BEESPOKE Frisian clay area: sticky trap protocol and analysis

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Background: In the framework of the BEESPOKE project, general insect biodiversity plays an important role. Pollinators are important in agriculture, but also other groups are functional, for example as pest control species. It is quite naive to think that promoting only certain species (such as pollinators) will sustainably result in a robust and resilient ecological community serving agriculture. Solutions for promoting resilient ecosystems entail development of reasonably stable situations, where a broad spectrum of functionally interchangeable species can develop. Measuring the development of these resilient insect communities thus should aim for a wide scope of target insects, i.e. measure biodiversity.

To inventory all species involved in any ecological trophic chain relation and intra-community interaction that occur in an ecosystem is too complex. It also requires continuous and long term expert involvement, which is expensive. An important practical problem that emerges is how to measure useful biodiversity parameters for use in the farmer business context in a fast and cheap manner, such that a farmer can potentially use the method as a part of his business information gathering. To get biodiversity information, many measurements need to be done, which needs to be manageable.

Any measurement system has its own set of problems. Any method has particular biases, related to subjective observer differences, and/or specific aspects of objective catching methods. Insect catches are always biased towards certain species groups of interest. To avoid bias in interpretation of effects it is useful to provide contrasting measurements (such as changes over time or contrasts between references) for a reasonably indicative species group under similar conditions, using a standardized method with a known and acceptable bias. Such monitoring method also needs to be cheap, target a wide variety of species, and preferably close to applicability by farmers themselves, or by other citizen scientists. That particular feature will increase the potential for adopting monitoring in the business workflow of end-users.

Flower strips are local and small scale biodiversity improvements, and thus need small scale biodiversity change indicators. This make large scale biodiversity indicator systems using large species less useful. Large scale indicator species groups will make success primarily dependent on the larger landscape context, which only makes sense when the particular area of interest is viewed as a small part in a larger landscape context.

Considering all this, we aimed to measure small scale biodiversity indicators (small flying Insects) with a cheap and standard method, that potentially can sample many families and species: yellow sticky traps.

Sticky traps: Sticky traps are highly standardized, and used in horticulture for pest insect assessments (although there are quality differences in stickiness and applicability with different manufacturers). From these standard sticky traps, we assessed quantitative biodiversity information by looking at variation at order and family taxonomic levels, and on species level for the relatively catchable and relevant species group hoverflies. Hoverflies contain pollinating species, as well as pest control species. Besides that, we assessed indices for trophic importance of the caught insects

Figure 1. *Example of a sticky trap placed in a rape seed field after 2 days of exposure.*

by assessing numbers of caught insects, measuring their length, and subsequently estimating biomass.

These measures can be used to contrast species richness and diversity by seeing if more orders or more families or more species make use of the local situation. The data can also be used to estimate species richness and species diversity. Also, it could be investigated if functional insects or insect groups are present. Additionally to that, the trophic value of the insect community can be investigated by looking if more insects and/or larger (more biomass) insects use the location. Longer and heavier Insects provide more biomass per effort for larger predator species, such as for amphibians, reptiles, mammals (shrews, bats) and birds. Also, higher abundance of larger insects may indicate a more stable environment, supporting the longer development processes of larger species. These indicators may be seen as information on the state of the location in allowing for (functional) insect populations to develop and ecological trophic possibilities of the local system.

Methods. Standard yellow stick traps of 10x25cm (Signaalplaat geel, Brinkman inc., see Figure 1) were mounted with notice board pins on wooden sticks (1x4x65cm) placed in the soil, with the sticky face approximately 20-45cm above the soil, facing South. The vegetation was opened up or cut away to prevent gras or vegetation to stick to the Sticky traps. The sticky traps were exposed to flying insects for approximately 2 days and then retrieved for processing. This timing was used to gather insects in reasonable amounts, and at any time of day.

Analysis: Sticky traps were analysed using different levels of visual analyses. First sticky traps were scanned on a photo scanner (Canon Canoscan 9000F Mark ll) at 1200dpi resolution. The scans were analysed by automatically detecting insects on the traps, using a custom trained object detection algorithm, generating separate images. These separate images were manually annotated. Body length was determined using a custom trained segmentation algorithm.

Insect object detection: The object detection algorithm (Boerema-Strijkstra Sticky Trap Insect Detection Algorithm, version 3.8) was implemented in python, based on the YOLOv7 algorithm reference implementation (Wang et al. 2022). Yellow Sticky traps obtained on locations throughout the Netherlands and Belgium were included in training (n=307; split for training: n=242 and validation: n=65).

Arthropods on the Sticky traps were annotated with bounding boxes, using the open source bio image analysis software ICY (de Chaumont et al. 2012). The algorithm was trained on a GPU (Nvidia 3090 RTX). Mean Average Precision (mAP) of the predictions after training was good (mAP@0.5=0.96; mAP@0.5:0.95 = 0.7). The optimum on the F1 curve was 0.92 at a confidence threshold of 0.34. This confidence level was used for subsequent object detections by the model.

Insect annotations: Manual determination scoring of separated images to the nearest easily attainable taxonomic level was done using an open source annotation and labelling platform ("Labelstudio"; version 1.7.0; available at https://labelstud.io). False positive images from the object detection algorithm (4.47%) and non-imago stage Insects (0.031%) were eliminated from the analysis at this stage.

Insect body length: Body areas of Insects were determined using a custom developed body segmentation algorithm (Boerema-Strijkstra Insect Body Segmentation Algorithm, version 1.0), based on the pyTorch variant of U-NET and trained using the NOUS AI platform (NOUS, Cosmonio Imaging BV.). Separated images (n=7139) of arthropods from Sticky traps obtained on locations throughout the Netherlands and Belgium were included in the training. On each image, body area was manually delineated with a polygon. The performance of the model after training was good, body areas of insects were generally detected quite well, (Dice/F1 value = 0.88), with the exception of Dragonflies (*Odonata*) and Butterflies (*Lepidoptera*). These taxonomic orders did not occur often on sticky traps and were excluded from automated body area analyses.

Length of the insect was calculated from the body area by calculating the longest distances within the body area, connected through the center point of gravity. The automated body length measurement correlated very well with manually measured lengths in a subset of the data (Linear Regression: n=10293; y=0.9296x, r^2=0.8995). The trained model and length calculations were implemented using a custom made Python script.

Biomass calculation: Biomass was calculated from body length using the length to mass relationship for terrestrial insects published by Sabo et al. (2002):

Biomass in mg = 0.03 * (body length in mm)$^{2.63}$;

We calculated biomass per sticky trap and also biomass per sticky trap per 1mm length category between 0-15mm and >15mm, as a potential source for information on the origin of the biomass: biomass can either be based on many small insects or fewer larger insects.

Measures of insect numbers and diversity: From the insect annotations, measures for insect biodiversity were derived. These could include numbers of individuals per class, order, family and species. For family and species level, also estimated numbers of species group (Chao-1; Chao, A. 1984) and estimated biodiversity of species groups (Shannon entropy; Chao et al. 2013) were made, using the biodiversity calculation program SpadeR (Chao et al. 2015).

Measures of insect biomass: From the length (in mm) and calculated biomass (in mg) data, numbers of individuals and biomass could be calculated per mm category. This yields an indication of the contribution of different sized insects to the available biomass of flying insects in the system.

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