A comparison between window trap and pan trap in monitoring flower-visiting insects in agricultural fields

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Abstract

Sampling flower-visiting insects in agricultural fields at large spatial and temporal scales is significant for understanding local insect pollinator communities. The most commonly used method, pan trap, has been criticized due to its attractant bias. Window trap (also referred to as the flight-intercept trap) is a non-attractant sampling method, which has been applied in forests and grasslands, but rarely in agricultural fields. We aimed to test whether we can replace pan traps with window traps in agricultural fields by comparing species richness and species composition between the two methods, and to show whether flower-visiting insects collected in the pan traps and window traps can reflect flower-visiting activity recorded by camera observation. We conducted a two-year study to compare the performance of pollinator sampling methods in an oilseed rape field. Results showed that the relative abundance of dominant flower-visiting species was highly correlated between window trap and pan trap samples, while window traps caught more individuals and higher (rarefied) species richness than pan traps. The species composition of window traps was more similar to each other than that of pan traps. The proportion of honey bees (*Apis* spp.) collected in both pan traps and window traps underestimated their flower-visiting activity recorded by camera observations, while sweat bees (Halictidae) and butterflies (Lepidoptera) were overestimated. Our study suggests that window traps have the potential to serve as an alternative sampling flower-visiting insects to pan traps. However, we need to be cautious when using specimens caught in both traps as a proxy of their flower-visiting activity.

Keywords: Pollinators; insect survey; flight intercept trap; flower visitation; Hymenoptera; Diptera; oilseed rape; wild bee

Introduction

Globally, more than 30% of crop production depends on animal pollination (Klein et al. 2007). Apart from managed honey bees, wild pollinators also play a significant role in providing pollination for crops (Bommarco et al. 2012, Garibaldi et al. 2013, Zou et al. 2017a), and the decline of wild pollinator has been of considerable concern (Potts et al. 2016). It is important to understand local flower-visiting insect communities. Not only does this provide a basic understanding of the different pollinator species that provide pollination services to specific crops (Howlett et al. 2009), but it also helps us to understand species distributions that are critical for biodiversity conservation.

There are a variety of methods that can be used to collect flower-visiting insects. To monitor pollinator communities at a large landscape scale or over a long-term period of time, those labor-intensive and difficult-to-standardize methods, such as sweep netting, may not be appropriate. Pan traps, a cost-effective method that can be deployed in the field over a relatively long period, is one of the most widely used methods that has been applied in monitoring the activity-density of local flower-visiting insects at a large landscape scale (Westphal et al. 2008, Kovacs-Hostyanszki et al. 2011, Zou et al. 2017b, McCravy 2018). A pan trap usually consists of colored containers that attract flower-visiting insects (Cane et al. 2000, Campbell and Hanula 2007, Westphal et al. 2008). However, the sampling efficiency of the pan trap may be influenced by surrounding floral resources (Baum and Wallen 2011) and biased towards a specific group of pollinators with similar physical features (Roulston et al. 2007). Validation of the pan trap in relation to local pollinator diversity has been criticized, since it is an attractant-based sampling method (Cane et al. 2000).

One of the non-attractant sampling methods that can be used in monitoring flight insect community is the window trap. Window trap is also called as flight-intercept trap, which consists of a large pane of glass or fine mesh that is invisible to flying insects and is used as a physical barrier in the potential flight path (Howlett et al. 2009, Sverdrup-Thygeson and Birkemoe 2009, Zou et al. 2012). As no attractant is involved, window traps may be

less biased than pan traps in exploring the overall pollinator taxa, which is recommended in monitoring local bees and wasps in the forest habitat (Rubene et al. 2015). Nonetheless, window trap has rarely been applied in the crop fields in monitoring flower-visiting insects.

In the agricultural pollination studies, pollinators' flower-visiting activity is an important index for measuring pollination services that are related to crop yield (Petersen and Nault 2014, Bartholomee and Lavorel 2019). Flower visitation of pollinators is usually conducted by direct observation, either by human observation or cameras (Banaszak-Cibicka et al. 2019, Liu et al. 2020). However, direct observation is usually time-consuming and can be affected by weather conditions and observation time, and therefore can hardly be standardized at a large spatial scale. Therefore, understanding whether and to what extent samples from traps reflect on-site insects' flower visitation activity is significant for agroecology (Liu et al. 2020). While the comparison between pan traps and direct observation (usually transect walking) has been conducted in several studies (Westphal et al. 2008, O'Connor et al. 2019, Templ et al. 2019), no study has validated pollinator samples collected in window traps with the on-site flower-visiting activity.

In this study, we conducted a two-year experiment in a field of oilseed rape (*Brassica napus* L.). Our study first aims to verify whether the relative abundance of each species collected in the window trap is positively correlated with the pan trap. We then tested whether we could replace pan trap with window trap by comparing the species richness (α-diversity) and species composition (β-diversity) of the two methods. Finally, we aim to explore whether flower-visiting insects collected by the pan trap and window trap could reflect their flower-visiting activity. If not, we then aim to investigate which taxa are biased in both sampling methods.

Materials and methods

Study area

This study was conducted in a 2700 m^2 (90m * 30m) field at the Jiangxi Agricultural University, Jiangxi Province, China (28°46.17'N, 115°49.99'E). The field was only used for rotation of oilseed rape (October to April) and rice (May to September) and no pesticides were applied since 2014. The experiment was conducted in 2018 and 2019 from the end of February to the end of April, which was the time of oilseed rape flowering. The field was devided into five ploughed plots (as five blocks) (Appendix 3) where the semi-winter cabbage type oilseed rape (Yangguang 2009, single traditional bred cultivar) was grown.

Pan trap and window trap

A pan trap consists of three cups (8.3 cm diameters, 13.5 cm in height and a volume of 450 mL) painted with three ultraviolet (UV) colors (UV-blue, UV-yellow and UV-white) to minimize bias from a single-color pan trap (Westphal et al. 2008). Traps were attached to a wooden stick. The height of the pan traps was 1.6m, which was approximately the height of the oilseed rape flower in the field. Two 3 mm-diameter holes were drilled in each caps at 2 cm from the edge of the cup to drain rainwater. We used saturated salt (NaCl) water with a mixture of several drops of detergent (to reduce water tension) as a killing agent for insects.

A window trap consists of a transparent acrylic plate (35*60 cm and 5 mm thick) as a barrier for intercepting flying insects. The plate was fixed on two wooden sticks, with its bottom containing two plastic trays that filled with the same killing agent with pan traps (Appendix 1). Window traps were fixed at a similar height as pan traps.

Because the surface area of a pan trap is much smaller than the barrier area of a window trap which can lead to the differences in sampling efficiency, we compared three pan traps and one window trap per field block to minimize this difference. In total, there were

15 pan traps and 5 window traps in the field (5 blocks). All traps were set at least 2 meters from edge of the field. Traps were set (end of February) and finished (end of April) on the same day, with emptying-refilling once a week and a total of 48 and 46 sampling days in 2018 and 2019, respectively. All specimens were pinned, sorted to morpho-species and identified by taxonomists. Overall, 93.1% of individuals were identified to species level and 99% to family level (Appendix 2).

Camera Observation

To monitor the activity of flower-visiting insects, we used three surveillance cameras (DFD®, Shenzhen) with a resolution of 1280*720 pixels per inch. To have a clear view, cameras were positioned to focus on the main branch of one flowering oilseed rape plant at a distance of about 40 cm, with a visible area of $35 \times 25\,cm^2$. We regularly changed the focused plant once its flowering ended. Recordings started from 8:00 am to 5:00 pm in the same sampling period with pan traps and window traps. We did not record on rainy days because flower-visiting insects were not active.

Three cameras, one of which was put in in the three middle blocks, were placed 5 meters from the field margins. The location of the camera is shown in Appendix 3. In total, we obtained recordings of 228 hours (131 hours in 2018 and 97 hours in 2019). We recorded all insects foraging on oilseed rape flowers. We managed to identify the insects that were recorded in the cameras into seven groups: Apis (*Apis mellifera* and *Apis cerana*), Andrenidae, Halictidae, other Hymenoptera, Syrphidae, non-Syrphidae Diptera, Lepidoptera and Coleoptera.

Data analysis

To explore how the relative abundance of each species collected in the window trap correlated with the pan trap, we applied a linear regression for the proportions of each species between two sampling methods. Data were square-root transformed to minimize the scale difference.

To compare α-diversity between the pan trap and window trap, we used the individual-based rarefaction–extrapolation curve to investigate the extrapolated number of species between the two methods (Colwell et al. 2012). The extrapolation was based on the doubled number of individuals in a sampling method, of which we pooled the individuals collected in the same year and compared them separately for each year.

To compare the difference in species communities between pan traps and window traps, we used the Chord-Normalized Expected Species Shared (CNESS) dissimilarity (Trueblood et al. 1994). The CNESS index, which is not sensitive to the sample size, measures the probability of obtaining the same species when a given number of individuals (the value *m*) were randomly drawn from two communities (Zou and Axmacher 2020). We used the modified version of CNESS by Zou and Axmacher (2020) with its value between 0 and 1. We used a small sample size (m=1) focusing on the difference of dominant species and a larger sample size (m=20) focusing on the overall species assemblages. CNESS dissimilarity matrices were then visualized using non-metric multidimensional scaling (NMDS). To obtain a robust sample size, individuals from the same field block were pooled, while analysis was separated between two sampling years.

All statistical analyses were conducted in R Version 3.5.2 (R Development Core Team, 2016). Package "vegan" (Oksanen et al., 2020) was used to calculate the rarefied species richness. Package "iNEXT" (Hsieh et al., 2014) was used to calculate the rarefaction–extrapolation curve. Function "ESS()" developed by Zou & Axmacher (2020) was used to calculate the CNESS value.

Results

In total, we caught 1392 individuals with 37 insect species, of which pan traps caught 387 individuals (33 species) and window trap caught 1005 individuals (34 species). The average sampling efficiency was 0.27 and 2.14 per trap per day for pan trap and window trap, respectively. Overall, the most abundant species were *Apis mellifera* (26.8%), followed by *Apis cerana* (22.8%), *Pieris rapae* (17.7%), *Osmia excavata* (4.7%), *Osmia pedicorns* (3.6%) and *Lasioglossum proximatum* (2.9%) (Appendix 2). There was a significant positive linear relationship between the proportion of species sampled in pan traps and window traps (beta=0.77±0.08, R^2=0.70, p<0.01, Fig. 1). There was no significant difference between the linear model and the y=x line (as indicated by the 95% CI, Fig. 1). *Andrena callopyrrha*, *Osmia microdonata*, *Osmia cornifornis*, *Episyrphus balteatus*, *Phytomia zonata* and a Diptera species only occurred in window traps, while species *Argynnis hyperbius*, *Pieris melete* and a species of *Nomada*, *Polistinae* and *Vespula* only occurred in pan traps.

Species rarefaction curves showed that window traps collected more rarefied number of species than pan traps, and this pattern was consistent for both years (Fig. 2). The extrapolation curves showed that the window trap (34.1±3.7 and 34.2±4.9 species in 2018 and 2019 respectively) would catch about 1.5 times more species than pan traps when both methods reaching their double sample size (16.9±1.5 and 21.8±2.2 species in 2018 and 2019).

The species composition differed between pan trap and window trap. When looking at the between-sample difference, the NMDS distance between-sample was relatively large for both methods for dominant species (m=1), but was larger in the pan trap than the window trap for the overall composition (m=20, Fig. 3). The overall species compositions (m=20) in the two sampling years were also distinctive, particularly for pan traps (Fig. 3).

The camera observation recorded 375 visits in two years. Both pan trap and window trap underestimated the flower visitation of the honey bee (Apis) in two years (Fig. 4a), while the proportion of the honey bee in pan traps was closer to the proportion in flower visitors (Fig. 4a). The proportion of sweat bees (Halictidae) and butterflies (Lepidoptera) were overestimated in the pan traps and window traps for their visitation to oilseed rape flowers (Fig. 4a). Hoverflies (Syrphidae) and mining bees (Andrenidae) could be reflected from both traps as flower-visiting activity (Fig. 4a). Excluding Apis, both the pan trap and window trap showed a similar proportion of sweat bees to its proportion in flower visitors, while there was still an overestimation of butterflies and under-estimation of non-Syrphidae Diptera (Fig. 4b). Relationships of the proportion of different groups between two traps and camera observation in 2015 and 2019 are shown in Appendix 4.

Discussion

While the window trap has been applied in forests (Wells and Decker 2006, Rubene et al. 2015), it has rarely been used in sampling flower-visiting insects in agricultural fields. The window trap we used here was much more efficient than the pan trap in terms of the number of individuals per trapping day, and the number of rarefied species. As a cost-effective method, the window trap has a good potential to replace the pan trap in sampling pollinator insects in mass-flowering cropland.

Our results are consistent with Rubene et al. (2015), who found that window traps performed better than pan traps in sampling Hymenoptera in forest habitat, although results might depend on the difference in terrain conditions (Rubene et al. 2015; Wells and Decker 2006). Sampling efficiency may be positively correlated with the area of the barriers (e.g. the glass panes in our study). Here we used a transparent acrylic plate with an area of about $0.2m^2$, whose sampling efficiency (on average 335 individuals per trap) was more than 10 times with a pan trap (about 26 individuals per trap). While a large barrier area of the window trap means higher cost and more interruption to the flight path of insects, the trade-off between barrier size and sampling efficiency needs to be considered in designing window traps.

The high correlation of the proportion of species abundance between the two sampling methods indicates that the relative abundance of species sampled in window traps can be representative for those in pan traps, although some species only occurred in the window trap. Nonetheless, if using window traps to replace pan traps, species (e.g. *Argynnis hyperbius*) that only occurred in pan traps require particular attention from researchers aiming at pollinator monitoring, as these species might be overlooked in the former traps. Nonetheless, as these species were also rarely found in pan traps, we do not know whether these species were biased away from window traps, or just coincidentally missing from our sample.

While samples in window traps showed slightly different compositions for the overall species assemblages, the more homogeneous composition than pan traps means that window traps were able to catch a more comprehensive species assemblage, which mainly resulted from the limited individuals sampled from pan traps. We suspect that the main reason for the different composition between the two traps was the inherent attractant bias, as we were comparing an attractant-based method (pan trap) with a non-attractant-based one (window trap). The effect of surrounding floral resources on the two methods will be different (Baum and Wallen 2011). In the oilseed rape flowering period, the insects' visitation to pan traps can be affected by the density of flower resources (Grindeland et al. 2005, Popic et al. 2013, Prendergast et al. 2020), while as the physical interceptions, window trap's sampling efficiency is less likely to be affected. Hence, considering its better performance in pollinator species and stable community composition, we recommend window traps if researchers are interested in understanding wild pollinator composition.

The insects collected in both traps cannot reflect flower-visiting activity for several taxa, but the proportion of hoverfly (Syrphiade) and mining bee (Andrenidae) was well represented. It is not surprising that specimens collected in sampling traps can be used effectively to monitor pollinator species biodiversity, but not flower-visiting activity for the targeted crops (Boyer et al. 2020). Flower-visiting activity can be influenced by floral

density (Grindeland et al. 2005), while different pollinators may respond differently when the floral resource differs (Sih and Baltus 1987). However, camera observations reflect a combination between the species density and their flower-visiting frequency, while individuals captured in traps only reflect activity density. This might be the reason why the camera slightly overestimates the true density for those with high flower-visiting frequency species such as Apis (Liu et al. 2020). We have to admit that the number of overall recorded pollinator visits was not high in our study, and thus we encourage a more comprehensive study with more cameras.

Conclusions

In conclusion, we found that window traps had higher efficiency at sampling insects, could catch more diverse insect assemblage and were more homogeneous between samples than samples in pan traps. Our results suggest that window traps can be used as a replacement for pan traps in studying the diversity of flowering-visiting insects in agricultural fields. Although results are consistent over two years, our study was only conducted in one landscape and one crop type. We therefore recommend further studies to be conducted with a variety of crop types and in different landscape contexts to comprehensively evaluate the performance of using window traps as a replacement for pan traps. Furthermore, we highlight that we should be cautious about using the pan trap sample as a proxy of pollinator's flower-visiting activity.

Authors' contributions

X.S. M.F, X.H. and Y.Z. designed the study; X.S., M.F. and X.H. performed the experiment; X.S., D.Y. and Y.Z. analyzed data; and X.S. and Y.Z. wrote the paper. All authors provided comments to the paper.

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Figures

Fig 1. The relationship of proportion (square-root transformed) of each insect pollinator species that collected in pan traps and window traps. The red line represents the linear regression model and the black line represents y=x. The grey shaded area represents the 95% confidence intervals of the regression.

Fig 2. Rarefaction and extrapolation curves for insect species collected in pan traps and window traps in 2018 (a) and 2019 (b). The solid lines represent interpolation and the dashed lines represent extrapolation predictions; shaded areas represent 95% confidence intervals.

Fig 3. Non-metric multidimensional scaling (NMDS) plots based on Chord-Normalized Expected Species Shared (CNESS) dissimilarity for m=1 (a, stress=0.08) and m=20 (b, stress=0.16) for pan trap and window trap of two sampling years.

Fig 4. The proportion of each pollinator group recorded in camera observation, and specimens caught in pan traps and window traps for (a) all taxa, and (b) the rest groups excluding Apis.

Figure 1

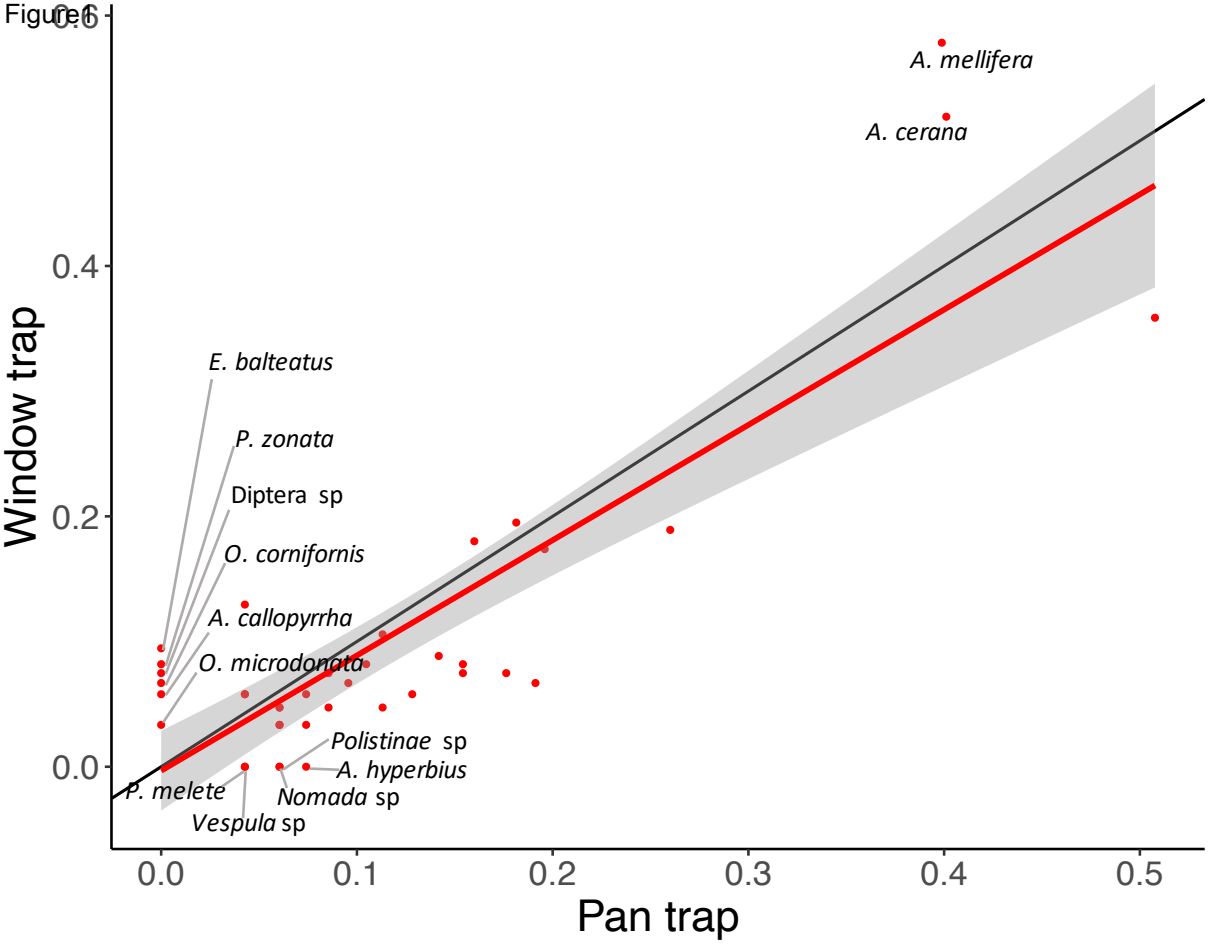

A. mellifera
A. cerana
E. balteatus
P. zonata
Diptera sp
O. cornifornis
A. callopyrrha
O. microdonata
Polistinae sp
A. hyperbius
P. melete
Nomada sp
Vespula sp
Window trap
Pan trap
0.0
0.2
0.4
0.6
0.0
0.1
0.2
0.3
0.4
0.5

Figure2

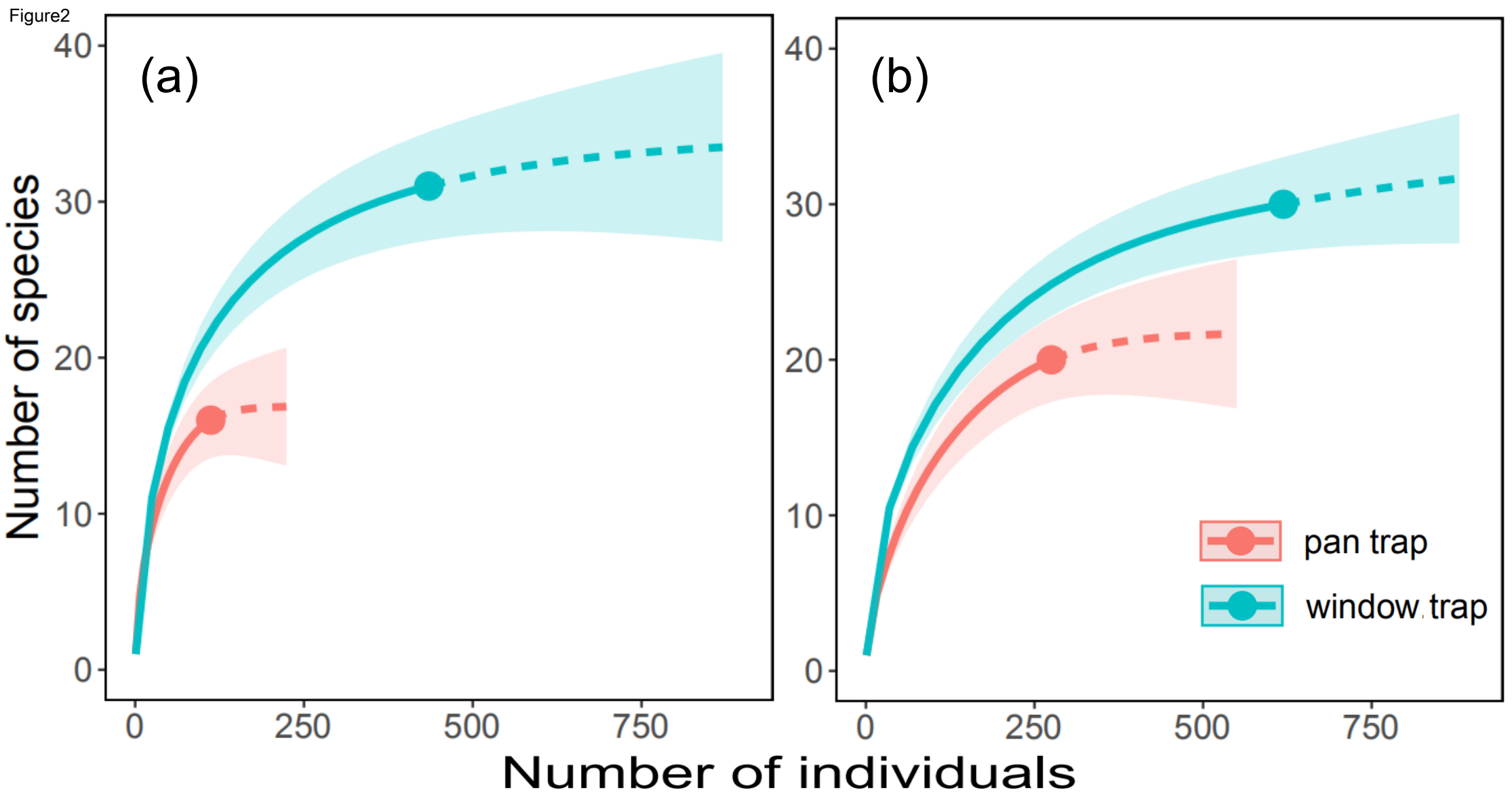

(a)
(b)
Number of species
Number of individuals
0
10
20
30
40
0
250
500
750
pan trap
window trap

Figure3

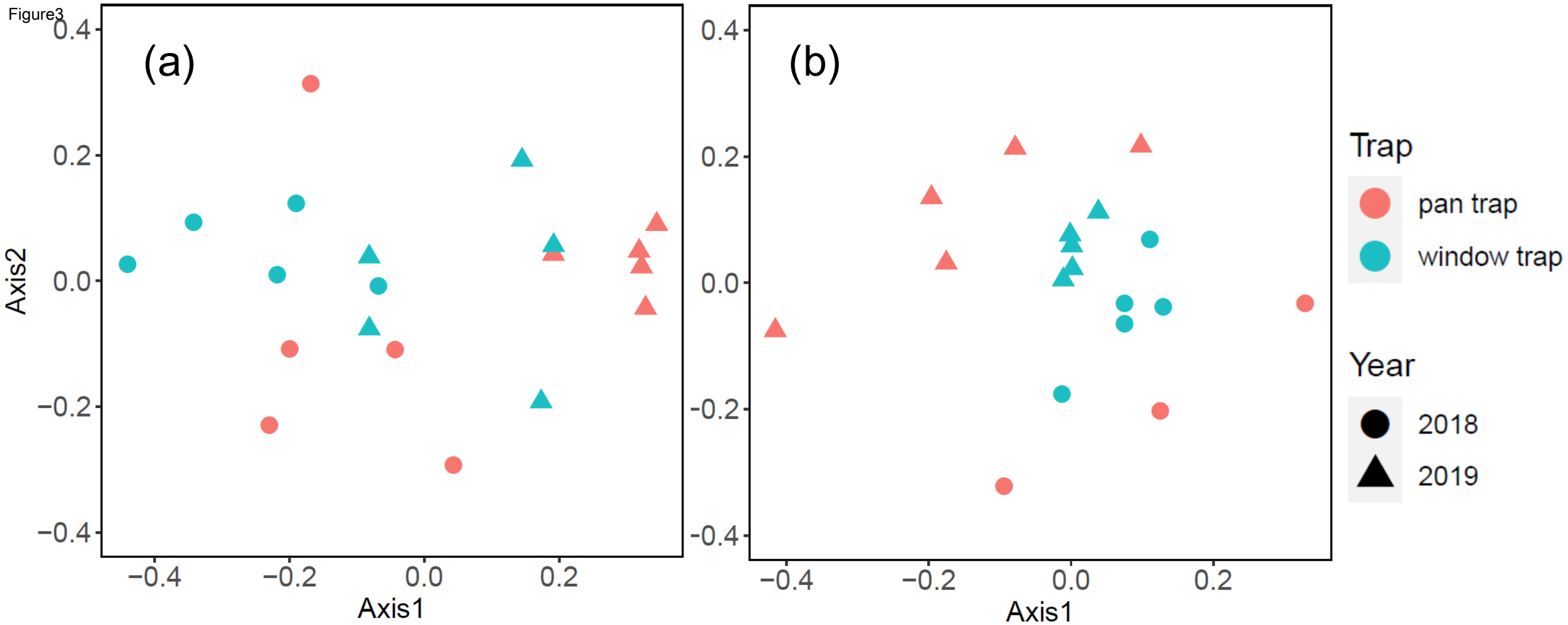
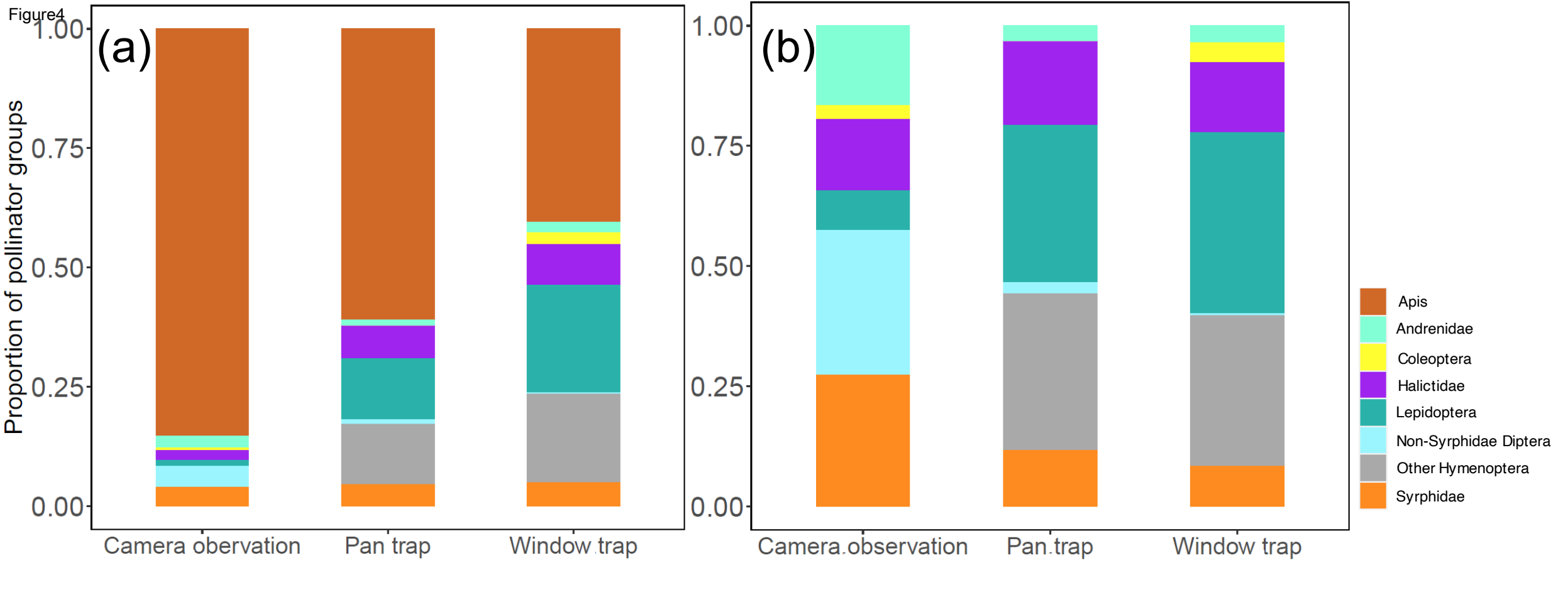

(a)
(b)
Axis2
Axis1
0.4
0.2
0.0
−0.2
−0.4
−0.4
−0.2
0.0
0.2
Trap
pan trap
window trap
Year
2018
2019

Figure4


(a)
(b)
Proportion of pollinator groups
1.00
0.75
0.50
0.25
0.00
Camera obervation
Pan trap
Window trap
Camera observation
Pan trap
Window trap
Apis
Andrenidae
Coleoptera
Halictidae
Lepidoptera
Non-Syrphidae Diptera
Other Hymenoptera
Syrphidae

Electronic Supplementary Materials for

A comparison between window trap and pan trap in monitoring flower-visiting insects in agricultural fields

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Appendix 1. The setup of a window trap.

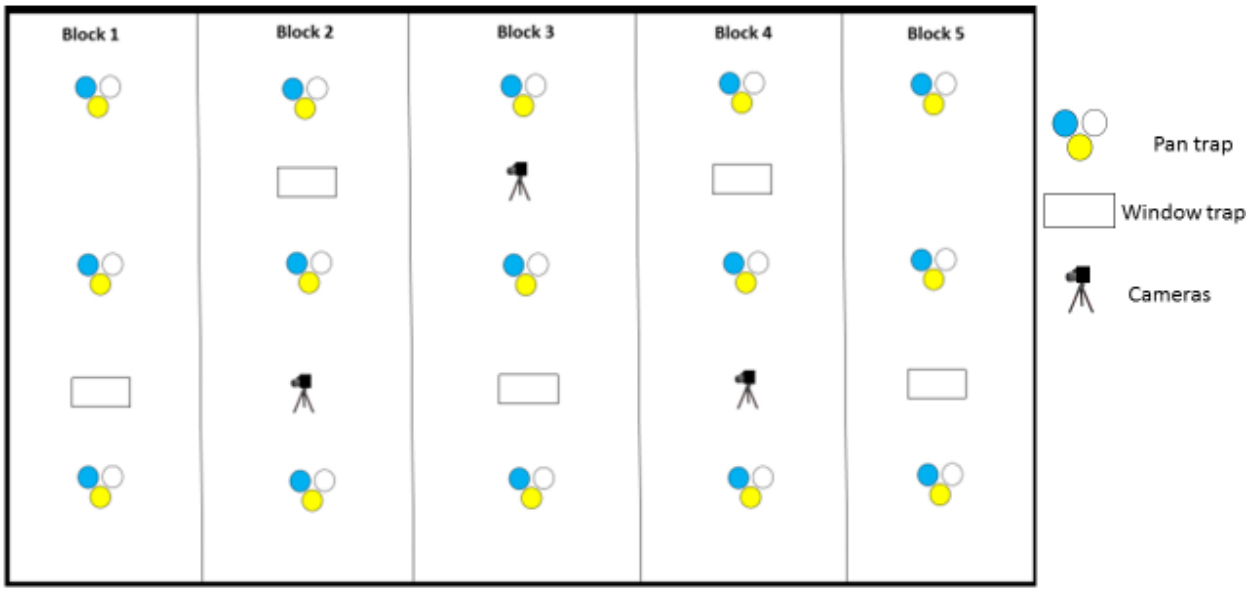
Appendix 2.

Table S1. The number of specimens collected in pan trap and window trap

Order	Family	Species	Pan trap	Window trap
Lepidoptera	Pieridae	*Pieris rapae*	141	115
Hymenoptera	Apidae	*Apis cerana*	88	241
Hymenoptera	Apidae	*Apis mellifera*	87	299
Hymenoptera	Megachilidae	*Osmia excavata*	37	32
Hymenoptera	Apidae	*Eucera floralia*	21	27
Diptera	Syrphidae	*Eupeodes corollae*	20	4
Hymenoptera	Megachilidae	*Osmia pedicorns*	18	34
Coleoptera	Scarabaeoidea	*Scarabaeoidea* sp1	17	5
Hymenoptera	Halictidae	*Lasioglssum proximatum*	14	29
Hymenoptera	Halictidae	*Lasioglossum* sp1	13	6
Hymenoptera	Apidae	*Ceratina okinawana*	13	5
Diptera	Syrphidae	*Syrphidae* sp1	11	7
Hymenoptera	Andrenidae	*Andrena* sp1	9	3
Hymenoptera	Halictidae	*Lasioglssum subopacum*	7	10
Hymenoptera	Andrenidae	*Andrena richardsi*	7	2
Hymenoptera	Apidae	*Anthophora melanog*	6	6
Hymenoptera	Halictidae	*Lasioglossum scitulum*	5	4
Diptera	Syrphidae	*Syrphus corollae*	4	5
Hymenoptera	Halictidae	*Lasioglssum kumejimense*	4	2
Hymenoptera	Halictidae	*Halictus aerarius*	3	3
Hymenoptera	Apidae	*Ceratina flavipes*	3	1
Lepidoptera	Nymphalidae	*Argynnis hyperbius*	3	0
Hymenoptera	Vespidae	*Eumeninae* sp1	2	2
Hymenoptera	Halictidae	*Lasioglossum occidens*	2	2
Diptera		Diptera sp1	2	1

Hymenoptera	Sphecidae	Sphecidae sp1	2	1
Hymenoptera	Halictidae	*Nomada* sp1	2	0
Hymenoptera	Vespidae	*Polistinae* sp1	2	0
Hymenoptera	Apidae	*Xylocopa tranquarorum*	1	15
Hymenoptera	Halictidae	*Lasioglossum* sp2	1	3
Hymenoptera	Halictidae	*Nomada* sp2	1	3
Hymenoptera	Pieridae	*Pieris melete*	1	0
Hymenoptera	Vespidae	*Vespula* sp1	1	0
Diptera	Syrphidae	*Episyrphus balteatus*	0	8
Diptera	Syrphidae	*Phytomia zonata*	0	6
Diptera		Diptera sp2	0	5
Hymenoptera	Megachilidae	*Osmia cornifornis*	0	4
Hymenoptera	Andrenidae	*Andrena callopyrrha*	0	3
Hymenoptera	Megachilidae	*Osmia microdonata*	0	1

Appendix 3. Field experimental design of pan traps, window traps and camera observations.



Appendix 4.

The relationship of the proportion of different groups between two passive traps and camera observation for 2018 (a and b), and 2019 (c and d); the black lines represent y=x.