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Declining abundance of pollinating insects drives falls in loquat (*Eriobotrya japonica*) fruit yields in the Pothwar region of Pakistan

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ABSTRACT

Insects perform numerous vital ecosystem services, so widespread reports of insect declines are of considerable concern. However, there are huge knowledge gaps with regard to the extent and scale of insect declines, with most studies being from Europe and North America and very few long-term data sets on insect population change in Asia, Africa or South America. The current study describes trends in abundance of insect pollinators visiting loquat (*Eriobotrya japonica*) over 13 years (2006–2018) in ten widely-spaced orchards in the Pothwar region of Pakistan. Results reveal a significant and concerning decline of 89.9% in overall abundance of flower-visitors across the study period. All pollinator species declined; for example numbers of *Apis dorsata* fell by 97%, *A. mellifera* by 96%, *A. cerana* by 93%, and *Bombus haemorrhoidalis* by 84%. Declines were non-linear, being most rapid in 2006–2009. In parallel, the average yield per loquat tree fell by 61%; sugar and organic acid content also declined. Total flower-visiting insect abundance was a strong predictor of crop yield and quality over time and across sites, while pollinator species richness was less powerful. Trees that were given supplemental pollination by caging them with small honeybee colonies during flowering showed no decrease in yield or fruit quality. These results indicate that declines in populations of wild pollinators are negatively impacting loquat production and the livelihood of the small farmers of the area. Further research to identify the cause of these insect declines is urgently needed, alongside development of conservation strategies for pollinators in this region.

1. Introduction

Pollinating insects are of vital importance as they provide valuable ecological services (Garibaldi et al., 2011, 2014). Approximately 90% of flowering plants species depend upon animal pollinators (Linder, 1998). Pollinating insects are necessary to achieve maximum yield in more than 75% of the world's crop species (Klein et al., 2007), and about 35% of total crop production depends directly on insect pollination (Klein et al., 2007), with the global extend of crops requiring insect pollination increasing (Aizen et al., 2009). It is estimated that crops worth 153 billion Euros can be attributed to pollinators around the globe (Gallai et al., 2009). Insect-pollinated crops are an essential source of vitamins, minerals, nutrients, and antioxidants for humans (Seeram, 2008; Eilers et al., 2011).

A recent global meta-analysis identified severe declines in populations of many groups of pollinators, as well as concomitant declines in the services they provide, although the authors highlight that very few long-term data sets on insect populations are available from outside Europe and North America (Sánchez-Bayo and Wyckhuys, 2019). Nonetheless it would seem that abundance and diversity of pollinating insects are under serious threat due to a range of factors including loss of habitat, pesticides, emerging parasites and diseases, climate change, introduced species, loss of floral diversity, and an increasing tendency towards monoculture agriculture (Biesmeijer et al., 2006; Carvell et al., 2006; Brittain et al., 2010; Godfray et al., 2015), with growing evidence for pollination deficit for some crops and in some regions (Lautenbach et al., 2012).

Loquat (*Eriobotrya japonica*) is an evergreen medium-sized fruit tree that starts flowering in autumn and early winter, at a time when few other flowers are available (Freihat et al., 2008). It is cultivated successfully in subtropical, Mediterranean, and mild temperate climates of the world (Crane and Caldeira, 2006; Sharpe, 2010). Flowers are small,

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white or yellowish, borne on panicles of 30–100 flowers, and they produce plentiful nectar and pollen (McGregor, 1976; Merino and Nogueras, 2003). The fruits develop in clusters during March and April when few other fresh fruits are available on the market in the Northern hemisphere (Cuevas et al., 2009; Hussain et al., 2011). It is a significant crop in Pakistan, with loquat orchards covering approximately 10,000 ha and producing an estimated 1280,000 tons of fruit per year (Khan, 2003).

Loquat is partially self-compatible, but cross-pollination improves fruit set and quality (Pérez, 1983; Cuevas et al., 2003; Freihat et al., 2008; Niska et al., 2010). Fruit size, fruit weight, number of seeds, flesh weight, and sugar contents are dependent upon the quality and quantity of pollination by insects (Freihat et al., 2008). Low fruit set and fruit size results when insect pollinators are excluded (Freihat et al., 2008; Cuevas et al., 2009).

The present study was conducted over thirteen years to assess changes in insect flower-visitor populations and the pollination services delivered by insects to small loquat orchards in the Pothwar region of Pakistan. We aimed to determine how yield has changed over time, which species are the main loquat flower visitors, and the relationship between insect abundance and the quantity and quality of the loquat fruit. By supplementing pollination to some trees we aimed to determine whether yield is limited by inadequate pollination, and whether changes in yield over time can be attributed to pollinator visitation or are being driven by other factors.

2. Methods

2.1. Study orchards

The study was carried out from 2006 to 2018, to ascertain population trends of pollinators in the area. The area is surrounded by mountains and lies between the Indus and Jhelum rivers. The elevation of study sites varies from 400 and 910 m (Fig. 1), with an average rainfall of 380–510 mm rain per year, and daytime temperature varying from 5 °C in winters to 45 °C in summer (mean temperature during winter and summer). Ten orchard sites (typically in the range 2.5–3 ha in size) were selected, with an average 20 km distance between them (Fig. 1). All orchards were planted with the loquat variety 'Tanaka' and received similar agricultural practices as follows: weeding was by hand hoeing; only the pesticide methyl eugenol was used, for the control of fruit flies; fertilizer was applied three times per year at the rate N:P:K 700:300:700 g/plant; 25 kg of farmyard manure per plant was also applied each year for the first 5 years, increasing to 50 kg per plant thereafter; trees were pruned in June. Tree height varied from 4 to 6 m. No commercial hives of *A. mellifera* were placed within 1 km of the experimental orchards, but wild colonies may have been present.

2.2. Pollinator surveys

During the loquat blooming period each year, ten trees from each site were randomly selected for this study, and pollinator surveys were carried out in each orchard twice a week for nine weeks (a total of 18 times in each orchard each year). As the blooming period of loquat in Pakistan starts in February and ends in March, the temperature in these months is low (occasionally below freezing at night), and the activity of insects starts after 8:00 AM and ends at about 4:00 PM. The observation period was divided into morning (8:00–10:00 AM) and afternoon (2:00–4:00 PM) on sunny days at each observation site. The survey at each site was done for about 20 min, once in the morning and once in the afternoon, and each pollinator was identified and counted. The trees were randomly sampled from the selected orchard. To do the survey, the researcher walked between the rows and counted the numbers of insects on all 10 selected trees, about 2 min were given to each tree and then the researcher moved to the next tree and so on. Flower-visiting insects were collected with a net and released after identification. To avoid the recounting of pollinators, each was marked with yellow paint (Uni-Posca, Japan; honey bee queen marking colour). If identification was not possible in the field, the insects were anaesthetized with ether and shifted to the laboratory where identification to species was performed. All recording was done by the same observers each year.

2.3. Loquat fruit quality and quantity

Fruit quantity and quality was measured in all study sites in the region. No thinning of flowers and fruits was practised in any loquat orchard at any experimental site. In each site, the same ten trees that had been observed for pollinators were assessed for yield each year. Fruit harvesting was done on the same day (from all sites) at full ripening for all trees selected. To measure the yield (kg) of the selected trees, all fruits were harvested and weighed using an electric balance.

Additionally, four branches per tree were selected and the size, sugar content and organic acid content of fruit were measured. Individual fruit weights were measured for about 20 randomly chosen fruits from each branch. To study the sugar content and acidity of the fruits we randomly selected 10 of these fruits from each tree from each site. The sugar

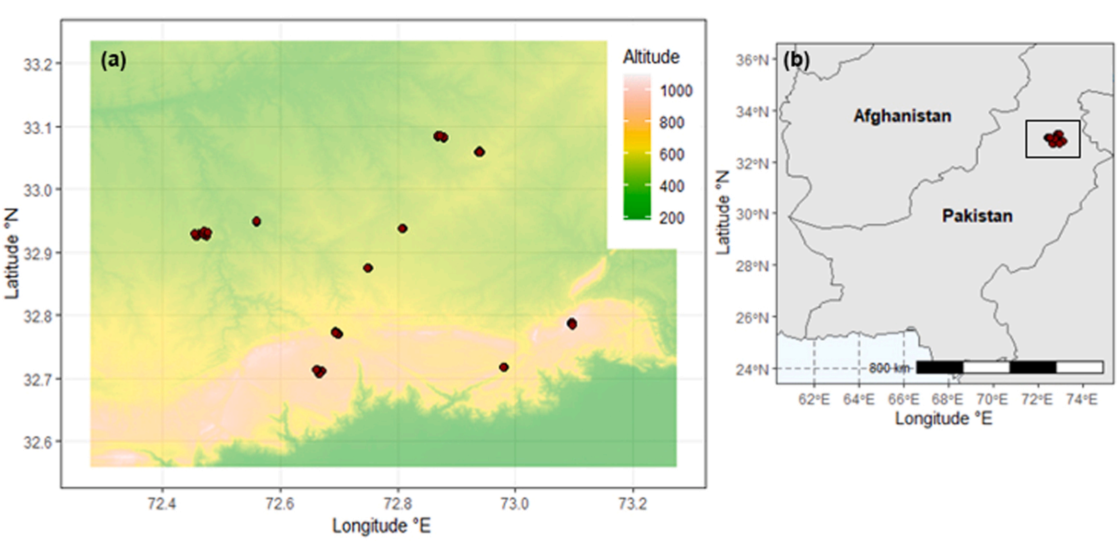

Fig. 1. Maps of 10 study sites (a) in the Pothwar region in Pakistan (b). Altitude is given in m. Overlapping dots indicate clusters of loquat trees within a site. Maps were created with the R packages 'ggplot2' and 'sf' using data from 'rnaturalearth'.

content was measured in Brix (% of sugar by mass) using a digital refractometer. Loquat juice acidity was determined using titration against NaOH with phenolphthalein as an indicator. It was expressed as mg/100 g FW of malic acid and was calculated following methods in Hong et al. (2008) and Garen et al. (2016).

2.4. Netted control trees

In each site, one additional loquat tree was randomly selected. The whole tree was covered with mosquito netting. After covering the tree, one 'mini nucleus hive' of *Apis mellifera* (a small hive containing about 300 worker bees + queen) was placed inside the net during the blooming periods to ensure pollination. As with un-netted trees, total yield from each tree was measured, and four branches were selected on each tree (40 branches in total across the ten sites) to assess fruit quality (20 fruits per branch were weighed, and a total of 10 randomly selected fruits used for assessing sugar and acidity).

2.5. Statistical analysis

The temporal dynamics across all insect taxa combined, pollinator species richness, loquat fruit quantity and quality as well as their associations were analysed using linear mixed models (LMMs), general additive mixed models (GAMMs), generalised least square (GLSs) and generalised additive models (GAMs). We conducted all models in R version 4.0.3 (R Core Team, 2020), using the R packages 'mgcv' (Wood, 2017) and 'nlme' (Pinheiro et al., 2020) for statistical analyses and 'ggplot2′ (Wickham, 2016) for some figures. We constructed various models per response variable and identified the best model using the Akaike's information criterion (AIC; Burnham and Anderson, 2004). The AIC of a model reflects both its goodness of fit and its number of parameters with better models having lower AIC values. Models with ΔAIC of ≤ 2 were interpreted of having the same explanatory power; models with ΔAIC of > 2 were interpreted as significantly different.

The total abundance of insects per year and per tree (*i.e.* 10 values per site per year) was the response variable in the first set of LMMs and GAMMs. To adhere to model assumptions, this response variable was square root transformed throughout. The variable 'year' was either included or excluded in models to examine the effect of time on insect abundance on loquat trees. We allowed for random intercepts for different sites. We also assessed other structures of random terms (including random intercepts for tree ID and tree ID nested within site) but these models had similar or higher AICs compared to models with 'site' as the only random term and are not presented here. We considered linear and non-linear effects of time using LMMs and GAMMs, respectively. In both approaches, we considered presence and absence of temporal autocorrelations of residuals. We used AR-1 autocorrelation (*i.e.*, auto-regressive model of the order 1, see Zuur et al., 2009), which assumes that pairs of residuals that are temporally further apart have lower correlations than those closer together. We set the structure of the AR-1 autocorrelation to resemble the temporal order of year on each level of the grouping variable (*i.e.* 'site'). In GAMMs we also assessed whether model fit was improved by a regression spline with shrinkage, which allows smoothers of non-linear trends to have zero degrees of freedom (Zuur et al., 2009). All models without 'year' as explanatory variable had lower explanatory power than the respective model with 'year' as explanatory variable and hence the above model specifications did not change the interpretation of the results.

In addition, we used GLSs and GAMs with interactions between 'year' and 'site' as explanatory variables to assess whether temporal changes in pollinator abundance on loquat trees were similar across sites (analyses presented in the Supplement). We compared these models to models that included additive effects of 'site' and 'year' or either of these main effects. We allowed for linear (GLSs) and non-linear effects (GAMs). The latter were only run for models including 'year' but not those including the factor 'site' only. We ran models with (GLSs only) and without autocorrelation (GLSs and GAMs).

The second set of models included pollinator species richness as response variable (number of pollinator species recorded on a loquat tree per year). The structure of the models was otherwise the same as for pollinator abundance.

To analyse whether pollinator abundance, pollinator species richness or other changes in the environment best predict loquat fruit characteristics (quantity and quality), we used sets of models with fruit yield (kg/tree per year), fruit sugar content (Brix, mean per tree per year) and total content of organic acids (hereafter fruit acidity; in mg/100 g FW; mean per tree per year), respectively, as response variables. Fixed terms included the year, pollinator abundance and pollinator species richness. Otherwise, the models were as specified above (*i.e.* we used LMMs with linear trends and GAMMs with non-linear trends, both with and without autocorrelation). For each of the fruit characteristics we also conducted GLSs and GAMs as outlined above (results in Supplement). It should be noted that pollinator abundance and species richness were highly correlated in some years (with a correlation coefficient of larger than 0.7, this applies to 7 of the 13 years). Therefore some of the results for abundance and species richness might not be completely independent.

Data obtained from netted control trees with supplemented pollination by honey bees were analysed with LMMs, GAMMs, GLSs (Supplement) and GAMs (Supplement) with the same model structure as outlined above. LMMs and GAMMs contained only 'year' as fixed term (or no fixed term) while GLSs and GAMs included 'year' and/or 'site' (or their interaction) as explanatory variables. Response variables were fruit yield, fruit sugar and fruit acidity (one control tree per site per year).

3. Results

3.1. Flower-visiting insect abundance and diversity over time

As *E. japonica* starts flowering in late winter, a relatively limited numbers of insect species visited its flowers. A total of 54,689 flower-visiting insects were recorded and identified over the 13 years. These comprised 31 species spanning four families (*Apidae*, *Syrphidae, Megachilidae,* and *Halictidae*) in two orders (Diptera and Hymenoptera) (Table 1**)**. The most common flower-visiting insects recorded were four honeybee species, *Apis florea, A. cerana, A. mellifera* and *A. dorsata*, which were all approximately equally abundant (Table 1), and collectively comprised 83% of insect visitors. Twenty-two other species of bee were recorded, all relatively scarce, including 8 genera, *Bombus*, *Thyreus*, *Osmia*, *Ceratina*, *Xylocopa*, *Amegilla*, *Anthophora*, and *Pseudapis*. Five species of hoverflies comprised the remaining 8.2% of visits (Table 1).

The abundance of flower-visiting insects decreased during the 13 years of the study non-linearly (Fig. 2a, Table 2). The abundance decreased most between 2006 and 2009, and more slowly afterwards. The total number of insects recorded in 2018 (1264) represented a fall in abundance of 89% compared to 2006 (12,504; Table 3). Sites differed in the rate of decline across time (Supplement: Fig. S1, Table S1). Sites 1, 5 and 10 showed a smaller decline in pollinator numbers than the other sites, the remaining seven sites showed drastic drops in pollinator numbers (Fig. S1).

Declines affected all insect flower visitors (Table 3). The most dramatic declines were amongst the honeybees, in particular *A. dorsata* and *A. mellifera* which declined by 97% and 96%, respectively, over the 13 years of the study. Of the more abundant flower visitors (the 12 species for which more than 400 individuals were recorded over the duration of the study period), *Amegilla niveocincta* showed the smallest decline, but still fell in abundance by 66% (Table 3). Four bee species and one hoverfly species that were present (though all relatively scarce) at the beginning of the study were not recorded at all in either of the last two years (*Thyreus himalayensis, Osmia cornifrons, O. caerulescens, Xylocopa collaris, Ischiodon scutellaris*).

Similar to the abundance, the species richness of flower-visiting insects decreased sharply over time (Fig. 2b, Table 2). The model that

Table 1
Pollinators on loquat trees during the period 2006–2018 in Pothwar region (Pakistan). The final column shows the number of years (out of 13) in which the species was recorded.

Groups/Species	Family	Abundance (%)	Years recorded
Honeybees		**83.1**	**13**
Apis cerana	Apidae	21.1	13
Apis dorsata	Apidae	15.4	13
Apis florea	Apidae	29.5	13
Apis mellifera	Apidae	17.2	13
Bumblebees		**1.0**	**13**
Bombus haemorrhoidalis	Apidae	0.8	13
Bombus asiaticus	Apidae	0.1	12
Bombus trifasciatus	Apidae	0.1	10
Cuckoo bees		**1.7**	**13**
Thyreus ramosus	Apidae	1.6	13
Thyreus himalayensis	Apidae	0.1	9
Mason bees		**0.2**	**7**
Osmia sp.	Megachilidae	0.1	6
Osmia cornifrons	Megachilidae	0.1	6
Osmia caerulescens	Megachilidae	0.1	6
Hoverflies		**8.17**	**13**
Eupeodes corollae	Syrphidae	2.5	13
Eristalis similis	Syrphidae	3.7	13
Eristalinus aeneus	Syrphidae	1.8	13
Ischiodon scutellaris	Syrphidae	0.1	9
Cheilosia albipila	Syrphidae	0.1	10
Carpenter bees		**2.1**	**13**
Ceratina sexmaculata	Apidae	1.1	13
Ceratina binghami	Apidae	0.2	13
Xylocopa basalis	Apidae	0.23	13
Xylocopa auripennis	Apidae	0.22	13
Xylocopa aestuans	Apidae	0.2	13
Xylocopa collaris	Apidae	0.1	11
Xylocopa fenestrata	Apidae	0.1	11
Blue banded bees		**2.4**	**13**
Amegilla niveocincta	Apidae	1.0	13
Amegilla insularis	Apidae	0.4	13
Amegilla confusa	Apidae	0.4	13
Amegilla cingulata	Apidae	0.5	13
Amegilla zonata	Apidae	0.1	13
Sweat bees		**1.0**	**13**
Pseudapis oxybeloides	Halictidae	1.0	13
Flower bees		**0.5**	**13**
Anthophora pulcherrima	Apidae	0.5	13

included a steady, linear decline had a slightly lower AIC than the model with a non-linear decline (Δ AIC = 2) but predictive power of both was very similar. Species richness decreased at different rates across sites over time, with about half the sites showing very steep declines across time (Fig. S2, Table S1).

3.2. Changes in fruit quantity and quality over time and with changes in flower-visiting insect abundance and diversity

For the open-pollinated trees, there was a marked non-linear decline in the overall fruit yield over time and with decreasing abundance of flower-visiting insects, with species richness being less important (Fig. 3a, Table 4). Sites did not differ in their rate of decline over time (Fig. S3, Table S1). Yield dropped from an average of 47.3 kg per tree in 2006 to 18.6 kg per tree in 2018 (60.7% drop). The weight of individual fruits declined by 53.6% from 35.1 g to 16.3 g.

Chemical characteristics of the fruits also changed significantly over time (Fig. 3c, e, Table 4). Sugar content fell markedly (61.8%), from 15.2 Brix in 2006 to 5.80 in 2018 with decreasing pollinator abundance. Models including and excluding pollinator species richness were similar in their explanatory power. The organic acid content fell from 843 mg/100 g FW in 2006 to 466mg/100 g FW in 2018 (44.7%) with both decreasing pollinator abundance and richness. Sugar content and organic acid content fell also with decreasing pollinator abundance (Fig. 3c, e, Table 4). The organic acid content additionally decreased with declines in species richness of flower-visiting insects (Fig. 3e, Table 4), while the models for sugar content with and without species richness were similar in their explanatory power (Δ AIC ≤ 2, see Table 4).

Importantly, there was no corresponding decline over time in the yield or other fruit characteristics of the netted control trees pollinated by *A. mellifera*, with yields per tree remaining more or less stable over time (Fig. 3b, d, f, Table 5). Fruit yields were similar to those recorded for the open-pollinated trees in 2006 (Fig. 3a, b). Sugar content and organic acid content of fruit from these netted trees also both remained stable over the 13 years (Fig. 3c-f).

4. Discussion

In 2006, loquats in Pothwar region attracted a diversity of pollinators, mainly wild bees with some hoverflies (Syrphidae). Over the thirteen years of the study, pollinator numbers fell dramatically, by 89%

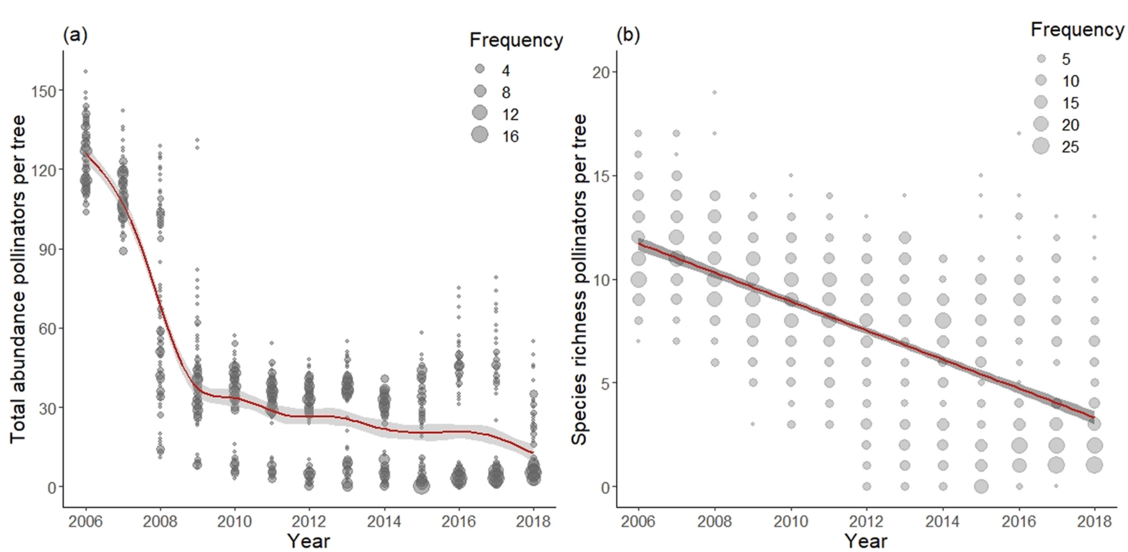

Fig. 2. Changes in (a) total pollinator abundance and (b) species richness over time across the ten study sites. Shown are raw data (dot sizes indicate frequencies), predicted means (red lines) and 95% confidence intervals (grey lines) obtained from the ‘geom_smooth’ function (method (a) ‘gam’ and (b) ‘lm’; ggplot2 package; Wickham, 2016). Model structures used for statistical analyses are presented in Table 2.

Table 2
Summaries of LMMs and GAMMs with the total number of pollinators per tree and year (abundance) and species richness as response variables. Models allowed for linear or non-linear trends, respectively, and were run with and without correcting for temporal autocorrelation. Random terms (random intercept) in all models included site. For models with shrinkage no null model was run. For each response variable, the model with the lowest AIC is highlighted in bold and with grey background; model(s) with Δ AIC ≤ 2 compared to the best model are also highlighted in grey.

Response	Model	Model type	x variable	Trend	Auto-correlation	df	AIC
Abundance (square root)	1a	LMM	Year	linear	no	4	5273.2
	1b	LMM	—	(linear)	no	3	6471.5
	2a	LMM	Year	linear	yes	5	3839.0
	2b	LMM	—	(linear)	yes	4	3961.2
	3a	GAMM	Year	non-linear	no	5	4724.5
	3b	GAMM	—	(non-linear)	no	3	6471.5
	3c	GAMM	Year	non-linear, shrinkage	no	4	4732.8
	4a	**GAMM**	**Year**	**non-linear**	**yes**	**6**	**3768.9**
	4b	GAMM	—	(non-linear)	yes	4	3961.2
	4c	GAMM	Year	non-linear, shrinkage	yes	5	3773.0
Species richness (SR)	1a	LMM	Year	linear	no	4	6135.8
	1b	LMM	—	(linear)	no	3	7083.1
	2a	**LMM**	**Year**	**linear**	**yes**	**5**	**5858.0**
	2b	LMM	—	(linear)	yes	4	6089.7
	3a	GAMM	Year	non-linear	no	5	6134.9
	3b	GAMM	—	(non-linear)	no	3	7083.1
	3c	GAMM	Year	non-linear, shrinkage	no	4	6139.9
	4a	GAMM	Year	non-linear	yes	6	5860.0
	4b	GAMM	—	(non-linear)	yes	4	6089.7
	4c	GAMM	Year	non-linear, shrinkage	yes	5	5866.1

Table 3
Total abundance of the most common flower-visiting insects, plus the grand total of all insects, from 2006 to 2018.

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	% decrease
Apis cerana	2791	3109	1679	830	554	440	472	433	432	237	167	182	186	93
Apis dorsata	2847	2298	926	434	361	319	279	257	201	167	137	103	87	97
Apis mellifera	2840	2040	1061	555	498	477	413	304	301	301	267	232	113	96
Apis florea	2734	2389	1754	1260	989	998	796	897	713	809	1153	966	652	76
Bombus haemorrhoidalis	67	71	53	55	49	21	28	22	12	21	13	12	11	84
Thyreus ramosus	111	133	89	101	91	118	41	41	21	35	22	22	27	76
Eristalis similis	266	231	156	125	148	104	118	224	214	161	132	81	44	84
Eupeodes corollae	157	129	105	81	140	136	112	154	86	92	90	81	20	87
Eristalinus aeneus	167	96	160	149	158	67	31	63	15	26	25	21	15	91
Ceratina sexmaculata	88	114	103	68	61	61	24	19	12	22	23	10	18	80
Amegilla niveocincta	65	84	75	54	42	19	57	50	30	13	25	21	22	66
Pseudapis oxybeloides	73	69	59	56	49	47	46	59	36	20	20	23	14	81
Other insects	298	305	225	150	133	133	121	127	74	68	81	61	55	82
Total insects	**12504**	**11068**	**6445**	**3918**	**3273**	**2940**	**2538**	**2650**	**2147**	**1972**	**2155**	**1815**	**1264**	**90**

overall, with honeybees being most affected but almost all flower-visiting insects showing sharp declines. Declines were not linear, being most rapid in the period 2006–2009. Over the study period, total yield of loquats per tree also dropped by 61%, with significant declines in measures of fruit quality such as sugar content. Again, the declines were not linear, with the sharpest falls in the period 2007–2009, approximately corresponding to the period of most rapid pollinator decline. Total pollinator abundance was a powerful predictor of yield and quality, while pollinator species richness had less predictive power. The positive relationship between pollinator numbers and crop yield across multiple sites strongly suggests a causative relationship, but from this alone we could not rule out the possibility of a third factor such as changing climate simultaneously driving both insect declines and yields. However, trees that were enclosed in netting with a small honeybee colony during flowering showed no drop in yield or fruit quality over time, leading us to conclude that by far the most likely explanation for the declines in fruit harvest is inadequate pollination.

The effect of pollination on loquat fruit physical characteristics has previously been reported (Freihat et al., 2008). Freihat et al. (2008) demonstrated the impact of different pollination methods on the fruit set and quality of loquat. They found that preventing pollinator access strongly affected the fruit size and weight of fruit as compared to open

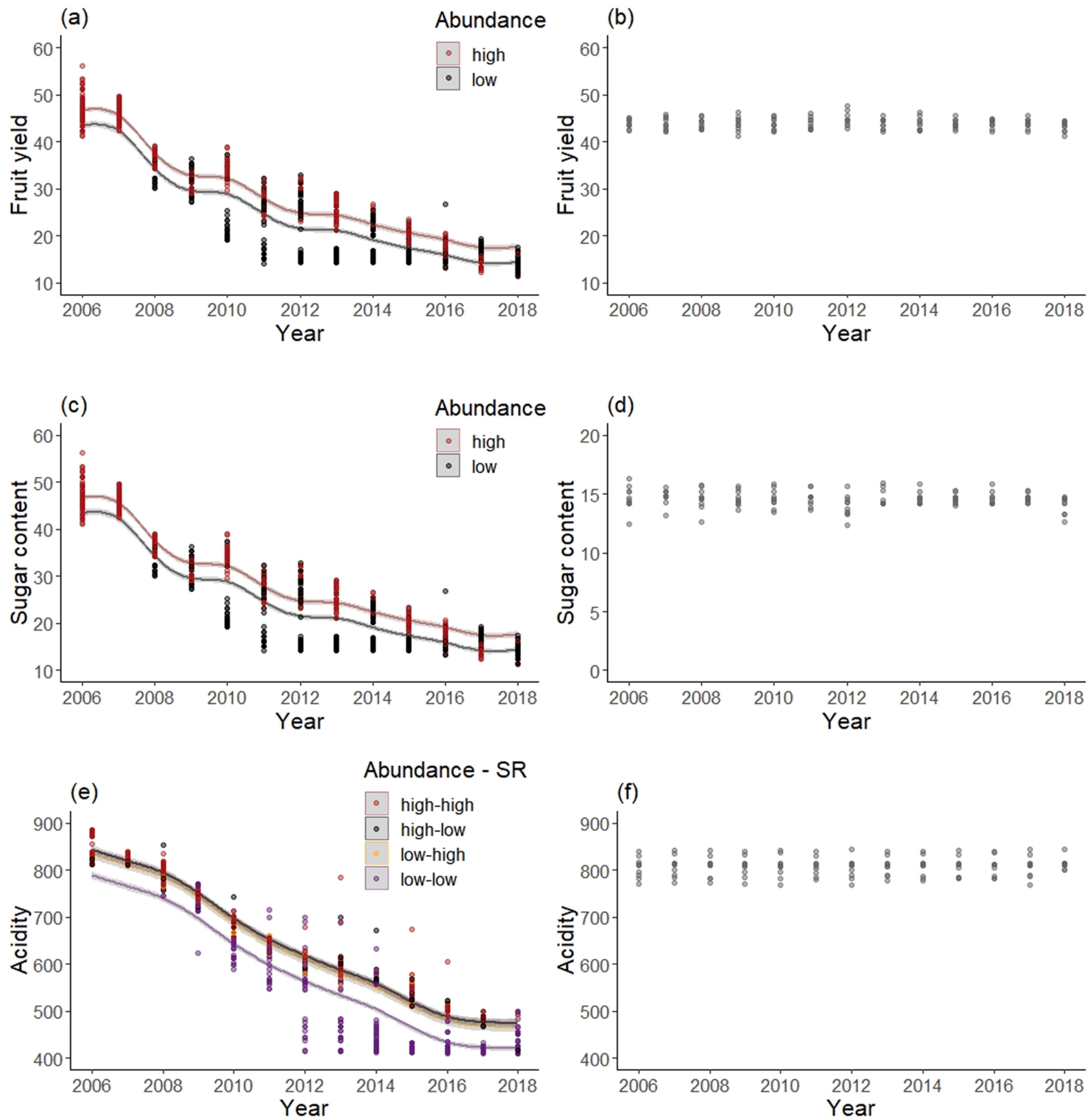

Fig. 3. Changing loquat fruit yield (kg/tree), sugar content (Brix) and content of organic acids (in mg/100 g FW) over time at the ten study sites: (a, c, e) Open pollinated trees; (b, d, f) netted control trees with supplemented pollination by honey bees. For graphical purposes predicted relationships are shown for high and low abundance of pollinators (cut-off point: median abundance; a, c) and for combinations of high and low abundance of pollinators and pollinator species richness (cut-off point: median species richness; e). Shown are predicted means (red lines) and 95% confidence intervals (grey lines) obtained from the 'geom_smooth' function (method (a, c, e) 'gam'; ggplot2 package; Wickham, 2016). In analyses, pollinator abundance and species richness were analysed as continuous variables (for model structures see Tables 4 and 5).

pollination (Garratt et al., 2014; Abrol et al., 2019). Various studies have reported that insect pollinators affect not only the quantity of production but also quality parameters such as sugar content and acidity in other fruits including apples (Brookfield et al., 1996; Garratt et al., 2014), mangoes (Rafique et al., 2016) and strawberries (Abrol et al., 2019).

A key question remains; what is the cause of the major decline in almost all of the pollinators in our study? Insect declines around the world are well documented, although with very few long-term data sets from Asia. However, few reported declines are as rapid as we describe here. For example, Hallmann et al., (Hallmann et al., 2017) described a 76% drop in flying insect numbers across Germany over 26 years, a report that gained considerable attention for revealing a pace of decline that had not previously been recognized. More recently, Seibold et al. (2019) describe a 78% drop in abundance of grassland arthropods in Germany over ten years, comparable with our finding of an 89% drop in 13 years. The causes of such rapid collapse of pollinator populations in Pothwar region are likely to include deforestation, habitat loss, the rapid spread of intensive monoculture cropping of wheat, mustard and maize and associated intensive pesticide use, and perhaps also climate change. In Maoxian, China, apple and pear farmers have been resorting to hand pollination of their crops since the late 1980s, with high levels of pesticide use thought to be the cause of inadequate pollination by insects (Partap and Partap, 1997; Partap and Ya, 2012). The rapid decline in pollinator abundance that we observed seems unlikely to be due to gradual change of the landscape, but is more likely to be driven by a switch in agricultural practice, such as adoption of a new pesticide (*e.g.* neonicotinoids). Unfortunately, detailed records of factors such as changing pesticide use are not readily available for Pothwar; obtaining such data represents a significant challenge for future research. The differential extent of the collapse of insect populations at the different study sites ought to provide a clue (sites 1, 5 and 10 suffered less sharp declines), for perhaps it could be related to differences in the patterns of land use change nearby.

Table 4
Summaries of LMMs and GAMMs with the different loquat fruit characteristics as response variables. Models allowed for linear or non-linear trends, respectively, and were run with and without correcting for temporal autocorrelation. Random terms (random intercept) in all models included site. For models with shrinkage no null model was run. For each response variable, the model with the lowest AIC is highlighted in bold and with grey background; model(s) with Δ AIC ≤ 2 compared to the best model are also highlighted in grey. SR, pollinator species richness (number of species).

Response	Model	Model type	x variable(s)	Trend	Auto-correlation	df	AIC
Fruit yield	1a	LMM	Year, Abundance, SR	linear	no	6	6767.1
	1b	LMM	Year, Abundance	linear	no	5	6765.3
	1c	LMM	Year, SR	linear	no	5	7347.5
	1d	LMM	Abundance, SR	linear	no	5	8061.2
	1e	LMM	Year	linear	no	4	7441.4
	1f	LMM	Abundance	linear	no	4	8181.6
	1g	LMM	SR	linear	no	4	8927.9
	1h	LMM	—	(linear)	no	3	9928.7
	2a	LMM	Year, Abundance, SR	linear	yes	7	5796.3
	2b	LMM	Year, Abundance	linear	yes	6	5794.5
	2c	LMM	Year, SR	linear	yes	6	5843.0
	2d	LMM	Abundance, SR	linear	yes	6	6043.9
	2e	LMM	Year	linear	yes	5	5843.9
	2f	LMM	Abundance	linear	yes	5	6042.4
	2g	LMM	SR	linear	yes	5	6095.9
	2h	LMM	—	(linear)	yes	4	6096.4
	3a	GAMM	Year, Abundance, SR	non-linear	no	9	6256.6
	3b	GAMM	Year, Abundance	non-linear	no	7	6287.8
	3c	GAMM	Year, SR	non-linear	no	7	6459.4
	3d	GAMM	Abundance, SR	non-linear	no	7	7963.1
	3e	GAMM	Year	non-linear	no	5	6614.3
	3f	GAMM	Abundance	non-linear	no	5	8040.9
	3g	GAMM	SR	non-linear	no	5	8905.0
	3h	GAMM	—	(non-linear)	no	3	9928.7
	4a	GAMM	Year, Abundance, SR	non-linear	yes	10	5584.4
	4b	**GAMM**	**Year, Abundance**	**non-linear**	**yes**	**8**	**5580.8**
	4c	GAMM	Year, SR	non-linear	yes	8	5619.4
	4d	GAMM	Abundance, SR	non-linear	yes	8	5992.6
	4e	GAMM	Year	non-linear	yes	6	5626.9
	4f	GAMM	Abundance	non-linear	yes	6	5989.4
	4g	GAMM	SR	non-linear	yes	6	6094.0
	4h	GAMM	—	(non-linear)	yes	4	6096.4
Fruit sugar	1a	LMM	Year, Abundance, SR	linear	no	6	3995.7
	1b	LMM	Year, Abundance	linear	no	5	4120.2
	1c	LMM	Year, SR	linear	no	5	4104.2
	1d	LMM	Abundance, SR	linear	no	5	5456.2
	1e	LMM	Year	linear	no	4	4352.7
	1f	LMM	Abundance	linear	no	4	5760.6
	1g	LMM	SR	linear	no	4	5925.5
	1h	LMM	—	(linear)	no	3	7111.3
	2a	LMM	Year, Abundance, SR	linear	yes	7	3594.0
	2b	LMM	Year, Abundance	linear	yes	6	3603.8
	2c	LMM	Year, SR	linear	yes	6	3632.7
	2d	LMM	Abundance, SR	linear	yes	6	3948.6
	2e	LMM	Year	linear	yes	5	3654.1
	2f	LMM	Abundance	linear	yes	5	3947.7

(continued on next page)

Table 4 (*continued*)

	2g	LMM	SR	linear	yes	5	3980.3
	2h	LMM	—	(linear)	yes	4	3986.1
	3a	GAMM	Year, Abundance, SR	non-linear	no	9	3581.0
	3b	GAMM	Year, Abundance	non-linear	no	7	3583.2
	3c	GAMM	Year, SR	non-linear	no	7	3716.2
	3d	GAMM	Abundance, SR	non-linear	no	7	5361.2
	3e	GAMM	Year	non-linear	no	5	4091.0
	3f	GAMM	Abundance	non-linear	no	5	5474.4
	3g	GAMM	SR	non-linear	no	5	5869.2
	3h	GAMM	—	(non-linear)	no	3	7111.3
	4a	GAMM	Year, Abundance, SR	non-linear	yes	10	3458.0
	4b	**GAMM**	**Year, Abundance**	**non-linear**	**yes**	**8**	**3456.5**
	4c	GAMM	Year, SR	non-linear	yes	8	3539.0
	4d	GAMM	Abundance, SR	non-linear	yes	8	3952.6
	4e	GAMM	Year	non-linear	yes	6	3588.0
	4f	GAMM	Abundance	non-linear	yes	6	3949.7
	4g	GAMM	SR	non-linear	yes	6	3982.3
	4h	GAMM	—	(non-linear)	yes	4	3986.1
Fruit acidity	1a	LMM	Year, Abundance, SR	linear	no	6	12970.0
	1b	LMM	Year, Abundance	linear	no	5	13128.0
	1c	LMM	Year, SR	linear	no	5	13054.3
	1d	LMM	Abundance, SR	linear	no	5	15067.4
	1e	LMM	Year	linear	no	4	13337.1
	1f	LMM	Abundance	linear	no	4	15377.9
	1g	LMM	SR	linear	no	4	15513.8
	1h	LMM	—	(linear)	no	3	16695.6
	2a	LMM	Year, Abundance, SR	linear	yes	7	12391.5
	2b	LMM	Year, Abundance	linear	yes	6	12402.1
	2c	LMM	Year, SR	linear	yes	6	12407.2
	2d	LMM	Abundance, SR	linear	yes	6	12655.5
	2e	LMM	Year	linear	yes	5	12426.6
	2f	LMM	Abundance	linear	yes	5	12655.8
	2g	LMM	SR	linear	yes	5	12667.9
	2h	LMM	—	(linear)	yes	4	12673.4
	3a	GAMM	Year, Abundance, SR	non-linear	no	9	12507.1
	3b	GAMM	Year, Abundance	non-linear	no	7	12530.3
	3c	GAMM	Year, SR	non-linear	no	7	12544.8
	3d	GAMM	Abundance, SR	non-linear	no	7	14959.1
	3e	GAMM	Year	non-linear	no	5	13026.8
	3f	GAMM	Abundance	non-linear	no	5	15122.7
	3g	GAMM	SR	non-linear	no	5	15453.6
	3h	GAMM	—	(non-linear)	no	3	16695.5
	4a	**GAMM**	**Year, Abundance, SR**	**non-linear**	**yes**	**10**	**12252.1**
	4b	GAMM	Year, Abundance	non-linear	yes	8	12257.5
	4c	GAMM	Year, SR	non-linear	yes	8	12296.9
	4d	GAMM	Abundance, SR	non-linear	yes	8	12658.9
	4e	GAMM	Year	non-linear	yes	6	12351.3
	4f	GAMM	Abundance	non-linear	yes	6	12655.9
	4g	GAMM	SR	non-linear	yes	6	12669.9
	4h	GAMM	—	(non-linear)	yes	4	12673.4

Table 5
Summaries of LMMs and GAMMs with the different fruit characteristics of the netted control trees (pollinated by honey bees) as response variables. Models allowed for linear or non-linear trends, respectively, and were run with and without correcting for temporal autocorrelation. Random terms (random intercept) in all models included site. For models with shrinkage no null model was run. For each response variable, the model with the lowest AIC is highlighted in bold and with grey background; model(s) with Δ AIC ≤ 2 compared to the best model are also highlighted in grey.

Response	Model	Model type	x variable	Trend	Auto correlation	df	AIC
Fruit yield	1a	LMM	Year	linear	no	4	411.5
	1b	**LMM**	**—**	**(linear)**	**no**	**3**	**410.0**
	2a	LMM	Year	linear	yes	5	413.5
	2b	LMM	—	(linear)	yes	4	412.0
	3a	GAMM	Year	non-linear	no	5	413.5
	3b	**GAMM**	**—**	**(non-linear)**	**no**	**3**	**410.0**
	3c	GAMM	Year	non-linear, shrinkage	no	4	412.0
	4a	GAMM	Year	non-linear	yes	6	415.5
	4b	GAMM	—	(non-linear)	yes	4	412.0
	4c	GAMM	Year	non-linear, shrinkage	yes	5	414.0
Fruit sugar	1a	LMM	Year	linear	no	4	287.5
	1b	LMM	—	(linear)	no	3	287.6
	2a	LMM	Year	Linear	yes	5	286.6
	2b	**LMM**	**—**	**(linear)**	**yes**	**4**	**286.5**
	3a	GAMM	Year	non-linear	no	5	289.5
	3b	GAMM	—	(non-linear)	no	3	287.6
	3c	GAMM	Year	non-linear, shrinkage	no	4	289.3
	4a	GAMM	Year	non-linear	yes	6	288.6
	4b	**GAMM**	**—**	**(non-linear)**	**yes**	**4**	**286.5**
	4c	GAMM	Year	non-linear, shrinkage	yes	5	288.3
Fruit acidity	1a	LMM	Year	Linear	no	4	973.4
	1b	LMM	—	(linear)	no	3	973.4
	2a	LMM	Year	Linear	yes	5	964.5
	2b	**LMM**	**—**	**(linear)**	**yes**	**4**	**964.2**
	3a	GAMM	Year	non-linear	no	5	975.4
	3b	GAMM	—	(non-linear)	no	3	973.4
	3c	GAMM	Year	non-linear, shrinkage	no	4	975.1
	4a	GAMM	Year	non-linear	yes	6	966.5
	4b	**GAMM**	**—**	**(non-linear)**	**yes**	**4**	**964.2**
	4c	GAMM	Year	non-linear, shrinkage	yes	5	966.1

Our findings are highly relevant to the farmers in the area and the companies that export loquats, since Pakistan holds a prominent place in the cultivation of loquat, producing 1280,000 tons in 2002 (Khan, 2003). Our data suggest that widespread and rapid declines of bees and other pollinators are very likely to be the cause of major declines in fruit quantity and quality. These declines are likely to affect other insect-pollinated crops in the region, which include peaches, citrus fruits, oilseed rape and linseed. We argue that research is urgently required to understand why pollinators are declining so rapidly in this region, and that in the meantime it would be wise to begin interventions to mitigate pollinator declines, such as reducing pesticide use and increasing floral diversity in the landscape.

We did not attempt to investigate which loquat flower visitors are the most effective pollinators. It is often assumed that visitation rate is a good predictor of pollination service delivery (see for example Kleijn et al., 2015), but this is not always true. In apples, *Osmia cornuta* have been found to deposit five times as much pollen as *A. mellifera* per flower visit, due to increased body contact with the reproductive parts of the flower (Vicens and Bosch, 2000). Clearly further investigation is needed to establish which of the insect visitors to loquat deliver most pollen transfer. Specific measures could then be introduced to encourage them, following the example of Gruber et al. (2011).

Author contributions

M.T. planned and started the experiments and surveys from 2006 to 2011; S.A.K. had joined the research and continued until 2018; S.A.K. wrote the first draft of the manuscript. M.M., S.A., M.N. and Z.N. helped in writing, review and editing. D.G., W.S. and C.D. performed the statistical analyses and edited the manuscript, W.S. and C.D. created the figures. All authors revised the manuscript and approved it for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.108138.

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