

POLLINATORS AND CROPS IN BHUTAN: INSECT ABUNDANCE IMPROVES FRUIT QUALITY IN HIMALAYAN APPLE ORCHARDS

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Abstract—Apples are one of the most important global crops that relies heavily on insect pollination, which has been shown to increase apple production and value. However, recent reports indicate that apple production has been declining in certain regions, including in Bhutan. One of the potential causes of declining production are pollination deficits driven by a low abundance and diversity of pollinators, a phenomenon that has received little attention in Bhutan to date. Here, we present the first study examining the diversity of flying insects in Bhutanese apple orchards in relation to apple quality. During the apple flowering season, 1,006 insects comprising 44 unique (morpho-)species from the orders Hymenoptera, Diptera, and Lepidoptera were recorded using a standardized method of passive and active trapping within nine different orchards in Thimphu, Paro, and Haa districts, in the western part of Bhutan. During the harvest season, 495 apples were collected from these nine orchards, and we measured five different parameters; weight, size, sugar concentration, seed number, and malformation score. The most dominant flower visitors across all orchards were honey bees (mostly *Apis mellifera*, followed by *A. cerana* and *A. dorsata*). Orchards with a higher abundance of flying insects (both managed and wild) had better apple quality (weight, size and sugar concentration). Contrary to reports from other regions of the world, flower visitor diversity did not correlate with the quality of the apples. This represents the first study reporting on apple pollination in Bhutan and highlights the importance of pollinators in apple production and reinforces the need to develop pollinator friendly practices to ensure sustainable apple production in Bhutan.

Keywords—Biodiversity; agroecology; community composition; Hymenoptera; Diptera; Lepidoptera

Journal of Pollination Ecology,
31(5), 2022, pp 39-52

DOI: [10.26786/1920-7603\(2022\)670](https://doi.org/10.26786/1920-7603(2022)670)

Received 12 October 2021,
accepted 05 May 2022

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INTRODUCTION

Pollination is an important natural process that is necessary for maintaining wild plant diversity and in providing vital ecosystem services to agriculture (Ollerton 2017). An estimated 75% of the world crops require animal-mediated pollination (Klein et al. 2007), and our reliance upon pollinator-dependent crops is increasing since the onset of the 21st century (Aizen et al. 2019). Insects are by far the most dominant pollinators, contributing between US\$ 235 and US\$

577 billion to the world agricultural crops economy annually (Potts et al. 2016).

Apple is one of the most important global, pollination-dependent fruit crops both in terms of yield and economic value (FAO 2020), with insect-dependency rate typically between 80-100% (Hein 2009). As in most other pollinator-dependent crops, insect-assisted pollination increases apple production, economic value, and fruit quality, as better pollinated apples are comparatively larger and with more seeds (Garratt et al. 2014). Garratt et al. (2016) also reported that pollination contributed to about 65% of the marketable quality

and quantity yield in apples in a UK survey. Several factors may contribute to the decline of apple production, such as orchard shrinkage, tree nutrition level, and incompatibility among cultivars (Sheffield 2014). However, low abundance of pollinators in the orchard (Rather et al. 2017), resulting in inefficient pollination (Garratt et al. 2016) may also have negative effects on production. Insect pollination in apples is understudied in many regions (Pardo & Borges 2020) and the insect communities responsible for crop production may vary between regions (Dymond et al. 2021). Insufficient insect pollinators in the community may lead to pollination limitation, whereby seed production is less than would be reached with supplemental pollen treatments. Diverse communities of wild pollinators are necessary to ensure sufficient pollination (Garibaldi et al. 2013; Reilly et al. 2020), including in apples (Osterman et al. 2021; Radzevičiūtė et al. 2021). Insect pollinators are in decline in some parts of the world (Biesmeijer et al. 2006; Nieto et al. 2014; Potts et al. 2016; Hallmann et al. 2017) but for many areas, insufficient baseline information of diversity and abundance is available (Didham et al. 2020).

In Bhutan, apples were introduced in the late 1960s and have been the leading cash crop in the country for over a decade (Choden & Shahnawaz 2015). Apples in Bhutan are grown in the temperate zone (1,800 – 4,500 m) and are planted even on steep mountainous terrain (Choden & Shahnawaz 2015). According to the Renewable Natural Resources (RNR) census report (2019), there are 5,533 apple growers in the country. The RNR census report and National Statistics Bureau (NSB) indicate that apple production in Bhutan has decreased dramatically from 7,051 metric tons (MT) in 2014 to 6,587 MT in 2016 (-6.6% compared to the 2014 baseline; Pelden 2015) and 3,684.42 MT in 2019 (-47.8% compared to the 2014 baseline; Wangmo & Dem 2020). In Bhutan there has been no research on pollinator abundance and richness at agricultural sites to understand its role in production declines.

In this study, we set out to identify the flying insects that are potentially involved in apple pollination in Bhutan and to link these to the

observed regional decline in apple quality. Although such a decline is likely to have a multifactorial origin, we suspect that inefficient pollination, a factor that has received little to no attention in Bhutan, might be one of the factors behind the phenomenon observed. Specifically, we aimed to quantify flower visitor diversity and abundance in nine apple orchards located in Western Bhutan and covering elevation, climatic, and landscape gradients to explore; What is the diversity and abundance of visiting insects in Bhutanese apple orchards, and how does this diversity vary between orchards? And does the diversity and abundance of insects in the orchard correlate with apple quality in the studied orchards?

MATERIALS AND METHODS

LOCATION OF THE STUDY SITE

The study was conducted between December 2019 and October 2020, in three major apple-growing districts in Western Bhutan, Thimphu, Paro, and Haa (Fig. 1). According to the RNR census report (2019), Paro is the largest apple-producing district with 2,417 MT (65.6%), followed by Thimphu at 755 MT (20.5%) and Haa at 293 MT (8.0%). The mean annual temperature in the study sites varies from 4.6 °C in the winter to 23 °C in the summer and the mean annual rainfall varies between 386.3 mm and 858.4 mm.

SITE OVERVIEW AND DATA COLLECTION

The protocol used in the study was developed as part of the CliPS (Climate change and its effect in Pollination Services) project (2018-2022). CliPS is a global project that focuses on apple pollination and pollinator communities and has been developed to ensure the highest level of replicability across different sites and regions (Prendergast et al. 2021; Leclercq et al. 2022; Weekers et al. 2022). Three orchards were selected from each of the three districts with a minimum distance of 2 km between orchards. The selected orchards ranged from 2,288 m to 2,712 m in elevation. We only selected orchards with a minimum of 100 trees. We selected the orchards so as to maximize the diversity of biogeographic conditions, elevation, and landscape type, among orchards (Fig. 2).

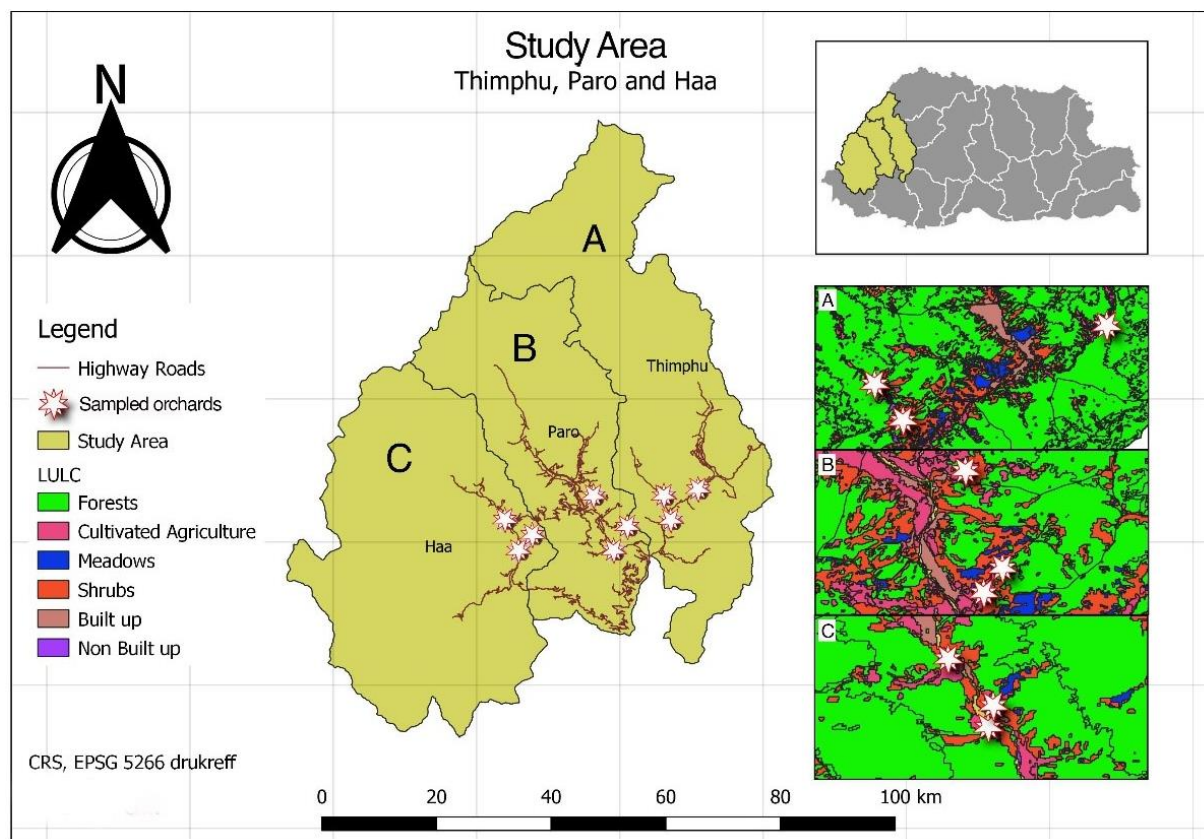


Figure 1. Location of the sampled orchards in each of the districts; (A) Thimphu, (B) Paro and (C) Haa, showing landscape context surrounding each orchard.

Flying Insect community sampling

Flying insect diversity and abundance in the orchard was recorded using pan trap and netting techniques. Field observations were made from 9:00 to 16:00 for three consecutive days during the peak apple flowering season (April-May 2020). Three triplets of pan traps, painted fluorescent yellow, blue, and white, were placed at 9:00 and were recovered at 16:00. A minimum distance of 2 m was maintained between pans and a distance of 30 m between each triplet. The pans were half-filled with soap water (10 ml of soap detergent/L of water) to lower the water tension and prevent the escape of insects visiting the pans. At the end of the day all the insects in the trap were collected; later they were washed, air-dried, and stored in a freezer.

Netting was standardized to 90 minutes before noon and another 90 minutes before 16:00 to ensure sampling of the total daily flying community occurred. During these 90 minutes of netting, the entire orchard was covered and only those insects observed visiting apple blossoms

were collected. The insects were put in a container containing 70% ethanol.

The specimens were then identified to species or morphospecies. Dr. John Smit from Naturalis Biodiversity Center (Netherlands) identified the Hymenoptera and Diptera while Mr. Phurba Dorji from the Royal Society for Protection of Nature (RSPN, Bhutan) assisted in the identification of Hymenoptera and Diptera. Mr. Thinley Phuntsho (RSPN, Bhutan) identified the Lepidoptera. At each site we also recorded the presence and species identity of any managed honeybee hives. The abundance and diversity figures were measured using the specimens collected by both netting and pan traps.

APPLE FRUIT SAMPLING AND MEASUREMENT

Apple sampling was conducted during the harvest period (September-October, 2020). From each orchard, 11 apple trees were selected at random and from each tree, five apples were collected from random branches (55



Figure 2. Overview of the diversity and management of a selection of the apple orchards sampled. In general, agricultural areas in Bhutan are found in high-quality landscapes and are much smaller and managed less intensively than in most other parts of the world. (A) Baytsho, Haa, 2690 m (a.s.l.). There was an *Apis mellifera* hive near this orchard which was introduced as part of an agriculture project by His Royal Highness DASHO Jigyel Ugyen Wangchuck. The bees here were introduced from the Bumthang district of Bhutan. (B) Youselfang, Thimphu, 2710 m (a.s.l.). The orchard was located in mountainous terrain, spread along the slope of the mountain. The owner also cultivated potatoes along with the apples in the apple orchard itself. They hired laborers to cultivate and harvest vegetables and apples as seen in the photo. (C) Jangtoena, Paro 2320 m (a.s.l.). The orchard was divided into three small plots with the help of a barbed wire. The orchard was surrounded mostly by settlements. (D) Chamzhingkha, Paro, 2440 m (a.s.l.). The orchard had a managed hive that has been repeatedly destroyed by bears. (E) Alternate angle of the orchard in (A) showing the mountainous terrain in which the orchard is situated. (F) Bjemina, Thimphu, 2440 m (a.s.l.). The owner has started cultivating cabbage below the apple trees in the orchard. All photos © Kinley Dorji.

apples/orchard, total of 495 apples). Two varieties of apples were grown across all sites, red delicious and golden delicious. Due to the propensity for red delicious to be more common across all sites, but also to incorporate the widely grown golden delicious, we sampled fruit from 7 red delicious

trees and 4 golden delicious trees at each site. Only the ripened apples were selected for measurement; pest-damaged apples were excluded. The apples were then measured for five different parameters: fruit weight (g), fruit size (mm), sugar concentration (% Brix; a measure of the percentage

of soluble sugar), number of seeds, and fruit malformation score. The weight of the apples was measured with a digital scale while the size was measured with the help of a digital Varner calliper. The sugar concentration of the apples was measured using a digital refractometer with the range of 0-60 % Brix. The number of seeds was counted manually. Fruit malformation score was given according to the state of the fruit: 1 – badly misshapen and unmarketable, 2 – somewhat malformed, and 3 – perfectly symmetrical and marketable quality. There are no standard criteria to check the shape and malformation of the apple fruits before export in Bhutan according to the Bhutan Agriculture and Food Regulatory Authority (BAFRA).

DATA PROCESSING AND ANALYSIS

Descriptive statistics were used to explore the total number of species recorded from each of the orchards and compare the measurements of apples in different orchards and by different collection techniques. The dominant species were visualised using a Whittaker plot and community analysis of species composition was conducted using rarefaction and a non-metric multidimensional scaling (NMDS) plot using the ‘vegan’ package (version 2.5-6; Oksanen et al. 2019). We conducted four statistical tests to determine the impact of diversity and abundance on apple quality. Firstly, (i) We tested whether the dominance of honeybees observed in the Whittaker plots were correlated to presence of hives, by fitting a linear model with honey bee abundance as the response variable and a binary measure of hive presence as the fixed effect. (ii) We used an ANOVA to determine if apple quality measures varied by orchard. (iii) We tested the relationship between all diversity metrics, (total, honeybee, hoverfly, bee and butterfly abundance and richness) and all apple quality metrics (apple weight, size, sugar concentration, the shape of the fruit, and seed number) using a Pearson’s correlation test. (iv) Based on the observed correlations we tested the effect of abundance and richness of flying insects on mean apple weight per orchard (which was correlated with all other measures) using multiple linear mixed effect regressions. District was used as a random effect to account for any spatial autocorrelation due to similar environmental conditions in the different districts. The best model

was selected as the model with the lowest Bayesian information criterion (BIC) (Schwarz 1978). All the data were analysed using the statistical software R, 4.0.4 (R Core Team 2021).

RESULTS

DIVERSITY AND ABUNDANCE ACROSS ORCHARDS AND COLLECTION METHODS

A total of 1,006 insects comprising 44 unique (morpho-)species belonging to three orders were recorded within apple orchards (Fig. 3A). Hymenoptera were the most diverse and abundant insect order (19 species, 780 specimens recorded), followed by Diptera (15 species, 181 specimens recorded) and Lepidoptera (10 species, 45 specimens recorded).

Netting collection was responsible for 649 specimens from 38 species recorded. Fewer specimens, but a similar species richness were collected with pan traps, 357 specimens from 39 species (yellow pan trap - 180 records for 31 species, white pan trap - 100 records for 29 species, and blue pan trap - 77 records for 24 species). Thirty-three species were collected by both methods (Fig. 2B) and five species were caught only by netting method and were not captured in the pan traps including one honey bee, *Apis dorsata*, and four butterfly species *Catopsilia pomona*, *Colias myrmidone*, *Everes huegelii*, and *Pseudozizeeria maha*. Six species were only collected in pan traps (including three *Lasioglossum* morpho-species, an *Andrena* sp., a species from the Eumeninae family and *Vespula flaviceps*). No species were unique to a particular pan trap colour (Fig. 2B). The three species of honey bees (in order of abundance *A. mellifera*, *A. cerana* and *A. dorsata*) were the most dominant species across all orchards and were far more likely to be collected by net than pan traps (Fig. 3C).

Honey bees (*A. mellifera*, *A. cerana* and *A. dorsata*) were the dominant insect visitor in all the orchards except for Gidakom, where the dominant flower visitor was a solitary bee (*Ceratina* sp2) (Fig. 4). Honey bees were more dominant in orchards located in Paro and Haa districts and there was lower species evenness in these orchards compared to the orchards in the Thimphu district that had less difference between the most abundant species and also overall greater species

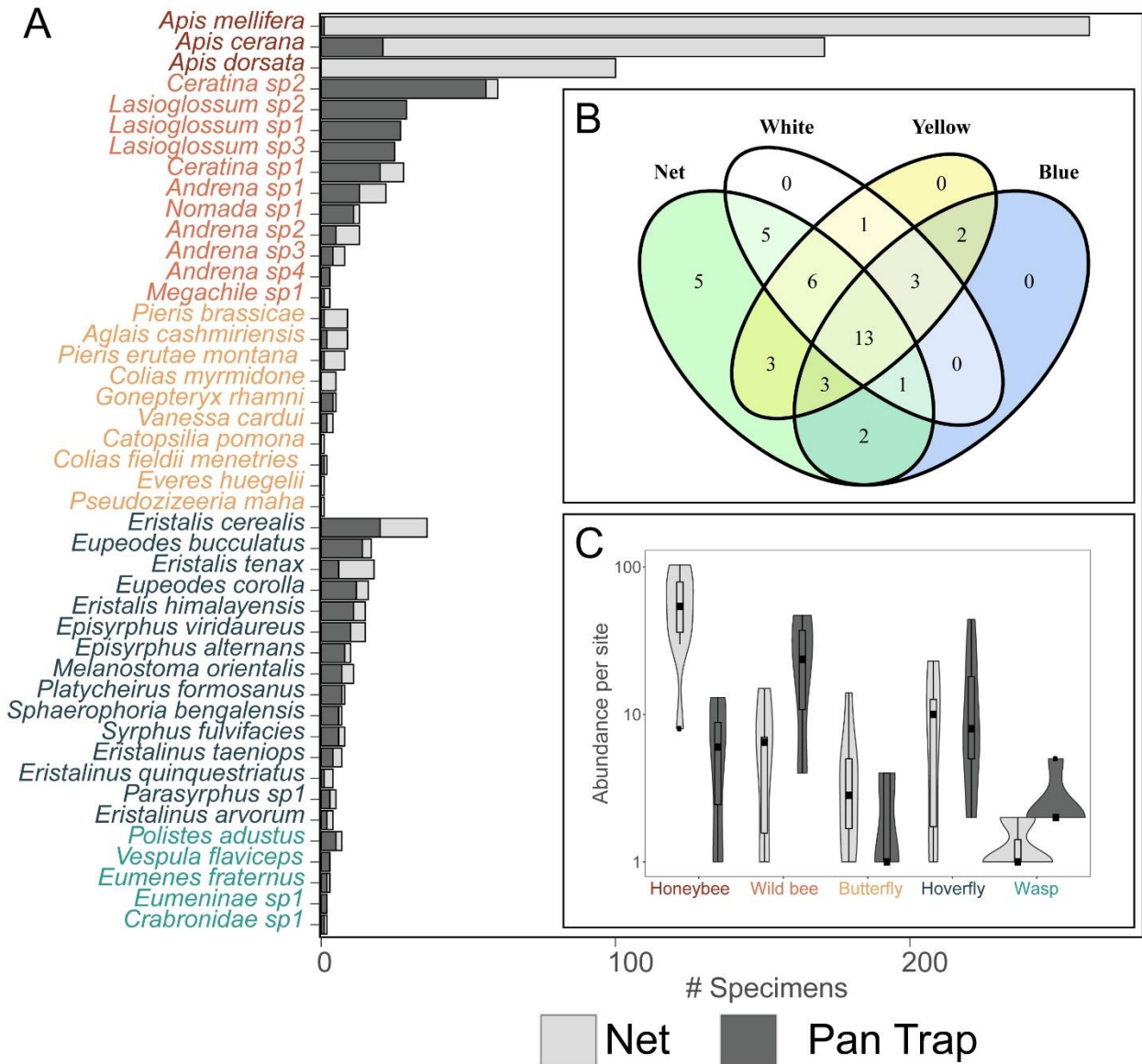


Figure 3. Overview of the complete inventory of pollinating insects found at each orchard. (A) Total number of specimens per (morpho-) species collected by netting or pan traps. The bars are stacked and represent the total number of (morpho-) species collected by both methods with the dark grey bar showing total (morpho-) species collected by pan traps and light grey total netted (morpho-) species. (B) Species richness shared by each collection method. Colours refer to the colours of the pan traps used. (C) Total abundance for each of the major insect groups by netting or pan traps.

richness per site (Fig. 4). Honey bee abundance was significantly higher in areas where hives were present in and around the orchard locality (linear model: $R^2 = 0.67$, $\beta = 28.03$, $T = 3.74$, $P = 0.007$). Chamzhangkha orchard in Paro had *A. cerana* hives while Baytsho and Tshelungkha orchard at Haa had *A. mellifera* hives.

The composition of communities in different orchards showed that there is heterogeneity within regions as the composition of species varies as much within regions as it does between regions (Fig. 5). However, overall, there was no significant

difference between the sites in terms of community composition (PERMANOVA; $R^2 = 0.51$, $F = 1.21$, $P = 0.21$). (Fig. 5). The collection method used resulted in greater differences in the community compared to the location. There was a significant difference in the community of insects collected by netting or pan traps (PERMANOVA; $R^2 = 0.21$, $F = 4.24$, $P = 0.002$). Most of the honey bees were caught by netting while pan traps were more effective in catching hoverflies and other wild bee species (Fig. 5).

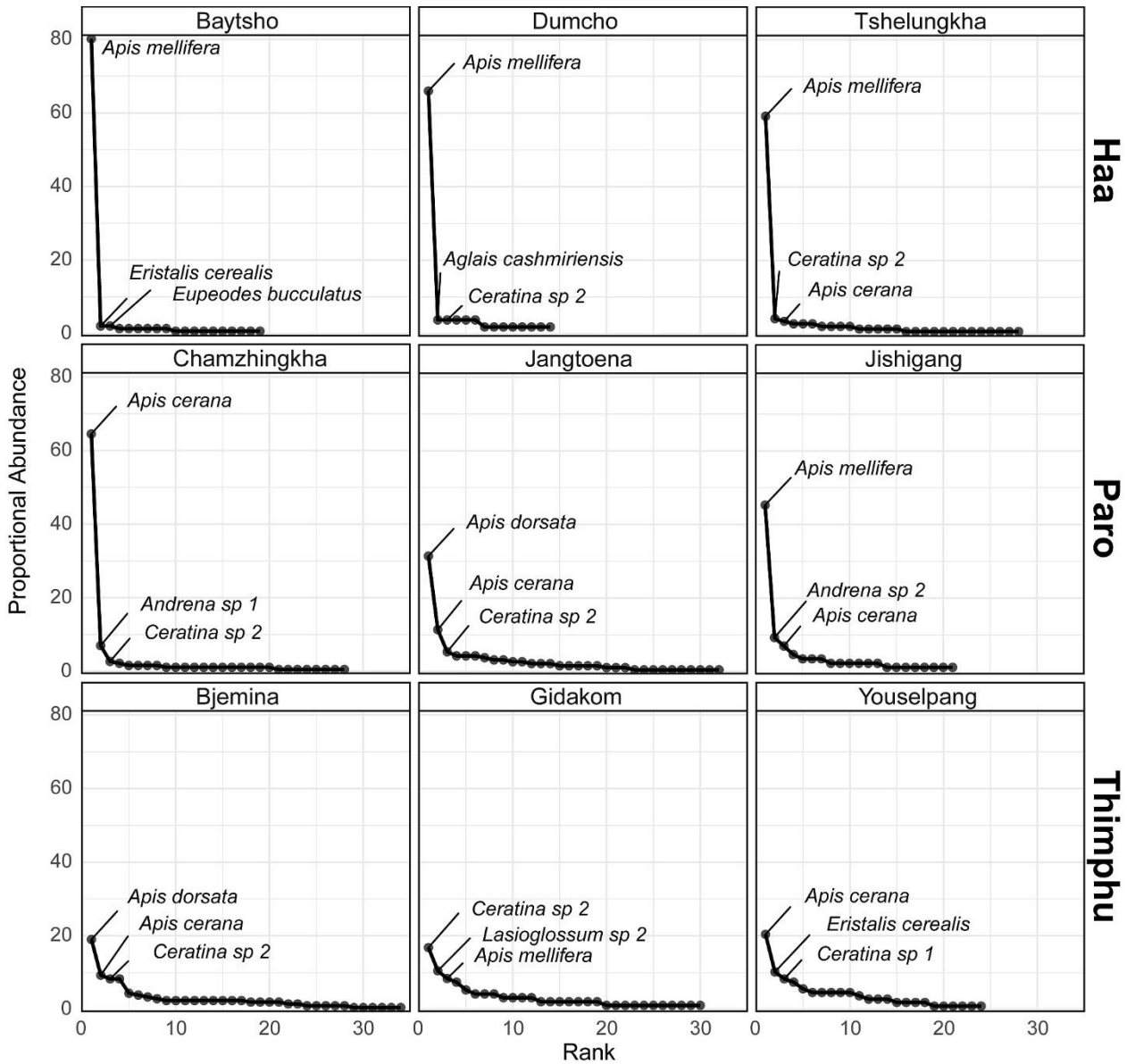


Figure 4 Whittaker plot showing relative species abundance and evenness per orchard site, top three most abundant species of the orchard are labelled.

APPLE PRODUCTION AND RELATIONSHIP TO DIVERSITY ACROSS ORCHARDS

There was a significant difference in the mean weight of the apples across different orchards. The mean size of the apples across different orchards was also significantly different ($F(8, 486) = 23.72, P < 0.001$). The difference in the mean sugar concentration of apples in the different orchards was significant ($F(8, 486) = 3.29, P = 0.01$), and the mean weight, size and sugar content per orchard were all significantly correlated with each other ($r > 0.7$) (Fig. 6). In particular, the mean number of seeds set in the fruit and the fruit quality (weight, size, sugar) was seen to be positively correlated

(Pearson's $r = 0.733$ (weight), $r = 0.736$, (size), $r = 0.767$ (shape) $r = 0.779$ (sugar concentration)) (Fig. 6). As the number of seeds in the apple fruit increases, the apples tend to be larger, sweeter and with better symmetry.

Therefore, we used mean weight per orchard as a proxy for the other apple measurements as the response variable in a linear mixed effects model to compare the correlation with richness and abundance. The best linear mixed effects model (lowest BIC) to explain variation in mean weight included only total abundance of insects ($\beta = 0.05, T = 17.12, P < 0.001$). Total abundance was significantly correlated with all other

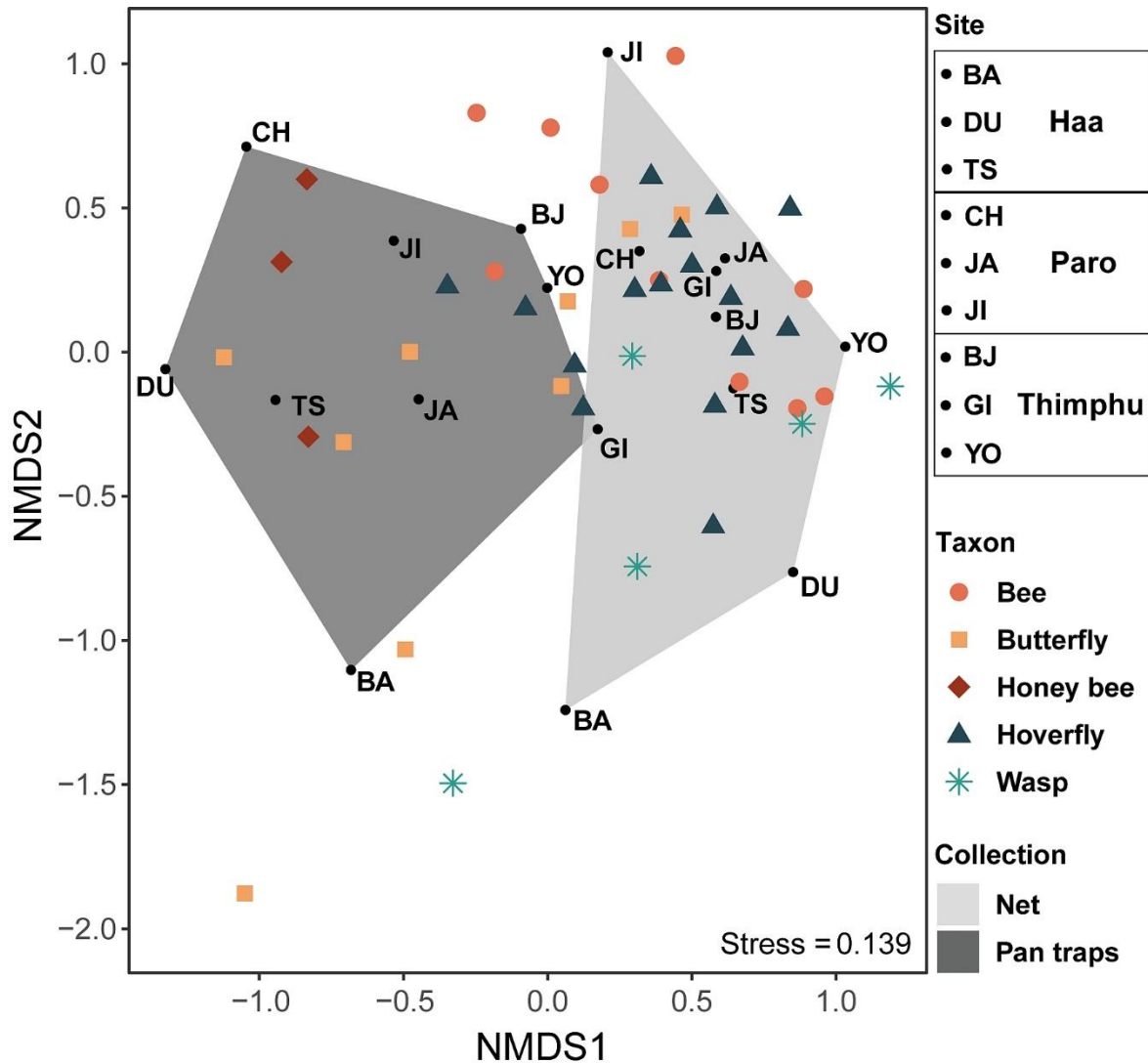


Figure 5 Non-metric multidimensional scaling (NMDS) showing the variability in community composition by site and collection method.

measurements, excluding the number of seeds and apple shape (Fig. 6). To see which groups were most influential in driving mean weight, we excluded total abundance and fit a model with the abundance of each major pollinator group. The best model in this case included abundance of honey bees ($\beta = 0.05$, $T = 14.38$, $P < 0.001$) and abundance of hoverflies ($\beta = 0.07$, $T = 10.33$, $P < 0.001$). Species richness did not have a statistical relationship with apple quality measurements (for all model results see Table S1).

DISCUSSION

This study represents the first survey of the diversity of apple pollinating insects in Bhutan. We found that in almost all cases, honey bees were the dominant visitor of apple blossoms in these

orchards. This pattern was consistent, especially in the orchards of Paro and Haa districts, where bee hives were present in and around these orchards. The non-native *A. mellifera* was the dominant visitor overall, as observed in apple orchards globally (e.g. Russo et al. 2015; Hutchinson et al. 2021; Prendergast et al. 2021; Weekers et al. 2022). *Apis cerana* was the second most common flower visitor across all orchards. *A. cerana* and *A. mellifera* have been shown to offer complementary pollination services to apples in the neighbouring Indian Himalayas (Verma & Dulta, 1986; Verma & Rana, 1994; Joshi & Joshi, 2010). *Apis mellifera* was seen to visit more flowers and have greater pollen loads and *A. cerana* was active for longer periods of the day (Verma & Rana, 1994; Verma & Dulta, 1986).

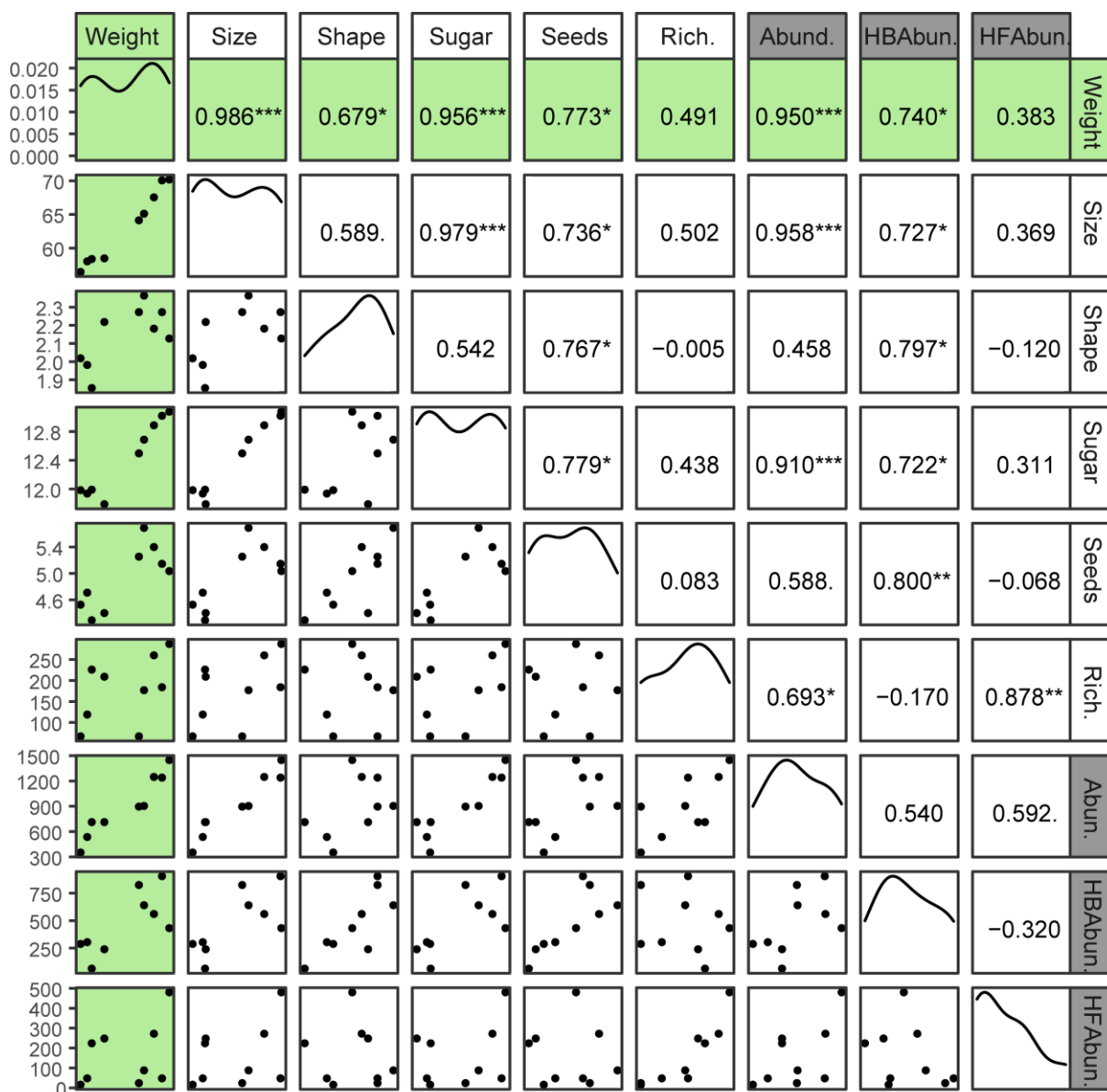


Figure 6. Correlation matrix showing the key apple quality measurements and measurements of per orchard (N = 9) abundance and diversity. Variables included, in order, are mean apple weight (g), mean apple size in (mm), mean apple shape which is a malformation score from; 1 – badly misshapen and unmarketable, 2 – somewhat malformed and 3 – perfectly symmetrical and marketable quality, mean sugar concentration (% BRIX), mean number of seeds per apple, total richness, total abundance, abundance of honeybees (HB) and abundance of hoverflies (HF). Mean weight (highlighted in green) was used as the response variables in a linear mixed effects model. Abundance was the only variable included in the best model (lowest AIC) and HB abundance and HF abundance were included in the best model if abundance was excluded (all highlighted in grey). Value on the upper triangle is the Pearson’s correlation coefficient and the * refer to the P-value significance level; * < 0.05, ** < 0.01, *** < 0.001.

A growing body of literature suggests it is likely that *Apis mellifera* may restrict other species through competition for resources (Geslin et al. 2013). Angelella et al. (2011) reported that there was a marked decrease in wild pollinator abundance, species richness, and fruit count on strawberry farms with bee hives and hives, and Weekers et al. (2022) recently showed that honey

bee dominance is statistically associated to overall lowered diversity of wild pollinators in apple orchards across Western Europe, irrespective of management practices. However, we cannot draw the same conclusion for Bhutanese apple orchards. So little is known about the diversity of pollinating insects in Bhutan making it difficult to know whether the diversity and abundances observed

reflect the wider community or if the apple orchards act as an environmental filter. In particular, because of the lack of knowledge of other pollinators and how to manage the landscape to promote them, previous recommendations to apple farmers in Bhutan suggested increasing managed honey bees (Partap & Partap 2001). There is sound evidence that a diverse community of pollinators improves crop production compared to only managed pollinators (Garibaldi et al. 2013). However, the promotion of wild pollinators in Bhutan can only begin once there is fundamental research to provide baseline knowledge in Bhutan. The results presented here provide evidence that, contrary to situations where apple production is supported primarily by honey bees in regions lacking alternative and efficient pollinators (see e.g. Prendergast et al. 2021), the communities of insects visiting apple blossoms in Bhutan, including wild bees (*Ceratina* and *Andrena*) and hoverflies, may contribute to a substantial share of the crop pollination service. Future research should investigate this issue with greater scrutiny, particularly to test the extent to which wild bees and hoverflies alone are sufficient to provide the pollination service in a context of commercial farms, or if honey bees are systematically required to fulfil the pollination demand.

Unlike apple orchards in other parts of the world (Pardo & Borges 2020; Hutchinson et al. 2021), no bumblebee species (*Bombus*) were recorded as visitors in the orchards surveyed here. Partap et al. (2019) reported bumblebees as pollinators in the Hindu Kush-Himalayan Region of India. However, many bumblebee species in Bhutan are likely to be found in very high elevation areas (Williams et al. 2010). Moreover, Nidup et al. (2017), reported that despite the importance of bumblebees in pollination, bumblebee diversity in Bhutan is fundamentally undocumented, and a diversity inventory is lacking in the country. Several hoverfly species were also found to be visiting apple blossoms, and globally their importance as crop pollinators may be underestimated (Rader et al. 2020). Hoverflies also may benefit crop production in another way, as most larvae in the subfamily Syrphinae and tribe Pipizini feed on aphids (Sommaggio et al. 1999).

The significant differences in communities collected by nets and pan traps suggest that only a subset of the entire community is actually visiting apple blossoms as potential pollinators; apple crops may act as a filter at the community level (Leclercq et al. 2022). Although, it can be difficult to record the entire range of visitors, especially when they are low in abundance (Russo et al. 2015). Overall, at the community level, there was no significant difference between composition across all orchard sites. Therefore, the filtering effects of the orchards may be stronger than any local variability in orchards resulting in a homogenous community of visitors. This may be because the overall quality of the landscape in Bhutan is very high, even in the agricultural areas that are much smaller and managed less intensively than in most other parts of the world.

Our results also suggest that a greater number of insects in the orchard increases the chances of efficient pollination, but that this is not dependent on richness. This reflects the results of recent research showing that apple pollination success may not be related to richness (Osterman et al. 2021), but can be dependent on abundance (Radzevičiūtė et al. 2021). Although, this is in contradiction to the findings of some recent studies (Garibaldi et al. 2013; Choi & Jung 2015; Blitzer et al. 2016; Nunes-Silva et al. 2020), which reported a key role of pollinator diversity (in addition to abundance) for improved pollination efficiency, especially as not all pollinators forage at the same time of the day. However, the low diversity overall and the limited number of orchards means we cannot draw detailed conclusions. Management is likely to play a large role in the potential for diversity to affect production (Nicholson et al. 2020); orchard owners in Bhutan report considerable variation in production between years and between sites, suggesting that there are several other factors affecting apple production related to management other than the presence of pollinators (Dorji et al. in press). Furthermore, here we only looked at apple quality and did not calculate fruit set per tree or yield per orchard. To do so would involve selecting branches during flowering and calculating the total number of flowers per tree in relation to the number of fruits harvested.

The abundance of honey bees, both native and introduced species, seems to be the most important

driver of fruit quality. Honey bees are the most dominant pollinators with their economic valuation being estimated at US\$ 217 billion globally (Park et al. 2018). Similar findings were also reported where honeybee dominance had a positive effect on apple weight (Nunes-Silva et al. 2020). The decline in both quality and yield in the Bhutanese apples over the years may be because of a pollinator deficit in the orchards and inefficient pollination. Identifying appropriate honey bee density thresholds and supporting wild pollinators in the orchard could significantly benefit apple growers (Angelella et al. 2011). This type of work and research should be conducted in Bhutan to not only help conserve the pollinators but also improve apple quality and yield and thus support farmers.

A study by Sheffield (2014) reported that the weight of the apple fruits increased linearly with the number of seeds per fruit, though this increase was minimal. A study by Buccheri & Vaio (2004), also reported a positive relationship between seed number, weight and shape of the fruit. Such differences in fruit quality are a result of the difference in pollen deposition, which reflects the pollination efficiency of different pollinators (Park et al. 2016). However, apple cultivar and pollinizer trees are equally important for fruit set. Nunes-Silva et al. (2020) also reported that the influence of pollinator diversity and abundance on apple quality and yield varies depending on the different apple cultivar. Future research in Bhutan would require analysing the contribution of pollinator diversity and abundance with different apple cultivars and of the pollinizer trees in the orchard (Carisio et al. 2020).

The results presented here serve as a preliminary study of the diversity of potential insect pollinators and apple pollination in Bhutan. The conclusions we can draw are limited due to low statistical power and the methodology used in this study should be replicated at a larger scale. More extensive research is required to obtain a true relationship between pollinators, efficient pollination, and its contribution to apple quality, especially in terms of apple quality for different varieties. However, this data can begin a process to help underpin future orchard management to involve pollinator health and conservation strategies. There are currently no standard criteria on apple quality for export in Bhutan. Therefore,

there is no added pressure on the farmers to produce better apples. This study can also be used to set standard criteria so that farmers are not only encouraged to produce better apples but will also improve the value and price of the Bhutanese apples in the export market, while acknowledging the contribution of native pollinators to crop production on the roof of the world, at the foot of Himalayas.

ACKNOWLEDGEMENTS

We would like to thank Dr. John Smit (Naturalis Biodiversity Center, Netherlands), Mr. Phurba Dorji (Royal Society for Protection of Nature (RSPN), Bhutan) and Mr. Thinley Phuntsho (RSPN, Bhutan) for their invaluable contribution to species identifications. We also thank Dr. Vincent Kalkman from (Naturalis Biodiversity Center, Netherlands), for his assistance in establishing the project, Dr. Timothy Weekers (Universite libre de Bruxelles, Belgium) for providing knowledge on apple sampling and the WWF Bhutan Program Office for the field work grant. We thank Kencho Tshering, Kunzang Wangle, Ugyen Wangchuk for their contribution to the fieldwork. Finally, we would like to thank all the farmers and land-owners who made this study possible by allowing us to enter and work at their orchards and assisted us in all our enquiries. NL, NJV and LM were funded by the FNRS/FWO joint programme 'EOS – Excellence Of Science' for the project 'CliPS: Climate change and its impact on Pollination Services (project 30947854).

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix 1. Figures and tables of model results.

REFERENCES

- Aizen MA, Aguiar S, Biesmeijer JC, Garibaldi LA, Inouye DW, Jung C, Martins DJ, Medel R, Morales CL, Ngo H, Pauw A (2019) Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global Change Biology* 25:3516-3527.
- Angelella G, McCullough C, O'Rourke M (2011) Honey bee hives decrease wild bee abundance, species richness, and fruit count on farms regardless of wildflower strips. *Scientific Reports* 11:1-12.
- Biesmeijer JC, Roberts S, Reemer M, Ohlemuller R, Edwards M, Peeters T, Schaffers A P, Potts SG, Kleukers R, Thomas C.D, Settele J, Kunin W (2006) Parallel Declines in Pollinators and Insect-Pollinated

- Plants in Britain and the Netherlands. *Science* 313: 351-354.
- Blitzer E, Gibbs J, Park M, Danforth B (2016) Pollination services for apple are dependent on diverse wild bee communities. *Agriculture, Ecosystem and Environment* 221:1-7.
- Buccheri M, Vaio C D (2004) Relationship Among Seed Number, Quality, and Calcium Content in Apple Fruits. *Journal of Plant Nutrition* 27: 1735-1746.
- Carisio L, Díaz SS, Ponso S, Manino A, Porporato M (2020) Effects of pollinizer density and apple tree position on pollination efficiency in cv. Gala. *Scientia Horticulturae* 273:109629.
- Choden J, Shah Nawaz S (2015) Apple Cultivation in Bhutan - Past Trends and Future Potential (Shah Nawaz U, Thinley, Eds.) Proceedings of the Conference on 'Climate Change, Environment and Development in Bhutan', Bhutan, pp 65-72.
- Choi S-W, Jung C (2015) Diversity of Insect Pollinators in Different Agricultural Crops and Wild Flowering Plants in Korea: Literature Review. *Journal of Apiculture* 30:191-205.
- Didham RK, Basset Y, Collins C. M, Leather SR, Littlewood NA, Menz MH, Müller J, Packer L, Saunders ME, Schönrogge K, Stewart AJ, Yanoviak SP, Hassall C (2020) Interpreting insect declines: seven challenges and a way forward. *Insect Conservation and Diversity* 13:103-114.
- Dorji K, Tashi S, Biesmeijer JC, Marshall L (in press) Farmers' perception on pollinators and apple pollination in western Bhutan. *Bhutan Journal of Natural Resources and Development*.
- Dymond K, Celis-Diez JL, Potts SG, Howlett BG, Willcox BK, Garratt MPD (2021) The role of insect pollinators in avocado production: a global review. *Journal of Applied Entomology* 145:369-383.
- Food and Agricultural Organization of the United Nations (FAO) (2018) The importance of bees and other pollinators for food and agriculture. Republic of Slovenia: Ministry of Agriculture, Forestry and Food.
- Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhaffer JH, Freitas BM, Ghazoul J, Greenleaf S, Hipolito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlöf M, Seymour CL, Schaep C, Szentgyörgyi H, Taki H, Tscharntke T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 340:1608-1611.
- Garratt MPD, Breeze TD, Boreux V, Fountain MT, Mc Kerchar M, Webber SM, Coston D.J, Jenner N, Dean R, Westbury DB, Biesmeijer JC, Potts S (2016) Apple Pollination: Demand Depends on Variety and Supply Depends on Pollinator Identity. *PloS one* 11:p.e0153889.
- Garratt MPD, Breeze T.D, Jenner N, Polce C, Biesmeijer JC, Potts SG (2014) Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. *Agriculture, Ecosystems and Environment* 184:34-40.
- Geslin B, Gauzens B, Baude M, Dajoz I, Fontaine C, Henry M, Ropars L, Rollin O, Thébault E, Vereecken NJ, (2017) Massively Introduced Managed Species and Their Consequences for Plant–Pollinator Interactions. *Advances in Ecological Research* 57:147-199.
- Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans, W, Müller A, Sumser H, Hörren T, Goulson D, Kroon H (2017) More than 75 percent decline over 27 years in total flying insects' biomass in protected areas. *PloS one* 12: p.e0185809.
- Hein L (2009) The Economic Value of the Pollination Service, a Review Across Scales. *The Open Ecology Journal* 2:74-82.
- Hutchinson LA, Oliver TH, Breeze TD, Bailes EJ, Brünjes L, Campbell AJ, Erhardt A, de Groot GA, Földesi R, García D, Goulson D, Hainaut H, Hambäck PA, Holzschuh A, Jauker F, Klatt BK, Klein A-M, Kleijn D, Kovács-Hostyánszki A, Krimmer E, Mc Kerchar M, Miñarro M, Phillips BB, Potts SG, Pufal G, Radzevičiūtė R, Roberts SP, Samnegård U, Schulze J, Shaw RF, Tscharntke T, Vereecken NJ, Westbury DB, Westphal C, Wietzke A, Woodcock BA, Garratt MP (2021) Using ecological and field survey data to establish a national list of the wild bee pollinators of crops. *Agriculture, Ecosystems & Environment* 315:107447.
- Potts SG, Imperatriz-Fonseca VL, Ngo HT, Biesmeijer JC, Breeze TD, Dicks LV, Garibaldi LA, Hill R, Settele J, Vanbergen AJ (2016) Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. <https://www.actu-environnement.com/media/pdf/news-26331-synthese-ipbes-decideurs-pollinisateurs.pdf>
- Joshi NC, Joshi PC (2010) Foraging Behaviour of *Apis* spp. on Apple Flowers in a Subtropical Environment. *New York Science Journal* 3:71-76
- Klein A.-M, Vaissière B, Cane J, Steffan-Dewenter I, Cunningham S, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274:303-313.
- Leclercq N, Marshall L, Weekers T, Anselmo A, Benda D, Bevk D, Bogusch P, Cejas D, Drepper B, Galloni M, Gérard M, Ghisbain G, Hutchinson L, Martinet B,

- Michez D, Molenberg J-M, Nikolic P, Roberts S, Smagghe G, Straka J, Vandamme P, Wood TJ, Vereecken NJ (2022) A comparative analysis of crop pollinator survey methods along a large-scale climatic gradient. *Agriculture, Ecosystems & Environment* 329:107871.
- Nicholson CC, Ward KL, Williams NM, Isaacs R, Mason KS, Wilson J.K, Brokaw J, Gut LJ, Rothwell NL, Wood TJ, Rao S (2020) Mismatched outcomes for biodiversity and ecosystem services: testing the responses of crop pollinators and wild bee biodiversity to habitat enhancement. *Ecology Letters* 23:326-335.
- Nidup T, Dema U, Phuntsho Y (2017) Diversity and Distribution of Bumblebee (Hymenoptera: Apidae: Bombini) in Kanglung Gewog, Trashigang District. Centre for Climate Change and Spatial Information Sherubtse College, Royal University of Bhutan.
- Nieto A, Roberts S, Kemp J, Rasmont P, Kuhlmann M, Criado M, Biesmeijer JC, Bogusch P, Dathe HH, De la Rúa P, De Meulemeester T, Dehon M, Dewulf A, Ortiz-Sánchez FJ, Lhomme P, Pauly A, Potts SG, Praz C, Quaranta M, Radchenko VG, Scheuchl E, Smit J, Straka J, Terzo M, Tomozii B, Window J, Michez D (2014) European Red List of Bees. Luxembourg: Publication Office of the European Union.
- Nunes-Silva P, Witter S, Da Rosa, J, Halinski R, Schlemmer L, Arioli C, Ramos JD, Botton M, Blochtein B (2020) Diversity of Floral Visitors in Apple Orchards: Influence on Fruit Characteristics Depend on Apple Cultivar. *Neotropical Entomology* 49:511-524.
- Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, Mcglinn D, O'Hara R.B, Simpson G.L, Solymos P, Stevens MHH, Wagner H (2019) vegan: Community Ecology Package. R package version 2.5-6. Retrieved from <http://CRAN.R-project.org/package=vegan>
- Ollerton J (2017) Pollinator Diversity: Distribution, Ecological Function, and Conservation. *Annual Review of Ecology, Evolution, and Systematics*. 48:353-376.
- Osterman J, Theodorou P, Radzevičiūtė R, Schnitker P, Paxton RJ (2021) Apple pollination is ensured by wild bees when honey bees are drawn away from orchards by a mass co-flowering crop, oilseed rape. *Agriculture, Ecosystems & Environment*.315:107383.
- Pelden T (2015) Apple orchards shrinking in Bhutan. Thimphu: Kuensel. Retrieved from <http://www.kuenselonline.com/apple-orchards-shrinking/>
- Pardo A, Borges P.A.V (2020) Worldwide importance of insect pollination in apple orchards: A review. *Agriculture, Ecosystems & Environment*. 293:106839.
- Park MG, Raguso RA, Losey JE, Danforth BN (2016) Per-visit pollinator performance and regional importance of wild *Bombus* and *Andrena* (Melandrena) compared to the managed honey bee in New York apple orchards. *Apidologie*, 47:145-160.
- Park MG, Joshi N, Rajotte E, Biddinger D, Losey J, Danforth B (2018) Apple grower pollination practices and perceptions of alternative pollinators in New York and Pennsylvania. *Renewable Agriculture and Food Systems*, 35:1-14.
- Partap T, Partap U (2001) Declining apple production and worried Himalayan farmers: Promotion of honeybees for pollination. Kathmandu, Nepal: International Centre for Integrated Mountain Development (ICIMOD)
- Partap U, Hussain S, Hussain E, Inayatullah M, Gurung M, Ismail M, Shah G (2019) Honeybee Pollination and Apple Yields in Chitral, Pakistan. Kathmandu, Nepal: International Centre for Integrated Mountain Development (ICIMOD)
- Prendergast KS, Leclercq N, Vereecken, NJ (2021) Honey bees (Hymenoptera: Apidae) outnumber native bees in Tasmanian apple orchards: Perspectives for balancing crop production and native bee conservation. *Austral Entomology* 60:422-435.
- R Core team (2021) R: A language and environment for statistical computing. R foundation for statistical Computing. Retrieved from <https://www.R-project.org/>.
- Rader R, Cunningham S, Howlett B, Inouye D (2020) Non-Bee Insects as Visitors and Pollinators of Crops: Biology, Ecology, and Management. *Annual Review of Entomology* 65:391-406.
- Radzevičiūtė R, Theodorou P, Schlegel M, Paxton R. J (2021) A two-part modelling approach reveals a positive effect of pollinator biodiversity in boosting the pollination of apple flowers. *Agriculture, Ecosystems & Environment* 306:107197.
- Rather G, Paray M, Javid R, Khursheed R, Yaqoob M, Rehman M, Majid F (2017) Pollination Problems in Apple of Kashmir Himalaya. *International Journal of Pure and Applied Bioscience* 5:817-823.
- Reilly JR, Artz DR, Biddinger D, Bobiwash, K, Boyle NK, Brittain, C, Brokaw J, Campbell JW, Daniels J, Elle E, Ellis JD, Fleischer SJ, Gibbs J, Gillespie RL, Gundersen KB, Gut L, Hoffman G, Joshi N, Lundin O, Mason K, McGrady CM, Peterson SS, Pitts-Singer TL, Rao S, Rothwell N, Rowe L, Ward KL, Williams NM, Wilson JK, Isaacs R, Winfree R (2020) Crop production in the USA is frequently limited by a lack of pollinators. *Proceedings of the Royal Society B: Biological Sciences* 287: 20200922.
- Renewable Natural Resources (RNR) Statistics Division (2019) RNR Census of Bhutan. Thimphu, Bhutan: Royal Government of Bhutan (RGoB), Ministry of Agriculture and Forests (MoAE)

- Russo L, Park M, Gibbs J, Danforth B (2015) The challenge of accurately documenting bee species richness in agroecosystems: bee diversity in eastern apple orchards. *Ecology and Evolution* 5: 3531–3540.
- Schwarz, G (1978) Estimating the Dimension of a Model. *The Annals of Statistics* 6: 461–464. <https://doi.org/10.1214/aos/1176344136>
- Sheffield C (2014) Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Annapolis valley, Nova Scotia, Canada. *Journal of Pollination Ecology* 12:120-128.
- Sommaggio D (1999) Syrphidae: can they be used as environmental bioindicators? *Agriculture, Ecosystems & Environment* 74:343–356.
- Wangmo C, Dem P (2020) Apple growers report poor fruiting. Thimphu: Kuensel. Retrieved from <https://kuenselonline.com/apple-growers-report-poor-fruiting/>
- Weekers T, Marshall L, Leclercq N, Wood TJ, Cejas D, Drepper B, Hutchinson L, Michez D, Molenberg JM, Smaghe G, Vandamme P, Vereecken NJ (2022) Dominance of honey bees is negatively associated with wild bee diversity in commercial apple orchards regardless of management practices. *Agriculture, Ecosystems & Environment* 323:107697.
- Williams PH, Ito M, Matsumura T, Kudo I, (2010) The bumblebees of the Nepal Himalaya (Hymenoptera: Apidae) Insecta matsumurana. *Journal of the Faculty of Agriculture Hokkaido University, Series Entomology* 66:115–151.
- Verma LR, Dulta PC (1986) Foraging Behaviour of *Apis Cerana Indica* and *Apis Mellifera* in Pollinating Apple Flowers. *Journal of Apicultural Research* 25:197–201.
- Verma LR, Rana RS (1994) Further studies on the behaviour of *Apis cerana* and *Apis mellifera* foraging on apple flowers. *Journal of Apicultural Research* 33:175–179.

