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Deliverable 2.4 Decision system for selecting a network of EU representatative sites

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Introduction

The scope of this deliverable is to provide some examples for using the selection matrix to guide the choice of sites for field testing, applying the methods and the rational described in Deliverable 2.3. These examples may enable applicants to support the choice of sites for field experiments during environmental risk assessment (ERA), but can also support risk managers in the appraisal of ERA conducted by applicants.

As stated in Deliverable 2.3, the scope of selecting sites can be for different purposes: field experiments for studying non target organisms (NTO), agronomic and phenotypic field trials, post market environmental monitoring, etc.
In this document we analyse two case studies, based on the data from AMIGA activities to show the procedure of site selection applied to NTO studies and to propose a rationale which leads to the definition of a possible network for EU representative sites.

The criteria and the rationale used in the selection matrix which were presented in Deliverable 3.2 are summarized in Table 1.

Steps	Goal	Data sources	Outputs
1. Growth defining, limiting and reducing factors of crop	identification of the potential area where the crop could be cultivated	existing agricultural data bases, expert opinions and/or modelling	
2. Potential and actual cropping area of GM crop	Identification of the impact of the intended GM trait on the distribution of the crop needs	A. Growth defining factors (e.g. CO2, radiation, and temperature requirements B. Growth limiting abiotic factors (e.g. irrigation, fertilizers) C. Growth reducing biotic factors (e.g. weeds, pests and diseases)	1) Maps of expected cropping area according to the crop/ trait combination
3. Biogeographical areas: institutional assurance	Selection of biogeographical regions	Existing biogeographical zoning maps www.eea.europa.eu	2) Map determined by overlapping output 1 and EEA geographical zones
4. Changes in agronomic practices	Define expected changes	Establish changes in data sources listed under point 2	
5. Regional and local protection goals	Define areas from Output 2) which include relevant protection goals	Legislation at different scale (Regional, National, European)	
6. Impact on indicator species and processes	Found similarities and differences in indicator species and processes	Distribution and relevance of relevant target and non-target species, and processes (from existing literature)	3) Identification of similarities and differences in NTOs between zones (to avoid duplication)
7. Site selection		All the outputs above	Final list of selected sites

Table 1. Matrix for field selection

Case example 1: Butterflies (Lepidoptera) and IR/HT crop

Here, site selection for ERA is exemplified by the evaluation of potential GM crop effects on butterflies (Papilionoidea et Hesperioidea), a recognised protection goal, also representative for biodiversity (VDI 2010). As a case study we chose a hypothetical IR/HT maize event producing a lepidopteran-specific Bt toxin and tolerant against the application of a broad-spectrum herbicide. Bt toxins have the potential to adversely affect butterfly populations as butterfly larvae may consume the Bt by feeding on larval host plants dusted with wind-dispersed GM maize pollen (Lang & Otto 2010). The application of broad-spectrum herbicides, such as glyphosate, is likely to change the herbicide regime, which can reduce the weed community within fields and in field margins, in turn adversely affecting larval and adult butterflies associated with such food plants (e.g., Haughton et al. 2003, Roy et al. 2003).

1st step. In assessing and monitoring adverse effects of IR/HT maize on butterflies, first, the present geographical distribution and intensity of maize cropping in Europe has to be determined. This approach is already described in more detail by a report delivered within the AMIGA work package 2.1 (Miklau et al. 2014). Precise data on European cropping area can be retrieved e.g. from the European Environment Agency and/or Eurostat (Fig. A1). Here, maize is widely distributed across Europe, with a concentration in the Steppic and Pannonian biogeographical areas.

2nd step. Then, it has to be assessed if the newly introduced traits may have a potential to change the future range of maize cultivation in Europe. For instance, in our case example one may conclude that introduction of IR/HT maize could result in a reduction of crop rotation, due to a more efficient and effective control of pests and weeds, also enabling to grow maize after maize. This may lead to an intensification of agricultural land use, but not necessarily to a change of the distribution range of maize growing area. Unless this leads to a substantial change in the core cropping areas of maize, it has less impact on the selection of the sites to be considered. Economic and political decisions may also strongly affect the distribution range of maize, such as subsidies for biofuel crops, leading to a possible increase in maize cultivation e.g. in the Mediterranean region or in South-East Europe.

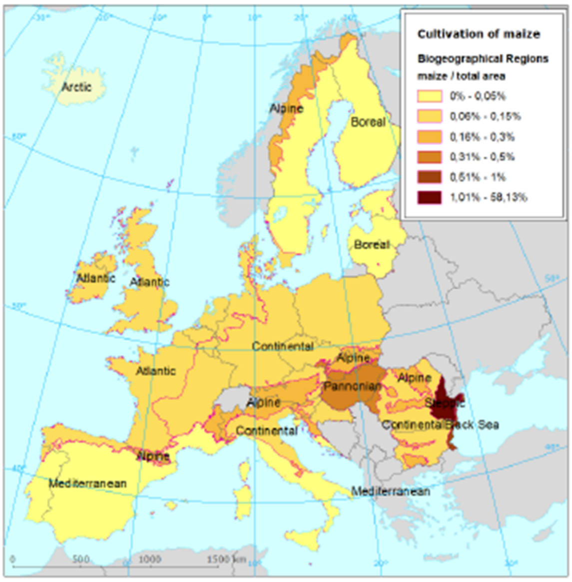

Fig. A1. Maize cultivation intensity per Biogeographical region (after Miklau et al. 2014).

3rd step. Following the first two steps, the indicator for ERA has to be selected and the relevant "receiving environments" to be chosen, i.e. the respective biogeographical zones, regions and sites. As outlined above, butterflies were chosen as indicators for being a protection goal due to the potential hazard of IR/HT maize. Now, the overlap between the distribution of the indicator and the growing area of current (and predicted) maize cultivation must be determined for the biogeographical zones. This can be done in various, differing approaches, not mutually exclusive but supporting each other, depending on the identified and detailed protection goals (see following text).

(i) Butterfly species identified in the ERA to be at risk by IR/HR maize can be the focal indicators determining site selection, e.g. because the species are highly sensitive to Bt and (intensely) exposed to maize pollen drift. Date of maize anthesis is affected by sowing date, climate and cultivar which will differ between different regions. For instance, the larvae of the Peacock Butterfly (*Inachis io*) are sensitive to Bt which will be distributed in the environment by wind when Bt maize sheds pollen (Felke et al. 2010), and the Peacock commonly flies and reproduces in agricultural landscapes (see AMIGA work package 7.2). However, this species might be differently exposed in different parts of European farmland. In Northern Europe, only one generation of the Peacock is produced, which does usually not overlap with the regional maize pollen shedding period, while in Central (and partly in Southern) Europe two generations occur of which the second one will be exposed to GM maize pollen (Fig. A2). In some hot and dry areas the Peacock may also produce only one generation and be possibly only slightly exposed (Constanti, pers. comm.). In this case, site selection will be determined by the occurrence of an overlap of the larval phenologies of possibly exposed species with the expected maize pollen shedding period. Several studies have estimated the degree of overlap for butterfly species in farmland with Bt maize pollen for different European countries, and can be used as guidance (e.g. Schmitz et al. 2003, Traxler et al. 2005, Lang et al. 2015).

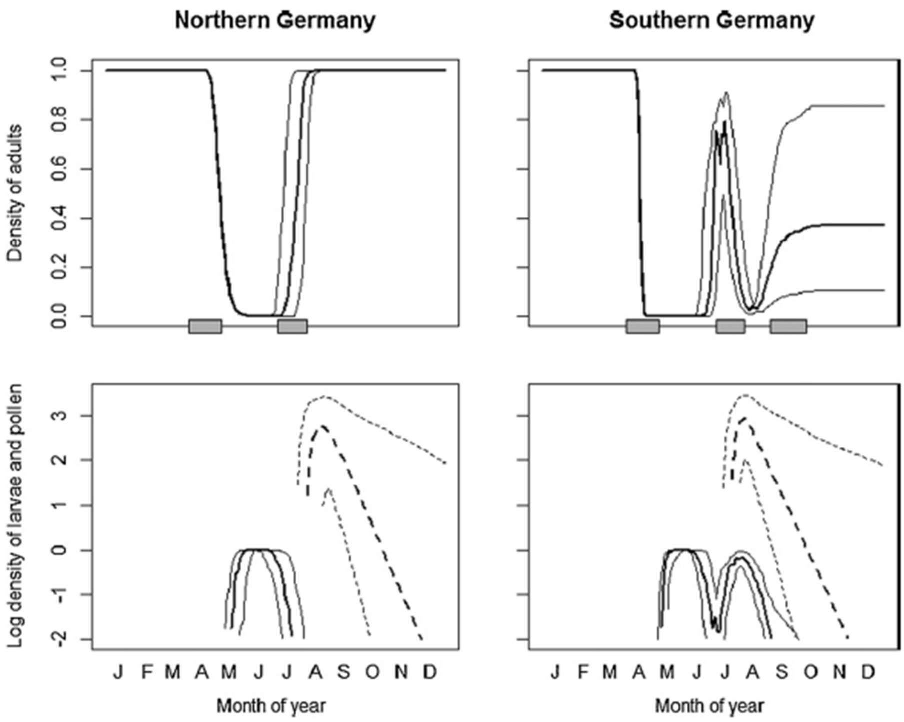

Fig. A2. Simulated phenologies of the Peacock Butterfly (*Inachis io*) in Northern and Southern Germany (after Holst et al. 2013). Density of *I. io* adults (top) and larvae (bottom, full lines), and of maize pollen (cm−2; bottom, broken lines). Thick lines show 50% percentiles, thin lines 10% and 90% percentiles of 1000 simulation runs. *I. io* densities are in nominal units. Horizontal bars (top x-axes) show typical periods when adults are observed flying.

(ii) Protected butterfly species can be chosen as focal organisms for the respective site selection. The protection status can refer to a European, national or regional level. An example for European protection status is the IUCN red list (Van Swaay et al. 2010) or the species listed in the Annexes of the Habitats Directive (Council of the European Union 1992). Usually, national red lists for butterflies exist for EU member states, albeit in varying quality and development stages. Often regional red list are also available, e.g. for federal states or some regions within EU member states.

European level. In AMIGA work package 2.1, Miklau et al. (2014) exemplified how to use protected species listed in the Annexes of the Habitats Directive for the selection of biogeographical regions by determining the overlap of maize cultivation with the distribution areas of a protected butterfly species (Fig. A3). In the example of Miklau et al. only the spatial overlap between species and maize growing areas was considered, and the identification of the temporal overlap with maize anthesis would be a necessary subsequent step (cf. Lang et al. 2015). The European Red List of Butterflies (Van Swaay et al. 2010) can and should also be used for the analyses of spatial and temporal exposure of protected butterflies. Species endemic to Europe are also recorded in this list, for which special responsibility can exist. The degree of overlap of maize cultivation with the red list and endemic species will again trigger site selection for ERA.

National and regional level. For the fine tuning of the site selection, national and regional red list should be accounted for. For instance, of the 202 butterfly species of Romania, “only” 43 species are listed on the European list (Van Swaay et al. 2010), but 105 species on the national Romanian list, and 122 species on regional lists in Romania (Rákosy 2002) (all butterfly species considered of IUCN categories: CR, EN, VU, and NT). This means that on an European level certain species may not be threatened, but they might be on a national or regional scale, thus depending on the objective of the exercise and on the risk managers involved consideration of additional sites should be included. This is also exemplified by the Peacock Butterfly (*I. io*), which is a species of “Least Concern” on European level (Van Swaay et al. 2010), but a protected species in Hungary (Darvas et al. 2004).

Fig. A3. Distribution of the butterfly species *Maculinea teleius* (Scarce Large Blue) and maize cultivation density per Biogeographical region (after Miklau et al. 2014).

(iii) The distribution of <u>protected habitats</u> can also be used for site selection, and should be incorporated when selecting sites. Taking into account habitats is a more integrative approach encompassing species communities (commonly including protected species) in contrast to the above described species-focused selection procedure. The Natura 2000 sites provide an ecological network of protected areas of the Habitats Directive comprising more than 26000 sites (EEA 2012), which can be viewed on the internet (http://natura2000.eea.europa.eu/). Maize cropping area (Fig. A1) can then be overlaid by the distribution of protected areas (Fig. A4), and support decisions about site selection.

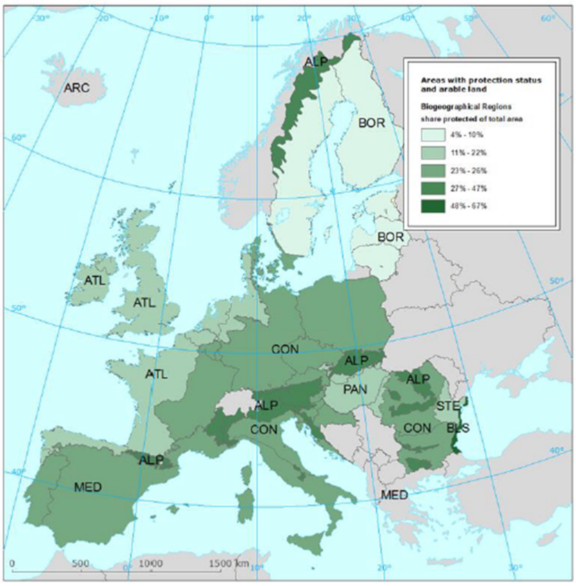

Fig. A4. Share of protected areas in Biogeographic Regions (after Miklau et al. 2014).

Like in the species example above, site selection must be fine-tuned on a national and regional level. For example, Lang et al. (2015) identified a core area in Switzerland where a high proportion of nature reserves are embedded within agriculturally managed land, with high numbers of rare and protected butterflies, which would then be much exposed to Bt maize growing (Fig. A5). Such sites with their species (communities) would also be focal sites to be considered in ERA and.

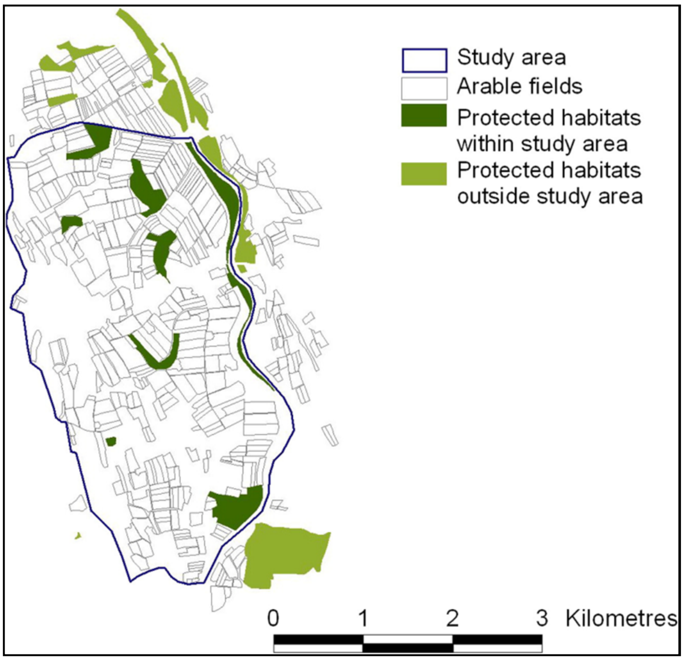

Fig. A5. Example for the potential exposure of protected areas within an arable landscape (Reusstal, Switzerland). Farmland with such high proportions of nature reserves (and protected species) embedded between arable fields are potential focus sites for ERA (after Lang et al. 2015).

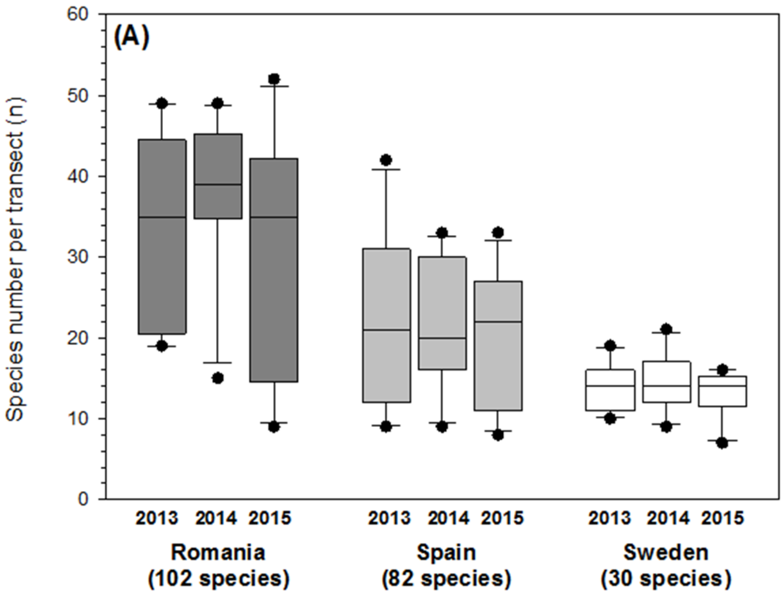

Fig. A6. Comparison of species richness of butterflies and burnet moths between three European countries demonstrating the butterfly hot spot in farmland of the region Transylvania, Romania (AMIGA work package 7.2). Boxes show the 25% and 75% quartiles, the horizontal line within the box is the median, while 10% and 90% percentiles are indicated by the whiskers and outliers by dots.

Another instructive example is the hot spot of butterfly biodiversity in the farmland region of Transylvania, Romania, which was identified by the AMIGA field monitoring (AMIGA work package 7.2). Traditional and extensive land management preserved and secured an amazingly high number of rare and protected butterfly species occurring in farmland (Fig. A6), thus such sites would be essential for the site selection of ERA of IR/HT maize.

With respect to protected butterfly communities, Prime Butterfly Areas (PBA) were identified and located across Europe (Van Swaay & Warren 2003, 2006). The distribution of PBAs can be used for site selection much like the above described examples for protected habitats, i.e. by overlapping PBAs with maize cultivation areas (in fact some of the PBAs are Natura 2000 sites, anyway). On the homepage of Butterfly Conservation Europe the list of PBAs is being kept up-dated (http://bc-europe.eu/).

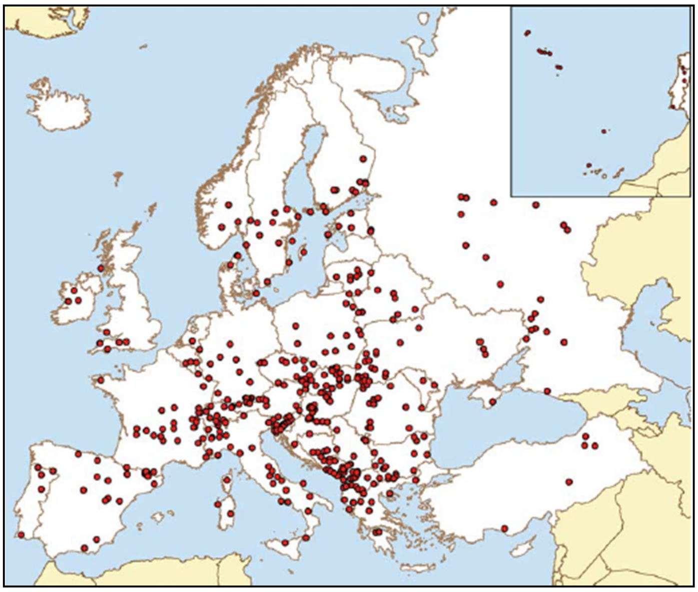

Fig. A7. The location of 431 Prime Butterfly Areas (PBA) across Europe for 34 identified target Lepidoptera species (after Van Swaay & Warren 2006).

Case example 2: Ground beetles (Coleoptera: Carabidae) and IR/HT crop

The target group, ground beetles, are a common and widespread family of beetles (Lovei & Sunderland 1996). They have several characteristics due to which entomologists and ecologists in general consider them a suitable group for environmental monitoring (Koivula 2011). They have soil-bound larvae, and variable dispersal power (Lovei & Sunderland 1996), and can be considered an ubiquitous insect group in cultivated areas in Europe (Kromp 1999). The standard method of surveying them is by the use of pitfall traps, which is also the method selected in work packages of the AMIGA Project. There are several species that are protected in Europe, but these are far not representative of the importance of the family, and many of the species are large-bodied specialists that do not live under cultivated conditions. Their monitoring is therefore, unlike the protected species of Lepidoptera, not a suitable target. Due to the ecological importance of the ground beetles as a group (Kotze et al. 2011), a suitable protection goal can be the integrity and diversity of the group at the sites of the cultivation of the GM crop. Similar to the previous section, we assumed the growing of a hypothetical IR/HT maize event producing a lepidopteran-specific Bt toxin and tolerant against the application of a broad-spectrum herbicide. Bt toxins have the potential to adversely affect butterfly populations as butterfly larvae may consume the Bt by feeding on larval host plants dusted with wind-dispersed GM maize pollen (Lang & Otto 2010, Holst et al. 2013). This may have an indirect effect on ground beetles, through the changes in larval density, and thus in food supply for ground beetles. The application of broad-spectrum herbicides, to which the GM crop is resistant, is likely to reduce the weed community within fields and in field margins, which changes the availability of weed seeds, an important food for several ground beetle species (Bohan et al. 2011).

1st step. In assessing and monitoring adverse effects of IR/HT maize on ground beetles, the present geographical distribution and intensity of maize cropping in Europe has to be determined. This approach is already described in more detail by a report delivered within the AMIGA work package 2.1 (Miklau et al. 2014). Precise data on European cropping area can be retrieved e.g. from the European Environment Agency and/or Eurostat (Fig. A1). Here, maize is widely distributed across Europe, with a concentration in the Steppic and Pannonian biogeographical areas (Dewar 2009).

2nd step. Then, it has to be assessed if the newly introduced traits may have a potential to change the future range of maize cultivation in Europe. Additionally and in tandem with other agricultural trends in Europe, there may be a reduction of crop rotation, increase in field size, as well as a reduction in insect and weed densities. This may lead to an intensification of agricultural land use, but not necessarily to a change of the distribution range of maize growing area. Unless this leads to a substantial change in the core cropping areas of maize, it has less impact on the selection of the sites to be considered. In other respects, the considerations already detailed in the previous example remain relevant here.

3rd step. Following the first two steps, the *indicator* for ERA has to be selected and the relevant "receiving environments" to be chosen, i.e. the respective biogeographical zones, regions and sites. It is also equally important to identify the *indicandum*, the parameter that reflects the phenomenon to be indicated (Lovei 2014). As argued above, the indicator would be the taxonomic group, Carabidae, in their entirety. It may pay to give detailed consideration to sub-groups, mainly on the basis of trophic groupings (e.g. specialised predators, mixed feeders, etc.), depending on the GM crop features. If an IR GM crop is to be monitored, the carnivorous or mixed feeder sub-group will be a more suitable target, while for monitoring the possible long-term environmental impact of a HR crop, the seed eating sub-group would be a more suitable and sensitive target than the whole group of Carabidae. The overlap between the distribution of the indicator and the growing area of current (and predicted) maize cultivation is not relevant here, because of the ubiquity of the target group: ground beetles will occur in all biogeographical zones, though some diversity at species level may be apparent.

(i) Site selection performed for butterfly monitoring can be usefully employed here, for logistical reasons. The monitoring methods to be used will also be general, independent of the possible trophic sub-group as monitoring target. The pitfall traps provide material that can be evaluated by various parameters, from individual response variables (such as average size or inequality (DiGrumo &Lovei 2016), fluctuating asymmetry (Elek et al. 2014) or condition) to assemblage/community characteristics such as taxonomic diversity (Lovei et al. 2013). Consequently, additional, special considerations with respect to site selection for butterfly monitoring are not needed. Trapping and monitoring can simultaneously be done at sites selected for butterfly monitoring. One particular, though, is important. During butterfly monitoring, as a feature of the method used, both cultivated areas and non-cultivated microhabitats can be (and are) surveyed. The pitfall traps to be used for monitoring ground beetles, on the other hand, collect individuals of a soil-bound or surface-moving group, so pitfalls should be placed not only into crops but also into non-cultivated edges or patches of (terrestrial) non-crop habitats, so that these areas are also monitored.

(ii) Protected carabid species, for reasons mentioned above, are not recommended as monitoring targets. For example, Annexes of the Habitats Directive (Council of the European Union 1992) have only 6 *Carabus* species in Annex II and 5 (partially overlapping) species in Annex III. Usually, national red lists are also lacking with respect to the ecological importance of this group. A similar, area-sensitive selection process that is possible for Lepidoptera is therefore neither practical nor feasible.

(iii) The distribution of protected habitats, similar to that suggested under the Lepidoptera example, can be used for site selection, and should be incorporated when selecting sites. Taking into account habitats in a more integrative approach, encompassing species communities is more suitable for monitoring this group as mentioned above. The network of Natura 2000 sites provide an ecological network of protected areas of the Habitats Directive comprising more than 26,000 sites (EEA 2012), which can be viewed on the internet (http://natura2000.eea.europa.eu/). Maize cropping area (Fig. A1) can then be overlaid by the distribution of protected areas (Fig. A4), and support decisions about site selection.

Overall, while the Lepidoptera as a group is rich in mobile protected species that can be frequent in cultivated areas of Europe (see previous section), and at least partially depend on these areas, ground beetles are important for different reasons, and the protection/monitoring aim is to follow the dynamics and composition of the group as a whole.

For Lepidoptera, the surveys performed in AMIGA found 30-102 species, forming a north-south gradient. The material collected by pitfall traps, including maize fields in Sweden, Denmark, Slovakia, Romania, Italy, and Spain, and from potato in Finland, Netherlands, Slovakia, Bulgaria and Romania require laboratory identification and is not yet fully available. Maize fields sampled for monitoring baseline in Denmark, for example, found 35 species (DiGrumo & Lovei 2016), which is a similar range as butterfly species richness found in AMIGA surveys in Sweden (see earlier). A Europe-wide standardised survey of ground beetles in forest fragments detected a north-south gradient, with 25 species in Finland, 43 species in Denmark, and 72 in Bulgaria (Magura et al. 2010) hinting at a gradient of similar magnitude as for butterflies. The analysis of this North-South gradient supports the step 6 of site selection indicated in the matrix.

Conclusions

The knowledge earned during the project has enabled AMIGA partners to consider several characteristics that are fundamental in order to establish a network of EU representative sites. The two examples presented above have shown that considerations on the geographical differences, the agronomic context and the legislative framework (protection goals), a grid can be built to concretely support species selection, according to the criteria suggested in EFSA (2010). Moreover, the experience with estimating biodiversity in different regions has led to additional important differences and similarities between species assemblages of NTOs in European zones. Starting from the existing zoning systems we have demonstrated a north-south trend in biodiversity, in work package 4 we have also highlighted differences and similarities in mesofauna and soil microorganisms which can guide site selection for NTO studies.

But in addition to this, in AMIGA we have experienced that in order to establish such a network, additional features are relevant.

AMIGA has built *de facto* a network of expertise. All field experiments in the project were based on:

- A number of protocols which were initially agreed and revised after the first round of field experiments;
- Experimental design and data analysis was supported by experienced statisticians since the planning phase;
- A number of experimental and commercial farms were part of the network;
- Continuous contacts with national Competent Authorities ensured a trustworthy framework which was essential for the completion of the studies;

What AMIGA has provided is a the foundation of a possible EU network which goes beyond the field sites and constitutes a network of expertise

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