



Low concentrations of fertilizer and herbicide alter plant growth and interactions with flower-visiting insects

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ABSTRACT

The increasing extent and intensity of agricultural land use has led to an increase in the volume of agrochemicals applied to the landscape, including those used to improve the nutritional quality of soils (fertilizers) as well as those used to control undesirable species in the agroecosystem (pesticides). Chemicals can disperse in the air and surface and ground water, leading to exposure of non-target organisms. Ruderal, disturbance-tolerant plants on which many flower-visiting insects rely are commonly exposed to these chemicals in agroecosystems. Our research questions were: 1) how does non-target exposure to agrochemicals affect plant growth? 2) what are the indirect effects of non-target exposure to agrochemicals on flower-visitation by insects? We designed a two year field experiment imitating field-realistic fertilizer run-off and non-target herbicide exposure scenarios to explore the impact of low concentrations of fertilizer and herbicide alone, and in combination, on communities of seven plant species, including six native perennials and one non-native annual commonly found in agricultural systems in Ireland (*Cirsium vulgare*, *Epilobium hirsutum*, *Plantago lanceolata*, *Origanum vulgare*, *Filipendula ulmaria*, *Hypochaeris radicata*, *Phacelia tanacetifolia*). We created field-realistic exposure scenarios by applying concentrations of mineral fertilizer similar to those detected in ground water, and glyphosate levels equivalent to 7.6 % of a standard field application, to the foliage and soil of the plots. We found low concentrations of fertilizer and herbicide affected plant growth: fertilised plants were taller when flowering, while plants exposed to herbicide flowered at shorter heights and produced shorter leaves. The size of the floral display had the largest effect on insect visitation, with larger floral displays significantly more likely to receive a visitor in a given sampling event. The size of the floral display also interacted significantly with the fertilizer treatment for both the abundance and species richness of floral visitors. Overall, our results suggest that there are direct and indirect effects of agrochemical exposure on plants in field margins, and that these effects change the interactions between ruderal plants and flower-visiting insects.

1. Introduction

Agricultural ecosystems currently comprise approximately forty percent of the terrestrial surface of the earth (Martin et al., 2012) and the impacts of agricultural practices extend beyond the target crop. Application of chemicals directly impacts the flora and fauna of surrounding habitats, and indirectly affects the species with which they interact. For example, the high concentrations of fertilizer regularly applied to maintain the high productivity of modern cropping systems have impacts on the soil microbiota (Jangid et al., 2008) and species diversity, community composition, and primary productivity of plants (Borer

et al., 2014; Gough et al., 2000; Leff et al., 2015). Research also shows that direct soil enhancement (i.e. with fertilizers) can cascade through food chains, from plants to herbivores (Staley et al., 2010), including pollinators (Cardoza et al., 2012; Russo and Shea, 2020), to predators and parasitoids of those herbivores (Pope et al., 2012). Chemicals are not confined to their area of application, and the effects of fertilizer run-off, typically of lower concentration, on terrestrial systems are less well established than in aquatic ones (Grimm et al., 2003). The direct and indirect effects of run-off levels of fertilizer on plant-insect interactions are even less well studied (but see Burkle and Irwin, 2010).

In addition to chemical fertilizers, agricultural land managers also

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apply pesticides to control unwanted species. In a recent survey of European soils, pesticide residues were detected in 83 % of soil samples; glyphosate and its derivative AMPA (aminomethylphosphonic acid) contributed the most to total pesticide content (Silva et al., 2019). Glyphosate, the active ingredient in the brand-name herbicide “Roundup” is the most widely used herbicide in the world, and its use has increased 15-fold globally after the release of “Roundup Ready” crops (Benbrook, 2016). Direct applications of herbicides, such as glyphosate, at recommended levels, are designed to kill a broad spectrum of plant species. However, there can be unintended impacts of herbicide use on non-target species. Even 1–5 % of the recommended application rate of glyphosate can have sub-lethal effects on non-target plant growth, including reducing floral density and plant species diversity (Schmitz et al., 2013; Strandberg et al., 2017), and a negative effect on mutualistic interactions with pollinators, including decreases in the visitation rates, species richness, and pollination services to *Tanacetum vulgare*, L. (Dupont et al., 2018). Moreover, it has recently been shown that glyphosate can have direct negative impacts on flower visiting insects, such as the honey bee (Motta et al., 2018).

In landscapes with high agricultural intensity, many non-target communities of plants and insects experience agrochemical exposure. The plant communities growing on the margins of arable and pasture fields are most likely to experience run-off concentrations of fertilizers and non-target exposure from herbicides. It is important to understand the critical role these field edges play in the maintenance of biodiversity in intensively managed agricultural systems, where they are sometimes the primary source of semi-natural habitat in the landscape (Marshall and Moonen, 2002), and contribute to the provision of ecosystem services such as pollination and pest control to croplands (Castle et al., 2019; Grab et al., 2018). For example, weedy plant species, such as those that survive on field margins, have been shown to be essential in supporting pollinators in agricultural systems, including both managed honey bees (Pettis et al., 2013) and a range of unmanaged pollinating insects (Grab et al., 2019; Park et al., 2015; Russo et al., 2016). The plant communities currently existing in these agroecosystems are almost certainly those that can tolerate a certain level of agrochemical exposure, given historical land-use (Brown, 2007); however, these plants are likely to continue to be affected by agrochemical exposure (Dupont et al., 2018).

Because the composition of plant communities changes in response to fertilizer (Clark and Tilman, 2008; Isbell et al., 2013) and herbicide exposure (Strandberg et al., 2017), and combinations thereof (Pellissier et al., 2014), it is difficult to study the responses of a fixed community of plants to low levels of agrochemical exposure over time. To overcome this, we designed a controlled experiment to examine how concentrations of fertilizer and herbicide typical of agrochemical run-off affect the growth of and visitation of flower-visiting insects to individual plants over two years across eight sites in Ireland. We created experimental communities with the same number of individuals of six native perennials commonly found on crop edges in Ireland, plus a non-native annual species common in pollinator plantings (including field margins and wild flower strips) in Europe. All of the species we include here have been shown to be attractive to pollinating insects (Russo et al., *in review*). Ireland has the largest proportion of land dedicated to agriculture in the EU-27, at 71.5 % (European Union, 2010) and a high density of field edges (Sullivan et al., 2013). While most of the agriculture in Ireland is pastoral grasslands, many weeds exist both on the margins of arable fields and within pastures, providing valuable resources to flower-visiting insects. At the same time, over 30 % of Irish bee species are regionally extinct, critically endangered, endangered, or vulnerable (Fitzpatrick et al., 2006). Thus, there are good reasons to be concerned about the direct and indirect impacts of non-target agrochemical exposure on weedy plant species and their pollinating insects in agroecosystems in Ireland, and these findings have implications for similar systems elsewhere.

We expect that plants exposed to run-off concentrations of fertilizer

will grow to a greater height and have a larger floral display, while plants exposed to low concentrations of herbicide will be shorter and have a smaller floral display, relative to plants exposed to just water. Moreover, we expect to see a higher visitation rate of insects on the inflorescences of plants exposed to fertilizer run-off, and a lower visitation rate on plants exposed to herbicides, relative to plants receiving just water. Finally, in the treatment that combines both run-off concentrations of fertilizer and low concentrations of herbicide, we expect positive and negative effects to cancel out and to see no significant difference relative to the control in both plant growth and insect visitation.

2. Methods

2.1. Experimental design

In the spring (April) of 2017, we established sixteen 2 × 2 m research plots across four sites (four plots per site) in Dublin, Ireland (Fig. S1). We selected urban, non-agricultural sites so that the research treatments would not be exposed to variation in background agrochemicals between sites. This allowed us to control the amount and concentration of herbicide and fertilizer each plot was exposed to, and also allowed us to have control plots that were not exposed to fertilizer or herbicide. These locations were selected based on the availability of space for the research plots within a fenced area so they would not be disturbed. Sites were separated by at least 1 km and all plots within a given site were separated by 50 m. Each plot was surrounded by a chicken wire fence to deter vertebrate herbivores. We established the plots by spraying the existing grass with the recommended dose of glyphosate in early April.

After this application, we waited two weeks before planting to allow for mineralization of glyphosate, which can be rapid in high pH soils with organic matter (Al-Rajab and Schiavon, 2010; Yu and Zhou, 2005) as these plots were (Table S1); glyphosate exhibits high adsorption in most natural settings, however, soils that are regularly treated with glyphosate may saturate available soil organic matter (Bai and Ogbourne, 2016). Thus, agricultural settings with regular, and repeated glyphosate applications exhibit a higher risk of non-target glyphosate exposure (Bai and Ogbourne, 2016). For this reason, we expect that the initial glyphosate application that was used to kill off the grass in our experimental plots would be unlikely to affect the plants later in the season. In addition, because all plots were treated at the same time, if there was an effect of initial glyphosate treatment, it would be equal across all plots.

We seeded each plot with 4.5 g (~608 seeds/m²) of *Phacelia tanacetifolia* (Benth.), an annual flowering plant commonly recommended in pollinator plantings in Europe (Rundlöf et al., 2018). Then we transplanted four individuals of each of *Plantago lanceolata* L., *Hypochoeris radicata* L., *Filipendula ulmaria* L., and *Epilobium hirsutum* L., two individuals of *Cirsium vulgare* Savi., and one individual of *Origanum vulgare* L. into each plot. Aside from *P. tanacetifolia*, all of the plant species in the plots were biennial/perennial and considered native to Ireland. In addition, all perennial individuals of a species were sourced from the same populations and were approximately the same size (within each species) upon planting. *P. lanceolata*, *C. vulgare*, *F. ulmaria*, *E. hirsutum*, and *H. radicata* individuals were sourced from populations in a wildlife conservation area in Kilkenny, Ireland, while *O. vulgare* individuals were sourced from a commercial supplier. After planting, the plots were watered for a month before treatments were initiated so the plants could establish. Throughout the season, plots were watered regularly and non-target plants (other than the species/individuals we planted) were removed by hand as needed. We also prevented *P. tanacetifolia* from competing with the perennial individuals with hand weeding; densities of this species were variable in the different plots, but we removed individuals when they overgrew the perennials. We considered *P. tanacetifolia* to be too close to the other plants when it blocked light from reaching them or when the plants were physically touching. We

randomly assigned treatments in mid-May to plots within a site (each site is a randomized block).

In the spring of 2018, we repeated the 2017 experimental design with sixteen new plots at four new research sites in Dublin. The plots were established in the same fashion and the perennials were obtained from the same source populations. The only differences in 2018 were that we increased the number of individuals of each of the perennial species because of low visitation rates in 2017 and that the plots were < 50 m apart at two of the sites where space constraints dictated they be closer. In 2018, we planted six individuals of each of *P. lanceolata*, *H. radicata*, *F. ulmaria*, and *E. hirsutum*, three individuals of *C. vulgare*, and two individuals of *O. vulgare*. The seeding rate of *P. tanacetifolia* was the same as in 2017 and treatments were applied in the same way as the previous year.

The plants in plots from 2017 did not receive treatment applications during the winter, but were treated again in their second year of growth (started in early May 2018). In plots where *P. tanacetifolia* had failed to flower in 2017, we seeded an additional 1 g of seed in April 2018. Thus, there were eight replicate sites of the first year of the experiment (4 sites in 2017 and 4 in 2018), and four replicate sites of the second year of the experiment (4 sites planted in 2017, treated and monitored again in 2018).

2.2. Treatments

Each plot received one of four experimental treatments and each site had all four treatments represented: 1) control (20 L water), 2) fertilizer (run-off concentration of NPK (Appendix 1) in 10 L water plus 10 L water), 3) herbicide (low concentration of glyphosate (Appendix 1) in 10 L water plus 10 L water), and 4) a combination treatment (same low concentrations of NPK in 10 L water and glyphosate in 10 L water) (Table 1). Thus, each plot received the same amount of water on each treatment day. Treatments were applied evenly across the foliage and soil in the plots once a week beginning in mid-May for three months throughout the growing season. For single treatment plots (fertilizer or herbicide), we applied the 10 L of untreated water first, and then the 10 L with the treatment. For combination plots (fertilizer and herbicide), we applied the 10 L with herbicide first, and then the 10 L with fertilizer. Fertilizer and herbicide concentrations were reduced through the season: we applied the first concentration once a week for four weeks, the second concentration once a week for four weeks, then the third concentration once a week for the final four weeks (Table 1). The decreasing concentrations of fertilizer and herbicide through the summer were designed to simulate true non-target exposure scenarios, given a likely spring application of fertilizer and herbicide in a typical agroecosystem, and the concentrations were selected based on published studies of fertilizer (Bertol et al., 2007; Craig and Mannix, 2009; Korsaeht and Eltun, 2000) run-off (Table 1, Appendix 1 for further description of chemical application).

Glyphosate is not mobile in the groundwater system and is rarely

Table 1

Decreasing concentrations of fertilizer and herbicide across the summer applications, as well as total annual application for treated plots. All treatments were mixed with 10 L of water and applied evenly across foliage and soil in the plots using watering cans.

	First month (once a week)	Second month (once a week)	Third month (once a week)	Total annual application
N (mg/l)	30	20	10	0.6 g/m ²
P (mg/l)	15	10	5	0.3 g/m ²
K (mg/l)	5.5	3	1	0.095 g/m ²
Glyphosate (mg active ingredient/l)	0.7	0.3	0.1	0.011 g/m ²

detected in high concentrations (Saunders and Pezeshki, 2015), but run-off levels can be very high (up to 7.6 mg/l, Jones et al., 2010) immediately after application, especially if it is sprayed over water (Edwards et al., 1980). In a recent survey of European soils, the maximum concentration of glyphosate detected was 2.05 mg/kg (Silva et al., 2019). Moreover, plants growing on field margins are also likely exposed to glyphosate drift after applications. Our maximum glyphosate application was based on the US EPA's Maximum Contaminant Level (MCL) for safe drinking water (US EPA, 2003) and decreased across the year. The highest concentration we applied was less than half the maximum level detected in Silva et al. (2019). If we sum across the entire season of applications, it was roughly 7.6 % of the standard annual field application (1440 g/ha) (Dupont et al., 2018).

2.3. Data collection

Once the plants began to flower, we sampled insects that came in contact with the reproductive parts of the inflorescences for at least 1 s. On each sample day at each site, we collected flower-visiting insects on each flowering plant species for five minutes at each plot within a given site using an insect vacuum (total of 96 sample days, or 623 date-plot-samples). Sites were visited between 12–14 times for collections in both the first and second year; variation in the number of site collections was due to variation in the timing of flowering between different sites. We conducted sampling events between the hours of 0700 and 1800, however, 84 % of the samples were collected between the hours of 1000 and 1600. The order in which we visited sites, plots within sites, and species within plots was randomized during each sampling event. In addition, we also recorded the number of inflorescences of each species during each sampling event. Insect species that could be identified in the field (specifically *Apis mellifera*, *Episyrphus balteatus*, *Bombus pascuorum*, *B. lapidarius*, and *B. pratorum*) were released alive at the end of the sampling event. All insects that could not be identified to species in the field were collected and stored in a freezer until they could be pinned and identified. Bees were identified using Falk and Lewington (2015), while syrphid flies were identified using Ball and Morris (2015). Species identifications were then validated and corrected by qualified taxonomists (see acknowledgements). For workers of the *Bombus lucorum/terrestris* complex, we left the identification at *Bombus lucorum agg.* Where we were unable to confirm identifications at the species level (2% total specimens), we sorted remaining specimens to morphospecies. Voucher specimens of all collected insects are stored at Trinity College Dublin.

To quantify plant growth, we measured the height of each plant individual that was flowering or had flowered during the season, and the longest leaf length of every plant individual that did not flower at the end of the 2017 growing season. We measured height for flowering plants and leaf length for non-flowering plants because the plants in our plots exhibited a tendency to bolt, or grow a taller stem for the purposes of flowering, and remained in a shorter, or rosette stage when non-flowering. For the annual species (*P. tanacetifolia*), we ranked the plant's density on a scale of 0–10 where 0 was absent and 10 was maximum percent cover possible for that plant species, given the presence of the other plant species in each plot (see above about preventing *P. tanacetifolia* from overgrowing the perennials). Although we applied the same volume of *P. tanaacetifolia* seed to each plot, there was substantial variation in the density of the plants. For each plant species, we used digital calipers to measure the diameter of twenty randomly sampled inflorescences. From these twenty measures, we used the average diameter to calculate inflorescence size, which multiplied by inflorescence number, yielded the total floral display during each sampling event. We also collected soil samples from each plot in April 2017 after the plants had been established and immediately before experimental treatments were applied and in September after all treatments were complete for the year. From these soil samples, we measured the pH, %N, %C, %P, %K, and % organic matter (see Appendix 1). Our

objective in measuring these soil attributes was to determine 1) if variation in soil quality contributed to plant growth and 2) whether our treatments significantly changed soil attributes across the season.

At the end of 2018, we conducted a destructive census on all plots. For the one-year plots (planted in spring 2018), we measured the height of any plant in flower or that had flowered during the season and the longest leaf length of any plant that did not flower. For the plots in their second year of growth (planted in spring 2017), we counted the number of flowering and non-flowering individuals of each species, and measured the tallest flowering individual of each plant species, and the longest leaf of the largest non-flowering individual of each species. Again, for the annual species (*P. tanacetifolia*), we ranked its density in each plot and recorded whether it had flowered or not.

2.4. Data analysis

First, we evaluated the effect of the treatments on the growth the plants, the soil quality of the plots, and the response of the flower-visiting insects. For the following comparisons, we used linear mixed effects models with the R package lme4 (Bates et al., 2014). A full list of all linear mixed effects models with the responses, random and fixed effects, is presented in Table S2.

2.4.1. Plant growth

We tracked the growth of 768 individual plants of the perennial species for year one of the treatment, and then tracked growth at the species level in the second year of the treatments (we monitored density of the annual species, *P. tanacetifolia*; see *Experimental Design* for the numbers of individuals of each species). We tested whether the treatments had a significant effect on the height of flowering plants (437 observations, 7 species, 8 sites), longest leaf length of non-flowering plants (308 observations, 6 species, 8 sites), probability of flowering (768 observations, 6 species, 8 sites), and density of *P. tanacetifolia* (32 observations, 8 sites) from our end-of-season measurements from year one plots in 2017 and 2018. For these analyses, we used species and site as random effects, except for *P. tanacetifolia*, for which we used only site as a random effect. We also tested year (2017 or 2018) as a fixed effect; when it was not significant, we removed it from the models (Table S2). We did not find any significant interactions between year and treatment. For the plots in their second year in 2018, we analysed whether the treatment had a significant effect on the number of flowering (95 observations, 6 species, 4 sites) or non-flowering stems (80 observations, 5 species, 4 sites) of each species, as well as the height of the tallest flowering plant, longest leaf length of the largest non-flowering plant, and density of *P. tanacetifolia* (16 observations, 4 sites).

We tested whether the treatments had an effect on the total display size (number of inflorescences * inflorescence size) produced by each plant species across the two flowering seasons (log-transformed to normalize the distribution). We conducted this analysis separately for year one (1400 observations, 75 sample dates, 7 species, 8 sites) and year two (636 observations, 36 sample dates, 7 species, 4 sites) blocks. We tested whether the treatments significantly altered the start of flowering in the plots by comparing the first day of flowering of each species in each plot (179 observations, 7 species, 8 sites). For these we also tested the year of the study (2017 or 2018) and removed it when it was not significant. We did not find any significant interactions between year and treatment. We tested whether treatment had a significant effect on individual plant mortality using treatment and year as fixed effects and site and species as random effects (224 observations, 7 species, 8 sites). We also tested for a significant interaction between year and treatment.

2.4.2. Soil analysis (2017 only)

To test whether treatments had significant effects on measured soil attributes we calculated the difference in pH, %N, C, P, K, and organic matter before and after treatment (Spring-Autumn), and tested whether

treatment type was a significant predictor of this difference (16 observations, 4 sites). We tested whether soil attributes had a significant effect on plant growth, using a model selection process including all uncorrelated measured (see below) soil attributes on the plant growth attributes: probability of flowering (320 observations, 7 species, 4 treatments), height (given flowering, 232 observations, 7 species, 4 treatments), longest leaf length (given no flowering, 196 observations, 5 species, 4 treatments), and number of inflorescences produced across the season. All models with $< 2 \Delta AICc$ from the top model (lowest AICc) were considered equivalently appropriate.

There was strong collinearity between %N and % organic matter (df = 14, Pearson correlation coefficient = 0.95, $P < 0.001$) and %N and % C (df = 14, Pearson correlation coefficient = 0.87, $P < 0.001$), so we chose to remove those correlated variables and to use CN ratio, pH, %K, and %P for the model selection process when relating soil attributes to plant growth. For the probability of flowering, the top model included %K and %P as fixed effects, with the random effects of species identity and site. However, two additional models had a $\Delta AICc$ of < 2 different from the top model (considered equivalent), which were a model including only %K as a fixed effect, and one including only %P as a fixed effect. We tested for an interaction effect between %K, %P, and the treatment, but there were no significant interactions ($P > 0.05$), suggesting the effects of the agrochemical treatments were not dependent on soil attributes within the measured range.

2.4.3. Insect response

We first determined whether insect abundance within a given sample correlated with the size of the floral display (number of inflorescences * average size of the inflorescence) using a Pearson correlation coefficient. Then, to determine whether the relationship between insect abundance and floral display was linear or polynomial, we compared these model types by comparing their AIC and adjusted R^2 values. We considered the model to have the best fit which had the lowest AIC and highest adjusted R^2 . We also compared insect visitation rate and species richness among plant species, and between the first and second years of the plots. Because the data on insect visitation to the flowers were highly zero-inflated (see below), we conducted two separate analyses on their response to the treatments. First, we conducted an analysis to determine whether the treatment had a significant effect on the presence or absence of a flower-visiting insect at the plot level during a sample (binary response) in year one and year two of plant growth. For this model, we include plant species, plot nested within site, and site as random effects to account for repeated measures (year one: 1400 observations, 32 plots within sites, 8 sites, 7 plant species; year two: 636 observations, 16 plots within sites, 4 sites, and 7 plant species). We included treatment and floral display (scaled and centered) as fixed effects, and tested for an interaction between these two variables. We also tested collection year (2017 or 2018) as a fixed effect, and included it in the final model if it was significant. Next, we tested the effect of the treatment, floral display, and collection year (2017 or 2018) on the abundance and species richness of flower-visiting insects at each plot on each sample day, given their presence, analysing the insect response at the plot level (aggregated among plant species). For this model, we also used site and plot nested within site as random effects (year one: 346 plot-level observations, 31 plots within sites, 8 sites; year two: 160 plot-level observations, 16 plots within sites, 4 sites). We similarly tested for an interaction between floral display (scaled and centered) and treatment in this model. Because insect abundance was so low across the two years, we also conducted a rarefaction analysis to compare the treatments, sites, and plants. This rarefaction analysis controls for sampling effort in terms of the number of insects collected, rather than the number of hours spent sampling (Chao et al., 2014).

Finally, we calculated the visitation rate for each sample. This was calculated as the number of flower-visiting insects in a given 5-min sample, divided by the size of the floral display (number of inflorescences * average inflorescence size for each plant species).

Initially, we tested whether the plant species in the plots had different average visitation rates. Then, we tested whether there was an effect of the treatment on visitation rate as a way to evaluate whether there was a change in plant attractiveness in the different treatments independent of the size of their floral display (year one: 346 observations pooled at the plot level, 31 plots within sites, 8 sites; year two: 160 observations pooled at the plot level, 16 plots within sites, 4 sites). We also tested the effect of sampling year on the visitation rate of year one plants and removed it from the final model if it was not significant.

3. Results

3.1. Insect collections

Over the course of the two years, we conducted a total of 2036 5-minute samples (approximately 170 h) and collected 2567 insect flower visitors, representing 110 species and morphospecies. While bees contributed by far the largest abundance of visitors (1755 individuals of 31 species), syrphid flies, which had the second highest abundance, contributed the highest number of species (676 individuals, 38 species). Other visitors included (in order of decreasing abundance): non-syrphid flies, butterflies, true bugs, wasps, and beetles (Table S3). These collection data were zero-inflated; 51 % of the samples (85.75 h) had zero visitors. On plots in their first year of growth, we conducted a total of 1400 5-minute samples (~117 h) and on plots in their second year of growth, we conducted a total of 636 5-minute samples (53 h).

3.2. Plant growth

We detected significant effects of the agrochemical treatments on the timing of flowering, probability that an individual plant would flower, longest leaf length of non-flowering individuals, and height of flowering individuals in their first year (Table 2). Plants exposed to fertilizer flowered significantly earlier in the year and had a higher probability of flowering as individuals. When fertilised plants flowered, they were taller than control plants. When plants were exposed to herbicide, they were shorter when flowering, and had shorter leaves when they were not flowering. Finally, plants exposed to both fertilizer and herbicide had a higher probability of flowering as compared to the control plants (Fig. 1). There was also a significant effect of year on the longest leaf length of non-flowering plants in their first year of growth, where plants in 2018 had significantly longer leaves than plants in 2017 (Table 2).

For plants in their second year, there was a significant effect of the agrochemical treatments on the number of flowering and non-flowering stems in the plots (Table S4). Specifically, plots exposed to both fertilizer and herbicide had fewer flowering stems than the control plots. Plots exposed to just fertilizer, or fertilizer and herbicide, had more non-flowering stems than control plots, while herbicide plots had fewer non-flowering stems (Fig. S4).

There was no significant effect of the agrochemical treatments on the timing of flowering, maximum height of flowering plants, or maximum leaf length of non-flowering plants in the second year of growth. Moreover, there was no effect of treatment on the size of the floral display for either year one or year two plants and floral display was similar between plants in their first and second year of growth (Fig. S2).

Table 2

A summary of model results for the plant growth of the first year plants (see sample sizes in Methods). See Table S2 for more details about the model structure.

Plants	Contrast	Estimate	t or z Value	P value
First day of flowering	F - C	-4.31	-2.27	0.02
	H - C	-2.72	-1.36	0.17
	HF - C	0.77	0.38	0.7
log(Display Size)	F - C	-0.14	-1.29	0.20
	H - C	-0.04	-0.35	0.72
	HF - C	0.02	0.17	0.86
	2018 - 2017	0.43	2.02	0.04
Probability of Flowering‡	F - C	1.5	3.92	<< 0.001
	H - C	-0.03	-0.13	0.90
	HF - C	0.81	2.2	0.03
Height Flowering	F - C	0.64	0.22	0.83
	H - C	-15.82	-4.98	<<0.001
	HF - C	2.03	0.66	0.51
Longest leaf length (non-flowering)§	F - C	3.16	1.57	0.12
	H - C	-7.81	-4.29	<<0.001
	HF - C	0.08	0.04	0.97
	2018 - 2017	15.96	4.54	<<0.001
<i>P. tanacetifolia</i> density§	F - C	0.09	0.48	0.63
	H - C	-0.23	-1.13	0.26
	HF - C	-0.02	-0.1	0.92
	Age (S - F)	-0.84	-4.12	<<0.001
Percent Mortality‡	F - C	-0.06	-1.70	0.09
	H - C	0.02	0.73	0.46
	HF - C	-0.02	-0.50	0.61
	2018 - 2017	-0.10	-3.05	0.002

The symbol ‡ indicates a binomial distribution and § indicates a Poisson distribution. Significant contrasts ($P < 0.05$) are in bold text, with negative effects in blue and positive effects in red.

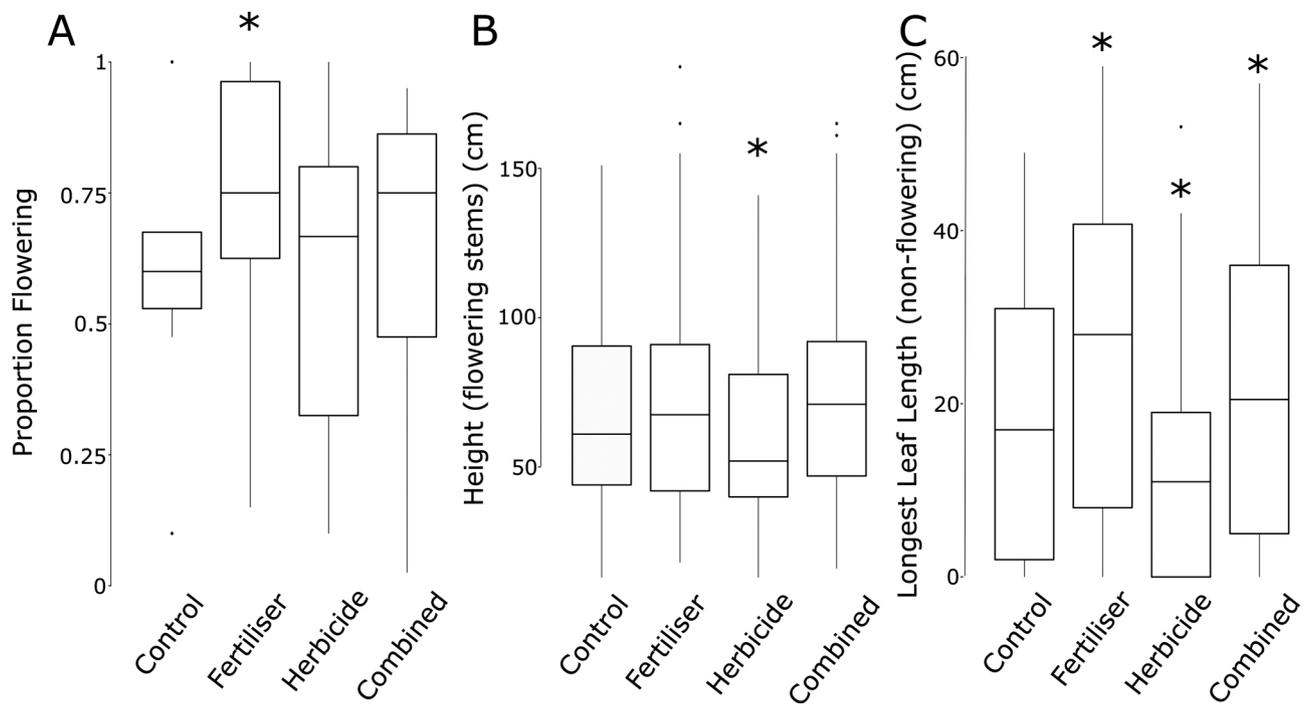


Fig. 1. Boxplots of the measured plant growth attributes for first year plants, including the proportion of flowering individuals (A), the height of flowering stems (B), and the longest leaf length of non-flowering stems (C). The asterisks indicate a significant difference between the treatment and the control.

However, plots initiated in 2018 had a significantly larger floral display than plants initiated in 2017, because we increased the number of individuals of the perennial species (Table 2). There was no effect of the treatment on the density of *P. tanacetifolia* stems in either year one or year two blocks, but there was a significant negative effect of plot age (Fig. S3, Table 2). Overall plant mortality was 8.7 % of individuals of the perennial plant species and there was no effect of treatment on plant mortality ($P > 0.05$, Table 2). There was no significant interaction between year and treatment on plant mortality, but there was a significant decrease in mortality in the second year (effect size = -0.10, $t = -3.05$, $P = 0.002$).

3.3. Soil analysis

There was no significant effect ($P > 0.05$) of the treatment on the

difference between the spring (before treatment) and autumn (after treatment) values of any of the measured soil attributes (pH, % organic matter, N, C, K, P). When comparing attributes of soil quality for plant growth, there were no significant effects of soil attributes on probability of flowering, number of inflorescences produced, or longest leaf length of non-flowering plants (Table S5). On the other hand, there was a significant positive effect of %P of the height of flowering plants ($P = 0.02$, Table S5).

3.4. Insect responses

The strongest predictor of insect visitation was the size of the floral display. The size of the floral display correlated significantly with the flower-visiting insect abundance across all collection events for year one (Pearson correlation coefficient = 0.69, $P < 0.001$) and year two

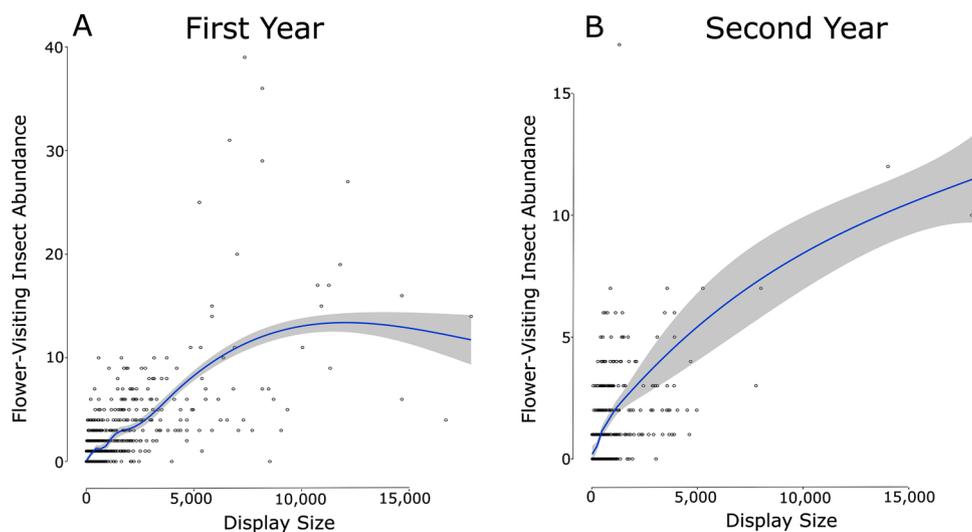


Fig. 2. The relationship between floral display at the plot level and flower-visiting insect abundance in the first (A) and second (B) year of plant growth during the experiment. The line is fit using a generalized additive model (Wood et al 2016).

(Pearson correlation coefficient = 0.59, $P < 0.001$) plots. A polynomial relationship fit better than a linear relationship for these data having a significantly lower AIC and an adjusted R^2 of 0.52 (Fig. 2).

There were significant differences in the visitation rates of insects to the flowers of the different plant species. When we pooled visitation data across the season for each plant species in each plot, the thistle, *Cirsium vulgare*, was visited at a significantly higher rate than the other plant species (Fig. 3). The next most visited species were *Hypochaeris radicata* (cat's ears) and *Origanum vulgare* (oregano), followed by the remaining species, which did not differ significantly from one another. There was no significant effect of the age of the plants (second vs first year average visit rate across plant species: effect size = -0.3, $t = -1.77$, $P = 0.08$). *H. radicata* had the highest values for species richness and Simpson's diversity, and was a close second behind *Plantago lanceolata* (ribwort plantain) for Simpson's diversity index according to a rarefaction analysis (Fig. S5). *P. lanceolata* also ranked closely to *H. radicata* in Shannon's diversity of flower visitors, but its 95 % confidence interval overlapped with *Filipendula ulmaria* (meadowsweet). Similarly, *F. ulmaria* had a confidence interval that overlapped with *H. radicata* for species richness (Fig. S5).

For both year one and year two plants, there was not a significant positive interaction between the size of the floral display and the fertilizer treatment on the binary presence/absence of a flower-visiting

insect during a sample and removing the interaction term improved model fit (significantly lowered AICc) (Fig. S6). For year one plants, collection year was not a significant fixed effect, and so we removed it from the model. The resulting models showed no significant relationship between treatment and the presence/absence of flower-visiting insects, but a significant positive effect of the floral display (year one: effect size = 3.73, $z = 10.53$, $P < 0.001$, Table 3; year two: effect size = 2.72, $z = 6.90$, $P < 0.001$, Table S6, Fig. S6).

For the abundance of flower-visitors to year one plants, there was a significant positive interaction between the fertilizer treatment and the size of the floral display (effect size = 0.33, $z = 3.56$, $P < 0.001$, Table 3, Fig. 4A). There was also a significant positive effect collection year (2018–2017) and the size of the floral display on floral visitor abundance (Table 3). For the abundance of flower-visitors to plants in their second year, there was a significant negative interaction between the combination treatment and the display (effect size = -0.35, $z = -2.00$, $P = 0.046$, Table S6, Fig. 4B).

For the species richness of flower-visitors to year one plants, there was a significant interaction between fertilizer treatment and display (Table 3, Fig. 4C). There was also a significant positive effect of collection year (2018–2017) on species richness, but no direct effect of display (Table 3). There was no interaction between display and treatment for the species richness of flower-visitors to year two plants

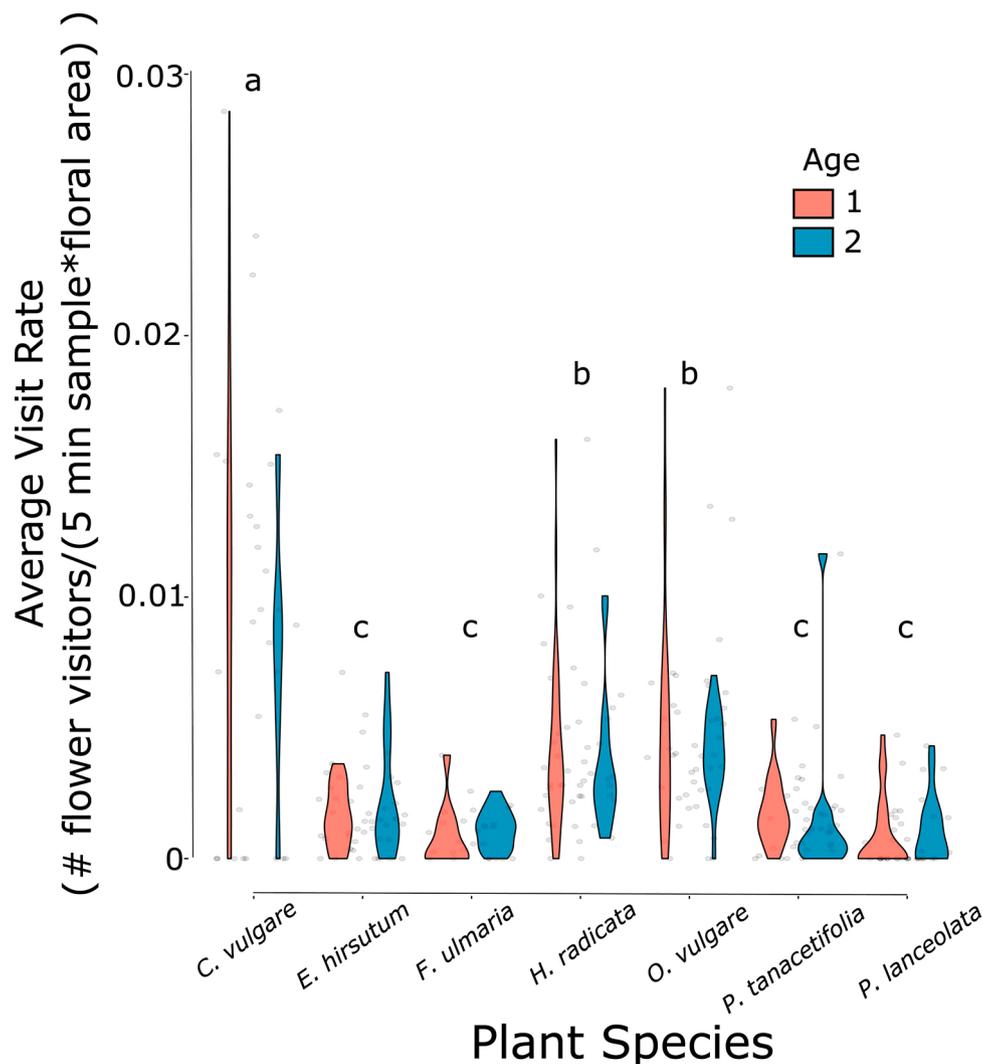


Fig. 3. Average visitation rates (# flower-visiting insects per unit floral area per sample), for the different plant species in the experiment. The violin plots show the distribution of the visitation rates. There was no difference between the first (1) and second (2) year plants, but there were significant differences between the plant species, as indicated by the letters.

Table 3

A summary of model results for the flower-visiting insect responses on the first year plants (see sample sizes in Methods). See Table S2 for more details about the model structure.

Flower-visiting insects	Comparison	Estimate	t or z Value	P value
Binary Pres/Abs ‡	<i>F - C</i>	0.25	1.27	0.21
	<i>H - C</i>	-0.34	-1.63	0.10
	<i>HF - C</i>	0.13	0.61	0.54
	Display	3.73	10.53	<< 0.001
Abundance §	<i>F - C</i>	0.17	1.14	0.25
	<i>H - C</i>	-0.27	-1.71	0.09
	<i>HF - C</i>	-0.12	-0.80	0.43
	2018 – 2017	0.57	2.60	0.009
	Display	0.31	4.48	<< 0.001
	F*Display	0.33	3.56	< 0.001
	<i>H*Display</i>	-0.02	-0.24	0.81
<i>HF*Display</i>	0.01	0.07	0.95	
Richness §	<i>F - C</i>	0.18	1.31	0.19
	<i>H - C</i>	-0.27	-1.86	0.06
	<i>HF - C</i>	-0.20	-1.38	0.17
	2018 – 2017	0.42	2.18	0.03
	<i>Display</i>	0.03	0.33	0.74
	F*Display	0.35	3.03	0.002
	<i>H*Display</i>	0.02	0.23	0.82
<i>HF*Display</i>	0.13	1.30	0.19	
Average Visit Rate	<i>F - C</i>	-0.0002	-0.29	0.77
	H - C	-0.001	-2.11	0.03
	<i>HF - C</i>	-0.0002	-0.30	0.76

The symbol ‡ indicates a binomial distribution and § indicates a Poisson distribution. Significant contrasts ($P < 0.05$) are in bold text, with negative effects in blue and positive effects in red.

(Fig. 4D), and none of the treatments had a significant effect on species richness. The rarefaction analysis (Chao et al., 2014) showed large confidence intervals for the extrapolated species richness in the different treatments, likely because of the low abundance of insects in general (Fig. S7). However, the fertilised treatment had the highest Shannon diversity, with a non-overlapping 95 % confidence interval with the other treatments. The combination treatment also had a significantly lower Simpson diversity than the control (Fig. S7).

The herbicide had a significant negative effect on the average visitation rate of flower-visiting insects at the plot level (effect size = -0.001, t value = -2.11, $P = 0.03$, Fig. 5A). There were no significant effects of the treatments on the visitation rate to year two plants (Table S6).

4. Discussion

In this study, run-off equivalent concentrations of fertilizer and low concentrations of herbicide had significant effects on plant growth and visitation by insects to inflorescences. These experimental agrochemical applications did not alter the chemical properties of the soil we measured, but rather had direct effects on plant growth. The changes in insect visitation rates were independent of the size of the floral display (number of inflorescences multiplied by inflorescence size), suggesting that changes in plant growth affected the relative attractiveness of the plant individuals. As a general rule, the changes in plant growth and insect visitation conformed to our hypotheses: herbicide applications had negative effects on height, longest leaf length, and visitation rate of floral visitors; there were positive effects of run-off fertilizer on the probability of flowering, with positive interactions between the fertilizer treatment and floral display for the abundance and species richness of floral visitors. Plants in the fertilizer treatment also flowered significantly earlier in the season than plants in the control treatment. In most cases, the combination treatment, with both fertilizer and herbicide, did

not differ significantly from the control, though plants in this treatment had longer leaves than the control and were more likely to flower.

In our study, exposure to even low concentrations of fertilizer or herbicide changed plant growth patterns. However, as our application method was applied both to the foliage and the soil in the plots, we were not able to determine the mechanisms of these effects (for example, toxicity to the roots, or changes in nutrient uptake or photosynthesis). When exposed to low concentrations of herbicide, plants were shorter when flowering and had shorter leaves, despite having the same floral display as the control. In contrast, the run-off concentration of fertilizer caused the plants to flower earlier in the year and they were significantly more likely to flower as individuals and to be taller when flowering. At the same time, they did not have a larger floral display than the control, suggesting they produced fewer inflorescences per individual. Plants exposed to fertilizer in the first year produced a larger number of non-flowering stems in the second year, while the plants in the herbicide treatment produced fewer of both flowering and non-flowering stems in the second year. Similar changes have been observed in other studies of sub-lethal glyphosate applications, in combination with fertilizer treatments (Dupont et al., 2018; Gove et al., 2007). Other studies have shown that sub-lethal doses of herbicides, including glyphosate, can trigger a short-term increase in biomass (Cedergreen, 2008a), though this is not maintained in the long term (Cedergreen, 2008b). Indeed, given that we eliminated competition from plants not included in the study, we cannot predict how the composition of the plant community may change over time in response to agrochemical exposure.

The concentrations of fertilizer and herbicide applied were based on published experiments on run-off (Bertol et al., 2007; Korsaeht and Eltun, 2000), surveys of groundwater run-off (Craig and Mannix, 2009), and government recommendations (US EPA, 2003). Because our estimates were based on groundwater run-off, and did not account for direct exposure through drift during application, our application rates were

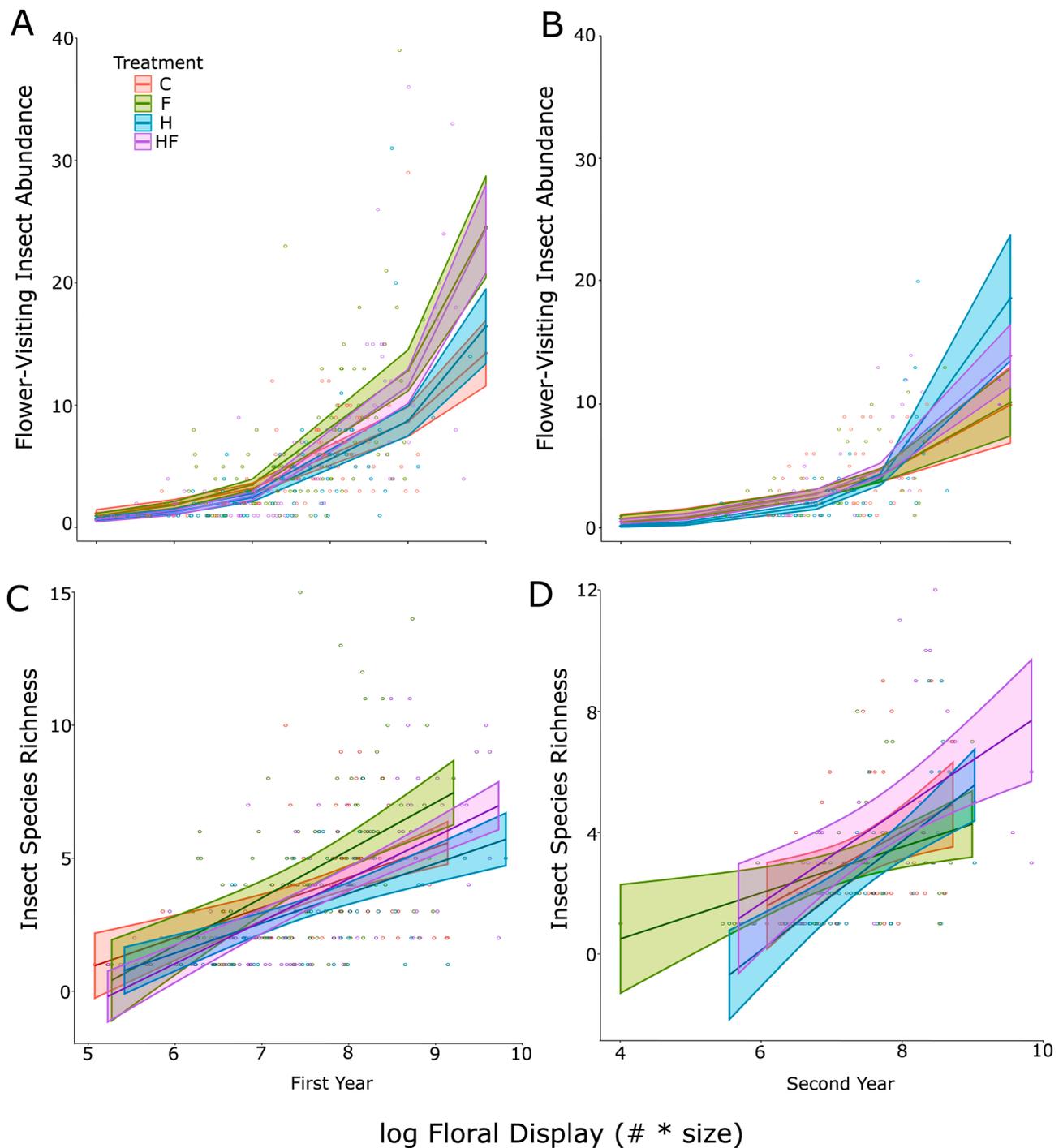


Fig. 4. Graphs showing the predicted effects of the relationship between the floral display and the abundance (A, B) and richness (C, D) of flower-visiting insects in the first (A, C) and second year (B, D) of growth. The shaded areas around the lines represent the 95 % confidence intervals around the predicted effects from the models. A significant interaction between display and the fertilizer (F) treatment combination (HF) treatment in the first year abundance (A). In the second year, there was a significant interaction between the "display and the combination (HF) treatment the herbicide (H) treatment in the second year abundance (B). For species richness to year one plants, there was a significant interaction between fertilizer (F) treatment and display. There was no interaction between display and treatment for the species richness of flower-visitors to year two plants.

likely conservative relative to what plants on crop margins may experience. We controlled for the source populations of the plants, their initial sizes, the number of individuals of each species (or the amount of seed used in initial plantings for the annual species), the amount of water they received, and their initial conditions. We eliminated any potential competition by removing contaminant plants throughout the growing season. Furthermore, we saw no significant changes in measured soil attributes among the treatments after the first season of

the experiment, though these soil attributes did explain some of the site-level variation in plant growth. Thus, we can be relatively confident that the plants were responding to the chemicals we applied. However, because our application rates were fixed across all plots, we were not able to test for the effects of different levels of chemical exposure, and thus did not have the resolution to determine whether higher or lower doses of agrochemicals would have resulted in different responses.

In the most realistic scenario, plants growing on the edges of crop

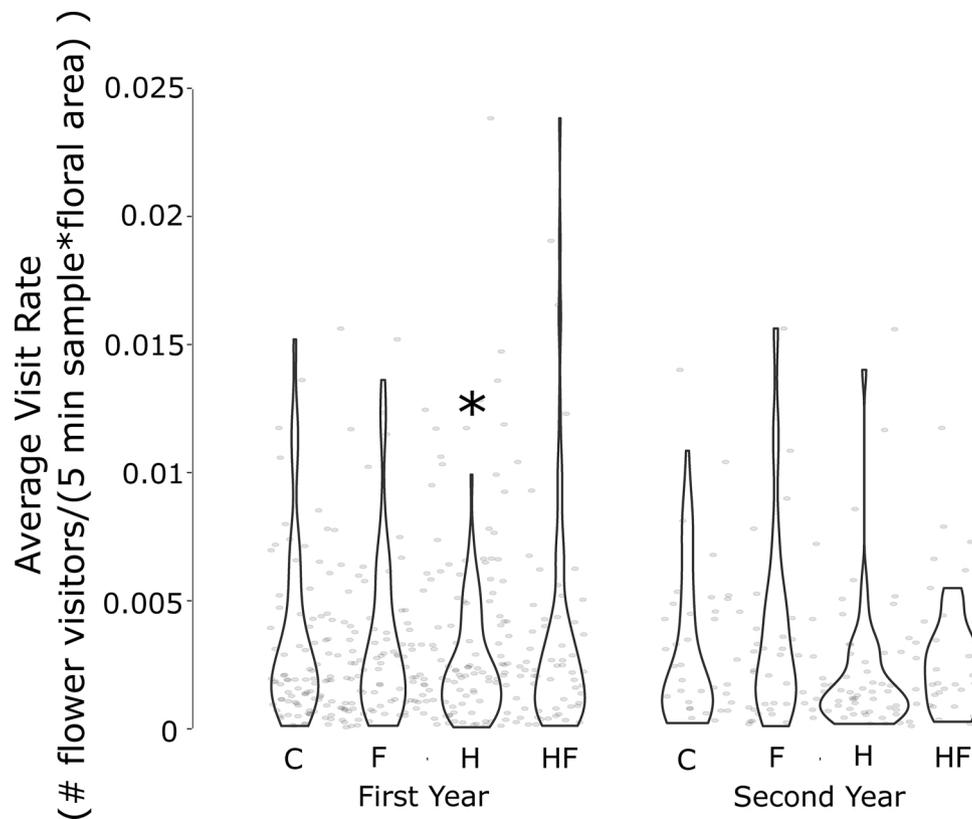


Fig. 5. The average visitation rate of flower-visiting insects to plants in their first and second year of growth, with violin plots to show the distribution of the data. The treatments are fertilized (F), herbicide (H), control (C), and combination of fertilizer and herbicide (HF). Asterisks mark where the treatments are significantly different from the control.

fields are likely to be exposed to both fertilizers and herbicides, and the interaction between these two could have some important implications for plant growth (Dupont et al., 2018). In many of the aspects we monitored here, the herbicide and fertilizer balance one another. For example, first year plants exposed to the combination treatment did not differ from the control in the timing of their flowering, size of their floral display, leaf length of non-flowering plants, or height of flowering plants. The herbicide and fertilizer effects did not balance one another completely over the long term, as plants in the combination treatment had a significantly lower number of flowering stems, and a higher number of non-flowering stems. The number of non-flowering stems in the second year of the combination treatment was intermediate between the control and the fertilised plots, suggesting the herbicide reduced the benefits of the fertilizer. Our results are similar to another study, which showed a significant interaction between glyphosate exposure and nitrogen fertilizer induced a delay in flowering, but that this interaction did not significantly affect the height, diameter of capitula, visitation rate, or seed set of *Tanacetum vulgare* (Dupont et al., 2018).

The size of the floral display was the strongest predictor of insect visitation, but did not differ among the treatments and did not directly affect species richness. However, there were significant positive interactions between floral display and the fertilizer treatment for the abundance and species richness of flower-visiting insects in the year one plants, and a significant negative interaction between the floral display and combination treatment on abundance in the year two plants. This suggests the effect of the treatments on flower-visiting insects depends on the size of the floral display. Moreover, the low concentrations applied in this experiment also affected the interactions between the plants and their flower-visiting insects independent of the floral display as the herbicide treatment had a negative effect on average visitation rate in year one. Other studies have found a negative effect of glyphosate exposure (Dupont et al., 2018) and positive effect of a low N fertilizer

treatment (Burkle and Irwin, 2009, 2010) on pollinator visitation rates. This change in insect visitation rate could relate to some change in the attractiveness in the plants, or potentially the quality of the floral resources (nectar and/or pollen) they are producing, as seen in other studies of soil amendment (e.g. Russo and Shea, 2020), including one where the ultimate fitness of the pollinating insects was also affected (Cardoza et al., 2012). This indirect effect of the agrochemical exposure likely interacts with the direct effects on plant growth to have implications for plant fitness.

The perennial plant species we selected for this study were all common in agricultural systems and native to Europe. They are among the species likely to be both regularly exposed to these low concentrations of agrochemicals, and also supplying pollinating insects with floral resources in agroecosystems. Historically, they are also the species most likely to have survived over a long history of exposure to agrochemicals; other plant species, including those which used to exist in these habitats, may be more sensitive. Although this study was intended to evaluate the impact of agrochemical exposure affecting non-target plants on crop edges, it is also likely that farmers are directly targeting many of these species to remove them from crop and forage fields. For example, *C. vulgare*, which had the highest visitation rate in our study, is considered a noxious weed by Teagasc (the Irish Agriculture and Food Development Authority), which recommends chemical control (O'Donovan, 2016). It is also important to consider the potential role of these plants in wider pollinator conservation in agricultural landscapes. For example, *H. radicata*, which attracted the highest diversity of visitors by several measures, is also recommended planting for the IUCN red-listed (in Ireland) bird species twite (*Linaria flavirostris*) conservation (Department of Agriculture, Food and the Marine 2016). *H. radicata* was also recently identified as a plant species that supports significantly more pollinators than expected in urban settings (Baldock et al., 2019). It is interesting to note that both *C. vulgare* and *H. radicata* support a

greater number of individual flower-visiting insects on a per-flower basis, and a greater diversity of insect species, than the non-native annual, *P. tanacetifolia*, which is commonly recommended for pollinator friendly plantings in Europe. It is also interesting to note that *P. lanceolata*, which is often considered a wind-pollinated plant (Tonsor, 1985), ranked highly in terms of the diversity indices, and actually had a similar visitation rate to *P. tanacetifolia*, *F. ulmaria*, and *E. hirsutum*, all with showy inflorescences and considered pollinator-friendly plants. *P. lanceolata* attracted a surprisingly large number of flower-visiting insect species, and is an example of a plant generalist that would appear to be a specialist if the sampling effort were lower (Dorado et al., 2011).

One significant challenge with this study was the very low abundances of flower-visiting insects we observed. In fact, we recorded zero flower-visiting insects in more than fifty percent of our observations, leading to zero-inflated data and, though we spent approximately 170 h observing these seven plant species, the rarefaction curves did not level off for most of them, because the visitation rate was so low. Moreover, the relationship between the size of the floral display and insect abundance was a saturating curve, which may suggest that large displays of inflorescences are pollinator-limited. Future work may further explore whether the indirect effects of agrochemical exposure on the interactions between these plants and their flower-visiting insects may also result in changes to the pollination services provided. Changes to the attractiveness of field edge plants may have implications for their fitness, especially where pollinating insects may already be a limiting factor.

Though the effects observed in our study may seem small at the scale of experimental 2×2 m plots, it is easy to see how they might scale up to a landscape level effect. All fields have edges, and chemical application in these fields inevitably leads to non-target exposure. Field edges can be refugia for biodiversity (Marshall and Moonen, 2002), including in Ireland, where pollinators are found to be more abundant and diverse on field edges than in the centre of fields (Power and Stout, 2011; Stanley and Stout, 2014). Many studies in agricultural systems demonstrate the importance of weedy species (Pettis et al., 2013; Russo et al., 2016) and of field edges (Carvalho et al., 2014; Garibaldi et al., 2011) to pollinating insects. They could be of critical conservation importance for pollinators in Ireland, where 50 % of native bee species are considered to be in decline and 30 % threatened with extinction (Fitzpatrick et al., 2006). The population trajectories of other pollinating insects, such as syrphid flies, which contributed an even greater species richness than bees in our study, are not well known. Pollinating insects are not only essential components of a functioning natural ecosystem, but they also provide economically valuable pollination services (Stanley et al., 2013). Our work suggests we should consider the broader impacts of agrochemicals on the interactions between weedy agricultural plants and native pollinators.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:<https://doi.org/10.1016/j.agee.2020.107141>.

References

- Al-Rajab, A.J., Schiavon, M., 2010. Degradation of 14C-glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils. *J. Environ. Sci. China (China)* 22, 1374–1380. [https://doi.org/10.1016/S1001-0742\(09\)60264-3](https://doi.org/10.1016/S1001-0742(09)60264-3).
- Bai, S.H., Ogbourne, S.M., 2016. Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. *Environ. Sci. Pollut. Res.* 23, 1898–19001. <https://doi.org/10.1007/s11356-016-7425-3>.
- Baldock, K.C.R., Goddard, M.A., Hicks, D.M., Kunin, W.E., Mitschunas, N., Morse, H., Osgathorpe, L.M., Potts, S.G., Robertson, K.M., Scott, A.V., Staniczenko, P.P.A., Stone, G.N., Vaughan, I.P., Memmott, J., 2019. A systems approach reveals urban pollinator hotspots and conservation opportunities. *Nat. Ecol. Evol.* 3, 363–373. <https://doi.org/10.1038/s41559-018-0769-y>.
- Ball, S., Morris, R., 2015. *Britain's Hoverflies: a Field Guide, second ed.* Princeton University Press, Princeton.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.
- Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* 28, 3. <https://doi.org/10.1186/s12302-016-0070-0>.
- Bertol, I., Engel, F.L., Mafra, A.L., Bertol, O.J., Ritter, S.R., 2007. Phosphorus, potassium and organic carbon concentrations in runoff water and sediments under different soil tillage systems during soybean growth. *Soil Tillage Res.* 94, 142–150. <https://doi.org/10.1016/J.STILL.2006.07.008>.
- Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M., Adler, P.B., Alberti, J., Anderson, T.M., Bakker, J.D., Biederman, L., Blumenthal, D., Brown, C.S., Brudvig, L.A., Buckley, Y.M., Cadotte, M., Chu, C., Cleland, E.E., Crawley, M.J., Daleo, P., Damschen, E.I., Davies, K.F., DeCrappe, N.M., Du, G., Firm, J., Hautier, Y., Heckman, R.W., Hector, A., HilleRisLambers, J., Iribarne, O., Klein, J.A., Knops, J.M.H., La Pierre, K.J., Leakey, A.D.B., Li, W., MacDougall, A.S., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Mortensen, B., O'Halloran, L.R., Orrock, J.L., Pascual, J., Prober, S.M., Pyke, D.A., Risch, A.C., Schuetz, M., Smith, M.D., Stevens, C.J., Sullivan, L.L., Williams, R.J., Wragg, P.D., Wright, J.P., Yang, L.H., 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508, 517–520. <https://doi.org/10.1038/nature13144>.
- Brown, A., 2007. Dating the onset of cereal cultivation in Britain and Ireland: the evidence from charred cereal grains. *Antiquity* 81, 1042–1052.
- Burkle, L., Irwin, R., 2009. The importance of interannual variation and bottom-up nitrogen enrichment for plant–pollinator networks. *Oikos* 118, 1816–1829. <https://doi.org/10.1111/j.1600-0706.2009.17740.x>.
- Burkle, L.A., Irwin, R.E., 2010. Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. *J. Ecol.* 98, 705–717. <https://doi.org/10.1111/j.1365-2745.2010.01648.x>.
- Cardozo, Y.J., Harris, G.K., Grozinger, C.M., 2012. Effects of soil quality enhancement on pollinator-plant interactions. *Psyche A J. Entomol.* 2012 <https://doi.org/10.1155/2012/581458>.
- Carvalho, L.G., Biesmeijer, J.C., Benadi, G., Fründ, J., Stang, M., Bartomeus, I., Kaiser-Bunbury, C.N., Baude, M., Gomes, S.I.F., Merckx, V., Baldock, K.C.R., Bennett, A.T. D., Boada, R., Bommarco, R., Cartar, R., Chacoff, N., Dänhardt, J., Dicks, L.V., Dormann, C.F., Ekroos, J., Henson, K.S.E., Holzschuh, A., Junker, R.R., Lopezzariza-Mikel, M., Memmott, J., Montero-Castaño, A., Nelson, L.L., Petanidou, T., Power, E. F., Rundlöf, M., Smith, H.G., Stout, J.C., Temitope, K., Tschamntke, T., Tscheulin, T., Vilà, M., Kunin, W.E., 2014. The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. *Ecol. Lett.* 17, 1389–1399. <https://doi.org/10.1111/ele.12342>.
- Castle, D., Grass, I., Westphal, C., 2019. Fruit quantity and quality of strawberries benefit from enhanced pollinator abundance at hedgerows in agricultural landscapes. *Agric. Ecosyst. Environ.* 275, 14–22. <https://doi.org/10.1016/J.AGEE.2019.01.003>.
- Cedergreen, N., 2008a. Herbicides can stimulate plant growth. *Weed Res.* 48, 429–438. <https://doi.org/10.1111/j.1365-3180.2008.00646.x>.

- Cedergreen, N., 2008b. Is the growth stimulation by low doses of glyphosate sustained over time? *Environ. Pollut.* 156, 1099–1104. <https://doi.org/10.1016/j.envpol.2008.04.016>.
- Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* 84, 45–67. <https://doi.org/10.1890/13-0133.1>.
- Clark, C.M., Tilman, D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451, 712–715. <https://doi.org/10.1038/nature06503>.
- Craig, M., Mannix, A., 2009. Groundwater quality, *Water Quality in Ireland*.
- Dorado, J., Vázquez, D.P., Stevani, E.L., Chacoff, N.P., 2011. Rareness and specialization in plant–pollinator networks. *Ecology* 92, 19–25. <https://doi.org/10.1890/10-0794.1>.
- Dupont, Y.L., Strandberg, B., Damgaard, C., 2018. Effects of herbicide and nitrogen fertilizer on non-target plant reproduction and indirect effects on pollination in *Tanacetum vulgare* (Asteraceae). *Agric. Ecosyst. Environ.* 262, 76–82. <https://doi.org/10.1016/j.agee.2018.04.014>.
- Edwards, W.M., Triplett, G.B., Kramer, R.M., 1980. A watershed study of glyphosate transport in runoff. *J. Environ. Qual.* 9, 661. <https://doi.org/10.2134/jeq1980.00472425000900040024x>.
- European Union, 2010. *Agricultural Census in Ireland*.
- Falk, S., Lewington, R., 2015. *Field Guide to the Bees of Great Britain and Ireland, first ed.* British Wildlife Publishing, London.
- Fitzpatrick, U., Murray, T., Paxton, R., Brown, M., 2006. *The State of Ireland's Bees.* Waterford, Ireland.
- Garibaldi, L.A., Steffan-Dewenter, I., Kremen, C., Morales, J.M., Bommarco, R., Cunningham, S.A., Carvalheiro, L.G., Chacoff, N.P., Dudenhöffer, J.H., Greenleaf, S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L.A., Potts, S.G., Ricketts, T.H., Szentgyörgyi, H., Viana, B.F., Westphal, C., Winfree, R., Klein, A.M., 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecol. Lett.* 14, 1062–1072. <https://doi.org/10.1111/j.1461-0248.2011.01669.x>.
- Gough, L., Osenberg, C.W., Gross, K.L., Collins, S.L., 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos* 89, 428–439. <https://doi.org/10.1034/j.1600-0706.2000.890302.x>.
- Gove, B., Power, S.A., Buckley, G.P., Ghazoul, J., 2007. Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys. *J. Appl. Ecol.* 44, 374–384. <https://doi.org/10.1111/j.1365-2664.2007.01261.x>.
- Grab, H., Danforth, B., Poveda, K., Loeb, G., 2018. Landscape simplification reduces classical biological control and crop yield. *Ecol. Appl.* <https://doi.org/10.1002/eap.1651>.
- Grab, H., Brokaw, J., Anderson, E., Gedlinske, L., Gibbs, J., Wilson, J., Loeb, G., Isaacs, R., Poveda, K., 2019. Habitat enhancements rescue bee body size from the negative effects of landscape simplification. *J. Appl. Ecol.* 1365-2664, 13456. <https://doi.org/10.1111/1365-2664.13456>.
- Grimm, N.B., Gergel, S.E., McDowell, W.H., Boyer, E.W., Dent, C.L., Groffman, P., Hart, S.C., Harvey, J., Johnston, C., Mayorga, E., McClain, M.E., Pinay, G., 2003. Merging aquatic and terrestrial perspectives of nutrient biogeochemistry. *Oecologia* 137, 485–501. <https://doi.org/10.1007/s00442-003-1382-5>.
- Isbell, F., Tilman, D., Polasky, S., Binder, S., Hawthorne, P., 2013. Low biodiversity state persists two decades after cessation of nutrient enrichment. *Ecol. Lett.* 16, 454–460. <https://doi.org/10.1111/ele.12066>.
- Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M. B., Endale, D.M., Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biol. Biochem.* 40, 2843–2853. <https://doi.org/10.1016/j.soilbio.2008.07.030>.
- Jones, D.K., Hammond, J.I., Relyea, R.A., 2010. Roundup® and amphibians: the importance of concentration, application time, and stratification. *Environ. Toxicol. Chem.* 29 <https://doi.org/10.1002/etc.240> n/a-n/a.
- Korsaeth, A., Eltun, R., 2000. Nitrogen mass balances in conventional, integrated and ecological cropping systems and the relationship between balance calculations and nitrogen runoff in an 8-year field experiment in Norway. *Agric. Ecosyst. Environ.* 79, 199–214. [https://doi.org/10.1016/S0167-8809\(00\)00129-8](https://doi.org/10.1016/S0167-8809(00)00129-8).
- Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A. C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10967–10972. <https://doi.org/10.1073/pnas.1508382112>.
- Marshall, E.J., Moonen, A., 2002. Field margins in northern Europe: their functions and interactions with agriculture. *Agric. Ecosyst. Environ.* 89, 5–21. [https://doi.org/10.1016/S0167-8809\(01\)00315-2](https://doi.org/10.1016/S0167-8809(01)00315-2).
- Martin, L.J., Blossy, B., Ellis, E., 2012. Mapping where ecologists work: biases in the global distribution of terrestrial ecological observations. *Front. Ecol. Environ.* 10, 195–201. <https://doi.org/10.1890/110154>.
- Motta, E.V.S., Raymann, K., Moran, N.A., 2018. Glyphosate perturbs the gut microbiota of honey bees. *Proc. Natl. Acad. Sci.* 115, 10305–10310. <https://doi.org/10.1073/PNAS.1803880115>.
- O'Donovan, T., 2016. *Herbicides for Dock Control in Established Grassland.*
- Park, M.G., Blitzer, E.J., Gibbs, J., Losey, J.E., Danforth, B.N., 2015. Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proc. R. Soc. London B Biol. Sci.* 282, 20150299 <https://doi.org/10.1098/rspb.2015.0299>.
- Pellissier, L., Wisz, M.S., Strandberg, B., Damgaard, C., 2014. Herbicide and fertilizers promote analogous phylogenetic responses but opposite functional responses in plant communities. *Environ. Res. Lett.* 9, 024016 <https://doi.org/10.1088/1748-9326/9/2/024016>.
- Pettis, J.S., Lichtenberg, E.M., Andree, M., Stitzinger, J., Rose, R., VanEngelsdorp, D., 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLoS One* 8, e70182. <https://doi.org/10.1371/journal.pone.0070182>.
- Pope, T.W., Girling, R.D., Staley, J.T., Trigodet, B., Wright, D.J., Leather, S.R., van Emden, H.F., Poppy, G.M., 2012. Effects of organic and conventional fertilizer treatments on host selection by the aphid parasitoid *Diaeretiella rapae*. *J. Appl. Entomol.* 136, 445–455. <https://doi.org/10.1111/j.1439-0418.2011.01667.x>.
- Power, E.F., Stout, J.C., 2011. Organic dairy farming: impacts on insect–flower interaction networks and pollination. *J. Appl. Ecol.* 48, 561–569. <https://doi.org/10.1111/j.1365-2664.2010.01949.x>.
- Rundlöf, M., Lundin, O., Bommarco, R., 2018. Annual flower strips support pollinators and potentially enhance red clover seed yield. *Ecol. Evol.* 8, 7974–7985. <https://doi.org/10.1002/ece3.4330>.
- Russo, L., Shea, K., 2020. Experimentally increased network connectance is associated with increased bee species richness and abundance in a plant–pollinator community. n.d. *Complex Networks*.
- Russo, L., Nichol, C., Shea, K., 2016. Pollinator floral provisioning by a plant invader: quantifying beneficial effects of detrimental species. *Divers. Distrib.* 22, 189–198. <https://doi.org/10.1111/ddi.12397>.
- Saunders, L., Pezeshki, R., 2015. Glyphosate in runoff waters and in the root-zone: a review. *Toxics* 3, 462–480. <https://doi.org/10.3390/toxics3040462>.
- Schmitz, J., Schäfer, K., Brühl, C.A., 2013. Agrochemicals in field margins-assessing the impacts of herbicides, insecticides, and fertilizer on the common buttercup (*Ranunculus acris*). *Environ. Toxicol. Chem.* 32, 1124–1131. <https://doi.org/10.1002/etc.2138>.
- Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils – a hidden reality unfolded. *Sci. Total Environ.* 653, 1532–1545. <https://doi.org/10.1016/J.SCITOTENV.2018.10.441>.
- Staley, J.T., Stewart-Jones, A., Pope, T.W., Wright, D.J., Leather, S.R., Hadley, P., Rossiter, J.T., van Emden, H.F., Poppy, G.M., 2010. Varying responses of insect herbivores to altered plant chemistry under organic and conventional treatments. *Proc. R. Soc. B Biol. Sci.* 277, 779–786. <https://doi.org/10.1098/rspb.2009.1631>.
- Stanley, D.A., Stout, J.C., 2014. Pollinator sharing between mass-flowering oilseed rape and co-flowering wild plants: implications for wild plant pollination. *Plant Ecol.* 215, 315–325. <https://doi.org/10.1007/s11258-014-0301-7>.
- Stanley, D.A., Gunning, D., Stout, J.C., 2013. Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland: ecological and economic incentives for pollinator conservation. *J. Insect Conserv.* 17, 1181–1189. <https://doi.org/10.1007/s10841-013-9599-z>.
- Strandberg, B., Boutin, C., Mathiassen, S.K., Damgaard, C., Dupont, Y.L., Carpenter, D.J., Kudsk, P., 2017. Effects of Herbicides on Non-target Terrestrial Plants, pp. 149–166. <https://doi.org/10.1021/bk-2017-1249.ch011>.
- Sullivan, C., Finn, J., Gormally, M., Skeffinton, M., 2013. Field boundary habitats and their contribution to the area of semi-natural habitats on lowland farms in east Galway, western Ireland. *Biol. Environ. Proc. R. Irish Acad.* 113, 187–199.
- Tonsor, S.J., 1985. Leptokurtic pollen-flow, non-leptokurtic gene-flow in a wind-pollinated herb, *Plantago lanceolata* L. *Oecologia* 67, 442–446. <https://doi.org/10.1007/BF00384953>.
- United States Environmental Protection Agency, 2003. Occurrence Estimation Methodology and Occurrence Findings Report for the Six-year Review of Existing National Primary Drinking Water Regulations. Office of Water (4606), EPA-815-R-03-006. www.epa.gov.
- Yu, Y., Zhou, Q.-X., 2005. Adsorption characteristics of pesticides methamidophos and glyphosate by two soils. *Chemosphere* 58, 811–816. <https://doi.org/10.1016/J.CHEMOSPHERE.2004.08.064>.