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Biodiversity in organic and low-input farming systems

Handbook for recording key indicators

Alterra Report 2308
ISSN 1566-7197

P. Dennis, M.M.B. Bogers, R.G.H. Bunce, F. Herzog and P. Jeanneret



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This research has been carried out in the framework of the EC FP7 project BioBio (EC-FP7 Contract KBBE-2B-227161).

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Handbook for recording key indicators

Edited by P. Dennis¹, M.M.B. Bogers⁴, R.G.H. Bunce⁴, F. Herzog² and P. Jeanneret²

Contributing authors

P. Dennis¹, F. Herzog², P. Jeanneret², M. Arndorfer³, D. Bailey², M.M.B. Bogers⁴, R.G.H. Bunce⁴, J.-P. Choisis⁵, N. Choisis⁵, D. Cuming⁶, O. Ehrmann³, W. Fjellstad⁷, T. Franck³, M.D. Fraser¹, J.K. Friedel³, I. Geijzendorffer⁴, T. Gomiero⁸, R. Jongman⁴, M. Kainz⁹, R. Kölliker², L. Last², G. Lüscher², G. Moreno¹⁰, P. Nicholas¹, M.G. Paoletti⁸, S. Papaja-Hülsbergen⁹, C. Pelosi⁵, P. Pointereau¹¹, J.-P. Sarthou⁵, M. Schneider², N. Siebrecht⁹, S. Targetti⁶, D. Viaggi⁶, J. Wilkes² and S. Wolfrum⁹

¹(ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK; ²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland; ³(BOKU) Division of Organic Farming, University of Natural Resources & Life Sciences, Vienna, Austria; ⁴(ALTERRA) Alterra, Wageningen UR, the Netherlands; ⁵(INRA) UMR Dynafor, INRA-ENSAT, Toulouse, France; ⁶(UNIBO) Imas Mater Studiorum, University of Bologna, Department of Agricultural Economics and Engineering (DEIAGRA), Italy; ⁷(NFLI) Norwegian Forest and Landscape Institute, Ås, Norway; ⁸(UP) Department of Biology, Padova University, Italy; ⁹(TUM) Centre of Life and Food Science, Weihenstephan, Technical University of Munich, Germany; ¹⁰(UEX) Forestry School, University of Extremadura, Plasencia, Spain and ¹¹(SOLAGRO) Initiatives and Innovations for Energy, Agriculture and Environment, Toulouse, France.

Alterra Report 2308

Alterra, part of Wageningen UR
Wageningen, 2012



Abstract

P. Dennis, M.M.B. Bogers, R.G.H. Bunce, F. Herzog and P. Jeanneret, 2012. *Biodiversity in organic and low-input farming systems. Handbook for recording key indicators*. Wageningen, Alterra, Alterra-Rreport 2308. 92 pp.; 17 fig.; 24 tab.; 30 ref.

This Handbook describes the methods required to measure the direct and indirect indicators of biodiversity in the field or through farmer interviews on organic, low-input and conventional (control) farms during 2010. It is the result of editing and revision of the BioBio deliverable D2.2.

A total of twelve Case Study regions were selected in eleven countries. A standard habitat mapping procedure for the European scale based on General Habitat Categories (GHCs) was applied. This method has been adapted further to deal with the assessment of organic/low-input farm holdings. An initial classification of farmed and non-farmed land has been used to direct the selection of the samples. After mapping the farm area, in each habitat type selected for flora and fauna surveys, all species indicators were sampled for vegetation, earthworms, bees and spiders. Farm practices and genetic diversity were measured through interviews with the farmer. A digitising protocol was provided to prepare all data for analysis.

The practicality and suitability of these methods for sampling plants and selected animals on very different farm types and habitats was evaluated. Lessons learned are described shortly after each chapter indicating difficulties encountered during the field work and giving practical suggestions. Further reports will be available in due course presenting the implication of analyses and results. The cost efficiency of the methodology was measured through reports on input of staff time and materials.

Keywords: habitat, indicator, organic farming, low-input farming of additional editing and revision of the D2.2

ISSN 1566-7197

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Alterra Report 2308

Wageningen, April 2012

Contents

Preface	7
1 General introduction and purpose of handbook	9
2 Participating countries of the case studies	13
2.1 Farm selection procedure within case study regions	13
2.2 Overall sampling strategy for each farm	14
2.2.1 Convention agreed for farm area to be surveyed on case study farms	15
3 Habitat mapping: the general habitat categories method	17
3.1 General habitat categories (GHCs) method	19
3.1.1 Timing of habitat survey	20
3.2 Habitat mapping: general rules	20
3.2.1 Mapping of individual elements	20
3.2.2 Recording of individual elements	21
3.2.3 Recording form	22
3.3 Mapping areal elements	22
3.3.1 Rules for separating map elements (i.e., new Alpha codes)	22
3.3.2 Determination of the General Habitat Category	23
3.4 Subdivision of general habitat categories	25
3.4.1 Field one: Rules for determining GHCs	25
3.4.2 Predefined list of linear elements and ponds	30
3.4.3 Field two: Environmental qualifiers	30
3.4.4 Field three: Site qualifiers	32
3.4.5 Field four: Management qualifiers	32
3.4.6 Field five: Detailed life form and species composition	33
4 Farm-level measurements and information gathering 2010	35
4.1 Convention for labeling samples and data records	35
4.1.1 Barcodes	36
4.2 Species-level measurements	37
4.2.1 Vegetation	39
4.2.2 Wild, domestic and bumble bees	47
4.2.3 Spiders	52
4.2.4 Earthworms	58
4.3 Genetic indicators – questionnaire	64
4.3.1 Indicators for plant genetic diversity	64
4.3.2 Sampling protocols	64
4.3.3 Data processing	65
4.3.4 Questionnaire Interview Sheet	65
4.3.5 Livestock genetic resources	70
4.4 Farm management indicators - questionnaire	71
4.4.1 Introduction	71
4.4.2 Defining requirements for the questionnaire	72

4.4.3	Structure of the farm management questionnaire	72
4.4.4	Data processing	73
4.4.5	Questionnaire Interview Sheet	74
4.5	Assessment of costs of measuring biodiversity indicators in BIOBIO project	76
4.5.1	Objective	76
4.5.2	Methodological proposal	76
4.5.3	Discussion	79
5	Data processing	81
5.1	BioBio habitat mapping - digitising protocol	81
5.2	Data transfer and organisation	89
5.2.1	Farm management indicators	89
5.2.2	Genetic diversity indicators	90
5.2.3	Species diversity indicators	90
5.2.4	Habitat diversity indicators	90
6	References	91

Preface

This Handbook presents the procedure for measuring indicators developed in the BIOBIO (Indicators for biodiversity in organic and low input farming) project of the EU FP7 framework. The content was first available as Deliverable Report 2.2 on the BIOBIO project website but this Handbook has been published to make the information more widely available, and includes a provisioned description on lessons learnt in the course of the project. The five editors have coordinated and edited the text in a format suitable for publication. However, each of the sections has been attributed to the relevant authors, so that their contribution can be cited as a reference and credited to their list of publications. Inevitably full details are not given and subsequent reports should be consulted describing the analyses and results from the various indications.

The report also includes the experience of the EBONE (European Biodiversity Observation network) project related to habitat mapping and the linked recording of vegetation. However, in contrast to the original BIOBIO D2.2 report, only an outline of this method is presented here since the EBONE manual is now published and available for consultation to find full details. A major conclusion from both projects was that adequate training is required, preferable at regional locations to ensure that the surveyors were fully conversant with the details of the classification rules. Also included are the protocols for the other directly, or indirectly, measured candidate biodiversity indicators together with the background justification for their selection.

These protocols were applied and evaluated in twelve case study regions in eleven BIOBIO partner counties, ranging from Norway to southern Spain. The data are currently being analysed and the results will shortly become available to draw conclusions about the suitability and effectiveness of each of the candidate biodiversity indicators for organic and low input farming systems. 'Down-to-earth' feedback after the application has been collated and is summarized at the end of each chapter.

Marion Bogers, Bob Bunce, Peter Dennis and Felix Herzog
January 2012

1 General introduction and purpose of handbook

Dennis, P.¹

¹(ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK

The aim of the BioBio project has been to develop a series of measured indicators of biodiversity associated with organic and low input farming systems. These indicators can then potentially be used to monitor the contribution that biodiversity makes to high quality food production as well as to assess the contribution of farming to the maintenance of biodiversity in areas of Europe under such farming systems. Agricultural production based on organic and low input farming systems is especially dependent on the organisms in healthy soils, natural enemies of pests, pollinators and dung-feeding invertebrates and often supports a rich wildlife and hence biodiversity.

Candidate biodiversity indicators for organic and low input farming systems were selected following a major review of indicator theory in the project and existing biodiversity indicators carried out in 2009 (Dennis et al., 2009). Direct indicators were chosen to represent livestock breeds, grassland and crop varieties (genetic diversity); domesticated and wild animal and plant species (species diversity); and the mixture of cultivated crops, pastures and semi-natural habitats on farmland (habitat diversity) (Table 1.1). The review included indirect biodiversity indicators based on farm management and farm accounts information where there is a proven connection between farm management information and the levels of genetic, species and habitat diversity (Table 1.1).

Indicators were ranked according to scientific criteria during the WP 2 workshop held in Aberystwyth, 9-10 September 2009. Subsequently, the remaining biodiversity indicators were assessed according to headline stakeholder 'usefulness' and 'cost-effectiveness' criteria. The 'usefulness' of the proposed biodiversity indicators was assessed by means of an online survey, where eighteen stakeholder criteria were applied. The results of the survey were discussed and confirmed during the second Stakeholder Advisory Board workshop in Brussels, 21-22 October 2009. Candidate indicators to be tested in field studies in BioBio were then short listed, accounting for the effort which the project partners can allocate to this field survey in 2010 (described in Dennis et al., 2009).

The purpose of this Handbook is to describe the methods required to measure the list of candidate direct and indirect indicators of biodiversity in the field or through farmer interviews on organic, low-input and conventional (control) farms during 2010. The practicality and suitability of these methods for sampling plants and selected animals on very different farm types and habitats across Europe and further afield will be evaluated. In particular, to determine whether the methods are sufficiently sensitive to distinguish between conventional, low input and organic farming systems.

Full instructions are given to undertake the evaluation of candidate indicators under the following headings:

- Summary of selection procedure for farms in each of the Case Study partner countries (full details in Deliverable 3.1 'Descriptive case study report')
- Farm level habitat mapping and associated stratified sampling design
- Farm level data collection
 - Field survey methods for vegetation, plant species and faunal indicators
 - Farmer questionnaires and interviews for genetic and farm management indicators
 - Cost of indicator measurement
- Indicator calculation, data analysis and scrutiny

Standardised procedures, apparatus and methods are described for each candidate indicator including sampling design, required equipment, data collection dates and the frequency and format of data for transfer to the co-ordinating centre for data recording and analysis. The evaluation will include a detailed economic assessment of the cost effectiveness of each of the indicator measurements. A comparison will be made between the costs of field sampling effort, equipment, data management and analysis and the perceived benefit of the information that is generated for farmers, conservationists, food industry and policymakers.

Table 1.1

Overview of indicators and data sources.

Level of biological organisation	Individual indicators	Source of data
A. Genetic diversity indicators	Animal husbandry	
	A1) Number and amount of different breeds per species (Breeds)	Farm questionnaire
	A2) Information on breeding practices ('on-farm' bull, artificial insemination,...) (Liveprac)	Farm questionnaire
	A3) Where available, pedigree of the herd (LivePedi)	Farm questionnaire
	Arable crops, legumes and trees	Farm questionnaire
	A4 + A5) Number, amount and origin of different cultivars / landraces / accessions per species (CultDiv)	Farm questionnaire
	A6) Information on seed propagation practices (on farm multiplication, sharing with neighbours, etc.) (seedmulti)	Farm questionnaire
	A7) Where possible, description of the cultivars based on IPGRI descriptors (through the farmer) (CropCuPheDiv)	Farm questionnaire
	A8) Where available, pedigree information on the cultivars grown (CropPedDiv)	Farm questionnaire
	Grassland species	Farm questionnaire
B. Species diversity indicators	A9) Where available, number and amount of different cultivars (GrassGenDiv)	Farm questionnaire
	A10) Information on seed propagation practices and amount of re-seeding (ReSeed)	Farm questionnaire
	B2) Flowering plants of semi-natural habitats	X-plots (patches) or rectangular plots (linear features) of vegetation survey
	B4) Earthworms	Soil samples in vegetation plots
	B6) Bird species richness	No field validation for this candidate indicator
	B8) Araneae - spiders	Suction sampling in vegetation plots
	B9) Hymenoptera, wild bees	Walked transects and net capture in vegetation plots

Level of biological organisation	Individual indicators	Source of data
C. Habitat diversity indicators	C1) Habitat Patch density (HabDensity)	Farm habitat mapping
	C2) Habitat richness	Farm habitat mapping
	C3) Habitat diversity (HabDiv)	Farm habitat mapping
	C4) Number of crops in rotation (CropRot)	Farm habitat mapping and farm questionnaire
	C5) Percentage area of arable land (ArableArea)	Farm habitat mapping
	C6) Percentage area of permanent grassland (GrassArea)	Farm habitat mapping
	C7) Percent of tree cover (Tree)	Farm habitat mapping
	C8) Cover of shrub layer (Shrub)	Farm habitat mapping
	C9) Availability of nitrogen, pH, moisture as Ellenberg values (Ellenberg)	X-plots (patches) or rectangular plots (linear features) of vegetation survey
	C10) Weeds in crops (Weed)	X-plots (patches) or rectangular plots (linear features) of vegetation survey
	C12) Vegetation composition: share of valuable habitats (ValueHab)	X-plots (patches) or rectangular plots (linear features) of vegetation survey
	C13) Linear elements: hedgerows, grassy strips between fields, streams, rivers and lakes, stone walls and terrace walls (Linear)	Rectangular plots (linear features) of vegetation survey
	C14) Multispecies grassland swards (Multigrass)	X-plots (patches) or rectangular plots (linear features) of vegetation survey
	C15) Grassland quality (GrassQ)	X-plots (patches) or rectangular plots (linear features) of vegetation survey
D. Farm management indicators	D1) Diversity of enterprises on the farm (DivEnt)	Farm questionnaire
	D2) Average stocking rates (grazing livestock units ha ⁻¹) on farm (AvStock)	Farm questionnaire
	D3) Area of land without use of mineral-based fertilisers (Minfert)	Farm questionnaire
	D4) N input (NitroIn)	Farm questionnaire
	D5) Input of Direct and Indirect Energy for crop production (EnerIn)	Farm questionnaire
	D6) Certified as Organic (CertOrg)	Farm questionnaire
	D7) IRENA Indicator 1: area under agri-environment support (AgrEnv)	Farm questionnaire
	D8) IRENA Indicator 15: intensification/extensification (IntExt)	Farm questionnaire
	D9) Pesticide Use - Treatment Frequency Indicator (PestUse)	Farm questionnaire
	D10) Area of land without or with reduced use of chemical pesticides (PestUse-Area)	Farm questionnaire
	D11) Frequency and timing of field operations (FieldOp)	Farm questionnaire
	D12) Frequency and intensity of livestock grazing (GrazInt)	Farm questionnaire
	D13) Productivity (cereal, milk or meat)	Farm questionnaire
	D14) Irrigation (practiced or not?)	Farm questionnaire

Feedback after application of the method in twelve case study regions: Synopsis on the entire sampling exercise including habitat mapping, species sampling (plants, bees, spiders, earthworms) and farm questionnaires (management, genetic diversity)

Strengths

Due to the different requirements regarding weather conditions of plants, earthworms, spiders and bees there was a continuous workload. One team could be employed over several weeks without lost time because of bad weather.

Difficulties

Long routes, scattered fields: transport costs were considerable! In the beginning, much time was needed for navigation and orientation in the field. Availability of cars was a limiting factor.

Practical hints

Planning the field work to get everything done required very good organisational skills.

Efficiency: For habitat and flora mapping, the efficient use of time in the field is a benefit. However, there were few synergies for faunistic indicator sampling because the three indicators were sampled with separate methods, two of them (bees, earthworms) rather time consuming. Sampling methods covering several indicator groups would improve the efficiency. E.g. it would have been easy to obtain additional taxa from the suction samples.

Proper training in the habitat mapping method is essential. Errors in mapping can mean errors in plot placement - which is much more costly than extra time used for training.

Cost of farm visits: Wild bee and spider sampling could be combined to save on journeys and time.

Separate teams of two persons were collecting bees and spiders. Easier orientation when locating the plots.

One person driving, the other navigating. Fewer cars were needed. However, there is the question of efficiency: two persons are not necessarily twice as fast.

It could be beneficial for some complex farms to separate description & mapping the 1st year (plus plant sampling in perennial vegetation plots), and all the direct and indirect indicators the 2nd year.

Contact taxonomists (spiders, bees, earthworms) before you start sampling and clarify how the material should be prepared (e.g. bees pinned or not, preservation of earthworms).

2 Participating countries of the case studies

Jeanneret, P.², Arndorfer, M.³ and Dennis, P.¹

¹ (ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK;

² (FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland;

³ (BOKU) Division of Organic Farming, University of Natural Resources & Life Sciences, Vienna, Austria

A total of twelve Case Study regions were proposed in eleven countries at the outset of BioBio (Table 2.1) to provide a wide variety of agricultural production systems across Europe with both organic options to conventional agriculture or enterprises based on low-input farming systems. Full details are given on the BioBio website (BioBio www.biobio-indicator.org).

Table 2.1

European case study countries listed by shared farming enterprise.

Case study no., region and country	Farming enterprise/ system
1. Marchfeld Region, Austria	Organic arable farming
2. Gascony Valleys and Hills, France	Organic arable farming
3. Southern Bavaria, Germany	Organic mixed farming
4. Rhodope mountains, Bulgaria	Semi-natural, low-input grasslands
5. Homokhatsag, Hungary	Semi-natural, low-input grasslands
6. Hedmark, Norway	Organic and low-input grassland with sheep
7. Swiss Alps, Switzerland	Organic mountain grassland with livestock
8. Welsh hill and uplands, United Kingdom	Organic mountain grassland with livestock
9. Extremadura, Spain	Mediterranean silvopastoral systems (Dehesa)
10. Extremadura, Spain	Organic olive plantations
11. Pleistocene, the Netherlands	Organic horticulture
12. Veneto & Friuli Venezia Giulia Regions, Italy	

2.1 Farm selection procedure within case study regions

Farm selection was separately determined and was reported in an output produced by BOKU (Deliverable 3.1 'Descriptive case study report'). Guidelines were provided to ensure that each of the twelve Case Studies was designed to focus upon the factor of interest, i.e., organic versus conventional or low-input versus intensive farming systems. Selection criteria were provided in the report to ensure that the factors of interest were not confounded with other factors known to potentially affect biodiversity. Two sets of potential confounding factors were recognized in BioBio:

1. Environmental conditions: biogeographical region, geomorphological and soil features, landscape situation, altitude.

2. Farm characteristics: type of farm (crops, forage, mixed farming, animal species), size, management intensity, uncultivated habitat types.

Examples of possible confounding effects and problems of interpretation caused by poor farm selection include:

- a) all (or most) of the organic farms are selected at high altitude in a region while all (or most) of the conventional farms are selected at low altitude. An observed difference by biodiversity indicators cannot clearly be attributed to the farming system because altitude is correlated with the farming system. It is then difficult to determine whether an observed difference in measurements of biodiversity indicators is due to the farming system or to altitude (see Figure 2.1).
- b) all (or most) of the selected organic farms have crops while all (or most) of the selected conventional farms have mixed farming or vice versa. An observed difference by biodiversity indicators cannot clearly be attributed to the farming system because the type of farm is correlated to the farming system. In this example it is difficult to determine whether an observed difference in measurements of biodiversity indicators is due to the farming system or to the type of farm.

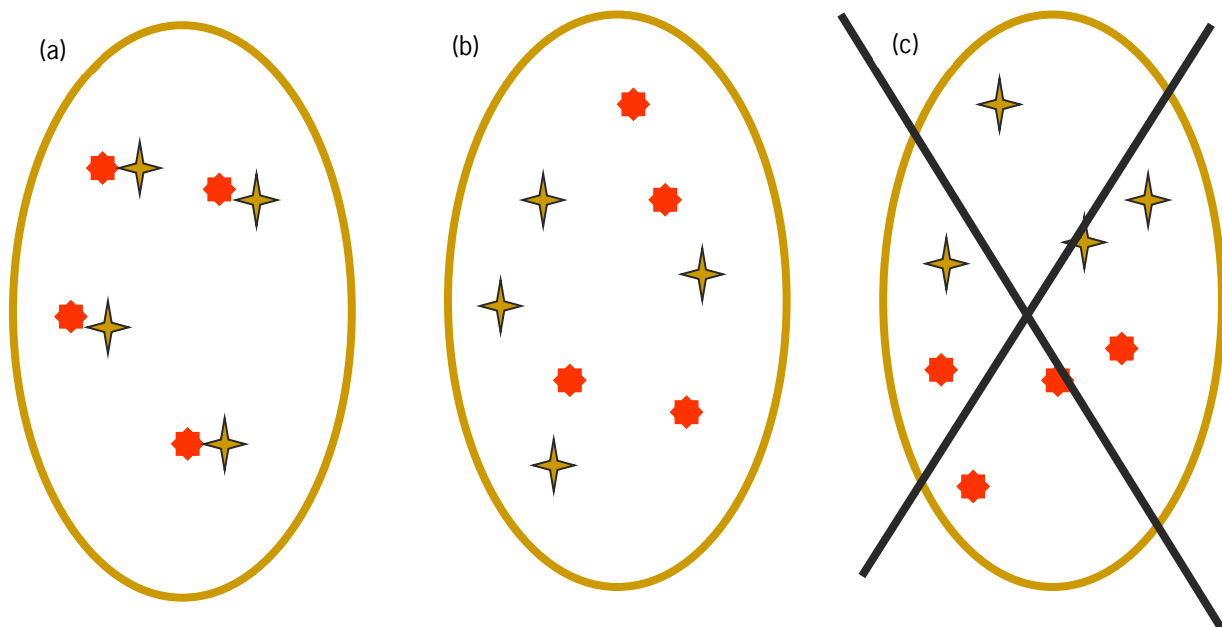


Figure 2.1

Acceptable patterns of farm selection for the comparison of organic and conventional farms (a) and (b). The systematic bias in option (c) must be avoided.

In each case study region, 16 - 20 farms were selected for the evaluation of candidate biodiversity indicators.

2.2 Overall sampling strategy for each farm

Farm selection was random assuming the consent of individual farmers was received to access and carry out sampling on their farm. Once the farms had selected, the following operations were carried out:

- 1) Habitat mapping across the entire farm of all parcels of habitat, linear features and adjacent non-farmed features such as hedgerows and walls (Section 3).

- 2) Random selection of one example of each habitat type recorded on the farm (up to fifteen different types; illustrated in Figure 3.1).
- 3) Surveys of vegetation, spiders, wild bees and earthworms on each example insular and linear habitat (Section 4.2).
- 4) Interviews with farmers about genetic resources (Section 4.3), management practices and inputs-outputs for 2010 reference year (Section 4.4).
- 5) Recording of the time spent on indicator measurements (Section 4.5).
- 6) Reporting of the data to the central database (Section 5).

2.2.1 Convention agreed for farm area to be surveyed on case study farms

The farm size constitutes the area of land under agricultural management by the selected farmer, including dispersed fields but generally excluding communal grazing land. In Norway and Wales, communal grazing land was included because it is critical to the livestock production systems practiced in those countries. All fields that are rented by the farmer were included in the farm area but land that is let by the farmer to third parties was not included in the farm area for investigation. There may also be a difference within the farm, especially where mountain grazing occurs in a separate location from the lowland area of the farm. The terms for this are as follows: in-fields and out-fields (Sweden and Norway), in bye and out bye (Northern England), fields and ffridd (Wales). In the context of BioBio, elements adjacent to the farm and affected by farming practices were also mapped, even if they were outside the actual farm property (Category 6, Table 3-1; e.g. the side of a hedge facing the field belonging to the farm).

3 **Habitat mapping: the general habitat categories method**

Bogers, M.M.B⁴, Bunce, R.G.H.⁴, Geijzendorffer, I.R.⁴, Jongman, R.H.G.⁴

⁴(AL TERRA) Alterra, part of Wageningen UR, the Netherlands

BIOBIO has adopted a standard habitat mapping procedure for the European scale developed in the BioHab project (Bunce et al., 2008) and later in the EBONE project (Bunce et al., 2011). The method of habitat/land use classification is based on an appropriate generic system of habitat definitions, General Habitat Categories (GHC). The habitat qualifiers, which characterize individual habitats with respect to their ecological features and quality, can include categories specifically related to agriculture and High Nature Value farming areas. The method has been adapted with refined GHC definitions to deal with the assessment of organic/low-input farm holdings that may vary in size, may not be a contiguous land area, often intertwined with other farms. An initial classification of farmed and non-farmed land has been described (Table 3.1), which builds on the work developed within a research project on non-farmed features carried out for the EU in 2008 (Jongman and Bunce, 2009) and has been tested in the EU FP6 SEAMLESS project. The application of this typology of areal, linear and point features is essential because much biodiversity is restricted to linear features which are not directly managed by farmers but remain influenced by farming practices (Bunce et al., 2005). Land uses such as urban and forestry are excluded. In difficult situations subsequent consultations of the base maps can be used to determine borderline cases and improve consistency.

Table 3.1.

Overview of farmed and non-farmed categories. Vegetation plots in BioBio will be placed in categories **1,3,4,5** and **6**.

1. *Fields managed only for agricultural objectives.* Such fields are usually intensively used but may also involve extensive systems. Usually there is a division between:
 - a. *Cultivated land used for arable (e.g., wheat) or perennial or woody crops (e.g., fruit trees, vineyards)*
 - b. *Grasslands used directly (grazing) or indirectly (hay, silage) by livestock*
2. *Fields managed regularly for non-agricultural objectives.* Usually these fields are used for horses or donkeys held for recreational purposes but could also include fields and mesotrophic grasslands managed for nature conservation and landscape objectives.
3. *Unenclosed land used regularly by stock, usually sheep and goats but also cattle and horses for meat.* This category has a wide range of use intensity and varies in character both regionally and locally. It includes many upland grasslands and heathlands but also Dehesas, Montados and wood pastures elsewhere. There is a potential overlap here with forests grazed by domestic stock where the tree cover is over 30%, so such land should be included here as the structure and character of the ecosystems present are determined by grazing.
4. *Unenclosed land used occasionally by sheep or goats but not in regular agricultural use and minimally affected by grazing* (e.g., some blanket bogs and mountain summits in Britain).
5. *Linear or point features on, or adjacent to, farmland that are managed directly or are likely to be highly influenced by farming activities* (e.g., hedges on farmland and grass strips between fields¹).
6. *Linear or point features on, or adjacent to, farmland that are indirectly influenced by current agriculture but are not managed actively* (e.g., field corners and small woodlands surrounded by agricultural land).
7. *Land not used by agriculture (usually urban herbaceous using the GHC definition) and managed usually by mowing* (e.g., roadside verges, recreation areas and sport fields).
8. *Land not used by agriculture but maybe managed for forestry, nature conservation except where grazing is involved or urban objectives*
 - α. *Abandoned fields and unenclosed land no longer used by agriculture.* Long term set-a-side could be included here. This category would also include semi-natural habitats under nature conservation management e.g., wetlands, some salt marshes and heathlands.
 - β. *Land which has never been used by agriculture or managed* e.g., steep roadside banks, cliffs and scree.
 - χ. *Forests.* These could be divided into three categories if a relationship was required with intensity of management
 - (i) Forests managed regularly often for nature conservation objectives using active management e.g., coppice woods for vernal flowers and for firewood
 - (ii) Commercial forests of planted species e.g., Sitka spruce in the UK and Norway Spruce in northern and central Europe. Small recent amenity plantations are not included here as they are still indirectly affected by agricultural practices
 - (iii) Forests that have not been managed in recent times, say about 50 years
 - δ. *Urban land within the definition provided by the BioHab project (Bunce et al., 2005; 2008)*

The separation of categories 5 and 6 is to some degree arbitrary, but was determined on the basis that class 5 actually had deliberately inputs from farmers, e.g. cutting hedges. Class 6 will have only indirect effects from farming, e.g. spray drift.

3.1 General habitat categories (GHCs) method

The BioBio project has, like the EBONE project (EBONE www.ebone.wur.nl), three tiers of recording of biodiversity with small deviation in the top level:

- A. The landscape level: km squares in EBONE = whole farms in BioBio.
- B. The habitat level where complexes of habitats form landscapes = habitat level in BioBio.
- C. The vegetation level; where different types of vegetation make up the habitats = vegetation level in BioBio.

Table 3.1 lists the farmed and non-farmed elements to which vegetation and faunal plots were assigned. Testing this typology in SEAMLESS firstly showed that the different classes had inherently different vegetation present and that any comparison of biodiversity had to be carried out within relatively homogeneous units. In the BioBio project, biodiversity recording was undertaken at the habitat (farmed/non-farmed categories) and vegetation & faunistic level with the landscape unit represented by the farm.

Prior to the mapping, the farm boundaries are obtained either from maps which delineate urban areas or from the farmer directly.

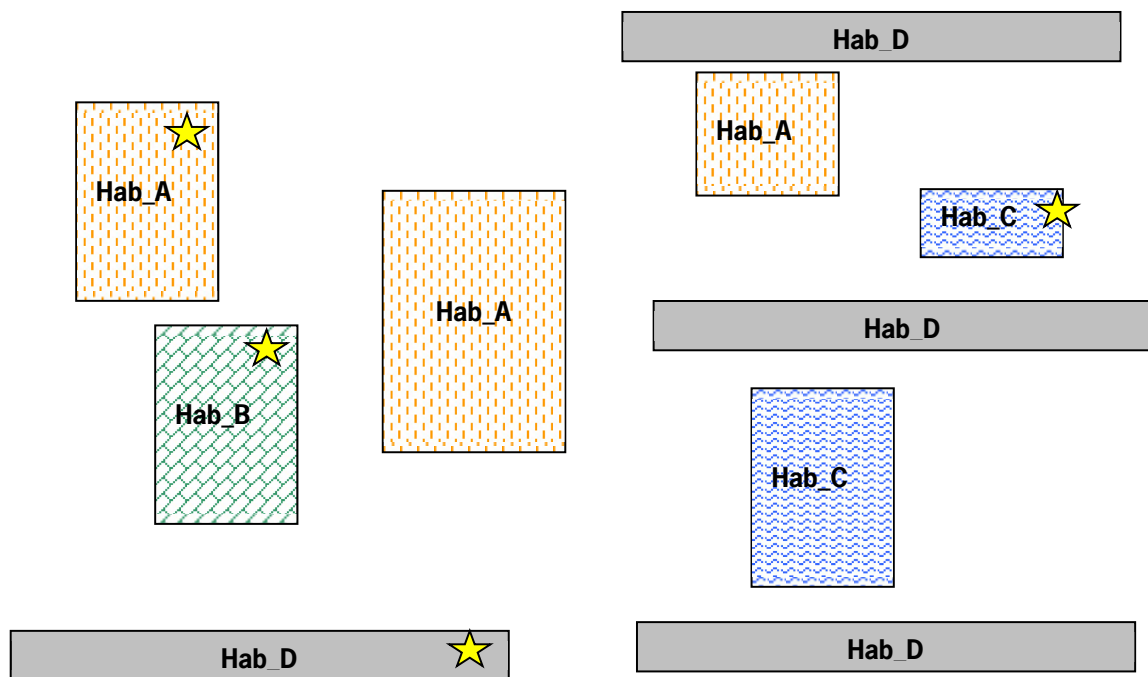


Figure 3.1

On this schematic farm, six areal and four linear habitats have been mapped. They belong to four different habitat types (A, B, C, D). From each habitat type, one specimen has been selected for species diversity measurements (marked with an asterisk).

The structure of the BioBio field recording is shown in Figure 3.1. It is important to locate the vegetation plots precisely on the habitat map so that destructive sampling of other groups, e.g., earthworms can be carried out adjacent to but not inside any vegetation plots. Each plot can be recorded using a GPS unit and with field notes of the character and location related to adjacent landmarks. Vegetation plots in BioBio are only recorded in the following types of land as defined in Table 3.1:

- 1a) Cultivated land

- 1b) Enclosed grassland used by livestock
- 3) Open land used regularly by agriculture
- 4) Open land used occasionally by agriculture
- 5) Features directly affected by farming
- 6) Features indirectly affected by farming

Categories 2 (Grassland used for non-agricultural purposes), 7 (Land not used for agricultural purposes, usually urban) and 8 (Land not used for agricultural purposes, usually forestry, except in Fennoscandia) are excluded because they do not belong to the farm.

3.1.1 Timing of habitat survey

According to Storkey et al. (2008), the timing of the sampling within a growing season is determined by:

- A. The stage in the life cycle of the indicator that is affected by the agricultural management activities.
- B. The phenology and behaviour of taxonomic groups.
- X. The heterogeneity of the life-histories in the taxonomic group: where species groups include a mixture of life-histories, multiple sampling dates across the growing season are required.
- Δ. The potential long-term effect of the new agricultural practices, inducing a time lag in the response of the indicators. This point is particularly important in the present program both for the choice of the farms (how long have organic farming practices been conducted?) and the choice of indicators.

Directly measured management indicators such as land cover is described when most of the crops and management activities are easy to identify. In practice as emphasized by Bunce et al. (2008) the best procedure is to sample at the height of the growing season.

3.2 Habitat mapping: general rules

Each field in the recording sheet is explained and decision rules are presented. The full definitions and code lists are provided in the EBONE manual (Bunce et al, 2011). The section below is a summary version concentrating on the sections most important to BioBio.

3.2.1 Mapping of individual elements

Separating map elements is based on strict rules. The mapping of areal elements adds to 100% of the land. The entire survey area defined by the farm property boundary must be mapped. It is important to consider that in general, larger elements should be mapped rather than attempting to map small patches which do not have distinct boundaries.

To determine what an element is, the decision rules are as follows:

- 1. The Minimum Mappable Element (MME) for an areal element is 400 m² with minimum dimensions of 5 x 80 m.
- 2. If the element is smaller than 5 m it is recorded as a linear element with a Minimum Mappable Length (MML) of 30 m.
- 3. Elements that do not pass the MME or MML criteria can be mapped and recorded as point elements or as a stated proportion of a larger element.

Elements with a total extent that passes the MME criteria for an areal element and lie across the farm property boundary should be recorded as areal elements even if the part of the element that is within the survey farm is below 400 m².

If a linear element has 20 m inside the target farm and at least 10 m on the adjacent farm (i.e. total length is >30 m) it should also be recorded. It is not uncommon for linear elements to form complexes, with several distinct linear elements adjacent to each other, such as a hedge next to a ditch next to a track. (e.g. Figure 3.2).

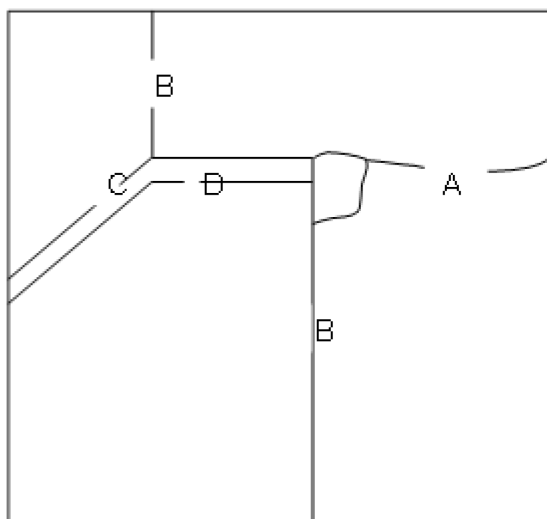


Figure 3.2

Map illustrating possible complexes of linear elements.

3.2.2 Recording of individual elements

The GHC method is based on Life Forms and Non-Life form categories with specific qualifiers. For European coherence in data, environmental conditions must be considered at a continental scale: e.g., 'dry' in Scotland may be 'mesic' compared with southern Italy (definitions are provided by EBONE on line). In order to avoid inconsistency field surveyors should make as many decisions as possible in the field and not postpone them to the laboratory. The creation of new categories is not encouraged, but when a major survey is underway surveyors should contact a central bureau to assign new classes. There are two types of data to be recorded: (a) the GHCs and (b) various qualifiers. Different sets of qualifiers can be developed for different regions and biomes.

The limited list of GHCs and specific rules to define them is designed to avoid a potential multiplicity of codes and mosaics and to provide a lowest common denominator for linking disparate datasets. The full spectra are recorded in field five. Elements are assigned **alpha codes** as identification codes that are the same on the map and on the corresponding recording sheet. All fields must have an entry in order to ensure that later database management can identify that an entry has not been omitted in error. In order to give as much information as possible about a GHC and the dominant species of mapped elements, field five of the data recording sheet is reserved to record these details for each alpha code that is used.

3.2.3 Recording form

A separate recording format and record sheet was used for areal and linear elements. The recording form for areal elements has an alpha identifier and eight subsequent recording fields (Bunce et al., 2011). The first entry is for the alpha code which links to the GHC. When recording, it is best to first fill in the alpha code, then fill in column 5 (full list of habitats) and then decide upon the GHC in column 2. The full list of 160 GHCs can be found in the EBONE manual (Bunce et al., 2011).

- The first field is for entry of the GHC.
- The second field is for entry of the global and the environmental qualifier, for expressing moisture regime and acidity variations between elements that otherwise may have the same GHC. Instruction on assessment of these qualifiers was included in the field training workshops (e.g., regional plant indicators).
- The third field is for entry of the site qualifiers to record other characteristics, e.g., geomorphology, geology, soil or archaeology, in order to express variation between elements that may have the same GHC.
- The fourth field is for entry of the management qualifiers to record managed characteristics, e.g., forest management, succession and recreation, expressing variations between elements that may have the same GHC,
- The fifth field is for entry of the full list of habitats within the GHC together with the major species and percentages,
- The sixth field is for entry of European Habitat classifications, including EUNIS, Annex I and other pan European classifications,
- The seventh field is for entry of Farmed and Non-Farmed features, if appropriate.

BioBio used a simplified form for linear features. It is likely that each project will develop a form appropriate to their requirements. Examples are given in Bunce et al. (2011).

3.3 Mapping areal elements

Areal elements are drawn on a separate map from the linear elements. Elements are assigned alpha codes as identification codes that are the same on the map and on the corresponding recording sheet. The detailed procedure depends on which recording sheet or field computer is being used.

Separate mapping elements that have identical data coding (i.e. entries in Fields 1 - 8) have the same alpha code; otherwise a new alpha code is used. Both the areal element registration and the linear/point element registration use the full alphabetic sequence for their alpha codes, i.e., both registrations can use 'A', 'B', 'C', etc. as their alpha codes. If using field computers the coding must be unique. In these cases the Codes A1, A2, etc.

3.3.1 Rules for separating map elements (i.e., new Alpha codes)

A new areal or linear element will be mapped and separated from adjacent or surrounding elements if any one of the following nine rules are true:

- A change in GHC.
- A change of more than 30% of a cover of a GHC.
- A change in environmental qualifier.
- A change in site qualifier.
- A change in the occurrence of point elements.
- A change in management qualifier e.g., a fence line or age of forest trees.
- A change of at least 30% in the cover of an individual species over the whole element.

- A change of at least 30% in any of the vegetated tree/ shrub (TRS) layers, if they are being recorded under forest canopies.
- A change in any other specified European habitat, especially the habitats of Annex I of the Habitats Directive.
- A change in the proportion in the Annex I habitats.

In lowland landscape separate fields are individually mapped, even though the boundaries may not be delimited by fence lines or grass strips. In most cases these are also marked as separated elements on the aerial photograph. These data are required for later spatial analyses.

3.3.2 Determination of the General Habitat Category

This section describes the rules for the determination of the GHC (i.e., the primary recording code) for areal elements. For the full list of GHCs see the EBONE Manual (Bunce et al., 2011).

Determination of the GHC is based upon a sequence of five dichotomous divisions (Figure 3.3) related to a set of six super-categories (Urban, Cultivated, Sparsely Vegetated, Tree and Shrubs, Herbaceous wetland and other Herbaceous) which determine the series of Non-Life Form Categories and Life Form Categories that can be used to identify the appropriate GHC. It is important to note that the GHCs are a restricted list for comparison between sites and not thousands of possible combinations. The full list of Life Forms and Non-Life Forms in Field five can be used for detailed comparisons.

The percentage cover of land surface for a given habitat is estimated from a vertical perspective that is the land cover is as seen from above, e.g., not that observed beneath a tree or shrub canopy.

3.3.2.1 Percentage rules for determining the GHC

For determining the GHCs there are only two percentage rules: over 70% for single GHCs or 40-60% for GHC's that are combinations of two habitats. An element with >70% cover of a single Life Form or Non-Life Form category is a GHC with a single code e.g., ART= Urban/Artificial or HEL= Herbaceous/Helophytes or a double code if the GHC belongs to the TRS supercategory e.g., FPH/CON and FPH/DEC.

Elements with 40-60% cover of two life forms or two non-life form categories belonging to the same super category or in case of TRS belonging to the same height category, are also GHCs, but with a double code, e.g., ROC/GVR or SHY/EHY or with a triple code if belonging to the TRS supercategory e.g. mixed Deciduous/Conifer Forest (FPH/DEC/CON). If there are equal proportions of life forms then rules to decide precedence are provided. The precedence will be given in the order of the GHCs as listed in Figure 3.4, e.g., if an element has a coverage of ART 30/NON 30/VEG 30/GRA 10, the GHC would be ART/NON with full percentages in Field 5.

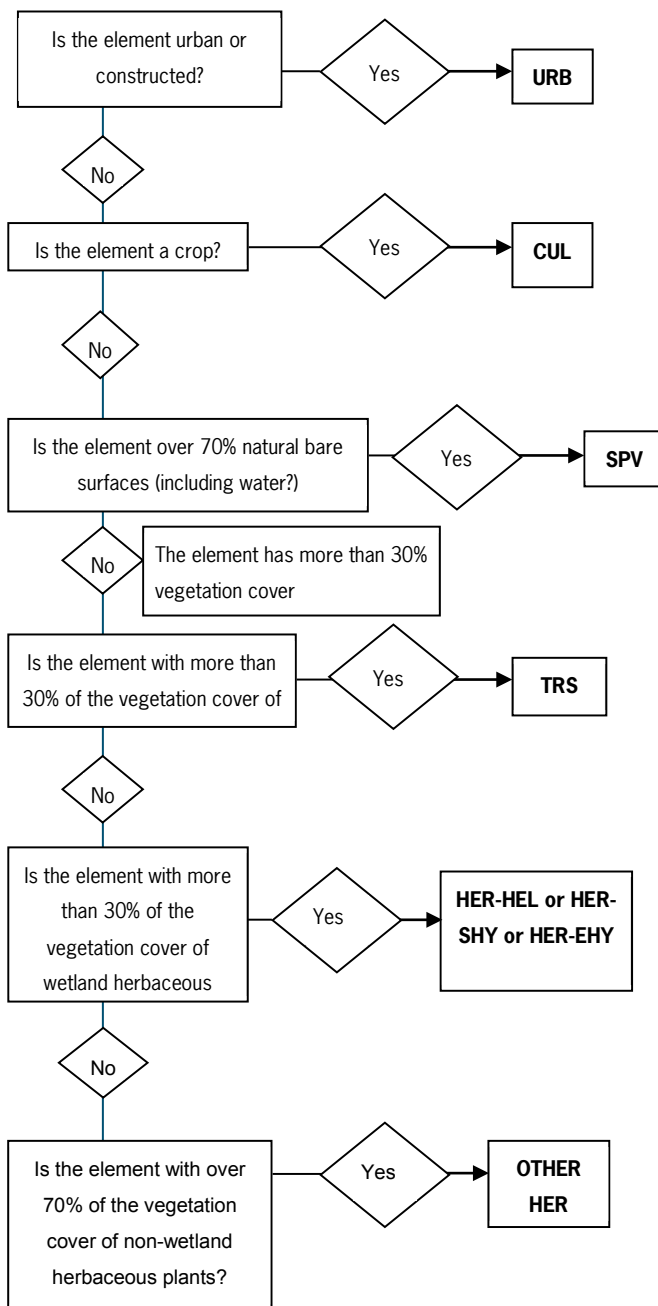


Figure 3.3
Decision tree for super categories.

3.4 Subdivision of general habitat categories

3.4.1 Field one: Rules for determining GHCs

All codes are unique. This means that on the recording form the first identifier URB, CUL, SPV, HER and TRS can be omitted to save recording time and space. GHCs may be Life Forms or Non-Life Form Categories, i.e. urban, cultivated or sparsely vegetated or combinations.

Non-Life Form Categories (Crops) will form an important part of the areal elements in the arable and horticulture areas. Note that the GHCs reflect the dominant plant cover. More complete information about the whole range of Life Forms can be obtained by analysis of the vegetation plots. Ellenberg values suggests that dominants can be more informative about the relationships between habitats and the vegetation. The Life Forms are based on the definitions available from plant morphology. Most users however, will not be familiar with the terminology involved so the descriptions have been made as general as possible. For example the 'leaves' of some *Acacia* species are actually modified shoots. In some cases also the strict morphological definitions have not been used in order to be as close as possible to the regression concept of Life Forms. The most widely used modification is of rhizomes, which in general act as organs of vegetative reproduction rather than overwintering.

There are further divisions in Non-Life Form Categories and Life form Categories with a subdivision in leaf type for the Tree and Shrub category is presented in Figure 3.4 (Bunce et al., 2011).

3.4.1.1 Urban/Constructed

The urban categories have aggregated life form, e.g. herbaceous (GRA) includes all herbaceous life forms e.g. Caespitose, Hemicryptophytes and Therophytes. The term urban applies to technically 'urban' or 'built-up' land, within the boundary of the land functionally related to buildings. The term is not based on life forms, but is a land-use division. Land is defined as urban, when it is *'an area of ground that is associated with a building and which has a use linked to that building e.g., garden'*.

The dominant function of the land should be considered, e.g. if an area is used as a camp site for two weeks a year and the other 50 weeks it is grazed by cattle and sheep then it is not urban.

Determining the urban boundary is difficult and the EBONE manual (Bunce et al., 2011) provides much detail. However it is a minor part in BioBio.

- **Urban artificial (ART):** includes all built up land, e.g. buildings, tarmac or other artificial material.
- **Urban Non-vegetated (NON):** includes all non-vegetated land that is within an urban boundary.
- **Urban Vegetables (VEG):** includes land that is under vegetables and/or fruit trees, within an urban area and includes, for example, allotments. Fruit trees over 2 m are included in TRE.
- **Urban Herbaceous (GRA):** within the urban definition and will include mainly grass e.g. playing fields.
- **Urban Woody (TRE):** includes fruit trees, as well as tall shrubs and trees. It may form an area around large houses.

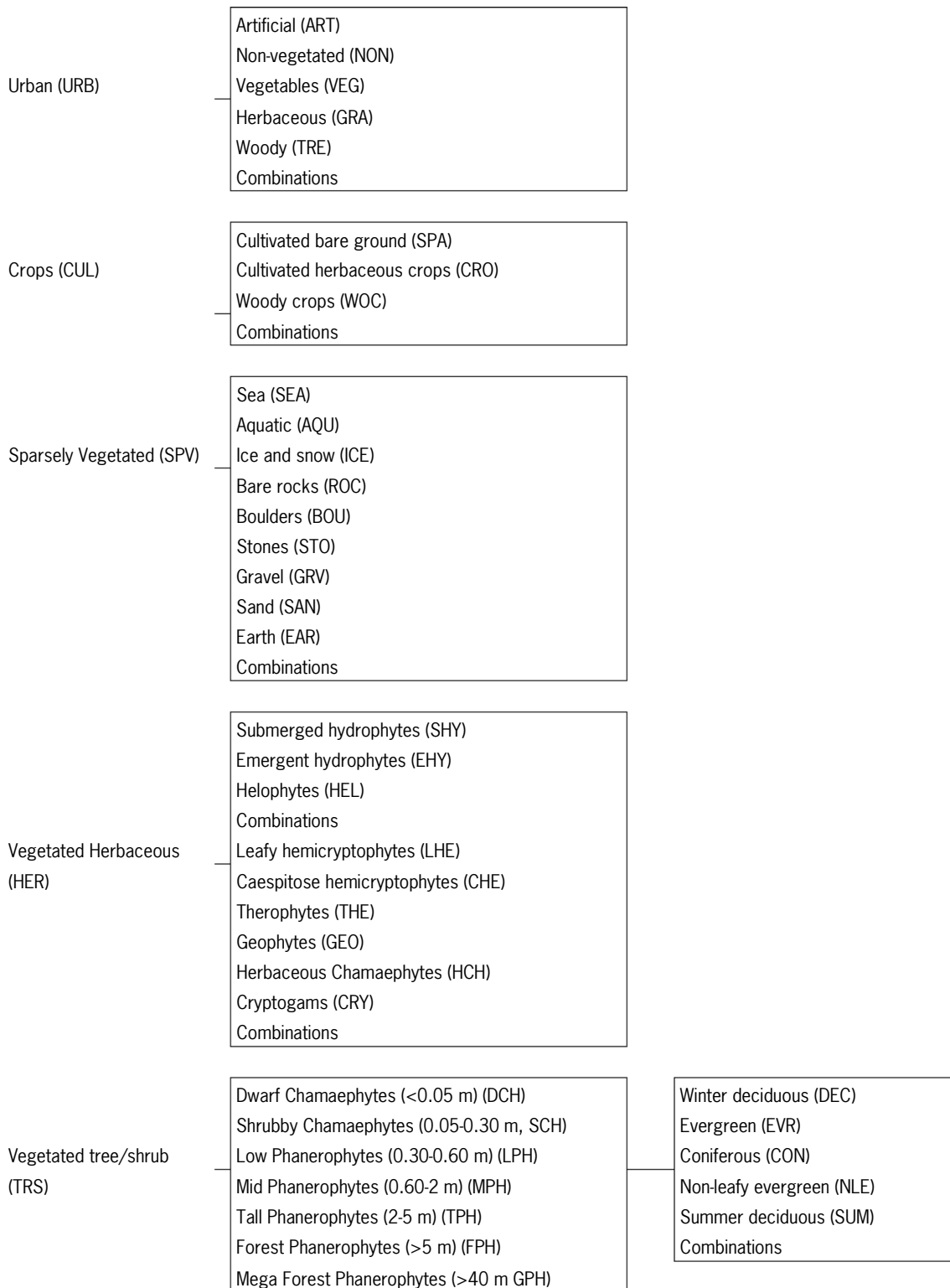


Figure 3.4

Diagrammatic representation of the GHC key.

3.4.1.2 Cultivated

Crops are mainly the product of plant breeding and are usually readily separated from their wild counterparts. Some native species such as walnut and carob are not distinct but should only be included as crops if they show definite evidence of having been planted. Wild species collected from semi-natural vegetation are excluded.

- The individual crops are recorded in the same way as plant species in field five. The percentages are not necessarily cover, but rather the percentage of the crop plants. If it is just recently sown or germinated the cover is a nominal figure. The percentages are needed because sometimes there are mixed crops, e.g., oats and barley.
- Land currently occupied by crops, or bare land with evidence of cultivation is recorded within the crop category with appropriate qualifiers.
- Crop land management is not always synchronic with maximum biomass. Therefore if the crop has been harvested recently and evidence of the actual crop is present, then it should be recorded as such. Dual cropping cannot therefore be recorded, but only the crop at the height of the season.
- If there is over 30% cover or crops in orchards, vineyards or olive groves it should be recorded in field five.
- If there is still evidence of cut stems in a crop even if there is over 30% cover of vegetation then it should still be recorded as crop. If the colonizing vegetation has smothered the crop stems, then it should be recorded as life forms only with a qualifier that there was evidence of former cropping e.g., plough lines
- Vines are regarded as abandoned if there is no evidence of pruning in the last five years.
- Olives and orchards are regarded as abandoned (see agricultural & semi-natural vegetation state management qualifiers) if there is no evidence of pruning, recent use, or collection of fruit.

The following GHCs have been defined to cover crop elements. The sequence provides the precedence rules as described below.

- **Cultivated bare ground (SPA):** elements with no crops planted or less than 30% cover of vegetation, including volunteers (self-seeded crop plants). Includes therefore only bare fallow or recently ploughed land which otherwise is recorded as a qualifier (EBONE Field Manual; Bunce et al., 2011) together with appropriate GHC. This code should not be used if the element has woody crops.
- **Cultivated herbaceous crop (CRO):** In BioBio a further division of four categories of herbaceous crops was made. (Table 3.2). The list of crops is not complete, so species can be added to the list when encountered. BioBio focuses on biodiversity at farm scale and therefore all biodiversity should be represented. The categories are now as narrow as possible and should yield meaningful results for comparison.

In BioBio the herbaceous crop category is sub-divided into four categories as the one crop category was considered to be too coarse. The division is based on two criteria: soil tillage affecting earthworm population and crops attracting insects (Table 3.2).

- **Cultivated woody crops (WOC):** includes all elements with trees or scrub, e.g., orchards, vineyards and olive groves. Cover cannot be used as a criterion to determine this GHC because of pruning. Therefore the rule is that there should be at least 20 trees/shrubs per ha, otherwise the scattered tree code can be used. Any vegetation cover, below or beneath the woody crop, over 30% should be recorded with appropriate life forms in field five.

Table 3.2

Division of crops in four BioBio categories.

Annuals, not entomophilic and/or bee attracting		Annuals, entomophilic and/or bee attracting	Perennials
Winter crops	Spring crops		
Winter oats	Spring oats	Oil seed rape	Fodder crops
Triticale	Beans	Sunflower	Lucerne
Winter barley	Spring barley	Maize	Asparagus
Beans	Peas	Soya	
Winter wheat	Lettuce	Cucumber	
Rye	Spring wheat	Tomatoes	
		Potato	
		Strawberries	

3.4.1.3 Herbaceous wetland

Examples of widespread species with short descriptions of all the following Life Forms are given in the Manual for Habitat and Vegetation Surveillance and Monitoring (Bunce et al., 2011).

- **Submerged hydrophytes (SHY):** plants that grow in aquatic conditions) the whole plant in water. This category includes marine species and floating species which overwinter below the surface.
- **Emergent hydrophytes (EHY):** plants that grow in aquatic conditions with the main plant above water.
- **Helophytes (HEL):** plants that grow in waterlogged conditions).

3.4.1.4 Herbaceous

Guidelines for the identification and details of widespread species with short descriptions are given in the Manual for Habitat and vegetation Surveillance and Monitoring (Bunce et al., 2011).

- **Leafy hemicryptophytes (LHE):** biannual or perennial broad leaved herbaceous species, sometimes termed forbs. Annual species are considered as **THE** (see below).
- **Caespitose hemicryptophytes (CHE):** perennial monocotyledonous grasses, sedges and rushes regardless as to whether they have rhizomes which in some floras are regarded as geophytes. Annual species are considered as **THE** (see below).
- **Therophytes (THE):** annual plants that survive during the unfavourable season as seeds.
- **Geophytes (GEO):** plants with buds below the soil surface, but without rhizomes.
- **Cryptogams (CRY):** bryophytes and lichens that are growing on the soil surface and some aquatic bryophytes, e.g., *Sphagnum* spp.
- **Herbaceous Chamaephytes (HCH):** cushion plants usually with perennial leaves.

The sequence above provides the precedence rules for equal proportions of life forms, i.e. CHE 30/THE 30/GEO 30/CRY 10, then the GHC is CHE/THE. The full formation is recorded in column five.

3.4.1.5 Trees and shrubs

Most of the following habitats are woody - the term usually used in habitat classifications - but some Chamaephytes e.g., *Phagnalon* spp., *Artemisia* spp. and *Asparagus* spp. do not have secondary ligneous

woody thickening in strict botanical terminology. However these genera have a shrubby form and have perennating buds above ground level. Height is therefore the only consistent arbiter (refer to Annex 2 of the Manual for Habitat and vegetation Surveillance and Monitoring (Bunce et al., 2011) for examples of plasticity).

There are seven divisions of trees/shrubs depending on height. Within each of these there are five divisions according to leaf type phenology. Detailed description are given by Bunce et al. (2011)

- **Dwarf Chamaephytes (DCH):** dwarf shrubs: below 0.05 m.
- **Shrubby Chamaephytes (SCH):** under shrubs: 0.05-0.3 m
- **Low Phanerophytes (LPH):** low shrubs, buds between 0.30-0.6 m.
- **Mid Phanerophytes (MPH):** mid shrubs, buds between 0.6-2.0 m.
- **Tall Phanerophytes (TPH):** tall shrubs, buds between 2.0-5.0 m.
- **Forest Phanerophytes (FPH):** trees between 5.0 and 40 m.
- **Mega forest phanerophytes (GPH):** trees over 40 m.

The following leaf subcategories are designed to fit into world biome systems and apply to the seven shrubs and trees categories. The groupings below are mandatory and are the major categories forming GHCs, as they are the lowest common denominators for classifying trees and shrubs.

- **Winter deciduous (DEC):** e.g., *Quercus robur*, *Fraxinus excelsior*.
- **Evergreen (EVR):** *Quercus ilex*, *Laurus nobilis*.
- **Conifers (CON):** *Pinus nigra*, *Juniperus communis*.
- **on-leafy evergreen (NLE):** e.g., *Sarothamnus scoparia*, *Ulex europea*.
- **Summer deciduous (SUM):** *Acacia* species, *Zyziphus lotus*

Precedence rules apply to **TRS** categories (Bunce et al., 2011).

The global codes **SCA** and **OPE** can be applied if the cover of trees and shrubs is below 10%.

The General Habitat Categories (GHCs) were designed as the lowest common denominator for integration of datasets of different national surveys. However it was realized when developing the EBONE protocols, that for correspondence with high spectral satellite imagery, some herbaceous categories needed further subdivision through information on environmental qualifiers, which is suitable and is also recorded in the standard EBONE procedure. It has been decided only to divide the pure grasslands (*Caespitose Hemicryptophytes* CHE) and the mixed-grasslands (*Caespitose* and *Leafy Hemicryptophytes*, LHE/CHE). This is not required for the other categories, because these have much more information on structure e.g., tall and dwarf shrub. The matrix is given in Section 3.2.3 of the field handbook (Bunce et al., 2011). Potentially this means that there could be up to 140 separate divisions of these two GHCs. In practice, in a given km square or farm there are only likely to be three or four such subdivisions. These subdivisions will be very important for biodiversity, e.g. mesic, neutral, mixed grassland will be very different in species composition from mesic, basic, mixed grassland. In Section 3.4.1.6 subdivisions of the GHCs will be considered as separate habitats and sampled accordingly.

3.4.1.6 Linear features and point features

1. In BioBio linear features are mapped based on a predefined list (Section 3.4.2).
2. The only point features that are identified in BioBio are ponds. They are marked in the field and marked with an X and a number.

3.4.2 Predefined list of linear elements and ponds

The list below defines the linear features to be recorded using the second procedure. The descriptions are based on the information in the field handbook of the Countryside Survey (2007), supplemented by European experience:

- **Ponds:** includes small areas of water below 400 m², both natural and artificial ponds. A temporary pond will have evidence of former water cover and is included in this category. In other surveys ponds are point features recorded optionally.
- **Walls (WAL):** includes dry stone, mortared and brick walls with or without capping, as well as earth walls and banks, but not levees.
- **Watercourses/water bodies (WAT):** includes seepage and spring lines with standing water, streams, rivers, canals, ditches of variable width with free standing water.
- **Lines of scrub (LSC):** includes lines scrub over 30 cm but under 5 m high with no evidence of management.
- **Hedges (HED):** has below 5 woody species per 30 m and includes lines of *woody tree and scrub vegetation* over 30 cm but under 5 m in height with evidence of positive management, whether coppicing, laying, flailing, cutting or pruning.
- **Species Rich Hedges (SRH):** The definition of a hedge is given above. Species Rich Hedges have 5 or more species per 30 m length.
- **Lines of trees (LTR):** includes lines of *trees over 5 m* in height whether spontaneous or planted. There may be an under-storey, but if this is managed, it should be treated as a hedge. They may have developed along field margins, beside walls, on steep banks or occasionally may be relicts of the original forest cover. They may also be present beside water courses/water bodies.
- **Herbaceous strips (HST):** includes grasses mixed with broadleaved plants or only broadleaved plants (LHE or THE) These comprise boundaries between crop fields as well as vineyards and olive groves. Strips of herbaceous vegetation under fences are included, if a different GHC to the surrounding land.
- **Grass strips (GST):** includes strips where grass is 70% of the vegetation cover.
- **Private roads and tracks with grass verges (TGS):** private roads and tracks are on farmland or within forests and are maintained by the owner.
- **Private roads and tracks with herbaceous verges (THS):** the definition is as above, but in this case the verge consist of mixed grass and herbs.

Note that recording the length of the hard surface of tracks is optional and can be done as a GIS exercise. Lastly note that neither GST nor HST are included under the canopy of trees and hedges. The EBONE Manual (Bunce et al., 2011) has modified this list on the basis of field experience in 2010 and 2011). There are also procedures for recording other point features.

3.4.3 Field two: Environmental qualifiers

Environmental qualifier codes are to be entered into the second field of the habitat recording sheets for areal and linear elements in order to express variation between elements that have the same GHC. They are not applied to urban/constructed, crop or sparsely vegetated elements. Global qualifiers may also be recorded in this field.

3.4.3.1 Moisture regimes

The categories below are based on the Concerted Action 'Water regimes for forest productivity' (Pyatt, 1999). The pF values are added for regional calibration of the used terms.

- **Aquatic:** covered in water over 70% of the time.
- **Waterlogged/water saturated:** water table at the surface with standing water for 60% of the year or with the soil completely saturated, only small patches may become only wet in mid-summer.
- **Wet:** water table with 40 cm of the surface and soil containing free water for most of the year.
- **Seasonally wet:** water table variable at the surface and waterlogged for the winter months or spring flooding season.
- **Mesic:** water table 40-100 cm of the surface, available water during most of the non summer period, may dry out during the mid-summer period.
- **Dry:** water table <100 cm of the surface, water available only during some periods.
- **Very Dry:** water table <100 cm of the surface, dry throughout most of the year.
- **Xeric:** water table <100 cm of the surface, dry throughout the year except in isolated rain events.

3.4.3.2 Other environmental conditions: Ellenberg values

Ellenberg et al. (1992) developed environmental indicators for Central Europe; they can be searched on the internet (Ökologische Zeigerwerte online). Ellenberg values have also been recalibrated for Britain (Centre for Ecology and Hydrology online). Some species change their ecological behaviour in different climate regimes. For many regions Ellenberg values are not available, so local experience of the ecological amplitude of species is needed, especially in the Mediterranean. The Ellenberg indicators used in the present context are fertility (eutrophy), acidity and salinity. The Ellenberg acidity value can be assessed by plant indicators, soil type or landscape context.

The matrix shown in Table 3.3 is the means of recording the environmental qualifier linked to a mapped element. The matrix consists of two primary axes, which largely determine vegetation composition i.e., humidity and nutrient content.

Table 3.3

Matrix and unique coding of environmental qualifiers. In general, acid is below pH 4.8; neutral is between pH 4.8 and 6.0; basic is over pH 6.0.

	Ellenberg values	Aquatic	Water-logged	Seasonally wet	Wet	Mesic	Dry	Very Dry	Xeric	Semi desert	Desert
Eutrophic	F > 7	1.1	2.1	3.1	4.1	5.1	6.1	7.1	8.1	9.1	10.1
Acid		1.2	2.2	3.2	4.2	5.2	6.2	7.2	8.2	9.2	10.2
Neutral		1.3	2.3	3.3	4.3	5.3	6.3	7.3	8.3	9.3	10.3
Basic		1.4	2.4	3.4	4.4	5.4	6.4	7.4	8.4	9.4	10.4
Saline low		1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5
Saline medium		1.6	2.6	3.6	4.6	5.6	6.6	7.6	8.6	9.6	10.6
Saline high		1.7	2.7	3.7	4.7	5.7	6.7	7.7	8.7	9.7	10.7

3.4.3.3 Global codes

Global codes for height/depth and substrate are codes that can be used as qualifiers in Feld 2. There are codes for absence of data in the EBONE manual (Bunce et al., 2011).

3.4.3.4 Other general codes

These codes can be applied to any GHC or element:

- BUR** = Burnt - can be applied to most life form categories. Use this code with the life form that was present according to residual material, e.g. forest trees or grasses.
- SCA** = trees/shrubs below 1% total cover but between 5 and 20 individuals/ha. Can also be applied to olives/fruit trees.
- OPE** = trees/shrubs 1-10% cover (e.g., *Dehesas*, *Montados* or parkland)

The appropriate GHCs should follow these codes. Note that cover of trees/shrubs over 10% but below 30% is included in field five.

Also note that where the vegetation cover is below 10% i.e., mainly in deserts then the percentage cover is of the actual cover present.

3.4.4 Field three: Site qualifiers

The site qualifiers are to be entered into the third field of the habitat recording sheets for areal and for linear elements to record characteristics of geomorphology, geology, soil, archaeology and life form complexity of elements, in order to express variations in these between elements that have the same primary code. Part of the definitions are provisional and need to be carefully researched further for pan-European application.

Geomorphologic classifications are in general made according to their relevance to the understanding of the genetic and historical development of the site, area or region. These morphological forms give limited information for assisting the understanding of the relationship between climatic/environmental conditions and the composition and distribution of plant life as indicators of climatic change.

Habitat complex site qualifiers are for use with elements that are widely recognisable and comprise a mosaic of patches of several GHCs of which the extent might be less than 400 m². These are situations where it would be difficult and time-consuming to make detailed mapping of each individual LF patch. They include some situations where this is also precluded by difficulty of access as for example in mires and fens. The primary codes for all the GHCs that occupy >30% of the element must also be recorded in the first field.

The definition of 'coastal' is that either there is a change in LF and management between the element next to the shore and inland or it is where the soil material has a recent marine origin. This definition separates coastal dunes from inland dunes and separates forests growing on rocks from those growing on marine sediments (sand, gravel and shingle). It is recognised that forests growing on bare rock surfaces would have to be covered by further qualifier e.g., wind pruned.

The list of Site qualifiers is given in the EBONE manual (Bunce et al., 2011), but other codes could be added if required.

3.4.5 Field four: Management qualifiers

The management qualifiers are organised in several levels, the first level being the time of the management, the second level are the general categories where management is taking place, e.g., forest or urban, and the third level is a more specific management activity. In some cases the third level is specified in a fourth level.

The list of Management qualifiers is given in the EBONE manual (Bunce et al., 2011), but other codes could be added if required.

3.4.6 Field five: Detailed life form and species composition

Field five of the areal element and the linear element recording sheets is to be used for recording of the full Life Forms and main plant and crop species associated with each recorded alpha code.

All Life Forms and Non-Life Forms that constitute at least 10% of the alpha code should be recorded, one per row, in the first column of Field-5, with the appropriate % code in the second column. Taken together, all recorded Life Forms and Non-Life Forms within a layer should add up to a total of 100%.

If there are several Life Forms with low % cover then the one with the highest % cover should be recorded.

The species that constitute at least 30% cover of the vegetation (as seen in vertical perspective) of each Life Forms that has been recorded in the first column of field five should be recorded in the third column of field five. If there is over 70% cover of the Life Forms by one species, just the one species is to be recorded. If more species have a cover over 30% then other species should be recorded. If no species reaches 30% then the two species with the highest cover should be recorded.

Separate rows in the recording sheet should be used for each species.

Flora Europaea nomenclature should be used if possible to name the species. (These can then be converted by database management into Flora Europea master codes (SynBioSys, www.synbiosys.alterra.nl).

If a plant species cannot be identified in the field, a specimen should be collected and identification later verified by an expert botanist.

Latin names are not to be used for crops but only the codes since the same species may refer to wild plants e.g., *Beta maritima* (sugar beet).

Other species should be recorded using the first three letters of the Genus name and the first three letters of the species name, e.g., *Galium aparine* as 'GAL APA', *Fraxinus excelsior* as 'FRA EXC'. Any ambiguities should be made clear by a comment in the 'Species codes and non-standard site and management qualifier codes' section of the recording sheet. For instance *Pinus pinea* and *Pinus pinaster* should be distinguished as 'Pin pin' and 'Pin pi1'. Cryptogams should be separated into percentage bryophyte and lichen cover.

The percentage cover of recorded species within each Life Form or Non-Life Form habitat should be recorded in the fourth column of Field 5. The % cover of the species should be given in each LF, i.e., **the percentages are of the Life Forms, not of the whole element.**

Feedback after application of the method in twelve case study regions

Strengths

Productive

General-purpose: comparable in a wide geographical perspective

Based on straightforward rules, but requires training!

Difficulties

The methodology can be quite complicated for mappers new to the system, so thorough training essential. The mapping handbook - in English - is a rather elaborate document for mapping experts who don't have English as their mother tongue, yet it is important to be familiar with the whole document to avoid mistakes and misunderstandings.

The mapping exercise has to be completed before any other work can be started.

Access to aerial photographs and good mapping software may be a limitation for some countries.

The decision to not include plots for species in the non-farmed features habitats (i.e. categories 2, 7, 8 in Table 3.1) caused a systematic bias and weakened the observed effect of habitat structure on species occurrence.

Practical hints

Training sessions in the field are needed and include all persons involved. On-site training in the specific areas concerned is preferable to a centralised training. The duration of the training is estimated at one week.

For real efficiency, habitat mapping requires two persons. One of them must be a competent field botanist or at least be able to identify the major plant species present. It is recommended that the other person is a GIS expert who will be digitising the maps later.

There is permanent field work in the mapping period, which could be made one year before the indicator sampling takes place.

A 'helpline' during the field season would be useful so that fieldworkers can phone in to check specific cases when they occur.

The use of a field computer would be a major advantage, if the mapping were to be a regular activity.

4 Farm-level measurements and information gathering 2010

4.1 Convention for labeling samples and data records

Wilkes J.², Herzog, F.², Lüscher, G.²

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland

A clear system for labelling all samples collected in the field survey under each protocol is essential. This must be hierarchical and requires the following elements:

- Date
- Summary code for each Case Study region and agricultural enterprise (Table 4.1)
- Farm code (unique identifier code to be provided by each CS partner)
- Habitat code based on description of farmland habitats in the EU (Table 3.1 derived from Jongman and Bunce, 2009)
- Sample code (abbreviated protocol names listed in Table 4.2)
- Name of personnel who collected the data

Table 4.1

Country codes to be used in field validation with associated identifier for agricultural enterprise.

Case Study country	Country and enterprise code
A: Austria	A_ ARA
F: France	F_ ARA
D: Germany	D_ MIX
W: Wales	W_ GRA
CH: Switzerland	C_ GRA
NL: Netherlands	L_ HOR
I: Italy	I_ VIN
E: Spain	E_ OLI
E: Spain	E_ DEH
BG: Bulgaria	B_ GRA
H: Hungary	H_ GRA
N: Norway	N_ GRA

Key to agricultural enterprises - ARA: Arable; GRA: Grassland; MIX: Mixed farming; OLI: Olive; DEH: Dehesa; HOR: Horticulture; and VIN: Vineyards.

Table 4.2*Sample codes for all vegetation and faunal survey samples and records.*

Protocol	Sample and associated indicator code
Vegetation	VEG - B2
Earthworms	EW - B4
Araneae - spiders	SPI - B8
Hymenoptera, wild bees	BEE - B9

4.1.1 Barcodes

In BioBio data for four indicators, such as vegetation (VEG), earthworms (EW), spiders (SPI), and bees (BEE), was collected for sixteen case studies in different EU countries and beyond. The indicators were identified centrally. In this sense and in order to be able to give each sample an ID, each sample was encoded with a barcode (windows font code 39). A barcode is an international encoding system, which can easily be applied.

In the barcode the following information was recorded: a unique ID number; country code (Table 4.1); farming system and/or agricultural enterprise (partner defined); number of farm (partner defined); code number of habitat patch, field or linear feature; code for sample protocol (Table 4.2) and associated indicator type (Table 1.1); number of indicator samples (e.g., earthworms (EW) were investigated in three samples in the same plot, in this example, a three samples from hand sorting and three from chemical extractant, a total of six samples will be taken) and date (see Table 4.3).

Table 4.3*Bar code composition.*

 *A1g_S1d2_157*		 *171Co1p6EW4bd1*	
A	Country	171	Unique ID number
1	Farm number (1-20)	C	Country code
g	plot (a-o)	o	Farming system
S	Indicator name (B: BEE, S: SPI, E: EW, V: VEG, G: GEN)	1	Farm number
1	sample number (E: 1-3, S: 1-15, B: 1-3, V: 1, G: still open)	p6	Number of field (plot)
d2	Date (d1, d2, d3)	EW	Indicator
157	Unique ID number (1-8100, 1-26100)	4b	Sample number
		d1	Date

4.2 Species-level measurements

Jeanneret, P.²

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland

On each habitat type selected for flora and fauna surveys, all species indicators were sampled:

- vegetation
- earthworms
- bees
- spiders

The spatial allocation for sampling aerial and linear plots is illustrated in the Figures 4.1 and 4.2, respectively.

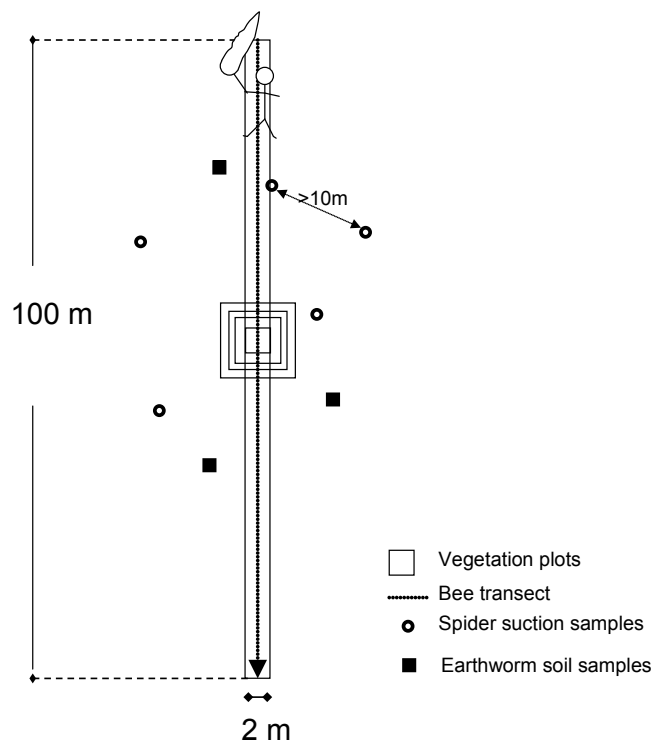


Figure 4.1

Flora and fauna sampling in areal plots.

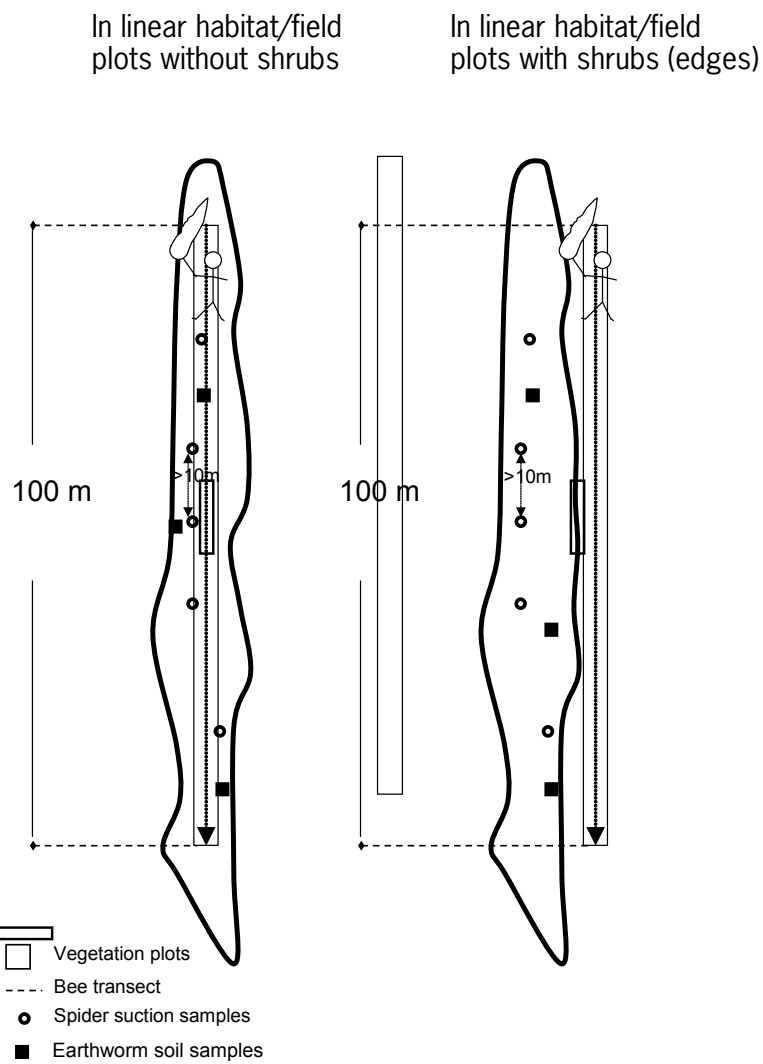


Figure 4.2
Flora and fauna sampling in linear plots.

4.2.1 Vegetation

Bunce, R.G.H.⁴, Geijzendorffer, I.R.⁴, Jongman, R.H.G.⁴

⁴(AL TERRA) Alterra, Wageningen UR, the Netherlands;

4.2.1.1 Preparation for vegetation recording

The Case Study farm is first mapped (Section 3) so that vegetation plots can be located. Preferably these are recorded immediately afterwards to save travelling time but in BioBio this was delayed as the mapping was carried out early in the season to enable early sampling of earthworms.

The procedure for recording vegetation plots uses two types of plots, square and linear plots. Square X-plots are placed in areal features (Figure 4.3) and linear L-plots are placed in linear features (Figure 4.4). The procedure below provides basic information on the species composition of vegetation within the GHCs in the sample squares and also allows estimation of quality for assessing future change.

If the position of vegetation plots is in dangerous terrain, then there are two possibilities. One is to move the plot to the nearest safe location within the element and the other is to recalculate a random position to select a different patch.

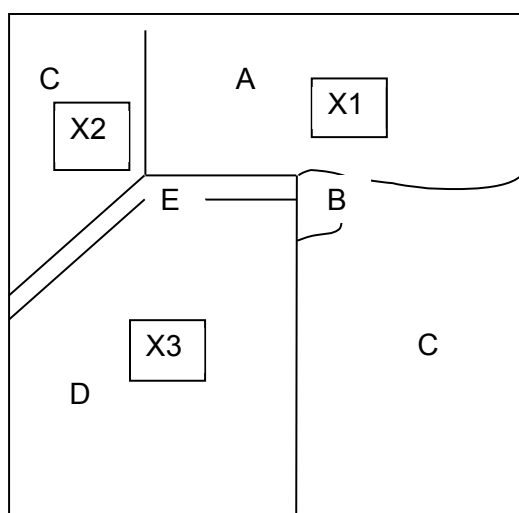
4.2.1.2 Procedure for random sampling for vegetation plots

1. Preliminary identification of fields of the farm based on the aerial photograph.
2. In the field, determine field boundaries and the GHCs of the fields.
3. For the GHCs that are only represented by one field, allocate the plot in the centre of the field or along the edge when a crop is present in the field.
4. If there are more fields of one GHC, number all the fields of one GHC, e.g. a_1 , a_2 , and a_3 . This should be done for all GHCs with multiple fields.
5. Randomly select one field per category using a randomization method and allocate the plot.
6. If there are less than five GHCs, take progressive random samples until five plots are selected for each farm. If there are less than five fields, randomly allocate additional plots in the fields present until five aerial plots are allocated.

4.2.1.3 Rules for allocating vegetation plots

The principle for allocating vegetation plots is to place **one plot in each GHC**, except in the case of grasslands (CHE and CHE/LHE) which need to be further subdivided according to the moisture and nutrient levels as indicated by the environmental matrix (see Table 3.3).

The subdivision in the grasslands is mainly because there are major differences in biodiversity between different types of grassland which therefore need vegetation data to define the detailed composition. In most farms there will be only one extra plot.



Location of vegetation plots

Example of location of X main plots

X1: in CHE field (A)

X2: random selecting from crop fields (C)

X3: in LHE/CHE field (D).

E and B do not have plots because they are Non-Life form habitats

Figure 4.3

Location of X main plots.

Dehesas can have ground vegetation dominated by Therophytes (THE) usually fallow, mixtures of LHE/CHE or herbaceous crops. Each one of these will be a different GHC if below 30% tree cover, but otherwise will be mapped as different elements because of different management. A separate X-plot should be put into each of such elements. See section on Trees and Shrubs in the EBONE manual (Bunce et al., 2011) for global codes to cover scattered shrubs, cultivated woody trees and shrubs and other trees.

In the following linear features vegetation will be recorded, if they are wider than 1 m:

1. Walls (including terrace walls)
2. Streams, rivers and lakes
3. Hedges
4. Grass strips between fields
5. Wood-hedges
6. Tracks on farmland

Plots should not be placed in any non-farmed land. Woodland grazed by domestic stock would therefore have plots but not ungrazed forest sites. Grazed woods will have a different alpha code from ungrazed woodlands as they are under different management regimes. This procedure is necessary to include grazed woodlands, which are integral features of many farm enterprises, e.g. in the UK and in Dehesas in Spain.

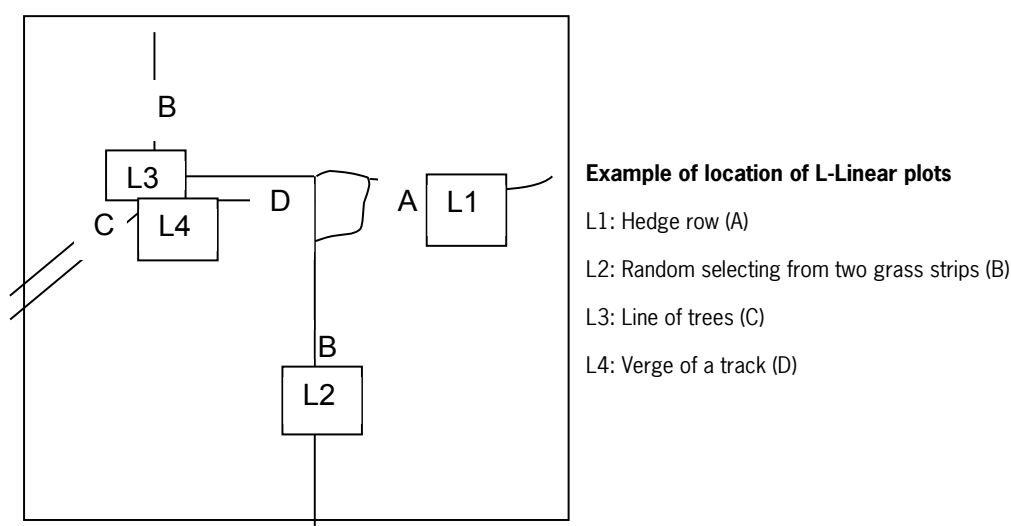


Figure 4.4
Location of L-linear plots.

4.2.1.4 Method for recording vegetation

The survey requires recording from different sizes of vegetation plots, depending on whether the plot is placed in an areal or a linear feature. The basic recording procedure is the same for all types of plots.

Samples are only included on land regularly or indirectly affected by farming as defined in Table 3.1. The location of the vegetation plots does not need to be recorded with GPS if monitoring is not part of the work schedule. Tracks on agricultural land are recorded and a plot should be laid out as in Figure 4.6. From the following categories in Table 3.1 no vegetation plots are recorded: categories 2, (Grassland used for non-agricultural purposes), 4 (Open land with casual grazing), 6 (Land indirectly affected by farming), 7 and 8. These categories are excluded from vegetation recording because they are not part of the main farm enterprise.

4.2.1.5 Rules for setting up X main plots

The X main plots (see Table 4.4, Figure 4.3) should be placed in the centre of the element concerned. The L linear plots (Figure 4.4) should be placed in the centre of the linear feature. In both cases to avoid edge effects. Examples are given here below:

- Header: information on the broad environmental and management attributes of the plot should be recorded using the environmental site and management qualifiers where appropriate.
- All vascular plants should be recorded, but no cryptogams (lichens or bryophytes). Epiphytes on rocks or trees should not be recorded.
- Species can be recorded using the first three letters of the genus and the first three letters of the species according to the Flora Europea, but always checking if species exist with the same abbreviations.
- On completion of recording of the whole plot, then the estimated cover % for the whole plot should be listed against each species, using 5% cover categories.

Table 4.4*Vegetation plot sampling strategy.*

Code	Name	Other names	Where	Size	BioBio	
Areal plots					On Farmland	Unfarmed land
X	Large	GHC plot	Centroid points in polygons	100 m ²	Yes	No
Linear plots						
A	Grass or herb strips		Arable field margins	10x1m	Yes	n.a.
H	Hedgerow		Alongside hedgerows	10x1m	Yes	No
S	Streamside		Alongside watercourses and water bodies	10x1m	Yes	No
T	Tracks		Alongside tracks on farmland	10x1m	Yes	No
O	Others: Walls		Alongside relevant features	10x1m	Yes	n.a

4.2.1.6 The main vegetation or X plot

A main vegetation or X plot is 100 m² in the centre of the GHC and is set up using survey poles with the strings forming the diagonals of the square as shown in Figure 4.5, Plate 4.1 left. This procedure was developed in the GB-Woodland Survey in 1971 and guarantees that the plots have an accurate size. The diagonals should be orientated carefully at right angles and the plot should be orientated with the strings on the north-south and east-west axes. The different nested plots are shown in Figure 4.5.

The strings or tapes should be of medium grade polyester that are unlikely to stretch. The half diagonals are 1.42 m, 3.54 m, 5.00 m and 7.07 m. and these should be laid out in the directions as shown in the diagram below. The objective of this layout is to ensure that the total area of the plots is always exactly correct, because trying to lay out square plots results in inaccuracies, as emphasised by Bunce and Shaw (1973). All species should be recorded from the inner nested plot first. When the inner plot has been completed the second nested plot should be examined and any **additional** species should be recorded. Each additional nested plot is examined in this way. Cover estimates are **only made for the whole plot** when all sizes have been completed. The standard practice in vegetation science is used i.e. only plants rooted in the plot are recorded, including trees and seedlings.

For estimates of cover it is necessary to constantly check between partners to avoid over estimates or under estimates. Total cover maybe over a 100% if several layers are present, e.g., *Pteridium* 100% over *Agrostis* 25%. Species with less than 5% cover are given a nominal cover of 1%. Bare ground includes leaf litter and rock.

If the plot falls in a field with a growing crop or hayfield, then the plot should be moved to the edge of the field. The new plot should be taken as a 100 m², (but estimated not measured, because the plot cannot be laid out) starting 3 m into the plot to avoid any edge effect. Access should be made using drill lines where possible and causing minimum disturbance to the crop or hayfield. A species list should be compiled from what can be seen in the crop.

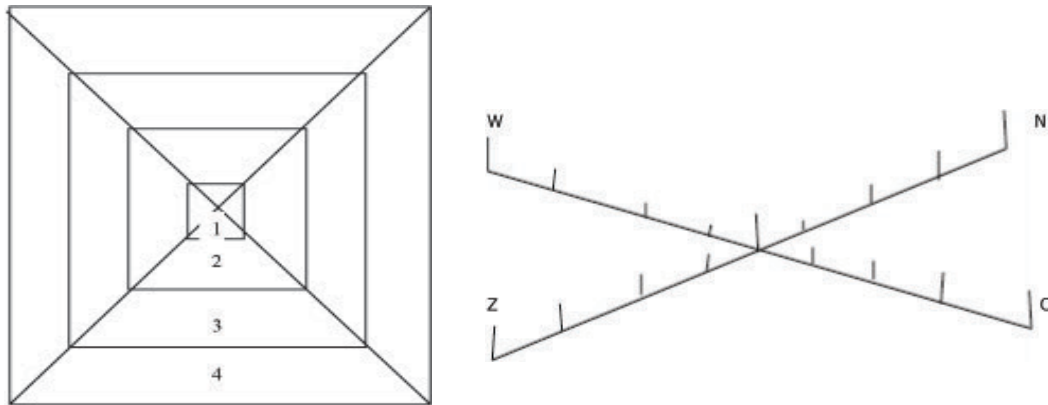


Figure 4.5

Design of the X plot (after GB Countryside Survey Handbook 2007). The length of the sides are in square 1: 2.00 m, 2: 5.00 m, 3: 7.07 m and 4: 10 m. This produces nested plots of respectively 4 m², 25 m², 50 m² and 100 m².

4.2.1.7 The linear plot

Plots from linear features are only recorded if the vegetation answers the criteria of a GHC which is different from the adjacent vegetation. For example, a strip of grass between crops could be LHE/CHE whereas the crop would be CRO. In the opposite case, a fence line between two grass fields would often have the same GHC as the fields themselves and will not be eligible for a linear plot, unless the strip of vegetation is different from the surrounding vegetation. The predefined list of linear features to be recorded is described in Section 3.4.2. In the case of a wall the width of the wall is not included.

In case of grass strips the plot is placed along the edge of the field and the plot is away from the crop edge into the strip. If the strip is over 2 m wide then the plot is placed as in a hedge plot.

The plot is placed according to the same randomization procedure as for the areal features. The side of the plot along the linear feature is determined according to the nearest large X-plot.

The plot is 1 x 10 m and is laid out along the feature as shown in Figure 4.6 and Plate 4.1 right. If the linear feature is less than 1 m wide, then the plot will extend into the field. In case of multiple boundaries a plot is placed in each linear according to the appropriate rules. However, plots cannot overlap; they should be placed 10 m apart.

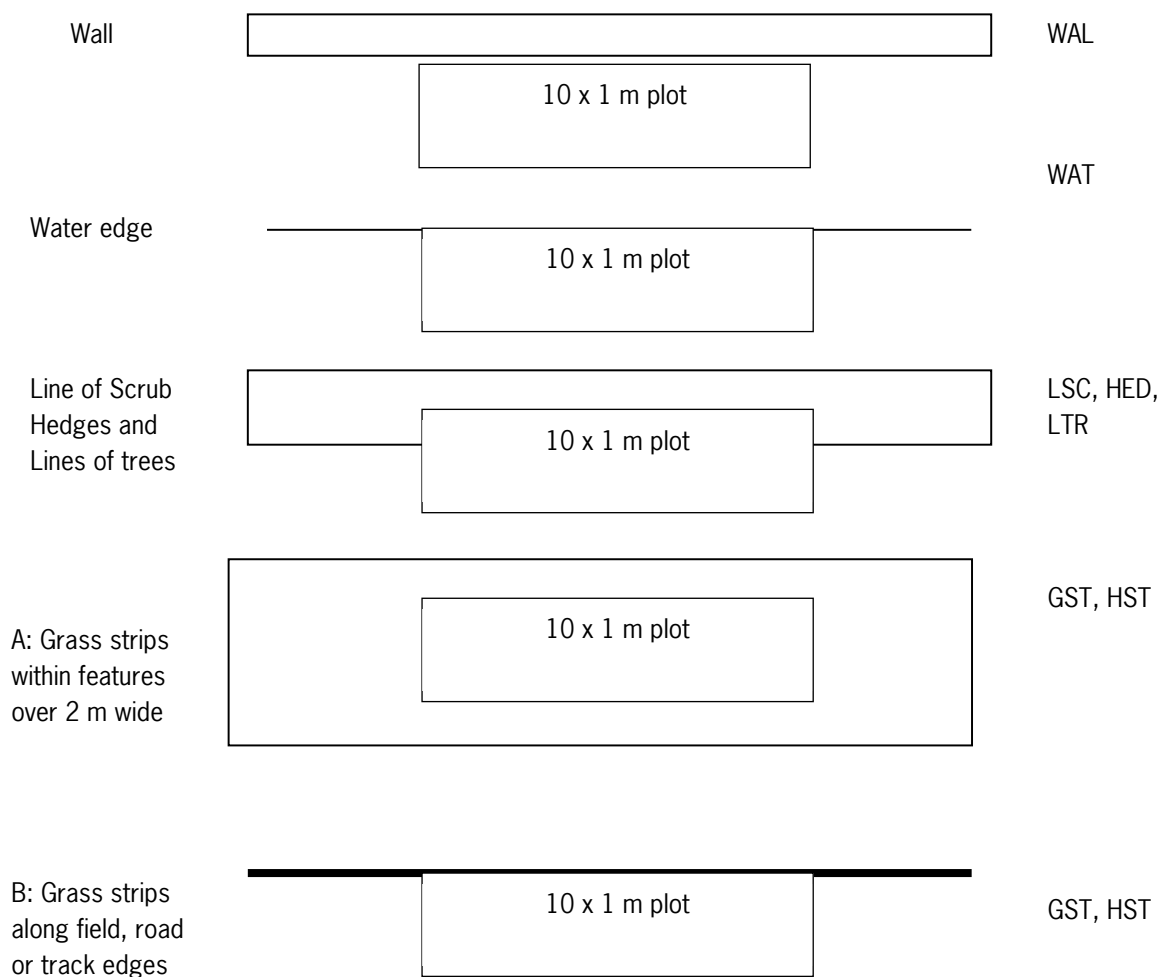


Figure 4.6

Location of plots along linear features, a hedge, wall, fence and grass strip. The plots are 1 x 10 m.

4.2.1.8 Laboratory processing of samples

The British Countryside Survey vegetation data has been analysed to produce a series of indicators which include some selected within BioBio. (Countryside Survey, 2007).



Plate 4.1

X plot and linear plot marked out ready for botanical assessment (Source: J.-P. Sarthou).

4.2.1.9 Format of data records

The format of the vegetation records will be an spread sheet with the following fields: code of the habitat/field plot according to the barcode composition, species list, cover of each species. In addition to the barcode composition, the surveyor name, the plot size (4, 25 and 100 m²) and the date have to be integrated (Bunce, 2011).

The spread sheet should get the name of the country, the farming system and include VEG (indicator). The vegetation data of each farm can be added on separate worksheets within the spread sheet file for quality control reasons. In that case all sheets should get a farm number. The unique identifier should be placed top row of the spread sheet. Selection and analysis of the vegetation plots will then be done when all data have been collected.

Feedback after application of the method in twelve case study regions

Strengths

The method is familiar to many field botanists and it is similar to the Countryside Survey method. The application is straightforward once trained and practised.

Representative of the local vegetation.

Flexible and weather independent. Rain will not affect the results, in contrast to sampling of other indicator species.

Difficulties

To give the complete list of plant species from a single field visit for certain native pastures is quite difficult.

A experienced botanist is required (e.g. more than 60 spp. in one grassland, over 500 spp. in total).

Not recommended to complete X plots in gorse and thorn shrub or tall bracken plots (health hazard).

Time consuming: four plots per day/person on average, but it increases with experience.

The assessment of % of ground covered by each species needs training and experience.

Frequent grass mowing in spring-summer and periodical herbicide application caused difficulties.

Practical hints

Some work needed to standardise names, but the website <http://www.synbiosys.alterra.nl> provided a very useful tool.

Use of expert staff with long experience of carrying out botanical surveys.

Plan a training period before the field work period, with a recognised skilled botanist.

Plausibility checks: Use existing species distribution maps to check the probability that a species can occur in the region.

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

Photograph N, E, S and W along botanical survey X plots or each direction of L plots so that invertebrate surveyors can accurately locate the plot according to alignment with landmarks given large error circle for handheld GPS.

4.2.2 Wild, domestic and bumble bees

Jeanneret, P.², Dennis, P.¹, Fjellstad, W.⁷, Franck, T.³, Sarthou, J.-P.⁵

¹(ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK;

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland;

³(BOKU) Division of Organic Farming, University of Natural Resources & Life Sciences, Vienna, Austria;

⁵(INRA) UMR Dynafor, INRA-ENSAT, Toulouse, France;

⁷(NFLI) Norwegian Forest and Landscape Institute, Ås, Norway

4.2.2.1 Introduction

Wild bees are widely used as indicators of change in land use and habitat quality and are sensitive to the timing and species composition of flowering plants in habitats. In addition, bees have specific requirements for nesting sites, such as dead wood, bare soil, plant stems or small rock cavities which must be close to feeding sites. Bees provide crucial ecological service in the agricultural landscape because they are considered to be the predominant and most economically important group of pollinators in most geographical regions. A full review of the characteristics of wild bees that makes them a suitable candidate biodiversity indicator is given in Dennis et al. (2009).

4.2.2.2 Summary of field sampling protocol

Sampling method: Transect walk with aerial netting.

Sampling location: In each habitat/field plots selected by the GHC method of case study farms.

Sampling location within the habitat/field plots:

100 m long x 2 m wide transect crossing the middle of the location of the vegetation relevé (see GHC method). When the plot is shorter than 100 m, 2 x 50 m (and 2 m wide) transects.

Sampling date: 3 surveys, dates depending on the CS region according to plant phenology.

Sampling procedure: Along the transect, bees are captured using a net during 15 minutes.

Timing: All habitat/field plots of the farms in each case study have to be sampled within 10 days.

4.2.2.3 Materials and methods

Bees are captured with a net (Plate 4.2). The aerial net method along transect ('belt') walks has been used for years in ecological studies (Banaczak, 1980; Westphal et al., 2008). Each habitat/field plot is surveyed by a slow walk along a 100 meters long and 2 meter wide transect crossing the middle of the location of the vegetation relevé (see GHC method, Section 3.1). In case of shorter plots than 100 m, 2 x 50 m transects are surveyed. The transect walk lasts 15 minutes (the speed of walking should then be of about 6-7 m per minute). While walking, the collector catches all individual bees seen within the 2 m wide 'belt' with a standard entomological aerial net (Plate 4.3). Captured specimens are immediately transferred into a kill jar, charged with ethyl acetate (or cooled rapidly in a cool-box if they can be transferred to a freezer within two hours; options detailed under Section 4.2.3 Spiders). The most direct approach is to bring the open kill jar into the

net and trap the bee against the netting¹. The killing jar should be a reasonably wide (ca. 10 cm diameter) jar of ca. 15 cm height with a sealed screw cap lid. A layer of 0.5 - 1.0 cm of cotton wool or lint can be packed into the base. A small volume of 1-2 ml ethyl acetate can be added directly to the cotton and once absorbed, a Whatman filter paper of the same diameter as the jar can be added to the surface of the lint to avoid wetting specimens in the interior of the jar. With the lid sealed closed, a lethal vapour will occupy the chamber. The trick is to transfer specimens quickly and reseal the lid before the vapour disperses. The jar will occasionally need to be recharged. The filter paper can be removed and a further 1 ml ethyl acetate added to the cotton before replacing the filter paper and continuing work.



Plate 4.2

Entomological aerial net ('student insect net', source: bioquip website).



Plate 4.3

Aerial net mobilised to capture bees encountered on the walked transect (Source: J. Wilkes).

¹ for more details about removing bees from the net, etc., download http://www.nbii.gov/images/uploaded/152986_1215796993084_Handy_Bee_Manual_Jun_2008.pdf

Specimens should be kept dry. When the transect is done, all bees are gathered in one jar with the label corresponding to the particular transect. In particular CS regions where collectors are trained to identify species in the field, bee species (e.g., bumble bees, domestic bees) will be recorded and then released. When bees cannot be identified immediately in the field or collectors are not willing to identify them or specimens resemble even vaguely bees, specimens are brought to the laboratory and then accumulated before dispatch to a taxonomist for identification. Particular attention must be put on bee species of Anthophoridae and to a lesser extent Megachilidae because they are wasp-like in appearance.

4.2.2.3.1 Field sampling protocol

Sampling should only be carried out between 10.00 and 19.00 hours on days that are sunny, not too windy and a temperature higher than 15 °C.

During the season, each plot of the farms is surveyed three times, the timing depending on local conditions (e.g., the Netherlands in May, June and July/August). It should be checked with a bee specialist what are the best three periods of sampling for bees in the region. Ideally, one habitat/field plot should be surveyed at different times of the day for each of the three sampling dates (the start point of the route has to be changed for each survey). If the transect walks are done by more than one person, habitat/field plots should not be visited by the same person three times (removal of systematic errors).

Basically, transect walks have to be carried out in habitat/field plots when vegetation is present. In hay meadows, transect walks should not be made shortly after meadows have been mown (> 15 cm vegetation height). In crop fields: transect walks should be made during the growing season of the cultivated plant.

The time-effort management is described in Table 4.5 for 20 farms.

Table 4.5

Effort per plot, per visit, per farm and estimation of total individuals collected for 20 farms.

BioBio Species indicators	Number of samples/plot ¹	Effort/sample (hr) ²	Effort/plot (8-hr d)	Number of visits	Number of plots/farm
B9) Hymenoptera, Wild Bees (BeeW)	1	0.33	0.041	3	15

BioBio Species indicators	Effort/farm (person.day)	N. of farms	Effort/visit (person.day)	Total (person.day)
B9) Hymenoptera, Wild Bees (BeeW)	1.85	20	12.3	36.9

BioBio Species indicators	N. of samples/CS	Sorting ³	Identification/CS ⁴	Cost Identification ⁵
B9) Hymenoptera, Wild Bees (BeeW)	900	14	3'600	2'340.

¹ One sample = wild bees collected along a transect walk of 100 x 2 m (or 2 x 50 x 2 m) during 15 minutes.

² Time allowance of 15 minutes + 5 minutes for transferring bees in vials.

³ No sorting necessary.

⁴ Estimated with 4 individuals per transect walk (Banaczak, 1980; Oertli et al., 2005).

⁵ Estimated with 0.65 EUR per specimen.

4.2.2.4 Laboratory processing of samples

In the lab, preparation of bees has to be acknowledged by the bee identifier. Some prefer having bees pinned, others not. Bees can be pinned directly from the jar into collecting boxes. Specimens are best pinned through the scutum between the tegula. If at all possible the pin should be to one side or the other of the mid-line. The midline of the scutum often contains features that are very useful in identification and these can be destroyed by the pin. If specimens are too small to be pinned they can either be placed on a point, glued to the side of a pin, or attached to minute double mounts². Bees have to be labelled so that the identification can without doubt be attributed to the specific GHC plot transect where the specimen was collected.

4.2.2.5 Format of data records

Two sets of records are provided after the transect walks have been done. In case all specimens collected are centrally identified, the second set of record does not apply:

- The field protocol of transect walks in form of an spread sheet with the following fields: transect walk code, observer's name, date, time of start of the netting, vegetation height, percentage cloud cover for that date, prevailing Beaufort wind code, Celsius temperature recorded, coverage of flowering plants (%) and main flowering species.
- The identification protocol in case specimens have been identified in the field, in form of an spread sheet with the following fields: transect code, date, identifier name, species list, abundance of each species.

If all specimens collected are centrally identified, only the field protocol will be provided by individual case study partner. A collecting box is then prepared with all the bees of each transect walk separately.

² for more details about pinning bees etc., download
http://www.nbii.gov/images/uploaded/152986_1215796993084_Handy_Bee_Manual_Jun_2008.pdf

Feedback after application of the method in twelve case study regions

Strengths

Good representation of the bee fauna.

Generally effective method if the observer was vigilant and observant.

Efficiency is fine: 8,8 plots per day and person, for each sampling round.

Equipment cheap and easy to use: Digital distanciometer, butterfly net, toolbox, containers, pins, labels.

Difficulties

Weather dependent so difficult to maintain to three 'tidy' sampling periods. When fields are at different altitudes and thus at different stages of vegetation development, this must be taken into account. The method needs good organisational skills.

Strongly depends on the worker's netting skill, and before on his/her aptitude for seeing the bees.

Due to intensive sampling during good weather periods, it was not possible to pin the bees within two days after capturing. Storing the bees in ethanol led to agglutinated hairs.

Identification has to be done by taxonomists. Difficult to find external specialists for identification of species. The minimum vegetation height of 15 cm evoked for some plots a race with the farmer. The time of mowing (depending on weather conditions) reigned our sampling schedule.

There are very few weeks in the summer exceeding the threshold temperature to carry out this protocol in northern Europe and in upland regions.

Practical hints

Useless to catch domestic bees, above all when very numerous and almost the single species. Instead it is better to count them. This is different for bumble bees: several species are very close to each other morphologically.

Avoid systematic sampling by field staff: Samples were randomly attributed to field staff so no systematic error could occur, i.e. a worker took samples of various habitats and farms. Avoid sampling one plot three times during the same time of day.

Judging the 100 m transect length was difficult. 50 m tape was used to complete the 100 m.

If there were too many bees taking too much time to put in the killing bottle, they were left in the net (its bottom closed with the free hand) until there were between 10 and 20 specimens in it, then the bottom of the net (like a little 'bee-ball') was put in the killing bottle in order to kill all of them at the same time. After that, the length of hunting was extended including the time used with the last killing task, and so on until hunting time reached 15 minutes.

Ask the taxonomist if the bees should be pinned or not. Pinning is very time consuming and for some species it makes identification more difficult.

4.2.3 Spiders

Jeanneret, P.², Dennis, P.¹ and Franck, T.³

¹(ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK;

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland;

³(BOKU) Division of Organic Farming, University of Natural Resources & Life Sciences, Vienna, Austria

4.2.3.1 Introduction

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).

4.2.3.2 Summary of field sampling protocol

Sampling method: Suction sampling with a modified vacuum shredder (Stihl SH 86-D, Andreas Stihl AG & Co. KG).

Sampling location: In each of the habitat/field plots selected by the GHC method on the case study farms.

Sampling location within the habitat/field plots:

Suction sampling comprises five sub-samples taken 'haphazardly' within each target vegetation plot. The sub-samples should be at least 20 m apart and ideally that distance but certainly no less than 5 m from a boundary with a different vegetation plot. The exception is for linear biotopes where sub-samples should be close to the mid-line but at least 10 m apart along the line feature.

Sampling date: Three surveys, two early summer and one late summer.

Sampling procedure: The sampling unit for comparison between vegetation plots is a single suction sample composed of material collected in five separate suctions or sub-samples that represent the extent of the vegetation plot. The ground area sampled by each sub-sample is 0.1 m² and material is collected with the modified leaf blower for 30 + seconds duration. The five suction sub-samples are collected in one gauze bag that is fixed into the end of the inlet nozzle to accumulate a single sample unit of total area 0.5 m². 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: The ambition is to complete the sampling of all areal and linear habitat/field plots of the full set of farms within a particular case study region within ten days for each of the three sampling periods.

4.2.3.3 Materials and methods

The method is adapted from Schmidt et al. (2005) and Schmidt-Entling and Dobeli (2009). Spiders are caught with a modified vacuum shredder powered by a two-stroke engine (Plate 4.4; Stihl SH 86-D, Andreas Stihl AG & Co. KG, D-64807 Dieburg, Germany, see Stewart and Wright 1995)(<http://www.stihl.de/>), each country has its own homepage, just substitute your country abbreviation as last two letters in the web address). A 50 cm

long, tapering gauze bag (mesh < 0.5 mm) is inserted into the 11 cm diameter intake nozzle to intercept the arthropods. This is retained by a ring of Velcro glued to the outside of the nozzle and also inside the hem of the bag. Please note that the nozzle end should be left at the angle provided by the manufacturer because the nozzle is held at an angle of ca. 35° when in operation.



Plate 4.4

Two-stroke engine (stihl sh 86-d).

On each of three sampling dates, a suction sample composed of five sub-samples is taken in each of the (up to) fifteen habitat/field plots selected from the GHC habitat map of each farm. Each of the five suction sub-samples is taken within a sample ring of 0.357 m internal diameter pre-placed on the target vegetation haphazardly within the habitat/field plot (each sample has a suction area of $0.1 \text{ m}^2 = \pi \times [0.357/2]^2$, total area per plot = $5 \times 0.1 = 0.5 \text{ m}^2$). The sample ring is 40 cm high³. In habitat/field plots with polygon form, the five suction sub-samples are located twenty meters apart from the border of the habitat/field plot and 10 meters apart from each other. In linear elements, the five suction sub-samples are taken along a line in the middle of the habitat and ten meters apart from each other. 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 minimum 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 visible (see Table 4.6). No samples are taken from bushes (edges) nor trees (orchards). The fabric net stays fixed to the nozzle of the leaf blower at all times unless wetted by rain or dew fall or torn from thorny vegetation or general wear and tear (Plate 4.5).

When a sample (consisting of the five pooled sub-samples) is completed, the engine is cut and the net contents inverted into a pre-labelled polyethylene zip-seal bag (Plate 4.6) and stored in a cool-box. For the purpose of the evaluation of the suction sampling method and to answer the question whether all five or perhaps as few as three sub-samples effectively represent the diversity of spiders in a plot, the five sub-samples from each plot should be stored in separate polyethylene zip-seal bags on at least one of the three sampling dates. The consistency of spider material in samples can later be investigated for specimens in three to five pooled sub-

³ 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.

samples. For this, the engine needs to be stopped and restarted after each sub-sample of 30 seconds. Spiders are sampled on three occasions (Table 4.6).



Plate 4.5

Fitting sampling net to leaf blower nozzle prior to sampling (Source: J. Wilkes).

Permanent habitats

Sampling 1: spring; the first sampling period starts two weeks after 90% of *Taraxacum officinalis* flowers are in bloom⁴ (or a similar species where it does not occur, e.g. in Spain);

Sampling 2: early summer; the second sampling period takes place four weeks⁵ after sampling 1.

Sampling 3: late summer; the third sampling period takes place 18 weeks⁶ after sampling 1.

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 and 2, like other habitat/field plots; Sampling 3, eight weeks after sampling 1.
- Beet, potato and corn ('late' crops): Sampling 1, six weeks after 90% of *Taraxacum officinalis* flowers are in bloom; Sampling 2, 9 weeks after sampling 1; Sampling 3, twelve weeks after sampling 1.

⁴ In the Swiss lowlands (500 m elevation), it corresponds to a period between 15th and 30th April.

⁵ In the Swiss lowlands (500 m elevation), it corresponds to a period between 15th and 31st May.

⁶ In the Swiss lowlands (500 m elevation), it corresponds to a period between 1st and 15th September.

Sampling is carried out by 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 ten days in all fields/habitat plots of the 20 farms.

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 Tscharrntke, 2005).



Plate 4.6

Suction sampling within guide ring and transferral of specimens from net to zip-seal polyethylene bag (Source: J. Wilkes).

The time-effort management for 20 farms is described in Table 6.8.

Table 4.6*Timetable for three sampling periods of spiders in different habitats.*

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														3
Cereals		1					2				3										
Rape		1					2			3											
Beet							1									2					3
Potato							1									2					3
Corn							1									2					3

Table 4.7*Effort per plot, per visit, per farm and estimation of total individuals collected.*

BioBio Species indicators	N. of samples/plot ¹	Effort/sample in hr ²	Effort/plot in d (8-hr d)	N. of visit	N. of plots/farm ³
B8) ARANEAE - SPIDERS (Spid)	1 (of 5 sub-samples)	0.025	0.016	3	15

BioBio Species indicators	Effort/farm (person.day)	N. of farms	Effort/visit (person.day)	Total (person.day)	N. of samples/CS ⁴
B8) ARANEAE - SPIDERS (Spid)	0.72	20	4.8	14.4	2'100

BioBio Species indicators	Sorting in d (8-hr d) ⁵	Identification/CS ⁶	Cost Identification /CS ⁷
B8) ARANEAE - SPIDERS (Spid)	70	12'375	8'044.-

¹ a sample = spiders collected with a vacuum shredder with 30 sec. suction within a 35.7 cm diameter ring with the 11 cm diameter intake nozzle (sampled area = of 0.1 m², total area per plot = 0.1 x 5 = 0.5 m²).

² estimated with 30 seconds suction and 60 seconds processing.

³ estimated according to tests with the GHC method.

⁴ estimated with separate sub-samples on one of three sampling rounds.

⁵ estimated with 7.5 minutes per sample.

⁶ estimated with 27.5 individual per m² (Schmidt and Tschardtke, 2005).

⁷ estimated with 0.65 EUR per specimen.

4.2.3.4 Laboratory processing of samples

Back to the lab, the five samples (= five zip-seal bags) per habitat/field plot 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 pencilled label with sample details can be added to the solution and the same information should be added to an external adhesive label.

4.2.3.5 Format of data records

If all specimen collected are centrally identified, only the field protocol will be provided by individual case study partners. The field protocol of the suction sampling in form of an spread sheet contains the following fields: habitat/field 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. If specimens are identified by individual case study partners, the identification protocol in the form of an spread sheet with the following fields will be provided: sample code (five different codes for each habitat/field plot and survey), date, identifier name, species list, abundance of each species).

Feedback after application of the method in twelve case study regions

Strengths

Straightforward and repeatable.

Relatively simple and quick.

No particular expertise is required for sampling.

Suction was carried out in a short period. One person could work alone efficiently.

About nine plots per day per person, for each sampling cycle.

Difficulties

Difficult in high vegetation.

To transport bags with litter plus spiders to the laboratory has logistical problems, because of the need for cold conditions in all the bags, and spiders can deteriorate.

Any residual moisture in the vegetation, either within wetland habitats or because of overnight dewfall or rain showers either halts or impedes sampling. Nets need to be changed over and specimens have to be carefully removed from wet netting to avoid cross-contamination between sample plots.

The leaf blower method incorporates large amounts of plant litter and debris which takes much time to sort in the laboratory.

It was difficult to find external specialists for identification of species.

The minimum vegetation height of 15 cm conflicted with the time schedule of the farmer. The time of mowing depends on weather conditions) and determine the sampling schedule.

The frozen spiders were sorted from plant bits and other debris in the laboratory. This task involves a considerable expenditure of time.

Practical hints

Training: all technicians and scientific staff need one day training for sampling spiders according to the protocol in different types of habitats and vegetation covers.

Two teams are necessary to maintain the sample needed interval.

Systematic sampling by field staff should be avoided. Samples should be taken randomly and attributed to individual field staff so that no systematic error can occur, i.e. field staff took samples of various habitats and farms.

The spiders were sorted after each extraction in the field. All the material from one extraction (out of five) into a large plastic box (more or less 50x40 cm and 35 cm deep) and the living so moving spiders were easy to see. Two or three persons were used to sample: one sucking up the material, one or two sorting the material (one plastic box per sorting worker).

4.2.4 Earthworms

Pelosi, C.⁵, Jeanneret, P.², Dennis, P.¹, Friedel, J.K.³, Ehrmann, O.³, Kainz, M.⁹, Moreno, G.¹⁰, Paoletti, M.G.⁸, Papaja-Hülsbergen, S.⁹, Sarthou, J.-P.⁵, Siebrecht, N.⁹ and Wolfrum, S.⁹

¹(ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK;

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland;

³(BOKU) Division of Organic Farming, University of Natural Resources & Life Sciences, Vienna, Austria;

⁵(INRA) UMR Dynafor, INRA-ENSAT, Toulouse, France; Italy;

⁸(UP) Department of Biology, Padova University, Italy;

⁹(TUM) Centre of Life and Food Science, Weihenstephan, Technical University of Munich, Germany;

¹⁰(UEX) Forestry School, University of Extremadura, Plasencia, Spain

4.2.4.1 Introduction

Earthworms are key soil detritivores, essential for composting and recycling soil nutrients whilst contributing to the maintenance of soil structure. The role of earthworms in enhancing soil fertility is well known and farming practices have considerable effects on both earthworm abundance and species composition. Earthworms can be divided into three eco-physiological categories: (1) leaf litter/compost dwelling worms (epigeic), (2) topsoil or subsoil dwelling worms (endogeics); and (3) worms that construct permanent deep burrows through which they visit the surface to obtain plant material for food, such as leaves (anecic). Anecic species which are large, vertically burrowing earthworms building up stable burrows play an important role in conservation and improvement of soil structure. Earthworms form the base of many food chains and all these aspects which led to their selection as a biodiversity indicator are reviewed in Dennis et al. (2009).

Earthworm sampling should preferably be carried out during cool and wet seasons. Most earthworm species are adapted to a particular habitat although earthworms can live in litter, soil, wet mud, submerged mud, organic manure, composts, dung, under bark and on rotted wood.. One active collection system consists of hand sorting from soil cores of 30 x 30 cm² dug to a depth of 20 - 50 cm with a spade. Digging deeper than 20 - 30 cm into the soil yields few specimens but sometimes reveals interesting deep-burrowing species. To assess populations of deep-burrowing and larger specimens, irritant solutions can be used to stimulate the earthworms to come to the soil surface, thereby facilitating collection. One particularly effective technique involves the application of aqueous formaldehyde solution onto 50 x 50 cm² of soil.

4.2.4.2 Summary of field sampling protocol

Sampling method: Extraction with an expellant solution (diluted allyl isothiocyanate: AITC) and then hand sorting.

Sampling location: In each habitat/field plots selected by the GHC method on case study farms.

Sampling location within the habitat/field plots: Three samples (30 cm x 30 cm x 20 cm deep) haphazardly.

Sampling date: One survey in spring when soil is moist.

Sampling procedure: Two litres of a solution of AITC is poured into a metal frame (30 x 30 cm) twice at 5 minutes interval. Earthworms appearing at the surface are collected. A soil core of 30 cm x 30 cm x 20 cm deep is extracted and earthworms are hand sorted from the soil during 20 minutes by one person.

Timing: All habitat/field plots have to be sampled within a 40 day period during spring 2010.

4.2.4.3 Materials and methods - field sampling protocol

In spring, three samples of 30 cm x 30 cm x 20 cm deep (up to) each are taken in each of the habitat/field plots selected by the GHC method of the farms. Soil needs to be humid. In habitat/field plots with polygon form, the three samples are located 20 m apart from the border of the habitat/field plot and 10 meters apart from each other. In linear elements, the three samples are taken along a line in the middle of the habitat and ten meters apart from each other. The three samples are located so that at least an area of 10 m x 10 m (linear elements: 1 x 10 m) in the habitat/field plot is not destroyed for future vegetation relevés.

The method is adapted after Zaborski (2003) and Pelosi et al. (2009). A combined method should be used to extract earthworms, namely extraction with an expellant solution and hand sorting of earthworms from a soil core.

Activities to be carried on before the field work:

- Prepare all materials needed (sampling equipment, depending on the number of persons; for two persons in the field: three metal frames, two scissors, one container to measure 2L, two spades or bar spades, containers with labels, two white plastic sheets, two plastic boxes (~60L), two tweezers, plastic gloves, two graduated rulers).
- Prepare an allyl-isothiocyanate (AITC) solution diluted with ethanol 70° to give a 5 g/l solution, shortly before going into the field (in the morning for instance, to prevent loss of irritating activity).

In the field:

- Locate plots and sampling sites (e.g., according to 'Placement of sampling sites' proposal, see below) but avoid trampling of sites.
- Dilute this solution with water to reach a concentration of 0.1 g/l (Plate 4.7 top right).
- Clean sampling site from vegetation or leaves carefully (with scissors, not by uprooting; Plate 4.7 top left).
- Place the metal frames (30 cm x 30 cm) on the soil and driven into the ground to a depth of approximately 1-2 cm to prevent the chemical from running off the sampling site. Avoid too much tremor if possible (Plate 4.7 top right).



Plate 4.7

Process of sampling earthworms by chemical extraction (Source: P. Dennis and J. Wilkes).

- Stir up AITC solution and apply 2 x 2L of AITC solution (two applications with 2 l at approx. 5 min. interval) per sampling site (Plate 4.7 lower left).
- Collect the earthworms that come to the surface during a 10 min. period after the first pouring. After the earthworm has left the soil completely use tweezers to put emerging specimens in a container with cold water to clean from AITC solution (Plate 4.7 lower right).
- NB. Use one container for earthworms collected with expellant application and another for hand sorted earthworms (two sub-samples).
- After 10 min. extract the soil cores of the sampling sites. Dig the exact dimension of the metal frame (30 cm x 30 cm) and a depth of 20 cm using a spade or bar spade (less damage to worms but more difficult to dig a straight hole; Plate 4.8 top left and right). In case this depth cannot be reached (stones, etc.), the depth should be recorded.
- Put the core on a white plastic sheet that is big enough to prevent earthworms from crawling away.
- Earthworms are hand sorted during 20 min by one trained person (Schmidt, 2001).
- Specimens are put in containers with cold water to clean from dirt. Use one container for each sample site (each sample has to be kept separated; Plate 4.8 lower left and right).
- Put specimens in labelled (name of the farm, habitat, sample, extraction method, name of collectors, notes if needed) containers with cold oxygenated water (Bartlett et al., 2006) or wet paper towels (Zaborski, 2003) and take them to the laboratory in a polystyrol container (no glass containers have be used), two for each sample site (each sample site and extraction method has to be kept separated).
- Put soil cores back in place.

To save time, it is possible to work on two or more samples in parallel: put two metal frames on the soil simultaneously; cut vegetation in both, pour expellant in both; move from one sample to the other during the ten minutes. After ten minutes, start digging the site that was poured with AITC first, put the soil in a plastic box and begin to hand-sorted the second site.



Plate 4.8

Soil sampling and sorting to extract earthworms (Source: P. Dennis and J. Wilkes).

The time-effort management is described in Table 4.8 for 20 farms.

4.2.4.4 Laboratory processing of samples

- Keep the sample in a refrigerator at 3-5 C°.
- Within one week after sampling each sample should be processed.
- The sampling has to be energetically washed using a kitchen colander under running water to remove remaining soil and gut content from the earthworms.
- Sorting, identification and counting is done under laboratory conditions by local experts.
- The surviving earthworms can be released but specimen copies should be kept for quality assurance.
- If earthworms are going to be identified by external taxonomist (centralized) 80% ethanol solution has to be used.

Adult earthworms can be identified to species level although it may not be possible to identify juvenile specimens with certainty. The numbers of each species will be aggregated for each part of the sample to achieve the best estimate of species richness but separate records of species and numbers will be kept to assess the efficiency of the combined method. So, each of the three 'soil core' samples and three 'AIRC' samples per habitat/field plot will be labelled separately.

4.2.4.5 Format of data records

If all specimens collected are centrally identified, only the field protocol will be provided by individual case study partners. The field protocol of the extraction and the hand sorting in form of a spread sheet contains the following fields: habitat/field plot code, observer's name, date, time of start of the AIRC application of the first sub-sample in a habitat/field plot (one record per plot), digging depth of each sub-sample, vegetation height (on average for the habitat/field plot), observation of nutrient input (yes/no, for example liquid manure), optionally the soil temperature and humidity.

If specimens are identified by individual case study partners, the identification protocol in the form of an spread sheet with the following fields is provided: sample code (six different codes for each habitat/field plot), date, identifier name, species list, abundance of each species. Sample data are transformed to record earthworm number (and biomass; optional) per square metre, so that comparison among different plots and farms can be carried out.

Table 4.8*Effort per plot, per visit, per farm and estimation of total individuals collected.*

BioBio Species indicators	N. of samples/plot ¹	Effort/sample in hr ²	Effort/plot in d (8-hr d)	N. of visit	N. of plots/farm ³
B4) EARTHWORMS (EW)	3	0.67	0.251	1	15

BioBio Species indicators	Effort/farm (person.day)	N. of farms	Effort/visit (person.day)	Total (person.day)	N. of samples/CS
B4) EARTHWORMS (EW)	3.77	20	75.3	75.3	900

BioBio Species indicators	Sorting in d (8-hr d) ⁴	Identification/CS ⁵	Cost Identification /CS ⁶
B4) EARTHWORMS (EW)	9	16'200	10'530.-

¹ a sample = a subsample of earthworms collected with expellant application within a 30 cm x 30 cm and a subsample of earthworms hand sorted from an excavated core of soil 30 cm x 30 cm x (up to) 20 cm deep. Subsamples are kept separately.

² for two samples, one person: ten min for installing + 15 min for chemical + 15 min to dig + 40 minutes for hand-sorting. Hand-sorting per sample = 20 minutes.

³ estimated according to tests with the GHC method.

⁴ estimated with five minutes per sample.

⁵ estimated with 18 specimen per sample (200 specimen per m²).

⁶ estimated with 0.65 EUR per specimen.

Feedback after application of the method in twelve case study regions

Strengths

A straightforward procedure that can be performed without special expertise.

The procedure was in general performed well. There were no specific demands on staff.

It can be carried out in any weather conditions, even though extended working days using head torches.

The protocol is straightforward and gives few opportunities for sampling biases caused by human error.

While AITC is draining away, it is possible to carry out other tasks in the field.

Twenty minutes to sort out the earth was at the right stage in the given situation (rich in earthworms).

Equipment was easy to use: metal frame, 30-L tank, 2-L bottle, toolbox, spade, plastic, alcohol, containers, gloves, AITC, maps.

Difficulties

The procedure was time consuming due to long period per plot. Heavy loads of water needed to be carried to each plot implicating staff health and safety. It was physically exhausting work. The fieldwork should not be done alone.

Water + AITC application is very uncomfortable in clay soils. The treatment takes a long time (up to fifteen minutes) for the liquid to seep into the soil. Very uncomfortable to separate the soil and find the earthworms (soil much more compact, earthworms hidden, takes very long).

There are difficulties on slopes. The heavy loads are a disadvantage at more remote sites.

The procedure is slow with about three plots per person per day.

AITC handling in the lab using a flue is a requirement, because of safety issues. Low shelf-life of the solution is a hindrance: solutions must be prepared all over again for each day of work. Unused material cannot be applied later.

It is difficult to find external specialists for identification of species.

Practical hints

Working in groups of three persons was more efficient, but two people are also possible.

The interval between habitat mapping and earthworm sampling is likely to be a serious drawback in dry areas of Central Europe, where periods for both surveys coincide in April. Ideally, earthworm sampling should be concluded by mid-April. An improvement would be to split sampling periods: spring sampling of farmed habitats (e.g. arable fields), autumn sampling of permanent habitats.

Filling the liquid in appropriate vessels was an important part of preparation before going to the field. Staff has to be aware of the time needed for preparation.

4.3 Genetic indicators – questionnaire

Last², L. and Kölliker, R.²

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland

A comprehensive set of indicators for the detection of biodiversity in organic and low input farming systems must include measures of genetic diversity within species. However, reliable detection of genetic diversity is generally laborious, often technically demanding and can be difficult due to the lack of information about breeding pedigrees and seed sources. Therefore, in the framework of the BioBio project, a detailed analysis of genetic diversity of all aspects concerning agricultural ecosystems is impossible. However, based on a PhD project we will evaluate the indicators outlined below mainly using on-farm surveys. The experimental part of the PhD thesis will focus on the detection of genetic diversity in grassland ecosystems based on a single model species (*Dactylis glomerata*) in order to provide information about the use of indirect indicators such as habitat diversity and / or management practices for estimating genetic diversity in grassland ecosystems.

4.3.1 Indicators for plant genetic diversity

A4 CultDiv-I	Number and surface covered by and origin of cultivars, landraces and wild species of arable crops, trees and vegetables grown on farm (Questionnaire)
A5 CultDiv-II	Number and surface covered by and origin of cultivars, landraces and wild forms of forage grass (grassland) grown on farm (Questionnaire)
A6 SeedMulti	Methods of seed management performed on the farm and to which crops it is applied (Questionnaire)
A7 CropCuPheDiv	Phenotypic diversity of selected crop species based on IPGRI descriptors (Questionnaire)
A8 CropPedDiv	Genetic diversity based on pedigree analysis (Questionnaire)
A9 GrassGenDiv	Molecular genetic diversity of model grassland species (Field and lab work)
A10 Reseed	Amount of reseeded of grassland (Questionnaire)

4.3.2 Sampling protocols

4.3.2.1 Questionnaire based evaluation of plant genetic diversity

Indicators A4-8 and A10 are evaluated using the specific questionnaire for plant genetic diversity developed at the start of the PhD project in early 2010. Specific questions should be developed and discussed with partners before to be included in the final questionnaire.

Questions for indicator A4 to A6 and A8 were developed as general as possible for all case studies areas, whereas questions for indicator A7 are specific for selected crops based on case study descriptions and crops grown on farms.

Initially, it was anticipated to evaluate this indicator not only based on a Questionnaire but also on surveys which could be performed by one or two persons in approximately two hours per crop, depending on the indicators selected. Since the indicators have to be selected for each crop species, it is not possible to determine these traits beforehand.

However, due to lacking descriptors for all crop species and the time and the time and labour required to assess phenotypic diversity of selected species directly on farm, the information for indicator A7 will only be collected for selected species and case studies areas and purely based on questionnaires.

Some crop species are very common in many of the case studies (e.g. wheat). Others are specific in single case studies areas (see Table 4.9: Descriptors for A7).

Table 4.9
Descriptors for A7.

Case studies	Descriptors for
Austria, Germany, France, (Ukraine)	Wheat
Italy	Grapes
Spain, (Tunisia)	Olives

The questionnaire for the assessment of plant genetic diversity is based on farmers' knowledge.

4.3.3 Data processing

In order to facilitate standardised data entry and calculation of indicator values, BioBio CS partners are provided with spread sheets for digitalisation of questionnaire data.

4.3.4 Questionnaire Interview Sheet

More detailed information can be found in the Appendix 7.5 of the BioBio Deliverable 2.2. (BioBio www.biobio-indicator.org)

Feedback after application of the method in twelve case study regions

Strength

Crops, and their cover, planted on farm can be easily surveyed.

Difficulties

Farmers do not always know the name of the cultivar, but only the commercial label.

Information of old species is lacking. Description of shape of trees, time of flowering, helps to distinguish different varieties of apples or cherries but is not sufficient for further analysis.

Complex farming systems, e.g. high cultivar diversity in mixed systems in Germany or horticultural farms in the Netherlands, need more time for interviews than homogenous farms.

Information of seed management on farm and re-seeding of grassland is inconsistent and requires more detailed definition.

Trees on rented land are often not managed by the tenant but by the owner of the land. The farmer does not know anything about the trees on his rented land.

Trees, e.g. cork oaks are mainly natural populations that have been managed over decades. Genetic information is therefore not readily available.

Definition of grassland types has to include more information to allow more efficient allocation.

Practical hints

The questionnaire should be adapted to the individual case study (e.g. arable, grassland, vine yards).

The crop categories need more precise definitions.

Comprehensibility of questions should be tested by performing test interviews. The general understanding should be clear and consistent to maintain reliable and comparable data sets from different studies. The subsequent feedback will then help to improve the questionnaire.

4.3.4.1 Molecular genetic analysis of a model forage grass species

Since evaluation of genetic diversity using molecular genetic or phenotypic markers is not suitable as an indicator routinely used to assess the quality of organic or low-input farming systems, habitat diversity may be used as an indirect indicator based on the following hypothesis: *At a given location (farm), genetic diversity of a grassland species can be predicted by the number of distinct habitats in which the species occurs.*

The main focus of the work on genetic diversity of grassland species is on the validation of the above hypothesis. Genetic diversity is evaluated in a subset of case studies, farms and habitats using molecular genetic markers and *Dactylis glomerata* as a model species. Molecular genetic analyses are supplemented by phenotypic analyses of key agronomic traits of selected populations if time and resources allow for this. Since for a conclusive characterisation of genetic diversity of outbreeding populations a larger number of individuals have to be investigated and due to the limited resources available, a total of 1920 plants from 60 populations are analysed as outlined in Table 4.10.

Table 4.10

Samples for molecular genetic analysis.

	Species	Case-Studies (species)	Systems (case study)	Farms (system)	Habitats (farm)	Number of plants (habitat)
Number	1	3	2	5	2	32
Description	<i>Dactylis glomerata</i>	CH, BU, NO	Organic (low input), conventional (high input)			
Total number of samples						1920

Sampling of 32 individuals per habitat are performed by the PhD student employed for the project at the occasion of farm visits for vegetation survey or as soon plant or leaf material is available. However, support by the involved partners is indispensable. Since the outcome of the project largely depends on the quality of and the differences among grassland habitats present on individual farms, particular care has to be taken when selecting suitable populations.

Genetic diversity of populations sampled in different habitats, farms and case-studies are analysed using molecular markers such as simple sequence repeats (SSRs or microsatellites).

SSRs are repeated sequences of DNA and consist of 1-6 repeated base pairs forming simple sequence repetitions of two, three or four nucleotide units occurring in tandems and randomly (Park et al., 2009). The number of repeats shows a high level polymorphism defining genetic differences within and between species. In combination with PCR, these length-polymorphisms can be detected by gel electrophoresis or capillary electrophoresis. Especially in plant genetics, SSR have advantages over other molecular marker. For example, (i) they are co-dominant, (ii) require a small amount of DNA (PCR-based), (iii) are highly abundant in almost all species and distributed through the whole genome, (iv) the identification of many alleles at a single locus is possible, (v) they are highly reproducible and, (vi) primer sequences are easily exchanged and accessible (Gianfranceschi et al., 1998; Rupp et al., 2009). For *D. glomerata*, a considerable number of SSR primer sequences has been published (Xie et al., 2010). In addition, other marker systems such as sequence tagged site (STS) markers or single nucleotide polymorphism (SNP) will be evaluated and used if appropriate.

4.3.4.2 Summary of field sampling protocol

<i>Sampling method:</i>	Rapid drying and preservation of plant tissue with silica gel as desiccating agent in sealable plastic tubes.
<i>Sampling design:</i>	Plant samples of <i>Dactylis glomerata</i> have to be sampled on five organic and five conventional farms per case study area. On each of these five organic and five conventional farms, two sampling plots have to be prepared in two contrasting habitats ⁷ (Figure 4.7). The sampling plots can be the same plots which have been prepared for the vegetation survey.
<i>Sampling location within the habitat/field plots:</i>	The sampling location within the habitat can be the same as selected for vegetation survey.
<i>Sampling date:</i>	During vegetation survey or afterwards - when young leave material is available
<i>Sampling procedure:</i>	five to seven leaves with a length of 4 to 5 cm of a single <i>Dactylis glomerata</i> plant are harvested. The leaves have to be inserted into a sealable plastic tube which is filled with silica gel (containing a moisture indicator dye). 32 plants have to be sampled per sampling plot.
<i>Timing:</i>	All habitat/field plots have to be sampled in 2010 whenever fresh leave material is available.

⁷ Contrasting according to management practice applied in this habitat/area. The difference between intensive and extensive management should be as big as possible.



Figure 4.7

Sampling design for model grass species (farm-scale).

4.3.4.3 Materials and methods - field sampling protocol

Preparation in the laboratory: 15 ml plastic tubes have to be filled with silica gel containing moisture indicator dye up to the mark '6ml. It is important to seal the tubes tightly.

Preparation in the office: After habitat mapping, *Dactylis glomerata* leave samples are collected in sampling plots within preselected habitats. These plots can be the vegetation plots prepared in aerial elements for the vegetation survey as far as *Dactylis glomerata* is present. The chosen habitats have to be as contrasting as possible. One should be located in an intensive managed habitat. The other should be in an extensive managed habitat on the same farm.

Sampling in the field: Preparation of a 10 x 10 m sampling plot in a habitat (plots prepared for the vegetation survey, can be used as far as *Dactylis glomerata* is present).

Sampling of plant material starts two steps from the middle () of the 10 x 10 m plot toward the edges Figure 4.8 Plant samples are taken every second step toward North, South, West and East, resulting in eight tissue samples per transect (N, S, E and W) and 32 tissue samples per plot.

- Plants that are sampled don't have to be directly on a straight line towards the edges. They can be up to 50 cm next to each side of this fictive line.
- Sampled plants are labelled with a marker (plastic stick ect.) to avoid duplicate sampling.
- If there are not enough plants within the sampling plot, the sampling continues around the sampling plot. Walk around the plot in a spiral and go on collecting.
- The distance between single plants is more than 1 m.
- Plants are not collected next to buildings, roads or other human made facilities (those plants could be part of reseeding mixtures after construction), but from areas representing the habitat.

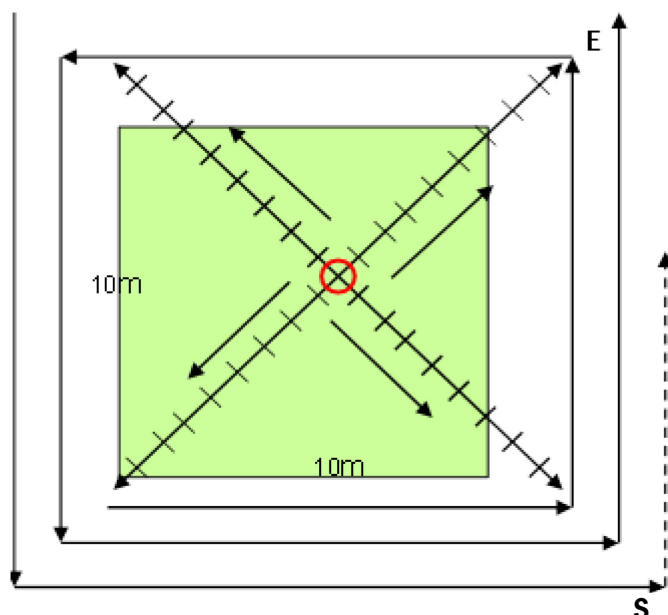
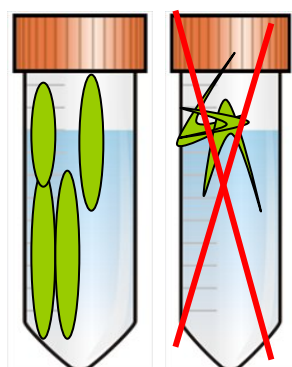


Figure 4.8

Sampling design for plant tissue of Dactylis glomerata (plot-scale).

5 to 7 young leaves (4 - 5 cm) are harvested per sample. Harvested leaf samples have to be straight in the tubes, not as a ball (Figure 4.9)



The plastic tubes are sealed tightly to avoid further moisture penetrating into the tubes. Shaking the tube after filling provides homogenous mixture of silica gel and plant material inside the tubes. The tubes have to be labelled immediately and tubes sampled from the same plot are stored together in a labelled plastic bag.

Figure 4.9

Position of leaf samples in the tubes.

4.3.4.4 Molecular marker analysis

DNA of individual plants will be extracting using the NucleoSpin 96 Plant Kit (Marchery Nagel). After quantification DNA of individual plants will be used for PCR amplification of ~33 SSR loci. Amplified fragments are separated on an ABI 3730xl genetic analyser (Applied Biosystems) and analysed using GeneMarker software (SoftGenetics). Genetic diversity is evaluated using multivariate statistics such as principle component analysis, cluster analysis and redundancy analysis using various statistical analysis tools, e.g. R or Arlequin.

Feedback after application of the method in twelve case study regions

Strength

Molecular marker based analysis provides an allele based tool giving a precise value on genetic diversity. It measures genetic diversity directly at the DNA level and is not influenced by the environment.

Molecular marker can be applied to wide range of species within the agro-ecosystem, e.g. wheat, rice, etc. Collection, preparation and storage of plant material was applicable with regard to preservation of DNA material for further analysis.

Difficulties

Within this study, only one species was investigated using molecular tools. This example gave an insight into genetic diversity of a small group of plants with similar characteristic, e.g. taxonomy system and breeding. The overall assessment of genetic diversity on a farm should include various species taking into account different groups of plants, e.g. grasses and trees, and their specific characteristics influencing genetic structure of populations.

In the case of grass species, the introduction of commercial seed products in grasslands, e.g. by partial over seeding by hand, might affect and confuse results on the genetic structure and differentiation of populations. For this reason, pre-evaluation and selection of plots should be performed very carefully, based on detailed information provided by the farmer.

Collection and analysis is labour and cost intensive.

Practical hints

The selection of grassland plots needs to be carefully structured. Definitions, e.g. permanent grassland, and information on plot management practices have to be clear and collected in great detail according to the information provided by the farmer.

In future, the application of sequencing methods will increase the number of samples and a high-throughput performances taking into account the analysis of several species at once.

4.3.5 Livestock genetic resources

The following livestock species and genetic diversity indicators are evaluated:

1. Number and amount of different breeds per species (**Breeds**)
2. Information on breeding practices ('on-farm' bull, artificial insemination,...) (**Liveprac**)
3. Where available, pedigree of the herd (**LivePedi**)

This is assessed from completion of livestock genetic resources table during the farmer interview.

Feedback after application of the method in twelve case study regions

It was easy to complete in terms of which breeds are used.

It does not capture the relevant information (the share of different genetics in a herd) in systems with only one livestock species (e.g. cattle) and hybrid breeding or mixed animals.

Breed status should be assessed centrally based on e.g. FAO data or CABI animal production compendium.

4.4 Farm management indicators - questionnaire

Arndorfer, M.³, Choisis, J.-P.⁵, Choisis, N.⁵, Dennis, P.¹, Jeanneret, P.², Friedel, J.K.³, Fraser, M.D.¹, Kainz, M.⁹, Pointereau, P.¹¹, Sarthou, J.-P.⁵, Schneider, M.³, Siebrecht, N.⁹ and Wolfrum, S.⁹

¹(ABER) Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK;

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland;

³(BOKU) Division of Organic Farming, University of Natural Resources & Life Sciences, Vienna, Austria;

⁵(INRA) UMR Dynafor, INRA-ENSAT, Toulouse, France;

⁹(TUM) Centre of Life and Food Science, Weihenstephan, Technical University of Munich, Germany and

¹¹(SOLAGRO) Initiatives and Innovations for Energy, Agriculture and Environment, Toulouse, France

4.4.1 Introduction

Along with habitat structure of the farms, farm management largely determines the pressure on species diversity that is assessed by direct biodiversity indicators, mainly on the managed area of the farms. Farming practices are therefore key points to maintain and restore biodiversity.

The Farm Management Questionnaire (BioBio Questionnaire 2⁸) is the basis for data collection to assess farm management of BioBio Case study (CS) farms. The selection of variables for the questionnaire is guided by the set of Farm Management Candidate Indicators, agreed at the PCC Meeting in Brussels. Farm management indicators representing different relevant categories of pressure were selected and tested in the BioBio project.

Indicators in relation to different thematic categories:

- Indicators related to farming intensity in general:
e.g. Total energy input (D5.1); Intensification/Extensification (D8).
- Indicators related to nutrient input and management:
e.g. Use of mineral N fertilizer (D3)9; Total nitrogen input (D4.0).
- Indicators related to farming practices:
e.g. Pesticide use (D9); Field operations (D11.1).
- Indicators for livestock systems:
e.g. Average stocking rate; Grazing intensity (D12.1).

Additional variables were subsequently included for the following reasons:

- To cover additional factors affecting direct indicator measurement on BioBio field plots
e.g., status of grassland (spontaneous vegetation or sown, rotational or permanent), winter soil cover and crop rotation in arable crops.
- To quantify organic matter flux and facilitate nitrogen balance (fodder, manure, crop yield).

⁸ This term was introduced earlier to distinguish between the questionnaire developed for the farm selection process (Questionnaire 1) and the questionnaire documenting farm management practices (Questionnaire 2).

4.4.2 Defining requirements for the questionnaire

The diverse data needs for the variety of farming systems investigated by BioBio were a particular challenge in the compilation of the questionnaire. The questionnaire is designed to cover the management practices of farms with and without livestock and takes into account different land use types such as grassland, arable crops and permanent crops (olives and vineyards) as well as semi-natural habitats (field margins, hedges etc.). Furthermore, data are recorded on different scales of measurement: farm level, crop level (standard operations for each crop), field level (plots of BioBio survey).

It is a basic requirement that one common questionnaire is used by all case studies and that data are kept as simple as possible. Complex datasets that need interpretation cannot be analysed due to the large number of farms in BioBio. As the data was collected from interviews with farmers, additional practical criteria guided the questionnaire design.

The duration of interviews must be limited to a maximum of two to three hours (including Genetic Diversity Indicators). One farm visit must suffice to collect all the data for the questionnaire.

The level of documentation of farm management differs from farm to farm and from region to region. Some farmers may keep environmental farm accounts on a routinely basis (e.g., Due to cross-compliance requirements), whereas other farmers can only provide data from their daily routine and from basic documents (e.g., receipts on energy consumption).

An initial proposal to ask farmers to document specific management practices for BioBio was eventually rejected. There was the general notion that farmers would be reluctant to keep additional notes and that data would finally be incomplete. Thus, gaps in data collection would make statistical analysis impossible. Therefore, all data collected in the BioBio farm management questionnaire should be deducted from the interviews based on the farmer's operational knowledge of his or her farm and on basic farm accounting.

4.4.3 Structure of the farm management questionnaire

The Farm Management Questionnaire is divided into four main Sections (A, B, C and D).

Form A General Farm Data concerns aggregated data collected on the farm level such as energy consumption, agri-environmental measures, organic matter fluxes etc.

Forms B1 and B2 survey variables that describe the plant production system of the farm. Based on **standard operations** such as fertilisation practices, plant protection measures and mechanised field operations, data are collected for each crop or grassland type. Due to similarities in the structure of management practices, form B1 covers **annual arable crops, olives and vineyards**, whereas form B2 focuses on **grassland and perennial fodder crops**. Data from forms B1 and B2 will be used to calculate nitrogen input and nitrogen balances and to assess the farming intensity based on grazing management, plant protection measures and mechanised field operations. The total of utilised agricultural area (UAA) will be calculated from these data. Therefore, the synthesis of data from forms B1 and B2 must reflect the management of the entire utilised agricultural area of the farm.

Forms C1, C2 and C3 concern **specific management of BioBio plots** where faunistic and floristic indicator sampling took place. Additional data are collected beyond standard operations, e.g. by estimating the timing of certain measures or by specifying grazing management and crop rotation. The forms are subdivided by categories used in the GHC method: **Areal Habitats** (C1 crops/olives/vineyards and C2

grassland/perennial fodder crops) and **Linear Habitats** (C3). Form C3 provides short information on the management of herbaceous and woody linear habitats.

Form D Livestock Management records the numbers of livestock on the farm broken down by livestock categories. Livestock units are calculated from this table. Additional variables concern meat production (indicator for productivity), use of pastures and common grazing land.

Due to the limited time available during the interviews, it is recommended that farmers are informed about certain data needs before the visit. Depending on the farming system, the farmer can be asked to prepare certain documents (e.g., on agri-environmental measures, energy consumption, purchase and sale of organic matter).

Table 4.11

General relevance of questionnaire sections for each BioBio case study.

	A	B1	B2	C1	C2	C3	D
A_ARA	X	X	(X)	X	(X)	X	
F_ARA	X	X	(X)	X	(X)	X	
D_MIX	X	X	X	X	X	X	X
B_GRA	X		X		X	X	X
H_GRA	X		X		X	X	X
N_GRA	X		X		X	X	X
C_GRA	X		X		X	X	X
W_GRA	X		X		X	X	X
E_DEH	X		X		X	X	X
E_OLI	X	X		X		X	
L_HOR	X	X		X		X	
I_VIN	X	X		X		X	

Remark: the 'x'-mark indicates that the respective section of the questionnaire is compulsory for the case study. Depending on the specific crops on a farm, additional sections may be relevant. e.g., a grassland farm may also grow annual fodder crops (form B1). Lucerne on arable farms will be recorded in form B2 ('perennial fodder crops'). A horticulture farm may also keep livestock, therefore form D must be filled.

4.4.4 Data processing

In order to facilitate standardised data entry and calculation of indicator values, BioBio CS partners are provided with spread sheets (forms A, B, D) and Access files (forms C1 and C2) for digitalisation of questionnaire data. The spread sheets are imported to a central database. Calculations of indicator values are done in the central database.

Table 4.12

Farm management indicators tested in BioBio as related to sections of the questionnaire.

n°	Name	Unit	Questionnaire
Farm-level Measurement			
D1) DivEnt	Diversity of Enterprises	N° of enterprises at farm level	B + D
D2.1) AvStock	Average stocking rate per ha utilised agricultural area (UAA)	N° of livestock units/ha UAA	D
D2.2) AvStock	Average stocking rate per ha forage area	N° of livestock units/ha forage area	D
D3) MinFert	Area without use of mineral N-fertiliser	% UAA	B
D4.0) NitroIn	Total nitrogen input	kg N per ha UAA	B (surface balance) + D
D4.2) Norg	Organic nitrogen fertiliser input	kg N per ha UAA	B+D
D5.1) EnerIn	Total direct and indirect energy input	Equivalent litre of fuel per ha UAA	A + B
D6) CertOrg	Organic farming: Certified as organic?	yes/no	A
D7) AgrEnv	Area under agri-environment support	Agri-environmental measures and area covered	A
D8) IntExt	Intensification/Extensification Expenditures on fertiliser, crop protection and concentrate feed stuff	€ per ha UAA	A
D9) PestUse	Pesticide use	N° of applications	B
D9.1)	Herbicide use	N° of applications	B
D9.2)	Insecticide use	N° of applications	B
D9.3)	Fungicide use	N° of applications	B
D10)	Reduced use of chemical pesticides	% UAA without use of chemical pesticides	B
D11.1) FieldOp	Field operations	N° of field operations	B
D11.2)	Mowing frequency	Number of cuts	B2
D11.3)	Mowing timing	Date of the first cut	B2
D11.8)	Soil cultivation: Ploughing	% arable land	A
D12.1) GrazInt	Grazing Intensity	N° of livestock units per ha grazing area	D
D14 Irrig	Irrigation	% UAA	A
Field-level measurement			
D4 NitroIn	Nitrogen input	kg N per ha UAA	C
D9) PestUse	Pesticide use	N° of applications	C
D9.1)	Herbicide use	N° of applications	C
D9.2)	Insecticide use	N° of applications	C
D9.3)	Fungicide use	N° of applications	C
D11.1) FieldOp	Field operations	N° of field operations	C
D11.2)	Mowing frequency	Number of cuts	C
D11.3)	Mowing timing	Date of the first cut	C

4.4.5 Questionnaire Interview Sheet

More detailed information can be found in the Appendix 7.4 of the BioBio Deliverable 2.2. (BioBio online). This version of the Questionnaire Interview Sheet comprises all BioBio farm management candidate biodiversity indicators.

Feedback after application of the method in twelve case study regions

Strengths

The questionnaires can capture all possible farm situations.

With good preparation material it is possible to carry out the interviews by telephone, which is much more cost-effective than farm visits, especially when the farms are far away.

Difficulties

Farm data: some sections require data that are often not recorded by farmers, such as electricity or gasoline consumption, therefore these data are often rough estimates by farmers.

In grassland systems involving in large fields where the treatment is similar but grazing may have unsystematic phases, farmers found it difficult to remember the days of grazing. The starting and end date will not give the exact dates of grazing. The biomass production and actual grazing is very much dependent on weather conditions.

The information on energy consumption is often unavailable, because many farmers have one energy measure for both farm and household.

Practical hints

It is best to avoid unnecessary questions to farmers. Therefore the questionnaire has to be tailor-made to each case study by reordering the questions and omitting the irrelevant parts.

Animals and practical farm operations are a good starting point for discussion. Environmental measures should be discussed later in the interview. The structure of the interview was explained in the beginning. The map of farm should be followed up and the plots surveyed were a helpful additional tool.

It is more effective to do the interviews after the maps are completed and the position of plots is known.

4.5 Assessment of costs of measuring biodiversity indicators in BIOBIO project

Targetti, S.⁶, Viaggi, D.⁶, Cuming, D.⁶

⁶(UNIBO) *Imas Mater Studiorum, University of Bologna, Department of Agricultural Economics and Engineering (DEIAGRA), Italy*

4.5.1 Objective

As long as the possibility of using indicators for the design, monitoring and evaluation of public policies is connected with costs and budget constraints, three main research issues arise for the needs of agri-environmental schemes:

- the identification of cost-effective indicators;
- the development of suitable procedures for using such indicators to elicit differing policy effects;
- the assessment of benefits from (more precise) environmental policies.

In this context, the availability of cost data concerning the measurement of biodiversity indicators is of significant importance both for the implementation of sound agri-environmental schemes and for the optimisation of funds for biodiversity monitoring and conservation (Ferraro and Pattanayak, 2010). Nevertheless, even though costs are clearly a central issue for long-term ecological monitoring programs, only a few studies exist which propose a methodological approach to the cost of assessing biodiversity measurement or provide empirical evidence about such costs. The cost-effectiveness of biodiversity measurement has therefore received relatively little attention (Screuder et al., 1999; Caughlan and Oakley, 2001; Franco et al., 2007; Qi et al., 2008).

The objective of this section is to describe a framework and illustrate an operational protocol for the evaluation of the costs of measurement of the biodiversity indicators in the BIOBIO project. The main targets include the implementation of a database composed by the collection of empirical data on time consumed and costs in the twelve case studies and the assessment of the costs of a biodiversity monitoring program based on the BioBio indicators.

4.5.2 Methodological proposal

4.5.2.1 Rationale

Biodiversity indicators use economic inputs (quantified by the cost of measurement) to produce an output represented by ecological information (in this context, the assessment of biodiversity). The cost of the measurement is the sum of the monetary value of resources consumed to undertake the measurement of the indicator and processing of data (Chambers, 1988). This cost can be estimated through direct information collection regarding resource use and unitary costs and can be used for cost-effectiveness analyses.

A long-term ecological monitoring includes two main phases (Caughlan and Oakley, 2001):

- Phase 1: Start-up and development
- Phase 2: Regular monitoring

The collection of cost-data during the BioBio project can be referred to the cost of a pilot study which is a specific part of the start-up and development phase. Indeed, we expect a consistent reduction of unitary costs and efforts related to the measurement of biodiversity during the regular monitoring phase. In fact, routine

measurements could benefit from economies of scale, the optimization of the sampling design, the availability of trained staff and the mechanisms underneath the call for tenders (i.e. competition between private monitoring agencies). The subsequent assessment of costs of a complete monitoring cycle shall consider the difference between the costs related to the two above-mentioned phases.

The data collection is based on the distinction of physical information and associated unitary prices. An important point is the need to identify fixed costs. Fixed costs are those that do not vary with the 'quantity of measures' performed. For example, some cost items can be fixed with respect to:

- Several indicators measured: e.g. a transport to a site to collect data for several indicators.
- Several data collections for the same indicators (e.g. the initial planning of the sampling/transects).
- Several analyses for the same indicator (e.g. the cost of machinery for laboratory analysis, purchased at the beginning and used several times).

This enables the testing of adjustments that could be necessary and/or the performance of a sensitivity analysis of the results on some variables (e.g. labour costs), in order to:

- Check how costs would change in different geographical areas (e.g. due to different labour costs).
- Check how the costs would change moving from experiments to routine measures (e.g. where fixed costs for preliminary operations are already paid for).
- Check if there are economies of scale and scope in the number of trials or data collection point.
- Adapt to real life (e.g. substituting salaries of the research institutions with everyday activities of a routine monitoring agency).
- Prevent/evaluate uncertainties in cost assessment.

An important point to be defined is the unit for cost calculation. The obvious candidate is the single indicator per farm. However, this may not be completely satisfactory in light of the previous concerns, e.g. when there are common fixed costs across indicators. Alternatives may concern disaggregation (single plots related to the same indicator), aggregation (e.g. bundles of indicators per farm) or hours of labour effort per indicator.

4.5.2.2 Data collection & organisation

The cost assessment is performed through the quantification of the cost of efforts and resources spent for the measurement of habitat mapping, vegetation, wild, domestic and bumblebees, spiders, earthworms and questionnaires, following the BioBio protocols of measurement.

The collection of data is split into three main parts:

- Recording of the activities carried out and the use of resources in physical terms.
- Inventory of the typologies of the main cost items, where descriptions and unitary costs are given.
- Information on the surveyed farms which includes N° of plots, N° of habitats, km and time of average travel from the research centre to the farms, etc.

The general workflow for data collection and organisation is summarized in Figure 4.10.

Each record includes the following information: date, identification of farm site, resource type and amount and is linked to the typology tables indicating the salary band of staff, the distance of the farm site from the research centre, transport time, equipment and consumable costs. After quality check, data are recorded in a relational database.

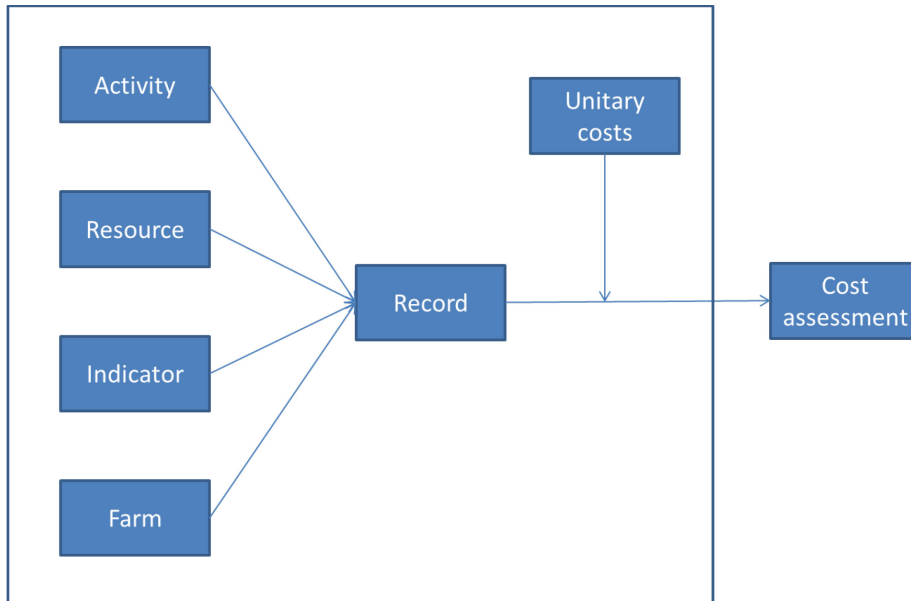


Figure 4.10

Organisation of data collection and cost assessment.

The database is organised in order to trace the effort costs per indicator, farm, activity and type of resource. This is used for cost calculation and, at a later stage, for cost simulation and sensitivity analysis (if relevant). This allows for an easy recording of activities and related costs over time and a clear source of data allowing for simulations, i.e. calculation of costs adapted to different conditions.

The data collection is based on the periodic (weekly) notation of resources used. This is important in order to build a reliable empirical database concerning time and resources spent in the measurement. The cost form templates proposed by UNIBO to support data collection (D2.2 at BioBio www.biobio-indicator.org) are intentionally not detailed (i.e. the categories of resources are not suggested a priori). This is thought to leave a rather free compilation by the research units in order to seize the variability of resource efforts and the different sampling organisation of the fieldwork. It is the responsibility of the leader of each case study to fill in these forms on a weekly basis.

The main categories of expenditure are as follows:

- Labour
- Equipment
- Consumables
- Transport (e.g. vehicle costs)
- Others (e.g. food and accommodations)

Equipment and consumables include all of the materials used for the measurement of the BioBio indicators of biodiversity. The unitary cost of the utilisation of equipment is calculated as the cost of the equipment purchase divided by its lifetime in the same measurement unit. Labour costs are expressed in € per hour or à forfait and include health insurance and taxes. Labour includes time devoted to measurement activities and the travel time of the field team to and from the farms. Labour includes also the cost of taxonomy performed by specialists for the identification of species. Vehicle costs are generally expressed per km and include fuel, car

insurance and vehicle depreciation. Other costs include accommodation and food for the fieldworkers and incentive payments to farmers.

Fieldwork activities include the cost of field sampling and the cost of transportation to and from the sampling plots (transportation refers to cost of vehicle and labour time spent in travel). Laboratory work includes sorting and preparation of species for taxonomic identification such as insect pinning. Deskwork includes tasks such as digitalisation of maps and data input.

Labour time spent in travel can be assessed directly from the cost forms considering for each travel:

- Number and type of field workers,
- Measured indicators,
- Travel time from the research centre to the served farms.

This type of analysis allows the distortions sourced by the different transportation costs in the 12 BIOBIO case studies to be determined.

The amount of used resources that were available at no cost for the project (e.g. volunteer labour and free accommodation) is also recorded. The cost assessment methodology is organised in such a way as to allow for an analytical assessment of actual costs and the subsequent simulation with standardised costs.

4.5.3 Discussion

The present database structure allows for a wide range of cost evaluation with BIOBIO information, which is important considering the current lack of empirical based research concerning the costs of measuring biodiversity. Sensitivity analyses and simulations are also needed in order to check the relevance of the present cost measures for the routine measurement and for conditions that differ from those of the BioBio case studies. Finally, it is necessary verify the possibility of relating costs with the value of information obtained. This will allow the economic analysis of benefits to be carried out.

5 Data processing

Bailey, D.²

²(FDEA-ART) Federal Department of Economic Affairs, Research Station ART, Zurich, Switzerland

5.1 BioBio habitat mapping - digitising protocol

To calculate the habitat indicators it is necessary to have the spatial data that were collected during the habitat mapping exercise. An image interpretation of the habitats surrounding those elements on the farm that were selected for the species sampling is also necessary. The digital maps enable the calculation of area and length data for the habitats recorded on the farms. The image interpretation provides a list of habitats that surround the sampled elements together with an estimate of their percentage covers.

The goal of this protocol is to produce standardised digital maps of the data collected during the habitat mapping exercise and to undertake a standardised image interpretation of the surrounding 250 m of the sampled elements on your farms. As the GIS programs are likely to vary between countries the protocol is unable to define the sequence of specific GIS operations.

To undertake this task templates have been provided with this protocol for the entry of the habitat mapping data collected in the field (D2.2 available at www.biobio-indicator.org).

The following products result:

1. One digital map of the areal habitats found on each of the farms
2. One digital map of the linear habitats found on each of the farms
3. A photo interpretation (spread sheet format) of the habitats surrounding each of the elements selected for species sampling on your farms.

General Requirements

Ortho aerial or satellite images: Ideally to digitise the maps ortho aerial images or ortho satellite images of the farm locations are required. The images should already be geo-referenced using the projection system of your country (detailed further below). Ortho images are aerial or satellite images which have been freed of their distortions and therefore show a uniform scale over their entire surface.

Other useful digital data: Other useful data are digital topographical maps and digital farm parcel information. For Spain see for instance: <http://sigpac.mapa.es/feqa/visor/> or for Austria, the site of BEV: <http://www.austrianmap.at/amap/index.php?SKN=1&XPX=637&YPX=492>. This can help to locate the farms and the different parcels belonging to the farm. These data are not essential; however, it may be possible to use them instead of aerial or satellite images if none are available for the region.

Spatial resolution (*pixel size*) of aerial or satellite images: Ideally, the spatial resolution of the images should be below the minimum mapping unit to be digitised. The minimum mapping unit to be digitised is defined by the smallest areal and linear elements that are recorded in the BioBio field mapping:

1. For areal elements this unit is 400 m². The element must have a minimum width of 5 m.
2. The minimum mapping unit of the linear elements is 30 m length.

This means that the spatial resolution of the aerial photographs, if possible, should be below 5 m because of the minimum width of the areal elements. If using satellite images, only high resolution images can be used.

Spatial extent of the ortho aerial or satellite images (area covered by the image): The extent of the images should cover the entire area of your farm including any scattered fields plus at least 250 m of the land surrounding your farms and scattered fields.

Production of Digital Maps

When digitising the habitat data collected during the field habitat mapping exercise, it produces two digital datasets. One dataset contains all the areal elements found on your farms and the other all the linear elements. For both datasets metadata need to be provided.

Metadata: The following metadata should be provided along with the digital maps.

1. The Projection (projected coordinated system) used in your country and the EPSG number from <http://www.epsg-registry.org/>.
2. The contact details of the person responsible for the maps.
3. Is the map based on satellite images or on aerial photographs?
4. Which GIS software was used.

Projection: Map projection systems allow the transformation of a three-dimensional image to a flat map sheet image. Their purpose is to provide a common basis for communication about a particular place or area on the earth's surface. For the production of the digital maps please use the projected (not geographical) coordinate system of the country¹⁰. In a projected coordinate system, locations are identified by x, y coordinates on a grid rather than latitude and longitude coordinated in a geographical coordinate system. Later the maps are transformed into a common European projection system to conform to the INSPIRE Directive.

When dealing with coordinate systems it is essential to know what the projection is and to have the correct coordinate system information associated with the dataset. **Please remember the projection metadata is essential (see metadata above).** For example the projected coordinate system of Switzerland is CH1903_LV03. The EPSG number is EPSG 21781 and can be found by searching under query by filter in <http://www.epsg-registry.org/> using the search terms Type = Projected CRS and Area = Switzerland. The following information is attached to this system:

Projection:	Hotine_Oblique_Mercator_Azimuth_Center
False_Easting:	600000.000000
False_Northing:	200000.000000
Scale_Factor:	1.000000
Azimuth:	90.000000
Longitude_Of_Center:	7.439583
Latitude_Of_Center:	46.952406
Linear Unit:	Meter (1.000000)

To conform to the INSPIRE Directive; it should be possible to convert your national projection system to the projected coordinate system ETRS 1989 LAEA (Lambert Azimuthal Equal Area). This system is recommended 'for pan-European spatial analysis and reporting, where true area representation is required'. More information can be found under:

¹⁰ In ArcGIS the standard folder for the installation of the projection is at: C:\ArcGIS\Coordinate Systems\Projected Coordinate Systems\National Grids.

(http://inspire.jrc.ec.europa.eu/documents/Data_Specifications/INSPIRE_Specification_CRS_v3.1.pdf). Thus, it may be easier to use directly the ETRS 1989 UTM system and it should be checked that it is possible to convert the data into this system. ETRS 1989 LAEA is divided into different zones, for example in Norway it is ETRS 1989 UTM32N. When using this system ensure to use the ETRS 1989 datum as this is the European geodetic datum which was introduced to uniform national reference systems. For ArcGIS users, the ETRS 1989 UTM zones can be found under the Projected Coordinate Systems - UTM - Other GCS. ETRS 1989 LAEA is an option amongst the predefined projected coordinate systems under 'Continental and 'Europe'.

Digitising Areal Elements:

1. The areal elements are to be digitised as polygons. The minimum size of the polygons is 400 m² with a minimum width of 5 m.
2. The polygon dataset should be exported as a polygon shape file including attribute table and projection. (Files: .shp, .shx, .dbf, .prj).
3. Care should be taken to avoid gaps between polygons that adjoin each other. This can be achieved by setting the snapping environment in your GIS program. The flexibility of your snapping environment may vary with your GIS program. Generally, the snapping tolerance defines the distance within which the feature that you are digitising will be snapped to an existing digitised feature. In ArcGIS, the snapping properties allows you to choose which part of the other feature the newly digitised feature should snap to whilst the snapping priority allows you to set the layer you want your feature to snap to (here the map that you are digitising).
4. Fig. 5.1 provides an example of digitised areal elements.

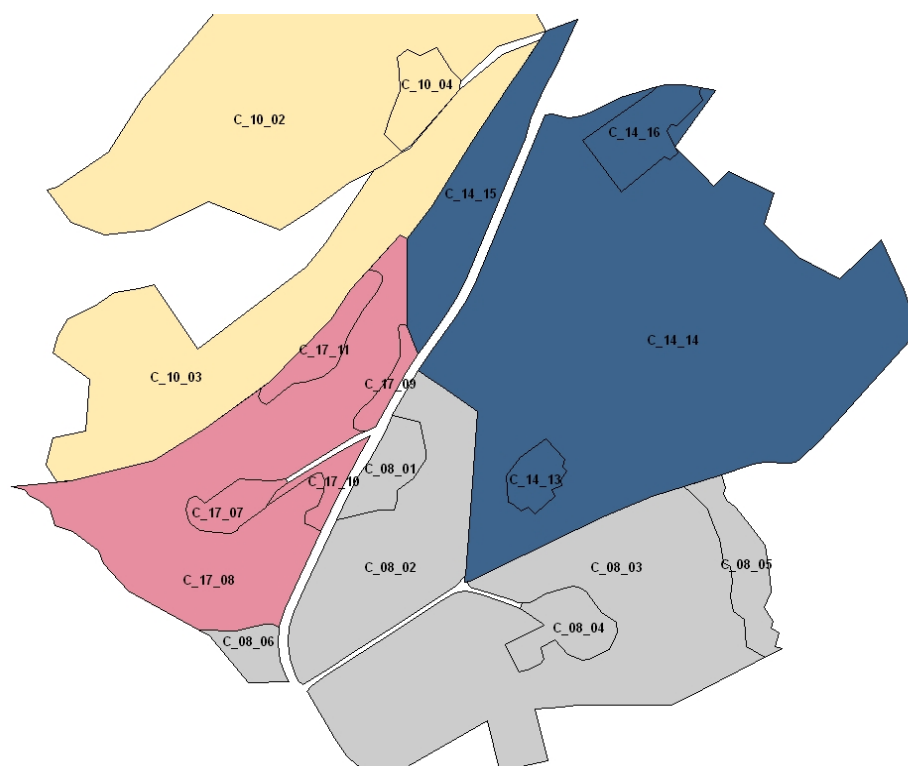


Figure 5.1

An example map comprised of digitised areal elements, labelled using the elementID for parts of farms 8, 10, 14 and 17 in Switzerland.

Table 5.1*Example of the areal attribute table related to Figure 5.1*

ElementID	Country	FarmNr	HabNr	Alpha_Code	GHC	Glob	Env	Site	Man	Man2	Annex1	FarmI_CI	Selected	PlotID	Shape_Leng	Shape_Area
C_14_14	C	14	14	K	LHE/CHE	OPE	5.1	0	A 1.5.2	0	0	1	0	C14g	1348.87	46606.31
C_14_15	C	14	15	G	LHE/CHE	0	5.1	0	A 1.5.2	0	0	1	1		473.40	5841.73
C_17_08	C	17	08	B	LHE/CHE	SCA	5.1	0	A 1.5.2		0	1	0		1018.30	17773.74
C_08_02	C	08	02	B	LHE/CHE	OPE	5.1	0	A 1.5.2	0	0	1	0		520.22	10032.19
C_08_03	C	08	03	C	LHE/CHE	SCA	5.1	0	A 1.5.2	0	0	1	0		1060.65	23323.03
C_14_13	C	14	13	A	ART	0	0	0	0	0	0	0	0		132.60	1064.39
C_14_16	C	14	16	A	ART	0	0	0	0	0	0	0	0		220.60	2326.16
C_08_01	C	08	01	A	ART	0	0	0	0	0	0	0	0		187.65	1706.08
C_08_04	C	08	04	A	ART	0	0	0	0	0	0	0	0		227.16	2004.61
C_08_05	C	08	05	D	FPH/DEC	LCO	0	0	A 3.15		0	8	0		286.43	2289.44
C_08_06	C	08	06	E	LHE/CHE	0	5.1	0	A 1.5.2	0	0	1	0		152.66	1108.85
C_17_07	C	17	07	G	ART	0	0	0	0		0	0	0		157.30	1186.00
C_17_10	C	17	10	I	WOC	0	5.1	0	A 1.12	A1.5.2	0	1	1	C17i	229.37	853.40
C_17_11	C	17	11	J	LHE/CHE	0	5.1	0	A 1.5.2		0	1	0		232.76	1552.85
C_17_09	C	17	09	H	LHE/CHE	0	5.1	0	A 1.5.2		0	1	0		161.74	681.25
C_10_02	C	10	02	B	LHE/CHE	0	5.1	0	A 1.5.2	0	0	1	0		965.10	35211.24
C_10_04	C	10	04	D	ART	0	0	0	0	0	0	0	0		173.67	1670.88
C_10_03	C	10	03	C	LHE/CHE	SCA	5.1	0	A 1.5.2	0	0	1	1	C10c	940.36	17001.57

Areal Attribute Table:

Each element must be attributed with certain data that you collected in the field. This information will be documented in the attribute table in the GIS environment (See example in Table 5.1). Table 5.2 details the columns that are required in the attribute table and how they should be defined. **It is essential that all partners use the same columns and column definitions.**

The ElementID in the areal attribute table is a unique number/letter combination for each areal element in your map. It is formed from the country code, the farm number and a habitat number, e.g., Country_FarmNr_HabNr. The country codes are listed in Table 5.3. The farm numbers in the ElementID should be written as 01,02,03,04,05,06,07,08,09,10 to20. The HabNr is a number that should be applied consecutively to all the elements that were found on your farm, starting with 01 through to X, i.e. corresponding to the number of elements that you found on the farm. Table 5.4 provides examples of ElementIDs and how they were formed.

Table 5.2

Attribute columns to be defined in the GIS attribute table for the areal elements.

Column heading	Data specification	Example	Description of column
ElementID	Text	C_01_01	This is a unique ID for each polygon element and will comprise the country, farm number and habitat number. See text above
Country	Text	C	The Country code
FarmNr	Long Integer	01, 02, 03....20	The Farm Number
HabNr	Long Integer	01, 02, 03,X	This is the continuous numbering system of the mapped elements within each farm
Alpha_Code	Text	A, B, C	The alpha code that was given to the different GHC that you identified in the field
GHC	Text	LHE, FPH/CON	The GHC classification
Glob	Text	OPE	The Global qualifier
Env	Double	5.1	The Environmental qualifier
Site	Text	1.1	The Site qualifier
Man	Text	A1.5	The Management qualifier
Man2	Text	A1.6	In case the element had more than one management qualifier, e.g. A1.5 and A1.6
Annex1	Long Integer	See mapping manual	Annex 1 habitat
FarmL_Cl	Text	1	The Farmland Class
Selected	Short Integer	1 or 0	One for selected (sampled) elements, zero otherwise
PlotID	Text	PlotID part of the bar code used for the species sampling, e.g. C1a, C1b	For those elements which are sampled: PlotID which appears on labels for the species sampling

Table 5.3*Country codes.*

Country	Code	Country	Code
Austria	A	Wales	W
Bulgaria	B	Hungary	H
Switzerland	C	Italy	I
Germany	D	Norway	N
Spain (Dehesa)	Ed	Netherlands	L
Spain (Olive)	Eo	Tunisia(Cork)	Tc
France	F	Tunisia (Olive)	To
Ukraine	K	Uganda	U

Table 5.4*Examples of elementID's for areal elements in Switzerland.*

ElementID	Country	FarmNr	HabNr
C_01_01	C	01	01
C_01_02	C	01	02
C_01_03	C	01	03
C_02_01	C	02	01
C_03_01	C	03	01
C_03_02	C	03	02
C_03_03	C	03	03
C_03_04	C	03	04

Areal Attribute Data entry

The data can be either entered by joining the attribute table to your existing data table. The joining technique will depend on your GIS environment. An empty table is provided for the entry of your areal data. This table includes columns related to the Habitat/Species data collected in Field 5. It is necessary to record this data in the file but it is not required in the areal attribute table. To join an existing data table (preferably MS Access format) with your polygon dataset both datasets will require the unique ElementID. The ElementID should be assigned to each element in the data table and correctly attributed to appropriate polygons in the polygon dataset.

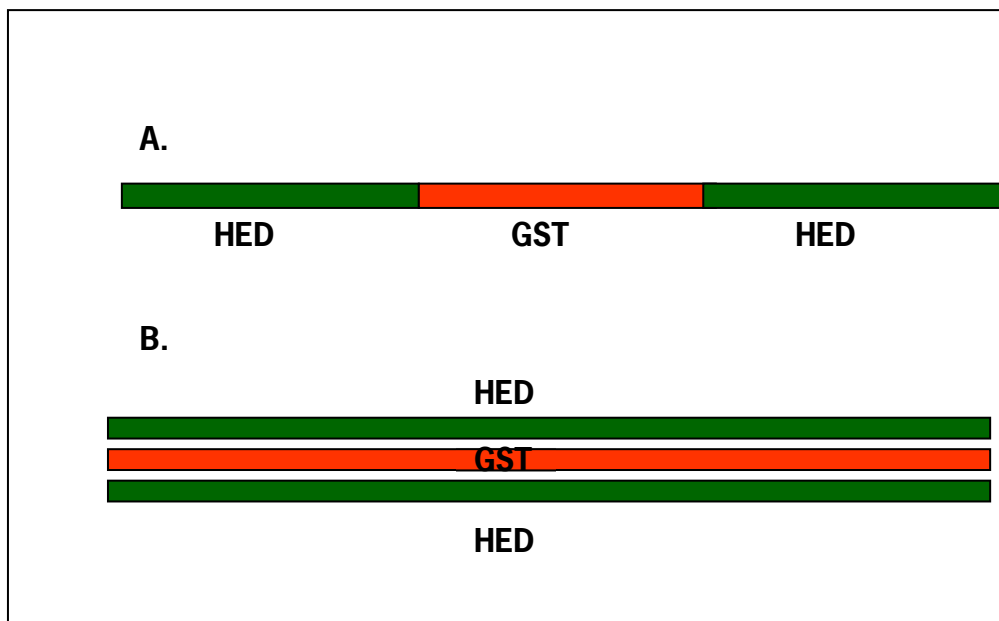


Figure 5.2

An example of digitized linear elements. A. Linear elements that adjoin each other and b. An example of a complex of linear elements.

Digitising Linear Elements

1. The linear elements are to be digitised as lines which have a minimum length of 30 m.
2. The line dataset will be exported as a shape file including attribute table and projection. (Files: .shp, .shx, .dbf, .prj).
3. If several linear elements are connected to each other, e.g. a linear element such as a hedge (HED) ends and becomes a grass strip (GST), care should be taken to avoid gaps between these adjoining lines (Figure 5.2a). This can be achieved by setting the snapping environment in your GIS program (see digitising areal elements).
4. Linear Elements that are part of a complex of linear elements can be digitised as a series of lines next to each other, e.g. a hedge (HED), then a water edge (WAT) and finally an herbaceous strip (HST). See Figure 5.2b.

Linear Attribute Table

Each element must be attributed with certain data that you collected in the field. This information will be documented in the attribute table in the GIS environment. Table 5.5 details the characteristics of the attribute table. **It is essential that all partners use the same definitions.** The data in the attribute table can be added by joining your data table to the GIS attribute table as described above (Area Attribute Data Entry). An empty table has been provided with the protocol for data entry.

The ElementID in the linear attribute table is a unique number/letter combination for each linear element. It is formed from the country code, the farm number and a habitat number, e.g. Country_FarmNr_HabNr. The country codes are listed in Table 5.3. The farm numbers in the ElementID should be written as 01,02,03,04,05,06,07 to20. The HabNr is a number that can be applied consecutively to all the elements that were found on your farm. **The numbering should start at 101 thus 101, 102, 103, 104 through to X**, i.e. corresponding to the number of elements that you found on the farm. The numbering here starts at 101 to make it unique from the areal elements. Table 5.6 provides an example.

Table 5.5

Attribute columns to be defined in the gis attribute table for the linear elements.

Column Heading	Data Specification	Example	Description of column
ElementID	Text	C_01_101	This is a unique ID for each linear element and will comprise the country, farm number and a habitat number. See Table 5.3
Country	Text	C	The Country code
FarmNr	Long Integer	01....20	The Farm Number
HabNr		101, 102, 103...X	This is the continuous numbering system of the mapped elements within each farm. The habitat number will start with 101, 102, 103 instead of 1, 2 and 3.
Alpha_Code	Text	A	The alpha code given to the different linear elements.
Line_Elem	Text	HED, GST	The linear element classification.
FarmL_CI	Text	5	The Farmland Class for linear elements
Selected	Short Integer	1 or 0	1 for selected elements (sampled), 0 otherwise.
PlotID	Text	Bar code used for the species sampling	For those elements which are sampled: PlotID which appears on labels of species sampling.

Table 5.6

Examples of element ID's for linear elements *Vraag: is het elementID of element ID.*

ElementID	Country	FarmNr	HabNr
C_01_101	C	01	101
C_01_102	C	01	102
C_01_103	C	01	103
C_02_101	C	02	101
C_02_102	C	02	102
C_03_101	C	03	101
C_03_102	C	03	102
C_03_103	C	03	103
C_03_104	C	03	104
C_03_105	C	03	105

Image Interpretation of the landscape surrounding the farm

The image interpretation does not require digitised maps. Instead, you will need to provide a data table (preferably a spreadsheet) that contains:

1. A list of the habitats that are within the surrounding 250 m of the boundary of the elements on your farm which were selected for species sampling.
2. Percentage cover estimates for each of the habitats listed which have a cover $\geq 10\%$.

To do the image interpretation you will need to:

1. Generate 250 m buffers (e.g. in the GIS environment) around the elements where species sampling was undertaken.

2. Use aerial or satellite images, list the habitats that you observe within each buffer (see list below of the habitats that should be identifiable on the image). The GHCs that you record should have a minimum coverage of 10% within the buffer.
3. Estimate by eye the percentage cover of these listed habitats. Taken all together the coverage of habitats in the buffer should add up to 100%.
4. The table in which you record the data should include the ElementID of the areal or linear element (see Tables 5.1, 5.3, 5.4 & 5.5) and then a list the observed habitats with percentage estimates (see Table 5.7).

It should be possible to recognize the following habitats from aerial or satellite images. The exact definitions of these habitats can be found in the Monitoring handbook (Sections 4.1.1 to 4.1.6, pages 34 to 42).

- URB (Urban, e.g. ART, NON, VEG, GRA,TRE and combinations)
- CUL (Cultivated herbaceous crops)
- WOC (Woody crops)
- AQU (Aquatic)
- SPV (Sparsely vegetated)
- FPH (Forest phanerophytes)
- TPH/MPH/LPH (Scrub)
- SCH (Heathland)
- EHY/HEL (Emergent hydrophytes/Helophytes)
- HER (Vegetated herbaceous, e.g. CHE/LHE)

Table 5.7

Image interpretation table: areal elements in a 250 buffer around two sampled elements in Switzerland (c), on farm 1 for habnr. 1 and 9.

ElementID	PlotID	GHC	Percentage
C_01_01	PlotID label of species sampling	WOC	10
C_01_01	As above	FPH	30
C_01_01	As above	HER	60
C_01_09	As above	URB	10
C_01_09	As above	FPH	90

5.2 Data transfer and organisation

Transfer of data from case studies to ART (responsible for the data management) will depend on the indicators in terms of material, format and layout.

5.2.1 Farm management indicators

Data collected from questionnaires is stored in the central database in form of spread sheets. Each case study gathers the questionnaires of its farms, digitalize them into spread sheets before transferring them to the central database. There is one spread sheet per farm. The first sheet contains the questionnaire data with respect to the whole farm. Each line in this table is a recorded indicator with its corresponding value (for instance 'Livestock units per ha UAA'). In a second sheet are data on standard operations of the habitat/field plots selected from the EBONE method for botanical and faunistic investigations. In the Table, each line is a

recorded indicator with its corresponding value (for instance Nitrogen - input (or N-Balance) in kg nitrogen per ha), and columns are the habitat/field plots.

5.2.2 Genetic diversity indicators

Data on genetic diversity indicators assessed with the farm questionnaires is collected together with the farm management indicators (Section 5.2.1).

5.2.3 Species diversity indicators

Data collected in the field on field conditions encountered during the field work (field data sheet) is digitised in a spread sheet and transferred to ART (details are given in corresponding sections of the indicators). The spread sheets are integrated in a central database so they can be linked to the species indicators for further analysis (if applicable). Either from individual partners in case of identification of species at case study level (decentralized identification) or from specialist identifying species for the consortium (or part of it, centralised identification), species lists will be established in form of spread sheets and transferred to ART (details are given in corresponding sections of specific indicators). These spread sheets will be integrated in a central database so that data can be analysed at the different levels, i.e. habitat type, farm, case study region, and all case study regions together.

5.2.4 Habitat diversity indicators

The basic dataset for the calculation of the habitat diversity indicators will be provided by the habitat mapping of the farms in each case study region. The maps will be digitized by individual partners and data transferred to NFLI for analysis (NFLI jointly with ART).

To calculate the habitat indicators it is necessary to have the spatial data that were collected during the habitat mapping exercise. An image interpretation of the habitats surrounding those elements on the farm that were selected for the species sampling is also necessary. The digital maps will enable the calculation of area and length data for the habitats recorded on the farms. The image interpretation will provide a list of habitats that surround the sampled elements together with an estimate of their percentage covers.

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