.02 (0.01)	2.3	*	5773	0.03 (0.02)	1.0	0.32

(p-value <0.001 = ***; 0.001 < p-value < 0.01 = **; 0.01 < p-value < 0.05 = *).

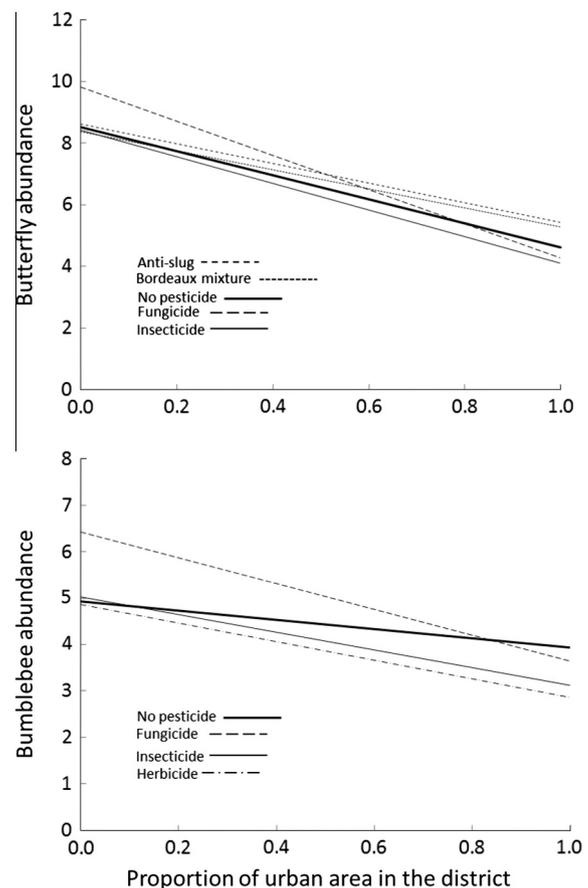
**Fig. 2.** Values of butterfly and bumblebee abundance with standard errors in gardens with and without use of pesticides ($n = 3722$ for butterflies, 1119 for bumblebees). Estimates resulting from linear mixed models; see Table 1 for figures.

effect of fungicides on butterflies and bumblebees was reduced in highly urbanized districts; for butterflies, the positive effect of snail pellets and Bordeaux mixture were more important in highly urbanized districts; for bumblebees, the negative effect of herbicides was more important in highly urbanized districts. Other interactions between pesticide use and urbanization were not significant (Fig. 3 and Table 1). Use of fertilizers had no significant effect on butterfly and bumblebee abundance.

4. Discussion

4.1. Limitations of the study

When the garden butterfly monitoring scheme was designed in 2005, it was decided that to promote participation, data requested should be as least intrusive as possible. For instance, no information such as age, sex or professional status was requested. Similarly, it was decided that the address of the observer would not be collected. For this reason, gardens are localized only through their district. This prevents a finer scale analysis, where the impact of landscape features in a buffer around the garden, such as urban

**Fig. 3.** Predicted effects of urbanization and pesticides use on butterflies and bumblebees. Estimates resulting from linear mixed models; see Table 1 for figures.

green spaces, semi-natural habitats, vegetation types or water bodies, could be precisely assessed. Our dataset only allows testing of the impact of the proportion of coarse habitat types in the

district as given by large databases, such as Corine Land Cover. A new version of the monitoring scheme will be released in 2015, and it will include interactive maps to locate gardens and provide potential for fine scale analyses in the future.

Another limitation of our study, also due to the design scheme, lies in the restricted list of plants observers recorded in their gardens. Even if this plant list is a good predictor of insect abundance and diversity, it only covers a small proportion of the range of plant species that may be encountered in the garden. Thus these data do not allow measurement of floral diversity in the garden, which may be another important predictor of insect abundance and diversity. Moreover, the available information may not be able to detect the potential effect of important plant species that would not be listed.

4.2. Landscape vs. garden

Private gardens represent a difficult habitat to study, not only because numerous biotic and abiotic factors may affect invertebrate abundance both at the landscape and local scales, but also because management decisions are usually not recorded. Moreover, garden features may have indirect effects on pollinator diversity/abundance. We showed that an increase of urban areas in the district was associated with a decrease of butterfly and bumblebee abundance. This is consistent with the study of Bergerot et al. (2011) who showed a decrease in mean butterfly species richness and feeding specialists in strongly urbanized sites. Moreover, Bergerot et al. (2010) explained that the decline in specialist species was not linked to a lack of resources in gardens but to landscape fragmentation, which hampers butterfly movements, because most sensitive butterflies cannot enter urban areas. Moreover, we revealed a high positive effect of garden area on butterfly and bumblebee abundance. Smith et al. (2006b) found that among a range of garden and landscape variables, garden area was the only factor significantly positively related to plant richness, as one could expect. We hypothesize that the greater floristic diversity in large gardens explains the positive effect of garden area on pollinators we observed. Other factors may also be important, such as the proportion of native plant species, already highlighted in other studies (e.g. Frankie et al., 2005; French et al., 2005; Burghardt et al., 2009; Pardee and Philpott, 2014), though the impact of native plants may be weak or depend on species (Hanley et al., 2014). However, this effect of garden area was dependent on landscape, i.e. very strong in urbanized districts and less important in more rural districts. In inhospitable urban landscape, where there are no natural or even semi-natural habitats, gardens, though heavily anthropized, represent the main patches of suitable habitats for flower-dependent insects. In this context, small gardens in highly urbanized areas are likely to be part of a network of adjacent gardens that provides more resources as a whole than small gardens taken individually. Conversely, in rural landscape, the insect populations also live in the matrix surrounding gardens, and the effect of garden area on observed insect abundance is thus less prominent.

We added to the complexity of the picture by taking into account for the first time the use of different types of chemical inputs in private gardens and their interactions with the larger environment. Insecticide effects decreased insect abundance locally, and this effect was less pronounced in a rural environment that could act as a source for the garden. Herbicide caused a decrease in insect abundance, because after such treatment, floral resources decrease in gardens, which leads to a decrease of flower-dependent insects. However, herbicide effects on butterflies were the same in rural or urban environment, whereas the negative trend was more pronounced in urban environment for bumblebees. This could be due to the fact that the small average

foraging distance for bumblebees (less than 1 km – (Knight et al., 2005; Osborne et al., 1999) implies that they are more affected by herbicide treatments in urban (i.e. highly fragmented) environment: the recolonization of gardens from the surrounding environment is less easy than in rural landscape, compared to butterflies, which have larger dispersal abilities, at least for migratory species.

For butterflies, the positive effect of Bordeaux mixture, snail pellets and fertilizers was more noticeable in urban areas, probably because alternative food sources are scarce in urban environment and improvement of these food sources has more impact there. We suggest that these interactions do not appear to be significant for bumblebees because of their lower dispersal abilities: they are more sensitive than butterflies to the type of landscape around gardens and less able to colonize urban gardens where chemicals protecting plants against pests are used. For butterflies and bumblebees, the use of fungicide is positive in rural districts, and negative in highly urbanized ones, a trend for which we have no straightforward explanation.

4.3. Garden management

The role of plant assemblages in private gardens on insects and birds has already been highlighted (Yahner, 2001; Frankie et al., 2005; French et al., 2005; Smith et al. 2006a). Studies in agricultural environments have shown negative direct effects of insecticides on pollinators (Brittain et al., 2010) and negative indirect effects of herbicides, related to the diminished abundance of their resources, i.e. weeds (Hawes et al., 2003). The effects of fungicides, Bordeaux mixture or anti-slug on flower-visiting insect abundances were poorly addressed (but see Cahill et al., 2008) and never in private gardens. We have shown here a negative effect of insecticides/herbicides, and a positive effect of fungicides/anti-slug/Bordeaux mixture on butterflies and bumblebees in private gardens. We hypothesize that the positive effects we observed are indirect, because plants protected from pests would be able to allocate more resources to produce nectar for insects. The behavior of garden owners is thus crucial to maintain floristic diversity, but also pollinator abundance in urban areas. These results send a strong message for gardeners, because they show that gardening practices can have a positive impact on flower-dependent insects, even in highly anthropized, urban landscape.

Our dataset is probably biased toward an underestimation of the use of pesticide at the national scale, for two reasons. First, only ca. 1% of the participants reported a regular (“often”) use of pesticides. We suspect this does not reflect the true use of pesticides at a nationwide scale. This is supported by Ahmed et al. (2011) who investigated individual perceptions and attitude to pesticide use in peri-urban areas in Sweden. While 80% of the interviewed people reported pesticide use in their home setting, 47% perceived themselves to be non-users of pesticides, thus their actual use of pesticides was higher than their perception. The situation is probably similar in our study, i.e. people’s perception of their pesticide use is an underestimation. Second, gardeners involved in our study are probably not representative of the whole French gardener population. Deciding to take part in a citizen science project implying counting butterflies and bumblebees in one’s garden probably denotes a broad interest for environment: it is likely that the sampled population uses fewer pesticides than the average gardener. Use of pesticides in gardens, and as a consequence, impact on pollinators is thus certainly more important globally in France than shown in our study.

Because management decisions depend mostly on the garden’s owners, it would be most useful to understand how such decisions are taken. In fact, studies on private gardens have generally been conducted from a single perspective, either ecological or social (respectively 68% and 34% of the papers reviewed by Cook et al.

(2011)) while interdisciplinary studies are rare (22% of the papers reviewed by Cook et al. (2011)). This is a constraint for an integrated understanding of private gardens biodiversity dynamics. Goddard et al. (2013) found that gardeners considered their garden as a place where they can be reconnected to nature. These gardens represent an ideal location to perform approaches integrating biological and social sciences on the links between biodiversity and urban citizens. This in turn would raise awareness on the role of private garden as biodiversity refuges in urban areas. In this context, the importance of citizen science should be promoted, because they represent a useful tool to understand large scale patterns of pollinator abundance, a group generally known to be difficult to study and that require a large amount of human power to get statistically meaningful data (Deguines et al., 2012). Citizen sciences provide a great way to study private gardens that are difficult to sample at a large scale because of access restrictions. Another benefit from studies such as ours could be modifying management practices, provided that academic results are properly communicated to a non-scientific audience. For instance, the French garden observatory newsletter and blog should be used to disseminate the main results of the present study. The monitoring scheme website would be another important communication tool, with the help of interactive graphs and maps allowing each observer to compare their own observations to other people's observation, and to compare insect communities from gardens with pesticide to those from gardens without pesticide. Because volunteers are already concerned about environment and biodiversity issues, such feedback on the monitoring program's results may prompt them to reduce their use of insecticide and herbicide.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2014.11.045>.

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