ORIGINAL ARTICLE

Pollinator diversity and density measures: survey and indexing standard to model, detect, and assess pollinator deficits

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Abstract

In response to the need to derive empirical data on the status of pollinators, we have developed a protocol to assess the pollinator population in managed and natural ecosystems, especially in Asia where there is no harmonized initiative for pollination research. This protocol is helpful in assessing organic and non-organic farming since pollinator persistence can be an indicator of good and sustainable practices. The three important steps in the protocol are planning, implementation of the survey, and computation of indices. In the planning step, experts rank and classify the pollinator hotspots in various ecosystem types based on the seasonality and likelihood of having high number of flowers and pollinators. Two-stage sampling and mapping of each hotspots are designed. The number of sample hotspots is computed according to sampling precision and confidence levels assigned per class per ecosystem type. In each sample hotspot, sample survey sites are randomly selected according to the agreed sampling precision and confidence levels. In the second step, for orchard or agroecosystem, a grid map with 100 m^2 divisions is surveyed. For natural vegetation, survey sites are randomly selected according to the location of pollinator nesting sites. All sampling surveys are conducted during the blooming period of the plants, with consideration of the peak of anthesis, because this is the period when the pollinators are actively foraging, the pollen viability is high, and the nectar secretion is at its peak. Moreover, a template is created to compute for diversity measures and our proposed pollination matching measures (P-to-P ratio and index).

Keywords Pollinators · Diversity · Indices · Harmonized method · Asia · Pollination matching

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Introduction

Majority of global primary food crops require some level of animal pollination (Potts et al. 2016; Champetier et al. 2015; Klatt et al. 2013; Winfree et al. 2011). In fact, it was estimated that 87.5% of flowering plant species are pollinated by animals (Ollerton et al. 2011) and the concern on how changing pollinator population affect pollination was identified as one of the key research questions by pollination ecologists (Mayer et al. 2011). Despite the importance of pollinators to agriculture and to the economy, data regarding pollinator diversity and abundance, particularly, in the Asian region have been limited. The diversity and richness of pollinator species, including native bee species, are estimated using different methods in different studies (van Strien et al. 2012; Lamb et al. 2009; Buckland et al. 2005).

In some parts of the world, there are many available and employed sampling methods, such as pan traps and net sampling (Popic et al. 2013; Nielsen et al. 2011; Westphal et al. 2008; Berenbaum 2007; Roulston et al. 2007). However, the

use of widely varying survey methods could lead to difficulty in comparing the temporal and spatial status of pollination. While standard survey procedures are costly and time-consuming, the benefits of a single monitoring protocol across an entire region can outweigh the investment of devising one.

In this paper, we have developed a harmonized method to survey the state of pollinators, especially in the Asian region, on a country-by-country basis. The survey method is coupled with a new index that can manifest plant–pollinator interaction and can be used for comparative studies. The results of the survey can be used to assess pollinator biodiversity, abundance, and health; estimate pollinator deficits; identify vulnerable scenarios and infer their causes (e.g., anthropogenic activities); and promote mitigation methods to conserve the pollinators. Here, we can answer the question: is there a decline of pollinators in Asia (Potts et al. 2010; Bauer and Wing 2010; Gallai et al. 2009)? We can also evaluate the status and recovery of pollinators in disaster-hit areas.

As a general rule, the survey design should be able to estimate temporal and spatial variations, and can easily detect occurrence of species. Examples of traditional survey methods are plots, quadrats, traps, transect lines, food baiting, camera surveys, search routes, timed counts, and pollinator nest survey. The Food and Agriculture Organization of the United Nations (FAO) has also recommended certain methods for agroecosystems (Vaissiere et al. 2011). More than one method can be used to complement and supplement each other (multiple survey methods) (Lonsdorf et al. 2009; Hill et al. 2005; Magurran 2004). Here, our proposed method balances simplicity and complexity without losing the desirable properties of a good pollinator deficit survey. Moreover, the services of systematists and entomologists (who can identify matching of plants and pollinators), and local experts (who can identify pollination hotspots) are important requirements of our proposed survey method.

Formulating and implementing long-term schemes to monitor pollinators is one of the ten policy recommendations for governments (Dicks et al. 2016). This is in response to the perceived global decline or deficit in pollination services. Moreover, the FAO established the International Pollination Initiative (http://www.fao.org/pollination) that includes five participating regions: Europe, North America, Latin America, Africa, and Oceania. We envision this research initiative to be the starting point for a similar initiative for the Asian region. This could help in narrowing the knowledge gap on global pollination, and improve the state of ecosystem services especially for organic farming.

There is no universal Pollinator Diversity and Density Measure (PDDM) that can comprehensively describe all pollinator ecosystems. Pollinator ecosystem comprises the community of pollinators and its interaction with both biotic and abiotic factors. Also, PDDMs should satisfy desirable properties, such as user friendly, practical, unbiased and precise, and error regulated (Iknayan et al. 2014; van Strien et al. 2012; Lamb et al. 2009). Examples of classical diversity and density measures are species richness, Simpson's index, Shannon index, arithmetic mean abundance, geometric mean abundance, and Sørensen's similarity coefficient (van Strien et al. 2012; Lamb et al. 2009; Buckland et al. 2005). However, these indices do not consider species interaction, such as plant–pollinator interaction (Soares et al. 2017; Geslin et al. 2013; Carstensen et al. 2016; Frund et al. 2010; Lonsdorf et al. 2009; Bosch et al. 2009; Olesen et al. 2008; Bluthgen et al. 2006).

In this study, we formulate a new index that is compatible to our proposed survey method that considers plant–pollinator interaction to monitor changes in pollinator deficits. This index does not consider only the pollinators or the plants, but it incorporates the matching between plants and pollinators. We call this new index as the Pollinator-to-Plant or P-to-P index. To compute for the P-to-P index, we are proposing a standardized survey method intended to be a benchmark for estimating pollinator deficits in Asia. A standardized survey method can minimize bias resulting from using different methods. This will enable us to appropriately describe and model the diversity and density of pollinators at the local, national, and regional levels, and to track temporal and spatial differences. As a result, this can be used to compare pollinator deficits among Asian countries and be a basis for policy-making. We note that our proposal is to establish a minimum standard but it does not inhibit one from using other methods as complements or supplements.

Pollinators are affected by pesticides. Reduced number of pollinators may lead to decline in fruit sets and consequently decline in production yield. Assessing pollinator deficits will provide measure how pesticide affects the ecosystem. In organic farming, we expect effective pollination due to the absence of pesticides. Organic farming practices are good for pollinator persistence, and in return, natural pollination services support the productivity of organic farming.

Survey methodology

Our proposed survey procedure consists of three steps (Fig. 1). The first step is the planning stage where we choose the location, carry out sampling and mapping of sites, prepare equipment, and perform initial observations of the sample sites. In the second step, different survey methods will be implemented depending on the sample sites. In the third step, the computation of indices is done. The results of these steps plus other relevant information will lead to insights
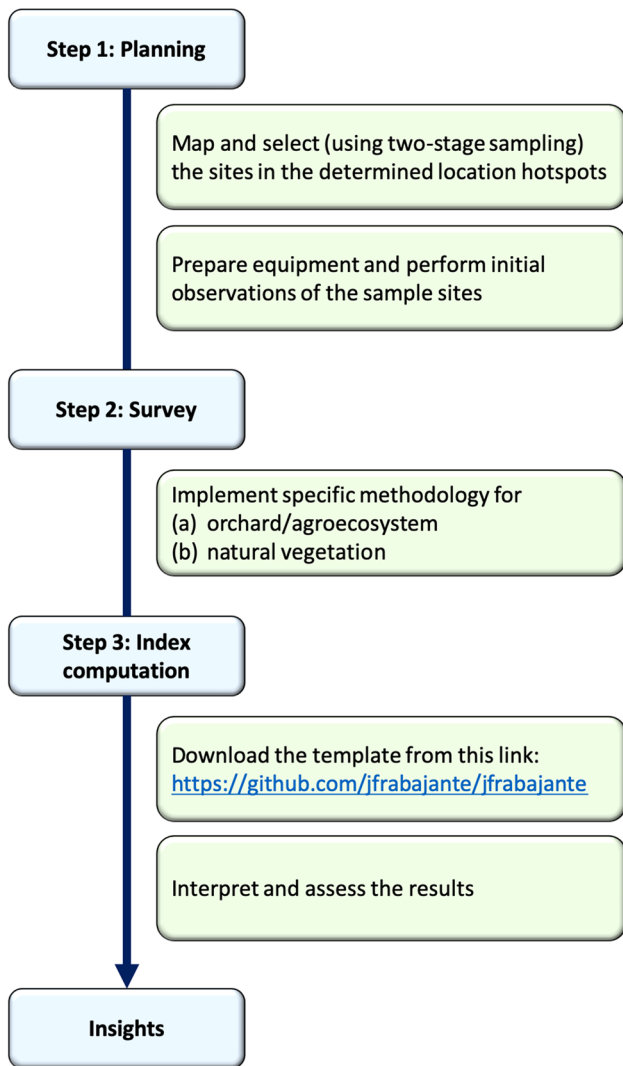
Step 1: Planning

We gather local experts' ranking of pollinator "hotspots" per location type. The local experts comprise entomologists and local farmers who had been trained to do sampling. We define "hotspots" as the potential locations with high abundance of pollinators and plants. The experts can do their ranking depending on the seasonality of flowering plants (e.g., some plants only bloom during summer). The ranking process, which is through classifying the hotspots as "very high pollinator occurrence" to "low pollinator occurrence", is qualitative. This qualitative ranking can be quantified using mathematical methods, with the guidance of statisticians or mathematicians. A suggested technique for ranking the hotspots is the Analytic Hierarchy Process (AHP) (Taha 2016; Triantaphyllou et al. 1998). AHP is a quantitative multi-criteria decision-making approach that derives priority scores from paired comparisons of alternatives and criteria. There can be different criteria used (such as a place cannot be visited due to political reasons). However, the ultimate criteria are the likelihood of high abundance of pollinators and plants. Moreover, we will also record the justifications why locations are included or not in the list of hotspots.

We consider different location types to be places with (1) orchard or agroecosystem, and (2) natural vegetation. After identifying and ranking the hotspots, we determine the area of each hotspot (e.g., using Google maps or local records). See Table 1 for an example. In this planning stage, we will also pre-validate the selected locations by visiting them and by estimating the diversity and abundance of pollinators and plants. The list and ranking of hotspots can be modified based on this pre-validation. On top of the list of hotspots, the survey can also be done to locations with low abundance of pollinators, for comparison purposes.

After identifying the hotspots and their abundance category, we perform a two-stage sampling and mapping of sites per hotspot. The first stage of the sampling procedure is to randomly select hotspots per class (rank) per location type. The minimum number of sample hotspots is computed according to the desired sampling error and to the total number of hotspots per class (termed as population size). A sample size calculator has been embedded in the Microsoft

Fig. 1 Steps in the proposed survey procedure. The goal of these steps is to draw insights from the gathered data to aid decision-makers in formulating policies and strategies for managing pollination services

where decision-makers can anchor their policies for managing pollination services. We give the details of the survey method in the following.

Table 1 Hypothetical example of information to be gathered from experts

Name of hotspot	Type	Seasonality	Area (100 m^2)	Category*
City 1	Agroecosystem	November–June	3×10^4	Class A
City 2	Agroecosystem	February–July	5×10^3	Class B
City 3	Natural vegetation	Year-round	4.2×10^6	Class A[a]

The column Category is the ranking made by the experts

[a]Likelihood of having high number of flowers and pollinators: Class A (very high); Class B (high); Class C (medium high); Class D (medium); Class E (medium low); Class F (low)
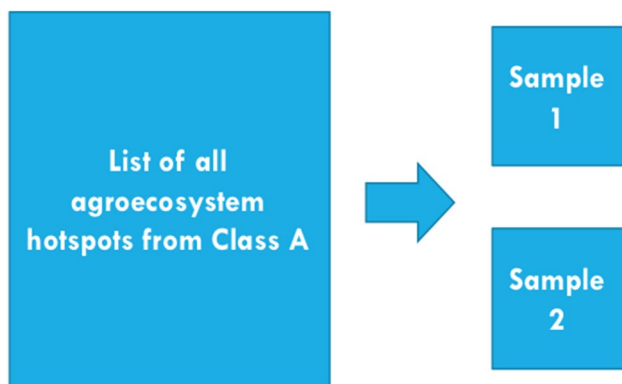
Fig. 2 An illustration of the first stage of sampling for a certain class (e.g., list of all orchard hotspots under Class A). The number of samples is computed using the sample size calculator
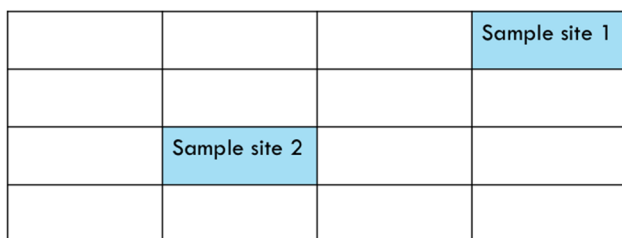

Fig. 3 An illustration of the second stage of sampling for orchards or agroecosystems. For each selected hotspot, a grid map is constructed with 100 m^2 divisions. Then, randomly select cells (sites) in the grid map, which will be the subjects of the survey

Fig. 4 An example of an orchard where the grid map contains rectangles. A rectangle can be the area between the plant rows

Fig. 5 An illustration of the second stage of the two-stage sampling for natural vegetation. For each selected hotspot, we determine the location of every detectable pollinator nesting site (circles). Then, we randomly select nesting sites that will be the centers of our survey

Excel file accompanying this paper (supplementary file). See Fig. 2 for an illustration.

In the second stage of sampling, we use different sampling methods for each location type. For sites with orchards or agroecosystems, we do the following: (a) for each selected hotspot, construct a grid map with 100 m^2 divisions. (b) Then, randomly select 100 m^2 sites which are the subjects of the survey to be implemented. The minimum number of sample sites is computed using the sample size calculator and according to the agreed sampling error. In the calculator, the population size is the number of cells in the grid map. Note that the grid map can have squares or rectangles (with 100 m^2 size) depending on the structure of the orchard or agroecosystem. See Figs. 3 and 4 for illustrations.

For places with natural vegetation, we do the following for the second stage of the sampling: (a) for each selected hotspot, determine the location of every detectable pollinator nesting site. (b) Then, randomly select nesting sites that will be the centers of our survey. If two nesting sites are within 250 m distance from each other, the nesting site selected first is chosen and the other is rejected as the center. The minimum number of sites is computed using the sample size calculator and according to the agreed sampling error. In the
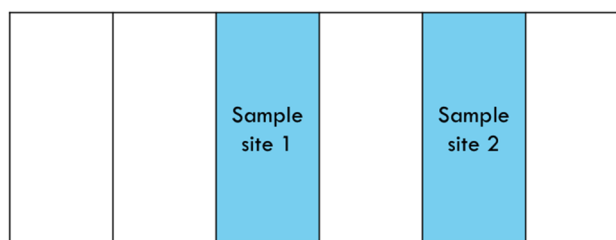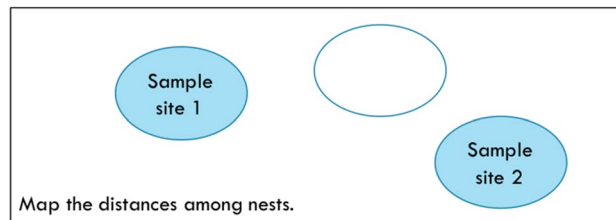
calculator, the population size is the number of nesting sites; see Fig. 5 for an illustration.

During the planning stage, we also prepare the equipment to be used (e.g., measuring instruments of temperature, sunlight intensity, wind, time, GPS) and survey materials (e.g., sweep nets, bags, counter, pen and paper, binoculars, camera, drone with camera). We also decide on the number of workers who will observe and will take note of the relevant information. The number of people depends on the number of sample sites to be surveyed simultaneously. We estimate the need of 1–2 workers per sample site.

One of the important parts of the planning stage is the initial observation of the sample site. Here, we survey available species of plants and pollinators (e.g., using sweep nets), determine possible matching between the plants and pollinators, and if available, survey the characteristics of nesting sites. This initial observation will be used in training the persons who will perform the survey. The expertise of systematists or entomologists is essential in this step.

Two important questions must be answered in the initial observation: (1) how many flowers can a pollinator species visit in a day (visitation rate)? (2) What is the possibility of a species to be a pollinator of a plant species (plant–pollinator matching)? The answers to these two questions are inputs to our Microsoft Excel file (supplementary file), which are necessary for computing the Pollinator-to-Plant (P-to-P) index. The value of the input is between 0 and 1. The value 0 means the insect is not a pollinator of the plant. The value

1 means that we are sure that the insect is a regular pollinator of the plant. The foraging behavior of the flower visitor should be closely observed to determine if they are "true biotic pollinators", that is, if they can effectively transfer the pollen grains from flower to flower of the same species. Also, frequency of visitation is correlated to pollination, and we need to determine that the flower visitation is not just random or by accident.

Step 2: Survey implementation

In this step, we apply different survey methods depending on the location type discussed in Step 1. Sampling should be done under weather conditions suitable for foraging activity of the insects leading to pollination, e.g., there is zero precipitation.

For orchards or agroecosystems, do the following: in each sample site, following the walking pattern shown in Figs. 6 or 7, count the number of plants and the flowers in them as well as their visiting pollinators near the walking path. Record the numbers per species of flowers and species of pollinators. The pollinator survey collection (equivalent to at least 300 sweeps per 100 m^2 site using a sweep net) should be done during pollination peak hours (e.g., from 8 to 10 am). The sum of the numbers recorded in all sample sites is our input to the pollinator abundance, plant abundance and number of flower worksheets in the Microsoft Excel file.

Usually pollination peak hours are consistent within geographical locations, depending on sunlight and weather. To verify this, there will be actual observation of the anthesis of flower and pollinator visitation. We will select a set of flowering plants, and every 15 min, we record the number of visiting pollinators to track the trend of visitation before, during and after the foraging peak period.
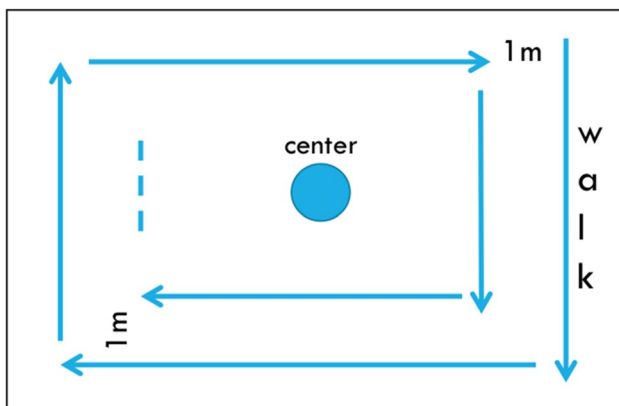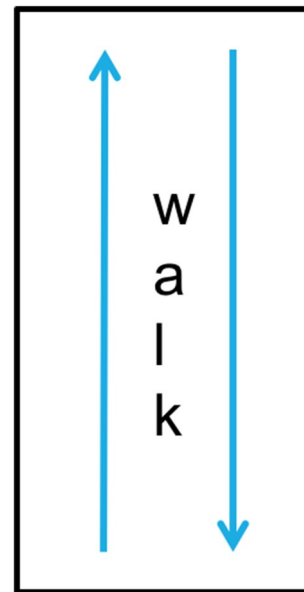

Fig. 7 Walking pattern (row rectangular grid) for surveying the pollinators in orchards or agroecosystems. Path is between plant rows

For natural vegetation, we survey and record the number of pollinators per species that forage inside an area with 250 m radius where the center is the nesting site (Fig. 8). If there are *X* number of nesting sites of one species inside the area, we divide the number of pollinators of this species by *X* in our record. We also count the number of plants per species and the flowers in them. The pollinator survey collection (equivalent to at least 600 sweeps per 250 m linear walk using a sweep net) should be done during pollination peak hours (e.g., from 8 to 10 am) focusing on the location with flowering plants. The sum of the numbers recorded in all sample sites is our input

Fig. 6 Walking pattern (square grid) for surveying the pollinators in orchards or agroecosystems. Walk in a spiral square manner from one of the corners of the 100 m^2 sample site to the center of the site. Distance between parallel paths can be 1 m
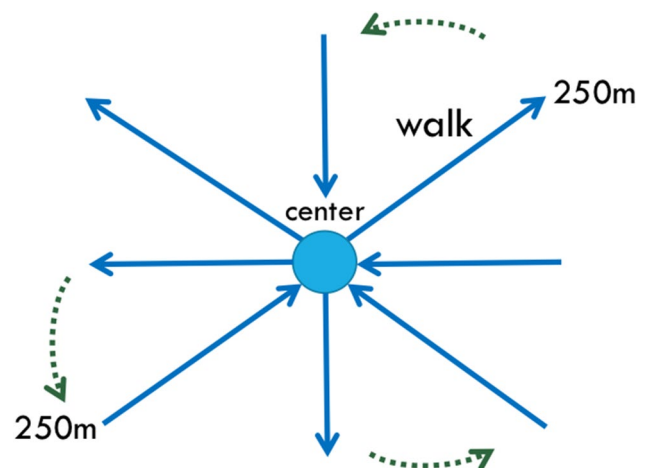

Fig. 8 Walking pattern, starting from the east, for surveying the pollinators in area with natural vegetation

to the pollinator abundance, plant abundance, and number of flowers worksheets in the Microsoft Excel file. We also have the option to select sets of flowering plants 10 m, 50 m, and 100 m away (in linear direction) from the nesting site, and every 15 min, we record the number of visiting pollinators in each set to track the temporal and spatial trend of visitation during the foraging peak period.

Step 3: Computation of indices

In this step, indices necessary for detecting and assessing pollinator deficits are computed. Existing indices that measure pollinator and plant diversity (i.e., Modified Shannon Index) are also computed together with the proposed Pollinator-to-Plant (P-to-P) index. These indices and measures can be computed using the Microsoft Excel calculator file. We use Microsoft Excel since this is a common spreadsheet package utilized worldwide. In the computation of the P-to-P index, simplicity and less modeling assumptions are favored to make explicit ecological interpretation.

The Pollinator-to-Plant (P-to-P) index requires input of estimated visitation rate of pollinators, that is, the number of flowers a pollinator can visit in a day. This can be estimated as the average total duration the pollinator is foraging in a day (in s) divided by the average duration the pollinator is visiting a flower (in s). The possibility value of being a pollinator of a certain plant species (e.g., $=0$ for non-pollinators of a flower; and >0 for pollinators of a flower, with $=1$ for the preferred pollinators) is also needed in the computation of the P-to-P index. The P-to-P ratio and index can be computed as follows (refer to the Microsoft Excel file in the supplementary material):

P-to-P ratio

P - to - P ratio for a pollinator species in a specific survey period

    = Abundance of the pollinator species

       $\times$ Visitation rate of the pollinator species

       $\div$ Flower assignment value,

where

Flower assignment value

    $= \sum_i$ (Possibility value of being a pollinator of plant species i

       $\times$ Number of flowers of plant species i) $\div$ Normalization factor.

Here, the normalization factor is

$$\sum_i \text{Possibility value of being a pollinator of plant species } i.$$

P-to-P index

P - to - P index in a specific survey period

    $= \sum_j$ (Abundance of pollinator species j

       $\times$ Visitation rate of pollinator species j) $\div \text{Max}\{A, B\}$,

where

$A = \sum_j$ Flower assignment value for pollinator species j

$B = \sum_i$ Number of flowers of plant species i.

The P-to-P ratio is computed for each pollinator species, while the P-to-P index is an aggregate measure for all pollinators.

Index interpretation

The Modified Shannon Index determines species dominance with respect to other species. A value close to zero for this index means there exist species that are relatively dominant. On the other hand, if the value of this index is high, there is no species that are relatively dominant.

The proposed Pollinator-to-Plant (P-to-P) ratio and index are measures of pollinator diversity with respect to plant diversity. A value that is less than 1 means that there is a deficiency in the number of pollinators. The ratio also gives the approximate number of times a flower is visited in a day.

Assessment

The indices are computed separately for each chosen hotspot sample site, meaning, individual Microsoft Excel index calculator must be made for each site. The computed indices, and their descriptive statistics, can be used to compare the different hotspots, temporally (e.g., annually) and spatially. In the same way, these indices can be used in different Asian countries, together with the recommended survey method, to determine pollinator deficits in Asia. Specifically, the P-to-P ratio and index can provide valuable insights into the issue of pollinator deficiency. A P-to-P ratio that is less than 1 shows that a flower, on an average, is visited by a pollinator less than once a day. Clearly, this implies that there is a deficit in the number of pollinators with respect to the number of flowering plants. In the Microsoft Excel file, one of the outputs is the pollinator deficiency matrix. This matrix shows the estimated number of pollinators needed to address the deficiency per species per year. This information together with the indices can guide decision-makers in formulating strategies to

address the shortage in the number of pollinators, especially for high-value crops.

For a specific sampling period, we suggest statistically acceptable number of samples (two-stage sampling). A sample size calculator is included in the Microsoft Excel file. To compare temporal data, classical statistical methods can be applied, such as comparing trends and geometric mean. Two temporal data points are enough to compute the slope representing change in pollinator deficits. Five years of annual monitoring is a good start to compare trends. Sampling periods in a year can also be increased (e.g., quarterly) to capture seasonality.

Sample actual surveys and further discussion

We applied our proposed survey procedure to various agroecosystem farms in the Philippines. In one of our survey sites (a bitter gourd farm with 400 crop rows located in Calamba Laguna, Philippines), we observed only 2 roaming carpenter bee *Xylocopa* sp. pollinating 48,000 flowers from 6 am to 11 am of 12 February 2017. The carpenter bees were estimated to visit each row every 15 min. During our survey, we observed 28,000 fruits. The P-to-P index was estimated to be equal to 0.0001 < 1, which means very few pollinators were present to pollinate a number of inflorescences. The bitter gourd fruits have curved appearance, an evidence of the lack of pollination (Fig. 9a). The farmers put hanging stones in the fruits to address this unpleasant appearance. We interviewed the farmers, and they informed us that they

Fig. 9 **a** Photos of the bitter gourd farm site in Calamba Laguna, Philippines. **b** Photos of the cucumber farm site in Calamba Laguna, Philippines

apply pesticides/insecticides anytime of the day, mostly during the morning.

Another survey site is a cucumber farm in Calamba Laguna, Philippines, 2.5 km away from the bitter gourd farm (Fig. 9b). This farm has 4000 flowers in 30 rows. We observed 600 *Apis cerana*, syrphid flies, *Amegilla* sp. and *Xylocopa* sp. The computed P-to-P index is $1.5 > 1$, which means enough number of pollinators are present to pollinate available flowers. We interviewed the farmers, and they informed us that they start spraying insecticides 3 pm in the afternoon. The results indicate that the schedule of the insecticide application may have an impact on pollinator density and consequently, on the harvestable crop yield.

Our formulated methodology, with other relevant information, provides insights that can be used to formulate hypothesis that can be further tested. Policy recommendations can also be drafted based on the results of the survey and other related studies. For example, the P-to-P indices computed in natural vegetation and in agroecosystems with and without bee pasture can be compared. From this comparison and other relevant information [e.g., from other mathematical models (Gavina et al. 2014) and the presence of bee diseases (Jatulan et al. 2015)], we can estimate the optimal area of bee pasture that could sustain the desired number of bee pollinators. This comparison is possible since we have developed a harmonized method.

Using our method, we can estimate the level of pollinator deficiency per species per year. Our survey design, if implemented in various locations and periods of time, can estimate spatial and temporal patterns of pollinator population dynamics. Implementing regular long-term monitoring of pollinators is one of the activities that can be done to assure sustainable pollination services.

Summary and conclusion

The protocol that we developed will be an important tool in quantifying the short- and long-term population density of pollinators in the tropics, especially in Asia. The harmonized method will yield empirical data that will be the basis in evaluating whether the pollinator population has increased, declined or remain stationary from year to year. Different ecosystem types such as agricultural, orchard and natural vegetation were considered in this study where we emphasized that sampling should be carried out during blooming period of the plant. In addition to the computation of diversity measures, the developed protocol includes the computation of pollination matching measures that determines the Pollinator-to-Plant ratio and index. A user-friendly Microsoft Excel template accompanies this protocol.

This study will help in monitoring the recovery of pollinators in disaster-hit areas. One of the effects of disasters is the devastation of plant communities and consequently, the extermination of pollinators. We observed that after a disaster, like typhoons in the Philippines, vegetation is wiped out and pollinators are almost absent. The rate of recovery of pollinators can be slower than the revival of plant communities. Plants rebound faster than pollinators that result in lower harvest especially for plants requiring outcrossing. Strategies for effective pollination services can be drafted based on the results of the surveys. Our survey can help in determining the rate or pattern of pollinator restoration vis-à-vis plant restoration.

Natural vegetation and an agroecosystem practicing organic agriculture are expected to yield higher pollinator diversity, because of the absence of chemical inputs, especially insecticides that directly kill the insects including pollinators and natural enemies. Diverse plants can be retained and serve as alternate food source of the pollinators, which will consequently result in higher productivity because of the sustained pollination services. Organic farming practices are good for pollinators. Also, natural pollination services support productive organic farming.

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