Diverse Response Diversity in Pollinators: Implications to the Resilience of Pollination Services in Buckwheat

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Abstract

Response diversity to environmental change among species is important for the maintenance of ecosystem services, but response diversity to changes in multiple environmental parameters is largely unexplored. Here, we examined how insect visitations to buckwheat flowers differ among species groups in response to changes in multiple weather variables and landscape structures.

We found differences in responses to changes in weather conditions among insect taxonomic groups visiting buckwheat flowers. Specifically, beetles, butterflies, and wasps were more active in sunny and/or high-temperature conditions, whereas ants and flies showed the opposite pattern. Furthermore, responses to weather conditions differed between large and small insects, which agreed with the expectation that optimal temperature for insect activity has a positive association with body size. Response diversity *per se* was also diverse. For instance, large insects were responsive to temperatures more than small insects while smaller insects were responsive to sunshine duration more than large insects. Responses to spatial variables also differed; large insects were more abundant in fields with surrounding forests and mosaic habitats, whereas small insects were not. We suggest that the “diversity” in “response diversity,” which is a higher-order response diversity, should be a focus of future studies of the biodiversity–ecosystem service relationships.

Introduction

Global environmental changes have led to concern about the deterioration of ecosystem functions and ecosystem services^{1,2}. The conservation of biodiversity may mitigate these effects to some degree, which is a central argument of the biodiversity–ecosystem functioning debate. One of the main rationale is that differences in responses to environmental changes among species provide a basis for preventing the deterioration of ecosystem functions and services through complementary mechanisms^{3,4}. Although this conceptual notion is now widely recognized, the extent to which response diversity contributes to the stability and enhancement of ecosystem services in the real world is not clear^{5,6}. Understanding response diversity to environmental change is essential to maintain bundles of ecosystem services in a changing world.

The mechanism underlying the relationship between diversity and ecosystem services is particularly well understood for pollination, both locally and globally⁷⁻⁹. This is not only because global food production and human health are highly dependent on pollination services, with 75% of crops requiring animal pollination^{10,11}, but also because diverse pollinators potentially play a role for the delivery of crop pollination services. Pollination services are currently facing major threats, including landscape change and climatic change¹²⁻¹⁵. A number of studies have revealed that heterogeneity in landscape structure and the existence of natural land increase the abundance of pollinators and maintain crop production¹⁶⁻¹⁸. However, responses to land use change vary among species and taxonomic groups^{6,19,20}. Dipterans

may partly compensate for decreases in bees and butterflies under land use intensification^{21,22}. This variability (i.e., response diversity) is thus likely to stabilize pollination services under landscape change.

Climatic change may also affect pollination services, not only through decline of pollinator abundance and richness^{15,23}) but also via changes in pollinator activity as well as phenological mismatches between pollinators and crop plants^{12,24,25}. However, as pollinator responses to changes in weather are diverse^{17,26,27}, complementary responses could mitigate the detrimental effects of climate change. For instance, honeybees are active at high temperatures on sunny days, whereas some native bees and hoverflies are active in cloudy and low-temperature conditions²⁸⁻³⁰, indicating the temporal complementation of pollination services. However, there are still few examples of the simultaneous evaluation of the temporal and spatial complementarity of pollinators at the landscape level³¹⁻³³. Furthermore, most studies of pollination services have focused on bees and hoverflies, with relatively few comprehensive analyses of visitors belonging to various taxa^{31,34}. Considering that extreme weather events that may affect pollination services have recently increased in frequency^{15,35}, clarification of spatiotemporal complementarity among diverse insects is an urgent task.

Here, we investigated how insect visitation to buckwheat flowers responds to spatiotemporal environmental change. Buckwheat is a self-incompatible plant with distylous flowers, and thus insect pollination is almost essential³⁶. Demand for buckwheat is increasing owing to the growing interest in health foods³⁷. Insects belonging to various taxa with a range of body sizes visit the flowers³⁸, and non-bee small insects may contribute to seed production³⁶. Therefore, buckwheat is well suited for the investigation of the responses of various flower visitors to spatiotemporal environmental changes and may provide empirical evidence for general issues related to response diversity and ecosystem services.

Our research consisted of two steps, namely, identifying insect response diversity to environmental changes and investigating the relationship between insect visitation and the seed set of buckwheat. The first step was aimed at determining whether different insect taxa exhibit different responses to changes in weather conditions (temperature, sunshine, and wind) and spatial structures of land use (Moran's eigenvector map; MEM) with regard to flower visitations. In particular, we tested the hypothesis that large insects are generally abundant in fine and hot weather and small insects are active even in cloudy and low temperature conditions, contributing to weather-dependent complementarity among groups. This expectation is based on the crude positive association between body size and thermal niches of flower visiting insects that include a broad taxonomic range; lighter insects generally prefer lower temperatures due to increased water loss derived from an increased surface/volume ratio^{22,39}. Although there are species- or taxon-specific differences in preferable weather conditions⁴⁰⁻⁴², trait-based approach across a broad taxonomic range, which is often used for ecosystem service assessment^{43,44}, may provide insight into the resilience of pollination services under climate change. Similarly, the responses to land cover are likely to differ among insects with different sizes, given that insect body size is known to decrease with land-use intensity^{15,22,45}. In the second step, we evaluate whether the number of visiting insects is translated into pollination services. We separately evaluated the contributions of large insects and small

insects to seed set, as in the first survey. For this evaluation, seed set in the control group (open pollination with unlimited access to flowers for all insects) was compared with that when inflorescences were covered with a bag, through which small insects could pass but large insects could not. We evaluated the hypotheses that 1) the seed set is correlated with the abundance of visiting insects and 2) the contributions of small and large insects differ with respect to spatial gradients.

Materials And Methods

Study Area

Field surveys were conducted in the town of Iijima, Nagano Prefecture in central Japan (35°38' to 35°43' N, 13°53' to 137°57' E). The average annual precipitation is 1421.9 mm and the annual average temperature is 11.3°C. Iijima is located in the narrow basin formed along the west side of the Tenryu River at an altitude of 600–700 m. The landscape has a fine-scale mosaic structure including farmlands (mostly rice paddy fields and buckwheat fields), residential areas, and fragmented forests (mixed with conifer plantations and broadleaved trees). Common buckwheat is cultivated twice a year: summer (May–July) and autumn (August–October). Cultivation and harvest are managed with the same schedule by a local agricultural association. The buckwheat fields are located on a flat terrace in an area of approximately 5 km × 5 km. In total, 14 buckwheat fields were selected as study sites with sizes ranging from 841 m² to 2419 m² (average 1866 m²) (see approximate locations of buckwheat fields in Supporting information 1). The distance between adjacent sites ranged from 260m to 1170m. All surveys and experiments were performed in the autumn of 2019.

Insect Observation

Visiting insects were observed at the 14 study sites on 11 days between September 8th and 22nd. An identical pair of observers visited all study sites (buckwheat fields) from 9 a.m. to 2 p.m. on 11 different non-rainy days. The order of sites visited was changed daily to reduce the effects of time. At each site, a 0.5 m × 5 m quadrat was established along each of the four edge of a rectangular buckwheat field, and observers recorded insects that landed on flowers in the quadrats for 12 minutes in total (3 minutes per quadrat). To prevent data bias due to differences in catching skills and handling time by the observers, the insects were not caught. Therefore, some insect records were not precise (for instance, non-syrphid Brachycera species (order Diptera) were recorded as “non-syrphid flies” and Syrphid dipteran species were recorded as “hoverfly”). Other Dipteran species were recorded in as much detail as possible. Ants and beetles were identified to the species level whenever possible. Bees, butterflies, and wasps were identified to the family level. Other insects, such as stink bugs and grasshoppers, were recorded in as much detail as possible. Body sizes of insects were categorized as small and large with the threshold of 4 mm body width, with a visual inspection. There were two reasons for dividing insects into these two size classes. First, this size threshold corresponds to the mesh size of bags used for covering inflorescences in the experiment; small insects would be able to pass thorough the mesh while large insects would not (see “Pollination Experiment”). Second, this size criterion corresponds approximately to

the middle of the body mass range of insects (about 10mg) reported in an earlier study²² (Fig.4) that examined the allometric relation between the body mass of flower-visiting insects and thermal niches (see Supplementary Fig. S5 for the relationship between body width and body mass of major insects in our study area). As a result of the classification, large insects consisted of about 38% of the total insects while the rest was small insects (see Results for details).

Weather data for the AMeDAS site (35°N, 138°E) in Iijima were obtained from the database of the Japan Meteorological Agency⁵⁵. It was assumed that insect visitations were affected by short-term weather conditions immediately before the observation. Hence, the mean sunshine duration, temperature, and wind speed were calculated for the 30-min period before each observation. Humidity was not used as a variable owing to its strong correlation with time of day ($r = -0.646$).

Pollination Experiment

Inflorescences were bagged to evaluate the separate contributions of small and large insects to buckwheat pollination. The bags (approximately 10 × 20 cm) were made of clear-colored polyethylene, with a mesh size of 4.5 × 4.5 mm, allowing only small insects to visit and contact the flowers. The bags were applied to inflorescences that had flower buds but no opened flowers. For control inflorescences, all insects had access to flowers, regardless of body size. At each study site, 6 and 12 inflorescences from different plants were selected, respectively, for the bagging treatment and control, consisting of equal numbers of thrum and pin inflorescences. The treatment and control inflorescences were marked with vinyl tape on September 3–5, when buds were about to open. These inflorescences were collected on October 7–10, when seeds ripened. The number of seeds and remnant wilted flowers were counted and the seed set was defined as (number of seeds)/(number of seeds + remnant flowers). The difference in seed set between the treatment and control groups represents the contribution of large insects, as wind pollination is rare³⁶. Therefore, the small insect contribution was defined as the average seed set from bagged inflorescences at each site. The large insect contribution was calculated by subtracting the average small insect contribution from the average seed set of controls at each site.

Analysis

Insects analyzed

Recorded insects were categorized into seven taxonomic groups: ants, bee, scarabaeid beetle, butterfly, non-syrphid fly, hoverfly, and wasp. These insects were considered potential pollinators for buckwheat^{56,57}, although no direct evidence for pollination contribution is provided. All of these were used for size-based analysis (see “*Response to weather and spatial structures*”), but bees were not included in the taxonomic-level analysis because they were rarely observed throughout the surveys and their abundance was not sufficient for statistical analysis. Other insects, including lady beetles, stink bugs, and grasshoppers, as well as spiders, were not included in the analysis, because of their lower abundance and/or predatory nature.

Spatial and landscape variables

To check the spatial autocorrelation of insect abundance across study sites, the significance of global Moran's I was tested for each taxonomic group and body size group. As a result, none of the autocorrelations turned out to be significant ($p > 0.05$). This, however, does not mean there were no spatial structures. Spatial structures of study sites were represented by Moran's eigenvector maps (MEM), which describe synthetic spatial patterns of study sites in a two-dimensional space. MEM is often used for multiple regression or canonical analysis to account for spatial dependence (or spatial filtering) and to infer large scale environmental factors affecting spatial gradients^{58,59}. MEM axes are automatically extracted from large- to fine-scale patterns, and each spatial pattern is quantified by the eigenvalue of the MEM axis⁵⁹. MEMs were constructed using the coordinate data for study sites, and Delaunay triangular and MEM scores were calculated using the R package *adespatial*. Significant MEM scores ($p < 0.05$ for Moran's I) were used in subsequent analyses.

The relationship between selected MEM scores and land cover was examined by a redundancy analysis (RDA). Land cover was categorized into five types; forest, buckwheat field, agricultural field (other than buckwheat), residential area, and others (areas that do not belong to any of the four types). The area of each land cover within a 200-m-radius buffer from the edges of each study site was calculated using QGIS 2.18.23. This buffer size was chosen to avoid substantial overlap between study sites. Additionally, this buffer size is appropriate for explaining the abundances of insects other than honeybees and bumblebees; 100–300 m buffer sizes are adequate for these insects^{16,57,60,61}. In addition to simple land cover area, the Shannon diversity index of land cover proportions, which represents the mosaic of surrounding land use, was also calculated for each site. RDA was performed using the R package *vegan*.

Response to weather and spatial structures

The responses of pollinators to differences in weather conditions and spatial structures were analyzed by two approaches. First, analyses were performed for each taxonomic group. Second, the difference in responses between small and large insects to weather and spatial variables was examined.

The response of each taxonomic group was estimated by GLM using Bayesian modeling. The abundance of each insect taxon in each site (number of individuals summed over four quadrats in a site) was used as a response variable. The three weather variables (temperature, sunshine duration, and wind speed), time, and MEM scores were used as explanatory variables. Note that none of the weather variables were highly correlated ($r < 0.367$) and thus no serious collinearity problems. Time was included to consider potential trends in pollinator abundance during the day. Time was quantified as the difference between the earliest observation starting time (8:32) and the starting time for each observation. In addition, the site ID was included in the model as a random variable. A negative binomial model was fitted to the abundance data to cope with overdispersion, using the R package "*brms*"²⁹. A non-informative prior distribution was used for all explanatory parameters in the model. Posterior samples

and distributions were obtained by MCMC sampling with 7000 iterations and 3500 burn-in samples. The number of chains was 2 and adapt_delta was set to 0.99. Convergence was confirmed by checking if Rhat was below 1.1⁶² for all estimates. Prior to the analysis, all explanatory variables were scaled into average 0 and variance 1, to save computation time.

The responses of small and large insects were also estimated by GLM using Bayesian modeling.

The abundance of insects in each size category (small or large) was used as a response variable. Body size (small or large), MEM scores, time, and three weather variables as well as two-way interactions between body size and environmental conditions (three weather variables, MEM scores, and time) were included as explanatory variables. A negative binomial model was used with a non-informative prior distribution for all explanatory parameters. The number of iterations, burn-in samples, and chains as well as adapt_delta were the same as those used in the first Bayesian model. All analyses were performed using R⁶³.

Insect visitation and seed set

To confirm the linkage between insect visitation and pollination services, the relationship between insect abundance and seed set was analyzed by a simple regression, i.e., GLM with a Gaussian error distribution. The average seed set of controls at each site was included as the response variable, and insect abundance averaged over all days at each site was used as the explanatory variable. Since the relationship between seed set and insect abundance appeared non-linear, showing a decelerated increase with insect abundance, log-transformation was performed for insect abundance.

In addition, to understand the spatial pattern in the relative contributions of small and large insects to seed set, a multiple regression analysis was conducted separately for small and large insects, as in the above GLM model. The seed set from small insects or from large insects was used as the response variable, and four MEM scores were used as explanatory variables. It was not possible to test how the relative contributions of small and large insects vary with weather conditions because seed set was derived from all flowers in a season, rather than from flowers that bloomed on a particular day or time. All analyses were performed using R⁶³.

Results

Observed insects

We recorded 6370 insect visitors in total, across sites and days. In terms of the taxonomic distribution, ants visited the flowers most frequently (2502 individuals), followed by monkey beetles (1596), hoverflies (697), non-syrphid flies (609), butterflies (259), wasps (219), and others (426). Only 62 bees were recorded. Small insects accounted for 3974 individuals and large insects accounted for the remaining 2396 individuals. Monkey beetles (1596) accounted for more than half of large insects,

whereas ants (2502) were most abundant small insects (Table. 1). All butterflies were categorized as large, and the majority of hoverflies and non-syrphid flies were categorized as small.

Spatial structure of study sites

Four MEM axes (referred to as MEM1–4 hereinafter) were significant according to Moran's I . The MEM scores for the study sites, representing different spatial structures defined by the four axes, are shown in Supplementary Fig.S1.

The results of the RDA linking MEM and land cover surrounding study sites are described in Fig. 1. Although the axes did not clearly represent land cover patterns, some general trends were observed. MEM1 was positively associated with forest. MEM2 was positively associated with the Shannon diversity index and negatively associated with residential area. MEM3 and 4 were positively associated with residential area.

Response to weather and spatial variables

The initial analyses based on taxonomic groups showed that responses to weather conditions and MEM scores varied among taxonomic groups. R^2 was below 1.1 for all estimates, indicating the convergence of estimated parameters.

With respect to weather variables, sunshine duration had a positive effect on the abundance of monkey beetles but negative effects on ants, non-syrphid flies, and hoverflies (Fig. 2). The abundances of butterflies and wasps were not affected by changes in sunshine duration (Fig. 2). Ants were less active with higher temperatures, whereas other groups (except non-syrphid flies) tended to be more abundant as the temperature increased (Fig. 2). Wind speed was generally associated with a lower abundance across taxonomic groups, although butterflies, non-syrphid flies, and wasps were more resilient to changes in wind speed (Fig. 2). Butterflies, non-syrphid flies, hoverflies, and monkey beetles were less abundant as time progressed from early morning to early afternoon, whereas ants and wasps were stable, regardless of time of day (Fig. 2). The response curves of each taxon to weather variables are shown in Supplementary Fig. S2.

Concerning spatial structures, MEM1 showed no clear associations with any insects; however, it had a weak positive association with monkey beetles (Fig. 3), indicating that these species may be more abundant in more heavily forested areas (Fig.1). MEM2 was positively associated with butterflies, hoverflies, and monkey beetles (Fig. 3), indicating that these groups are more abundant when the surrounding land use is mosaic (Fig.1). MEM3 was positively related to flower visitation frequencies of non-syrphid flies and hoverflies (Fig. 3); thus, these taxa may increase in sites with more residential areas (Fig. 1).

The second analyses based on body size showed that size, temperature, wind, time, and MEM2 have significant effects on the overall abundance of pollinators (Table 2). R^2 values were less than 1.1 for all estimates, again indicating parameter convergence. Several interaction terms between body size and

weather or spatial variables were significant based on the 95% credible intervals, including body size $\times$ temperature, body size $\times$ solar radiation length, body size $\times$ MEM1, and body size $\times$ MEM2 (Table 2). Small insects increased as the sunshine duration decreased but were not related temperature, MEM1, or MEM2 (Fig. 4). In contrast, large insects were more abundant with higher temperatures and higher values of MEM1 and MEM2 but showed no response to sunshine duration (Fig. 4). Overall, large insects visited flowers more actively in fine or hot weather and became more abundant when the surrounding landscape was forested or mosaic structure. In contrast, small insects were more abundant when the weather conditions were rather inclement and were not strongly influenced by spatial variables. Wind speed had a negative influence on both large and small insects. The response curves of each body size group to weather variables are shown in Supplementary Fig. S3.

Insect visitation and seed set

The seed set from control plants averaged over all sites was 0.27 (27% of the total flowers). The contribution of small insects was 0.12, whereas that of large insects was 0.11, indicating that small and large insects made nearly equal contributions to buckwheat pollination services (Fig. 5).

The seed set showed significant positive relationships with the abundances of the following insects visiting buckwheat field (Supporting information 4); ants ($p = 0.016$), butterflies ($p = 0.025$), monkey beetles ($p = 0.046$), and wasps ($p = 0.048$). With regard to body size, seed set had a positive association with both large insects ($r = 0.395$, $p = 0.016$) and small insects ($r = 0.284$, $p = 0.050$) (Supplementary Fig.S4). The seed set had also a positive association with total abundance of observed insects ($r = 0.450$, $p = 0.009$) (Supplementary Fig.S4).

A multiple regression analysis showed that only MEM2 was positively correlated with the contribution of large insects to seed set (Table 3). This indicates that the contribution of large insects to pollination increases when the surrounding land cover is mosaic, with fewer residential areas, as shown in Figure 1. However, none of the MEM scores were significantly related to the contribution of small insects to seed set (Table 3), indicating contribution of small insects to pollination is insensitive to surrounding land cover.

Discussion

We detected differences in responses to changes in weather conditions among insect groups visiting buckwheat flowers. In particular, the responses of beetles, butterflies, and wasps differed from those of ants and flies; the former group was more active in sunny and/or warm conditions, whereas the latter taxa showed the opposite pattern. Responses to weather conditions varied with insect body size as well; large insects were more active in high temperatures, whereas small insects were more active in cloudy weather. This contrasting pattern agrees with a previous study showing that insect body size is an important determinant of niche positions at ambient temperatures²². Such a trait-based pattern was

observed likely because insects visiting buckwheat flowers consisted of diverse taxa and hence idiosyncratic responses within a taxon might have been masked.

Many studies have reported response diversity of pollinators to weather conditions among bee species visiting crops. Honeybees are generally active in high temperatures, while wild bee such as *Bombus*, *Osmia*, and *Anrena* are more active under low temperature or windy weather^{20,26,40,42}. Moreover, dipterans have different responses to temperatures and sunshine duration than those of honeybees and wild bees, with dipterans being active even under anomalous weather conditions^{26,30,42}. To our knowledge, our study is the first to show diverse responses to weather conditions in pollinator communities visiting a single crop species, including Hymenoptera (bees, wasps, and ants), Diptera, Lepidoptera, and Coleoptera (but see Kühnel and Blüthgen (2015)²² for wild flowers, rather than a crop species). Most earlier studies focused on crops that are highly dependent on bee pollination, such as blueberry, strawberry, watermelon, pumpkin, and almond. As buckwheat was visited by a variety of insect taxa in addition to bees, response diversity might have been more clearly demonstrated in this system. However, some earlier studies have shown that honeybees and bumblebees are major buckwheat pollinators^{25,46}, similar to their contribution to other well-studied crops. It is therefore surprising that only a few bees were observed in our study area. This small number of bee visitation was also confirmed in a former study conducted in this region⁴⁷. It is worth noting that, despite low bee visitations, seed set in our study fields was larger than that reported in a previously study (ca. 25% vs. 20%) showing frequent bee visitations³⁶. This suggests that diverse insects other than bees should have overcompensated the deficit of bee pollination services.

Thus, the insect diversity is apparently effective for the pollination of buckwheat and seed production in our study area. Of particular note is that ants were positively correlated with seed set, and they accounted for a substantial portion of small insects. Ants are major pollinators of some wild plants, in addition to bees^{48,49}. However, ant pollination of crop plants has not been reported, except for mangoes in the tropics⁵⁰. Given that ants visiting buckwheat flowers had pollen grains attached to their bodies, they were potential buckwheat pollinators³⁶. Ants do not move long distances, and pollination may be limited to a small spatial range⁵⁰. However, given that buckwheat is cultivated densely and two types of distylous flowers are close by, pollen transfer by ants is likely to be prevalent.

Although it is not possible to quantify the exact degree to which response diversity to weather conditions affects the pollination service of buckwheat, its contribution is likely to be significant because the contributions of large and small insects to seed set were nearly equivalent, as evaluated by the bagging field experiment. Small insects were mainly ants and dipterans, whereas large insects were mainly beetles and butterflies, and these groups appear to have independent contributions to seed production in buckwheat. In addition, given that there were significant statistical interactions between the body size of insects visiting flowers and weather conditions (both temperature and sunshine duration), response diversity is likely to have an important role in the stabilization of seed set of buckwheat. Further studies are required to link visitation frequency with seed set for various insect groups. As a caveat, bags could be visible to some flying insects and prevented access to flowers even for small insects, which might

have underestimated contribution of small insects. This does not alter the conclusion, however, that small insects had a significant contribution to seed set of buckwheat, as it was certain that only small insects were able to get access to flowers in the bagging treatment.

It is noteworthy that we simultaneously estimated the response to three weather variables and time of day with a single statistical model, and the pure responses to weather variables were therefore extracted, without the confounding effect of time. In this analysis, temperature disproportionately affected large insects, whereas sunshine duration tended to affect small insects. This implies that patterns of response diversity differ between environmental parameters. Different effects of weather variables on response diversity were also observed at the taxonomic level. Although the patterns of response diversity among species group were qualitatively similar for temperature and sunshine duration, the response intensities, as measured by the estimated parameter values, were different. For instance, the activity of hover flies increased under high temperatures, but decreased under long sunshine durations. Additionally, butterflies and wasps were more active under higher temperatures, but a similar trend was not found for sunshine duration. In general, temperature and sunshine duration are correlated; however, this correlation appears to be relatively weak when warm air and cold air are frequently exchanged, such as in the spring and autumn. Moreover, temperature is more stable within a given day compared with sunshine duration, which can change over short time periods; accordingly, separate effects of the two weather variables on insect visitation may have been detected.

These findings have important implications for our general understanding of response diversity to environmental changes other than weather variables. Earlier studies have focused on response diversity along a single environmental gradient. However, multiple environmental changes (e.g., increased CO₂, nitrogen, and grazing intensity) affect particular ecosystem functions. This indicates that a greater species richness is required to maintain ecosystem functioning in comparison with that required for a single environmental change⁵¹. Moreover, an environmental change could be accompanied by changes in multiple environmental parameters. In the case of climate change, changes in temperature and precipitation are the two major determinants jeopardizing biodiversity and ecosystem services; however, the sensitivity to changes may differ for the same species^{32,52}. In addition, other variables, including sunshine duration and humidity, are also expected to change^{53,54}. If patterns of response diversity differ between weather variables, a higher biodiversity is required to ensure resilient ecosystem services compared with that required when response diversity patterns are the same. We therefore suggest that the “diversity” in “response diversity,” which is a higher-level response diversity, deserves more attention in future studies of the biodiversity–ecosystem service relationship.

In addition to response diversity to weather, responses to spatial structures also differed between large and small pollinator insects; large insects tended to be more abundant in fields with more surrounding forest (MEM1) and mosaic habitats (MEM2), whereas small insects did not exhibit such patterns. This suggests that small insects are less sensitive than large insects to habitat alterations, which may compensate for a decrease in large insects visiting buckwheat fields. This is consistent with a previous study showing that smaller insects are less sensitive to land-use intensification in agricultural

landscapes^{15,21,22,27}. As a caveat, the buckwheat fields examined in this study were distributed on a narrow spatial scale, and landscape-level replication was not possible. Instead, we used MEM variables to represent spatial structures. MEM variables are purely dependent on the positions of the buckwheat fields in a 2-dimensional space. Given that the spatial structures are not based on particular land cover types, their interpretation with respect to environmental gradients is not straightforward. In addition, the observed effects of landscape structures may simply reflect chance events occurring at a local scale, irrespective of land cover types. Despite these limitations, the different responses of insect groups with different body sizes to spatial structures provides evidence for spatial complementation, even if such a difference arose by chance. It should be noted that the contribution of large insects to seed set was positively related to MEM2 (habitat mosaic), whereas that of small insects was not. This suggests that the spatially different visitation rates of large and small insects were translated to pollination services of buckwheat, and that small insects may sustain crop production under habitat alterations, as suggested previously^{27,34}. Further studies on buckwheat in other regions, as well as on other crop species, are required to generalize the importance of response diversity of pollinators for ensuring resilience to environmental changes.

In conclusion, we detected temporal and spatial response diversity of various insects visiting buckwheat, which contributed to the stability of seed production in buckwheat, as suggested partially by a bagging experiment. A particularly important finding is that response diversity per se is also diverse in relation to different weather variables. There are many temporal dimensions, including yearly, seasonal, and daily changes, and these should be considered when attempting more precise future predictions of pollination services under climate change. The final point to note is that, due to the limited number of study fields, we could not test the interactive effects of spatial and temporal factors or the interactive effects of weather factors (temperature and sunshine). Given that interactive effects could mitigate the detrimental effect on pollinators caused by single factor^{32,33}, further studies should investigate how higher-order interactive effects of environmental variables stabilize or destabilize pollination services.

Declarations

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Authors' contributions :

TM and HT conceived and designed the field observation and experiments. SH performed data collection. SH and TM analyzed the data. TM and SH wrote the manuscript.

Competing interests:

The authors declare no competing interests.

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Availability of data and material:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics declarations:

Not applicable

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Not applicable

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Tables

Table 1. Taxonomic composition of large and small insects observed visiting buckwheat flowers combined at all sites (14 buckwheat fields).

Large Insects		Small Insects	
Taxon	Abundance	Taxon	Abundance
Monkey beetle	1596	Ant	2502
Butterfly	259	Hoverfly	538
Hoverfly	159	Fly	463
Fly	146	Others	313
Others	113	Wasp	115
Wasp	104	Bee	43
Bee	19		
Total	2396	Total	3974

Table 2. Results of GLM using Bayesian analysis, showing the effects of insect body size (large or small), three weather variables, and four spatial variables on the abundance of insects visiting buckwheat flowers. Spatial variables were represented by four MEM axes (Moran's Eigenvector Map). Variables with bold letters indicate their 95% credible interval do not overlap with zero.

Variable	Estimate	-95%CL	+95%CL
Intercept	2.40	2.11	2.68
Size	0.61	0.52	0.71
Sunshine duration	0.04	-0.05	0.13
Temperature	0.23	0.14	0.32
Wind speed	-0.11	-0.20	-0.03
Time	-0.13	-0.20	-0.05
MEM1	0.26	-0.03	0.54
MEM2	0.29	0.01	0.57
MEM3	0.09	-0.19	0.39
MEM4	0.12	-0.16	0.41
Size× Sunshine duration	-0.20	-0.32	-0.09
Size×Temperature	-0.25	-0.37	-0.13
Size×Wind speed	-0.02	-0.13	0.09
Size×Time	0.10	-0.01	0.20
Size×MEM1	-0.27	-0.37	-0.17
Size×MEM2	-0.33	-0.44	-0.23
Size×MEM3	-0.09	-0.19	0.01
Size×MEM4	0.01	-0.09	0.10

Table 3. Results of multiple regression analysis relating seed set of buckwheat to spatial variables represented by four MEM axes (Moran’s Eigenvector Map). The analysis was conducted separately for seed set contribution of large and small insects. The variable with a bold letter indicates statistical significant.

	Large Insects		Small Insects	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
MEM1	-0.347	0.737	1.215	0.255
MEM2	2.813	0.020	0.297	0.773
MEM3	0.005	0.996	0.323	0.754
MEM4	0.968	0.358	0.323	0.222

Figures

Figure 1

Results of RDA relating four spatial variables (MEM axes: Moran's Eigenvector Map) to five landcover variables, as represented by the two RDA axes. MEM scores were used as response variables and a set of landcover variables were used as explanatory variables (area proportions of 5 land cover types and Shannon diversity index of land cover types at around each site).

Figure 2

Results of GLM using Baysean analysis showing how weather variables and time infuence abundance of various insects visiting backwheat flowers. Lines indicate 95% credible intervals of the estimated coefficients of variables, and dots are the means of the coefficients.

Figure 3

Results of GLM using Baysean analysis showing how saptial variables (MEM axes) influence abundance of various insects visiting backwheat flowers. Lines indicate 95% credible intervals of the estimated coefficients of variables, and dots are the means of the coefficients.

Figure 4

Figure 4

Graphical representation of how insect body size and weather variables or MEM interact to affect insect abundance visiting buckwheat flowers (see Table 2 for significant interactions). Different symbols indicate different positions along each of the explanatory variables; 0 is the mean, and -1 and 1 are 1 standard deviation below or above the mean. Bars indicate 95% credible intervals. Only variables for which the interaction with body size are evident (95% credible intervals not overlapping with 0) are shown. Note that this figure is for graphical illustration that does not directly match with GLM analysis (Table 2).

Figure 5

Figure 5

Seed sets of buckwheat (number of seeds / number of flowers) of control inflorescences (open to all insects), bagging inflorescences (only small insects had access), and the subtraction of bagging seed set from the control seed set. The three seed sets represent, respectively, the contributions of all insects, small insects, and large insects.

Supplementary Files

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