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Alfalfa seed yield across different landscapes depends on the joint effects of pollinators and climate in north-eastern China

Sachurula Bao[1,2,3], Xuerong Lei[2,3], Chao Gao[4], Duofeng Pan[4], Yanchun Zhou[5], Zhijian Li[2], Qinfeng Guo[6], Chuan Ma[1], Bingzhong Ren[2,3] and Hui Zhu[1,2,3]

[1]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China; [2]School of Life Sciences, Key Laboratory of Vegetation Ecology of the Ministry of Education, Jilin Songnen Grassland Ecosystem National Observation and Research Station, Northeast Normal University, Changchun, China; [3]Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization, Northeast Normal University, Changchun, China; [4]Institute of Forage and Grassland Sciences, Heilongjiang Academy of Agricultural Sciences, Harbin, Heilongjiang, China; [5]Institute of Grassland and Ecology Sciences, Jilin Academy of Agricultural Sciences (Northeast Agricultural Research Center of China), Gongzhuling, Jilin, China and [6]USDA – Southern Research Station, Asheville, NC, USA

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Corresponding author: Hui Zhu;
Email: zhuh824@nenu.edu.cn;
Bingzhong Ren; Email: bzren@nenu.edu.cn

Abstract

Alfalfa is a widely cultivated forage crop with high nutritional value, and it relies heavily on pollinators for successful reproduction owing to its keel petal structure. Numerous studies have shown the differences in pollinator diversity at different landscapes and/or regions, subsequently impacting plant reproduction. However, there is still insufficient evidence on variation in pollinator diversity at different sites with different landscapes and its effects on alfalfa reproduction. In this study, we investigated the diversity and abundance of floral visitors, and their impacts on alfalfa seed yield, across three sites represented by different landscapes, i.e. urban (Gongzhuling), semi-natural grassland (Changling), and farmland (Harbin) in north-eastern China. Our results showed that the diversity and abundance of all visitors, including bees in Changling were significantly higher than those of the other two sites. Changes in visitor diversity and abundance of alfalfa were closely related to climate variables, with positive effects of air temperature, and complex effects of precipitation. Meanwhile, the differences in pollinators may also be attributed to landscape types due to different habitat heterogeneity. Alfalfa seed yield in Changling was also significantly higher than that in the other two sites. Among these factors, air temperature, and diversity of floral visitors, particularly bees were positively related to alfalfa seed yield, indicating that pollinators combined with climate have an important contribution to changes in alfalfa seed yield. The findings from this study emphasise the importance of conserving pollinators to sustain and even enhance alfalfa yields, especially in the context of climate change and alterations in landscape types.

Introduction

Alfalfa (*Medicago sativa* L.) (Fabales: Leguminosae) is the most extensively cultivated forage crop globally and is planted in over 80 countries (Cecen *et al.*, 2008; Al-Kahtani *et al.*, 2017). It serves as a rich source of high-quality proteins and amino acids for livestock (Shi *et al.*, 2017). As the demand for alfalfa increases due to the growing livestock industry, the area dedicated to its cultivation continues to expand. However, to date, the actual supply of seed yield is still lower than expected (Haedo *et al.*, 2022). Alfalfa is a gynomonoecious and cross-pollinated species, its seed yield heavily depends on pollinators due to tripping agents; that is, pollination is only completed when pollinators open the unique keel petals of alfalfa flowers (Bohart, 1957). Thus, pollinators play a pivotal role in alfalfa seed yield, and effective pollination is essential for maximising seed yields (Pitts-Singer and Bosch, 2010). Given this, it is necessary and urgent to deepen the understanding of the interactions between alfalfa and its pollinators, especially in relation to seed yield.

Approximately 70% of flowering plants in both natural and agricultural ecosystems rely on pollinators for reproduction (Ollerton *et al.*, 2011). For alfalfa, the relationships between seed yield and their wild pollinators have received attention. Most studies to date have focused on the community composition of pollinators and found that bees were the main pollinators of alfalfa (Requier *et al.*, 2023). Several wild bee species have been recognised as effective pollinators of alfalfa, including *Megachile abluta, M. cephalotes*, and *M. lanata*, all of which are closely associated with alfalfa seed yield (Wang *et al.*, 2012; Rauf *et al.*, 2021). Among these bee species, megachilids have been shown to have remarkable effects on increases in alfalfa seed yield (Shebl *et al.*, 2008; Bao *et al.*, 2025). Despite their crucial role in plant reproduction, wild pollinators have been reported to decline due to climate change, habitat loss, and pesticide use (Greenleaf and Kremen, 2006; Goulson *et al.*, 2015). Domestic bees, particularly honeybees (*Apis mellifera*), have been widely introduced to maintain crop yields (Robinson *et al.*, 2023), including alfalfa. Research on alfalfa pollination has shown that seed yield increased with higher bee density, with a yield peak at approximately 20 individuals/m^2 (Al-Kahtani *et al.*, 2017; Bao *et al.*, 2025). However, honeybees have relatively low open tripping rates, limiting their effectiveness for alfalfa pollination (Bauer *et al.*, 2017). As a result, alternative pollinators such as *M. rotundata* and *B. terrestris* have been introduced owing to exhibiting higher tripping rates, which could improve seed yields of alfalfa (Cane, 2002; Robinson *et al.*, 2023; Messellem *et al.*, 2025). For example, alfalfa seed yields have been shown to increase by 1.8–2.2 fold under the visitation of *M. rotundata*, and increased by 14% in the treatments of *B. terrestris*, compared to honeybee-systems (Cecen *et al.*, 2008; Renzi *et al.*, 2022). Additionally, the effects of domestic megachilids and bumblebees on alfalfa yield are also density-dependent. Maximum yields have been observed at approximately 150,000 *M. rotundata* per ha, while *B. lucorum* performed best at a density of 10 individuals/m^2 (Pitts-Singer and Bosch, 2010; Pitts-Singer, 2013; Bao *et al.*, 2025). The above studies highlight the importance of domestic bees in achieving optimal densities for effective pollination of alfalfa. However, researchers found that wild bees enhance crop yield more, and domestic bees only serve as a supplement rather than a replacement for wild pollinators (Garibaldi *et al.*, 2013; Klein *et al.*, 2018). Fewer studies indeed provide a clear understanding of the effects of these wild pollinators on alfalfa seed yield.

Wild pollinators have been shown to vary their diversity and abundance across different landscape types in a local site (Gaspar *et al.*, 2022). For instance, meadows supported higher abundance and richness of wild bees than woodlands (Maurer *et al.*, 2022). Similarly, there are differences in pollinator diversity across different landscapes at a regional scale. Changes in pollinator diversity at the regional scale are influenced by not only the difference in climatic factors (Nielsen *et al.*, 2017) but also the synergism of landscape type and climate (Cohen *et al.*, 2025; Ganuza *et al.*, 2025). Woodland and grassland landscapes support the higher pollinator abundance and diversity, while agricultural and urban landscapes cause declines in pollinator abundance and diversity across different European biogeographic regions (Bottero *et al.*, 2023). In regions with higher air temperatures, pollinator communities exhibited an increase in species richness and a decrease in abundance relative to regions with cooler temperatures (Oldham *et al.*, 2025; Zanini *et al.*, 2026). Given the importance of pollinators on plant reproduction, the differences in pollinator diversity across different landscape types at varied regional scales may likely drive plant reproduction (Laterza *et al.*, 2025). Meanwhile, plant reproduction can also be directly or indirectly affected by landscape types and climate factors (Toro-Manríquez *et al.*, 2023). Alfalfa is widely planted in different landscapes across a regional scale (Wang *et al.*, 2012; Shi *et al.*, 2017). However, whether and how geographic variation in alfalfa reproduction is affected by the combined effects of climate and pollinators, especially for different landscape types remains poorly understood.

In north-eastern China, alfalfa is often cultivated across heterogeneous landscapes, we hypothesise that pollinator richness and abundance differ significantly among diverse planting sites, and that these differences in pollinator diversity, together with climatic factors and landscape context, affect alfalfa seed yield in this study. Accordingly, we addressed two specific questions: (1) How do the diversity and abundance of floral visitors vary among alfalfa plantation sites with different landscape types? (2) How do changes in pollinator communities, in combination with climatic variables, influence alfalfa seed yield?

Materials and methods

Study site

This study was conducted in three experimental stations in north-eastern China: (1) Gongzhuling (GZL) of the Institute of Plant Protection, Jilin Academy of Agricultural Sciences; (2) Changling (CL) of the Jilin Songnen Grassland Ecosystem National Observation and Research Station; and (3) Harbin (HB) of the Institute of Forage and Grassland Sciences, Heilongjiang Academy of Agricultural Sciences (Figure S1). The distance among these three sites was greater than 150 km. All of the sites were located in the temperate zone with a continental monsoon climate but had distinct climate conditions (air temperature and precipitation amounts) (Table S1). The GZL site was located at the outskirts of GZL county, and alfalfa was cultivated over an area of approximately 4.5 ha at the experimental station. Other species of the alfalfa plantation include the perennial grass *Leymus chinensis, Plantago asiatica*, and *Setaria viridis*. The CL site was classified as a semi-natural grassland dominated by the *L. chinensis, Phragmites australis, Calamagrostis epigejos, Kalimeris integrifolia*, and *Potentilla flagellaris* (Wang *et al.*, 2019). Nearby, an alfalfa cultivation area covered approximately 5 ha. The HB site is categorised as farmland, with an alfalfa cultivation area of approximately 4 ha. Other crop plants include *Zea mays, Glycine max, Solanum tuberosum*, and *Oryza sativa*.

Study organism

Alfalfa is a cross-pollinating species, and its flowers have an explosive system where the anthers and the stigma are exposed only when the flower is tripped by a visitor. Tripping is an indispensable step for alfalfa reproduction because the process of pollination is completed only when the flowers are tripped (Bohart, 1957). The non-tripping flowers have only about a 5% probability of pods (Haedo *et al.*, 2022). Thus, the production of alfalfa strongly relies on pollinators. The flowering period of alfalfa occurs from early June to late July in north-eastern China, whereas the blooming period is longer, with approximately 25–30 days. Meanwhile, the pollination efficiency of alfalfa is higher at the blooming stage, when floral visitors are also more diverse. In practice, pollinators are often introduced into alfalfa fields at the blooming stage to maximise pollination and enhance seed production (Hagler *et al.*, 2011; Zhang *et al.*, 2022).

Floral visitor sampling

Floral visitor surveys at each experimental site were conducted during the blooming stage when visitor abundance was the highest, accounting for approximately 60% of the total number of visitors throughout the entire blooming period in our study (H. Zhu, unpublished data). At each site, three sampling transects (80 m long and 2 m wide) were set up with a distance of 30–50 m between them. We used the sweep net survey method with a light muslin net (40 cm in diameter) along each transect to sample floral visitors, which were identified as those in contact with the reproductive parts of alfalfa flowers. Specifically, we conducted two sweeps per

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meter while walking slowly (1 m/min) along each transect line. After completing the sweeps for each 1-meter segment, the net was emptied into a vial containing ethyl acetate and stored in a refrigerator at 4°C for later identification. Sampling of each transect at each site lasted 80 min within a sampling round, with one in the morning (9:00–10:30) and the other in the afternoon (13:30–15:00), and the sampling was repeated twice on each sampling date. Visitor surveys were conducted over 2 consecutive days, a total of 960 survey minutes per site. Visitors were collected under sunny and weak windy conditions. The captured insects were pinned, labelled, and identified in the laboratory, referring to reference books and entomology experts. Adult insects were identified as recognisable taxonomic units using published keys with the help of reference specimens, and further classified into bees and hoverflies, respectively. The insect species richness per transect was cumulated by the number of visitor, bee, and hoverfly species, and insect abundance was accumulated by the sum of all individuals for visitors, bees, and hoverflies throughout two sampling dates with morning and afternoon per date in the given year. We pooled floral visitor data from the three sites for further analysis of the relative abundance. The relative abundance of each species was calculated using the formula $pi = ni/N$, where pi represents the relative abundance of species i, ni is the number of specimens for species i, and N is the total number of specimens (Table S2).

Measurements of alfalfa seed yield

In early August 2021, the pods and seeds of alfalfa were harvested when ripping in the three sites. We randomly selected 20 alfalfa individuals from each transect (the same transect with visitor sampling) at each site, ensuring a distance of at least 4 m between plant individuals. One basal branch of each plant individual was selected, and the number of pods for the alfalfa branch was counted. The number of seeds per pod, with random five pods per branch, was further counted, for a total of 100 pods per transect. The number of pods per branch was calculated by the average of pod number per 20 branches per transect, and the number of seeds per pod was calculated by the average of 100 pods per transect.

Climate variables

Climate data for the three experimental sites were obtained: the climate data for CL and HB were acquired from the records of the local meteorological station, and the climate data of GZL were sourced from the WorldClim database (www.worldclim.org/). We used the mean air temperature and the total precipitation amount during the growing season in the experimental year (April–September 2021) as environmental variables. Mean temperature was calculated as the average of the monthly mean temperatures, and total precipitation amount was obtained by calculating the sum of monthly precipitation amounts from April to September (Table S1).

Statistical analyses

Community composition of floral visitors at the three sites was examined by principal coordinate analysis (PCoA) on the Bray–Curtis dissimilarity index, and the permutational multivariate analysis of variance (PERMANOVA) with Adonis function using the 'vegan' package (Oksanen *et al.*, 2013). All data were tested for normality and homogeneity of variances, but no transformations were needed. The differences in the richness and abundance of visitors, bees, and hoverflies among the three experimental sites were analysed using generalised linear mixed models in the R package 'lme4' with a Poisson distribution, while the number of pods per branch and the number of seeds per pod were evaluated using the same models with a Gaussian distribution (Hothorn *et al.*, 2008). For post hoc comparisons, the 'agricolae' package was used with Least Significant Difference tests, and results were considered significant at the level of $P < 0.05$. Linear and quadratic models were used to examine the relationships between richness and abundance of floral visitors and climatic variables, and the relationships between richness and abundance of floral visitors and alfalfa seed yield, as well as the relationships between climate variables and alfalfa seed yield, respectively. Both linear and quadratic models were selected based on Akaike information criterion (AIC) values and P-values, with the model exhibiting the lowest AIC and P-values deemed the best fit (Goulnik *et al.*, 2020). To assess the importance of climate variables and floral visitors for alfalfa seed yield, we performed feature selection using a 'random forest' algorithm with the R package 'Boruta' (500 trees). Boruta determines whether the importance of each variable is significantly higher than that of a random variable (shadow features) ($P < 0.05$) by assigning randomness to the data and creating shuffled copies of all parameters as shadow features (Kursa and Rudnicki, 2010). All data analyses were performed with the software R (version 4.4.1 × 64-bit, 2024; The R Foundation for Statistical Computing).

Results

Diversity and abundance of alfalfa floral visitors

A total of 3017 visitors belonging to 5 orders, 32 families, and 87 species were recorded (Table S2). Diptera (27 species) and Hymenoptera (27 species) were the most abundant visitors of alfalfa, representing 53.40% and 21.94% of all the visitors sampled, respectively (Figure S2a, b). At the order level, the community composition of visitors varied among the three sites. The richness (36.51% of the total number of species) and abundance (28.27% of the total number of individuals) of Hymenoptera were greater in CL than those in GZL and HB, with *A. mellifera, Eutricharaea argentata*, and *Megachile* sp. being the main species. The Diptera showed the lowest richness (28.57% of the total number of species) and abundance (30.10% of the total number of individuals) at the CL site (Figure S2c).

From the Venn diagram, 23 species of visitors were shared by the three sites (Figure S3a). Principal coordinates analysis clearly showed that visitor community composition significantly ($P = 0.004$, PERMANOVA) differed among the three sites. The first principal component (PCoA1) could explain 43.17% of the total variance, and the second principal component (PCoA2) could explain 43.05% of the total variance (Figure S3b).

There are significant differences in the richness and abundance of floral visitors observed among the three experimental sites, with the highest numbers found in CL and the lowest in GZL (fig. 1a, b). The proportion of bee and other species richness in CL was higher than that of GZL and HB, whereas the opposite pattern was observed for the hoverfly richness (fig. 1c). The proportion of both bee and hoverfly abundance was higher in GZL than in CL and HB, with the opposite pattern observed for the abundance of other visitor species (fig. 1d). Bee richness in CL was notably greater than in GZL and HB, and the hoverfly richness of HB was significantly higher than that of GZL and CL (fig. 1e). The number of hoverflies showed significant differences among

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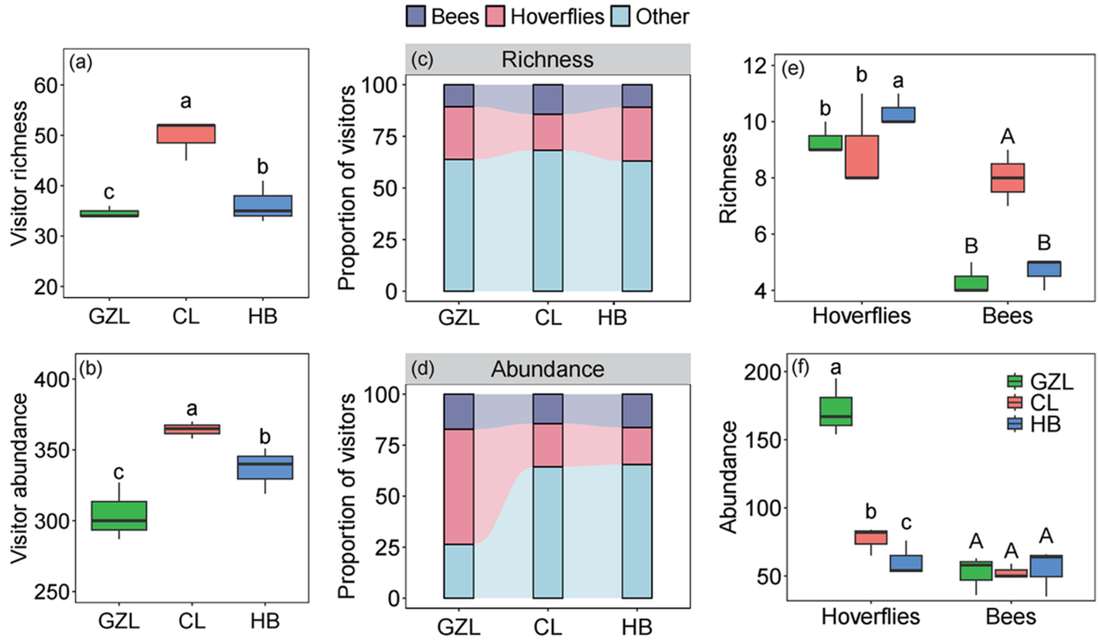

Figure 1. The difference in richness and abundance of visitors, bees, hoverflies, and visitor composition among the three experimental sites: Gongzhuling (GZL), Changling (CL), and Harbin (HB). The difference in visitor richness(a), visitor abundance (b), richness of bees and hoverflies (e), and abundance of bees and hoverflies (f) among the three experimental sites. The proportions of richness (c) and abundance (d) of bees, hoverflies, and other visitors in the three experimental sites. Different lower or capital letters indicate significant differences among the sites.

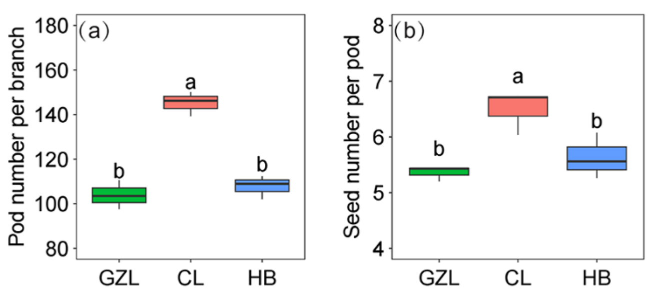

Figure 2. The comparison of alfalfa seed production among the three experimental sites: Gongzhuling (GZL), Changling (CL), and Harbin (HB). The difference in the pod number per branch (a) and seed number per pod (b) among the sites. Different lowercase letters (a and b) indicate significant differences among the sites ($P < 0.05$).

different sites, while there was no difference in the number of bees (fig. 1f).

Alfalfa seed yield

Significant differences in alfalfa seed production were observed among the three experimental sites. Specifically, the number of pods per branch and the number of seeds per pod in CL were significantly higher than in GZL and HB (fig. 2a, b).

Effects of climate variables and floral visitors

The richness of visitors and bees showed a pattern of first decreasing and then increasing in relation to the mean air temperature during the growing season (fig. 3a, e). No relationship was found between hoverfly richness and mean air temperature (fig. 3i). The richness of both visitor and bee species increased and then decreased with the increasing gradient of total precipitation amount during the growing season (fig. 3b, f). Hoverfly richness was not related to the total precipitation amount (fig. 3j). The abundance of visitors decreased and then increased with the increasing gradient of mean air temperature, whereas hoverfly abundance exhibited an opposite pattern (fig. 3c, k). No relationship was detected between bee abundance and mean air temperature (fig. 3g). The dynamics of the abundance of visitors firstly increased and then decreased with the increasing gradient of total precipitation amount, the abundance of hoverflies significantly increased with total precipitation amount, and there was no relationship between bee abundance and the total precipitation amount (fig. 3d, h, l).

The pod number per branch and the seed number per pod were positively correlated with the mean air temperature during the growing season (fig. 4a, c). With the increase in total precipitation amount, the pod number per branch and the seed number per pod increased first and then decreased (fig. 4b, d). The number of pods per branch was positively associated with the richness of visitor and bee species (fig. 4e, i), and it firstly decreased and then increased with the increasing diversity of hoverflies (fig. 4m). The number of pods per branch initially decreased and then increased with the increasing abundance of visitors (fig. 4f), and it was not correlated with the abundance of bees and hoverflies (fig. 4j, n). The number of seeds per pod was significantly correlated with the richness of visitor and bee species, and the abundance of visitors (fig. 4g, k, h), but not with the richness of hoverflies or the abundance of bees or hoverflies (fig. 4o, l, p).

Relative importance of climate variables and floral visitors

The importance score ranking shows that air temperature, visitor richness, visitor abundance, and bee richness are all quite important for the number of pods per branch of alfalfa, as they have relatively high importance scores (fig. 5a, b). For the number of

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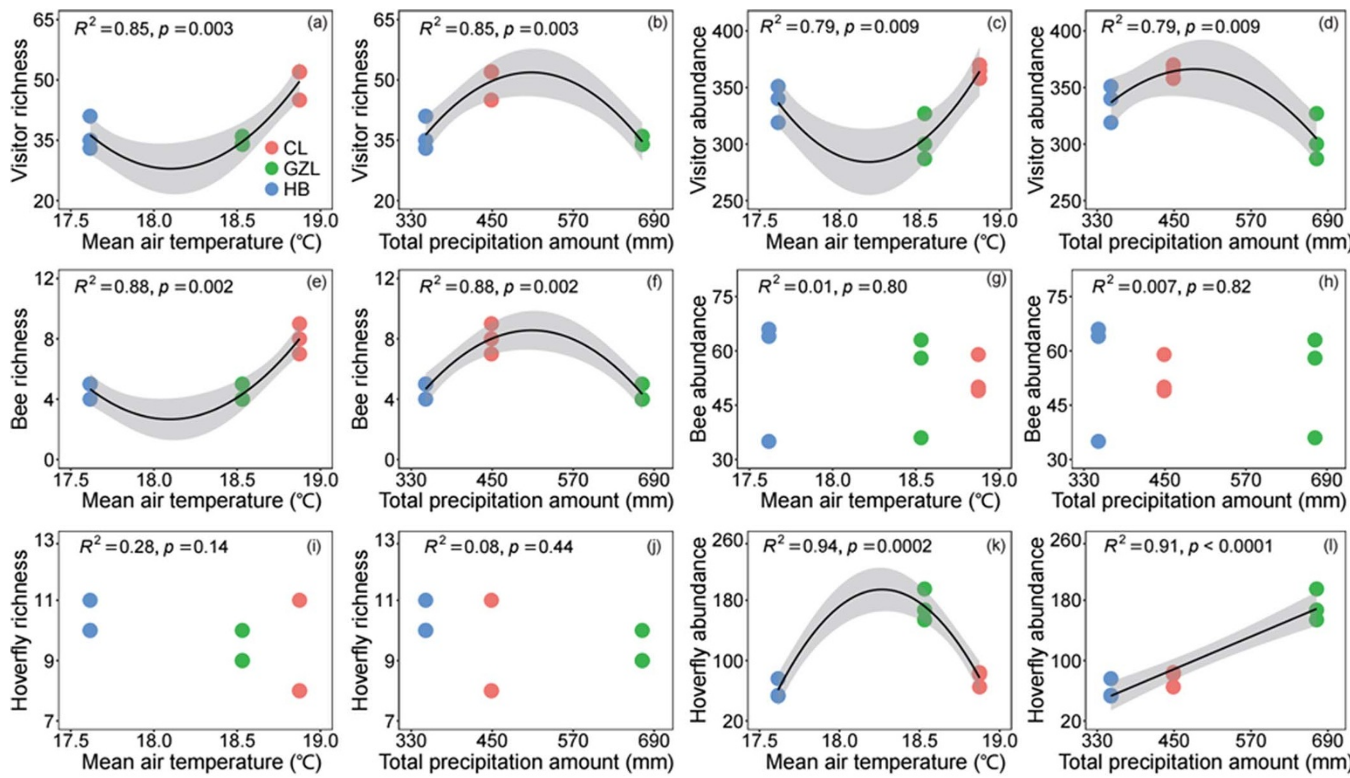

Figure 3. Relationships between the mean air temperature or total precipitation during the growing season of the experimental year and the number of floral visitors (a–d: visitor richness and abundance; e–h: bee richness and abundance; i–l: hoverfly richness and abundance). Black lines show the ordinary least squares regression fits. The grey-shaded areas represent the 95% confidence intervals. GZL, Gongzhuling; CL, Changling; HB, Harbin.

seeds per pod, visitor abundance has the highest importance score and serves as the key factor affecting alfalfa seed yield (fig. 5c, d). Meanwhile, the relatively low importance score of other factors was considered to have a poor effect on the seed yield of alfalfa.

Discussion

Variation in the pollinator community and their consequences for plant production and crop yield are extensively focused (Gibbs *et al.*, 2016; Park *et al.*, 2016; Laina *et al.*, 2022). In our study, using the alfalfa plantation system model, we found differences in the community composition, diversity, and abundance of alfalfa pollinators across the three experimental sites with different landscape types, which are mainly driven by landscape types along with climatic variables. Changed pollinator diversity, combined with climatic factors in turn affected alfalfa seed yield. Our study provided new experimental evidence that the effects of pollinators on alfalfa reproduction are more complex than previously thought.

As expected, we found significant differences in the community composition, richness, and abundance of visitors to alfalfa across three experimental sites. The CL site, situated in a semi-natural grassland in this study, supported greater richness and abundance of pollinators than the two other sites, which is consistent with most previous studies (Gaspar *et al.*, 2022; Maurer *et al.*, 2022). Semi-natural landscapes consistently maintain higher richness and abundance of pollinators than farmland landscapes (Ganuza *et al.*, 2025), because semi-natural grasslands are often accompanied by more flowering forb plants, such as *Kalimeris integrifolia, Potentilla flagellaris*, and these flowering plants could provide sufficient food resources for pollinators (Khalil *et al.*, 2023). Meanwhile, abundant plant species richness and heterogeneous structure of grass-forb community could also increase habitat complexity, subsequently offering more nesting options for pollinators and helping minimise predation risk by providing refuges (Gaspar *et al.*, 2022). Compared to semi-natural grasslands, lower pollinator richness and abundance in the HB site with a farmland landscape were found. As the farmland habitats were cultivated by non-flowering crops, such as *Zea mays* and *Oryza sativa*, resulting in habitat simplification that provided fewer food resources and habitat for pollinators (Udy *et al.*, 2020). Meanwhile, the use of chemical pesticides could also reduce pollinator richness and abundance by directly causing acute or chronic toxicity (Goulson *et al.*, 2015). With the rapid spread of urbanisation, pollinator diversity has been increasingly threatened (Liang *et al.*, 2023). In our study, the results found the lowest pollinator richness and abundance at the GZL site of the urbanised landscape, compared with the CL and HB sites. Although diverse ornamental flowering plants are often used in the urban landscape, habitat simplification, extensive buildings, and impervious surfaces severely limit opportunities for pollinators to find nests and food resources (Baldock *et al.*, 2015; Bennett and Lovell, 2019; Fijen *et al.*, 2022). Thus, it is suggested that semi-natural grassland ecosystems are more beneficial to the conservation of wild pollinators.

Generally, climatic variables (i.e. air temperature and precipitation patterns) are important abiotic factors in influencing pollinator diversity through direct and indirect effects (Fourcade *et al.*, 2019). In this study, the higher mean air temperature recorded at the CL site corresponded to increased pollinator richness and abundance, which is consistent with the observations from the

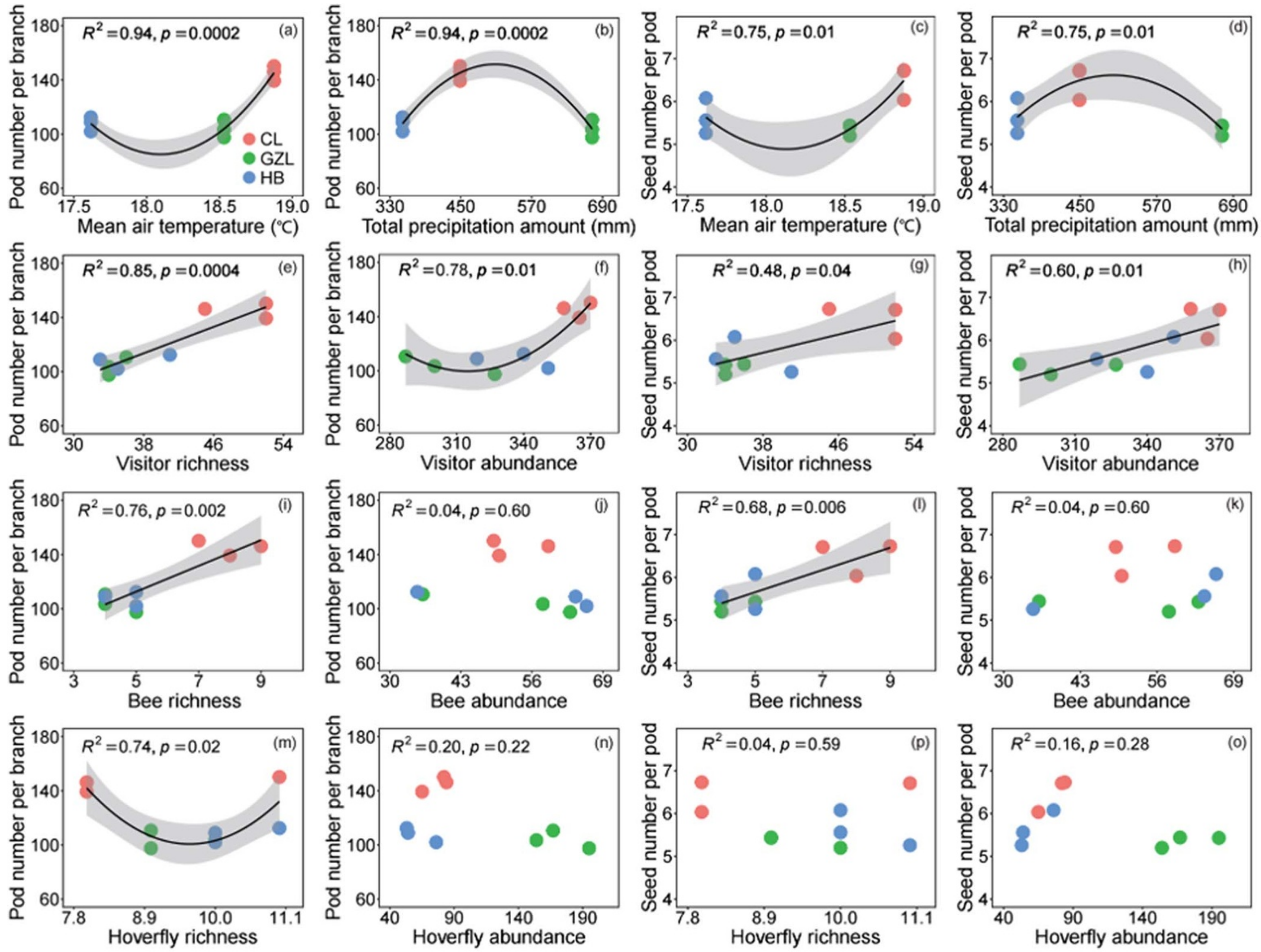

Figure 4. Relationships between the mean air temperature or total precipitation during the growing season of the experimental year and alfalfa seed yield (a and b: pod number per branch; c and d: seed number per pod). Relationships between the pod number per branch and the number of floral visitors (e and f: visitor richness and abundance; i and j: bee richness and abundance; m and n: hoverfly richness and abundance). Relationships between the seed number per pod and the number of floral visitors (g and h: visitor richness and abundance; k and l: bee richness and abundance; o and p: hoverfly richness and abundance). Black lines show the ordinary least squares regression fits. The grey-shaded areas represent the 95% confidence intervals. GZL, Gongzhuling; CL, Changling; HB, Harbin.

European Alps (Corcos *et al.*, 2018). The positive effects of air temperature on pollinators may be driven by its dual role: direct enhancement of the development duration, reproductive capabilities, and survival rates of pollinators (Chick *et al.*, 2019), and indirect modulation of floral availability for pollinators (Lessard *et al.*, 2011). Besides air temperature, precipitation also plays a central role in influencing pollinator richness and abundance (Zhu *et al.*, 2014; Fourcade *et al.*, 2019). Our results found that higher precipitation amount was closely related to lower floral visitor richness and abundance. The negative relationships were likely attributed to multiple pathways: an unfavorable habitat with excessive humidity, direct damage to the wings, and the dilution of pollen and nectar nutrients (Frampton *et al.*, 2000; Lawson and Rands, 2019; Frigero *et al.*, 2025).

A growing number of studies have confirmed that variations in the richness and abundance of pollinators significantly affect seed production in flowering plants (Woodcock *et al.*, 2019; Robinson *et al.*, 2023). In this study, alfalfa seed yield exhibited distinct variations across the three experimental sites, with higher yields at the CL site, and lower yields at the GZL site. The alfalfa yield pattern was likely attributed to changes in the richness and abundance of floral visitors. Our results found that the species richness and abundance of total visitors and bees were significantly higher at the CL site, and diverse visitor species played an important role in the higher alfalfa seed production. Firstly, diverse pollinator species may enhance pollination efficiency through complementary or synergistic effects (Garibaldi *et al.*, 2016). Particularly, among these floral visitors, bees are recognised as efficient pollinators and play a vital role in increasing seed yield for most plant species (Garibaldi *et al.*, 2013; Zhang *et al.*, 2022). Higher bee richness, mainly leaf-cutting bees in the CL site enhanced the seed yield of alfalfa. In fact, leaf-cutting bees are commonly used to improve alfalfa seed yield in agricultural systems (Pitts-Singer, 2013; Haedo *et al.*, 2022). In addition to richness, visitor abundance is also a critical factor affecting seed yield of crops (Eeraerts, 2022; Gaspar *et al.*, 2022; Bao *et al.*, 2025), and these studies have shown that high pollinator abundance could increase the probability of pollen transfer by enhancing floral visit frequency, and consequently for plant

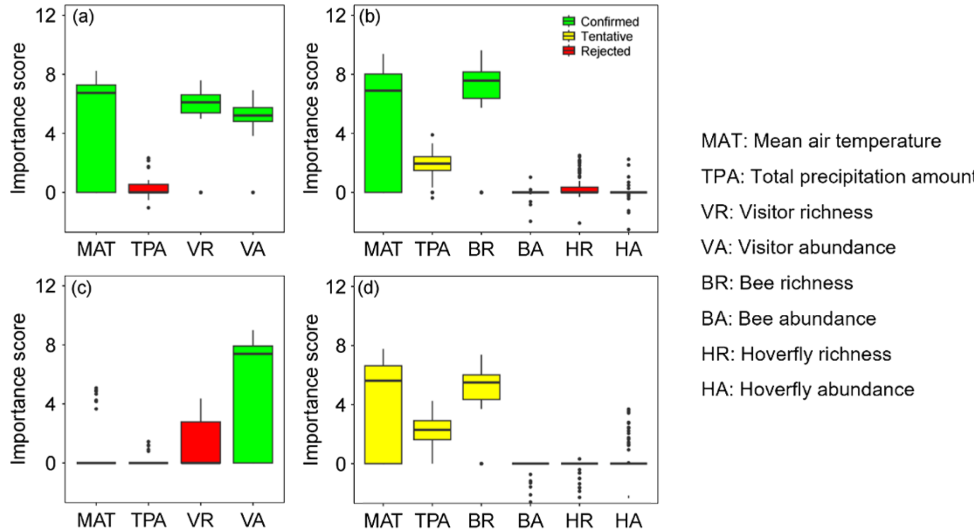

Figure 5. The relative importance of floral visitors and climate variables for alfalfa seed production based on Boruta analysis (a and b: pod number per branch; c and d: seed number per pod). Green boxplots correspond to factors that are marked as 'confirmed,' indicating that they are highly significant factors ($P < 0.05$). Yellow and red boxplots correspond to factors marked as 'tentative' and 'rejected' respectively, indicating that these factors are unimportant ($P > 0.05$).

reproductive success (Woodcock *et al.*, 2019). In this study, more abundant visitors resulted in the higher seed yield of alfalfa in the CL site, while the lower seed yields in the other two sites were linked to lower abundance of visitors and bees. Although hoverflies were relatively abundant at GZL and HB sites, they did not contribute to higher alfalfa seed yields. This contradicts the findings of other studies where hoverflies promoted seed yield of strawberries and caraway (Toivonen *et al.*, 2022; Van Oystaeyen *et al.*, 2022), which may be likely due to the mismatch between the specific tripping mechanism of alfalfa and the visiting behaviour of hoverflies. Therefore, it is implied that hoverflies may be less effective as pollinators of alfalfa.

While pollinators exert significant impacts on plant reproduction, climatic variables also serve as key drivers regulating crop yield (Alsajri *et al.*, 2020). In this study, higher air temperatures were observed to positively affect alfalfa seed yield, consistent with conclusions from previous research that air temperature promotes plant seed production (Bogdziewicz *et al.*, 2019). Increase in air temperature could directly accelerate pollen germination and enhance reproductive efficiency, and indirectly regulate pollinators, thereby influencing the seed yield of crops (Pahadi *et al.*, 2025; Zanini *et al.*, 2026). In contrast, precipitation in this study showed a negative correlation with alfalfa seed yield. This stands in contrast to the findings that higher rainfall amounts increased plant yield (Hu *et al.*, 2025). This phenomenon is likely linked to the ecological adaptability of alfalfa in arid and semi-arid regions (Tlahig *et al.*, 2025). Excessive rainfall amounts can induce yield loss through restricted root development, and poor uptake of water and nutrients (Parent *et al.*, 2008; Li *et al.*, 2019), and by decreasing both the frequency of flower visitations and the abundance of pollinators (Lawson and Rands, 2019). Notably, the effects of climatic variables and pollinators on crop seed yield are often non-independent, they may typically exhibit additive or combined effects (Olsen *et al.*, 2022; Zanini *et al.*, 2026). Our results revealed the combined effects of climate and pollinators on alfalfa seed yield, where diverse pollinator richness/abundance jointly with high air temperatures significantly increased alfalfa seed production. Although the findings are not tested by a controlled experiment, these results confirmed that both pollinators and climate variables are important factors in influencing alfalfa yield.

Conclusions

Numerous studies have established the critical role of pollinators in alfalfa seed production (Cecen *et al.*, 2008; Pitts-Singer and James, 2008), but there is a notable scarcity of research examining the integration of landscape, climate, and pollinators. This study clarifies the relationships among climate, floral visitors, and alfalfa seed yield across different landscape types, revealing that visitor variations are driven by climatic variables, and that changed visitors, in conjunction with climate, influence alfalfa seed yield. These results enrich the understanding of climate-mediated pollinator dynamics and their combined effects on crop yield. It suggests that protecting wild pollinators is crucial for enhancing alfalfa seed yield, especially under different landscape types and climatic changes.

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