



# Mustard plants distant from forest fragments receive a lower diversity of flower-visiting insects

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## Abstract

Mustard fields (*Brassica campestris*: Brassicaceae) are mass flowering crops attracting a wide diversity of flower-visiting insects. Many studies have shown that the diversity of insects is higher near forest fragments than farther away from the forest edge and that *Apis*-bees numerically dominate such ecosystems. In this study, we investigated how insect diversity changes with distance from the forest edge (100 m, 1100 m, 2100 m) in mustard crop fields in Nepal. The effects of distance on both the abundance and richness of insects were evaluated using generalized linear mixed models, while Hill numbers were used to describe species diversity. We performed ordination analysis and PERMANOVA to examine the dissimilarity between insect communities at different distances. Finally, percent similarity was used to describe which insect species contributed most to the dissimilarity among distances. Our findings suggest that richness and abundance of the flower-visiting insects differed between distances of 100 m and 2.1 km from forest fragments. We found that values of all diversity measures were higher nearer to the forest fragments and moderately distant from the fragments. Accordingly, the ordination analysis corroborated the GLMMs, showing that insect community composition near to and moderately far from the forest fragments differed from that farther in the field. Additionally, we detected that solitary bee species (*Andrena* spp., *Halictus* spp. and *Megachile lanata*) outnumbered *Apis*-bees, greatly contributing to community discrimination among distances. Overall, we demonstrated that insect diversity within mustard crops is not homogeneous in Nepal. Cultivated plants farther from forest fragments are facing a deficit of diversity of flower-visiting insects. Furthermore, the role and the nesting habitat of solitary bees sampled here should be investigated, as they may contribute to mustard pollination in Nepal.

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**Keywords:** Agriculture; Crops; Forests; Insect community; Solitary bees

## Introduction

Worldwide, forest areas have been reduced by 129 million ha since 1990 (FAO, 2015). More than 40% of the earth's land surface is already used for agriculture and pasture

(Foley et al., 2005). This has caused the loss of natural habitats through deforestation and ecological degradation, resulting in a decrease of biodiversity (Ricketts et al., 2008; Steffan-Dewenter & Westphal, 2008). As forest biodiversity supports a wide variety of ecosystem services, such as biological control, seed dispersal and pollination (Thompson et al., 2011), this degradation may impact ecosystem functioning, which is especially concerning in the

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face of global environmental change (Elmqvist et al., 2003; Hooper et al., 2012; Tilman, Reich & Isbell, 2012).

Thus, if a pollinator population was negatively affected by environmental disturbances, then food supply and security may be compromised for human beings (Gallai, Salles, Settele & Vaissière, 2009). The pollinating animals are responsible for approximately 80% of the reproduction of wild and cultivated plants (Ollerton, Winfree & Tarrant, 2011). As such, they provide meaningful ecological and economic contributions to the global agriculture (Garibaldi et al., 2016; Kleijn et al., 2015; Potts et al., 2016)

The ecological service of pollination provided by these organisms to crop production has been recognized mainly in cultivated plants growing in close proximity to natural vegetation as compared to those growing at a distance far from it (Bailey et al., 2014; Blanche, Ludwig & Cunningham, 2006; Halinski, dos Santos, Kaehler & Blochtein, 2018; Hipólito, Boscolo & Viana, 2018; Ricketts, 2004). It is believed that most pollinating organisms (e.g., bees) nest either in crop soils or, more commonly, in natural or semi-natural areas adjacent to these crops (Bailey et al., 2014; Blanche et al., 2006; Halinski et al., 2018; Hipólito et al., 2018; Ricketts, 2004). Consequently, pollinator diversity is consistently higher near these natural habitats than away from them (Bailey et al., 2014; Blanche et al., 2006; Halinski et al., 2018; Hipólito et al., 2018; Ricketts, 2004). The main reason for this asymmetrical diversity of insects in crops, that is, near or far forest fragments, is because most of them need natural substrates for nesting that are commonly found in or near forest remnants (Fahrig et al., 2011; Garibaldi et al., 2014; Halinski et al., 2018).

However, mass flowering crops may, at least temporarily, provide abundant food resources for flower-visiting insects (Diekötter, Peter, Jauker, Wolters & Jauker, 2014). For example, mustard fields (e.g., *Brassica campestris*, *Brassica juncea*, *Brassica napus*) are mass flowering crops that are quite attractive for a large diversity of generalist insects (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal, Amin, Rahman & Akanda, 2018; Mishra, Kumar & Gupta, 1988; Pudasaini, Thapa, Chaudhary & Tiwari, 2015; Stanley, Sah & Subbanna, 2017). As with most *Brassica* spp., the morphology of mustard flowers (open) makes it easy for multiple insects like flies (Diptera), butterflies (Lepidoptera), wasps and bees (Hymenoptera) to feed from them (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal et al., 2018; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017).

Therefore, even though mass flowering crops, such as mustard, may be pollinated by all of the insect groups mentioned above, they are particularly favoured by social bees, such as *Apis mellifera*, *A. cerana*, *A. dorsata* and *A. florea*, as they are dominant in such ecosystems once they nest or are managed near such crops (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal et al., 2018; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017). The solitary bees and, to a lesser extent, flies and butterflies, have a

similar role as *Apis*-bees (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal et al., 2018; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017). However, *Apis*-bees may forage over wider distances (revised by Abou-Shaara, 2014). They often require trees as nesting sites, but their nests have thousands of forage workers that commonly outnumber other bee species in flowers, and they can efficiently recruit (via their communication system) a large number of nestmates for resources of interest (Dornhaus, 2002).

The mustard (*Brassica campestris* L. var. toria) is a dominant winter season oilseed rape crop that occupies approximately 85% of the total oilseed rape area in Nepal (Basnet, 2005). However, its production has declined over the last years, compelling Nepal to import a huge quantity of oilseeds for domestic demand (Basnet, 2005). To overcome this situation, Nepalese farmers might be forced to adopt agricultural practices as expanding their cultivated areas to compensate for the unsatisfying productivity of mustard grains.

Since large agricultural areas may not be visited by enough pollinators, we hypothesize that the spatial extent of mustard crop fields, and specifically distance to forest fragments, will modify the diversity within the crop. To evaluate this premise, here, (i) we investigate whether the richness, abundance and species composition of flower-visiting insects in mustard crops vary with distance from natural vegetation. Furthermore, (ii) assuming that *Apis*-bees are the dominant taxon in mustard crops (see above), we predict that they will be more abundant, thereby allowing to differentiate between the insect communities found at the three distance classes and (iii) that they will be the major contributors to discriminating the different insect communities at every distance established below.

## Materials and methods

### Study location

Fieldwork was carried out in 2016 on the mass flowering crop mustard (*Brassica campestris* var. toria: Brassicaceae). We selected commercially growing areas of mustard crop in the months of December-January during the blossom period in four localities in Nepal: Bachauli, Gitanagar, Padampur, and Pragatinagar sharing a similar cropping pattern in vicinities of the Chitwan National Park. The farmers adopted the same agricultural practices for the cultivation of the mustard crops.

At each of the four localities, we selected a mustard crop field adjacent to a forest fragment and established a transect with three sampling quadrats. The first quadrat was located at a distance of 100 m from the forest fragment; the second quadrat was established 1100 m and the third quadrat 2100 m away from the forest fragment (see Appendix A: Fig. 1). Since we sampled in four localities  $\times$  three distances

within mustard crops, we obtained 12 sampling units. We repeated the insect sampling in these units three times during the main flowering period of the mustard. At each distance described above, quadrats were 1250 m<sup>2</sup> (50 m × 25 m) in size and used for sampling flower-visiting insects following a protocol to detect and assess pollination deficits in crops as suggested by the Food and Agriculture Organization of the United Nations (Vaissière, Freitas & Gemill-Herren, 2011), as described below.

## Insect sampling

Sampling began when more than 10% of the mustard crop had begun to bloom. In each quadrat, we sampled all insects visiting the mustard crops. In the 50 × 25 m area we used six 25-m-long, 2-m-wide transect walks, each 5 min long, totalling 150 m over 30 min (Vaissière et al., 2011). To quantify how many insects visited the target crop, we measured by scan sampling a fixed number of open floral units in each experimental unit. Five hundred flowers or flowering units of mustard crops were assessed by scan sampling, where an insect will be recorded or not depending on whether it is present at the time in a given flower is first seen, which is the most reliable way to assess the abundance of insects that reside on flowers (Levin, Kuehl & Carr, 1968; Westphal et al., 2008). The scan sampling was performed by walking slowly along each transect line and recording the numbers of flower-visiting insects seen when looking at the individual floral units one by one in sequence (Vaissière et al., 2011). We performed the transect walks between 09:00 and 16:00 h in the daytime when temperatures were at or above 15 °C, with no precipitation, dry vegetation and a low wind speed < 40 km/h, as recommended by Westphal et al. (2008). This one-day sampling effort was repeated three times for each study site during the flowering period of the mustard crop. Insects captured with aerial nets were pinned, labelled and taxonomically identified in the entomology laboratory by Prof. Dr. Resham Bdr Thapa of the Agriculture and Forestry University (Nepal).

## Statistical analysis

### Generalized linear mixed models

All statistical analyses were performed using R version 3.3.2 (R Core Team, 2016; Ihaka & Gentleman, 1996). We first evaluated the effect of distance (independent variable) on both the abundance and richness of insects (response variables) visiting flowers of mustard crops. For this analysis, we fitted two generalized linear mixed models (GLMMs). These models were chosen because our experimental design incorporates repeated measures, i.e., four localities where three repetitions were performed within every sampling unit. Consequently, the random effects were structured as crossed random effects, since they were spatial (localities) and temporal (repetitions).

Since the Poisson family distribution (link=log) is adequate to count data, both GLMMs (abundance, richness) were performed using the function *glmer* from the package ‘lme4’ (Bates, Mächler, Bolker & Walker, 2015). However, after testing for over-dispersion using the function *overdisp. glmer* in the package ‘RVAideMemoire’ (Hervé, 2015), we detected that the abundance (2.83), but not richness (0.33) was over-dispersed. We, therefore, re-run the abundance GLMM with negative binomial distribution. After that, we performed pairwise comparisons between the three distances using least-squares means in the function *lsmeans* from the package ‘lsmeans’ (Lenth, 2016).

### Accumulation curve and Hill numbers

We were also interested in evaluating whether our sampling effort was sufficient to observe as many insect species as possible that were visiting the flowers of the mustard crops. Thus, we computed a species accumulation curve and its respective interpolation/extrapolation of Hill numbers based on the sample size and the abundance of individuals, with diversity values ( $q=0$ ) estimated from Chao1 (Chao et al., 2014) after 2000 replications. This analysis was carried out using the individual-based abundance data in the function *iNEXT* from the package ‘iNEXT’ (Hsieh, Ma & Chao, 2019). Additionally, we calculated the extrapolated species richness from our data matrix to estimate the number of unobserved species. This estimate was performed using the function *specpool* from the package ‘vegan’. The extrapolated richness was calculated from Chao1 equations that gives more weight to the low abundance species to estimate the number of missing species (Chao et al., 2014).

While accumulation curve and number of unobserved species demonstrate whether our experimental design was suitable for capturing a wide diversity of insects within mustard crops, the traditional diversity indices help to compare the variety of organisms between groups. A manner to do this easily is plotting a diversity profile according to the diversity order of Hill numbers (Hill, 1973). This approach gives the possibility of comparing how insect diversity dynamically changes along scale parameters (Rényi diversity) between different communities (Chao et al., 2014). Thus, if diversity values on that scale are all higher for a specific community, then it is considered as more diverse than another (Tóthmérész, 1995). Additionally, such an approach allows us to extract some usual diversity indices from Hill numbers as richness [ $q=0$ ], Shannon-Wiener [ $q=1$ ] and Simpson [ $q=2$ ]. To perform such an analysis, we used our data matrix of flower-visiting insects to generate the diversity profile using the function *renyi* (Hill = TRUE) from the package ‘vegan’ (Oksanen et al., 2018).

### Non-metric multidimensional scaling

To assess the dissimilarity of the insect composition between the three different distances within mustard crops,

we performed an ordination analysis using non-metric multi-dimensional scaling (NMDS). Before performing this analysis, we standardized our data matrix using the function *decostand* (method = 'log'). Consequently, we used the Bray–Curtis dissimilarity index (which is appropriate for abundance data) using the function *vegdist*. Both the *metaMDS* and *stressplot* functions from the package 'vegan' were used to fit the NMDS and to find the goodness of fit measure. If the stress value was near or less than 0.2, we considered our NMDS adequate. However, to assess whether insect community compositions differed between the three distances while controlling for locality, we fitted a type II permutational analysis of variance (PERMANOVA) using the function *adonis.ii* from the package 'RVAideMemoire' (Hervé, 2015). Type II PERMANOVA calculates the sum of squares regardless of how the model terms are ordered, i.e. the order in which the individual terms are fitted will not matter as much as the interaction between them. After that, we performed pairwise comparisons between the three distances (2000 permutations, statistics test by the Pillai method and *p*-value adjustment after 'fdr') using the function *pairwise.perm.manova* from the package 'RVAideMemoire'.

### Similarity percentages

We also calculated the average individual contribution of sampled insect species to help discriminate between pairs of distances using similarity percentages (Simpser analysis). We ran 2000 repetitions using the function *simper* from 'vegan'. This function displays the most important species for each pair of groups (distances from forest fragments). Results suggest that these species must contribute at least 70% of differences between groups.

## Results

We sampled 2154 individuals from 23 species belonging to three insect orders: bees, wasps (Hymenoptera), flies (Diptera) and butterflies (Lepidoptera) – see Appendix A: Table 1. Interestingly, the abundance of solitary bees was 1.4-fold higher than that of social bees (see Appendix A:

Table 1). Overall, we demonstrate that the diversity of flower-visiting insects within mustard crops is not homogeneous.

Both abundance and richness of flower-visiting insects differed significantly between distances of 100 m and 2100 m, while communities recorded for the 1100 m quadrats were intermediate, without differing significantly from the other distances (negative binomial GLMM<sub>abundance</sub>,  $\chi^2 = 6.92$ , *p*-value = 0.03, Fig. 1A; GLMM Poisson<sub>richness</sub>,  $\chi^2 = 10.6$ , *p*-value = 0.004, Fig. 1B).

We found that our sample size (number of individuals) was satisfactory to collect as many insect species as possible with Chao1 estimation achieving 23 species (Fig. 2A). These data were consistent to show that the overall diversity of flower-visiting insects was highest near the forest fragments rather than far away from it (Fig. 2B). For example, some diversity indices that can be extracted from Hill numbers (richness [*q* = 0], Shannon-Wiener [*q* = 1] and Simpson [*q* = 2]) were always higher near to rather than far from forest fragments (Fig. 2B).

Accordingly, our ordination analysis was adequate to detect how the insect composition changes as the distance from the forest fragments increases (NMDS, stress = 0.22, non-metric fit [*R*<sup>2</sup>] = 0.94, Fig. 3). Thus, we had evidence that the insect compositions were significantly different each other mainly between the insect community nearest to the forest remnants (100 m) rather than furthest from it, i.e., 2100 m (PERMANOVA,  $F_{(1, 34)} = 3.43$ , *p*-value = 0.001, Table 1).

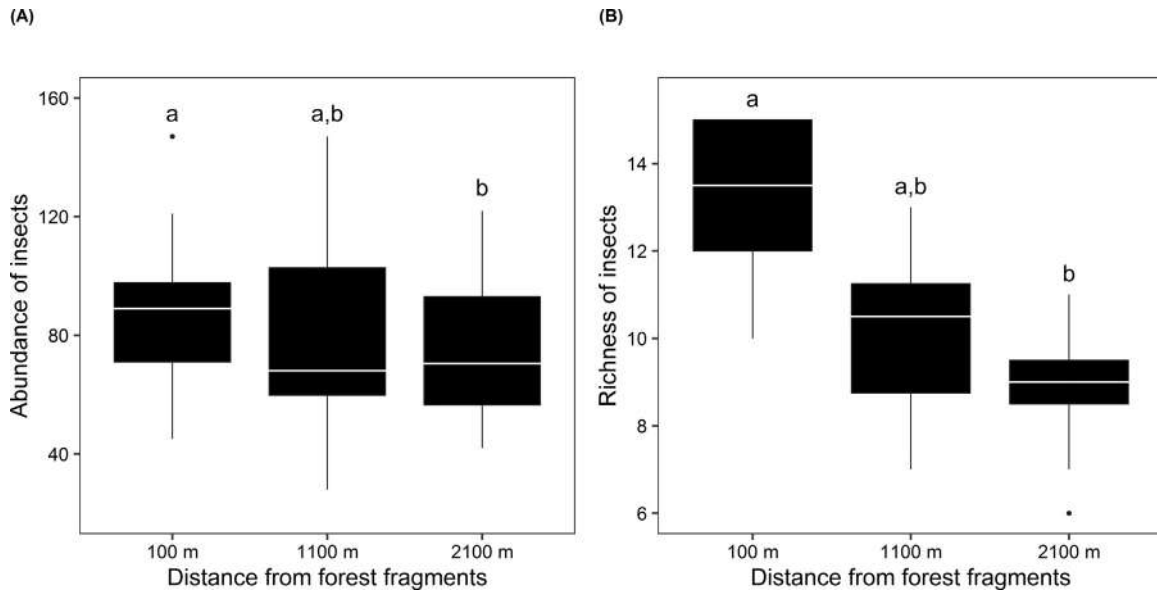
Finally, according to our SIMPER analysis, we detected substantial contributions of three solitary bee taxa (*Andrena* spp., *Halictus* spp. = underground nesting bees, *Megachile lanata* = aboveground nesting bees) plus four social bee species (*A. cerana*, *A. dorsata*, *A. florea*, *A. mellifera*) and one fly species (*Musca domestica*) characterizing (~70%) the insect communities at the three different distances within mustard crops in Nepal (Fig. 4, Table 2).

## Discussion

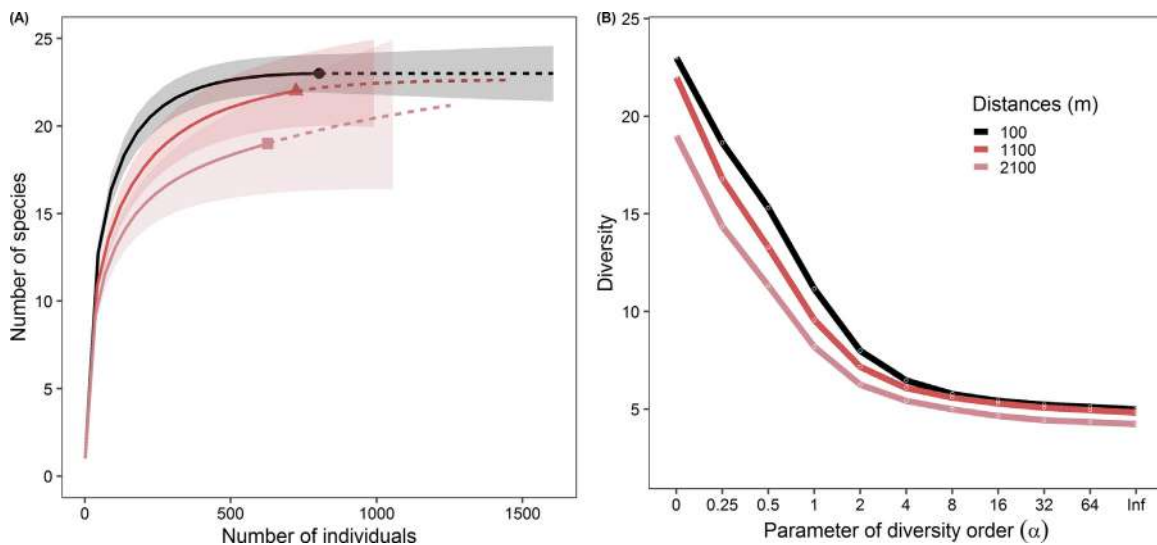
Our findings demonstrate that a large diversity of insects as bees and, to a lesser extent, flies and butterflies, visit the

**Table 1.** Results of the PERMANOVA evaluating the dissimilarity of the insect communities at three different distances (100 m, 1100 m and 2100 m) from forest fragments within mustard crops in Nepal.

	Sum of squares	Mean squares	Degree of freedom	F	<i>p</i> -value
Distances	0.20	0.20	1	3.43	< 0.001
Residuals	2.00	0.05	34		
Total	2.20		35		
<i>Pairwise comparisons between distances from forest fragments (p-values)</i>					
	<b>100 m</b>	<b>1100 m</b>	<b>2100 m</b>		
<b>100 m</b>	–	0.168	0.001		
<b>1100 m</b>	0.168	–	0.172		
<b>2100 m</b>	0.001	0.172	–		



**Fig. 1.** Effects of distance from forest fragments on the (A) abundance and (B) richness of potential pollinator insects on mustard crops.

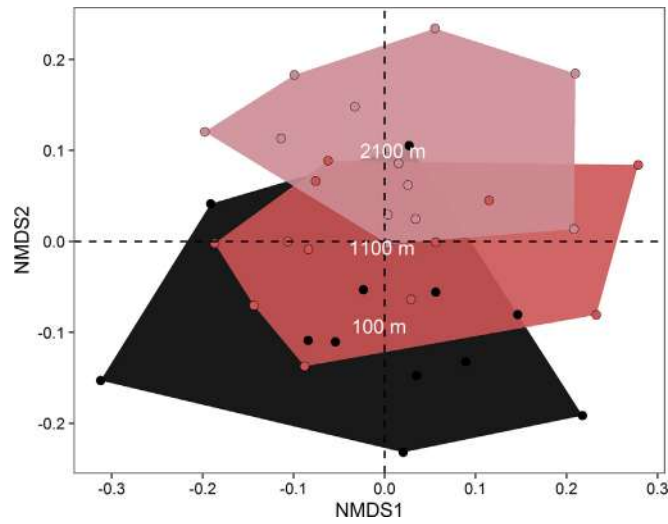


**Fig. 2.** (A) – Species accumulation curves: average number of species found and the number of expected species against average number of individuals by sampling unit. Interpolation (solid lines) and extrapolations (dashed lines). Note: The shadow indicates the confidence intervals (95%). (B) - Diversity profile of Hill numbers: insect diversity based on three distances from the forest on mustard crops in Nepal. The  $x$ -axis is the index of the Hill number, while the  $y$ -axis displays the level of diversity for each of the measures. When the profiles of two communities do not cross, we assume that the upper assemblage is more diverse than the lower. Left-hand side of the  $x$ -axis represents the rare species becoming more important, while the right-hand side indicates the abundance of bee species. The diversity indices on the  $x$ -axis are as follows: a) 0 = species richness; b) 1 = Shannon-Wiener index; c) 2 = Simpson index and; inf = Berger-Parker index.

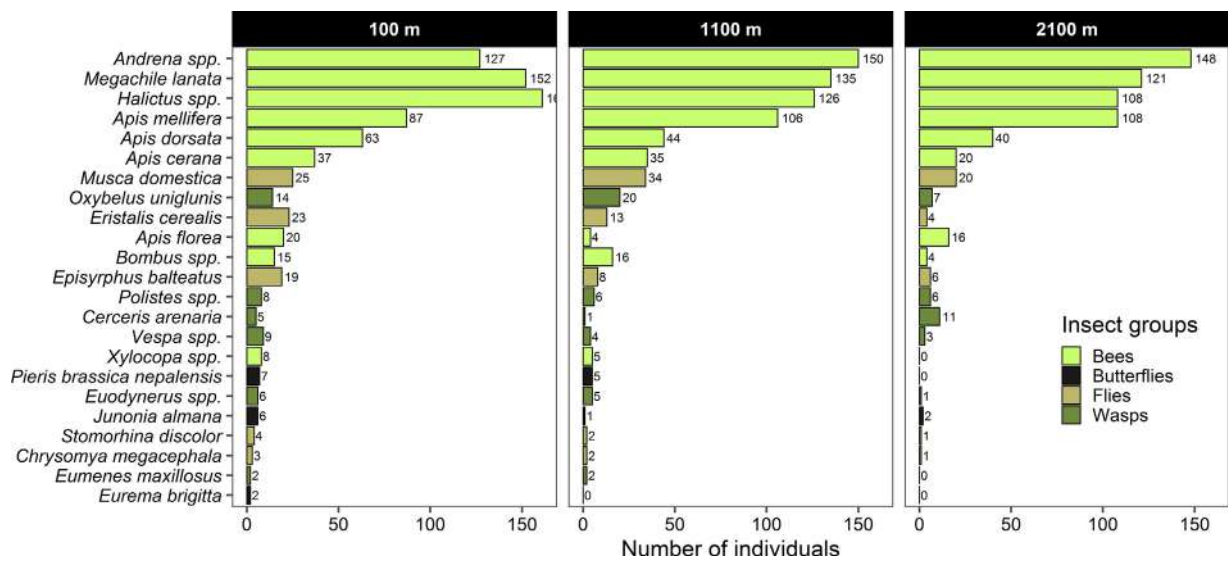
flowers of mustard crops in Nepal. Most bee taxa identified here are important pollinators of mustard crops such as *A. mellifera*, *A. cerana*, *A. dorsata* and *A. florea* and, to a lesser extent (abundance), *Andrena* spp., *Halictus* spp. and *Megachile* spp. (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal et al., 2018; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017). Flies and butterflies may also pollinate mustard crops, but their contribution is negligible (Bajjiya & Abrol, 2017; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017). However, contrary to these

studies suggesting that *Apis*-bees commonly dominate mustard fields, we observed that the three taxa of solitary bees (*Andrena* spp., mining bees; *Halictus* spp., sweat bees; *M. lanata*, leafcutter bees) were present in greater proportions than *Apis*-bees.

The dominance of *Apis*-bees, mainly *A. mellifera*, found in other studies seems to be attributable in part to the presence of hives near mustard crops (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal et al., 2018; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017). Nevertheless, in



**Fig. 3.** Non-metric multidimensional scaling: composition of flower-visiting insects on mustard crops at different distances from forest fragments. Note: Numbers inside the polygons indicate the distance from the forest fragments; points represent the sample units.



**Fig. 4.** Number of individuals by insect taxa caught at three distances from forest fragments in mustard crops.

**Table 2.** Cumulative contributions of the most important insect taxa in mustard fields, differentiating pairs of flower visitor communities at recorded at three distances from forest fragments in Nepal. Note: columns for each comparison represent the taxon names, the taxon’s individual contribution and the cumulative values.

100 m vs. 1100 m		100 m vs. 2100 m		1100 m vs. 2100 m	
<i>Andrena</i> spp.	0.14	0.14	<i>Andrena</i> spp.	0.15	0.15
<i>Apis mellifera</i>	0.14	0.28	<i>Apis mellifera</i>	0.14	0.29
<i>Halictus</i> spp.	0.11	0.39	<i>Megachile lanata</i>	0.12	0.41
<i>Megachile lanata</i>	0.11	0.50	<i>Halictus</i> spp.	0.11	0.52
<i>Apis dorsata</i>	0.10	0.60	<i>Apis dorsata</i>	0.09	0.61
<i>Apis cerana</i>	0.06	0.66	<i>Apis florea</i>	0.05	0.66
<i>Musca domestica</i>	0.05	0.71	<i>Apis cerana</i>	0.05	0.71
			<i>Apis mellifera</i>	0.18	0.18
			<i>Andrena</i> spp.	0.15	0.33
			<i>Halictus</i> spp.	0.10	0.43
			<i>Apis dorsata</i>	0.10	0.53
			<i>Megachile lanata</i>	0.08	0.61
			<i>Musca domestica</i>	0.07	0.68
			<i>Apis cerana</i>	0.05	0.73

our study, we did not evaluate the presence of apiaries around our sampling units. Thus, we can not assert if there would be any natural nest or any honeybee hives being managed near to the study areas. We can only affirm that the diversity (abundance, richness) of solitary bees outnumbered that of *Apis*-bees.

The experimental design of this study did not permit us to evaluate whether the species of solitary bees sampled here nest in the soil of mustard crops or in forest fragments. Soil-nesting bees, such as *Andrena* spp. and *Halictus* spp., forage near their nests because they do not fly farther than necessary, resulting in short foraging distances (Evertaars, Settele & Dormann, 2018). Similarly, *M. lanata* nests in stems, twigs, and existing cavities (Cane, 2003). Thus, we propose that these leafcutter bees need substrates found near forest fragments to nest. Therefore, we suggest that future studies assess the different functional diversities of solitary bees that occupy the agricultural landscapes of mustard in Nepal. Additionally, the substrates used by butterflies and flies to nest could be investigated since their low availability in or adjacent to the crops may restrain the presence of these insects in flowers due to their small flight ranges (Kendall et al., 2019).

Our data reinforce previous findings that the diversity of pollinator insects in crop fields is higher closer to forest fragments than far from them (Bailey et al., 2014; Blanche et al., 2006; Gutiérrez-Chacón, Dormann & Klein, 2018; Halinski et al., 2018; Hipólito et al., 2018; Ricketts, 2004). It suggests that the plants cultivated near and far of forest fragments may be differently pollinated. Therefore, regardless of the fact that mass-flowering crops attract large numbers of insects interested in their floral resources, the extension of crop fields may affect the insect diversity visiting them (Holzschuh et al., 2016; Krewenka, Holzschuh, Tschamtker & Dormann, 2011; Steffan-Dewenter, Münzenberg, Bürger, Thies & Tschamtker, 2002). As shown in this study, the sizes of *B. campestris* fields in Nepal allow most farmers to cultivate mustard as far as 2100 m from forest fragments. Thus, based on well-established literature that demonstrate that higher crop yields are achieved near natural vegetation patches due to larger pollinator diversity (Bailey et al., 2014; Halinski et al., 2018; Hipólito et al., 2018; Kleijn et al., 2015; Le Féon et al., 2013; Morandin & Winston, 2005), we assume that mustard plants located over such far distances are most likely facing a pollination deficit. Thus, the management of honeybee hives within crops could be thought as an alternative to supply higher pollination levels (Lindström, Herbertsson, Rundlöf, Smith & Bommarco, 2016).

The forest fragments may play an important role in conserving pollinator communities (Proesmans, Bonte, Smaghe, Meeus & Verheyen, 2018). Isolation and destruction of such places are among the major causes of the decline of the richness and abundance of pollinating insects across the globe (Winfrey & Kremen, 2009; Potts et al., 2010; Williams et al., 2010). Therefore, we recommend that

forest fragments adjacent to mustard crops in Nepal remain supported by local farmers and policy-makers.

In summary, while we confirmed that pollinator insect diversity and composition would be different (non-homogeneous) over different settled distances from the forest fragments within mustard crops, we revealed that solitary bees, such as *Megachile lanata*, *Halictus* spp. and *Andrena* spp., outnumbered the usually dominant *Apis*-bees. Therefore, even though *Apis*-bees commonly dominate *Brassica* spp. fields in Asian regions (Bajjiya & Abrol, 2017; Devi et al., 2017; Mandal et al., 2018; Mishra et al., 1988; Pudasaini et al., 2015; Stanley et al., 2017), we found that some solitary bee species can also be relevant in mustard crops in Nepal. As a result, we suggest that local farmers could modify their agricultural practices to preserve the regional insect diversity especially those bee species with solitary lifestyles.

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## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.baae.2020.05.005.

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