The Impact of Biotic and Abiotic Factors on Pollination of *Vicia faba.*

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BB30042

January 2021

Word count: 5997

Contents

Lay Summary

Pollination is an essential service for food production and has been shown to increase crop yields by an average of 43%. Insects such as bumblebees (*Bombus sp*.) and honeybees (*Apis mellifera*) are the most efficient pollinators but over the last few decades they have declined in abundance which threatens ecosystem stability and global food security. To reverse this decline, we need to understand how factors such as temperature, surrounding habitat and interactions between species effect pollinator abundance and crop pollination on Spring beans (*Vicia faba*).

We used data on current environmental conditions and Met office climate predictions to predict how climate change may affect our current pollinator populations. We found that pollinator abundance could decrease by 25% if no intervention to increase abundance occurs. To inform recommendations for increasing abundance, we compared pollinator abundance in response to different boundary habitats; Woodland, Treeline, Hedgerow, and other artificial habitats such as fences and roads, either with or without additional margins. The type of boundary habitat was found to effect pollinator abundance within the boundary with the highest abundance observed when floral margins were present. This suggests that enhancing floral habitat surrounding crop fields could increase pollinator abundance which would make them more resilient to changing environmental conditions.

Two foraging behaviours on bean flowers were observed during the study; legitimate visitation where the bee enters through the front of the flower to collect nectar and pollen or robbing behaviour where the bee bites into the side of the flower to directly collect nectar without transferring any pollen. Robbing behaviour does not contribute to crop pollination as no pollen is picked up or transferred between plants. Different species showed different levels of legitimate visitation and robbing behaviour with Garden bumblebees (*Bombus hortorum*) legitimately foraging 97% the time whereas honeybees legitimately forage just 34% of the time. These different behaviours were found to correspond with tongue length of the bees, with long-tongued species foraging more frequently while short-tongued species robbed. If floral mixes can be developed that increase abundance of long tongued species adjacent to bean crops, then pollination efficiency could be maximised to enhance yield from bean crops.

Abstract

Pollination is a crucial service for maintaining healthy ecosystems and for increasing agricultural productivity. However, in recent decades, pollinator abundance has declined putting strain on global food security. Furthermore, climate change threatens to uncouple plant-pollinator mutualisms that many crops such as *Vicia faba* (Spring Beans) rely on. To reverse this decline and mitigate future effects of climate change, we need to understand how abiotic factors such as temperature and biotic factors such as interspecific interactions and surrounding habitat can be used to reverse pollinator declines and preserve pollination services. Here we show that boundary habitat significantly effects pollinator abundance with floral abundance and pollinator abundance showing a significant positive correlation. We also found that pollinators exhibit species specific foraging behaviours on *V. faba* dependent on proboscis length; long-tongued pollinators are more efficient for pollination of *V. faba* than short-tongued pollinators. Optimising field boundaries to enhance pollinator abundance combined with targeting floral mixes to attract long-tongued pollinators onto *V. faba* crops could increase pollinator abundance and maximise pollination efficiency. Repeating this experiment across multiple crop species could maximise pollination services for crops and inform landscape scale management practices to allow pollinators to move between crops throughout the flowering season. Therefore, improving pollinator habitat provision has the potential to reverse pollinator declines, maintain *V. faba* food security and mitigate the effect of climate change on pollinator populations.

Introduction

Pollination is a vital service for many terrestrial ecosystems and is responsible for increasing agricultural productivity and yield by an average of 43% in animal pollinated crops (Eilers et al., 2011). The annual global value of pollination services is estimated to be worth £180-£440 billion and an estimated 5-8% of global crop production would be lost without pollination services (Bishop et al., 2020; Eilers et al., 2011). Invertebrates, specifically members the Hymenoptera family such a *Bombus sp.,* are the most efficient and widespread pollinator but 46% of *Bombus sp*. are declining in abundance and 23.5% of species are considered threatened in Europe (Nieto et al., 2014). Urgent action is required to preserve the health of agricultural ecosystems and the services they provide (Williams et al., 2007). To reverse pollinator declines and stabilise global food security, we first need to understand how abiotic factors such as temperature and biotic factors such as species interactions effect pollinator abundance and crop pollination.

Pollinator habitat

Wild pollinators are dependent on nectar and pollen for survival hence pollinator communities tend to establish in florally rich and diverse habitats. However, habitat fragmentation caused by agricultural intensification isolates wild pollinators from the crops they are needed to pollinate (Potts et al., 2010). Creating flower rich habitat throughout agricultural landscapes will reverse habitat fragmentation and provide essential resources for pollinators.

At the local level, habitat provision for pollinators has numerous secondary benefits such as attracting natural enemies for biological control of crop pests, reducing wind damage to crops and enhancing nitrogen fixation in the soil. Further secondary benefits such as reduced soil erosion, secondary sources of income and increase carbon sequestration can be achieved by employing pollinator habitat management at the farm and landscape scale (Fig. 1) (Wratten et al., 2012). The Farmer Cluster System has been developed to bring together local landowners for landscape scale habitat management and conservation projects. This system facilitates implementation of complementary management techniques that create wildlife corridors to link areas of rich natural habitat between multiple adjacent farms. Therefore, wild pollinators are enabled to move around the landscape, providing maximum benefit for crops and pollinators across the flowering season (Glibert-Norton et al., 2010). Although planting flower rich habitat around fields is common practice, success in increasing abundance and biodiversity of pollinators is varied (Gallai et al., 2009).

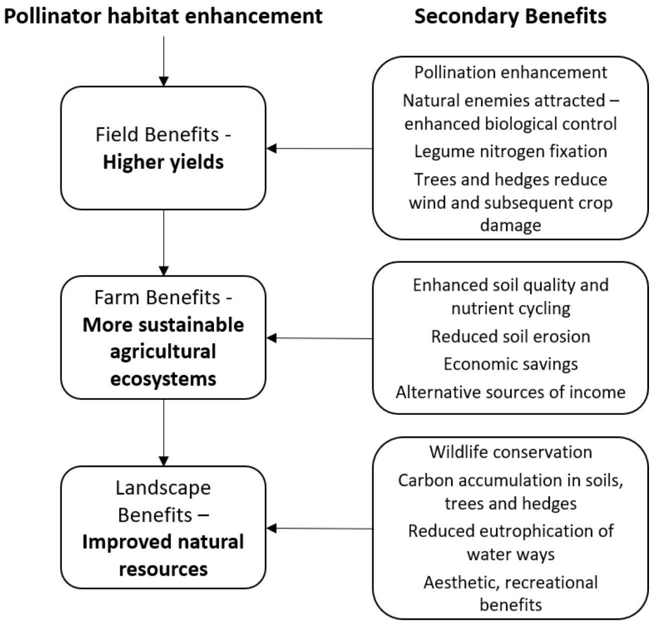

Figure 1: **A hierarchy of primary and secondary benefits from pollinator habitat management**. (Adapted from Wratten et al, 2012)

Pollination behaviours and interactions

Pollinators exhibit a range of foraging behaviours that are effected by numerous factors such as proboscis length, energy content per flower and availability of alternative nectar resources (Corbet et al., 1995). Foraging behaviours are categorised as legitimate foraging visits or robbing visits. During legitimate visits, pollinators enter through the front of the flower to collect pollen and/or nectar whereas robbing pollinators use their maxillae to make a hole directly into the nectar store of the flower (Fig. 2). Pollination requires contact with the anthers and stigma to transfer pollen therefore pollination only occurs during legitimate visits (Irwin et al., 2010). Some pollinator species are physiologically restricted and robbing is their only way to access nectar. However, many pollinators are facultative robbers which abandon legitimate visits and learn to become secondary robbers, given the opportunity (Irwin et al., 2010). Such behaviour is commonly seen in *Apis mellifera* (Western honeybee) and *Bombus terrestris* (Buff-tailed bumblebee) although long-tongued species such as *Bombus hortorum* (Garden bumblebee) are less likely to switch to facultative robbing (Newman and Thomson, 2005).

Figure 2: **Different species exhibit different foraging behaviours**. A) *A. mellifera* legitimately foraging on *V. faba* flowers exposing anthers and stigma for cross pollination. B) Nectar robbing of *V. faba* flowers by *Bombus terrestris*. Maxillae are used to bite a hole directly into the nectar store at the bottom of the corolla tube. Photos are copyright to the Author.

Increasing the relative abundance of species that predominantly carry out legitimate visits could increase pollination efficiency in agricultural systems. Prior research has shown that community structure can alter the abundance of different species. For example, research by Wermuth and Dupont (2010) showed that long tongued *Bombus sp.* are negatively affected by the presence of *A. mellifera* hives. Further concerns over our dependence on *A. mellifera* hives arise from newly emerging challenges to hives such as parasites, pathogens and exposure to pesticides (Kalayu et al., 2018). Increasing abundance of wild pollinators and reducing our dependence on *A. mellifera* hives should restore natural pollinator community structure and will make pollination services more robust to future threats.

Vicia faba pollination requirements

Vicia faba (Spring beans) are one of the most globally important legume crops (Karkanis et al., 2018). High protein and nutrient content make them a valuable food and they are efficient at symbiotic fixation of nitrogen which improve soil fertility, contributing to the sustainability of agricultural ecosystems. Higher yielding individuals are usually the product of outcrossing (Fyfe and Bailey, 1951) but the volume of pollen produced and the angle of the style to the ovary means that self-pollination is possible (Stoddard, 2017). *V. faba* flowers consist of two banner petals and two wing petals (Fig. 3A) which must be depressed to reveal the anthers and stigma, therefore large pollinators such as *Bombus sp.* are required for cross pollination (Stoddard and Bond, 1987). *V. faba* flowers also contain a long corolla tube therefore pollinators with a long proboscis are best adapted to reach the nectar stored at the bottom of the corolla (Stoddard and Bond, 1987). Marzinzig *et al. (*2018) showed that long-tongued species such as *B. hortorum* are the most efficient pollinator of *V. faba* whereas short-tongued bees are more likely to rob the flower by biting a hole at the base of the corolla directly into the nectar store (Fig. 2B).

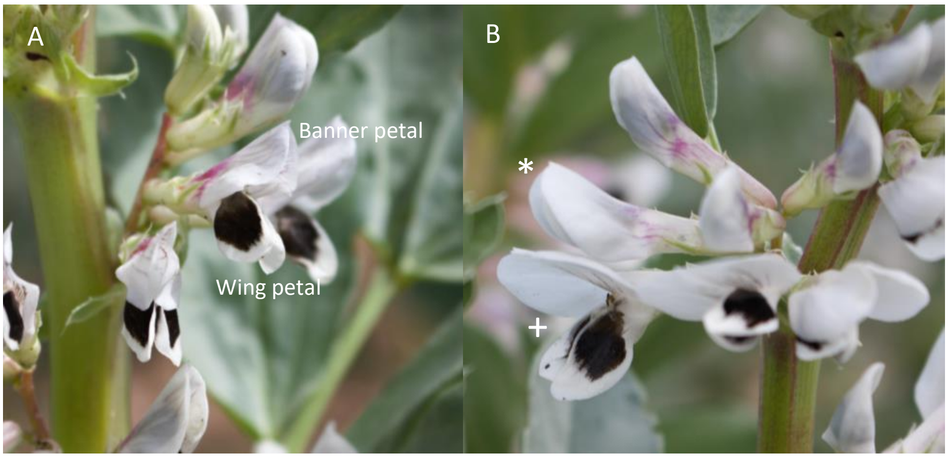

Figure 3: **Floral anatomy of V. faba flowers.** A) Floral anatomy of V. faba illustrating banner and wing petals. B) Floral anatomy before and after pollinator visit. * shows a closed flower with the anthers and stigma hidden. + shows an open flower after pollination revealing the anthers and stigma. Photos are copyright to the Author.

Heat stress during floral development is known to reduce pollen fertility in *V. faba* which limits their ability to self-pollinate (Bishop et al., 2016). Under these conditions, double the number of outcrossed pods are produced which suggests that increasing temperatures and extreme weather events associated with climate change could increase *V. faba* dependence on wild pollinators. Climate change poses a second threat to global food security with potential uncoupling of plant-pollinator mutualisms as changes in temperature effect the phenology of first flowering and emergence of worker bees in the spring (Settele et al., 2016). In landscapes with high abundance and diversity of pollinators, services may withstand this shift but populations that are already under threat from agricultural intensification and habitat fragmentation are unlikely to have such resilience (Settele et al., 2016).

Project Aims

During my placement with the Game and Wildlife Conservation Trust, I collected data on the pollination of *V. faba* crops and the factors that affect their pollination. These factors include abiotic variables such as temperature and wind speed, and biotic factors such as interspecific interactions within the pollinator community. The large quantity of abiotic conditions that were recorded and identification of pollinators to species provided a unique opportunity for a large-scale analysis of the factors effecting pollinator abundance and pollinator behaviours on *V. faba*.

Given the numerous challenges facing pollinators, a better understanding of pollinator interactions with the environment and species-specific behaviours will inform management practices for reversing the decline of invertebrate pollinators and enhancing pollination services. In this project, I will use generalised linear models to assess the importance of environmental factors on pollinator abundance within *V. faba* fields and predict how future climate scenarios will impact pollinator abundance. I will then determine how morphological traits of pollinators effect interactions and foraging behaviours on *V. faba* and I will analyse the impact of pollinator community structure and composition on crop yield. This will inform management recommendations for enhancing pollinator conservation and develop crop specific management recommendations for maximising pollination services.

Methods

Data for pollinator abundance in *V. faba* fields was obtained from the BEESPOKE project. Eight *Vicia faba* (Spring Bean) fields with a mixture of woodland, treeline and hedgerow field boundaries were selected for sampling across eight farms in Hampshire, Susses and Dorset. A 50x50m sampling grid was established across each field and a maximum of 20 points were sampled per field. At each sample point, temperature, wind speed and cloud cover were recorded. All observed pollinators were identified to species and foraging behaviours were recorded. Further information on the sampling protocol can be found in the appendix, section 1. Processing and analysis of the data was carried out in Excel and R Studio 3.5.0 (RStudio Team, 2020).

To investigate the spatial distribution of pollinators across the field, distance to the nearest margin was calculated in QGIS (QGIS, 2020) using GPX data for sample grids and field boundaries. Field boundary polygons were transformed into point files and a distances matrix was created to measure the distance between each sample point and the field boundary.

Diversity indexes for pollinators were calculated using Simpsons index (Simpson, 1949) and Shannon's index (Shannon, 1948) to assess the influence of pollinator diversity on crop yield. Indexes were calculated per farm, both within *V. faba* crop and within the boundary habitat. Although both Shannon's and Simpson's diversity index were calculated, I chose to present Shannon's index as it gives a better representation of species richness without being skewed by overabundance of one common species such as *A. mellifera* which, in some cases, is artificially augmented by the addition of hives.

Shapiro Wilk tests were used to check for normality in the data; when $p>0.05$ parametric tests were used and when $p<0.05$ non-parametric tests were used. Two-way ANOVAs were used to investigate the relationship between survey location – within the field or within the boundary – and boundary habitat on pollinator abundance. Kruskal-Wallis tests were used to compare crop yield between pollination treatments and farms and to compare pollinator abundance between different boundary habitats. Where appropriate, post-hoc Dunn tests (Dunn, 1964) from the Dunn.test package in R were used with Bonferroni correction for multiple comparisons. Spearman's rank tests were used to investigate biotic interactions such as the relationship between foraging behaviour and proboscis length, interspecific relationships, and relationships between floral abundance and pollinator abundance.

To assess the influence of factors such as temperature, wind speed, cloud cover and boundary habitat on pollinator abundance, generalised linear models were created and simplified using

Akaike's information criterion (AIC) automated stepwise backwards progression. Due to Covid-19 disrupting fieldwork, surveys were independently carried out by multiple individuals which could create observer bias in the results. A generalised linear model was created to check which variables influenced pollinator abundance; no reporter bias was found (Appendix Table 7).

Results

Pollinator community composition and abundance were compared between farms to understand how surrounding habitat impacts pollinator populations. A total of ten species of pollinator were observed and five species were present across all sites – *Apis mellifera*, *Bombus lucorum* (White-tailed bumblebee), *Bombus lapidarius* (Red-tailed bumblebee), *Bombus terrestris* (Buff-tailed bumblebee) and *Bombus pascuorum* (Common Carder). Pollinator community composition and total abundance varied greatly between farms (Fig. 4). Farm 1 contained the highest abundance but over 75% of observed pollinators were *A. mellifera* due to the presence of commercial hives on the site. Farm 7 also contained *A. mellifera* hives, but overall abundance was lower and higher species evenness was observed. Farm 6 had the lowest pollinator abundance and also had a black bean aphid infestation which damaged many of the plants and caused reduced flowering. Farm 8 had a large percentage of land covered by wildlife areas compared to other farms and had the second highest level of pollinator abundance, the highest without the presence of hives. Farm 2 and Farm 8 were also part of a Farmer Cluster aiming to employ complementary management techniques for enhancing biodiversity although different boundary habitats surrounded each field (Table 1). There was limited overlap in pollinator community composition between Farm 2 and Farm 8; Farm 2 had the highest diversity of pollinators across all farms with ten species present whereas Farm 8 had seven species present but a much lower species evenness.

Table 1: **Each farm has a unique composition of boundary habitat.** Boundary habitat surrounding each *V. faba* field. X denotes the presence of that habitat.

Farm	Hedgerow	Hedgerow with margin	Treeline	Treeline with margin	Woodland	Woodland with margin	Other e.g. fences	Margin only
1	x		x			x		x
2	x		x	x		x		
3	x	x	x	x	x	x		
4		x		x		x		
5		x				x		
6	x						x	
7		x				x		
8				x				x

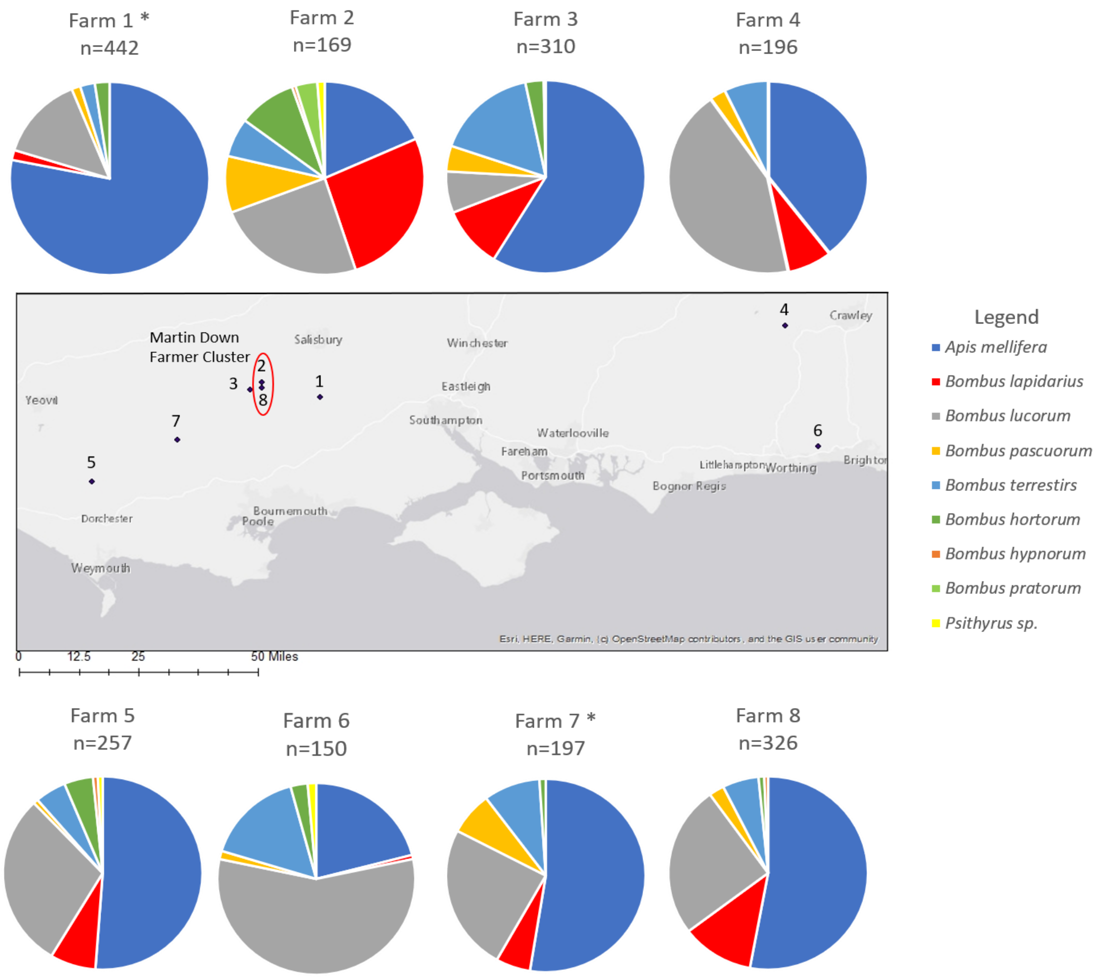

Figure 4: **Pollinator abundance and community composition varies between farms**. A map shows the eight sample locations and pie charts show the abundance and community composition of pollinators on each farm. * indicate the presence of *A. mellifera* hives.

Environmental conditions effect pollinator abundance

Understanding the effects of climate on pollinator abundance is vital for predicting how plant-pollinator interactions may change over the coming decades. To investigate how pollinator abundance changes with abiotic factors I assessed the effect of temperature, wind speed and cloud cover on pollinator abundance. Pollinator abundance exhibited a normal distribution in response to temperature with a clear peak in abundance at 19 °c with 12.4 ± 1.6 bees per survey. Current temperatures peak at 20 °c on average during June but under different climate change scenarios average peak temperature could increase by up to 4 °c which would see pollinator abundance halve during peak afternoon temperatures (Fig. 5A). Cloud cover showed a large increase in pollinator abundance at 2 oktas although the average temperature recorded at 2 oktas was 18.8°c (Fig.5B). This corresponds with the maximum abundance observed at 19°c. Wind speed also influenced pollinator abundance; speeds of up to three Beauforts showed consistent levels of abundance but as wind increased to four and above, pollinator abundance decreased (Fig. 5C). One sample point on farm 2 was completely sheltered from the wind by treeline and woodland habitat. At this sample point, there were 24 pollinators observed in a single survey compared to an average of 5.1 pollinators observed across the other surveys carried out that day.

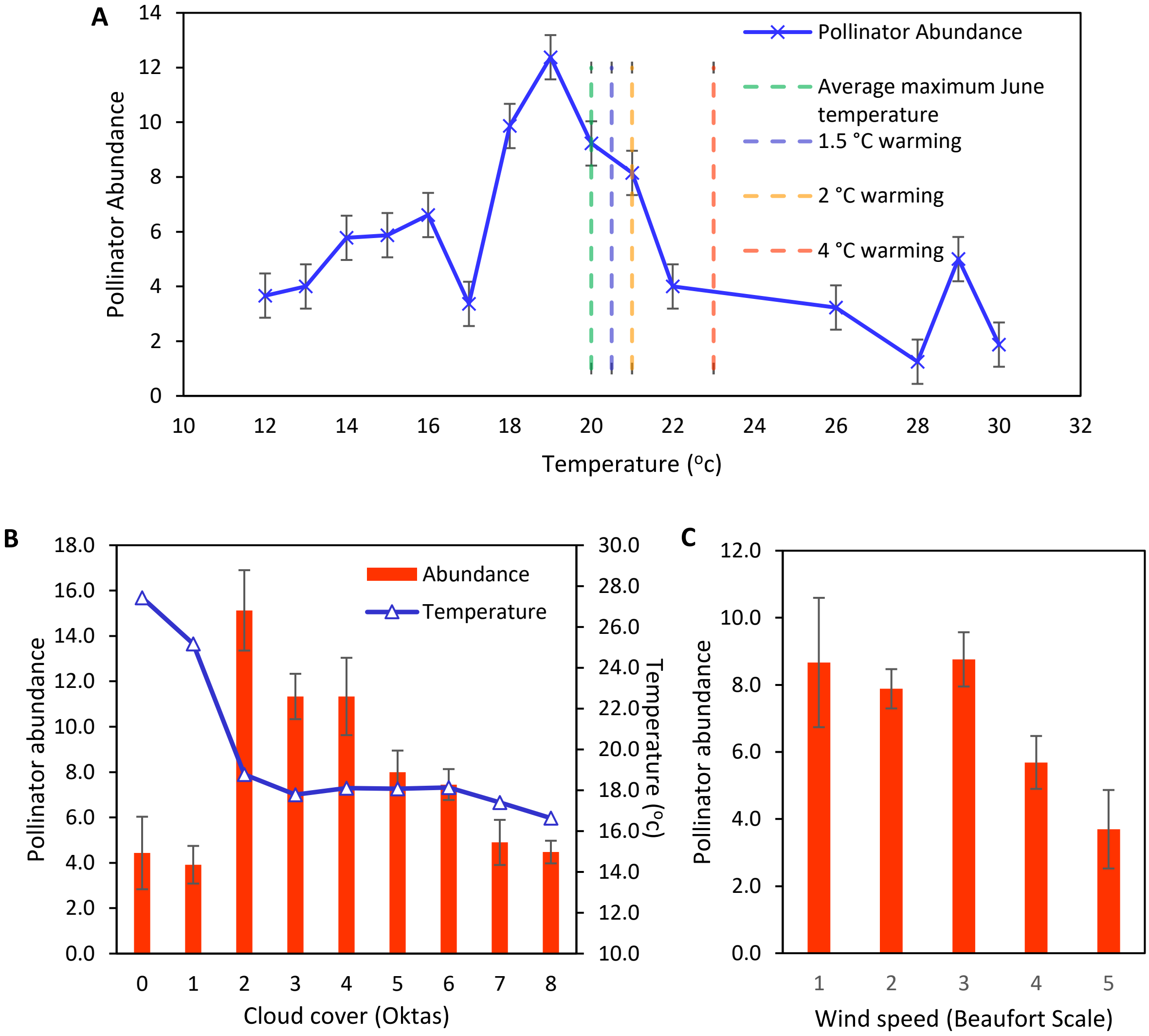

Figure 5: **Environmental conditions effect pollinator abundance within *V. faba* fields**. A) Average pollinator abundance within *V. faba* crop by temperature. Dashed lines illustrate current and future climate scenarios B) Relationship between cloud cover and average pollinator abundance within *V. faba* fields. Secondary axis shows the relationship between cloud cover and temperature. C) Average pollinator abundance within *V. faba* fields by wind speed. Error bars represent standard error.

Generalised linear models were created to investigate the effect of each environmental variable on pollinator abundance. The model showed that temperature, cloud cover and wind speed are all individually significant at determining pollinator abundance (Table 2). The average conditions observed during surveys were 18.5 °c, 5 oktas and 3 Beauforts therefore equation 1 predicts seeing an average of 7.8 bees per survey. This model can be used to predict how climate change will impact pollinator abundance under current habitat management. Under a high emissions scenario, average temperature would increase to 23 ^{o}c causing predicted average pollinator abundance to drop to 4.3 bees per survey. Pollinator abundance can be approximated by the equation

$$P = 32.518 – 0.774T – 1.304C – 1.313B \quad \textbf{(1)}$$

Where P= pollinator abundance, T= temperature in ^{o}c, C=cloud cover in oktas and B=Beaufort Wind speed.

Table 2: **Environmental conditions effect pollinator abundance**. Results from a Generalised linear model simplified using Akaike's information criterion automated backwards stepwise progression. The model predicts in field pollinator abundance based on environmental conditions.

	Estimate	Standard error	t-value	p-value
Intercept	32.5177	3.2347	10.053	<0.0001
Temperature	-0.774	0.1203	-6.435	<0.0001
Cloud cover	-1.304	0.1849	-7.053	<0.0001
Wind speed	-1.313	0.3544	-3.704	0.0003

Habitat effects pollinator abundance within boundaries but not within field

Understanding the relationship between boundary habitat and pollinator abundance both within the boundary and within the crop can inform management recommendations for increasing pollinator abundance. Within field boundaries, pollinator abundance is higher in the presence of a margin unless in a woodland boundary (Fig. 6A). For woodland, the trend is reversed, and more pollinators are present without a margin. Maximum pollinator abundance was observed in boundary habitats where only a margin was present with an average of 12.4 pollinators per 100m transect. Conversely, the lowest pollinator abundance of 0.5 bees per 100m transect was recorded in alternative boundary habitats such as fences and roads where margins were absent (Fig. 6A). A Kruskal-Wallis test showed that habitat significantly effects pollinator abundance within the boundary (p=0.044). Although post-hoc analysis did not show significant differences between any two habitats, further analysis into the composition and quality of margins showed that pollinator abundance was significantly correlated with the number of flower heads (p=0.044, R^2=0.4247) but diversity of species in flower did not show a significant correlation with abundance (p=0.113, R^2=0.1876).

To investigate the effect of boundary habitat on pollinator abundance within the field, surveys were categorised based on the nearest boundary habitat. The highest average pollinator abundance (5.8 bees) was observed when a treeline without a margin was the nearest habitat and the lowest abundance (3.4 bees) was observed when alternative boundaries without a margin such as fences or roads were closest (Fig. 6B). A Kruskal-Wallis test showed there was no significant difference between pollinator abundance within the field based on the nearest boundary habitat (p=0.055). Although, this result is not significant at the threshold of p<0.05, the result is close enough to merit attention and therefore I suggest more samples are collected for a definitive conclusion.

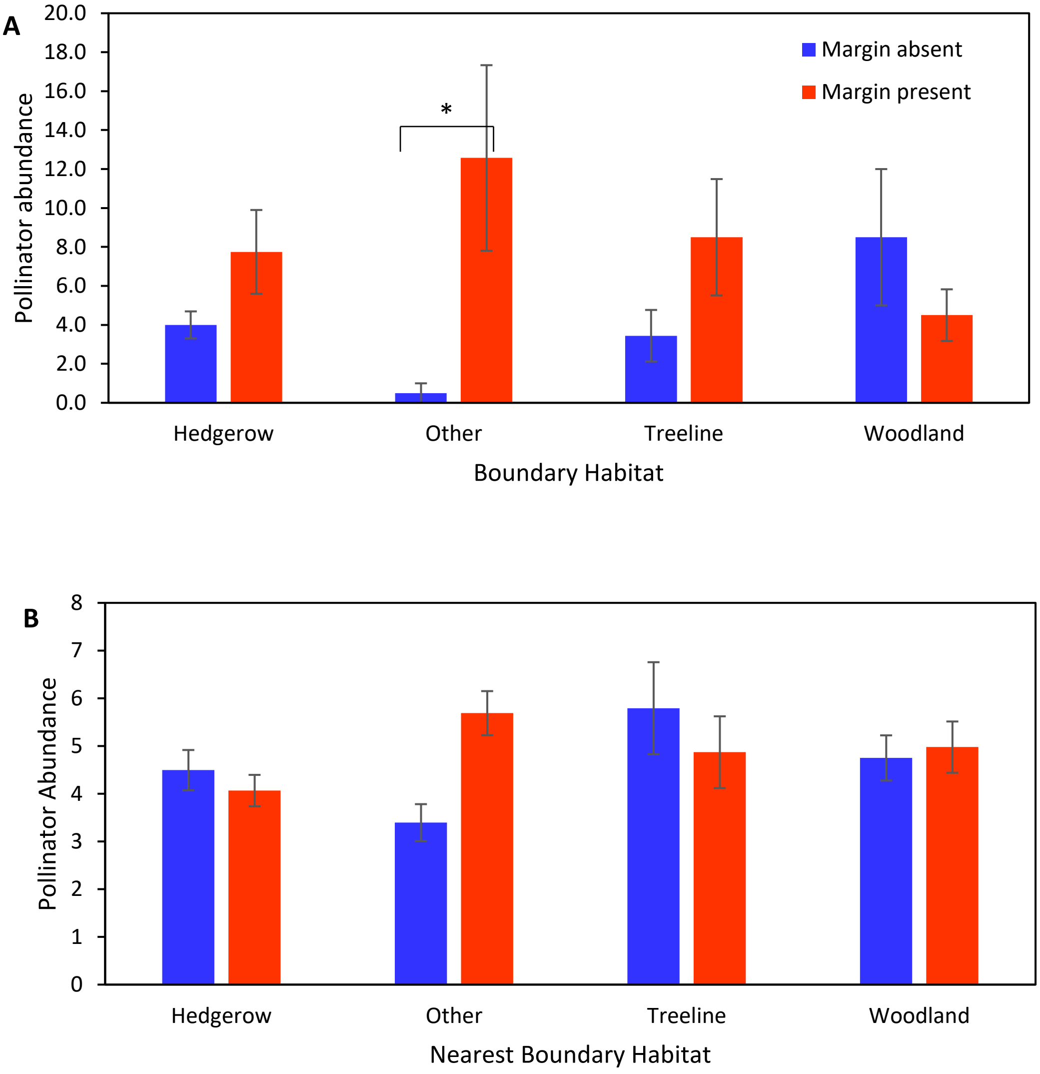

Figure 6: **Habitat effects pollinator abundance within boundary habitat but not within field**. A) Pollinator abundance within the boundary. Significant comparisons from post hoc Dunn tests are marked with an * B) Pollinator abundance within the field categorised by the nearest boundary type. Error bars represent standard error.

Generalised linear models were created to predict which boundary habitat would increase pollinator abundance within the field. The presence of any individual habitat was not significant at predicting pollinator abundance within the field (Table 3) but if all surrounding habitats are known then pollinator abundance predictions can be made. Based on the 34 boundary habitats sampled, equation 2 was produced to predict which combination of boundary habitats, entered into the equation as present (1) or absent (0), would maximise pollinator abundance within the field. Pollinator abundance can be approximated by the equation

$$P = 6.21H + 6.27W + 5.06M - 1.62T - 2.79\,T_m - 1.71A - 0.34 \quad \textbf{(2)}$$

where P= pollinator abundance, H=hedgerow (with or without margin), W=woodland (with or without margin), T=treeline, T_m= treeline with margin, A= artificial boundary habitat, M= margin only.

Table 3: **Boundary composition around fields effects pollinator abundance.** Results from a generalised linear model refined using automated backward stepwise progression. The model predicts pollinator abundance based on surrounding boundary habitat.

Boundary Habitat	Estimate	Standard error	t-value	p-value
Intercept	-0.34	1.07	-0.305	0.811
Hedgerow	6.21	1.58	3.93	0.159
Woodland	6.27	0.95	6.86	0.096
Treeline without margin	-1.62	0.95	-1.70	0.34
Other without margin	-1.71	0.95	-1.8	0.32
Treeline with margin	-2.79	0.67	-4.14	0.15
Standalone margin	5.06	0.95	5.31	0.12

Boundary habitats effect spatial distribution of pollinators

Boundary habitats have been shown to increase pollinator abundance within the boundary but simply increasing abundance does not guarantee improved crop pollination. The flow of pollinators between boundary and field was investigated using interaction plots that showed the average abundance of *A. mellifera* and *Bombus sp*. within boundaries and within the field (Fig. 7). Boundaries where only a margin was present acted as a sink for *Bombus sp*., however *A. mellifera* abundance increased both within the margin and at the nearest points in the field. Treelines without margins showed the opposite effect on *Bombus sp.* where more individuals were present near the margin than within the margin. Within field abundance of *A. mellifera* correlated with abundance within the boundary for most boundary types. *A. mellifera* showed high abundance in woodland without margins although abundance within the field did not appear to act as a source for this. To investigate the effect of survey location (within field or boundary) and the effect of boundary habitat on pollinator abundance, a two-way ANOVA was used. Neither the habitat or the location of the survey was significant for *A. mellifera* abundance (p=0.198 and p=0.378, respectively). *Bombus sp.* showed less correlation between boundary and in field abundance (Fig. 7). However, a two-way ANOVA showed that the type of boundary and survey location made no significant difference to *Bombus sp.* abundance within the field (p=0.765 and p=0.112 respectively).

Although pollinator abundance did not show spill over between boundary and field, the relationship between pollinator diversity within the boundary and within the field was also investigated (Table 4). Farm 2 has the highest pollinator diversity in both boundary and field, but Farm 1 had the third highest diversity in the boundary but the lowest diversity in the field. The remaining farms show a relatively constant level of pollinator diversity within the field despite variation in diversity within the margin.

Table 4: **High pollinator diversity in boundaries does not always correspond to high pollinator diversity in fields**. Shannon's diversity index for pollinators within the field and within the boundary for each farm.

Farm	Field Diversity	Boundary Diversity
1	0.31	0.66
2	0.80	0.93
3	0.56	0.81
4	0.55	0.41
5	0.57	0.54
6	0.52	0.42
7	0.56	0.54
8	0.50	0.46

Unequal spatial distribution of pollinators could cause pollination deficits within areas of the field where pollinator abundance is low. Our data shows that *Bombus sp*. abundance remains constant up to 100m away from boundaries, but *A. mellifera* show a decline in abundance as distance from the boundary increases (Fig. 7C).

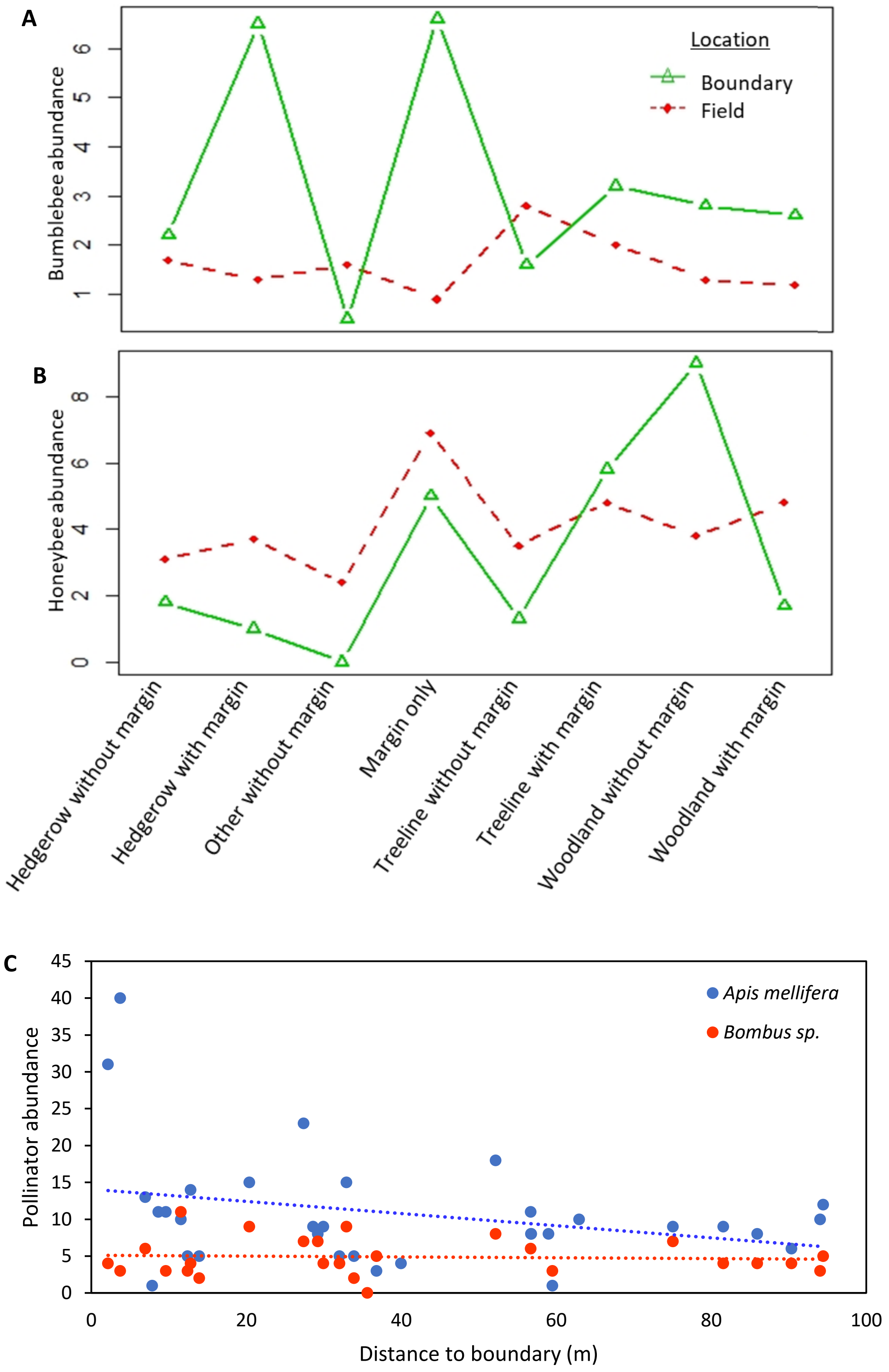

Figure 7: ***A. mellifera* and *Bombus sp*. show different spatial distribution trends.** A) Interaction between abundance within field boundaries and abundance within the field for *A. mellifera* B) Interaction between abundance within field boundaries and abundance within the field for *Bombus sp.* C) Scatter graph of pollinator abundance by distance to boundary habitat using a subsample of environmental conditions (19ºc, 2-5 oktas and 1-3 beauforts). Linear lines of best fit were added for each taxa.

Foraging behaviours show interspecific variation

Understanding foraging behaviours and specialisations of individual species will identify the most efficient pollinators for each crop and help produce crop specific management recommendations. Legitimate visitation of flowers results in cross pollination whereas robbing behaviours do not therefore, species that carry out legitimate visitation will be the most efficient pollinators. Individual species show vastly different foraging behaviours on *V. faba* crops; *B. hortorum* legitimately forages 96.6% of the time compared to the *B. lucorum* which forages on just 24.2% of flower visits and *A. mellifera* which forages on just 34.3% of visits (Fig. 8A).

I hypothesised that the difference in foraging behaviours observed between different species was due to physical constraints in their proboscis anatomy with short-tongued species unable to reach nectar through legitimate visits. Tongue length measurements were plotted against percentage of legitimate flower visits and analysis using a one tailed Spearman's rank test for positive correlation showed a significant correlation between tongue length and foraging behaviour ($P=0.028$) with an R^2 value of 0.74 (Fig. 8B). As *B. hortorum* exhibits the highest percentage of legitimate foraging visits*,* it is important to understand whether interactions with other species could be influencing the abundance of *B. hortorum*. Existing literature suggests there is a negative correlation between *B. hortorum* and *A. mellifera* abundance, therefore I tested the relationship observed in our data using a one tailed Spearman's rank test for negative correlation (Fig. 8C). The test returned an R^2 value of 0.42 and p-value of 0.058. Although, this result is not significant at the threshold of $p<0.05$, the result is close enough to merit attention and therefore I suggest more samples are collected for a definitive conclusion.

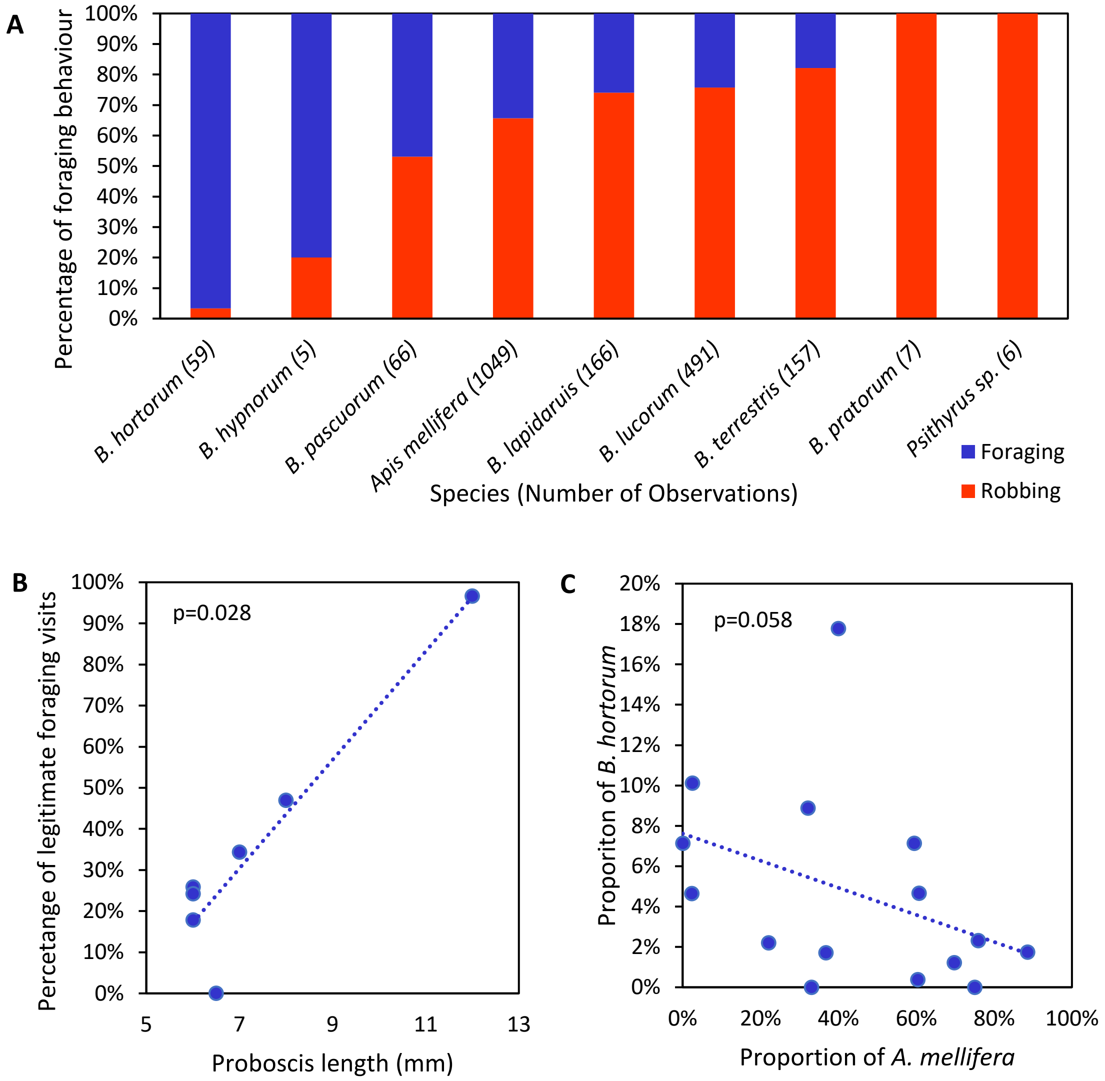

Figure 8: **Foraging behaviour on V. faba shows interspecific variation that is correlated with proboscis length.** A) Stacked bar chart showing percentage of legitimate foraging visits and percentage of robbing visits by species. B) Scatter plot with linear line of best fit for correlation between tongue length and percentage of time spent legitimately foraging. C) Scatter plot with linear line of best fit for correlation between relative abundance of *B. hortorum* and relative abundance of *A. mellifera*.

Pollination increases *V. faba* yield

The degree of dependence on cross pollination for crop yield and quality has long been debated in *V. faba*. Cross pollination occurs during open (pollinator) pollination and hand pollination treatments. Hand pollination was used as a control representing complete cross pollination to test whether there was a pollination deficit in open pollinated pods. Number of pods, number of beans and dry weight of beans from hand-pollinated, open pollinated and self-pollinated seed sets were compared (Fig. 9). Of the 160 self-pollinated bagged plants, 122 (76%) produced no pods on the marked trusses. Statistical analysis showed the number of pods produced significantly varies with pollination treatment, p<0.0001. Further analysis with a Dunn test showed that there was a significant difference (p<0.0001) between cross pollination and self-pollination treatments but there was no significant difference between hand and open pollinated treatments (p=1). The effect of pollination treatment on number of beans per pod and dry weight of beans per pod was also tested but pollination treatment did not significantly affect either (p=0.3849 and p=0.1795 respectively). There was also significant inter-farm variation in pod set (p<0.0001) although both hand and open pollination produced the same yield on each farm (Fig. 9D). A Dunn test showed that farm 3 had significantly less pods per truss than all other farms. Farm 8 also had significantly more pods per truss than farm 4 and farm 6 but the remaining farms were not significantly different (Table 5). To investigate the cause of inter-farm yield variation, I tested the correlation between pollinator abundance and diversity with yield; neither showed a significant correlation to yield (p=0.429 and p=0.463 respectively).

Table 5: **Farm 3 has significantly lower *V. faba* yield.** Bonferroni corrected p-values from a post hoc Dunn test comparing number of pods per truss between farm. * indicates significant comparisons.

Farm	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	1						
3	0*	0*					
4	0.1430	0.2607	0.0308*				
5	1	1	0*	0.1888			
6	0.2155	0.3951	0.0013*	1	0.2665		
7	1	1	0*	0.0761	1	0.1015	
8	1	1	0*	0.0024*	1	0.0019*	1

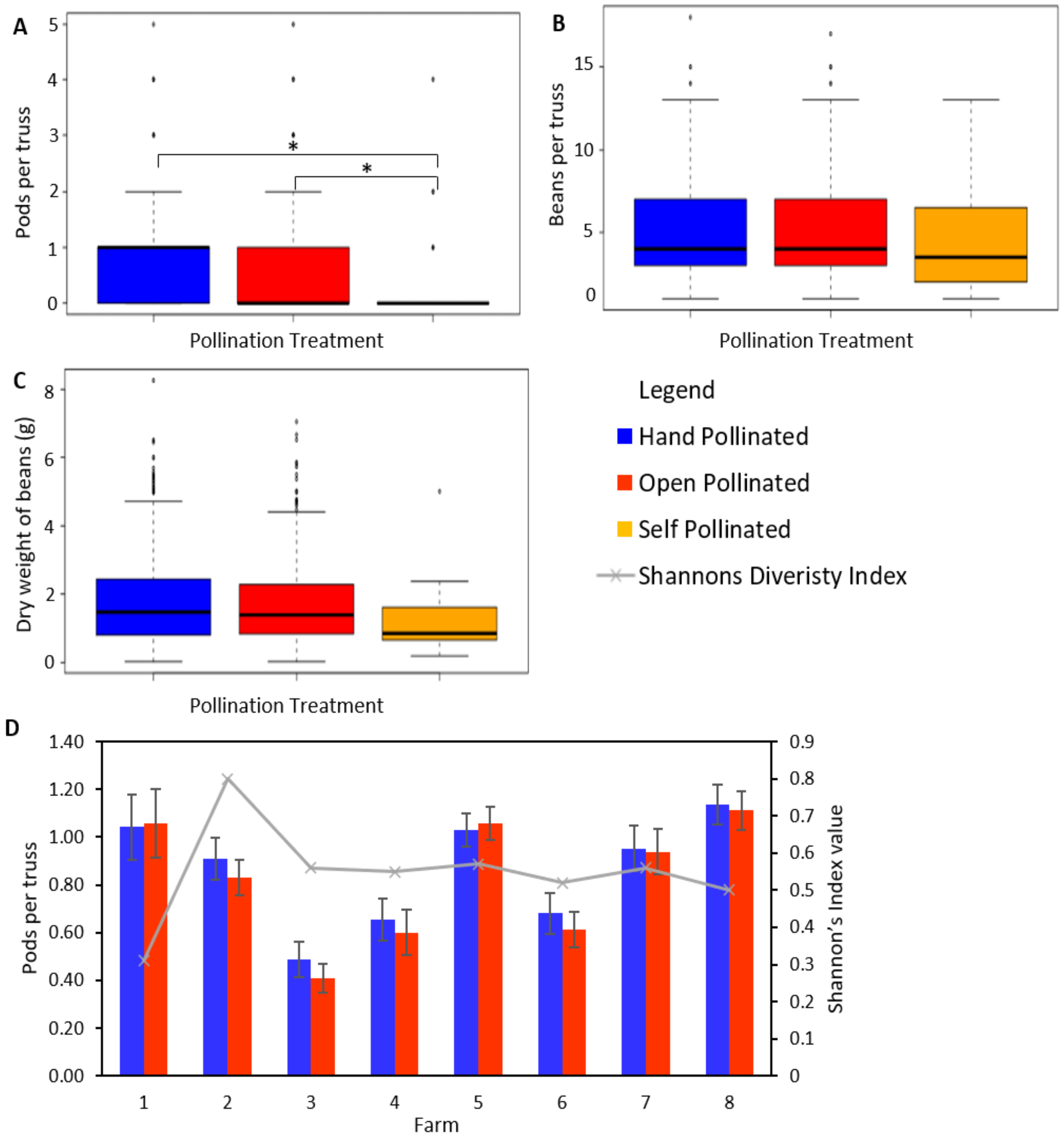

Figure 9: **There is inter-farm variation in yield although cross pollination increases yield across all farms**. Boxplots show the interquartile range (IQR), and the bold line shows the median value. Minimum and maximum bounds are 1.5 times the IQR and outliers are shown as dots. A) Difference in number of pods per truss based on pollination treatment. Brackets and * illustrate significant comparisons from a Dunn post hoc test. B) Number of beans per truss by pollination treatment. C) Dry weight of beans per truss by pollination treatment. D) Number of pods per truss for hand and open pollination by Farm. Shannon's diversity index was used to test for a relationship between number of pods and pollinator diversity. Error bars represent standard error.

Discussion

Impact of climate change on pollinator abundance

Understanding how environmental conditions influence pollinator abundance is crucial to predicting and mitigating the effects of climate change on pollinator diversity and pollinator dependent food crops. This study found that environmental conditions such as temperature, cloud cover and wind speed significantly affect pollinator abundance in *V. faba* fields and abundance declines outside of optimal foraging conditions. *Bombus sp.* flight is thermoregulated therefore pollinators require an optimal thoracic temperature of 40°c to operate (Goulson, 2010). The maximum thoracic temperature tolerated is 44°c but infrared imaging has shown that the flight muscles in the thorax can be 20°c hotter than ambient temperature. This creates a risk of overheating at high ambient temperatures hence low pollinator abundance was observed at high temperatures in this study. Current daily temperatures peak at an average of 20 °c in June in South England (The Met Office, 2016). However, UK climate predictions suggest that high emission scenarios, currently with 90% probability, could see summer heatwaves becoming more frequent and even hotter, pushing peak ambient temperature to around 30°c (Met Office, 2019). Many pollinators would overheat during flight in these temperatures which would reduce pollination if heatwaves occur during the flowering season. This increase in temperature would reduce current pollinator community activity by 25% (Fig. 5). The effects of climate change on pollinators is well studied and it is suggested that species will shift their ranges North to find cooler temperatures (Settele et al., 2016). However, predicting the end result on plant-pollinator mutualisms is much harder because the outcome depends on the densities and phenologies of other species within the community (Gilman et al., 2012). Such climate shifts could cause spatial and phenological uncoupling of plant-pollinator mutualisms. Research by Bishop et al., (2017) showed that heat stress in *V. faba* increases dependence on pollinators for seed production. Without a plan for pollinator recovery, declines in pollinator abundance and increased pollinator dependence for seed production is doubly problematic for food security. Reversing declines in pollinator abundance and increasing diversity through improved habitat provision will create robust pollinator populations that have a better chance of continuing pollination services during extreme and variable environmental conditions.

Pollinator habitat management

Field margins offer resources and habitat for pollinator reproduction making them valuable habitat for pollinators (Bäckman and Tiainen, 2002). Understanding how pollinators use arable margins and how this influences pollinator abundance across arable landscapes can inform management practices to reverse pollinator declines such as the farmer cluster scheme. Short-tongued generalist pollinators such as *B. terrestris, A. mellifera* and *B. lucorum* were present across all farms in this study but specialist long-tongued species such as *B. hortourm* were relatively rare (Fig. 4 and Fig. 8). This could be due to limitations in the availability of suitable plants for specialist species to forage on. Although many farms had habitat in common, the exact combination and composition of habitat was unique to each farm (Table 1). Farm 2 and Farm 8 were carrying out complementary management practices through the Farmer Cluster management scheme but there was little similarity in pollinator community composition between these farms (Fig. 4). This suggests that surrounding habitat may have an effect on the outcome of field level habitat management. There is currently a lack of information surrounding this topic, but analysing survey data from farmer clusters presents a unique opportunity to assess the importance of surrounding habitat on management techniques and discern the relative value of field and landscape level management for pollinator abundance and diversity. Farmer cluster surveys would provide data on the lag time between implementing management and observing field level increases in abundance and landscape scale continuity in pollinator communities. As our data only covers two of the eleven farms in this cluster, further analysis is required to draw reliable conclusions both from within this cluster and from other clusters.

Pollinator abundance and diversity is not only affected by the wider surrounding habitat but also by the local habitat surrounding each field. In this study, pollinator abundance increased in the presence of woodland, hedgerows, and standalone margins (Equation 2) with each habitat offering a unique set of benefits. For example, Jha and Kremen, (2013) showed that natural woodland is valuable for attracting *Bombus sp.* to nest while Cranmer et al., (2012) showed that linear hedgerow features influence bumblebee flight paths across farmland landscapes. Furthermore, employing a diverse range of boundary habitats could provide shelter from wind that would benefit both pollinators and crops (Gurr et al., 2003). Floral abundance and diversity were used as a measure of habitat quality and it was shown that floral abundance positively correlates with pollinator abundance. Previous studies by Scheper et al., (2013) show similar trends between pollinator diversity and abundance in relation to both floral diversity and abundance. A florally enhanced, heterogenous landscape would encompass the benefits offered by each habitat and would enhance pollinator abundance and movement at the landscape scale.

The effect of habitat management on crop pollination must be considered to maximise pollination potential. The relationship between field boundary abundance and in-field abundance is complex. This study found that *A. mellifera* abundance is correlated between boundary and field, but this trend was not observed in *Bombus sp.* abundance between boundaries and field (Fig. 7A and 7B). Holland et al., (2015) found that florally diverse boundary habitats can act as a sink for pollinators therefore increasing pollinator abundance within boundaries does not necessarily create a source of pollinators for crop pollination. A meta-analysis by Zamorano et al., (2020) showed no consistent spill over of pollinators from field boundaries into crops which agrees with our results (Fig. 7). Pollinator diversity between margins and field was also compared but no consistent trend was observed (Table 4). This suggests that pollinator habitat provision aids pollinator conservation but does not improve pollination services which presents an interesting problem for balancing optimal habitat for pollinator abundance whilst ensuring pollination services continue. Limitations on movement into the crop could be due to numerous factors such as surrounding landscape composition, size of margin or crop management practices but further studies are needed to isolate these variables and understand the individual interactions with pollinator abundance and movement (Zamorano et al., 2020). Pollinator abundance within fields could be improved by using florally enhanced strips to increase floral diversity and break up crop monoculture. Pollinators will travel over crops whilst moving between floral strips providing opportunities for crop pollination.

Previous research has shown that *A. mellifera* routinely forage multiple kilometres away from the colony whereas *Bombus sp.* often only travel up to 800m (Minahan and Brunet, 2018). As colonies are likely to be nesting in field boundaries, I compared abundance of *A. mellifera* and *Bombus sp.* based on distance from the nearest field boundary. I found that *Bombus sp.* abundance remains constant whereas *A. mellifera* abundance decreases as distance from the field boundary increases (Fig. 7C). However, sampling only covered 100m from field boundaries and the exact locations of colonies were unknown. Differences between *A. mellifera* and *Bombus sp.* foraging distance could arise due to differences in foraging behaviours, colony size, division of labour and communication of resource location. *A. mellifera* are thought to be more efficient at locating patches of high reward with their waggle dance whereas *Bombus sp.* use trapline foraging to repeatedly visit patches of high reward in a predictable route (Minahan and Brunet, 2018). The discrepancy between our results and the literature could be due to differences in resource availability at individual sites or due to using field boundaries as a surrogate for colony location. Resolving the nature of this trend would be beneficial for predicting the effectiveness of landscape scale management practices on pollinator abundance; species with a short foraging range will primarily benefit from field level habitat management whereas species with a large foraging range could benefit from landscape scale habitat management.

Pollination of *Vicia faba*

Many species of pollinator exhibit a unique set of foraging behaviours and interactions. These behaviours and interactions can be utilised to maximise pollination for specific food crops. Each species of bumblebee exhibited a unique proportion of robbing and legitimate visitation behaviour whilst foraging on *V. faba (Fig. 8A)*. *B. hortorum* carried out the most legitimate foraging visits which agrees with other studies (Marzinzig et al., 2018). Foraging behaviour has often been linked to tongue length (Ranta and Lundberg, 1980), therefore I hypothesised that long tongued species were able to forage more efficiently due to the long corolla length of *V. faba* flowers. Our results supported this hypothesis with a strong positive correlation between percentage of legitimate visits made by each species and their average tongue length (Fig. 8B). Enriching margins with flowers coadapted for long tongued pollinators such as members of the Labiate family could increase *B. hortorum* abundance within *V. faba* fields (Fussell and Corbet, 1991). Due to similar floral anatomy between legumes, *B. hortorum* is likely to be an efficient pollinator for other legume crops. However, there is a deficit in species level pollinator studies for other crop families, therefore further investigation into other pollinator dependent crops will help identify which suite of pollinator species are best suited to each crop. Bespoke margin mixes could be created to attract the most beneficial suite of pollinators for each crop and optimise pollination across all crops.

Many farms employ *A. mellifera* hives on their farms to enhance wild pollinator populations, but previous research has highlighted that there is a negative correlation between the presence of *A. mellifera* hives and *B. hortorum* abundance (Wermuth and Dupont, 2010). Our data showed the same trend with a negative correlation between *B. hortorum* abundance and *A. mellifera* abundance (Fig. 8C). *A. mellifera* robbing behaviour could reduce nectar volume making legitimate visitation less favourable for *B. hortorum* causing them to move to a different foraging patch. However, other species such as *B. terrestris* also rob but a negative correlation is not observed between these two species. Alternatively, the relationship could be an artefact of the environment; *A. mellifera* hives are usually deployed to compensate for a pollinator deficit therefore *B. hortorum* may not be present due to lack of suitable habitat. Uncovering the true reason for this relationship will be vital for enhancing *B. hortorum* populations to maximise *V. faba* pollination. Recording *B. hortorum* abundance before and after the addition of *A. mellifera* hives across multiple farms would provide insight into the true cause of this relationship.

There is an ongoing debate about how dependent *V. faba* is on cross-pollination for seed production (Bishop et al., 2020; Scriven et al., 1961; Fyfe and Bailey, 1951). Our data showed a significant increase in number of pods after cross pollination compared to pollinator exclusion treatments suggesting cross pollination enhances crop yield (Fig. 9A). Similarities in yield from hand and open pollinated treatments suggests there is no pollination deficit limiting crop yield (Fig. 9A). Similar results were found in numerous other studies. (Bartomeus et al., 2014; Nayak et al., 2015; Marzinzig et al., 2018). However, due to the nature of bagging the whole plant for pollinator exclusion, some plants showed growth deformities where the bag rested on top of the stem. The stress of this weight on growth could have caused pod formation to be abandoned although this is unlikely as pods formed below and occasionally above the marked truss in bagged plants. To avoid any doubt, future replicates for self-pollination should bag individual trusses or use large cages that encompass multiple plants without impeding on plant growth. Crop yield significantly differed between farms (Fig. 9D) although there was no correlation between yield and pollinator abundance or diversity. Nayak et al., (2015) also found no correlation between pollinator abundance and yield but Bartomeus et al., (2014) suggests that pollinator visitation rate did enhance yield. This is an ongoing debate caused by difficulties in comparing data; the benefits of pollination vary with site, cultivar and year (Bishop et al., 2020). Yield is also limited by other factors that are unique to each farm such as temperature stress, resource availability or disease/pest damage to crops (Alharbi and Adhikari, 2020). For example, Farm 6 had a black aphid infestation which damaged *V. faba* plants and likely caused the low crop yield on that farm. Therefore, it is important to interpret the effect of pollinator abundance on crop yield in relation to other yield effecting factors; temperature stress increases the effect of pollinator abundance on *V. faba* yield whereas pest damage to crops overrides the effect of pollinator abundance on crop yield.

Although pollination is not currently a limiting factor for *V. faba* yield, future declines in pollinator abundance and increased dependence on cross pollination could change this. The incoming agri-environment scheme increases emphasis on preserving natural resources such as soil quality and water retention, potentially removing current limitations on yield (Alharbi and Adhikari, 2020).Therefore, it is important we know how pollinator interactions with crops can be enhanced to boost yields if pollination become the yield limiting factor.

Concluding remarks

Current pollinator management is focussed on mitigating past problems however, we have a unique opportunity to pre-emptively manage pollinators to reduce the effects of future climate change. Our investigation has shown that environmental conditions play a significant role in determining pollinator abundance. Our predictions show that climate change is likely to contribute to pollination declines in UK *V. faba* crops, therefore we need to increase pollinator populations now to give them the best possible chance of surviving. Increasing pollinator abundance can be achieved by increasing floral abundance and diversity across agricultural landscapes and providing heterogenous boundary habitats to maximise resource availability. Pollination services can be optimised by providing species-specific floral margin mixes adjacent to crops to maximise pollination for each crop such as attracting *B. hortorum* for *V. faba* crops. It would be beneficial to replicate this study across numerous food crops to build a picture of ideal pollinator communities for each crop and maximise pollinator movement across crops throughout the flowering season. There is currently a lack of information about how landscape context impacts success of pollinator habitat provision (Heard et al., 2007) therefore investigating pollinator movement at the landscape scale by utilising ecological surveys carried out across Farmer Clusters could better inform management practices and recommendations for the future.

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Acknowledgements

I would like to thank Dr John Holland at the Game and Wildlife Conservation Trust for kindly allowing me to use this data from BEESPOKE, an Interreg project supported by the North Sea Programme of the European Regional Development Fund. I would also like to thank Professor Ed Feil for his guidance and support throughout the project, and for sharing my enthusiasm for bees. Finally I would like to thank friends and colleagues for providing feedback on the finished manuscript.

Appendix

Fieldwork Protocol

Sampling was carried out 10 days and 20 days after the start of flowering in June and sample points were marked with a flexicane. At each grid point, four plants, approximately 2m apart, were selected at random and on each plant one truss of flowers was hand pollinated and the other was open (pollinator) pollinated to act as a control. All selected trusses required at least three open flowers and the number of flowers per truss were recorded. To avoid unequal distribution of nutrients to the developing beans, two plants had the open pollinated truss below the hand pollinated truss and the other two plants had the hand pollinated truss below the open pollinated truss. Ten self-pollination control plants per field were selected and bagged with a fine mesh to exclude all pollinators.

Hand pollinated trusses were marked with pink wool immediately below the target truss and a label was added detailing grid location, plant number, sampling round and hand-pollination code e.g. F1-A1-H. Flowers to be hand pollinated were opened by gently pulling down on the wing petals and the stigma was brushed with anthers from a different plant. Each set of anthers were used up to three times before being discarded and replaced until all selected flowers were pollinated.

Each open pollinated truss was marked with yellow wool and care was taken to avoid touching the flowers as this could trigger self-pollination. Each plant was labelled describing the grid location, plant code, survey round and the open pollination code e.g. F1-A1-O.

Closed buds were selected for self-pollination trusses to ensure no pollination had taken place prior to the plants being bagged. The target truss was marked with orange wool and labelled with grid location, plant code and survey round.

Adjacent to each of the grid points, a five-minute pollinator observation was carried out along a 5 m transect. Surveys were conducted between 1000h and 1700h when the temperature was above 10°c, wind levels were below 4 on the Beaufort Scale (Table 6) and there was no heavy rain or thick fog. Time of day, ambient temperature, wind speed and cloud cover were recorded for all surveys. All pollinators observed were identified and behaviours were recorded as legitimate foraging or nectar robbing. *Bombus* were identified to species and *A. mellifera* were also recorded.

Table 6: **Description of Beaufort wind speed scale**.

Force	Wind speed (km/h)	Description
1	1-5	Direction of wind can be seen by smoke drift
2	6-11	Wind felt on face, leaves rustle
3	12-19	Leaves and small twigs in constant motion
4	20-28	Raises dust and loose paper, small branches are moved
5	29-38	Small trees in leaf begin to sway
6	39-49	Large branches in motion

At harvest, the number of beans produced by each marked truss was recorded and the pods from each truss were removed and stored in paper bags labelled with the grid code, plant letter, pollination treatment and sample round. In the laboratory, the number of pods per truss, number of beans per pod and weight of beans per pod were recorded.

Pollinator transect surveys were conducted around the field boundaries which were broadly categorised into woodland, hedgerow and treeline with or without a margin. Each transect was 100m long and each habitat was surveyed once per field. Pollinator surveys were conducted under the same range of conditions as in the field. All bees that were seen foraging or actively nest searching within 2 m of the observer were recorded. *Bombus sp.* and *A. mellifera* were recorded to species and caste. Along the same transect used for the pollinator survey, the density of flower heads and number of species in flower was measured every 10m using a 0.5 m quadrat. At each location, the quadrat was placed in the middle of the margin and the number of flowering units and the number of different species in flower were recorded. Umbellifers were recorded separately; one umbel was recorded as one flower head due to the time-consuming nature of counting individual flowers within an umbel.

Generalised Linear Model

Table 7: **Observer does not affect pollinator abundance.** P-values from a generalised linear model indicate which variables significantly affect pollinator abundance. Only variables in bold remained in the model after refinement using automated backward stepwise progression.

Variable	P-value
Farm	**<0.001**
Survey Round	**<0.001**
Observer	0.22
Time of day	0.85
Temperature	**<0.001**
Cloud cover	**<0.001**
Wind	0.11