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ORIGINAL PAPER

Higher richness and abundance of flower-visiting insects close to natural vegetation provide contrasting effects on mustard yields

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Abstract

The conservation of pollinating insects in agriculture is a global concern since the diversity of such organisms may affect the productivity of pollination-dependent crops. In this study, we assessed (i) how distances from natural vegetation affect the diversity (guilds, richness, abundance) of flower-visiting insects within mustard crops in Nepal, (ii) how insect richness and abundance are related to mustard yields (weight of seeds) and (iii) the contribution of flower-visiting insects to mustard pollination by conducting pollinator exclusion experiments. To analyse these data, we carried out (i) hierarchical clustering followed by a Procrustes analysis as well as a generalized linear mixed model and (ii), (iii) linear mixed models. We found that the guild composition was not similar near and far from natural vegetation, indicating a tendency for *Apis* bees to displace in opposite directions relative to non-*Apis* bees. Nevertheless, while both richness and abundance were higher nearer natural vegetation, the former showed a stronger and more positive effect on mustard yields than the latter. Furthermore, we found that mustard flowers have a significant requirement for insect pollination since productivity increased by ~70% with insect visitation. Overall, our data suggest that the diversity of flower-visiting insects enlarges nearer to natural vegetation. However, the results indicate that species richness may be more relevant than abundance to mustard production. As such, we suggest that the maintenance of natural vegetation could be considered a strategy for ensuring the presence of multiple pollinator species within mustard fields to promote its long-term sustainability in Nepal.

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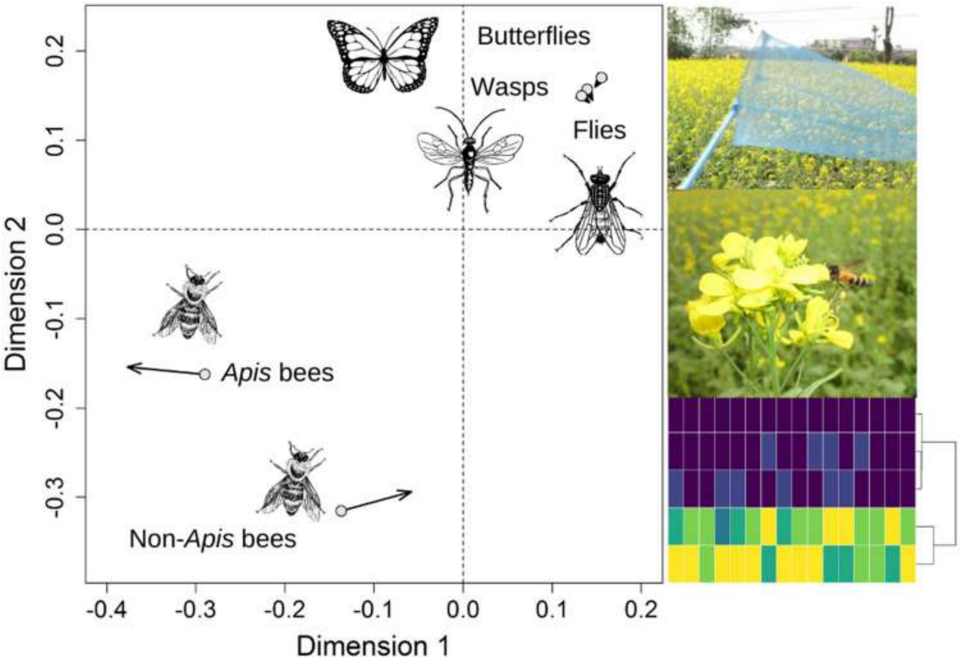
Graphic abstract



Keywords Bees · *Brassica campestris* · Crop system · Diversity · Habitat · Oilseed rape · Pollination · Production

Introduction

The global decline in pollinating organisms such as bees and other insects is of serious concern (Biesmeijer et al. 2006; Bartomeus et al. 2013; Koh et al. 2016), as 88% of wild angiosperm species rely on pollinators for sexual reproduction (Ollerton et al. 2011). The decline in pollinators may also negatively impact agriculture, as the productivity of 75% of crops worldwide benefits from this ecological service (Klein et al. 2007). As a result, the decline in pollinators may compromise both food security and the economy. The decrease in wild pollinator populations seems to be mainly attributable to anthropogenic disturbances or activities such as (a) climate change; (b) spread of pests and pathogens; (c) invasion by non-native species; (d) threats from agricultural development and intensification and particularly (e) land-use alteration leading to habitat loss, fragmentation and degradation (Steffan-Dewenter and Westphal 2008; Brown and Paxton 2009; González-Varo et al. 2013; Ollerton et al. 2014).

Some pollinator-dependent crops may benefit from their spatial proximity to natural areas (hereafter, natural vegetation) because they may provide a higher number of wild pollinators among other benefits (Garibaldi et al. 2011; Halinski et al. 2018; *but see* de Palma et al. 2016). However, if crop fields possess extensive cultivated areas, then the diversity of pollinators may gradually decrease with increasing distance from natural areas (Steffan-Dewenter et al. 2002; Ricketts et al. 2008; Garibaldi et al. 2011; Cariveau et al. 2013; Halinski et al. 2018). This asymmetry in pollinator distribution within crop fields results in lower pollination in some parcels and, in turn, a pollination deficit (Morandin and Winston 2005; Bailey et al. 2014; Bartomeus et al. 2014; Hipólito et al. 2018; Halinski et al. 2018). Therefore, a robust method to infer the importance of natural areas in enhancing agricultural productivity is to assess, for example, the pollinator diversity from the edge of natural vegetation to different distances within crop fields to evaluate whether the spatial distribution of beneficial insects is related to crop yields (Morandin and Winston 2005; Bailey et al. 2014; Bartomeus et al. 2014; Hipólito et al. 2018; Halinski et al. 2018).

In Nepal, Southeast Asia, oilseed mustard (*Brassica campestris* L. var. Toria: Brassicaceae) is a dominant winter crop (Basnet 2005). Currently, it occupies approximately 85% of the total oilseed rape cultivation area, and more than 80% of people in Nepal use mustard oil for cooking (Basnet 2005). The production of mustard in Nepal continues to increase each decade (Online Resource 1), and thus, it is necessary to consider the long-term sustainability of this crop, which requires a wide range of flower-visiting insects to enhance its pollination rate. The contribution of insects to pollinating *Brassica* spp. crops may reach 50% (Bommarco et al. 2012; Stanley et al. 2013; Garratt et al. 2014;

Lindström et al. 2016; Zou et al. 2017). Therefore, even though *Brassica* spp. crops may be self-pollinated or wind pollinated, insects seem to play an important role in boosting crop yields (Morandin and Winston 2005; Bommarco et al. 2012; Bartomeus et al. 2014).

The yellow flowers of *Brassica* spp. produce nectar and pollen and are highly attractive to most flower-visiting insects (Kunin 1993; Partap 1999). Several studies in Asian regions have found that the main pollinating insects of *Brassica* spp. are social bees such as *Apis mellifera*, *Apis cerana*, *Apis dorsata* and *Apis florea* and, to a lesser extent, solitary bee species such as *Andrena* spp., *Halictus* spp. and *Megachile* spp. (Mishra et al. 1988; Pudasaini et al. 2015; Bajiya and Abrol 2017; Stanley et al. 2017). The *Apis* bees may have some attributes not found in most non-*Apis* bees that seem to favour their greater presence in such crop systems. For example, (a) their nests are commonly populous and may shelter thousands of workers; (b) they may forage over wide distances even some kilometers; and (c) they have an efficient communication system able to recruit a large amount of foragers to mass-flowering plants (Dornhaus 2002; revised by Abou-Shaara 2014). Furthermore, honeybees such as *A. mellifera* can be managed into beehives, which may contribute to increasing their number on flowers (Mishra et al. 1988; Pudasaini et al. 2015; Bajiya and Abrol 2017; Stanley et al. 2017). Other insects, such as butterflies, flies and wasps also forage on *Brassica* flowers, but their contribution to boosting yields is negligible (Mishra et al. 1988; Pudasaini et al. 2015; Bajiya and Abrol 2017; Stanley et al. 2017). The diversity of these insects within such crops may be raised if attributes such as restricted use of pesticides, wide availability of food and nesting resources and large-scale landscape (3 km) heterogeneity are present in these areas (Samnegård et al. 2016; Landaverde-González et al. 2017; Theodorou et al. 2017; Stiles et al. 2019).

Since the proximity to natural vegetation and its putative higher diversity of insects beneficial to crop fields is questionable (e.g. Garibaldi et al. 2011; de Palma et al. 2016; Halinski et al. 2018), more studies are needed to disentangle this issue. Thus, using mustard fields in Nepal as a model, in the current study we evaluated: (i) whether two different distances from the edge of natural vegetation affect the diversity (guild composition, richness, abundance) of flower-visiting insects within mustard crops; (ii) how insect richness and abundance are related to mustard yields (weight of seeds); and (iii) the contribution of flower-visiting insects to mustard pollination by conducting pollinator exclusion experiments. We hypothesized that proximity to natural vegetation may alter the composition of guilds and positively influence the richness and abundance of flower-visiting insects within mustard crops. Furthermore, we predicted that the weight of mustard seeds would increase with the richness and abundance of flower-visiting insects and, finally, that the presence of flower visitors will increase mustard yields demonstrating their efficiency as pollinators.

Materials and methods

Study area

We carried out flower-visiting insect sampling and measured the effect of pollination on oilseed rape mustard crops in the Chitwan (27°35′N 84°30′E) and Nawalparasi (27°32′N 83°40′E) districts of Nepal. The selected study sites are well known for their production of oilseed mustard crops. They lie in the tropical zone and are characterized by similar topographies, vegetation, and agricultural landscapes and practices.

We selected eight mustard fields in the two districts mentioned above with a completely randomized design using two distances from the natural vegetation (i) four near the forest (100 metres) and (ii) four far from the forest (3 km), both within mustard crops (Fig. 1). Since most wild bees have a maximum foraging range under three kilometres (Greenleaf et al. 2007), this latter distance was chosen to avoid as much spatial pseudoreplication among sample units as possible. Within each of the eight mustard fields, we established a 50 m × 25 m study area with homogeneous and continuous crop cover according to the protocol for assessing pollination deficits within crop fields (Vaissière et al. 2011).

Insect sampling

In each of the eight mustard fields, we assessed the species richness and abundance of flower-visiting insects. For insect sampling, we used transect walks with sweep nets (Fig. 1) through six 25 m transect lines for 5 min per line for a total of 30 minutes (Vaissière et al. 2011). Then, we recorded the number of visiting insects in an individual floral unit, defined here as five hundred flowers as suggested in a specific protocol (Vaissière et al. 2011). Thus, to establish such floral units within mustard crops, they were assessed using a scan sampling technique, which is the most reliable way to assess pollinator density on flowers (Levin et al. 1968); the units were assessed in sequence on each transect line by walking slowly along it (Vaissière et al. 2011). We performed transect walks between 0900 and 1600 h on days with temperatures at or above 15 °C, with no precipitation, dry vegetation, and low wind speed (< 40 km/h) (Westphal et al. 2008). Insect specimens were pinned, labelled, and subsequently identified to the genus and species levels in the entomology laboratory at Agriculture and Forestry University, Nepal.

Therefore, our sampling effort comprised insect sampling in eight mustard fields (four near the forest = 100 metres; four

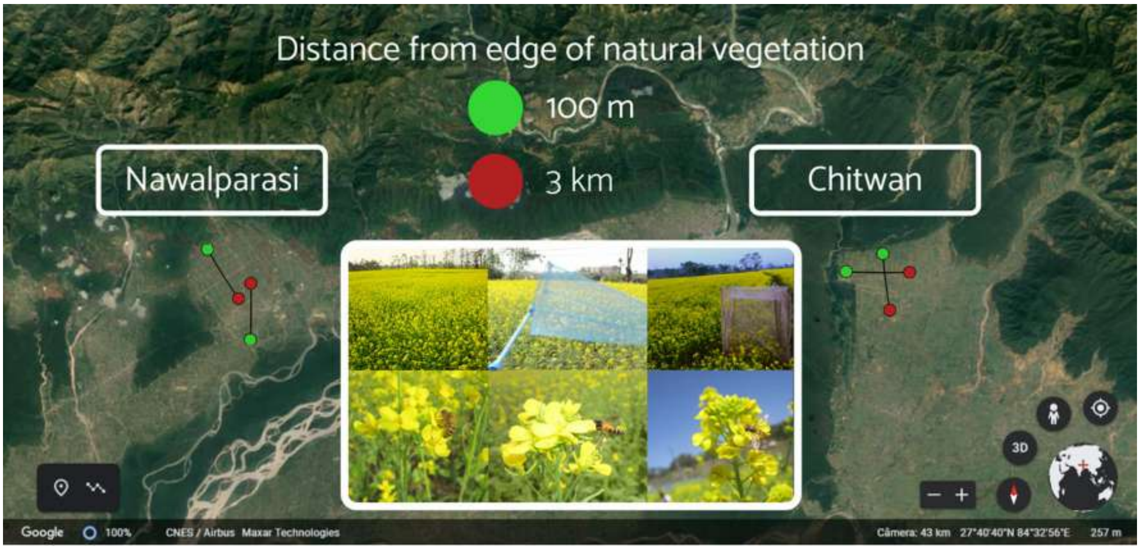


Fig. 1 Study areas (Nawalparasi and Chitwan) showing sampling sites in oilseed rape mustard (*Brassica campestris* var. Toria) fields in Nepal. Inner plot depicts from left to right: *upper* (i) blossoming mustard field, (ii) the harvesting of flower-visiting insects using a sweep net and (iii) a closed cage being installed for the pollinator exclusion experiment; *lower* (iv) an *Apis mellifera* worker, (v) an *Apis dorsata* worker and (vi) a hoverfly. Map source: Google Earth (https://earth.google.com/web/)

far from the forest = 3 km) from two districts where we performed two repetitions totalling 32 sampling units during the main flowering period of the target crop.

Pollinator exclusion experiments and crop yield analysis

In each of the eight mustard fields, we demarcated an experimental area with four divisions. Each division had two treatments, and five tagged contiguous plants were monitored per treatment. The first treatment (open cages) was open pollination, in which all flowers of the mustard plants were accessible to autonomous self-pollination, wind pollination and insect pollination. In the second treatment (closed cages), a plot (1 × 1 m) with mustard plants was covered with a nylon mosquito net before the onset of flowering. In the closed cage, all flowers were exposed to wind pollination and self-pollination, such that the difference between the first and second treatments represented the real contribution of insect pollination. After blooming was finished, we removed the cages and left the fruits to ripen.

After harvesting and threshing, we recorded the agronomic units (= total seed weight [g] per plant) within both open and closed cages to attain a more precise level of pollination in both experiments. The crop yields as a function of pollination level were compared by using the following formula (Vaissière et al. 2011, p. 11):

$$Y = F(X) + A$$

where Y is the total crop yield measured in agronomic units, F(X) is the yield resulting from the level of pollination in the pollinator exclusion treatment, and A is the yield resulting from the open experiment, with both X and A measured in the same unit as Y (Vaissière et al. 2011, p. 11).

Data analysis

Insect diversity: Guild composition, richness and abundance

The sampled insect community was grouped into four groups: bees, butterflies, flies and wasps. However, since honey bees most often dominate *Brassica* crops (as previously indicated), we subdivided bees into (a) *Apis* bees belonging to this genus and (b) non-*Apis* bees (all other bee taxa), as we shall reference later. Then, we performed two hierarchical clustering analyses to evaluate the similarity in the structure of insect guild composition between the nearer distances (100 m) and those farther (3 km) from natural vegetation. Both matrices were scaled and centred to zero mean and unit variance using the function *decostand* (method = standardize) of the 'vegan' package (Oksanen et al. 2018). After that, they were transformed into Euclidean distance matrices using the unweighted pair group method with arithmetic mean (UPGMA) as a method of hierarchical clustering. The results were plotted as clustered heatmaps using the 'pheatmap' package (Kolde 2019). The goodness-of-fit of the resulting dendrograms was evaluated with cophenetic a correlation index using the functions *cophenetic* and *cor* in R.

The congruence between both matrices was assessed with a Procrustean superimposition approach (Gower 1971). This analysis is a least-squares orthogonal mapping useful to compare two multivariate sets of data in which the ordination is scaled and rotated to find an optimal superimposition that maximizes its fit (Gower 1971; Peres-Neto and Jackson 2001). As a result, the sum of the squared residuals between configurations in their optimal superimposition is used as a degree of concordance, i.e., M^2 (Gower 1971). This metric of association varies from 0 to 1, where lower values of M^2 indicate greater concordance among configurations (Peres-Neto and Jackson 2001). Consequently, if both matrices are similar to each other the points in the rotated configuration should be as close as possible in the same subspace (i.e., small length of residuals). Since Procrustes analysis exhibits the corresponding displacement between target and rotated matrices, we considered the direction of residuals to infer the trajectories of guilds if near or far from natural vegetation. The congruence between both matrices was tested with a permutation test (protest). We used the functions *procrustes* and *protest* (1999 permutations), both in the 'vegan' package (Oksanen et al. 2018) for these analyses.

To evaluate how the distance to the edge of natural vegetation (near = 100 m; far = 3 km) affects the diversity (richness, abundance) of flower-visiting insects within mustard crops in Nepal, we fit two generalized linear mixed models (GLMMs) with the Poisson distribution family (link = log). Since we repeated the insect sampling at both study sites, i.e., the selected districts (Chitwan and Nawalparasi), the structure of our data included repeated measures. Therefore, the variable "districts" was included as a random effect in both GLMMs. The models above were fit using the function *glmer* in the package 'lme4' (Bates et al. 2015).

On the other hand, to assess how the richness and abundance of flower-visiting insects and the interaction between them affect mustard yields (total weight of seeds), we fitted a linear mixed model (LMM). Since districts (Chitwan and Nawalparasi) and distances (near = 100 m; far = 3 km) might have their own environmental particularities that could improve model fit, but assuming that its underlying variables were not harvested here, they were included as crossed random effects. This model was also fit using the function *lmer* in the package 'lme4' (Bates et al. 2015).

Finally, we analysed the importance of flower-visiting insects to mustard pollination (pollinator exclusion experiments) by comparing yields (total weight of seeds) in open cages with those in closed cages using LMM. Again, both districts and distances were included as crossed random effects in the model. This analysis was performed using the function *lmer* in the package 'lme4' (Bates et al. 2015). The LMM was chosen after evaluating assumptions of normality of residuals using the function *shapiro.test* and homogeneity of variances using the function *leveneTest* in the package 'car' (Fox and Weisberg 2011). Since the residuals of the LMM presented normality ($W = 0.97$, p value = 0.18) and equal variances ($F_{(1,62)} = 0.30$, p value = 0.57), this model was kept. The goodness-of-fit of all GLMM and LMM were analysed with a coefficient of determination (R^2) that was computed by applying a standardized generalized variance approach using the function *r2beta* of the 'r2glmm' package (Jaeger 2017). All analyses were carried out in the statistical programming language R (Ihaka and Gentleman 1996; R Core Team 2016).

Results

Flower-visiting insect composition, richness and abundance

In total, we sampled 1986 flower-visiting insects ($n = 1{,}046$ at 100 m; $n = 940$ at 3 km) from 24 taxa within mustard crops belonging to four taxonomic groups: bees ($n = 9$ spp.), butterflies ($n = 4$ spp.), flies ($n = 4$ spp.) and wasps ($n = 7$ spp.) (Fig. 1d–f for examples, Table 1).

The structuring pattern of the flower-visiting insect community between near (100 m) versus far (3 km) distances from natural vegetation clustered bees (*Apis* and non-*Apis*) closer to each other, whereas butterflies, flies and wasps formed another group (Fig. 2a). Both coefficients of cophenetic correlation were equal, i.e., 0.98, indicating that dendrograms exhibited substantial two-dimensional representation of the calculated distances. However, the Procrustes analysis did not demonstrate congruence between both community matrices, suggesting a mismatch in the pattern observed for guilds at 100 m compared to those 3 km away from natural vegetation ($M^2 = 0.02$, correlation = 0.98, $p > 0.05$, Fig. 2b). While the low M^2 would indicate a similarity between two configurations and high correlation, the non-significance of this analysis suggests that some displacements of guilds were larger than expected by chance (Fig. 2b). Therefore, there seems to have been considerable and opposite displacements exhibited by guilds of *Apis* bees vs non-*Apis* bees (Fig. 2b). On the other hand, butterflies, flies and wasps presented nearly no movement.

Our findings demonstrate that the richness and abundance of insects were significantly higher close to the edge of natural vegetation than farther from natural vegetation (Fig. 3a and b). We found that close to natural areas, the richness ($n = 14$) of flower-visiting insects was significantly higher than that farther from such areas ($n = 11$ species), as predicted by our model (GLMM Poisson; estimate = 0.21, z value = 2.21, p value = 0.02, $R^2 = 0.28$, Fig. 3a). Similarly, the abundance of flower-visiting insects within mustard crops was higher

Table 1 Insect species identified visiting flowers of oilseed rape mustard (*Brassica campestris* var. Toria: Brassicaceae) in Nepal, South Asia.

Species	Order, family	Guilds	Amount at:		Proportion of drop or rise
			100 m	3 km	
Bees					
1. *Apis cerana*	Hymenoptera, Apidae	*Apis* bees	87	107	↑ 9%
2. *Apis dorsata*	Hymenoptera, Apidae	*Apis* bees	56	28	
3. *Apis florea*	Hymenoptera, Apidae	*Apis* bees	145	134	
4. *Apis mellifera*	Hymenoptera, Apidae	*Apis* bees	127	186	
		Subtotal:	415	455	
5. Bombus spp.	Hymenoptera, Apidae	Non-*Apis* bees	11	0	↓ 19%
6. Andrena spp.	Hymenoptera, Andrenidae	Non-*Apis* bees	137	176	
7. *Halictus* spp.	Hymenoptera, Halictidae	Non-*Apis* bees	147	82	
8. *Megachile* spp.	Hymenoptera, Megachilidae	Non-*Apis* bees	139	95	
9. *Xylocopa* spp.	Hymenoptera, Apidae	Non-*Apis* bees	18	16	
		Subtotal:	452	369	
Butterflies					
10. *Danaus chrysippus*	Lepidoptera, Nymphalidae	Butterflies	7	6	↓ 41%
11. *Eurema brigitta*	Lepidoptera, Pieridae	Butterflies	13	10	
12. *Pieris brassicae nepalensis*	Lepidoptera, Pieridae	Butterflies	14	0	
13. *Junonia almana*	Lepidoptera, Nymphalidae	Butterflies	3	3	
		Subtotal:	37	19	
Flies					
14. *Chrysomya megacephala*	Diptera, Calliphoridae	Flies	7	4	↓ 34%
15. Hoverflies	Diptera, Syrphidae	Flies	29	15	
16. *Musca domestica*	Diptera, Muscidae	Flies	33	27	
17. *Stomorhina discolor*	Diptera, Rhiniidae	Flies	9	6	
		Subtotal:	78	52	
Wasps					
18. *Athalia lugens*	Hymenoptera, Tenthredinidae	Wasps	6	0	↓ 30%
19. *Cerceris arenaria*	Hymenoptera, Crabronidae	Wasps	12	5	
20. *Eumenes maxillosus*	Hymenoptera, Vespidae	Wasps	7	0	
21 *Evania appendigaster*	Hymenoptera, Evaniidae	Wasps	3	2	
22. *Oxybelus uniglumis*	Hymenoptera, Crabronidae	Wasps	22	19	
23. *Polistes* spp.	Hymenoptera, Vespidae	Wasps	8	12	
24. *Vespa* spp.	Hymenoptera, Vespidae	Wasps	6	7	
		Subtotal:	64	45	
		Total:	1046	940	

[§] Distances from natural vegetation and number of sampled individuals per taxa

nearer to natural areas (*n* = 281 individuals; median) than farther from such areas (*n* = 209 individuals), as predicted by our model (GLMM Poisson; estimate = 0.11, z value = 5.17, *p* value < 0.001, $R^2 = 0.38$, Fig. 3b).

Effect of flower-visiting insects on mustard yields

Overall, our model had a moderate power of explanation ($R^2 = 0.46$). However, each variable (richness, abundance) individually showed a different slope and effect. Thus, richness had a positive effect (LMM; $\chi^2 = 24.6$, *p* value < 0.001, $R^2 = 0.25$), while abundance had a negative but negligible effect on mustard crop yields (LMM; $\chi^2 = 4.88$, *p* value = 0.02, $R^2 = 0.01$), as shown in Fig. 3c and d. By contrast, we did not find a significant effect of the interaction between richness and abundance of flower-visiting insects on mustard yields (LMM; $\chi^2 = 0.20$, *p* value = 0.65).

Our data also indicated that mustard plants in the field condition, i.e., not caged (free access to flower-visiting insects), had higher yields (weight of seeds) than netted plants (closed cages) with restricted insect visitation (LMM, estimate = 23.2, $R^2 = 0.42$, t value = 6.95, *p* value < 0.001,

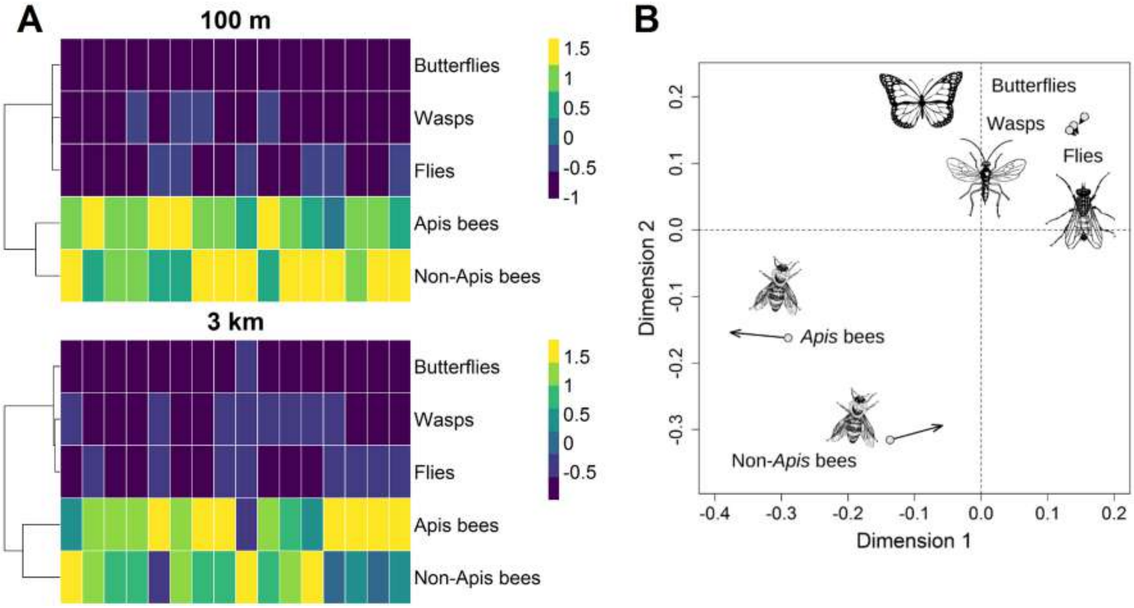


Fig. 2 **a** Clustered heatmaps based on the number of guilds of insects sampled within mustard fields at 100 m (near) and 3 km (far) from natural vegetation. The hierarchical cluster analysis was performed with the UPGMA method and Euclidean distance measure. **b** Procrustean superimposition analysis exhibiting the lack of concordance ($M^2 = 0.02$, correlation = 0.98, $p > 0.05$) between both matrices (near = target; far = rotated). Notes: in B, points indicate the position of the guilds of insects in the first clustering ("100 m"), while arrowheads point out the corresponding displacement towards the "3 km" rotated matrix. The lengths of residuals (i.e., straight lines) indicate how closely matched both configurations are after optimal fit

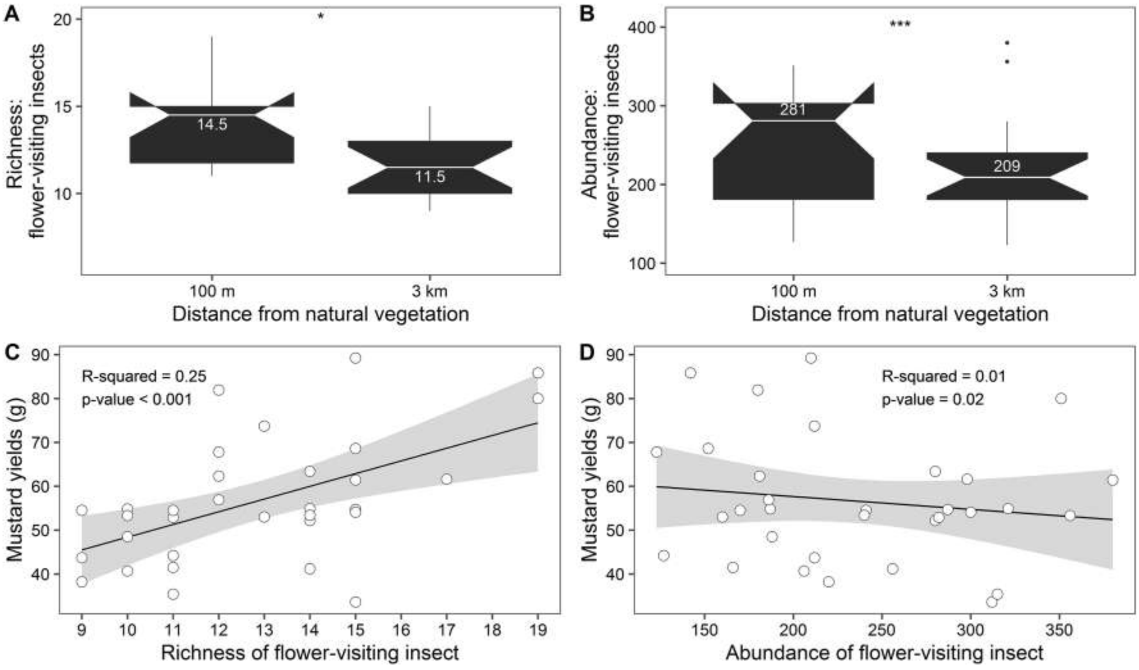


Fig. 3 Richness (**a**) and abundance (**b**) of insects within mustard crops at different distances from natural vegetation. Effect of flower-visiting insect richness (**c**) and abundance (**d**) on mustard crop yields. Note: *box* = 1st and 3rd quartiles, *whiskers* = the minimum and maximum range of variation, *median* [white line] = 2nd quartile, *notches*= the confidence interval around the median. *p* values: *< 0.05, ***< 0.001. White numbers in boxes indicate the median value. *Dots* are observed values, and *solid lines* show the model-predicted fits with 95% confidence intervals (shaded areas, dashed lines)

Fig. 4). Thus, the real contribution to mustard pollination ascribed to flower-visiting insects is 69.4% (56.6 g; median) for mustard flowers exposed to local entomofauna compared with flowers that do not receive such visits (33.4 g; median, Fig. 4).

Discussion

Insect diversity within mustard crops

We found that the proximity of natural vegetation surrounding mustard fields had a positive effect on flower-visiting insect diversity (richness, abundance) compared with those at farther distances. Our results suggest that there may be a decline in pollinating insect diversity within crop fields with increasing distance from the edge of natural vegetation as observed in other studies (Morandin and Winston 2005; Garibaldi et al. 2011; Bailey et al. 2014; Bartomeus et al. 2014; Halinski et al. 2018; Hipólito et al. 2018). However, this reduction in the diversity of beneficial insects far from natural vegetation may not be found anywhere since each place has its own local particularities (de Palma et al. 2016).

Our results indicate that the surrounding natural areas seem to be a relevant driver that sustains the richness and abundance of native pollinators within crop systems (Kremen et al. 2004; Fabian et al. 2013; Forrest et al. 2015). Commonly, natural areas provide key resources to pollinator populations, such as perennial forage areas, nesting substrates and breeding sites, which are otherwise unavailable in or temporarily offered by crop fields (Steffan-Dewenter and Tscharntke 1999; Chacoff and Aizen 2006; Ricketts et al. 2008; Knight et al. 2009; Halinski et al. 2018). The forest edge provides habitats to pollinators, creating nesting sites for cavity nesting and ground nesting bees, which increase the pollination of mass flowering crops (Le Féon et al. 2011). The forest edge provides a diversity of floral resources throughout the bee activity period (Bailey et al. 2014). Therefore, it would be expected that pollinator diversity declines within crop fields whose cultivated plants are more isolated from natural habitats because most insects have a limited flight range (Greenleaf et al. 2007).

However, contrary to other studies carried out within *Brassica* spp. crops in Asian regions (Mishra et al. 1988; Pudasaini et al. 2015; Bajiya and Abrol 2017; Stanley et al. 2017), our data suggest that honey bees may not be the dominant pollinators in mustard fields, at least for those observed here. For example, *Apis* bees (*A. mellifera*, *A. cerana*, *A. dorsata*, *A. florea*) have been identified as the dominant taxonomic group of insects (> 80%) in similar crops while non-*Apis* bees such as *Andrena* spp., *Halictus* spp. and *Megachile* spp. have been observed to a lesser extent or in negligible quantities (Mishra et al. 1988; Pudasaini et al. 2015; Bajiya and Abrol 2017; Stanley et al. 2017). Conversely, our data indicate that, as a whole, both guilds of bees were represented at comparable quantities, but the number of *Apis* bees tended to increase far away from natural vegetation, while the number of non-*Apis* bees decreased at the same spatial distances. This fact may be due to, among other things, *Apis* bees being able to forage hundreds or even thousands of metres from their nests (Dornhaus 2002; revised by Abou-Shaara 2014). On the other hand, non-*Apis* bees such as *Andrena* spp., *Halictus* spp. and *Megachile* spp. are expected to forage over short distances since their small body size and/or solitary lifestyle may restrain their flight range (Greenleaf et al. 2007; Everaars, Settele and Dormann 2018).

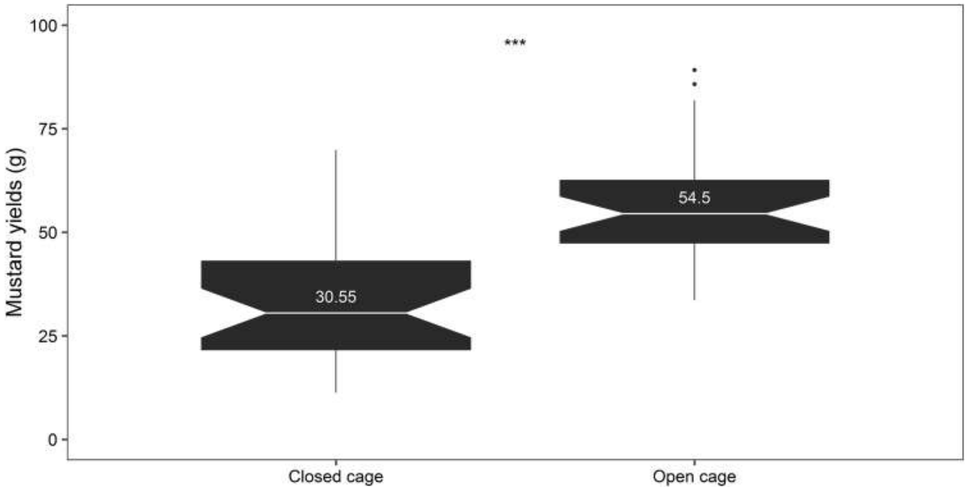


Fig. 4 Pollinator exclusion experiments evaluating the real contribution of flower-visiting insects to mustard pollination. Note: *box* = 1st and 3rd quartiles, *whiskers* = the minimum and maximum range of variation, *median* [white line] = 2nd quartile, *notches*= confidence interval around the median. *p* values: ***< 0.001. White numbers in boxes indicate the median value

Insect diversity and its contribution to mustard yields

The richness and abundance of pollinators are two diversity indices that are widely used by researchers to investigate how agriculture yields shift with changes in the variety and quantity of such organisms at spatial scales within pollination-dependent crops (Kremen et al. 2004; Morandin and Winston 2005; Garibaldi et al. 2011; Bommarco et al. 2012; Bailey et al. 2014; Bartomeus et al. 2014; Zou et al. 2017; Halinski et al. 2018; Hipólito et al. 2018). In our study, we found that while both richness and abundance of flower-visiting insects had significant effects on mustard yields, the former index had a more predictive power ($R^2 = 0.25$) than the latter ($R^2 = 0.01$).

Even though the predictive power of the abundance of flower-visiting insects was negligible, as shown above, this model had a negative effect on mustard yields, suggesting that a larger quantity of such organisms is not necessary to increase mustard production. Many studies have shown that the richness and abundance of pollinators can have either converging or contrasting effects on crop yields (Klein et al. 2003; Kremen et al. 2004; Morandin and Winston 2005; Zou et al. 2017; Bommarco et al. 2012; Bartomeus et al. 2014; Landaverde-González et al. 2017; Halinski et al. 2018). These studies suggest that a wider variety (richness) and quantity (abundance) of such organisms at a given spatial scale do not necessarily correspond to higher production (Klein et al. 2003; Morandin and Winston 2005; Kremen et al. 2004; Bommarco et al. 2012; Bartomeus et al. 2014; Zou et al. 2017; Landaverde-González et al. 2017; Halinski et al. 2018). In other words, depending on locality and the cultivated plant species, only the richness but not the abundance of pollinating insects may have a positive effect on the final productivity of target crop. Our study suggests that while both richness and abundance of flower-visiting insects were higher near natural vegetation, the latter diversity measure, even significant, was not reliable since it fails to accurately explain the variability of the mustard yields.

We believe that the richness of flower-visiting insects had a greater effect (positive) on mustard production than the corresponding abundance of such organisms because each species possesses behavioural particularities that could optimize the chances of mustard flowers to be adequately pollinated. It is known that different species of pollinators may show different behaviours when visiting the same flowers (forage period, time per visit, floral resources harvested, stigma contact and so on), which may augment the pollination rate due to complementary or synergistic activities of such organisms (Rader et al. 2009; Brittain et al. 2013; Witter et al. 2015). Nevertheless, the abundance of pollinators in similar situations does not necessarily contribute to increased crop yields in a linear way. Plants have pollen limitations; for example, the density and visitation rate may restrain pollen deposition on the stigma (Morandin and Winston 2005; Morris et al. 2010; Rogers et al. 2014; Garibaldi et al. 2020). Since mustard plants greatly benefited from the presence of flower-visiting insects, we assume that the abundance of such organisms in the studied fields may not have been a limiting factor to enhance pollination, while richness seems to have better predictive power to explain the positive effects on the weight of mustard seeds.

Finally, in our study we found that the presence of flower-visiting insects accounted for ~ 70% of the increase in the weight of mustard seeds. Such a proportion ascribed to the majority of organisms in the wild is relevant for at least two reasons. First, if a single pollinator species is allowed to access mustard flowers (e.g., beehives of *Apis mellifera*), then the weight of seeds drops to 45% (Devkota et al. 2016). This is not a small value, but compared to the pollination service naturally provided by this species plus the other flower-visiting insects identified here, mustard production may still be more enhanced. Second, some studies have found that multiple pollinating insects may contribute differently to enhance yields (e.g. 18 to 71%) depending on the crop (Bartomeus et al. 2014; Kleijn et al. 2015; Landaverde-González et al. 2017). Accordingly, in the current work this contribution was elevated demonstrating that if mustard crops are well managed the community of flower-visiting insects will provide a sufficient pollination rate.

Conclusion

The mustard crop fields in Nepal may receive a great variety of flower-visiting insects, such as bees, butterflies, flies and wasps. If bees are subdivided into groups as *Apis* bees vs. non-*Apis* bees it is possible to observe a pattern opposite in community structure with *Apis* bees being more representative far away from natural vegetation, while non-*Apis* bees tend to be more widely observed near natural vegetation. As the richness and abundance of flower-visiting insects were higher close to natural vegetation, but only the former had a great and positive effect on the weight of mustard seeds, the maintenance of the diversity of these organisms within mustard crops should be encouraged since they contribute to an increase of ~ 70% in the weight of seeds. To promote this, we suggest that large natural areas surrounding mustard crops continue to be conserved and that pollinator-friendly practices such as offering nest substrates and cultivating a wider variety of plants consortiated with mustard could be adopted by Nepalese farmers. We believe that such practices have the potential to keep mustard production sustainable for the long term in Nepal.

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Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

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