

## Differential Response of Pumpkin Pollinator Density to Habitat Loss in Southeastern Yunnan (Postprint)

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### Abstract

Response diversity theory posits that different pollinator bees exhibit spatiotemporal variation in their response scales and processes to environmental stressors; however, the response patterns of pollinator bees in important agricultural and forestry ecosystems of China to environmental stressors remain unclear. This study examined a pumpkin agroecosystem in Guangnan County, Wenshan Prefecture, Yunnan Province, selecting pumpkin sample plots based on gradients in the quantity and area of natural and semi-natural habitats within the farmland landscape and distance gradients from natural and semi-natural habitats, and conducted sampling surveys of bumblebee density and honey bee density within the pumpkin plots. Satellite imagery was interpreted to obtain land cover data, the correlation coefficients between bumblebee density and honey bee density and the percentages of natural and semi-natural habitat area and cropland area were analyzed, and the response patterns of honey bee density and bumblebee density to habitat loss were explored at the spatial scale corresponding to the maximum correlation coefficient. The study found that bumblebee density increased significantly with increasing percentage of natural and semi-natural habitat area, whereas honey bee density was not affected by natural and semi-natural habitat area. Meanwhile, bumblebee density decreased significantly with increasing percentage of cropland area, whereas honey bee density was not affected by cropland area. Furthermore, bumblebee density decreased significantly with increasing distance from natural and semi-natural habitats, whereas honey bee density was not influenced by distance. Thus, pumpkin-pollinating bumblebees and honey bees in Wenshan Prefecture, Yunnan Province exhibited diverse response patterns to habitat loss. The differential responses of bumblebees and honey bees to habitat loss may represent an important mechanism ensuring pumpkin pollination in this region.

## Full Text

### Preamble

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### **Response of summer squash pollinator density to habitat loss in Southeast Yunnan, China\***

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**Abstract:** The response diversity theory claims that functional pollinators differently react to environmental change across a range of spatial and/or temporal scales. Diverse responses of pollinators could contribute to the stabilization and resilience of ecosystem pollination functions through the reorganization of pollinator communities. To date, however, little remains known about the responses of Chinese pollinators to environmental changes, especially considering the rich pollinator diversity represented in China. We selected 18 summer squash fields in Guangnan County, Yunnan Province, to study the reaction of bee communities to habitat loss. In our study, habitat loss was represented with two change gradients—one gradient of natural and semi-natural habitats surrounding the squash fields in landscapes (used to investigate landscape effects on pollinators) and one gradient of distance away from natural and semi-natural habitats (used to investigate distance effects on pollinators). While 8 fields were selected to explore the landscape effect, 12 fields were used to explore the distance effect, and two fields were simultaneously used for both experiments. Flower visitors were sampled once in the 18 fields on sunny days from June to July 2015. Land use and land cover were derived from Landsat satellite imagery and were identified as secondary forest, grassland, water, dryland, wetland, and building. Secondary forest and grassland were treated as natural and semi-natural habitats, which were beneficial for managed and wild bees. Dryland and wetland were treated as farmland, which were believed to have a diminishing effect on wild bee populations. The Pearson correlation coefficient was calculated between pollinator density and percentages of semi-natural habitats and farmlands at a range of nested spatial scales (250 m, 500 m, 750 m, 1,000 m, 1,500 m, 2,000 m, and 2,500 m). Responses of honey bee density and bumble bee density along the percentages of natural and semi-natural habitats and farmlands were

explored at spatial scales with the highest correlation coefficient. Honey bee and bumble bee density changes along the distance away from natural and semi-natural habitats were also explored. We found that bumble bees and honey bees were the dominant pollinators for squash flowers in the study area. Ants and wasps also visited the squash flowers, but they were not analyzed due to their extremely low densities. Bumble bee density and honey bee density were associated with natural and semi-natural habitats and farmlands at different spatial scales. The percentage of natural and semi-natural habitats was significantly negatively correlated to that of farmlands at spatial scales ranging from 500 m to 2,500 m. Bumble bee density increased along the gradient of natural and semi-natural habitats in landscapes, but honey bee density was not influenced by surrounding natural and semi-natural habitats. While bumble bee density declined as surrounding farmlands increased in landscapes, honey bee density was not influenced by farmlands. Moreover, bumble bee density declined along increasing distance away from natural and semi-natural habitats, but honey bee density was not influenced by the distance to natural and semi-natural habitats. The different responses of honey bees and bumble bees to habitat loss could be related to pollination services of squash. Our findings indicated the need for special attention by farmers and researchers to understanding and managing crops that are reliant on wild pollinators.

**Keywords:** Summer squash; Pollinator; Bumble bee; Honey bee; Spatial scale; Land use type; Response diversity

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## Introduction

Pollination service is a crucial ecosystem function, and revealing the mechanisms that maintain this service represents a current research priority. The prevailing view holds that response diversity plays an important role in stabilizing ecosystem pollination. This theory posits that different pollinators exhibit distinct response characteristics, processes, and scales to environmental stress in time and space, thereby ensuring the stability of ecosystem pollination services. Consequently, exploring pollinator response patterns to environmental stress carries significant scientific and ecological importance.

Research indicates that habitat loss constitutes a primary threat to pollinating bees, primarily manifested through the reduction of natural and semi-natural habitats. Previous studies have revealed the impacts of natural and semi-natural habitat reduction on pollinating bees at both local and landscape scales. At the local scale, pollinator diversity is higher near natural and semi-natural habitats and decreases with distance from them. At the landscape scale, reductions in natural and semi-natural habitat quantity cause declines in pollinator diversity. Simultaneously, different pollinators show varied response processes to changes in natural and semi-natural habitats. For instance, when wild bee diversity (excluding *Apis* spp.) declines with decreasing natural and semi-natural habitat

quantity, honey bee abundance (including managed colonies) does not necessarily decrease.

China possesses extremely rich diversity of wild pollinating insects. While Europe and America have conducted relatively more research on pollinator diversity and influencing factors in agricultural and forest ecosystems, such studies remain scarce in China. The mechanisms maintaining ecosystem pollination service functions are not well understood. These theoretical and research gaps may affect pollinator conservation and could even impact food security. Therefore, it is necessary to conduct research on specific agroforestry ecosystems to understand pollinator diversity characteristics, environmental stresses they face, and the resulting dynamics of pollination service functions.

The Yunnan-Guizhou Plateau represents one of China's richest regions for wild bees. Previous studies have shown that wild bees (such as *Bombus* spp.) in this region have experienced diversity declines due to natural and semi-natural habitat loss, causing insufficient pollination services in some ecosystems. As a hotspot of wild bee diversity, the stresses on pollinators, their intensity, and their response patterns to habitat loss remain unclear. This study examines the pumpkin (*Cucurbita moschata* Duch.) agroecosystem and its pollinating insects—in the karst landscape of Southeast Yunnan to explore how dominant pollinators—honey bee density and bumble bee density—respond to changes in natural and semi-natural habitat quantity in farmland landscapes and to distance from these habitats. This work aims to establish a foundation for revealing the stability mechanisms of ecosystem pollination services in the Yunnan-Guizhou Plateau of China.

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## 1. Materials and Methods

### 1.1 Study Site Selection

The experiment was conducted from mid-June to July 2015 in Guangnan County, Wenshan Prefecture, Yunnan Province (Fig. 1 [Figure 1: see original paper]A, 23°47' -24°20' N, 104°34' -105°22' E). We selected 18 pumpkin fields, with 8 fields used to study the response of pumpkin pollinator communities to farmland landscapes (referred to as “landscape effects”) and 12 fields used to study changes in pollinator community composition at different distances from natural and semi-natural habitats (referred to as “distance effects”). Two fields were simultaneously used for both experiments (Fig. 1B-C). To investigate landscape effects on pumpkin pollinator communities, we selected pumpkin fields based on a gradient of natural and semi-natural habitat quantity (secondary forest + grassland, see “Landscape Analysis” section) surrounding the fields (0-91.40%) (Fig. 1B). To ensure spatial independence, the distance between any two fields was at least 5 km. To investigate distance effects on pumpkin pollinator communities, we selected pumpkin fields along a gradient of distances from natural and semi-natural habitats (0-2.55 km: 0 indicates

adjacent to natural and semi-natural habitats, and 2.55 km represents the maximum distance from natural and semi-natural habitats in the study area) (Fig. 1C). Pumpkin field areas ranged from 497 to 1,604 m<sup>2</sup>, determined by planting conditions. All fields were in full bloom during sampling. No pesticides or herbicides were applied to pumpkin fields from planting to harvest. Although surrounding crops may have used pesticides or insecticides for pest control, no pesticide application was observed in pumpkin fields or surrounding farmland during sampling.

### 1.2 Pollinator Survey

Depending on pumpkin field size, we established either one 100 m × 3 m transect or two 50 m × 3 m transects within each field, with transects located at least 5 m from field edges. Within each transect, we randomly placed 20 1 m<sup>2</sup> quadrats, with at least 2 m between quadrats. Observers recorded pollinator species and abundance within quadrats from the center point. Each quadrat was observed for 2 minutes, resulting in 40 minutes of observation per field.

During observation periods, flower visitors primarily included honey bees, bumble bees, wasps (Hymenoptera: Vespidae), and ants (Hymenoptera: Formicidae). Wasps were extremely rare, flying among flowers to search for and prey on other insects, with minimal flower contact and poor pollination effectiveness. Ants were observed on few flowers. Since pumpkin has separate male and female flowers, we did not observe ants moving between them, indicating weak pollination effectiveness. Honey bees and bumble bees were the main pollinators, so observers recorded species abundance of these two groups. During the 2-minute observation period, observers recorded pollinators entering the quadrat; once a pollinator left the quadrat, it was no longer recorded.

Bumble bee species included *B. montivagus*, *B. breviceps*, *B. atripes*, and *B. ignitus*. Although *B. montivagus* and *B. breviceps* belong to different subgenera (*Megabombus* and *Alpigenobombus*, respectively), their similar color patterns made field identification difficult, so they were counted as *montivagus* + *breviceps*. After quadrat observations, at least 10 minutes of field collection was conducted at each pumpkin field, confirming the coexistence of *B. montivagus* and *B. breviceps*. Honey bees were dominated by *Apis cerana*, with only a few *A. mellifera* visitors.

### 1.3 Landscape Analysis

We used maximum likelihood classification to interpret ETM+ aerial imagery from May 20, 2009 (cloud cover <3%). Land types were classified into six categories: secondary forest, grassland, dryland, paddy field (including irrigated land), buildings (including roads), and water bodies. Training points were derived from field sampling locations, with spectral separability >1.80 between all land type pairs and classification accuracy >94.38%, meeting requirements for spatial data analysis.

Since different pollinators respond to landscape structure at different scales, we first analyzed correlations between honey bees/bumble bees and landscape structure, then explored response patterns at scales showing maximum correlation. We calculated the ratio of “secondary forest + grassland” (natural and semi-natural habitats) and “dryland + paddy field” (farmland) areas to total land area within circular buffers centered on pumpkin fields at seven spatial scales: 250 m, 500 m, 750 m, 1,000 m, 1,500 m, 2,000 m, and 2,500 m (Fig. 2 [Figure 2: see original paper]). Using geographic information system software, we established circular buffers at different spatial scales and calculated the area percentages of natural and semi-natural habitats (Fig. 2A) and farmland (Fig. 2B) within each buffer. Previous studies have shown that wild bee diversity positively correlates with natural and semi-natural habitat quantity and negatively correlates with farmland area. By establishing relationships between land type percentages and pollinator density, we explored correlations between pollinators and natural/semi-natural habitats and farmland, then calculated pollinator response patterns at scales showing maximum correlation.

Pumpkin field area showed no significant correlation with natural and semi-natural habitat percentage ( $r < 0.33$  at all scales, Pearson correlation test  $P > 0.42$ ) or farmland area percentage ( $|r| < 0.41$  at all scales, Pearson correlation test  $P > 0.31$ ), indicating that field area did not vary with landscape structure.

#### 1.4 Statistical Analysis

Pearson correlation coefficients were calculated between bumble bee density/honey bee density and natural and semi-natural habitats/farmland at different spatial scales, testing whether correlations differed significantly from the null hypothesis (zero correlation). Pearson correlation was also used to assess relationships between natural and semi-natural habitat percentage and farmland percentage at different scales.

Simple linear models (LM) tested effects of pumpkin field size (FS), landscape-level natural and semi-natural habitat percentage (PNSH), or farmland percentage (PF) on bumble bee and honey bee density. Simple linear regression also examined effects of distance to natural and semi-natural habitats and farmland area on pollinator densities. Four candidate models were constructed: (1)  $Y = X1 + X2$ ; (2)  $Y = X1$ ; (3)  $Y = X2$ ; (4)  $Y = X1 + X2 + X1 \times X2$ , where  $X1$  is field size (FS) and  $X2$  is natural and semi-natural habitat percentage (PNSH) or farmland percentage (PF). Bumble bee and honey bee densities were log-transformed to meet homogeneity of variance and residual normality assumptions. The best model was selected based on the lowest AICc value. The best model determined effects of natural and semi-natural habitat percentage or field size on pollinator densities, with  $P < 0.05$  considered significant. When the best model excluded field size or the interaction term, we further tested models 1 or 4 to verify that excluded variables had no significant effect, thereby confirming model validity.

## 2. Results

### 2.1 Differential Responses of Honey Bee and Bumble Bee Density to Natural and Semi-Natural Habitats in the Landscape

Bumble bee density showed significant correlation with natural and semi-natural habitat percentage at the 500 m scale ( $t = 5.77$ ,  $df = 6$ ,  $P = 0.001$ ). At the 2,500 m scale showing the strongest correlation, honey bee density showed no significant relationship with natural and semi-natural habitat percentage ( $t = 0.72$ ,  $df = 6$ ,  $P = 0.50$ ) (Fig. 3 [Figure 3: see original paper]A). At the 500 m scale, bumble bee density increased significantly with natural and semi-natural habitat percentage (Fig. 3B, Table 1), while at the 2,500 m scale, honey bee density did not increase significantly with natural and semi-natural habitat percentage (Fig. 3C, Table 1).

### 2.2 Differential Responses of Honey Bee and Bumble Bee Density to Farmland in the Landscape

Farmland percentage and natural and semi-natural habitat percentage showed no significant correlation at the 250 m scale ( $P = 0.13$ ) but were significantly negatively correlated at scales  $>250$  m ( $P < 0.05$ ). Bumble bee density showed significant correlation with farmland percentage at the 1,000 m scale ( $t = -3.52$ ,  $df = 6$ ,  $P = 0.01$ ). At the 500 m scale showing the strongest correlation for honey bees, no significant relationship was detected between honey bee density and natural and semi-natural habitat percentage ( $t = -0.90$ ,  $df = 6$ ,  $P = 0.40$ ) (Fig. 4 [Figure 4: see original paper]). At the 1,000 m scale, bumble bee density decreased significantly with increasing farmland percentage (Fig. 4B, Table 2), while at the 500 m scale, honey bee density did not decrease significantly with farmland percentage (Fig. 4C, Table 2).

### 2.3 Distance Responses of Honey Bee and Bumble Bee Density to Natural and Semi-Natural Habitats

Distance to natural and semi-natural habitats significantly affected bumble bee density but not honey bee density (Table 3). Bumble bee density decreased with increasing distance from natural habitats (Fig. 5 [Figure 5: see original paper]A), while honey bee density showed no significant change (Fig. 5B).

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## 3. Discussion and Conclusion

Bumble bees and honey bees are the primary pollinators of pumpkin in South-east Yunnan, but they exhibit different response patterns to habitat loss. First, their correlations with natural and semi-natural habitats in the landscape differ. Bumble bee density showed the highest correlation coefficient with natural and semi-natural habitat percentage at the 500 m scale, whereas honey bee density showed the highest correlation at the 2,500 m scale. Additionally, bumble bee

density showed the highest correlation with farmland percentage at the 1,000 m scale, while honey bee density showed the highest correlation at the 500 m scale. Previous studies suggest that pollinator spatial response scales may relate to body size and foraging range. However, in our agroecosystem, honey bee density may be confounded by managed colony numbers, potentially affecting its relationships with natural and semi-natural habitats and farmland.

Second, at scales showing maximum correlation, bumble bee and honey bee densities responded differently to natural and semi-natural habitats in the landscape. Bumble bee density decreased with declining natural and semi-natural habitat percentage, while honey bee density showed no clear response. The pumpkin-pollinating bumble bees in Southeast Yunnan are wild species, and natural and semi-natural habitats provide abundant nectar and pollen resources, limiting bumble bee density. In contrast, honey bees may originate from either wild or managed colonies. Since managed honey bee populations are not limited by natural and semi-natural habitats, honey bee density remains unaffected when natural and semi-natural habitat area decreases and bumble bee density declines.

In the agroecosystems of Southeast Yunnan, bumble bee density showed significant negative correlation with farmland percentage, indicating that farmland expansion in the landscape causes bumble bee declines. Southeast Yunnan is a major agricultural production region in Yunnan Province. Our study confirms that agricultural activities cause bumble bee population declines in this region, consistent with findings from other agroecosystems and collectively revealing how land use conversion affects wild bumble bee diversity. Agricultural practices alter landscape structure by converting natural and semi-natural habitats to farmland, reducing food and nesting resources for wild pollinators and driving pollinator diversity declines. Additionally, chemicals such as pesticides and herbicides negatively impact wild pollinator reproduction and population maintenance. Previous studies indicate these agricultural practices may act synergistically to reduce wild bumble bee populations. Notably, agricultural land in the study area is mostly distributed in karst depressions, while peak-cluster land suffers from severe rocky desertification and is difficult to cultivate. Consequently, karst peak-clusters remain in natural and semi-natural states, providing essential food and nesting resources for wild bees. Although our study shows agricultural activities cause bumble bee abundance declines, the intensity, scope, trends, and consequences of this decline require further investigation.

Distance from natural and semi-natural habitats also affected bumble bee density. The study found that bumble bee density decreased with increasing distance, consistent with findings from other researchers. The distance effect on bumble bee density can be understood as another manifestation of habitat loss, where reduced natural and semi-natural habitats at distant locations cause bumble bee density declines. Although distance significantly reduced bumble bee density, its effect on honey bee density was not significant. Similar to landscape-scale processes, managed honey bees at distant locations likely confound the

distance response of honey bee density.

The study found that while bumble bee density declined with habitat loss, honey bee density remained unaffected. This differential response of bumble bees and honey bees to habitat loss carries important ecological significance. Functionally redundant bumble bees and honey bees are important pumpkin pollinators in Southeast Yunnan, providing pollination services. Studies in other ecosystems have shown that bumble bees deposit more pollen on stigmas per flower visit than honey bees. However, bumble bee populations are vulnerable to landscape structure and decline with habitat loss. Fortunately, honey bee density, which provides equivalent pollination function, is not affected by natural and semi-natural habitats. Therefore, when bumble bee density declines, honey bee visitation can compensate for the pollination deficit, maintaining ecosystem pollination function within certain limits. Thus, the differential response patterns of bumble bees and honey bees to habitat loss in Southeast Yunnan may represent an important mechanism ensuring ecosystem pollination service stability, conferring resilience to pollination functions when ecosystems are disturbed. However, how pollinator response diversity to land use conversion ensures pumpkin pollination services in Southeast Yunnan requires deeper investigation.

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