Experimental framework and standardised protocols for EBAs described in first version of a living document

Deliverable D1.2

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SHOWCASE

SHOWCASing synergies between agriculture, biodiversity and Ecosystem services to help farmers capitalizing on native biodiversity

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Summary

The decline of biodiversity raises concerns about the loss of farmland species in general and of ecosystem services that are crucial for agricultural productivity, such as pest control, soil fertility and pollination. The sustainability of farming systems is dependent of these services and thus the effect of agricultural practices on farmland biodiversity has to be evaluated with relevant indicators, reflecting the status of farmland biodiversity and inform on its evolution, acting as guides for a transition to a more sustainable agriculture.

The European project SHOWCASE aims to deliver tools to facilitate the transition towards more biodiversity-friendly farming practices. Its first work package aims to develop a multi-disciplinary approach - including farm production, biodiversity protection and social impacts - that will be tested and evaluated in a network of Experimental Biodiversity Areas (EBAs). In this context, the present Deliverable (D1.2) aims at identifying a set of relevant biodiversity, ecosystem service and farm indicators to be measured in the different EBAs. It is a living document, thus can be updated over time. The first part of the document presents the general framework (farm interventions, selection of indicators), then we provide a list of core and optional indicators, then we describe in detail the protocols. The deliverable also includes a short section on how interventions in each EBA were chosen in regard to biodiversity objectives. D1.2 compiles all the finalized protocols.

Based on previous projects, and on an iterative process of bilateral discussions, workshops and circulated drafts between the EBA project partners, we propose here a minimum set of core biodiversity indicators, which are being measured in all EBAs based on a standardized measurement protocol. The present document provides detailed protocols for the three core biodiversity indicators: plants, bees and spiders. In addition, agronomic yield measures will be added at a later stage. Other protocols, that are not mandatory but options which each EBA could choose to apply, are also provided. These additional protocols and indicators may be appropriate for some of the EBAs, depending on their farm type and type of intervention. All indicators discussed in detail in this report are grouped in three main categories: (i) habitat and species, (ii) ecosystem services and (iii) management. Socio-economic indicators are the topic of another task within SHOWCASE.

List of abbreviations

EU	European Union
EBA	Experimental Biodiversity Area
ES	Ecosystem Service

Introduction

Aims

In SHOWCASE, each EBA is to serve both as a local testbed for developing and implementing novel interventions and incentives, and act as a knowledge exchange hub. The EBAs are located in 10 different countries (CH, EE, ES, FR, HU, NL, RO, PT, SE, UK) and have been selected based on their representativeness of the diversity of European farming systems, as well as on already existing local or regional multi-stakeholder structures (see Deliverable 1.1 – Network of EBAs established across Europe).

This multi-actor community identifies and prioritizes local or regional challenges of biodiversity-agricultural production trade-offs, and ii) co-formulate potential solutions. SHOWCASE is a place-based research initiative, meaning that within farm interventions, which are co-designed or at least discussed with farmers, are locally designed. However, to add value at the European level and allow up-scaling and out-scaling of solutions, it is essential to have a common framework and set of core standardized methodologies and measures used by all EBAs. D1.2 aims at describing how relevant biodiversity interventions in each EBA will be monitored using a standardized core methodology, despite their differences in design and set-up. The biodiversity interventions will subsequently be tested for impacts on biodiversity, ecosystem service benefits and associated costs in T2.5 and T3.2.

In task 1.2, a standardized study design to determine biodiversity and intervention related management adaptations in relation to the standard operations on farm was elaborated. Hence, T1.2 also delivers important information for further tasks that assess the interventions effects at farm and landscape level, e.g. socio-economic effects, and ecosystem services (WP2). The indicators used in WP2 for analysis beyond the specific interventions and beyond plot scale, e.g. at farm and landscape scale, will be separately described and surveyed in the respective deliverables (D2.3, D2.5, D2.7).

Intervention study designs will include issues such as having sufficient replication and suitable controls. We have organized several online workshops for this task, since both design and methodology need to be flexible enough to be useful in different landscape types and socio-economic settings yet consistent enough to allow for cross-continental analyses. Results of these two workshops are summarized here and produce the final document with agreed-upon designs and protocols for assessing biodiversity, biodiversity-based ecosystem services, and productivity.

Methodology

In addition to workshops, literature was reviewed (especially from relevant European projects) and most importantly, experience gained by scientists in various EBAs already operating allowed to select easily between available methods. We targeted simple systems, standardized, cheap and fast methods (as a general rule, one or a few days to operate the complete scheme). Particular attention focused on the feasibility of running protocols in highly diverse contexts and crops, and allowing maximum flexibility since in some of EBAs, experiments had not yet been designed when we were discussing protocols.

The monitoring of the interventions in EBAs and of their effects will be harmonized, as well as the protocols for collecting biodiversity data. Our overall strategy is:

-Collect a core set of indicators following a common protocol

-Complement the core set with additional indicators that account for the specificities of individual EBAs. EBA site managers will decide on the recording method
-Indicator categories comprise:
    -Species and habitat indicators
    -Ecosystem service and ES provider indicators
    -Management indicators
    -Socio-economic indicators

The focus of this Deliverable is on the species, habitat, and ES indicators. Also, plot level management indicators for interventions and controls are in the focus.
Core socio-economic indicators will be gathered in the WP2 Task 2.3 farm survey. Specific additional socio-economic indicators for further analyses in WP2 will be tailor-made to each EBA in close consultation with each EBA, taking into account the specific economic research question.

The framework: EBA farm interventions

Before establishing standardized protocols, we set up local multi-actor initiatives between SHOWCASE scientists and stakeholders, farmers or cooperatives. The number of representatives from different stakeholder groups that the SHOWCASE partners interacted with differed per EBA, but farmers were always the core group (Table 1). The way SHOWCASE scientists interacted with farmers to select the biodiversity interventions differed also between each EBA depending upon local historical engagement, context and geographic situation.

Table 1. The number of stakeholders SHOWCASE partners interacted with during the establishment of the EBAs in the different countries

	Farmer organizations	Farmers	Policy makers	NGOs	Other
CH	2	20	3	1	0
EE	1	20	0	2	20
ES	8	24	3	1	3
FR	1	35	0	2	0
HU	0	16	1	0	1
NL	1	40	1	3	2
PT	4	15	2	0	0
RO	0	3	1	1	0
SE	3	43	1	0	1
UK	1	30	1	1	7

In the UK and Sweden, a full co-design approach identifying together with farmers what biodiversity intervention to aim for, was initiated late 2020 (UK) or spring/summer 2021 (Sweden). These latter two countries are the only one where such full co-design approach was used since the start of the SHOWCASE project. For instance, several meetings with farmers were organized in the UK, to co-develop interventions before a final design was acceptance. In Switzerland and France, a co-design approach was also used, but was initiated before SHOWCASE started. For instance in France, scientists have been collaborating intensively with farmers to co-design socio-

ecological experiments since 2012. In Hungary also, an experiment was designed with farmers (on their grasslands) before SHOWCASE started, but discussions with a single large state-owned farm to implement wildflower strips resulted in a new intervention, which was suggested by the researchers. In contrast, in the Netherlands, SHOWCASE scientists decided to work on existing interventions (hedges) and interacted with farmers to ask what kind of variables they were interested in and whether they would agree to work on their land. A similar situation was found in Estonia. In Spain and Portugal (orchards), farm interventions were discussed with farmers, in a partial co-design process where scientists suggested the intervention type and farmers providing feedback on how, when and where they would like to see this implemented. Finally, in Romania, farm interventions was proposed by SHOWCASE scientists and managers. The variety of approaches, while potentially making cross EBA comparisons more complex, do however allow us to explore a diversity of different approaches to the design of biodiversity focused interventions, thereby providing useful insights into a wide range of farming systems across Europe. In all EBAs, whether using a full co-design, a mixed or a mainly scientist/manager-led approach, the choice of farm interventions involved meetings with 3-40 farmers, and in most cases, one or more one-to one meetings in order to establish the protocols in detail.
Biodiversity itself was seldom the main target of the farm intervention (except in the Netherlands, Hungary, Romania and Estonia; notably the countries that had an approach that was not strongly co-design based). More often, agroecological targets, which include combined agronomic, economic and ecological (i.e., biodiversity) aims, were discussed. Therefore, the links between farm interventions and biodiversity were as diverse as the farm interventions themselves. This diversity required additional (numerous) meetings between SHOWCASE scientists and EBAs PIs in order to elaborate standardized protocols.

Some definitions

Field

A field is an agricultural parcel, or plot. It can be an arable field, a grassland (or meadow), or an orchard. A field contains the core field, the field margin (i.e., typically the first meter or less, quite often without crop between the external border and the first sowed row). Next to the field there is in some countries a grassy margin, but sometimes there is nothing like in France, where the next crop starts.

Focal Field

The focal field is the field where surveys are being conducted. It can be the Biodiversity Intervention Field, but can also be a field next to an intervention.

Biodiversity Intervention “Field”.

The Intervention field is the very precise field or plot where the intervention is being conducted. Depending on the situations, it can be an entire field (excluding field margin in arable, including field margin in grasslands), part of a field, it can also be a grassy strip out of the field but next to it. Or some other boundary feature, so that hedges, ditches and other features can be included.

Control “Field”

Each Biodiversity Intervention “Field” is paired with a Control “Field”, except in cases

with multiple interventions, where the fields without interventions are included but not in a pairwise fashion. Ideally, Intervention and Control are 1 km distant from each other to avoid spatial autocorrelation. At the same time, the basic abiotic conditions should be similar, i.e. similar elevation, exposition, soil type, climate. Make sure that there is no systematic bias between Intervention and Control (e.g. not to place all intervention fields in coastal edge and all control fields in the hinterland).

Selection of indicators

A first list of potential biodiversity indicators was obtained by screening the rich body of literature and evidence from previous projects and monitoring initiatives on indicators selection (e.g. Dennis et al., 2012; Herzog et al., 2012). These indicators were grouped into four main categories:

- Habitat and species indicators
- Ecosystem service indicators
- Management indicators
- Socio-economic indicators

The different indicators listed were then evaluated and rated by all EBA project partners based on their 1) scientific support, 2) relevance at the European scale, 3) ease of data collection, 4) cost effectiveness, 5) ecological meaning and 6) relevance for stakeholders. This resulted in the identification of a set of core indicators, which will be measured in all EBAs following common protocols. In complement, a group of optional indicators was also proposed to account for the EBA site specificities (i.e., farm type or intervention), and that will be implemented by the EBA managers.

Habitat and species indicators

A core set of four habitat and species indicators was selected to be measured in all EBAs and is described below. Five optional indicators have been added to the list and will be measured according to each EBA site location, farm type and scale of interest. Table 2 summarizes the four core and five optional habitat and species indicators.

Habitat type

Habitat is itself an important component of biodiversity (e.g., Bailey et al., 2007) and a good indicator of biodiversity at the species level. Habitat mapping is the first step to monitor habitat type and diversity. The QuESSA standardized approach will be used to map the habitat (Holland et al., 2014), in combination with the use of new monitoring methods based on remote sensing (i.e. satellite-based images).

Vascular plants

These are the primary producers in farmland and are at the basis of the food chain, being thus essential to the maintenance and stability of higher trophic levels. Vascular plant diversity or richness is particularly sensitive to specific field management, but also to the presence of pollinators or seed dispersers. Therefore, they are good bioindicators of agricultural management and practices, and they are widely studied and well documented.

Wild bees

This indicator groups essential pollinators of farmland ecosystems. Their recent decline has attracted public attention and raised awareness to the link between biodiversity and ecosystem services (e.g., Sutter et al., 2018). The factors behind their decline seem to be multiple and complex, but habitat destruction, the spread of chemical compounds and the loss of floral resources (and year-long availability) have been shown to be important.

Spiders

They are a large group of predator species, with several of them preying on agricultural

pest insects and thus reducing crop damages. Sensitive to farming practices, vegetation composition and structure, they are good indicators of management at the plot level.

Ecosystem service and ecosystem service provider indicators

The agronomic yield (quantity and quality) was the only ecosystem service indicator selected to be measured in all EBAs. The main objective of farmers is to maintain, or even increase, yield and it is thus relevant to measure as well yield increasing effects of the services provided by biodiversity (e.g., pollination, pest control, decomposition)as well as yield reduction (e.g. due to competition).
The agronomic yield indicator is accompanied by five optional indicators that will be collected depending on the EBA site and farm type. They are summarized in table 2.

Management indicators

Farm management affects biodiversity and the three core management indicators selected reflect the intensity of farming practices, with variations in indicator measurement depending on the EBA site (e.g., farming system, type of intervention). Additionally, a set of three optional management indicators has been defined, their collection depending on the EBA site. Table 2 synthesizes the core and optional management indicators, and the three indicators composing the core set are briefly described below.

Field operations

It characterizes the disturbance caused by farming operations on farmland. Variations in indicator types and monitoring methods are planned in relation to the type of farming and intervention of the different EBA sites (i.e., mowing frequency in grassland, or plowing depth in crops).

Nitrogen input

Nitrogen is a key production factor and quantitatively the most essential plant nutrient for biomass production. Therefore farmers raise through fertilization the level of nitrogen in soils to increase yields. High levels of nitrogen supply directly affect biodiversity, habitats and ecosystems likewise as leaching and gaseous losses.

Pesticide use

Pesticide application is commonly associated with a loss of biodiversity in agricultural landscapes. By being relatively non-specific, the application of herbicides, insecticides and fungicides has negative effects on numerous species and disrupts the ecosystem trophic web at different scales and levels.

Grazing intensity

For interventions on grassland sites with respective management, this indicator evaluates the intensity of grazing on the pastures of the intervention/ control and of the farm.

Crop rotation

Crop rotation on arable land is the practice of alternating crops grown on a specific field in a planned pattern or sequence in successive crop years so that crops of the same species are not grown without interruption on the same field.

Socio-economic indicators

Farms socio-economic conditions have a strong impact on the farmers' motivation and feasibility to implement biodiversity interventions. The purpose of gathering socio-economic indicators (e.g. indicators related to the farmer (age of the farmer, gender, training/education), to the farms (farming type, farm size, type of management, farm income, ownership), to biodiversity management (biodiversity related practices, subsidies/AES, conservation advice received) etc.) should therefore help to understand the context of the farms within the individual EBAs, mostly in relation to the motivation of the farmers, to their economic situation and to the larger policy context. In SHOWCASE, partaking of intervention farms in the WP2 Task 2.3 large scale farm survey should be guaranteed by the EBA leads, thus socio-economic indicators can be gathered along the T2.3 survey questionnaires.

General overview of the Deliverable

The next sections provide detailed protocols for the three biodiversity indicators that have been retained, plants, bees and spiders. In addition, agronomic yields will be added at a later stage. Other protocols, that are not mandatory, are provided for those EBAs who would like to use them.

For each protocol, the background framework is summarized, then we describe the goal of the indicator, and then the methodology to collect the relevant data. Where appropriate, a map of the field, with the various options for the intervention (whether in or off field) are also provided.

Figure 1 provides a guideline on what page which indicator can be found. The complete set of indicators is presented in Table 2.

Figure 1. A schematic overview of the deliverable.

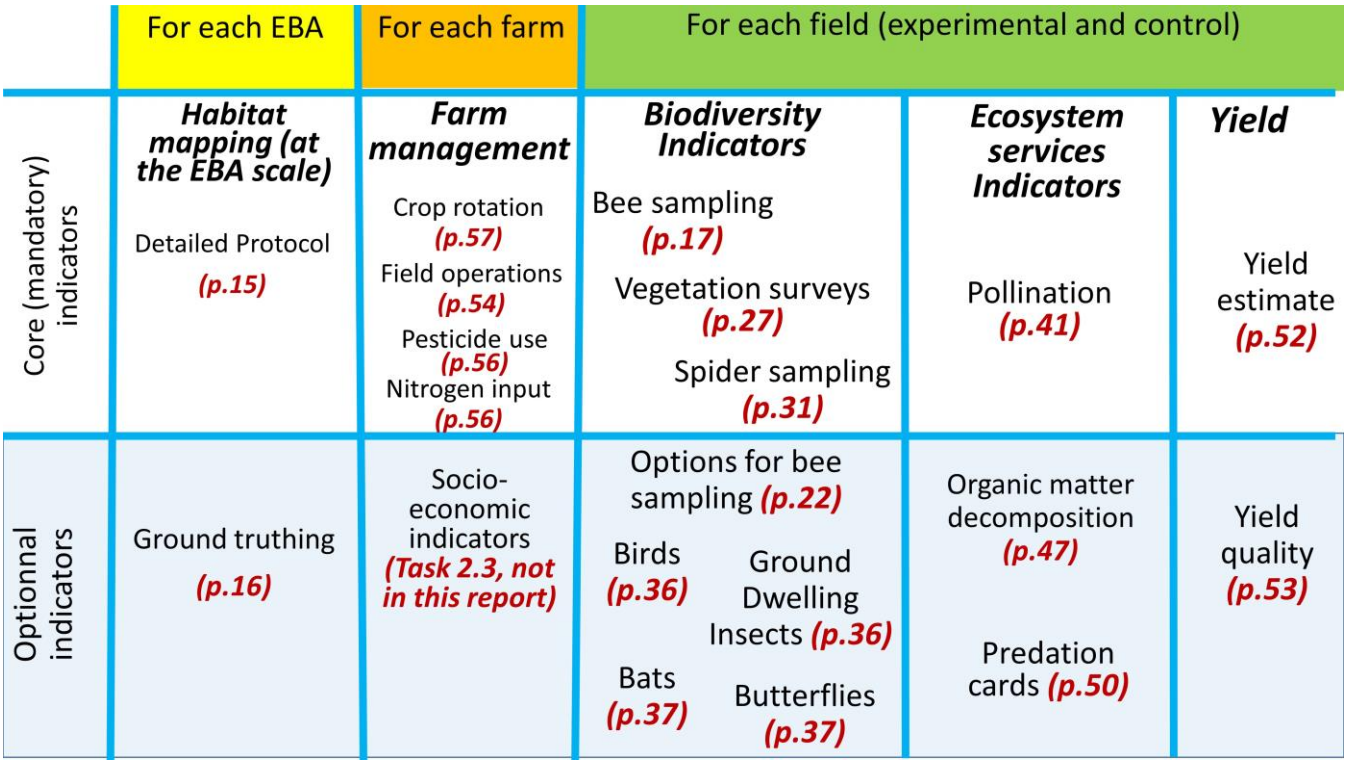

Table 2. An overview of the set of indicators that was selected for use in SHOWCASE

Indicator type	Rationale (framework)	Indicator variables	Protocols and method	Authors
Core indicators all EBA				
Habitat type / mapping	Record the habitat type of the focal field and of the surrounding landscape (circle with 500m radius) according to a standardized terminology and with standardized rules. Explanatory variables that are relevant for the intervention are computed from habitat map.	Habitat diversity / Target habitat / %SNH	Habitat mapping with satellite RS, or in some cases, aerial photos	Felix Herzog Duccio Rocchini
Vascular plants	We want to know plant species diversity and abundance (1) of the focal field as a whole and (2) as related to the intervention depending on where in the focal plot the intervention takes place.	Vascular plant richness / Target plant species / Flagship species Diversity and abundance	10 1x1m squares	David Kleijn
Wild bees (incl. Honeybee)	Wild bees (including bumble bees) are important ecosystem service providers and at the same time a good biodiversity indicator	Same as for plants	Transect walks	Matthias Albrecht & David Kleijn
Spiders	Spiders are important ecosystem service providers. As biodiversity indicators, they react to the structure of the habitat.	Same as for plants	Suction samples	Felix Herzog & Philippe Jeanneret, adapted from http://www.biobio-indicator.org/factsheets/spiders.pdf
Optional indicators			Leads for implementation	
Butterflies	Iconic species group, taxonomy well established, potential for involving citizen scientists	Same as for plants		EBA site managers
Syrphids	High species diversity, providing both pollination and predation ecosystem services	Same as for plants		EBA site managers
Carabid beetles	Well known species group, important ecosystem service provider. Good indicator in arable systems.	Same as for plants		EBA site managers
Pests	Crucial need for farmers to know when applying an intervention whether it is a reduction in pesticides and/or habitat creation (should ask growers what pests risks are they concerned about with any given intervention	Abundance, biomass, species richness	rapid assessment can be used (e.g. counts of aphids, slugs, white butterflies etc.)	EBA site managers
Nesting birds	Birds are a landscape scale indicator. If a specific EBA intervention aims at promoting birds, it may also be necessary to monitor them at field scale (e.g. number of nests of soil breeding birds). Also, in some EBA it may be interesting to evaluate their role as predators of insects. High potential for involvement of citizen scientists	Same as for plants		EBA site managers
Earthworms	In addition, the total biomass is of interest as a proxy for the potential service provided. Earthworms should only be sampled if the EBA intervention is expected to affect soil properties. High interest from farmers.	Same as for plants, also biomass	Extracting soil monoliths (30x30cm x20cm deep) & hand sorting in field. 2-3 per plot. Not using irritants.	EBA site managers, e.g. http://www.biobio-indicator.org/factsheets/earthworms.pdf

Protocols for Showcase core indicators

Habitat mapping

1. Characterizing Landscapes

Author/ contributor: Felix Herzog, Duccio Rocchini

Background & Purpose

Characterization of the EBA landscape for the task 1.3 (Identifying relevant biodiversity and ecosystem service indicators at the farm, national or European scale), by characterization of the landscape surrounding the focal field and record coarse explanatory variables on habitats in a 500m circle around the center point of the focal field.

Goal

Obtain information about the share and type of semi-natural habitats in the 500m circle, possibly subdivided into woody habitats (forest edges, hedgerows, lines of trees), permanent grasslands, cropland. Possibly, also mass-flowering crops will be identified. In addition, heterogeneity indices will be computed on both field data and remote sensing data and their explanatory power will be analyzed. Different types of classification (supervised, unsupervised, fuzzy…), based on the use of remote sensing data will be tested in order to characterize the EBA landscape.

Protocol

Optical and LiDAR data will be used to characterize the EBAs. Sentinel-2 data (freely available with a spatial resolution of 10m) can be used for our purpose in particular: to describe various vegetation patterns, to derive different remote sensing vegetation variables (e.g. NDVI used to characterize the green areas, or to estimate vegetation biomass), to identify smaller and point/linear biodiversity elements typical of forest and agricultural landscapes (forest edges, permanent grasslands, hedgerows, shrubs...) and to assess more in general the environmental heterogeneity through the spectral heterogeneity approach.
LiDAR data could be used for assessing the 3rd dimension of vegetation. 3D information could give us advantages in order to characterize different habitats within a considered EBA.
Satellite information will be used for all EBA in order to have a standardized approach. In addition, EBA partners are free to investigate the surrounding habitats more precisely with either aerial photographs and / or with field mapping.

Information required

GPS location of center point of focal field, GIS polygon of focal field (in WGS-84 projection) with land-use type (type of crop, grassland, etc.), GIS polygons of directly adjacent fields with land-use type (type of crop, grassland, etc.) and of directly adjacent linear structures, which may be difficult to identify by RS (hedgerows, permanent herbaceous strips that are at least 3m wide and 10m long, vegetation patterns under the canopy of trees or bushes). See for example, Fig. 2.

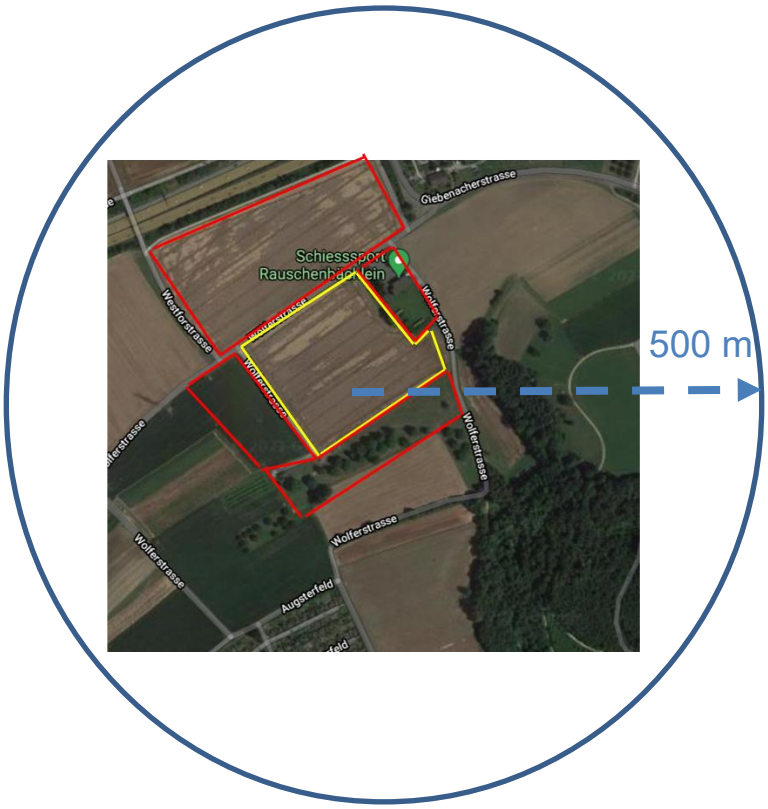

Figure 2. Provide a small GIS map with land use / land cover information about the focal field (yellow, in the center, intervention field or control field) and about the adjacent fields (red, on all sides). Habitat information will be made available in a 500m radius around the center point.

Ground truthing

In order to achieve the task 1.3 through the use of Remote Sensing data, information related to the spatialization of the different patterns as described in the point 4 are needed. This can be done also using high resolution remote sensing data.
In order to validate the possible results a ground truth of the spatialization of the small vegetation patterns - hedgerows, permanent herbaceous strips - (as stated in point 4) in some EBA is needed.

Biodiversity

2. Bee Sampling

Author/contributor: Matthias Albrecht, David Kleijn, et al.

Background & Purpose

Bees are one of several biodiversity indicator taxa studied in the Showcase EBAs across Europe (Task 3.2). Bees will be sampled using a common protocol in all EBAs to allow for integrated overarching analysis using raw data. The different EBAs operate in different agro-ecosystems and are located in different climatic zones. Furthermore, interventions can be implemented in-field and off-field. This protocol is therefore designed to produce meaningful results under all these conditions and types of vegetation (including arable crops, grasslands, horticulture, herbaceous and shrubby semi-natural vegetation etc.). The spatial design of the bee sampling protocol is designed to match with other protocols, e.g. transects aligned to the plots of the vegetation survey where possible to ensure consistent and integrative data analysis.

Goal

The present sampling protocol is the common protocol to be used in all EBAs (irrespective of whether your focal intervention is targeted for pollinators or not or whether the focal crop is insect-pollinated or not), and it should be regarded as the common minimum amount of sampling done in all EBAs. However, EBA partners are of course always free to do more sampling (more transects, more sampling rounds etc.) if they think this would be useful for their specific system or to meet additional objectives. However, it is important not to change the core sampling protocol described here, but rather to add additional sampling. For example, if you would like to do more sampling in your off-field intervention, please do not change the length of the transects, but rather walk the transects with length, width and time exactly as specified in the protocol here, and simply add further transect(s), making sure the same core set of data sampled the same way can be analysed across all EBAs.

Protocol: sampling methodology

Bees (Hymenoptera: Apiformes) will be sampled using standardized transect walks (e.g. Westphal et al. 2008). This method is flexible enough to be used in different types of vegetation (e.g. herbaceous vegetation, shrubby vegetation, or in horticultural systems). As an area-based method it should accurately reflect the local (foraging) bee communities, and is therefore preferred over e.g. pan traps, which may attract bees from the surroundings, differ in sampling efficiency depending on local floral resource availability, and their efficiency may differ across bee taxonomic groups. Through the transect walk method described in the following all bees, including wild and managed bees, such as honey bees (*Apis mellifera*), shall be sampled.

Transect walks

Bees will be collected within several different linear belt transects of 50m length and 1.5m width during 7.5min (7min and 30 sec) pure sampling time for each walked belt

transect (see below). This seems to be a bit an odd number, but it ensures that exactly the same time is spent per belt transect area as in several previous projects all based on the STEP protocol for performing bee sampling, which is a protocol proven robust, and following to this protocol facilitates comparison of data with studies that already used it. Collectors should walk at a slow speed and record/collect all bees encountered within the belt transect (those clearly visible/ can be captured with the net right in front of the collector), irrespective of whether bees are visiting flowers, are in flight etc. Bees which can be identified in the field at species level can be recorded instead of collected (e.g. honey bees, *Apis mellifera*). All bee individuals that cannot be identified in the field at species level have to be collected for later identification in the lab. Make sure that you do not count the same individual twice if you did not collect it. Bees which were neither successfully caught nor identified to the species level in the field should be recorded nevertheless together with as much taxonomic information as possible (e.g. at least "bumble bee" etc.) and recorded together with the information "not caught" so that they can be included in abundance estimates. Bumble queens should not be captured but only recorded, along with as much taxonomic information as possible.
For all recorded or collected bees, information about the unique transect ID (ID of several linear transects walked at a site, e.g. the different transect walked at different within-field distances from the intervention, see below) should be recorded (e.g. labels on vials should contain this information for each collected individual bee, or the information of transect ID should be included on the recording sheet for for recorded specimens, respectively). The information about transect ID will be required for the analysis of the data, e.g. to be able to fit decay functions from intervention at the field edges with increasing distance towards the field center. Further information should include date, time of the day, site, temperature and cloud clover (for recording of temperature and cloud cover see below).
To capture bees a "butterfly net" with small mesh size should be used. Captured bees should then be put into a vial (or a freezer bag with a zipper). The collector should stop walking and the clock should be stopped (by using a stop watch) during the time insects are handled (i.e. transferring bees from nets into vials, eventually labelling of vials, taking notes etc.). Thus, the sampling time per transect indicated below refers to the pure time spent for searching and catching the pollinators with the net, WITHOUT handling time of captured bees. Otherwise the number of bees would be underestimated in sites with high abundances.
The labelled vials or zipper bags with captured bees are put in a transportable cooling box with a sufficient amount of ice/cooling elements immediately after a transect walk has been carried out, or bees are killed immediately with Ethyl Acetate. Back in lab after a sampling day collected bees can be stored in a freezer until pinning them for identification.

Positioning of transects: sampling design of in-field interventions and controls and within crop.

To align the sampling design for bees with that of vegetation surveys and the sampling of other indicators, linear 50m long belt transects of 1.5m width (7min 30sec sampling time per transect) should be positioned at different distances from the same field side as chosen for the transect into the field for the vegetation surveys (see vegetation survey protocol and below). The first transect will be positioned in the outer 1.5m of the field, parallel to the respective field edge (e.g. the edge with an adjacent off-field intervention such a flower strip), and the fourth transect in the centre of the field (again parallel to the field edge where the first transect is located; Fig. 3a below). The two other transects will be distributed proportionally between these extremes (Fig. 3a). For

example, if the field is 60 m wide and the center of the field thus at a distance of 30m from the field edge, the four transects should be located parallel to the field edge at roughly 1m, 10m 20m and 30m from the field edge. However, use as much as possible available tramlines for the transect walks; in particular in densely growing annual crops (e.g. oilseed rape), this can facilitate walking the transects in those fields. Thus, if the distance of the tramline is not exactly at the right place (e.g. not exactly at 20 or 30 m in the example above) that's fine, but than the exact distance should be written down so that this distance information can be used in the analysis. In fruit orchards, walk along the tree lines (rows) of intensive fruit orchards. If there are off-field interventions, the transect will be located next to the intervention in the middle of the transect to sample bees (with the middle quadrat characterizing plant diversity in the vegetation survey in the off-field interventions (see below). If possible, try to keep a minimum distance of at least 10m from the two short sides of the focal field to minimize potential edge effects from adjacent habitats at those sides. As each of the four transects will be walked for 7min 30sec the total pure sampling time per field is 30min covering a total area of 300m^2. This is exactly the same total area and sampling time used in the STEP protocol (but using 4 instead of 2 transects, but which have half the length of the STEP transects).

In addition to the field next to off-field interventions, that will be surveyed similarly as the on-field interventions as described above, bees need to be also sampled in the off-field interventions and off-field controls. Off-field interventions consisting of predominantly annual plant communities (e.g. annual wildflower strips) two 1.5m wide belt transects of each 50m length should be each walked for 7min 30sec. For narrow linear off-field interventions (e.g. narrow flower strips, hedgerows) the two transects can be arrange in a line of a total length of 100m. In less than 100m long off-field interventions and/or wide (areal) off-field interventions the two belt transects may be arranged as two more or less parallel transects. In special circumstances four 1.5m x 25m transects would also acceptable as long as the total surface area and time spent for the off-field intervention (and control habitats) per sampling round remains the same (150m^2, 15min). Transects should be at least 1m apart from each other. The transects should be aligned as well as possible to the 10 vegetation survey plots; Fig. 3b below). For hedgerows or other interventions where the main or typical flowering "horizon" is vertical, walk along a randomly selected side of the hedgerow and collect/record bees from the vertical shrub flower horizon, respecting the 1.5m width of the belt transect (height, main flowering horizon). Bees sampled in off-field interventions will have to be compared to those sampled in suitable control habitats. What represents a suitable control is context dependent and has to be carefully decided and justified in each EBA. For example, a perennial wildflower strip intervention can be compared with a pre-existing field boundary. A hedge intervention can be compared with a herbaceous field boundary because without the intervention the hedge might have been not planted or removed. If multiple off-field interventions are studied at a site, this protocol (with total sampling area of 150m^2 during 15min) should be done of each intervention type (e.g. if two types of off-field interventions are studied, twice 150m^2 during 15min, thus 300m^2 during 30min should be sample etc.; see also Fig 3b).

Sampling rounds

Each site (both, sites with and without interventions) should be sampled during at least two different sampling rounds. Two sampling rounds are the absolute minimum, but we encourage EBA partners to perform more, ideally up to four sampling rounds. Sampling rounds should be chosen according to relevant time period of the focal crop and

implemented intervention, and should ideally cover the main activity periods of all relevant bee taxa in the study region/ system. Make sure that sites (e.g. fields) sampled in the morning in first round will be re-sampled in the afternoon in the second round and vice versa because activity of bees might change during the day. Should this not be possible logistically, ensure that sampling daytime is randomized across sites. This is required because activity of bees can vary during different times of the day.
Bee sampling should generally be carried out from 10.00h – 17.00h, ideally during sunny weather, or when weather conforms to the following standards, respectively: temperature above 13°C with at least 60% clear sky and above 17°C in any sky conditions, apart from rain low wind velocity (ideally < 2.5 m/s; Pollard & Yates 1993). These conditions may have to be locally adapted depending on climatic region of the EBA. However, conditions need be standardized within an EBA region. However, these are the ideal conditions, but if they are not fully met but you think conditions are close to these optimal conditions enough that flower visitors are active, you may decide to sample anyway. Generally, it is better to collect data even if the weather conditions are not perfect (given that the same conditions prevail at all sites) than to have no data at all. Especially during the typically short flowering period of the focal crop try to collect data whenever bees are flying and thus to collect as much data as possible. If you have the possibility to collect data under conditions, which are more suitable, you can neglect those data, but this should be decided prior to analyses. This method might make data collection more time-consuming but it maximizes the chance to get data, which can be analysed even under difficult conditions such as short flower periods.

Recording of temperature and cloud cover (all EBA partners)

Please measure and record air temperature as precisely as possible (at least one decimal point, two decimal points if possible) in the shade (no direct sunline exposure, could be the shade of yourself), c. 1.5 m above ground, before starting a transect walk for bee sampling using a precise high-quality hand thermometer (or a portable weather station, if available). If possible, also record temperature at 1.5m height additionally in a not shaded (i.e. sun-exposed) condition using a second hand thermometer (it is generally better to use one thermometer for shade and the other for sun-exposed situations, because it takes otherwise potentially quite long time until the thermometer provides a constant, correct temperature measure. As an example of such a thermometer see e.g.: https://www.testo.com/de-CH/testo-175-h1/p/0572-1754?gclid=Cj0KCQjwyN-DBhCDARIsAFOELTmNP6OWVUsHLdRu7TklwkbthrWetYqEcEY6rpFTjGck99ElNLpCAc8aAvrqEALw_wcB#. But any other similar product can be used of course.. Please note to each record of temperature whether it was the one under shaded or the one under unshaded conditions at the beginning of each transect walk. Additionally, please record cloud cover as in % before starting with the bee sampling transect (rough estimates sufficient). For both measures please also record daytime (5min precision, e.g. 10.25, 14.15 etc.; data regarding site ID etc. should anyway already be recorded on recording sheet). Ideally also record whether 1) no wind or 2) very low wind speed (c. <2m/sec) or 3) windy (c. >2m/sec) before starting a transect walk. (If possible, measure wind speed using a hand anemometer, but this is optional and not part of the basic protocol).

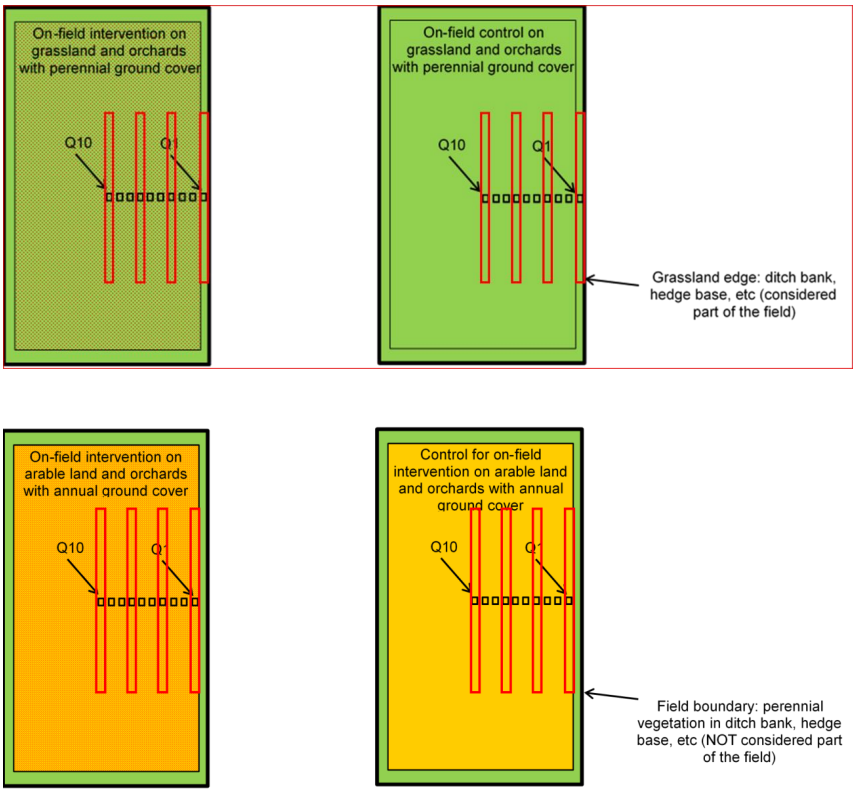

Figure 3a. Rough schematic of sampling design of on-field interventions:

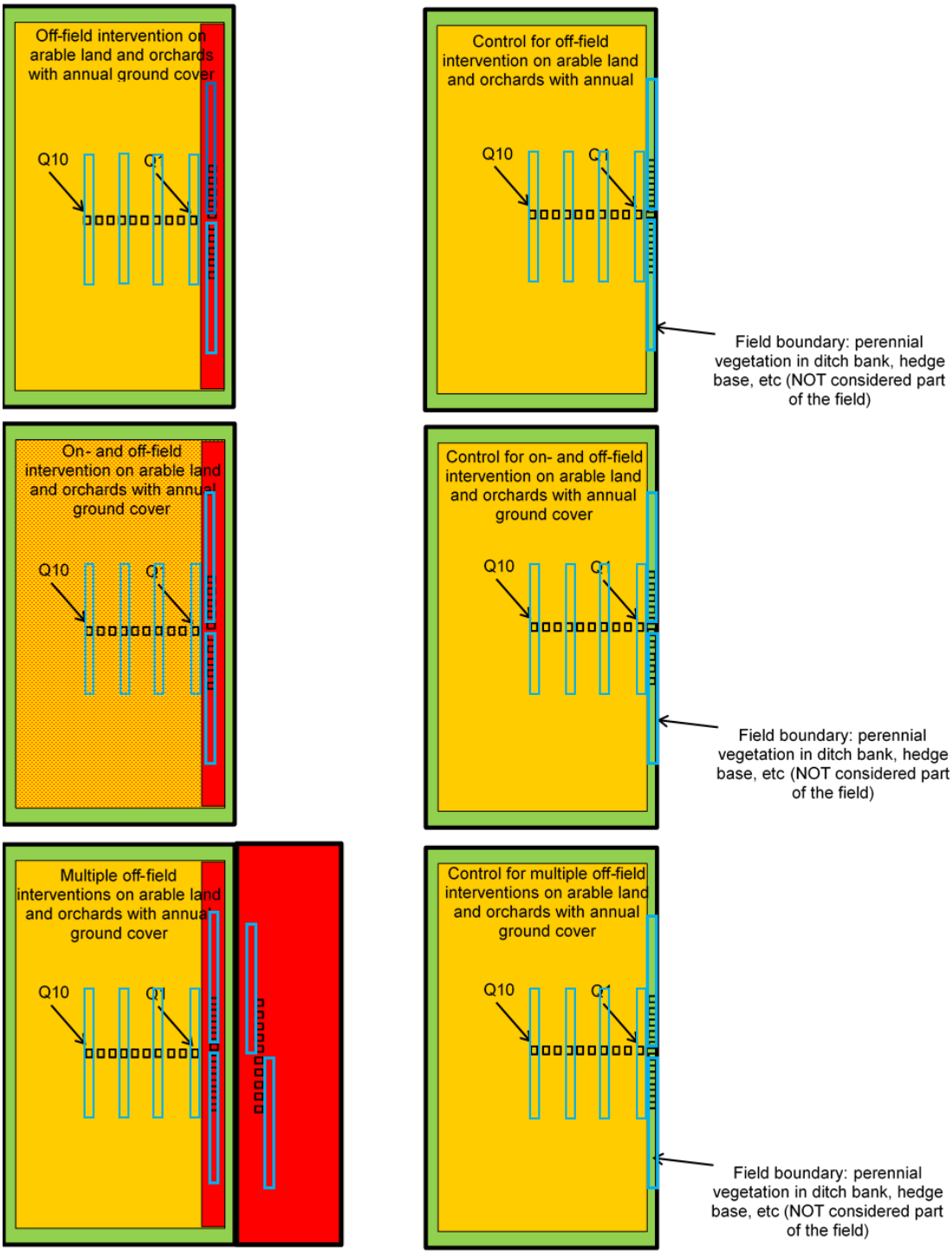

Figure 3b. Examples of schematics for sampling designs of examples of off-field interventions and multiple interventions.

3. Optional additions to bee sampling

Additional species to be surveyed

Butterflies (to species) and hoverflies too (simple counts only or to species) can also be easily surveyed using exactly this technique with little additional time needed or

training (Note that for species level ID for hoverflies requires additional training/expertise). For example, one can first survey bees along the transect when walking in one direction, and then survey butterflies along the same transect along the way back. A butterfly sampling scheme is available in section 6.

Additional sampling during "sub-optimal" weather conditions

Main goal

Determining climatic niches of bees/ crop pollinators.
Focus: temperature and cloud cover. This can be done when sampling bees during the basic general sampling of bees as outlined in the protocol above. For task 3.4, additional sampling of bees during extreme and potentially "sub-optimal" climatic conditions (low/high temperature, cloudy weather/ high cloud cover) is required. However, additional sampling should not be performed during rain/snowfall, or during very windy conditions.

Protocol

The methods of bee sampling are exactly the same (same protocol, same transects etc) as outlined in the basic protocol above. The only difference is some additional sampling of bees under sub-optimal weather conditions (in additional to sampling under "ideal" or almost ideal conditions as described above in the protocol for task 3.2. However, this additional sampling should only involve a limited amount of work (see below) so that it should be possible to integrate this additional sampling in the field work schedule. The good thing is that it targets mainly weather situations during which some other samplings are not advised normally. Ideal for the additional sampling for this task are for example:
Temperatures are lower than c. 13°C considered for "normal" pollinator sampling under optimal conditions (i.e., 5-12°C; no need to sample if temperatures are below 5°C) during the sampling period between 10.00 hours and 17.00 hours (not necessary during this entire period, on the temperatures during a particular sampling event is relevant).
Temperatures are exceptionally high (i.e. >30°C in temperate/northern regions, or even higher temperatures in Mediterranean regions) e.g. during a heat wave) during the sampling period between 10.00 hours and 17.00 hours (not necessary during this entire period, on the temperatures during a particular sampling event is relevant).
Cloudy days (high percentage of cloud cover during the sampling period between 10.00 hours and 17.00 hours (not necessary during this entire period, on the temperatures during a particular sampling event is relevant). However, sampling should not be done during rainfall/snowfall or at very high wind speeds (see above).

Work load, number of sites and sampling events

The more sampling and data collection under such "sub-optimal" conditions the better! As a minimum at least one additional sampling event under "sub-optimal conditions of each of 6 crop fields with interventions and 6 crop fields without interventions. Ideally all sites (crop fields) will be sampled at least once under sub-optimal conditions, but this not a must. If more than one additional sampling round could be done under sub-optimal conditions, this would be extremely valuable, but is not a must.

Additional sampling: Estimation of flower cover

In each vegetation survey plot assess flower cover and floral species richness. Perform vegetation survey and assessment of flower cover and floral species richness subsequently to, rather prior to the pollinator sampling protocol in order not to disturb and chase away bees during these plant surveys. Flower cover estimates should ideally be done on the same day after the bee sampling of a transect. However, if there is a high number of flowering species which are difficult to identify, it might be worth to record them in the morning or in the evening when it is too cold for conducting the bee survey. This will result in additional driving if you cannot do the bee sampling subsequently, but it will save time for the bee survey during the valuable warm hours of the day. Flowering plant species that cannot be quickly identified in the field could also get a provisional code on the recording sheet in the field, collected, and identified later on in the lab.

For estimation of flower cover the STEP protocol will be used, which has proven feasible for many different vegetation types producing reliable results. Instead of assessing flower cover in many separate small plots, flower cover will be estimated for the entire belt transect area. This should from experience take just as much time but should result in more robust and representative data matching exactly with the area bees are sampled.

Flower cover will be estimated as the number of flower units of each flowering plant species within the belt during each sampling round (e.g. Pywell et al. 2006). Flower units (inflorescences) can be a single flower or, in the case of multi-flowered stems, umbels (e.g. *Daucus carota*), flower heads (e.g. *Trifolium pratense*), spikes (e.g. *Rhinanthus minor*) or capitula (e.g. *Centaurea jacea*). Considering the large survey area, it is sufficient to roughly estimate the number of flower units; thus there is no need to spend a very large amount of time individually count each flower unit in the belt transect area. But it may help to count flower units of certain subplot area and the extrapolate to the larger area, if a plant species is flowering more or less homogenously throughout the transect area. Please always record a single number of estimated flower units rather than recording classes: e.g. record an estimated 6 or 60 flower units, rather than recording classes of e.g. 1-9, 10-100 etc., even if you think your estimates may not be super precise. This number should be the number of flower units for the entire belt transect area.

Together with number of flower units estimated for each flowering plant species (without grasses, mosses, etc.) the flower unit type used to record it should also be recorded on the recording sheet, as well as the estimated area of the flower unit (you can also record the radius or diameter to later calculate the area). There is of course no need to measure all flower units present in the survey area individually. It is sufficient to always use the same mean flower unit area estimate of the flower unit of a species; this may be the average of 5 separately measured/estimated flower units of each flowering species.

Table 3 illustrates how this data will be used then to estimate flower cover:

Table 3. An illustration how the collected data will be used to estimate flower cover.

Year	Transect_Code	Round #	Date	Genus	Species	Estimated no. flower units	solitary / heads or umbels (s/h)	average flower number per head or umbel	Area flower unit (cm2)	Total flower area (cm2)
2018	CF10	1	16-5-2018	Alliaria	petiolata	45	h	5	0.64	144.00
2018	CF10	1	16-5-2018	Bryonia	dioica	800	s		1.44	1152.00
2018	CF10	1	16-5-2018	Geum	urbanum	2	h		1.27	2.55
2018	CF10	1	16-5-2018	Sambucus	nigra	11	h	177	0.28	550.51
2018	CF10	2	3-7-2018	Bryonia	dioica	312	s		1.44	449.28
2018	CF10	2	3-7-2018	Epilobium	hirsutum	1	s		4.91	4.91
2019	CF10	1	4-6-2019	Geranium	dissectum	6	s		0.20	1.18
2019	CF10	1	4-6-2019	Matricaria	chamomilla	45	s		2.41	108.24
2019	CF10	2	26-6-2019	Matricaria	chamomilla	50	s		2.41	120.26
2020	CF10	1	26-5-2020	Bellis	perennis	5	s		3.14	15.70
2020	CF10	2	18-6-2020	Capsella	bursa-pastoris	2	h	5	0.05	0.51
2020	CF10	2	18-6-2020	Geranium	dissectum	8	s		0.20	1.57
2020	CF10	2	18-6-2020	Matricaria	chamomilla	108	s		2.41	259.77
2020	CF10	2	18-6-2020	Matricaria	discoidea	20	s		0.33	6.64
2020	CF10	2	18-6-2020	Taraxacum	officinale	2	s		15.90	31.81
2019	S3	1	5-6-2019	Lotus	corniculatus	25	h	4	0.73	72.75
2019	S3	1	5-6-2019	Medicago	lupulina	430	h	15	0.10	645.00
2019	S3	1	5-6-2019	Pilosella	aurantiaca	11	s		3.14	34.56
2019	S3	1	5-6-2019	Ranunculus	acris	322	s		4.91	1580.61
2019	S3	1	5-6-2019	Ranunculus	repens	288	s		4.91	1414.08
2019	S3	1	5-6-2019	Sisymbrium	officinale	10	h	3	0.07	2.10
2019	S3	1	5-6-2019	Trifolium	pratense	11	h	20	0.30	66.00
2019	S3	1	5-6-2019	Trifolium	repens	17	h	20	0.30	102.00

In Table 3 only three types of flower units where used: single flowers (solitary), umbels (e.g. Apiaceae) and flower heads (e.g. Asteraceae). You may record additional types of flower units (e.g. spikes). In such type of flower units for which flower do not form a clear horizontal area as flower heads or umbels, a good approach would to first clearly define the flower unit and what belongs to one unit and what not (not always straightforward) and then to count/estimate the number of single flowers making up such a flower unit for the species of 5 different flower units to calculate the average. It is crucial to clearly define and record the type of flower unit used for a species. For example for Apiaceae building “composite” umbels (umbels consisting again of several smaller umbels) it is crucial to clearly define what was used as umbel for the recordings (e.g. the smallest umbel unit of the species). For many species in many European regions such information about number of flowers and/or area is available in plant trait databases. However, if anyhow possible, this data should be collected as specified above, especially if is not clear whether such information is available in a certain trait database.

For shrubby/woody vegetation (e.g. hedgerows), in which the main “flowering horizon”

has mainly a vertical dimension, exactly the same methodology as describe above can be used to estimate flower cover. The only difference is that the 1.5m width of the belt transect represents a vertical dimension (e.g. in the case of hedgerows, flower cover of shrubs and potentially herbaceous plants at the lower part of the belt transect and flowers of trees probably in upper part of the 1.5m wide belt transect will be estimated in the entire area of the transect, analogue to the sampling of bees).

4. Vegetation Surveys

Author/contributor: D. Kleijn

Background

The objective of doing vegetation surveys in Showcase Task 3.2 is to be able to estimate the effect on plant diversity and cover of the biodiversity interventions that are examined in the 10 EBAs across Europe. Plant diversity is one of three biodiversity indicators that will be collected using exactly the same protocols in all EBAs to allow for integrated analyses using raw data. The different EBAs operate in different agro-ecosystems and are located in different climatic zones. Furthermore, interventions can be implemented on-field and off-field. This protocol is therefore designed to produce meaningful results under all these conditions. At the same time, the vegetation survey protocol shouldn't be too time-consuming since additional biodiversity, ecosystem service, agronomic and economic variables have to be collected in each EBA. The current protocol represents a good balance between reliability and robustness on one hand and efficiency on the other hand.

Protocol

Survey methodology.

We will characterize plant diversity and cover using at least 10 quadrats per site, where a site represents a field or an intervention. In arable fields these quadrats measure 1x1 m. Because plant density is much higher in grasslands or other types of perennial vegetation, here we use quadrats of 50x50 cm (0.25 m^2). Plant diversity will be surveyed once per year only, at a point in time that allows for reliable estimates of plant diversity. This best time for doing vegetation surveys may differ between EBAs. In each quadrat all vascular plant species will be recorded using a nationally accepted identification guide. The cover of each species will be estimated visually in percentage. Although visual cover estimates are less accurate than, for example, more objective point count methods they are much faster and we can therefore survey more quadrats in the same time. For the purpose of this study sample size trumps accuracy. Cover of bare soil/moss/rock will be an additional category for which the percentage cover will also be recorded. All species that occur in negligible cover will be scored with 0.01% cover. All estimated percentages per quadrat should add up to approximately 100% (so no multiple vegetation layers).

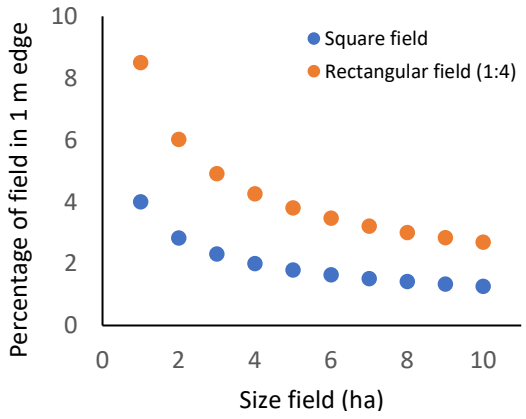

Figure 4. The relationship between field size and percentage of the field surface that is occupied by the outer 1 meter of a square and a rectangular field.

Sampling design of on-field interventions and controls

In the more intensively managed fields, plant diversity is mostly restricted to the field edge but field edges make up a relatively small proportion of a field (typically much less than 10%; Fig. 4). To sample plant diversity proportionally, we will position the first quadrat in the outer meter of the field and the last quadrat in the centre of the field. The

eight other quadrats will be distributed proportionally along a transect between these extremes. Fig. 5a gives a graphical representation of the sampling design. Quadrats will always be numbered from edge (quadrat 1) to centre (quadrat 10). Quadrat 1 will start 10 cm from the border of the field (e.g. the uncultivated boundary, ditch bank, base of hedgerow, etc). The transect will be located at the centre of a long side of the field. If there are off-field interventions, the transect will be located next to the interventions in the middle of the transect of quadrats characterizing plant diversity in the off-field interventions (see below).

Sampling design of off-field interventions and controls

In addition to the field next to off-field interventions, that will be surveyed similarly as the on-field interventions using a transect perpendicular to the crop edge of one of the long sides of the field, plant diversity needs to be also surveyed in the off-field interventions and off-field controls. This will allow us to assess their contribution to biodiversity conservation and to be able to relate intervention quality to ecosystem service providers or provision in the neighbouring crop field. Off-field interventions consisting of predominantly annual plant communities (e.g. annual wildflower strips) will be surveyed using 10 1x1 m quadrats. Perennial off-field interventions will be surveyed using 10 50x50 cm quadrats. Quadrats will be located in a transect parallel to and at 1 m distance from the crop edge. Within the intervention transect, quadrats will be spaced 5 m apart, covering a total distance of 45 m. Plant diversity in off-field interventions will have to be compared to plant diversity in suitable control habitats. What represents a suitable control is context dependent and has to be carefully decided and justified in each EBA. For example, a perennial wildflower strip intervention can be compared with a pre-existing field boundary. A hedge intervention can be compared with a herbaceous field boundary because without the intervention the hedge might have been not planted or removed.

Maps

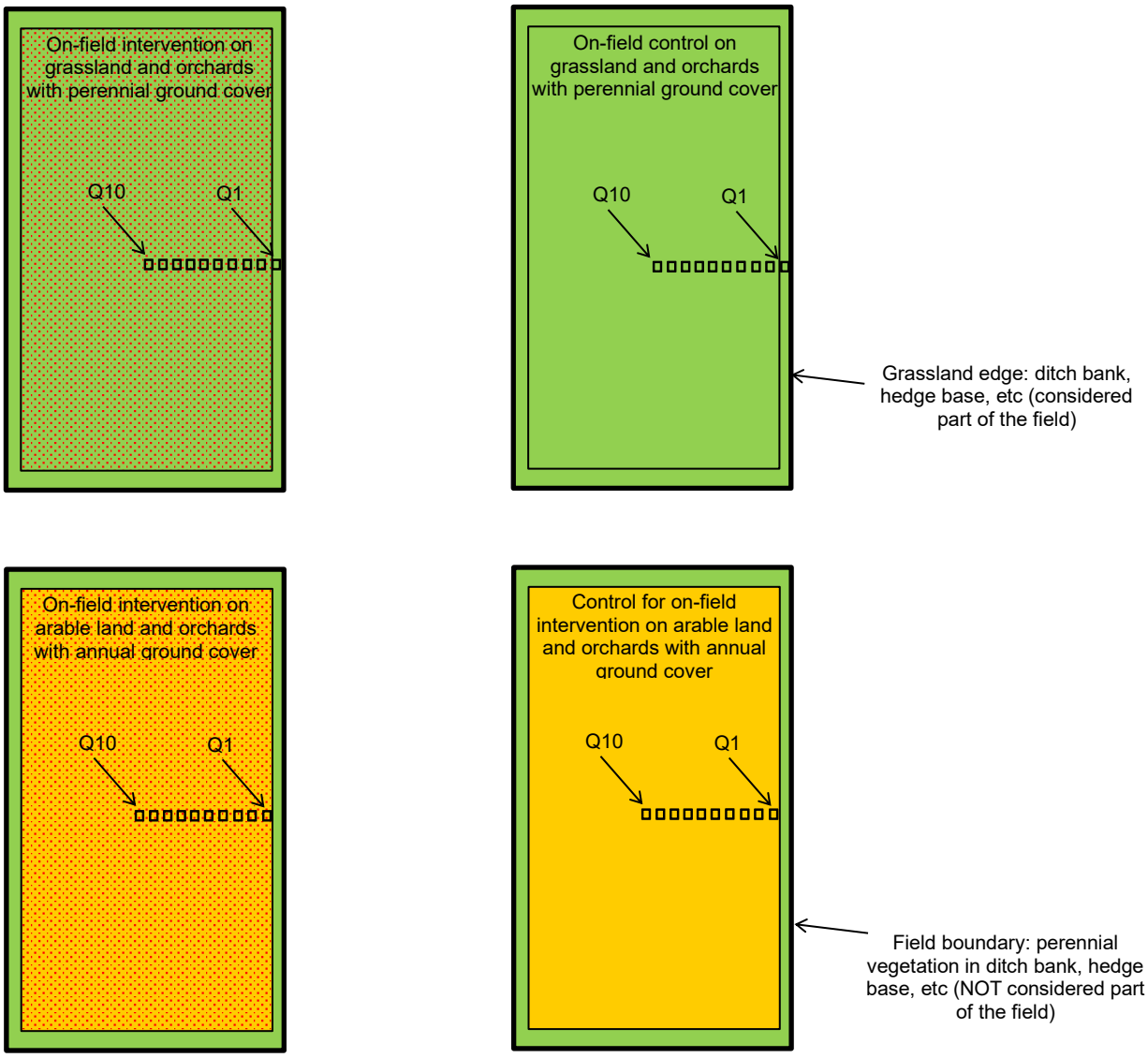

Figure 5a. Rough schematic of sampling design of on-field interventions:

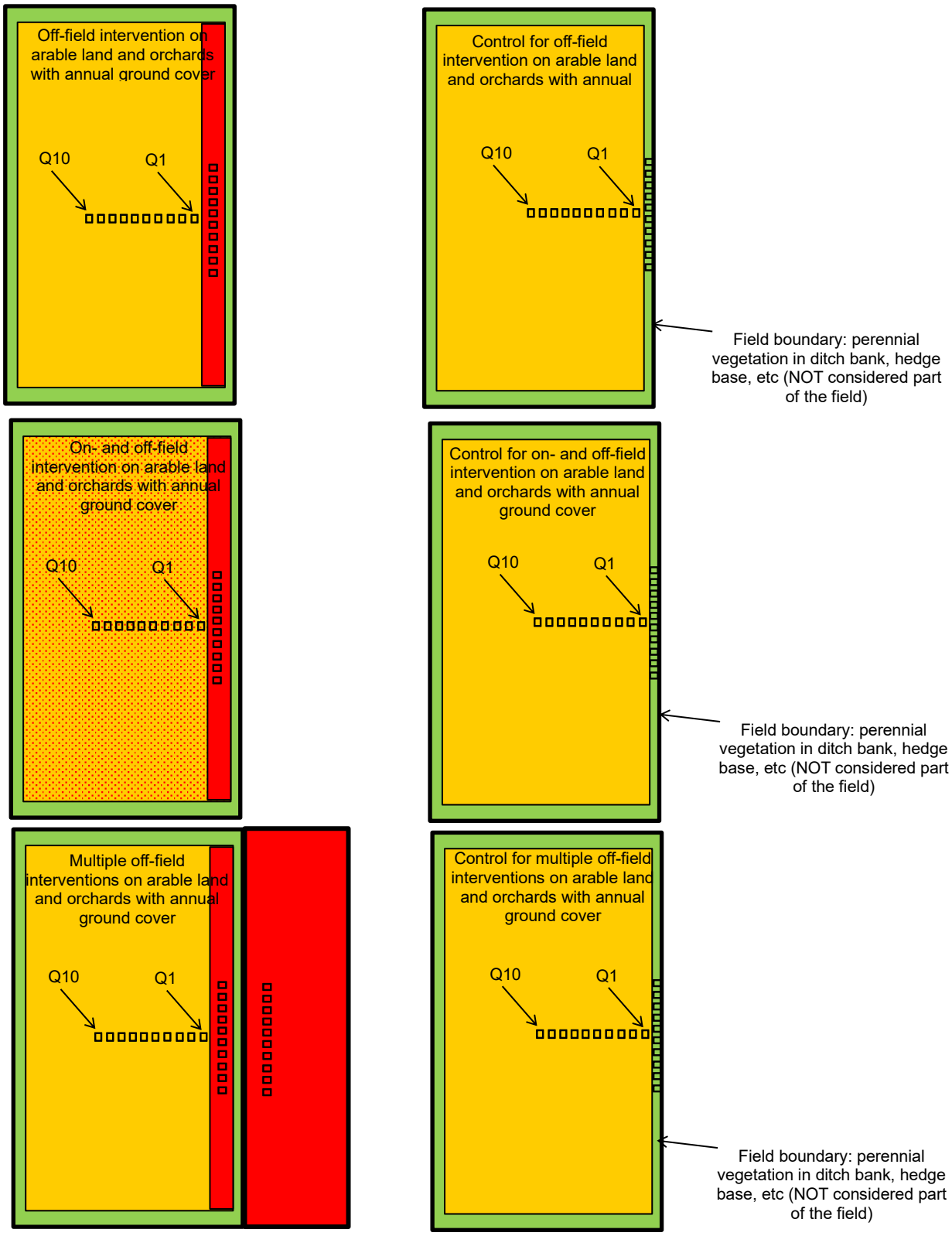

Figure 5b. Examples of schematics for sampling designs of examples of off-field interventions and multiple interventions

5. Spider Surveys

Author/contributor: Felix Herzog, Philippe Jeanneret, et al. (Adapted from: Dennis P., Bogers M.M.B., Bunce R.G.H., Herzog F., Jeanneret P. (2012) Biodiversity in organic and low-input farming systems. Handbook for recording key indicators. Wageningen, Alterra-Report 2308. 92 pp. www.biobio-indicator.org)

Background

Spiders are widespread, abundant and form a species-rich taxon of predators which have been intensively investigated in agro-ecosystems because of their potential role in the control of agricultural pests. In agricultural fields, responses of farmland spiders to agricultural practices and management intensity are well known and documented. A full review of the characteristics of spiders that makes them a suitable candidate biodiversity indicator is given in Dennis *et al*. (2009).
The method is adapted from Schmidt et al. (2005) and Schmidt-Entling and Dobeli (2009).
On each of two sampling dates, a suction sample composed of three sub-samples is taken in each bee transect: One in the center, the other two on either side at about 25 m distance. Each of the three suction sub-samples is taken within a sample ring of 0.357 m internal diameter pre-placed on the target vegetation (each sample has a suction area of 0.1 $m^2 = \pi \times [0.357/2]^2$, total area per plot = 3 x 0.1 = 0.3 m^2). The sample ring is 40 cm high[1]. The suction nozzle is placed down firmly over the low vegetation, so as to sample from both the low vegetation and litter layers as far as possible for a total duration of 30 seconds. In hay meadows, samples are not taken shortly after mowing but when the vegetation height is > 15 cm or less if the aftermath is grazed. In crop fields, the first survey is made when plants are already visibleable 2). No samples are taken from bushes (edges) nor trees (orchards). When a sample (consisting of the three pooled sub-samples) is completed, the material is transferred into a pre-labelled polyethylene zip-seal bag and stored in a cool-box. Spiders are sampled on two occasions.

Protocol: sampling method

Suction trap

Spiders are caught with a modified vacuum shredder e.g. Stihl SH 86-D, Andreas Stihl AG & Co. KG or a local equivalent. ('Vortis' insect suction samplers can also be used http://burkard.co.uk/product/vortis-insect-suction-sampler/ but pre-tests in the Netherlands showed that these yield a bit fewer spiders when used in grassland).

Sampling location within the fields

Suction sampling aligns to the bee transects. In each bee transect, three suction samples are taken, one in the center, one about 25m to the left and one about 25 m to the right. The three suction samples per transect can be pooled directly in the field and will be analyzed together.

[1] The ring can be made of a sheet of flexible plastic rolled. The length of the plastic sheet is then 1.222 m (0.4 m high) with 0.1 m overlap area to fix both ends of the plastic sheet together with A double row of pop rivets to produce the circle (the effective circumference of the circle is 1.122). Two sheets of aluminium of 0.1 x 0.4 m may be required to sandwich the overlap and to support the rivets.

Sampling procedure

The sampling unit for comparison is the same as the transects for the bee sampling. It consists of the three pooled suction samples (or sub-samples) per transect. The ground area sampled by each sub-sample is 0.1 m^2 and material is collected with the Vortis' insect suction sampler for 30 seconds duration. The three suction sub-samples are pooled to accumulate a single sample unit of total area 0.3 m^2. The material of each sample is transferred to a zip-seal polyethylene bag of 43 cm length x 27 cm width by inverting the gauze bag into it after switching off the leaf blower engine.

Timing

Two surveys, one early summer and one late summer.
The ambition is to complete the sampling of all areal and linear habitat/field plots of the EBA within 10 days for each of the two sampling periods.

Protocol: materials and methods

Permanent habitats

Sampling 1: spring; the first sampling period starts two weeks after 90% of *Taraxacum officinalis* flowers are in bloom[2] (Table 3; or a similar species where it does not occur, e.g. in Spain).
Sampling 2: late summer.

Non-permanent habitats

Special sampling periods take place for crops due to non-permanent vegetation occurrence. This should ensure that plants are already visible by the first survey:
Cereals and rape ('early' crops): Sampling 1, like other habitat/field plots.
Beet, potato and corn ('late' crops): Sampling 1, 6 weeks after 90% of *Taraxacum officinalis* flowers are in bloom.
Sampling is carried out during dry, warm weather. To avoid effect of seasonal succession of spider species to occur during one sampling date in a region, spiders should be caught within 10 days in all transects.
Suction sampling provides abundance data for spiders, but individuals in soil crevices or dense layers of vegetation or litter may be undersampled (Topping and Sunderland 1994). However, as the highest spider abundances will probably be observed in habitats with dense vegetation and litter, the results and conclusions could only be weakened by resulting bias (Schmidt and Tscharntke 2005).

***Table 3**. Timetable for 2 sampling periods of spiders in different habitats. Note that dates are given as an example, and can be changed. Note that the starting date is Taraxacum officinalis bloom.*

Week	0=90% T. officinalis in bloom	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Permanent habitats			1																		2
Cereals			1								2										
Rape			1								2										
Beet							1														2
Potato							1														2
Corn							1														2

[2] In the Swiss lowlands (500 m elevation), it corresponds to a period between 15th and 30th April.

Laboratory processing of samples

Back in the lab, the samples per transect are kept separately all along the process of sorting the spiders out from the zip-seal bags. Adult and juvenile spiders are sorted out from the material that has been collected with the suction engine (plant material, sand, soil, etc.) and put in vials with 70% alcohol. A penciled label with sample details can be added to the solution and the same information should be added to an external adhesive label. Taxonomy: Adult spiders are identified to the species level. Juvenile spiders are not identified but counted and recorded as “juveniles”.

Format of data records

The field protocol of the suction sampling in form of an Excel sheet contains the following fields: habitat/field code, plot/transect code, where appropriate off-field intervention code, observer’s name, date, time of start of the first suction sub-sample (one record per plot), vegetation height and percentage cloud cover for that date, prevailing Beaufort wind code, Celsius temperature recorded.

Practical hints

Training

All technicians and scientific staff, preferably spend one day sampling spiders according to the protocol in different type of habitats/groundcover conditions before doing the actual field work.

Working with two teams may be necessary to keep withing the sampling interval.

Avoid systematic sampling by field staff. Samples to take were randomly attributed to field staffs so that no systematic error could occur, i.e. a field staff took samples of various habitats and farms.

The spiders were sorted after each sucking up in the field, pouring all the material from one sucking into a big plastic box (more or less 50x40 cm and 35 cm deep) and the living so moving spiders were very easy to see. 2 or 3 persons were used to sample: one sucking up the material, one or two sorting the material (a plastic box per sorting worker).

Maps

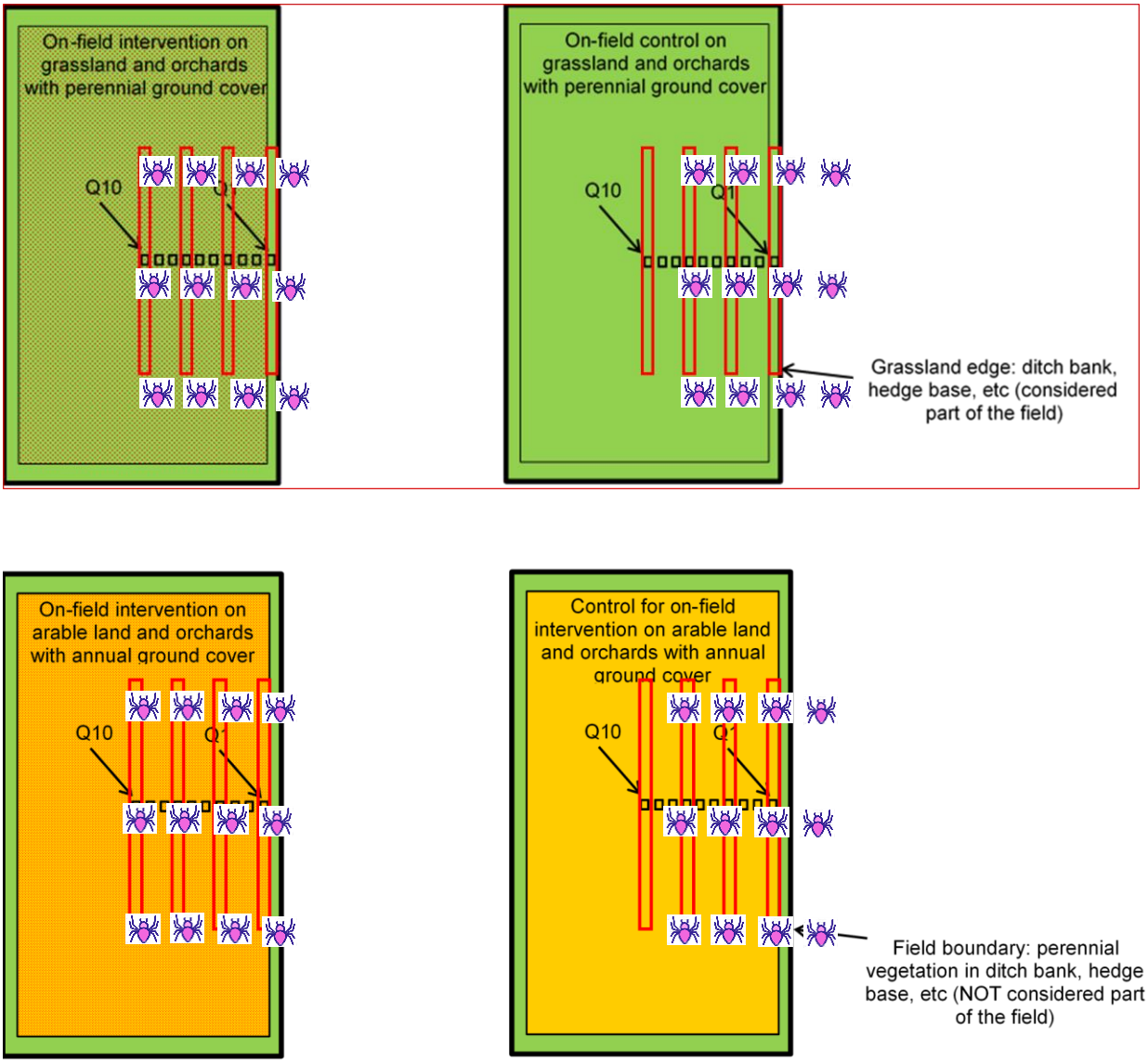

Figure 6a. Rough schematic of sampling design of on-field interventions. Spider suction sampling aligns to the bee transects. In each bee transect, three suction samples are taken, one in the center, one about 25m to the left and one about 25 m to the right. The three suction samples per transect can be pooled directly in the field and will be analyzed together.

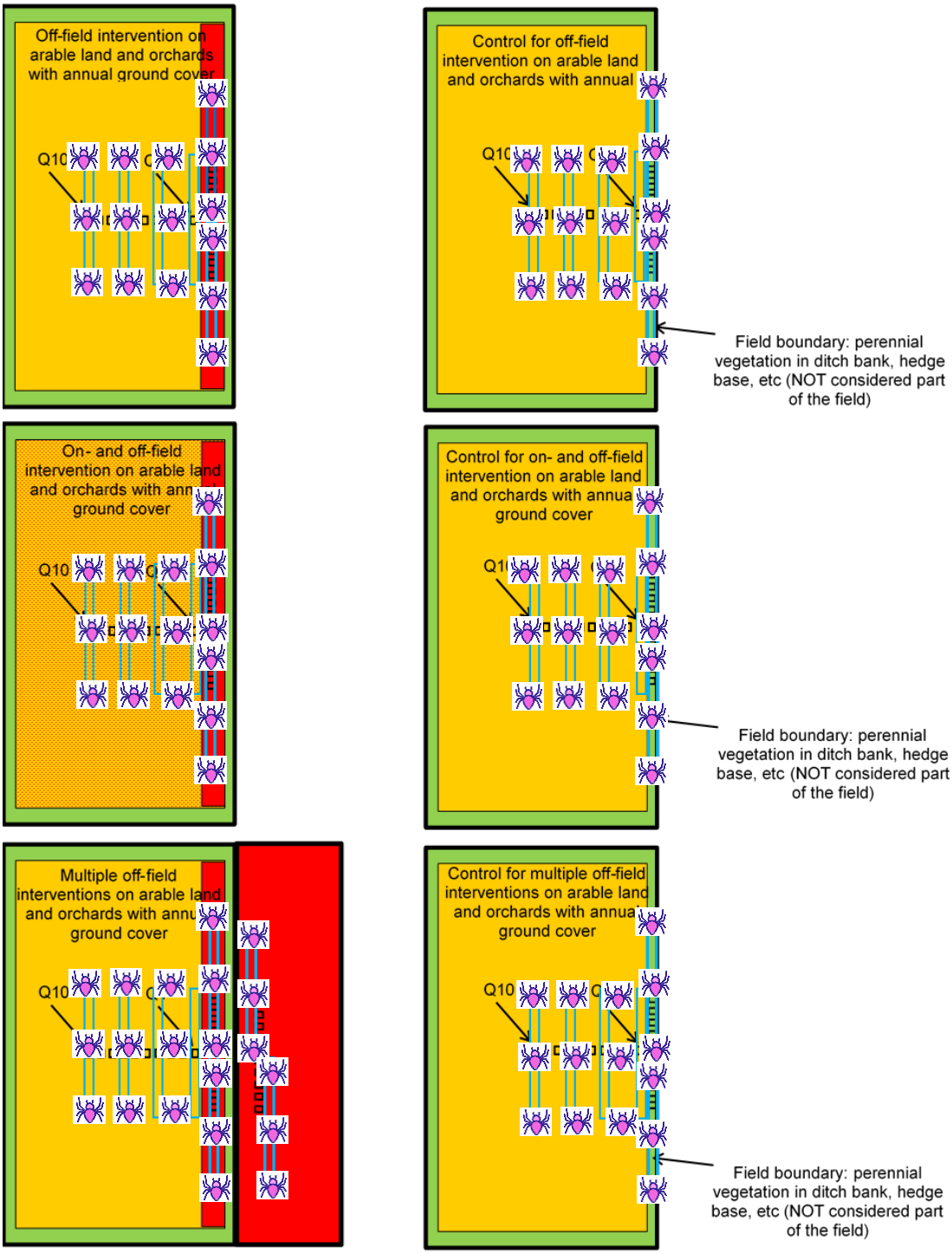

Figure 6b. Examples of schematics for sampling designs of examples of off-field interventions and multiple interventions.

Other protocols for measuring biodiversity (optional)

6. Ground dwelling arthropods (carabid beetles)

Author/contributor: V. Bretagnolle

The activity-density of ground dwelling arthropods will be assessed using pitfall traps. Pitfall traps are particularly relevant to survey ground-dwelling arthropods such as carabid beetles. This survey primarily targets the carabid beetle community, but can eventually broadened to all invertebrates captured, including spiders. It can also catch many pest species. Four pitfall traps are placed: two traps set in the margin (first crop row) and two others at 10 and 25m from the edge. Pitfall traps consist of plastic cups (8.5 cm in diameter and 7 cm deep), buried at the ground level, and filled with a solution to improve insect preservation. Traps are filled with ten drops of odorless soap and 10g of salt per liter of water. Traps should have a cover to prevent flooding from rain, and they should not be left out during periods of heavy rain for the same reason. Pitfall traps are left in place for four effective days (96h). Arthropods are then stored in 70% ethanol and later identified to species level.
In relation to timing of trapping, carabid beetles are present all seasons from spring up to fall (and even in winter in temperate regions, though less active). Trapping is recommended in spring (e.g., april to july), when crops are growing. Avoid trapping after harvest, since beetles disperse. In southern regions, trapping may start in March.

7. Bird counts

Author/contributor: V. Bretagnolle

Passerine populations are surveyed with point counts. Observer is at the center of a circle, 200m radius. Observation radius is restricted to 200m from the observer to reduce any bias in detectability and avoid overlap of observations between two neighbouring points (ie, neighbour points must be 500m from each other at least). All birds observed, as well as their behavior (singing/perching/flying/on ground) are recorded on a field map. Counts lasts 10 min per sampling point. Counts are performed in the morning (typically from 7:00 to 11:00 am), not too early in order to allow skylark displaying, twice in the breeding season (e.g. April and May). The 10 minutes are split into 5 x 2-minute sessions, in order to increase the detection probability. Points should be randomly resampled. Ideally, counts should be replicated twice in the breeding season, the first for early breeders (resident species and early migrants), i.e. March in Spain and Portugal, April in France, the UK and Central Europe, May in Sweden. And a second count should be performed for late migrants, typically one month later. Contact V Bretagnolle for further details.

8. Bats

Author/contributor: José M. Herrera, Sílvia Barreiro et al.

Bat activity will be assessed by acoustic methods using bat detectors equipped with microphones with sensitivity range from 10 kHz and 150 kHz, covering the complete range of species-specific echolocation calls from the regional bat assemblage (e.g. D500x, Pettersson Elektronik AB; AudioMoth, Open Acoustic Devices; SM4, Wildlife Acoustics). All sites will be monitored using the same bat detector model and settings (Bat detector with trigger functionality: auto-recording mode for 3 seconds without pre-trigger, digitized at least at 250 kHz; Bat detectors without trigger: 3 s recordings with an interval of 3s between recordings, digitized at least at 250 kHz).

Bat detector will be placed in the middle of field margin (and corresponding location of paired control site) and set at 1.5 m above the ground, with the microphone facing upwards at a 45º angle, in a parallel position and at a distance of at least 1 m from perennial vegetation (e.g. shrubs, trees).

Acoustic surveys will be carried during a relevant time period of the implemented interventions, simultaneously at each intervention and respective paired control site. Each site will be sampled once during 3 consecutive nights starting 30 minutes before sunset and ending 30 minutes after sunrise.

Recorded echolocation call sequences will then be used to determine the identity of occurring bat species and to contrast bat flight activity (number of bat passes: search-phase echolocation sequences of at least 3 consecutive echolocation call pulses of one individual bat) between intervention and control sites. Thus, recorded echolocation calls sequences will be identified to the lowest taxonomic level possible. When the identification of bat calls is not possible at the species level, they will be assigned to single or multi-genus complexes.

9. Butterflies

Author/contributor: Erik Ockinger, Michiel Wallis De Vries

Butterflies, in common with some other groups of insects, have declined in many places in recent decades. We need their monitoring to understand the causes of the decline and to support conservation measures. One of the best networks to get useful information and important results of butterfly monitoring is with citizen science. Thanks to thousands of volunteers we know the status of butterfly species and see where is necessary to apply conservation actions. Butterflies are frequently used as indicator species in different contexts. Most species are easy to identify in the field, they are charismatic and hence attractive to the general public and land owners, and the diversity of butterflies often correlate with the diversity of other insect groups.

Butterflies can be sampled using a similar design as the bee transects (see section 1.4 and 1.5 for in-field and off-field interventions, respectively), i.e. four transects of 50 m length per field, but with modified transect belt width and sampling effort. The principles for the placement of the butterfly transects should identical to what is described for bees. However, the sideways distance between transects should be least 5 m (and ideally transects should be at least 10 m apart). If this is not possible, transects can instead be made longer, i.e. 100 or 200 m, but still maintaining the same total transect length per site.

Transect walks

The observer walks slowly along the transect, at a constant speed, approximately 2 minutes for a 50 m long transect. N.B. that this is faster than the bee transects, but this

refers only to the effective walking time. The time for identifying and recoding observed butterflies is added to the walking time. The observer records all butterflies observed within an imaginary "box" of 2.5 m to each side (N.B that this is wider than for the bee transects) and 5 m in front of the observer. In the case of narrow linear off-field interventions, for example narrow flower strips, the width of the transect can instead be equal to the width of the flower strip. If so, measure and record the width of each transect.
Some butterflies can be identified immediately without catching them, while others need to be caught be a hand-held butterfly net. A few species can be difficult to identify in the field, and can either be photographed, or caught and put in a small box which is placed in a transportable cooling box for later identification. If photographed, make sure to get photos of both sides of the wings of each individual. Stop the transect time for any handling of butterflies or making records, and do not count any additional butterflies while stationary. Record the number of observed individuals of each species, separately for each 50 m transect.
If it is impossible to identify a butterfly individual to the species level, for example a butterfly that is flying fast across the transect and cannot be caught with the net, identify it as close as possible (e.g. genus, family or other suitable grouping) and record as for example "*Pieris sp.*"

Sampling rounds and weather conditions

Each site (both, sites with and without interventions) should be sampled during at least four sampling rounds. As for bees, the timing of the sampling rounds should be chosen according to relevant time period of the focal crop and implemented intervention, as well as the flight periods of all relevant butterfly species in the study region.
Butterfly sampling should generally be carried out from 10.00h – 17.00h, ideally during sunny weather, or when weather conforms to the following standards, respectively: temperature above 17°C with at least 60% clear sky and above 20°C in any sky conditions, but never during rain. The wind velocity should ideally be low (< 2.5 m/s; Pollard & Yates 1993). These conditions may have to be locally adapted depending on climatic region of the EBA, and described for bees in section 1.7.
Make sure that sites (e.g. fields) sampled in the morning in first round will be re-sampled in the afternoon in the second round and vice versa because activity of bees might change during the day. Should this not be possible logistically, ensure that sampling daytime is randomized across sites.

Butterflies monitoring under citizen science data (**eBMS)**

The following text is from European Butterfly Monitoring Scheme (https://butterfly-monitoring.net/bms-methods). In eBMS, all the data collected involved a monitoring effort that provides incredible information for the science (species abundance).
The basic and more important methodology of eBMS is the transect counts where we get the most robust information on the butterfly population. All the Butterfly Monitoring Schemes are made by many different transects in their country. However, due to the difficulty of applying transects in some countries and remote areas, a new methodology 15-min Counts was created to reinforce the monitored data and give more flexibility in its collection. With 15-min Counts is expected to increase the butterfly monitored data in Europe, but a BMS should always have as a basis an important number of transects.

Transect Counts - Pollard Walks

A transect is a fixed route (walk) established at a site where butterflies are recorded,

ideally weekly, over a number of years following some basic rules. The majority of transects are chosen by the walker and they decide which route to choose. Some schemes provide advice about areas to record to get even coverage of land cover/ habitat types as well as ensuring that a good range of the species present in a country is sufficiently monitored.
There are just a few rules to follow for doing a transect:

1. *Length?* Transects are typically about 1km long and divided into sections that correspond to different habitat areas, or are a fixed length (e.g. 50m).
2. *When to count?* Butterflies are counted when adults are present - e.g. during the flight period of butterflies. Depending on the country and the region, the flight period of butterflies will be longer or shorter (normally during spring and summer).
3. *Frequency?* At least 10 visits per year. The transect is ideally walked every week during the butterfly flight season. If it is not possible, count as often as possible, every two weeks or 10 days. Don't leave more than 3 weeks between one visit and another. If not possible to follow this regime, your visits can be concentrated in a few months (only in spring/summer) to cover the flight period of some species.
4. *How to count?* Count all butterfly adults present in an imaginary box of 2.5m to each side, 5m high and 5m ahead (see the image) while walking a constant pace.
5. *Which weather?* The visits should be done with good weather: sunny and warm, with no rain and not too windy. Count when butterflies are more active, in the central hours of the day, at least with 13°C if it is sunny or 17°C with cloudiness. See the manual for more explanation regarding the weather.

Download the Butterfly Transect Counts Manual for more explanations, it is available in several languages: Portuguese, Polish (a Manual adaptation for Poland), Spanish, Italian and Bulgarian

15-min Count

This method consists of counting butterflies in a specific area for a fixed amount of time, in eBMS we use 15 minutes. This more flexible methodology is used to obtain butterfly abundances and it can be used to get more information of rare species, butterflies with specific behaviours, or butterfly communities of remote places but it can be used anywhere, so also in agricultural landscapes, city parks, or gardens. We recommend this type of method for people with some knowledge of butterflies who like to visit different areas and habitats. A 15-minute count is a simple method that allows recording in many places:

- on a path, route
- in a given area: a meadow, park, a garden
- at a fixed point: sitting on a balcony or terrace

The method is simple, during 15 minutes you record all the butterfly species and individuals seen. The rules to follow are similar to the ones for transects: record butterflies inside of the imaginary box (5x5x5m), with good weather, sunny and warm, with no rain and not too windy. Visiting the same area several times with a certain frequency will provide us with better data, so try to do repeated visits to the same places.

This methodology is really useful for eBMS and Butterfly Monitoring Schemes, filling gaps in areas for some countries where transects have not been set up. Keeping the same time in areas with similar extensions will produce standardised results which help us to obtain butterfly abundances. A complete list of the butterflies seen during 15-min Counts will give absence information (non-detection) that is highly valuable for statistical analysis.
All the data submitted with 15-min counts can be downloaded via your account on the project website (www.butterfly-monitoring.net), you just need to go to the page My 15-min counts and you will find the routes, samples, and records.
Discover more information on how using the ButterflyCount app on the page: eBMS data -> eBMS - mobile application

Showcase Protocols for measuring Ecosystem Services

10. Pollination Services

Author/contributor : Matthias Albrecht, David Kleijn, et al.

Background

In addition to biodiversity indicators, indicators for ecosystem services and dis-services, yield and economic indicators will be measured in the Showcase EBAs across Europe (Task 3.2). The present document describes a common protocol for assessing pollination services in a subset of EBAs for which pollination services are relevant, which should allow for integrated overarching analysis using raw data. However, the different EBAs operate in different agro-ecosystems with different focal crop species including arable crops and horticulture. Furthermore, interventions can be implemented in-field and off-field. This protocol is therefore designed to produce meaningful results under all these conditions, crop species and types of interventions. The spatial design of the pollination protocol is designed to match with other protocols, e.g. the bee sampling and crop yield protocol to allow integrative data analysis.

General approach

The main goals are (i) to quantify impacts of interventions on the delivery of crop pollination services, and (ii) to assess to contribution of pollination services to agronomic yield (assessing consequences on crop yield quantity and quality) in focal fields with vs. without interventions. Typically, assessments of pollination services also include a bagging treatment (i.e. preventing flowers from access to pollinators through nets, but still ensuring wind pollination or autogamous selfing of flowers, see below). However, bagging has also disadvantages/issues: 1) it is time consuming; 2) it can change micro-climatic conditions or preventing also herbivores/ florivores, 3) it could affect wind pollination, it could harm plants mechanically. The Showcase protocol for addressing objective (i) aims to avoid bagging if possible, to save time and to avoid the above-mentioned issues. A bagging treatment could produce relevant additional information (1) animal dependence of the crop is highly variable across varieties or (2) if animal dependence of the crop is not or poorly known. In such cases it would be recommended to include a bagging treatment in addition to the open pollination treatment, but individual EBAs are free to make this decision.

Protocol: measuring crop pollination services (open pollination)

Measurements of pollination services should in principle be taken at roughly the same locations/ in-field distances as bees are sampled within the four belt transects per site (see bee sampling protocol). However, to avoid any potential influence of the transect walks on pollination success of arable crop plants, focal arable crop plants to measure pollination services should be chosen right adjacent, but not within, the belt transects to avoid that walking transects will affect pollination of focal plants. In orchards, however, trees of the same tree lines used for the transect walks for the bee sampling should be chosen. Pollination services will only be measured in insect-pollinated crop

fields (no measures of pollination in non-crop areas, such as off-field interventions). Within or right adjacent to the four belt transects (see above and bee sampling protocol) a total of at least 3 separate crop plants (e.g. individual trees in orchards) should be randomly chosen for each of the four belt transects, thus 12 plants in total per field (or orchard). Chosen focal plants should be marked (e.g. with light cable ties, coloured tape, ribbons etc.). For each focal plant, mark at least 2 randomly (or stratified randomly, see example below) chosen branches or inflorescences, e.g. for tree crops. For arable crops, however, it is strongly recommended that whole plants or branches are bagged and not just individual flowers as the scale of bagging can have big impact on yield/quality. Of course the number of focal plants per transect and/or number flowers/inflorescences per focal plant can be chosen to be considerably higher by EBA partners if adequate and feasible in your study system, the numbers given in this protocol just represent the minimum numbers. Chosen focal plants or branches (or flowers/inflorescences, if not possible to bag whole plant/ branches) should be marked (e.g. with light cable ties, coloured tape, ribbons etc.). It some case this may not be necessary (EBA partners will know if necessary or not for their study system). Marking should not attract or deter potential pollinators. Number of flowers should be counted if flower units (inflorescences, clusters of flowers) are marked rather than individual flowers (see example below). Of these flowers, the number of fruits and/or seeds will be recorded to be able to calculate fruit or seed set, depending on whether animal pollination can contribute to fruit set, seed set or both in the study crop species. Fruit set should be measured at all pollination and/or yield relevant time points, e.g. immediately after bloom (early fruit set, e.g. before the thinning treatment in fruit trees such as apple), at harvest (late fruit set) and potentially at further relevant points in time (optional see example below). Early fruit set measures may however not be necessary in all crop species and only measuring fruit set at harvest is required. However, in all crops where thinning is used or where it is important to quantify fruit abortion rate etc., it is important to additionally measure early fruit set. In addition to the quantitative measures of pollination services and pollination dependent yield components such as fruit and seed set, animal pollination depending relevant measures of yield quality must be measured. These measures may include: fruit size, fruit weight, seed weight, percentage malformed/deformed fruits, oil content/quality, sugar content etc. EBA partners have to measure all pollination dependent yield components that are of significant economic importance. Protocols for such measures tailored to the specific crop/system studied are the responsibility of EBA partners, which are strongly encouraged to share protocols with partner studying the same crop species or crop type (e.g. top fruits, berries, oilseed crops etc.)

As an example, for intensively managed orchards (e.g. apple, cherry, etc.), the following approach may be chosen, if adequate for the study system: of each randomly selected focal tree two branches are chosen (one in the “lower part 50cm-150cm”, one branch in the “upper part” (150-250cm); within the two “height classes”, selection of branches is random). Cluster of flowers are marked with coloured ribbons and all flowers of each marked cluster of flowers are counted. Open pollination treatment will consist of counting the number of set fruits (out of recorded number of initial flowers counted):

Once at the end of bloom (initial fruit set) (where relevant, see above)

Once after thinning / natural abortion period (optional)

Once before harvest (final fruit set)

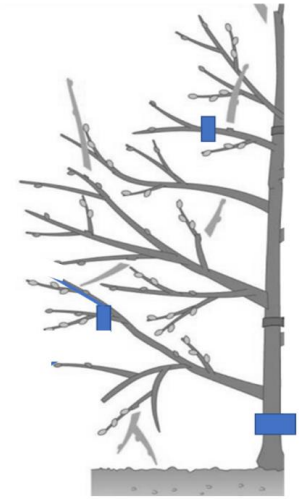

Fig. 7. Example of marking of two branches at different heights in intensive fruit orchards.

Bagging treatment (optional, see above)

Within each of the four belt transects at each site (see bee sampling protocol for design and spatial location of belt transects) at least two randomly selected plants will be marked for the bagging treatment. In orchards, the same trees should be used for measuring open pollination and bagging, as trees may differ quite a lot in size, vigour, number of flowers etc. In arable crops, however, different plants should be chosen to avoid that open pollination of the often relatively few flowers/inflorescences per plant may be affected by the bagging treatment, and to avoid potential resource allocation issues. Bags need to be installed before focal flowers have opened and need to be in place during the entire flowering period until focal flowers have withered. Bags should be removed as soon as possible after flowering. Focal flowers/inflorescences may need to be marked (EBA partners will decide whether this may be necessary and how this works best for the studied crop). Mesh size of bags to prevent pollinators from flower visitation must at least 1mm to avoid/reduce potential effects on wind pollination (e.g. Sacchi & Price 1988). Bags should be large enough to prevent flowers touching bags. They may need to be "stabilized" by fine wire or similar to avoid that bags collapse and flowers touch bags. However, bags should not be too heavy resulting in pulling flowers/inflorescences down, bending shoots to much or even damaging plants. Any EBA team who is not used these methods on their target crop before should set up a meeting with a group who is experienced in these methods.

Recording of flower visitation rate

To be able to better understand impacts of interventions on the delivery of pollination services and their contribution to crop yield we will measure flower visitation rates. Visitation rates will be measured for each of the marked focal plant or branch for measures of pollination services (see above). Strategic selection of marked plants for yield estimates will make it possible to observe multiple plants or branches at the same time. This approach has proven to work well and to yield robust results in terms of relationships with crop yield in pollinator attractive crops. However, should you expect low visitation rates by pollinators to single focal plants (even if multiple pants or branches are observed simultaneously, e.g. in oilseed rape), more flowering plants may be required to observe together and around the focal plants. This can be done using plots of adequate size (e.g. 1 x1m; or larger/smaller, depending on your focal crop/system) around the focal plants /flower units in the center of plots. EBA partners

should decide whether they can use the suggested focal plant approach, or whether additional plants within a plot around focal plants be required for their focal crop/system. In doubt, this issue should be discussed with EBA partners measuring pollination services. Always use the same approach/plot size for all observations in each field and record e.g. plot size). If the plot approach is used: plots should be as large as possible to achieve representative and robust data avoiding very low numbers or many zero visits per observation time, but still manageable in terms of keeping the overview of visits to ensure accurate data. Generally, data of too low visitation rates and many zeros are worse than not perfectly accurate measures under high visitation rates. Irrespective of whether the focal plant or plot approach is used, it is crucial to count/estimate the number of open (not yet withered) flowers per observation plot (or plant). Should it not be possible to record the number of single flowers, but rather only flower units (e.g. flower heads of Asteraceae), please record the number of flower units; count the number of single flowers building a flower unit of at least 10 flower units ideally from different plants and fields to estimate the mean number of flowers per flower unit; should there be large variation across field e.g. due different varieties, flowers of more flower units should be counted).
During a standardized amount of time (e.g. 10 min; but again depending on the crop species/system and expected flower visitation rates; however use the same time period for each observation and record it) the number of visits to flowers to focal plants (or flowers of plants within the plots, if the plot approach is used, respectively) by flower visitors per time period will be recorded, irrespective of whether visits are from the same flower visitor or different flower visitors. Visits should be recorded separately for different flower visitor taxa/groups. Taxa/groups should be identified to the highest taxonomic resolution possible in the field (e.g. *Apis mellifera*, *Bombus*, *Osmia cornuta*, *Osmia bicornis*, Halictidae, *Andrena* xy, *Andrena*, non-*Bombus* bee, Episyrphus balteatus, *Eristalis*, hoverfly, other fly, wasp, beetle, *Pieris rapae*; butterfly etc. These are only examples, of course use groups relevant for your system; include visits of all flower visitor taxa you observed to visit flowers of your focal crop). It is optional to collect flower visitors after having recorded their visits so that diversity of flower visitors can be estimated. If you decide to do this take utmost care not to damage the flowers.
Flower visitation rates measurements should be performed at least twice during the crop flowering period during the major flowering period of the focal crop, avoiding times when only a relatively small proportion of crop plants are flowering. Flower visitation rate measurements should be conducted during the same day time periods and weather conditions as specified in the bee sampling protocol. Field that were visited in the morning in the first sampling round should be visited in the afternoon in the second sampling round and vice versa. Record for each flower visitation observation: date, time of day, temperature and cloud cover (exactly as specified in the bee sampling protocol), field ID, transect ID, focal plant ID (or plot ID if plot approach used, respectively), number of open flowers (or flower units, see above) per observed plot or plant.

Additional variables to measure/ record:

Crop variety
Crop flowering period (start, peak, end)
Further important variables will be available from the bees sampling protocol: e.g. densities of managed honeybees and wild bees etc.

Maps

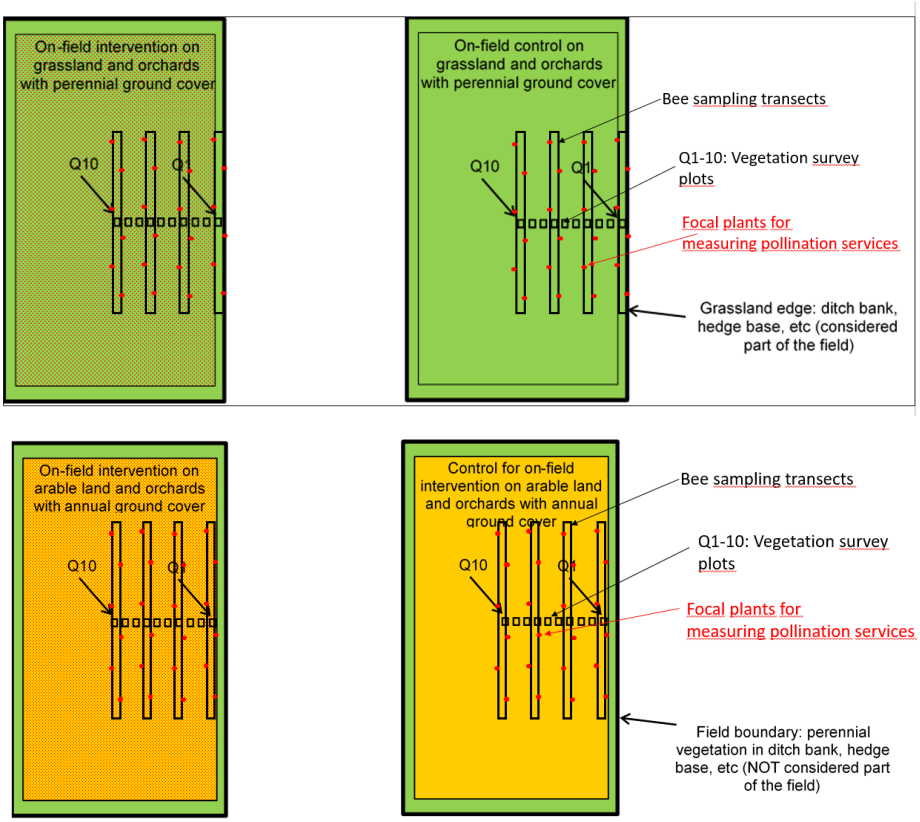

Figure 8a. Rough schematic of sampling design of on-field interventions. In these examples, 6 focal plants for measurements of pollination services (e.g. fruit and/or seed set under open pollination) are illustrated with red dots right adjacent to each belt transect for bee sampling. The minimum number of focal plants per bee transect should be ≥3 plants (≥12 plants in total per field/orchard). As pollination of focal plants in arable crops may be affected by transect walks for bee sampling, focal plants should be select right adjacent to belt transects rather than within the belt transects. In orchards, the same tree lines used for bee sampling should be used for the selection of focal trees for measuring pollination services. Depending on the focal crop additional bagging may be required of plants right adjacent/within belt transects for pollinator sampling (see protocol).

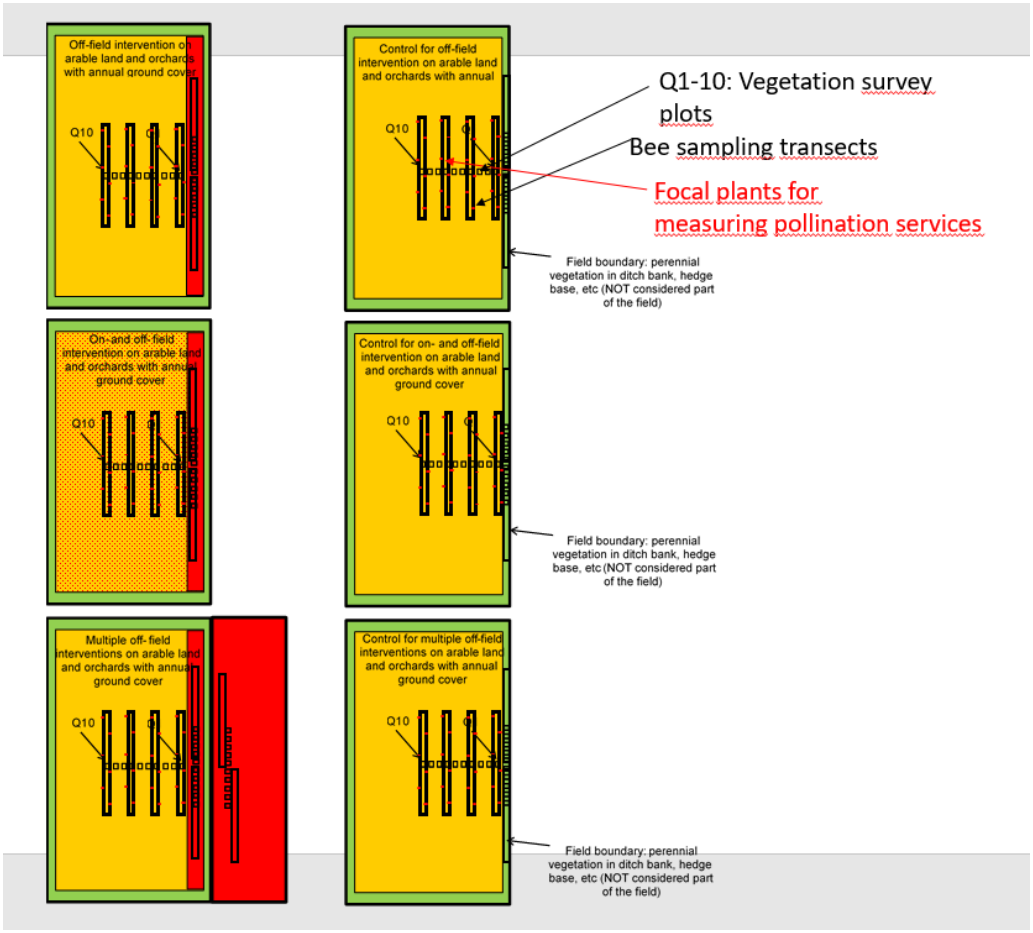

Figure 8b. Examples of schematics for sampling designs of examples of off-field interventions and multiple interventions. In these examples, 6 focal plants for measurements of pollination services (e.g. fruit and/or seed set under open pollination) are illustrated with red dots right adjacent to each belt transect for bee sampling. The minimum number of focal plants per bee transect should be ≥3 plants (≥12 plants in total per field/orchard). As pollination of focal plants in arable crops may be affected by transect walks for bee sampling, focal plants should be select right adjacent to belt transects rather than within the belt transects. In orchards, the same tree lines used for bee sampling should be used for the selection of focal trees for measuring pollination services. Depending on the focal crop additional bagging may be required of plants right adjacent/within belt transects for pollinator sampling (see protocol).

11. Other protocols for measuring services (optional)

Measuring organic matter decomposition (Tea Bags)

Author/contributor: V. Bretagnolle

The approach described here uses a standardised plant litter to measure decomposition and stabilisation. The full and detailed protocols are to be found in https://besjournals.onlinelibrary.wiley.com/doi/epdf/10.1111/2041-210X.12097

The key component of the approach is the use of commercially available tea bags as highly standardised test kits containing tea as representative dead plant material. Two types of tea material with distinct qualities are being used; the Green tea with green leaves (*Camellia sinensis*) and high cellulose content and expected fast decomposition, and rooibos tea (*Aspalanthus linearis*) with high lignin content and expected slow decomposition. The bag material is made of woven nylon and has a

mesh size of 0.25 mm allowing access of microfauna, microbes and very fine roots. Before the start of the incubation all tea bags are oven-dried at 70 °C for 48 h and the initial weight recorded (overall mean = 1.81 g, s.d. = 0.10). Each bag is identified with a unique number and buried in the upper 5 cm of the top soil layer during spring season. At least two homogenous areas (plots) are selected (at least 1 m apart) at each site. Two replicates of the two litter qualities (Green tea and Rooibos tea) are installed in each of the two blocks, resulting in 4-12 bags of each tea type per site and sampling time. Tea bags are collected at all sites after a field incubation period of three months (this can be modified according to crops).

When back, the tea bags are cleaned from soil and roots, oven dried (70 °C for 48 h), and the weight of the remaining tea (without bag) recorded. Instead of weighing incubated tea bags (as often damaged, tag dissolved or rope missing) an averaged bag weight (40 empty tea bags; 0.248 g per bag) can be used to estimate the amount of the tea before the incubation. If the collected tea bags is visibly contaminated with soil, ash content (refers to the mineral residue after removal of organic matter by ignition) is determined by heating in a muffle oven at 500° C for 16h, in order to correct for the mineral part.

In summary

Use one bag of Lipton green tea (EAN: 87 22700 05552 5) and one Lipton rooibos tea (EAN: 87 22700 18843 8) per replicate. To obtain better estimates of TBI, bury more replicates per site.

Measure the initial weight of the tea bag and subtract the weight of an empty bag to determine the initial weight of the tea.
Mark the tea bags on the white side of the label with a permanent black marker.
Bury the tea bags in 8-cm deep, separate holes while keeping the labels visible above the soil and mark the burial site with a stick.
Note the date of burial, geographical position, ecotype and experimental conditions of the site.
Recover the tea bags after c. 90 days
Remove adhered soil particles and dry in a stove for 48 h at 70°C (not warmer!).
Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation. To get a more precise estimation, open the bag and weigh its content; combust the content at 550°C and subtract what is left from the content weight.
Calculate stabilisation factor S and decomposition rate k using eqn 1b in https://besjournals.onlinelibrary.wiley.com/doi/epdf/10.1111/2041-210X.12097

Showcase Resin Bag Protocol

Author/contributor: A. Hood

Ion-exchange resin bags are an accessible way to measure nutrient leaching in soils. Since the resin attracts dissolved ions in the soil, resin bags are not suitable to quantify the amount of leaching in a soil, but when applied in a standardized they allow to compare nutrient leaching across locations. Therefore, standardize the materials used (notably resin and fabric type), the amount of resin per bag, burial depth and placement time, and place at least 2-3 replicate bags per field or treatment. Bear in mind that next to nutrient concentration, e.g. weather and soil conditions may influence leaching and ion absorption by the resin bags. This protocol subsequently describes the required materials and steps for preparation, placement & retrieval, and analysis of ion-exchange resin bags in agricultural soils.

Bag Preparation- Preparation materials

Ion Exchange Resin: Multiple products are available. They can be non-regenerable (i.e., they have to be disposed of after use) or regenerable through acid/base baths. Mixed-bed resins absorb anions and cations (e.g. Nitrate, Ammonium, Phosphate, Potassium) while anion-only resins are also available. Some procedures recommend pre-washing the resin before deployment in the field to remove impurities. For washing: prepare 10% (1.2M) HCl using de-ionised water. Use safe lab practices for washing, prepare suitable transportation (e.g. ziploc bags) and avoid touching the bags with bare hands after they have been washed until placement.

Nylon/lycra Swimsuit material: Nylon/lycra swimsuit is a finely woven, non-degradable and durable, but water-permeable material (e.g. (e.g. https://www.textielstad.nl/badpak-lycra-off-white.html). Other non-degradable fabrics, e.g. nylon stocking material, may work as well.
Wire string or alternative: Wire or other; use a non-degradable, durable, easy to tie and best brightly colored material. Tag, metal or alternative When no above-ground markings can be placed, metal tags can be used and relocated with a metal detector. Using easy-to-find tags (e.g. a tent peg or big screw) is advisable. In the Netherlands, metal tags were not necessary in loose arable soils when accurate GPS coordinates

were used (a colourful belowground tag would be helpful), but in more compact soils (e.g. grassland) metal tags were indispensable next to accurate GPS points.
Paper bags for storageString and tag
Lab gloves
Measuring scoop, scale
Fabric scissors or alternative
Plastic zip ties

Stepwise preparation

- Cut the nylon fabric in squares of e.g. 12-13 cm (suitable for e.g. 3-5 gram resin) or 20*20 cm for larger bags. Too small squares risk leakage of the resin.
- Cut wire strings of the right length; i.e. fit to bury the bags at the desired depth under a 60° angle and to attach a tag either above or just under (metal tag) ground level. Attach the tags to one end of the wire.
- Attach the wire to the zip tie below the head of the zip tie and make a circle of each zip tie by connecting its end.
- Measure the appropriate amount of resin with a scale. This depends on the duration of placement and the expected leaching, thus on land use intensity as well as on weather conditions. Applying too little resin may cause saturation of the exchange capacity and thus incorrect results. In the Netherlands, working on heavily fertilized fields, we used 3 – 3.3gram/month (source: DOI: 10.1111/gcb.14123), which was enough. Note that the resin loses weight when the bags dry during storage.
  Weighing does not need to be exactly accurate since the samples are again weighted before extraction of the absorbed ions, but the bags should be approximately the same weight.
- Invert the resin into a tidy pile in the center of the fabric square. Be careful to avoid spoiling.
- Carefully bundle the corners and edges of the fabric, not leaving any gaps at the edges. Tie off the bag by securing the bag below where you hold the fabric together with your fingers, but above the pile of resin. This step likely involves two persons.
- Check if the bag is not leaking and pull the zip tie tightly.
- Label and store the bags. They are now ready for placement or for acid wash if desired.

Placement

- Digging gear, e.g. a soil corer and hand shovels
- Marking material
- GPS device, preferably high-accuracy (e.g. GNSS).
- Optional: metal detector
- Storage & labelling material
- Timing: Place the bags at crop seeding or shortly thereafter. Record burial dates.
- Depth: In order to capture leached ions unavailable to plant roots, place the resin bags below rooting depth. In the Netherlands, we placed the bags at 20-25 cm depth in grasslands but at 60 cm depth in cereal and lupin fields.

- Digging: Dig at an angle of approximately 60° to avoid disturbance of the soil immediately above the resin bag. Minimize soil disturbance during placement, e.g. by using a soil corer. It is helpful to mark the desired depth on the soil corer. Replace the soil in the same order it came out while holding the marker.
- Marking: sustain the metal tag just below the surface or place a tag above surface. When working with metal tags, you may want to check with a metal detector if your spot is not heavily polluted with scrap metal. In the Netherlands, this caused difficulty during retrieval in about 10% of cases.
- Locations: Carefully save location coordinates with a high-accuracy GPS device (e.g. GNSS system). This way, accuracy is about 20 cm while a mobile phone or handheld GPS is easily a few meters off. If a high-accuracy GPS is unavailable, belowground tags may prove impossible to retrieve.

Retrieval

- Timing: Collect the bags at or shortly before crop harvest. Collecting the bags more than a few days after harvest, you risk the bags registering nitrate leaching from the post-harvest fallow period, which can have rapid leaching.
- Relocation: Retrieve the tags of the resin bags either by accurate GPS coordinates, with a metal detector or by another method.
- Digging: use a handheld trowel to retrieve the bag once you have located the wire. Avoid breaking the string and especially striking the resin bag, although the swimsuit fabric is durable. Avoid pulling on the fabric.
- Labelling: Carefully register a unique number or ID for each resin bag, e.g. on a collection bag, to avoid confusing bags from different locations. Record collection dates.
- Storage: Store the bags prior to analyses in a fridge or dry them at 30°C for 24-48h.

Predation Cards

Author/contributor: V. Bretagnolle

Sentinel preys are used to estimate biological control potential. It is a standard and efficient method related to predator activity (Lövei and Ferrante, 2017; Boetzl et al., 2020a) and pest regulation (Perez-Alvarez et al., 2019). In each focal field, natural pest control will be measured as the realised predation rate on two common pests: the aphid *Acyrthosiphon pisum* (Ximenez-Embun, Zaviezo, & Grez, 2014), and the weed *Viola arvensis* (Petit, Trichard, Biju-Duval, McLaughlin, & Bohan, 2017). *V. arvensis* is a relatively common weed in cereal crops while *A. pisum* is often used to estimated aphid predation rate in fields (Winqvist et al., 2011; Ricci et al., 2019) as many predators as ground beetle predated on number of aphids species (Bilde and Toft, 1997). Aphids can be bought or raised in colonies on peas *Pisum sativum* in the laboratory. If in some EBA regions these species are not common or even not occuring, other aphids and/or weed seeds of species (maybe seeds of similar size) that are common in these regions will be used.

Predation rates are quantified using sentinel cards, on which either 3 dead aphids or 10 weed seeds are glued (with organic glue) on the rough side of 5x6cm sandpaper

cards (Boetzl, Konle, & Krauss, 2020; McHugh et al., 2020). Cards are placed 24 hours in the freezer at -20°C before the experiment to avoid attractive or deterrent effect of predator due to glue evaporation (Boetzl et al., 2020a). Two parallel transects of 20m are selected per field, distant by at least 10m from each other to ensure independence between transects. To limit potentially confounding field margin effects, set up transects at least two tractor bays (i.e. approximately 25-35m) away from the field border. On each transect, 4 cards of each prey type are set on the ground, held a pin (Winqvist et al., 2011; Boetzl et al., 2020a), each being 7m apart (Ricci et al., 2019; Boetzl et al., 2020b). Seed and aphid cards were put on the same position on the transect and spaced 40 cm apart. Cards were folded in half to provide a tent-like shelter with aphids facing to the ground and limit the deterioration of the aphid or seed gluing by climatic conditions (rain, sun, wind…) as advised by (Winqvist et al., 2011). The position of cards in the fields was recorded with a GPS.
Each field has to be sampled twice over the spring and summer seasons to account for temporal variation of predation rates throughout the season (Ximenez-Embun et al., 2014). Seed cards were left 4 days in the field whereas aphid ones were collected after one day (24 h) because of much higher predation rates (see results, Ximenez-Embun et al., 2014). This period is standard in studies using predation prey card (Lövei and Ferrante, 2017; Perez-Alvarez et al., 2019; Boetzl et al., 2020a) **We count the number of aphids or seeds remaining on the cards to estimate predation rates, and then removed cards from fields.**

Showcase Protocol for estimating Crop Yields

Author/contributor: V. Bretagnolle

12. Estimating yields in various crops

Yields should always be asked to farmers in which experiments are carried out. In addition, EBAs should also collect their own measure of yields. Since measure and components of yield vary with crops, we provide below dedicated methods for the main crops. Note that yields are to be measured in both experimental and control units, be it at plot, field part or field levels. Yields should be measured just prior to harvest, to get an as close as possible measure referring to farmer's yield. Yields are also to be measured in several quadrats in the field, that can be arranged spatially either in transects (but not necessarily, depending on tractor tracts), and avoiding atypical places in the field, such as close to hedges.

OilSeed Rape

Data are collected at the end of cropping season (typically late June in France), preferably 1 week before actual harvest (this should be established with the farmer). Early in the season (e.g., in March or April), OSR plants are counted in 1 m² quadrats, from field border to field centre, in 10 quadrats. For instance place 10 quadrats from the intervention-crop boundary into the crop, and 10 quadrats form the control-crop boundary into the crop (quadrats should be spaced by at least 5-10m). Plant density at field scale is then estimated by averaging the number of plants over the 10 quadrats. Yield in OSR is measured at OSR plant level, from a minimum of 12 individual plants per plot (i.e., in control and experimental areas). Plants are collected and plants/branches are brought back to the laboratory, each part being stored in individual paper bags. All bags are left 48 hours in a climate chamber at 60°C, to standardize hygrometry. Three traits are measured at branch scale: the fruiting rate obtained as the ratio of pods per branch out of the number of flowers per branch (note that even if the flower is unsuccessful, the caudal peduncle is still present and visible), the individual seed weight (select randomly 3 seeds/branch), and the number of seeds per pod for a sample of pods from the branch (at least 5 pods). For the latter, we assume that four to six pods per branch are enough to assess the number of seeds per pods (average 3.51-4.84 pods in France). Variation in number of pods per branch is due to variability in branch length (hence number of pods) between years. Individual seed weight is obtained using three randomly selected seeds per branch, individually weighed to the nearest .01 mg.

Wheat (and winter sowed cereals)

Crop yield and quality are assessed using grain yield biomass and grain protein content measured in a 1m² quadrat. Grain yield is estimated by harvesting 1m² quadrat per experimental unit (at least 6*1m² quadrats, preferably 8-10, per plot). For instance place 10 quadrats (if you sample 10 quadrats) from the intervention-crop boundary into the crop, and 10 quadrats form the control-crop boundary into the crop (quadrats should be spaced by at least 5-10m). Harvests are performed one week before the fields are harvested by the farmers. Samples are oven-dried at 60-80° C for 48h and weighed. Plant sward and grain are separated, and weighed each. Then, grains are extracted and counted. A classical measure of yield is the weight per 1000 grains. You need to have a seed counter to have the 1000 seeds, then weight them.

Sunflower (and Maize)

Data are collected at the end of cropping season (typically late August in France). Just prior to harvest, sunflower plants are counted in 1 m² quadrats, from the field border and every 5-10m from the field edge. For instance place 10 quadrats from the intervention-crop boundary into the crop, and 10 quadrats form the control-crop boundary into the crop (quadrats should be spaced by at least 5-10m). Plant density at field scale is then estimated by averaging the number of plants over the quadrats. Sunflower plants are collected five days before harvest. In the laboratory, head diameter (in mm) is measured twice and averaged for analyses and then heads are stored in individual bags and left into a heat chamber at 60°C for 48 hours. Seeds are removed mechanically from the heads, and fertilized seeds are separated from empty seeds (arbitrary threshold of 9 mg) by seed density with a wind machine (e.g. Batteuse petites graines, ATID, France). Then fertilized seeds are counted twice with a seed counter (e.g. Contador 2, Pfeuffer, Germany). The repeatability between the two measurements is extremely high (less than 0.1% difference), so use the average. Total seed mass (using only fertilized seeds) is measured (nearest 0.1 mg) and three individual fertilized seeds are randomly chosen and weighed to provide the individual unit mass (average of the three weights).
The same technique can be used for Maize, i.e. count plant per quadrat (1m²), and then collect plants, dry them, weigh them, remove the fruit and seeds, count seeds, and weigh them.

Alfalfa and grass

Remove above-ground plant biomass from 1m² quadrats (at least 6, preferably 10), dry all plants, and weigh them. This will allow to get a measure of dry plant biomass, as an index of harvest.

Showcase Protocols for measuring farm management indicators

13. Indicators of farm management

Author: Felix Herzog

In order to evaluate the impact of farm management on biodiversity and ecosystem services, the following core management indicators will be recorded: Number of Field Operations, Grazing Intensity, Pesticide Use, N-Input, and Crop rotation.
The indicators have been taken and simplified from the EU FP7 research project BIOBIO (Biodiversity indicators for organic and low input farming systems, KBBE-227161). More detailed factsheets and examples of indicator results can be found on the BIOBIO website
Not all management indicators are relevant for all EBAs. On grasslands, there will normally be no pesticide applications, for example. On the other hand, the grassland related management indicators do not apply to crop EBAs.
Data collection can be implemented by technical staff (farm interviews, retrieval from databases). For data validation, skills in the interpretation of farm balances and background knowledge in agriculture are necessary to examine the plausibility of both the input and output variables.
Some of these indicators will in addition be supplemented through related (sub-)indicators at farm level, in order to collect information on the level of average management intensity in relation to the crop specific practices in the intervention and control plot, and to inform related tasks in WP2 (2.3, 2.5, 2.7, 3.2)

Number of field operations

Description

Quantifies the number of mechanised field operations in crop fields and grassland. The **unit** of measurement is the total number of field operations.

Sub-indicators

The indicators 'Mowing frequency', 'Mowing timing' and 'Soil Cultivation: ploughing' are no genuine sub-indicators because they use different input variables. However, they are thematically related.

Data collection method

In surveys, farmers must be interviewed using a structured questionnaire.

Calculation method

Total number of field operations (FieldOp)
Input variables:
Number of mechanised field operations from

- Soil cultivation and seeding (S_i)
- Fertilisation (F_i)
- Mechanical weeding (W_i)
- Pesticide treatments (P_i)
- Mowing / harvesting (M_i)
- Other operations (O_i)

The number of operations must be added up for each crop or grassland.

$FieldOp = \Sigma(S_i + F_i + W_i + P_i + M_i + O_i)$

Mowing Frequency of Grassland or Perennial Fodder Crops (MowFreq)
Input variables:

- Number of cuts per year (differentiated by grassland type) (C_i)

$MowFreq = \Sigma\ C_i$

Mowing Timing (for grassland or perennial fodder crops) (**MowTime)**
Input variables:

- First cutting (calendar week)

$MowFreq = \Sigma Wk_i$

On grassland of mixed mowing / grazing, the grazing intensity in combination with the mowing frequency should be taken into account. As those situations can be manifold and complex, it is suggested to note the actual practices on the EBA grasslands and then discuss the integration into an index or indicator for the situations, where this actually occurs.

Grazing intensity

Description

This indicator evaluates the intensity of grazing on the pastures of the intervention/ control.
Unit: Number of livestock units (LU) per hectare grazing area. The indicator takes into account the actual time that livestock spends on grazing land.

Calculation method

Input variables:

- livestock categories (L_i)
- livestock units for each livestock category (LU_i)
- average number of animals by livestock category on the farm (for one calendar year) (N_i)
- number of days per year that a particular livestock category spent on the farm (d_i)
- proportion of presence time that a livestock category spent on farm-owned grazing land (g_i), e.g. 80% of presence time on grazing land = 0,8
- grazing area on the farm (A_g).

$Graze = \Sigma\ (N_i\ LU_i\ d_i\ g_i\ /\ 365)\ /\ Ag$

For the estimation of presence time on grazing land (g_i), a method from the tool DIALECTE was adopted. Thereby, for each livestock category the average daily hours on grazing land are estimated for each month of the year.
On grassland of mixed mowing / grazing, the grazing intensity in combination with the mowing frequency should be taken into account. As those situations can be manifold and complex, it is suggested to note the actual practices on the EBA grasslands and then discuss the integration into an index or indicator for the situations, where this actually occurs.

Pesticide use

Description

This indicator measures the frequency of pesticide use on the intervention / control field. **Sub-indicators** differentiate specific classes of pesticides: 'Herbicide Use', 'Insecticide Use' and 'Fungicide Use'.

Calculation method

Categories of pesticides (P_i):

- Herbicide – Number of Treatments
- Fungicide – Number of Treatments
- Insecticide – Number of Treatments
- Retardant – Number of Treatments
- Molluscicide– Number of Treatments
- Nematicide– Number of Treatments
- Other Measures (to be specified) – Number of treatments

In practice, farmers may apply different types of pesticides as mixtures. In the interviews, such operations are recorded as separate treatments.
e.g. 1 application with a combination of a fungicide and an insecticide = 2 pesticide treatments
but: 1 application with 2 different fungicidal substances = 1 fungicide treatment
The pesticide treatments are recorded for each crop or grassland type. They are summed up for each crop/grassland. Also the timing of pesticide use is to be recorded (spring, autumn)

$$PestUse = \Sigma N_i,$$

where N_i is the number of treatments with a certain pesticide type (P_i).

We may want to consider to account for the toxicity of different pesticides or relate the number of applications to the number of "standard applications" (Indicateur de fréquence de traitements phytosanitaires IFT) (https://agriculture.gouv.fr/indicateur-de-frequence-de-traitements-phytosanitaires-ift). Yet, this should only be done if the pesticide applications on EBA fields with intervention differ from the control fields.

Nitrogen input

Description field level

The **unit** of measurement is average input of nitrogen on the intervention/control field (kg N per ha and year)
Subindicator field level are organic (manure, slurry, compost) and mineral nitrogen fertilizer input measured as kg N/ha*a.

Calculation method field level

Input variables:

- Quantities of mineral nitrogen used on the field (kg N/ha) (Nmin)
- Quantity of organic fertilizer used on the field (kg N/ha) (Norg)*
- N_2 fixation by legumes (crops, grassland) (Nfix)

Some of the variables cannot be assessed directly from interview data. For N_2 fixation and organic nitrogen approximations are made, as described below.
N2 fixation of the pre-crop (preceding crop) is not taken into account.
N_2 fixation is estimated as the equivalent of the nitrogen content of the harvest (grain or forage). The input data used are the yield of leguminous crops and the average

nitrogen content of the plant material. For example, 1 ton of peas will fix 32.5 kg N and 1 ton of alfalfa 39 kg N. The nitrogen available in the soil is not assessed.
*Moreover, potentially, farmers will not know how to express particularly Ntotal (kg N7ha Norg) of organic fertilisers. In this case, the kind of organic fertilizer and application amount (t/ha, fresh matter) need to be assessed, and average data must be used for calculation.

Nitrogen Input (NitroIn)
NitroIn = Nmin + Norg + Nfix
The N deposition is not taken into account.

Crop rotation

Crop rotation on arable land is the practice of alternating crops grown on a specific field in a planned pattern or sequence in successive crop years so that crops of the same species are not grown without interruption on the same field. This indicator is proposed in addition to the above mentioned BioBio indicators. We should evaluate, for the arable crop EBAs, if it is applicable at the field scale and if crop rotations between intervention and control fields differ.

Description

At farm level, the indicator (1) **'crop share'** measures the amount of different crops on arable land within a farm.
Sub-indicators indicate at field level (2) '**crop order'** the sequence of alternating crops grown on a specific field in a sequence in successive crop years
(3) **'crop type'** indicates the crop or a planned pattern of crops at the moment of sampling the biodiversity indicators (spatially explicit for the intervention fields and control fields) and (4) **'crop position'** indicates the order within the sequence/ rotation according to (2) (spatially explicit for the intervention fields and control fields).

It is a **state indicator,** e.g. listed and defined by JRC for the strategic monitoring on agri-environment (https://esdac.jrc.ec.europa.eu/projects/agri-env-indicators).
Purpose of a crop rotation is that crops of the same species are not grown without interruption on the same field (https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Glossary:Crop_rotation), what being typical for monocultures increases the pressure of plant diseases from infected residues, pests or seeds from weeds that remain in or on the top soil and thus with time can develop a competitive advantage against the crop, what again may urge to increase pesticide use.
Direct effects of the crop rotation are increased genetic diversity of the crops themselves, increased diversity as a habitat, as source of food, as root soil functional system positively impacting soil biodiversity.
Indirect effects are related to reduced pressure for fungicide, insecticide and herbicide use. In organic farming systems a broad crop rotation is a key management practice to stabilize yield, soil fertility, plant health and weed management.

Calculation method

(1) Crop share
The **unit** of measurement is the area-weighted number of crops on a farm (Area for each crop as share of the total UAA of the farm)

(2) Crop order
Indicate the crop type per cultivation (= harvest) year in its sequence until the repletion starts.

Examples:

Organic farming crop rotation

1. Clover grass
2. Winter wheat
3. Winter rye, intercrop
4. Faba beans
5. Winter wheat, intercrop
6. Oats with clover grass underseed

Conventional farming crop rotation

1. Winter rape
2. Winter wheat
3. Winter barley

(3) Crop type

Spatially explicit for each intervention field and control field: name of crop (e.g. winter wheat)

(4) Crop position

In addition to (3): on the example organic farming crop rotation: 5. (means winter wheat after faba beans)

Synergies with other indicators

Causal links to the biodiversity indicators and the farm level indicators (N-input, field operations, pesticide use).

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