



Pollination services in the North China Plain measured using buckwheat sentinel plants; is there a deficit?

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ABSTRACT

The transition of food systems for healthy diets and sustainable food systems calls for greater production of fruit and nuts, i.e. pollinated crops. The North China Plain (NCP) is a cereal dominated area that needs to transition to a more sustainable food system with a nutritionally more diverse output. Buckwheat pairs high nutritional quality with low input requirement, but it is pollination dependent. There is little knowledge on the pollinator communities and pollination service in the NCP. We selected Quzhou county as a study region on the NCP, and used potted plants of buckwheat (*Fagopyrum esculentum* M.) to measure pollination in three treatments: insect exclusion, open pollination, and honeybee supplementation. Measurements were made at 24 sites across the county in 2021 and 2022. At each site, we directly observed the activity of flower-visiting insects and measured the pollination rate, seed set, seed abortion rate and yield-related indicators. Most flower visits were made by hoverflies (Syrphidae) and flies from the families Muscidae, Sarcophagidae, and Calliphoridae. Compared with open pollination, insect exclusion reduced buckwheat pollination rate and seed set by more than 90 %. Without honeybee supplementation, the pollination rate in different experimental sessions was reduced by 12–21 %, and the seed set was reduced by 22–33 %. Honeybee supplementation increased harvest index and seed weight per plant with factors of 1.6 and 2.0, respectively. Hence, a pollination deficit would exist if only natural pollinators were relied upon. Managed honeybees provide an efficient means for enhancing pollination of buckwheat in the North China Plain.

1. Introduction

Pollination is a critical ecosystem service that profoundly impacts human well-being. Three-quarters of global food crops depend at least partially on pollination, and more than one-third of the global crop production volume is derived from crops reliant on pollinators (Klein et al., 2007). Globally, from 1961 to 2016, the percentage growth in area of pollinator-dependent crops was greater than that of pollinator-independent crops, and the proportion of pollinator-dependent crops in total agricultural area increased by 70 % (Aizen et al., 2019). In China, the area of pollinator-dependent crops has increased by 50 % from 1961 to 2018, while pollinator-independent crops expanded by 14 % (Mashilingi et al., 2021). The demand for a healthy plant-based diet rich in fruits, vegetables, and nuts (Smith et al., 2015) is thus driving an increase in demand for pollination services, both globally and in China.

While the need for pollination services increased over the last decades, insect abundance and diversity show a declining trend (Hallmann et al., 2017; Sánchez-Bayo and Wyckhuys, 2019; Simmons et al., 2019). The long-term losses in honeybee colonies in the US and the downward population trend of pollinators in some EU countries caught public attention (Althaus et al., 2021). Despite an increase in managed beehives, the density of honeybees per hectare of pollinator-dependent crops in China has remained constant and it has fallen below the average colony density in other countries (Teichroew et al., 2017).

Semi-natural habitats in the farming landscape are beneficial for pollinator diversity, while land consolidation reduces pollinator diversity (Shi et al., 2021). A low proportion of non-crop areas in the landscape, such as the woodland, is associated with lower diversity and abundance of wild bees (Gaines-Day and Gratton, 2016). Misuse and overuse of pesticides in crop production (Mu et al., 2022) negatively affects the living environment for both wild bees and managed

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honeybees. Insect decline is thought to be driven by a combination of factors such as habitat loss, insecticide pollution, nitrification (resulting in loss of flowering plants), urbanization, and global warming, i.e. "death by a thousand cuts" (Wagner et al., 2021).

In the USA, declines in wild and managed bee populations have led to decreased yield for five of seven major pollinator-dependent crops (Reilly et al., 2020). Field experiments conducted in six European countries demonstrated a pollination deficit resulting in an average yield loss of 2.8 % for oilseed rape, pear, sunflower, and pumpkin (Holland et al., 2020). While evidence of pollinator decline and pollination deficit have been found globally, focusing on Europe and America, limited information is available from China. The North China Plain is the largest food production base in China. The region is marked by simplified landscapes, dominated by cereals, with intensive irrigation, high inputs of nitrogen fertilizer and chemical protectants, resulting in greenhouse gas emissions, pesticide contamination, and groundwater depletion (Liang et al., 2022). About 59 % of the arable land in this region is covered with wheat-maize double-cropping (Li and Lei, 2021). Although being predominantly a cereal production region, the North China Plain has seen a continuous increase in the proportion of pollinator-dependent crops, such as fruit trees and vegetables, and also including buckwheat, even though buckwheat is a minor crop in this area (Zhang et al., 2019). There is very little information on the status of pollinator communities and the pollination services provided by them in this region.

Here we measured pollination services in the North China Plain using potted plants of common buckwheat (*Fagopyrum esculentum* M.). Common buckwheat belongs to the family *Polygonaceae*. Buckwheat is becoming more popular in Asia and most Western European countries due to its nutritional benefits, in relation to the high concentration in the grain of protein and minerals (Jacquemart et al., 2012). Furthermore, buckwheat is favoured as a candidate crop for environmentally friendly agriculture due to its low requirement for fertilizer. And the species may serve as a service plant that supports natural enemies and wild bees due to its corolla type flowers with easily accessible nectar and pollen (Penca

et al., 2017). Buckwheat used to be widely planted in the North China Plain. Nowadays, it has almost been replaced by maize and wheat because maize and wheat have much higher yields. We selected common buckwheat as an indicator plant because, on the one hand, it is a self-incompatible species with distylous flowers with two distinct flower morphs: pin flower and thrum flower (Cawoy et al., 2008, 2009). Due to its self-incompatibility, buckwheat depends greatly on insects for pollination (Adhikari and Campbell, 1998). On the other hand, the North China Plain is facing the transition to a more sustainable cropping system that provides healthier diets in a green way. Buckwheat might be a candidate crop.

We selected a representative county in the North China Plain to investigate two main questions: (a) Is there a pollination deficit in this region when buckwheat is used as a test species? (b) What are the common flower visitors of buckwheat in this region? Additionally, we are also interested in the question: How do pollination treatments affect buckwheat yield? We don't know whether the relationship between seed set and seed weight is the same between different pollination treatments and whether honeybee supplementation increases seed weight by increasing seed set or both seed set and single seed weight. We compared pollination services in three treatments: insect exclusion, open pollination, and honeybee supplementation, and observed flower visitation of buckwheat. We determined the effects of treatments on seed set and determined the relationship between seed set and the seed yield per plant.

2. Materials and methods

2.1. Experimental area and materials

Quzhou County (114°50'E–115°13'E, 36°35'N–36°57'N) is located in the south of the province Hebei, in the North China Plain (Fig. 1). It typifies agriculture in the North China Plain, with large area of the winter wheat-summer maize double cropping system, as well as cotton.

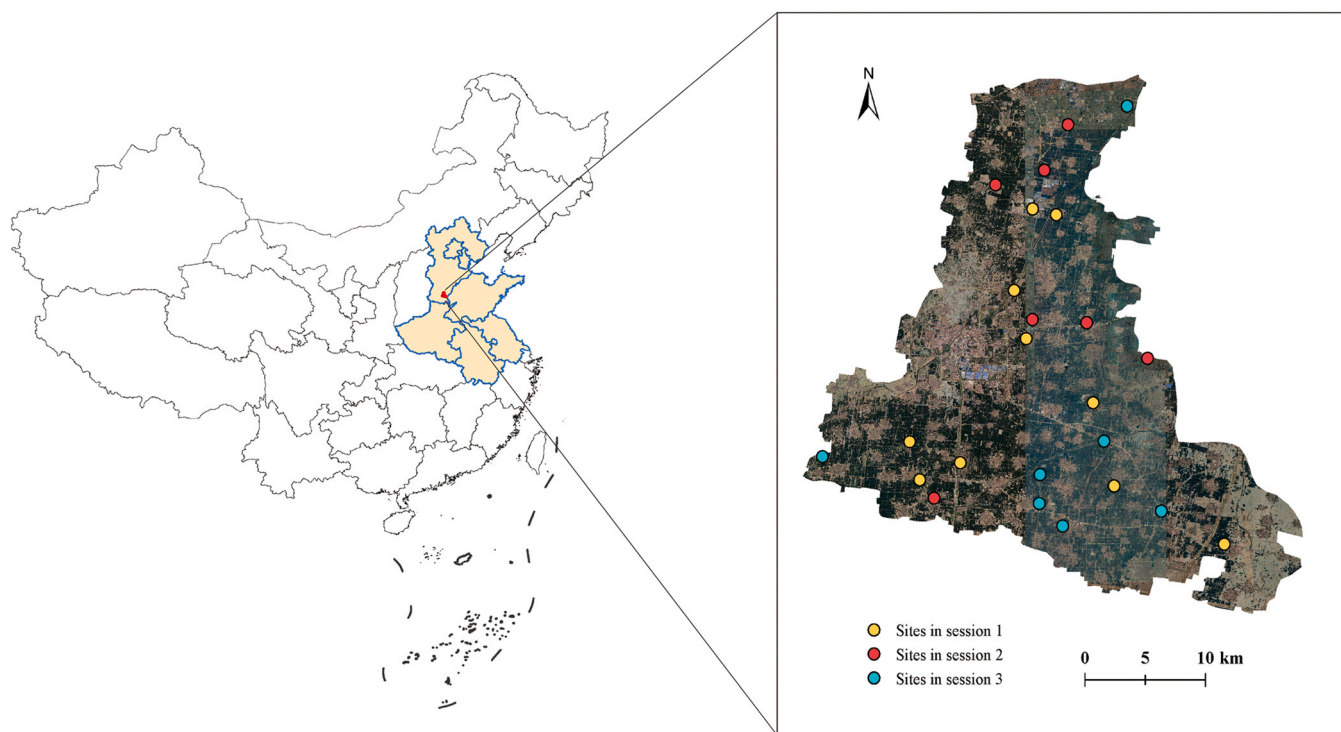


Fig. 1. Quzhou County (red dot) is situated in the North China Plain, which covers the provinces of Hebei, Shandong, Henan, Anhui, and Jiangsu, and the city areas of Beijing and Tianjin (yellow shading). The map on the right depicts Quzhou County. Yellow dots represent the sites selected for measurements during session 1 (autumn 2021) while the red and blue dots represent additional sites selected for measurements during sessions 2 and 3, in spring and summer 2022, respectively. Seven of the ten sites used in session 1 were reused in session 2, and seven sites used in session 2 were reused in session 3.

Quzhou County has a warm summer monsoon climate with a mean annual temperature of 13 °C and yearly precipitation of 556 mm, and more than half of the precipitation is between June and September. Most of the farmland in the county is cultivated by smallholders (Xu et al., 2024). The county was selected in 2020 as a demonstration county for the National Agriculture Green Development Strategy in China.

The study covered 24 sites across the county over two years during three measurement sessions. These sessions were held in the fall of 2021 and the spring and summer of 2022 (Fig. 1). To ensure the independence of data, the distance between any two experimental sites was at least 1.5 km, considering that the foraging range of most solitary wild bees typically does not exceed 1 km (Ricketts et al., 2008). The average landscape composition within a 500 m radius circle at the 24 sites was 69 % arable land, 8 % semi-natural habitat, and 23 % other land uses (Fig. S1).

2.2. Experimental design

We grew the buckwheat in 20 cm high pots with upper and lower diameters of 28 and 18 cm, respectively. Each pot had three plants. The pots were filled with substrate (Pindstrup company, product code: LV1167/021-LV12871), consisting of 80 % peat and 20 % perlite, with the pH adjusted to 5.5–6.0. The number of pots per site ranged from 6 to 15 (see Table 1 for details). Seedlings were grown in the greenhouse and transported to the field after 25–30 days shortly before they would start flowering. The plants with pin and thrum flowers at each site maintained an approximate 1:1 ratio. We engaged local farmers at each site to water the plants regularly and maintain consistent soil conditions for all the plants across sites.

Measurements were done during three sessions (Table 1). The first session was carried out at 10 sites in the autumn of 2021 and involved two treatments at each site: insect exclusion (Exclusion) and open pollination (Open). The second session was conducted at 14 sites in the spring in 2022. It included seven additional sites for open pollination and reused seven sites from the first session for honeybee supplementation (Supplementation). The third session was performed at 14 sites in the summer in 2022. It included seven additional sites for the Open treatment while seven sites that were used for the Open treatment in the second session were reused for the treatments Exclusion and Supplementation (Fig. S2). We avoided having Open and Supplementation treatments at the same site to prevent the honeybees maintained at the site from pollinating plants of the Open treatment. Due to the traffic limitations during the COVID-19 pandemic, we could not carry out observations on flower visitation in the Open treatment during session 2. However, we were able to retrieve all the plants placed in the field during session 2 and evaluated their seed yield under different treatments.

In the Open treatment, plants were exposed to natural insect pollinators. In the Supplementation treatment, a beehive was placed at the

site at no more than 3 m from the buckwheat plants. In the Exclusion treatment, plants and pots were enclosed in a 1.5×1.5×1.5 m³ cage covered completely with nylon mesh of 1×1 mm², and insects were therefore unable to reach the plants (Fig. S2). The cage was placed when plants were moved into the field and removed when all plants were moved from the field to the greenhouse after approximately four weeks as no more new flowers were opening. We tended the plants in the greenhouse until the seeds matured. The exposure time for potted plants in the field was 26 days, 27 days, and 28 days for the three sessions, respectively.

2.3. Indicators related to pollination services and seed yield

A buckwheat floret normally has one of three fates: the floret will wither if it is not pollinated, it results in an aborted seed if the flower is pollinated but the seed does not fill, or it can develop into a mature and filled seed (Cawoy et al., 2007). After seed maturation, we counted for each buckwheat plant the number of filled seeds, aborted seeds, and withered florets. The total number of florets at the final evaluation was calculated as the sum of filled seeds, aborted seeds, and withered florets. For the buckwheat floret, even if it gets pollinated and has a sufficient resource supply, it may still abort due to female organ sterility, which is related to the position of female floret in the raceme and the age of the plant (Cawoy et al., 2009). We used the following metrics to evaluate the results of pollination service:

- 1. Pollination rate: proportion of pollinated florets (i.e., filled plus aborted seeds over all florets).
- 2. Seed set: proportion of filled seeds relative to all florets.
- 3. Abortion rate: proportion of aborted seeds relative to all florets.

The pollination rate serves as a direct indicator of pollination success. It is influenced by pollinator abundance, diversity, and their efficiency. The seed set determines the seed yield and is affected by pollination but also by factors related to the plant, such as flower position, plant age, and resource supply. We dried the aboveground straw and seeds from different treatments at various sites in an oven at 65 °C until they reached a constant weight. We used the resulting data to calculate the harvest index (HI), i.e. the total seed weight per plant divided by the total biomass (including seeds) of the plant.

The pollination contribution (PC) of insects was estimated by measuring the difference in pollination service between open pollination and insect exclusion. The pollination deficit (PL) was determined by measuring the difference between open pollination and honeybee supplementation. Herein, we used honeybee supplementation to represent the maximum pollination potential for buckwheat in production. Our metrics for PC and PL are defined as:

$$PC = 1 - X_{\text{exclusion}} / X_{\text{open}}$$

$$PL = 1 - X_{\text{open}} / X_{\text{supplementation}}$$

where $X_{\text{exclusion}}$, X_{open} , and $X_{\text{supplementation}}$ represent the pollination rate or seed set (the pollination rate was not measured in session 1) in the insect exclusion, open pollination, and honeybee supplementation, respectively (Olhnuud et al., 2022). A PC value close to 1 suggests a strong reliance on insect pollinators for crop pollination. A PL value of 1 indicates a substantial pollination deficit, with few seeds produced in natural open pollination.

2.4. Direct observations of pollinator activities

We conducted observations of flower visitation at 25 sites in three sessions (Table 1). In session 1, we made observations of flower visitation at all 10 sites with open pollination. In session 2, we made observations at a single site with honeybee supplementation due to traffic

Table 1
Details of the experimental design.

Session	Season (Year)	Treatments	Pots for each treatment	Direct observation
1	Autumn (2021)	Open & Exclusion (10 sites)	Open (10) + Exclusion (5)	10 sites, 4 periods per day
2	Spring (2022)	Open (7 sites)	9	-
		Supplementation (7 sites)	9	1 site, 2 periods per day
3	Summer (2022)	Open (7 sites)	6	7 sites, 2 periods per day
		Supplementation & Exclusion (7 sites)	Supplementation (6) + Exclusion (4)	7 sites, 2 periods per day

control during a COVID-19 outbreak. In session 3, we made observations at 14 sites: seven sites with open pollination and seven sites with honeybee supplementation. All observations were done on rain-free days with wind speeds below 8 m/s (Fijen and Kleijn, 2017). In session 1, we scheduled observations during four times of day: 9:00–10:00 (P1), 11:00–12:00 (P2), 13:00–14:00 (P3), and 15:00–16:00 (P4). As we observed few flower visits during P3 and P4 in session 1, we made observations on flower visitation during sessions 2 and 3 only in the morning at P1 and P2. During session 1, each site was observed in total for eight hours (two rounds $\times$ four times of day per site per round). During session 3, each site was observed for two hours (one round $\times$ two times of day per site per round).

Before the experiment, we did a pilot experiment to obtain local insect specimens and practice observation of flower visitation. We used pan traps to collect the insect specimens. Then, we sent all specimens to experts for identification and also learned methods of insect identification from them. We identified some flower visitors at the genus or family level when they could not be identified to species. There were two groups with two persons in each to complete all observations on flower visitation during the three sessions. To avoid observer bias, the observation groups were rotated between observing dates, times of day, and sites to ensure that each group made observations at a site and time of day. Each team visited each site the same number of times. We used a standard protocol for observations on flower visitation (see below).

During each observation period of one hour, we observed flower visitation during three sessions of 20 minutes. In each 20-minute observation, flowers from the middle and top positions of the plant were randomly selected, and the observation unit was a flower cluster with approximately 50 small florets. Once an insect made a contact with the target flower cluster, a cluster visit was recorded, during which the insect could visit several florets before moving away from the flower cluster. We recorded each touch-down of an insect on a floret as a separate floret visit, even if the same floret was visited more than once (i.e. it was revisited after having been left). The total number of floret visits in each 20-minute observation was defined as the sum of floret visits by all insects visiting the floret cluster.

2.5. Statistical analysis

All calculations and statistical analyses were conducted using R version 4.2.3 (R Core Team 2022). We firstly analysed the effect of pollination treatments on indicators related to pollination services and seed yield, including pollination rate, seed set, abortion rate, seed weight per plant, straw weight, single seed weight, harvest index, total floret number, and filled seed number. As the plants in a pot are pseudoreplicates, the mean value per pot was calculated for statistical analysis. For the ratio data, the mean over three plants (e.g. for harvest index; HI) was calculated as $(x_1+x_2+x_3)/(y_1+y_2+y_3)$ where x_1 , x_2 , x_3 , y_1 , y_2 and y_3 are the values for each individual plant (in the case of HI, x is seed weight, and y is plant weight). The treatment effects were analysed separately for each of the three sessions since there were major differences in weather and insect abundance and community between the sessions, which were associated with major differences in pollination rate and seed set.

In a first analysis, we used linear and generalized linear mixed-effect models to analyse the response variables mentioned in the previous paragraph, treating pollination treatment as a fixed effect and site as a random effect (see Table S1). Open pollination was the reference treatment to assess treatment effects. Visual diagnostic plots (Zuur et al., 2010) were used to check that model residuals adhered to the assumptions of normal distribution and homogeneity of variance. For response variables involving ratio and count data, after using natural logarithm or square root transformations, the model residuals met the assumptions of normal distribution and variance homogeneity. Therefore, we chose to use parsimonious but well-fitting models with the assumption of normally distributed homoscedastic residuals.

In a second analysis, we employed linear regression models using plant-level data to analyse the relationship between pollination services and seed weight of buckwheat in the Open and Supplementation treatments. The total seed weight per plant served as the response variable, and three models having as fixed effects, respectively, treatment $\times$ seed set, treatment $\times$ pollination rate, and treatment $\times$ total floret numbers. In these three models, we used pot nested within site as a random effect to allow for correlation between results for plants sharing the same pot (Zuur et al., 2009). We conducted likelihood ratio tests to assess the significance of the fixed effect and select the optimal models (i.e. with or without interaction).

Mixed effects models were fitted using the function `lme` from the package `nlme` (Pinheiro et al., 2022) and generalized linear mixed-effects models were fitted using the function `glmer` from the package `lme4` (Bates et al., 2015). To estimate the mean value of treatments in the fitted models, we employed the `emmeans` function from the package `emmeans` (Lenth, 2023). We used the `r.squaredGLMM` function from the package `MuMIn` (Bartoń, 2023) to calculate the marginal and conditional R^2 of the mixed effect model, representing the variance explained by fixed effects and both fixed and random effects, respectively. We used the package `ggplot2` (Wickham, 2016) for data visualization.

3. Results

3.1. The pollination contribution and deficit

Insect exclusion reduced seed set by 91% (Confidence interval (CI): 88% - 93%) in session 1 (Fig. 2A) and by 93% (CI: 88% - 100%) in session 3 (Fig. 2B), compared to open pollination (Table S1). Furthermore, the pollination rate was reduced by 92% (CI: 84% - 100%) in the absence of insect pollination in session 3 (Fig. 2B).

Compared to honeybee supplementation, open pollination reduced pollination rate by 12 % (CI: 0.3 % - 23 %) and 21 % (CI: 30 % - 72 %) in sessions 2 (Fig. 3A) and 3 (Fig. 3B), respectively, while seed set was reduced by 22 % (CI: 10 % - 35 %) and 33 % (CI: -11 % - 78 %). The effect size of honeybee supplementation was consistent across the two sessions, but due to the greater variability during session 3, the effect was only significant during session 2 ($p < 0.01$ for pollination rate and $p < 0.001$ for seed set, Table S1).

3.2. Pollination services and seed yield

In sessions 1 and 3, insect exclusion significantly decreased the number of filled seeds, total seed weight per plant and harvest index compared to open pollination (Table 2, Table S1), with more than 80 % reduction in the number of filled seeds. The total floret number, single seed weight and straw weight were higher in insect exclusion than in open pollination, while the effect was only significant in session 1 ($p < 0.001$), not in session 3 (Table 2).

In sessions 2 and 3, honeybee supplementation significantly increased the number of filled seeds, seed weight per plant, and the harvest index compared to open pollination (Table 2, Table S1). In these two sessions, compared to open pollination, honeybee supplementation, on average, increased the harvest index and seed weight per plant with factors of 1.6 and 2, respectively. Honeybee supplementation reduced seed abortion by 25 % compared to open pollination ($p < 0.001$, Table 2) in session 2, but this effect was not found in session 3. The total floret number was higher in honeybee supplementation than in open pollination in session 3 ($p < 0.05$), while this effect was not observed in session 2. There was no significant difference in single seed weight between open pollination and honeybee supplementation in sessions 2 and 3 (Table 2).

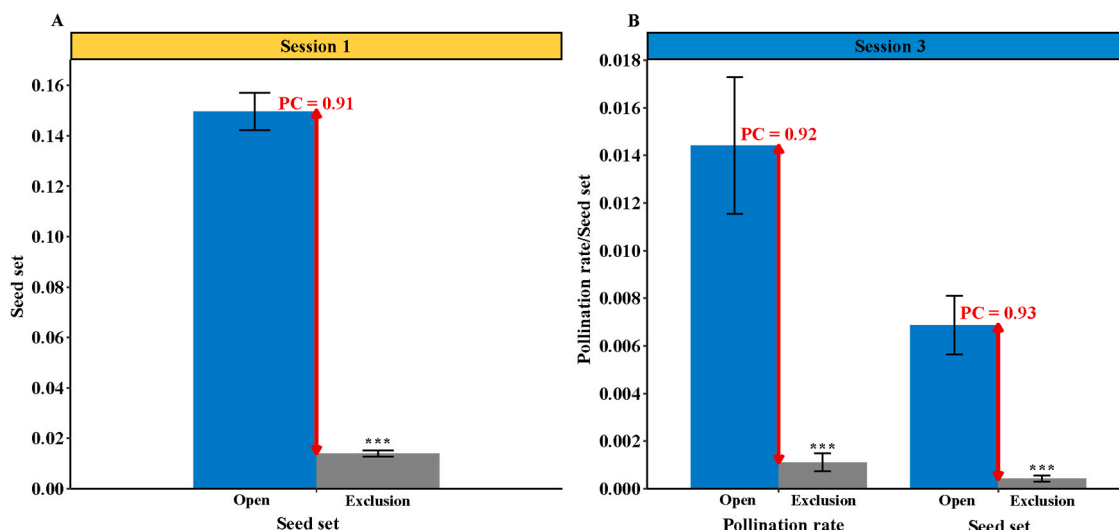


Fig. 2. Pollination rate and seed set of buckwheat with open pollination (Blue bars) and insect exclusion (Grey bars) during sessions 1 (A) and 3 (B). Session 2 did not have the insect exclusion treatment, and the pollination rate was not measured in session 1. The bar represents the mean $\pm$ standard error, with red arrow and values indicating the pollination contribution (PC). Asterisks denote significant differences (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) between insect exclusion and open pollination, as determined by the results of the linear-mixed effect models (Table S1).

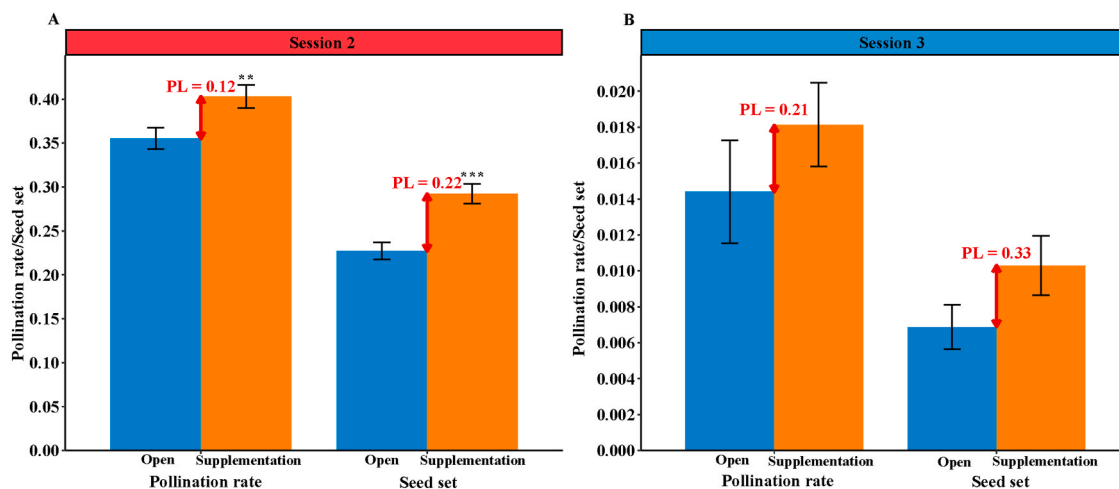


Fig. 3. Pollination rate and seed set of buckwheat with open pollination (Blue bars) and honeybee supplementation (Orange bars) during sessions 2 (A) and 3 (B). Session 1 did not have the honeybee supplementation treatment. The bar represents the mean $\pm$ standard error, with red arrow and values indicating the pollination deficit (PL). Asterisks denote significant differences (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) between honeybee supplementation and open pollination, as determined by the results of linear mixed-effect models (Table S1).

3.3. The relationship between pollination service and seed weight per plant

The seed weight per plant increased as the pollination rate and seed set increased both with open pollination and honeybee supplementation (Fig. 4 and Fig. S5). In session 2 (Fig. 4A), honeybee supplementation had higher seed weight per plant than open pollination, given the same pollination rate and seed set, indicating heavier seeds with honeybee supplementation. In session 3 (Fig. 4B), a significant interaction between seed set or pollination rate and treatment indicated that higher seed weight with honeybee supplementation was related to a higher filled seed number per plant.

Seed weight per plant increased with total floret number, both with open pollination and honeybee supplementation in session 2 (Fig. 5A), but this trend was not observed in session 3 (Fig. 5B). In both sessions 2 and 3, honeybee supplementation resulted in significantly greater seed production per plant for a given floret number than natural pollination ($P < 0.001$, Fig. 5A; $P = 0.049$, Fig. 5B) (significant different in intercept,

but same slope).

3.4. The composition of flower-visiting insects and their contribution to flower visits

The number of individual flower-visiting insects was higher in session 1 (1995 individuals) than in session 3 (1209 individuals) but the number of species caught was similar (21 in session 2 and 20 in session 3) (Table 3). Hoverflies (Syrphidae) and other flies (Muscidae, Sarcophagidae, and Calliphoridae) were the most frequent visitors to buckwheat, while wild bees (Halictidae) and wasps (Vespidae, Crabronidae, Ichneumonidae, and Chrysididae) accounted for only a small proportion (3.7 %) of the total visitors. Butterflies and ladybeetles were observed visiting flowers occasionally (Table 3).

Hoverflies accounted for over 60 % of the total flower visits in session 1, and for 16 % and 31 % of the total flower visits without and with beehives, respectively, in session 3 (Fig. S4). Other flies, notably Muscidae, frequently visited flowers, accounting for more than half of total

Table 2

Seed yield-related indicators in three treatments in three measurement sessions.

Indicator	Treatment	Session 1	Session 2	Session 3
Total number of florets per plant	Open	1058±37	686±22	3361±325
	Supplementation	- ^a	707±23	4785±264 ^{*b}
	Exclusion	2023 ±114 ***	-	4527±607
Filled seed number per plant	Open	148±6	150±6	19±3
	Supplementation	-	203±9 ***	43±5 **
	Exclusion	27±2 ***	-	2±1 ***
Abortion rate (%)	Open	-	36.2±1.4	41.7±3.8
	Supplementation	-	27.2±1.4 ***	43.6±4.0
	Exclusion	-	-	51.6±11.5
Single seed weight (cg)	Open	2.35 ±0.04	2.88 ±0.06	2.06±0.13
	Supplementation	-	3.15 ±0.05	2.43±0.09
	Exclusion	3.38 ±0.13 ***	-	2.83±0.71
Total seed weight (g/plant)	Open	3.45 ±0.15	4.30 ±0.19	0.45±0.08
	Supplementation	-	6.39 ±0.29 ***	1.06±0.14 **
	Exclusion	0.94 ±0.10 ***	-	0.04±0.02 **
Straw weight (g/plant)	Open	8.33 ±0.29	7.43 ±0.21	10.42 ±0.64
	Supplementation	-	7.17 ±0.23	13.55 ±0.65
	Exclusion	9.84 ±0.29 ***	-	13.62 ±1.29
Harvest index	Open	0.29 ±0.01	0.36 ±0.01	0.04±0.01
	Supplementation	-	0.46 ±0.01 ***	0.07±0.01 *
	Exclusion	0.09 ±0.01 ***	-	0.003 ±0.001 ***

^a A dash (-) means a variable was not measured in a treatment in this session.^b Asterisks (***) $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) indicate significant differences between a treatment (Supplementation or Exclusion) and the open pollination reference. Details of underlying statistical analyses are given in Table S1. Values are presented as mean ± standard error, and the confidence interval can be calculated as mean ± 1.96 times the standard error.

flower visits in session 3, irrespective of the presence of beehives (Fig. S4). Honeybees infrequently visited flowers in session 3, regardless of the presence of a beehive. However, *Apis mellifera* emerged as the dominant flower visitor in session 2, comprising 92 % of flower visits when introducing a beehive (Fig. S3).

4. Discussion

In this study, we used supplementary honeybee hives to measure maximum seed set, revealing that potted buckwheat plants at sites without supplementary beehives had 22–33 % lower seed set (Fig. 3) and 33–58 % lower seed weight per plant than sites with supplementary honeybees (Table 2). Predominant flower-visiting insects at our sites were hoverflies and flies, with *Episyrphus balteatus* and *Eristalinus aeneus*. Jointly, these two species accounted for 51.4 % of the flower visits in session 1 and 13.8 % in session 3. Flies from the families Muscidae, Calliphoridae and Sarcophagidae made 32.7 % of the flower visits in session 1 and 65.1 % in session 3.

While we did not measure pollen deposition for the flower-visiting species, evidence from Liu et al. (2020) suggests that any insect visiting buckwheat flowers may contribute to pollination, albeit with varying efficiency. Jacquemart et al. (2012) state that buckwheat attracts a diverse pollinator community, including honeybees, bumblebees, solitary bees, hoverflies, blowflies and butterflies. The most abundant flower-visiting insects vary across regions but are most commonly from Apoidea and Syrphidae. Our study results are in agreement with this. Our results are also in agreement with a study by Chen et al. (2022) showing that hoverflies were frequently visiting watermelon flowers in Quzhou (unpublished results), resulting in 74 % higher fruit set with open pollination than insect exclusion.

4.1. The pollination deficit in the North China Plain

Our results highlight the existence of a moderate pollination deficit in the North China Plain when buckwheat is used as a test species. Apples and pears are locally important pollinator-dependent crops. Wu et al. (2021) found a 15 % reduction in apple yield due to pollination deficit in a large apple-growing region near Beijing, which is in the northern part of the North China Plain. Li et al. (2022) reported a 68 % decrease in pear fruit set with open pollination compared to honeybee supplementation in Xinjiang. A farmer's survey conducted in Quzhou county in 2021 indicated that 60 % of farmers who were growing insect-pollinated crops thought the effectiveness of pollination services was intermediate to very good (Cheng et al., 2023). Thus, farmers are

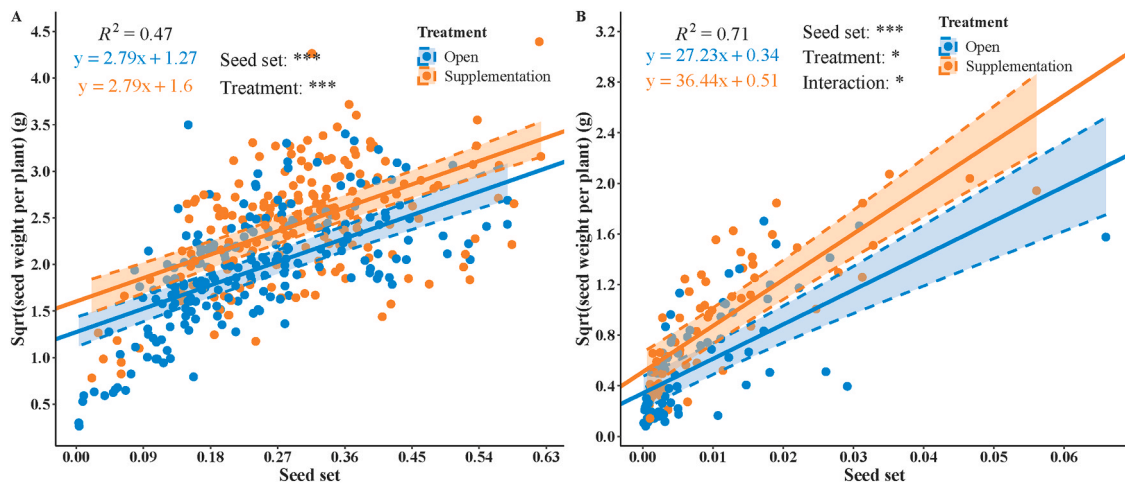


Fig. 4. The relationship between seed set and seed weight per plant in session 2 (A) and session 3 (B). Each point represents a single plant. The shaded bands represent the 95 % confidence interval derived from the linear mixed-effect models (Table S2). Asterisks denote significant differences (***) $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

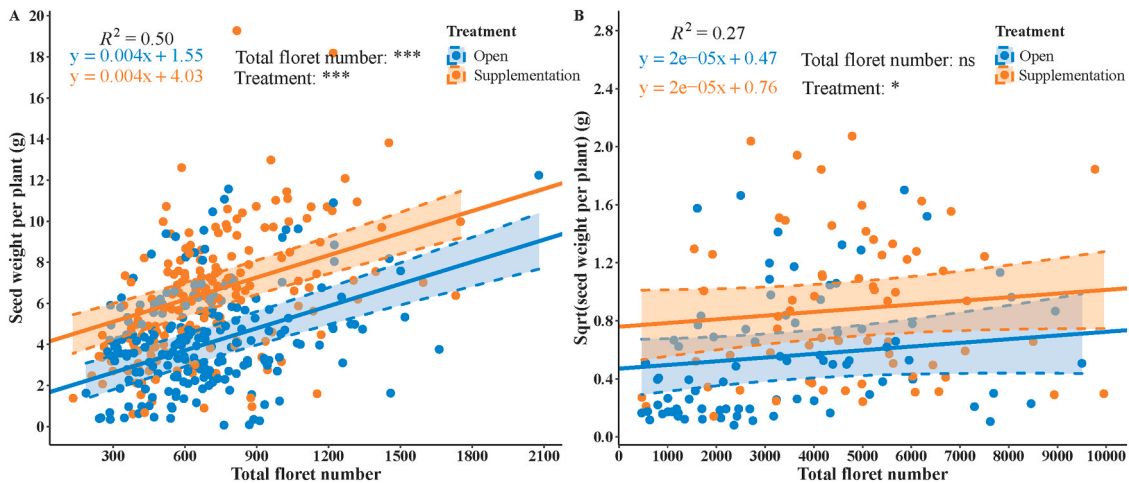


Fig. 5. The relationship between total floret number and seed weight per plant in session 2 (A) and session 3 (B). Each point represents a single plant. The shaded bands represent the 95 % confidence interval. Asterisks denote significant differences (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: not significant) derived from the linear mixed-effect models (Table S2).

Table 3

The composition and number of flower-visiting insects in two treatments in two measurement sessions (1 and 3). Families are ordered according to the total number of visiting individual insects per family.

Order	Family/Species	Session 1 (Open)	Session 3 (Open)	Session 3 (Supplementation)	Session 3 (Total visitors)	Total
Diptera	Syrphidae	1243	121	256	377	1620
	<i>Eristalinus aeneus</i>	540	53	105	158	698
	<i>Episyrphus balteatus</i>	559	14	3	17	576
	<i>Syrpita orientalis</i>	7	48	108	156	163
	<i>Eristalis tenax</i>	51	5	3	8	59
	<i>Sphaerophoria scripta</i>	13	0	28	28	41
	<i>Phytomyia zonata</i>	34	1	0	1	35
	<i>Eupeodes corollae</i>	19	0	8	8	27
	<i>Helophilus continuus</i>	20	0	1	1	21
Diptera	Muscidae	156	288	319	607	763
	<i>Musca</i> spp.	156	288	319	607	763
	Sarcophagidae	287	17	32	49	336
	<i>Sarcophaga</i> spp.	287	17	32	49	336
Diptera	Calliphoridae	175	51	19	70	245
	<i>Lucilia</i> spp.	152	51	15	66	218
	<i>Chrysomya megacephala</i>	23	0	4	4	27
Hymenoptera	Halictidae	21	23	17	40	61
	<i>Lasioglossum</i> spp.	21	10	16	26	47
	<i>Lipotriches ceratina</i>	0	13	1	14	14
Diptera	Tachinidae	44	2	13	15	59
	<i>Linnaemya comta</i>	44	0	0	0	44
	<i>Tachina nupta</i>	0	2	13	15	15
Hymenoptera	Vespidae	47	0	0	0	47
	<i>Stenodynerus bluethgeni</i>	41	0	0	0	41
	<i>Rhynchium quinquecinctum</i>	6	0	0	0	6
Diptera	Rhiniidae	0	22	10	32	32
	<i>Stomorhina obsoleta</i>	0	22	10	32	32
Hymenoptera	Apidae	10	6	1	7	17
	<i>Apis mellifera</i>	10	6	1	7	17
Hymenoptera	Crabronidae	0	4	3	7	7
	<i>Crabronidae</i> spp.	0	4	3	7	7
Coleoptera	Coccinellidae	2	0	5	5	7
	<i>Coccinella septempunctata</i>	2	0	0	0	2
	<i>Harmonia axyridis</i>	0	0	3	3	3
	<i>Propylea japonica</i>	0	0	2	2	2
Lepidoptera	Nymphalidae	5	0	0	0	5
	<i>Polygonia c-aureum</i>	5	0	0	0	5
Hymenoptera	Ichneumonidae	3	0	0	0	3
	<i>Diplozon laetatorius</i>	3	0	0	0	3
Hymenoptera	Chrysididae	2	0	0	0	2
	<i>Stilbum cyanurum</i>	2	0	0	0	2
	Total	1995	534	675	1209	3204

perhaps unaware of pollination deficit even if it exists, a phenomenon which may be regarded as an example of a shifting baseline syndrome (Papworth et al., 2009). Measuring pollination deficit of local pollinator-dependent crops is essential to bridge the knowledge gap and get a comprehensive understanding of pollination service in this region. Our results underscore the need for further research on pollinators and pollination services in China and more interaction with farmers to build better knowledge on the adequacy of pollination services in main crops and inform farmers and learn from their experiences.

We found that the seed weight of buckwheat was positively associated with pollination services (pollination rate and seed set), while the effect of honeybee supplementation and open pollination on seed weight differed between seasons. In the summer (session 3), given the same seed set, honeybee supplementation increased the single seed weight compared to open pollination. This indicates that the honeybee pollination either enhanced the seed filling, or somehow, honeybees pollinated more often those florets with a position on the rachis favouring seed filling. Our result illustrated the advantage of honeybee supplementation in improving crop yield. It would help enhance pollination services and improve crop yield by holding workshops to deliver knowledge to farmers about the benefits of honeybee supplementation and how to use honeybees properly.

4.2. The composition and activities of pollinators

The most abundant flower-visiting insects in our experiment were hoverflies and flies, predominantly *Episyrphus balteatus* and *Eristalinus aeneus*, and other flies from Muscidae, Calliphoridae and Sarcophagidae. *Episyrphus balteatus* is considered the most common hoverfly in agroecosystems worldwide, and its larvae feed on more than 200 aphid species, especially cereal aphids (Leroy et al., 2010). The prevalence of maize and wheat in our research region provides suitable resources for the development of the aphidophagous larvae of *Episyrphus balteatus* as wheat is a reliable source of aphids in the spring. *Eristalinus aeneus*, *Musca* spp. and Sarcophagidae utilize a variety of environments, including rotten plants, animal excrement, ponds, slow-moving rivers, streams and irrigation ditches (Campoy et al., 2020). Such environments are ubiquitous in Quzhou as it harbours both plant and animal production. *Musca* spp. and Sarcophagidae also occur in decaying meat and other organic substances, manure and household garbage (Moon, 2019; Sreejith et al., 2021). Common landscape elements in Quzhou, such as small ponds and weedy field ditches, combined with widespread animal production, may thus contribute to supporting high abundance of hoverfly and fly species in our experiment.

We rarely observed honeybees visiting flowers during session 3, contrasting with the higher frequency observed in session 2 at the same time of day (Fig. S3). This is likely influenced by weather differences. Honeybees are generally more active with cooler weather in early spring than with higher temperatures in summer (Kendall et al., 2021). The temperatures in the morning of session 3 ranged from 24 °C to 33 °C, potentially unfavourable for honeybee activity, which is higher between 12 °C and 27 °C (Kendall et al., 2021). Buckwheat can attract various flower visitors, including honeybees, hoverflies, bumblebees, and solitary bee wasps among others, and the flower visitors vary by season, region and year (Cawoy et al., 2009). We observed a few wild bees visiting buckwheat flowers, but pan traps caught many wild bees from Halictidae in this region (unpublished data). The literature on buckwheat mentions a variety of pollinating species, comprising not only honeybees and hoverflies, which are major flower visitors, but also wild bees belong to the genera *Bombus* and *Andrena*, while Halictidae were less reported as flower visitors (Campbell et al., 2016; Fijen et al., 2022; Liu et al., 2020). Considering the circadian system of bees, it is plausible that some insect visits would occur near sunrise or sunset, outside the observation times of our flower observation protocol. This underscores the importance of adjusting observation times between seasons based on the sunrise and temperature to avoid underestimating insect activity.

Moreover, observing the same sites at the same periods in different seasons can also give a more comprehensive understanding of insect activity. Based on our flower visitation observation results, and coupled with existing literature, conserving the diversity of wild pollinators, including wild bees, also non-bee groups, such as hoverflies and other flies is important for pollination services.

4.3. The strengths and limitations of using potted buckwheat plants as indicators and honeybee pollination to assess pollination deficit

Most pollination studies use hand pollination to represent maximum pollination (Gazzea et al., 2023), but we used honeybee supplementary pollination instead. We did this because hand pollinating buckwheat is cumbersome as the plant has many florets that open over time. Furthermore, hand pollination may overlook dynamic changes in pollen viability, availability and the complexities of plant-pollinator interactions in natural environments (Ashman et al., 2004; Mitchell et al., 2009). Nevertheless, using honeybees also carries uncertainties. For instance, honeybees may be attracted by surrounding crops, not the target crop, and overpollination by honeybees may result in pollen tube clogging due to an overload of non-viable or incompatible pollen (Subasinghe Arachchige et al., 2022). Nevertheless, honeybees are regarded as key pollinators of buckwheat (Campbell et al., 2016), and buckwheat yield depends on honeybee density (Fijen et al., 2022). Therefore, the method is deemed justifiable for measuring pollination deficit, especially for numerous flower crops.

In our experiment, we used potted buckwheat plants as a "phytometer" (Woodcock et al., 2014) to measure pollination services across landscapes, offering several advantages, such as attributing seed set differences solely to pollination treatments, enhancing the estimation accuracy by eliminating variations in soil fertility. Alternatively, when using field grown plants, a pollination service index (PSI) could be calculated to account for site-specific variation in pollination, and hence more accurately calculate treatment effects (Zou et al., 2017). The flexibility of using potted plants permits assessments at various intervals throughout the year, improving opportunities for evaluation. Nevertheless, phytometry has limitations, including susceptibility to plant loss due to lodging, disease, and pests. To mitigate this, a sufficient number of pots (e.g., 1164 plants in three sessions with 953 surviving plants after field exposure in our study) is crucial. Moreover, a smaller population of potted plants and isolation distance between potted plant patches may be less attractive to insects, particularly in landscapes with mass-flowering plants (Artz and Waddington, 2006; Lazaro and Totland, 2010), potentially leading to an underestimation of pollination services. To address this, it is recommended to increase the number of sample sites.

5. Conclusion

The study revealed that Quzhou county, an intensive cereal production region in the North China Plain, experiences a pollination deficit, resulting in a 22–33 % reduction in the seed set of buckwheat when compared to pollination with honeybee supplementation. The supplementation of honeybees significantly reduced the seed abortion rate, increased the harvest index, and boosted seed weight per plant. The pollination rate and seed set, and the effectiveness of honeybee supplementation, varied greatly between sessions. The most frequent flower-visiting wild insects were *Episyrphus balteatus* and *Eristalinus aeneus*, belonging to the hoverfly family (Syrphidae), while other flies, mostly from the Muscidae, Sarcophagidae, and Calliphoridae, contributed also substantially to flower visits. The most frequent flower-visiting insects, hoverflies, depend on aphid prey during part of the life cycle while most other flower visiting insects, other flies, utilize waste material, exemplifying potentially complex relationships in the landscape. Wild pollinators contribute substantially to pollination of buckwheat in the study area, but supplementation with honeybees is required to

achieve maximum yield.

CRediT authorship contribution statement

Yanjie Chen: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Chaochun Zhang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Wopke van der Werf:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2024.109129](https://doi.org/10.1016/j.agee.2024.109129).

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