

Deliverable 3.1: LL methodology for organic and mixed farming systems (version 1)

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Associated Work Package(s)

WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8
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Version History

Version number	Implemented by	Notes
1.0	CICYTEX	with CTIFL

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Abbreviations

Living Lab(s)	LL(s)
Living Lab board(s)	LL board(s)
Agroecological Weed Management	AWM
Work Package	WP
Grant Agreement	GA
Key performance indicators	KPI
Arbuscular mycorrhizal fungi	AMF
Unmanned Aerial Vehicle	UAV

1. Introduction

The GOOD project will foster LLs aimed at promoting agroecological weed management strategies and reducing the use of herbicides in different annual and perennial crops. Co-creation activities and experimental trials will be developed in conventional, organic and mixed systems for the testing of Agroecological Weed Management (AWM) strategies.

Work Package (WP) 3 is oriented to the development of experimental activities in pilot sites in organic and mixed systems. The objectives of this WP are:

O3.1 To provide standards and typologies for the agroecological promotion in organic and mixed farming systems to be adopted at national level.

O3.2 To develop feasible AWM solutions tailor-made to organic and mixed farmers.

Organic systems are considered to be those in which no synthetic chemicals (herbicides, pesticides or chemical fertilizers) are used but a combination of strategies following the European regulation for organic production (Regulation (EU) 2018/848). Organic farming principles are based on closing nutrient and energy cycles at farm and landscape level, maintenance of fertility and health of soils, preservation of biodiversity, respect to natural processes and care of the planet and human health. Organic and mixed pilot sites in GOOD do not need to be certified under the organic farming regulation but to comply with this management.

Mixed systems are those that combine agricultural production with livestock farming and/or those that combine organic production with conventional production under transition to become organic (in different parts of the farm).

The main innovation points of GOOD will be to use **cover crops** combined with various practices in an AWM context in order to reduce weed pressure and reliance on herbicides.

Task 3.1 establishes the creation of guidelines and protocols to be followed in a common and coordinated way by all the experimental sites in order to implement the AWM strategies, trial design and assess the most relevant indicators. These protocols will be updated up to three times throughout the project (M6, M18, M30).

The description of Task 3.1, as written in the Grant Agreement (GA) on page 82, is the following:

Task 3.1: Establishment of guidelines and protocols for the organic and mixed sites of each LL

This Task will develop the framework of the pilot operations along with the guidelines for establishing and managing the organic and mixed experimental sites of LLs providing instructions about the methods and tools (D3.1). It will also include the template received from T1.1 and protocols received from T1.2 (key timelines, cover crop species, AWM strategies, and termination techniques of cover crops). The LL boards established (T1.1) will monitor, update and provide feedback for improving the guidelines and protocols of the pilot farms annually.

The Task 3.1 is led by CICYTEX (María Ramos).

The following partners are participating in Task 3.1: UC, LSSV, AUA, COSMOCERT, AIAB, CNR, UNICT, CICYTEX, USC, CUT, MRZIP, HUMOFERT, DELPHY and LLKC.

The Deliverable D3.1 “LL methodology for organic and mixed farming systems (version 1)” is due in Month 6 of the project (i.e., 31 October 2023).

1.1. Objective of the task and scope

The overall objectives of Task 3.1 are:

- Encourage discussion among WP3 partners on the best AWM techniques and the most appropriate methodologies.
- Develop guidelines on the methodologies to be applied in each LL for experimental designs and measurement of effectiveness indicators of the different strategies for weed control, crop development, environmental and socioeconomic indicators.
- Include inputs and protocols coming from other WPs in successive versions.
- Successful implementation of pilot sites and co-creation activities.

Target numbers and Key Performance Indicators (KPI) related to the establishment of organic and mixed sites:

Table 1. Expected results and target values in conventional and organic and mixed pilot sites.

Result	KPI – Target value
Design, assess and demonstrate combinations of AWM strategies in conventional, organic and mixed farming systems to enhance user acceptance	20 assessed cover crop species combined with 15 main crops & 48 assessed AWM solutions combined with cover crops (3 per LL)
	At least 14 assessed cover crops combined with inoculation (1 per LL) & at least 40 weed species identified using AI from the drone images
	32 Best combinations of AWM practices (2 per LL)
	15 N° of crops that AWM solutions will be tested
	40 N° of AWM strategies included in the repository
Stakeholders engaged	160 (10 per LL) N° of stakeholders engaged in the co-creation of LL boards
	1600 (100 per LL) N° of stakeholders engaged in the AWMN
Reporting	16 Life Cycle Assessment report (incl. Social, Economic and Environmental LCA results) (1 per LL)
	80 Factsheets (5 per LL)
	80 Practice Abstracts (5 per LL)

Experimental sites of organic and mixed systems

GOOD will develop, test and demonstrate context-specific AWM strategies in organic and mixed systems through the establishment of 15 large field scale LLs in six different pedo-climatic conditions. Emphasis will be given on the diversity of farming systems and the wide range of crops (14), both annual (9) and perennial (5) (Table 2).

The main crops selected for testing in each LL belong to the most economically important ones in the LL's country and/or those considered vulnerable to weed infestations and difficult to manage. The combinations of AWM solutions will be designed considering factors like the respective weed suppression, the technical and economic feasibility, the societal acceptability, the operational capacity, and the potential impact on soil health properties and diversity.

Table 2. Experimental sites of Organic and Mixed systems.

Annual crops			Permanent crops		
Crop	Country/Partner	LL code number	CROP	Country/Partner	LL code number
RYE/PEA	Latvia/LLKC	LV rye-pea/11	OLIVES	Portugal/ LSSV	PT olives/22
ONION	Netherlands/DELPHY	NL onion/12	CITRUS	Italy/AIAB	IT citrus/23
SOYBEAN	Serbia/MRIZP	RS soybean/13	GRAPES	Italy/AIAB	IT grapes/24
MAIZE	Serbia/MRIZP	RS maize/14	GRAPES	Greece/AUA	GR grapes/25
TRITICALE	Italy/CNR	IT triticales/15	OLIVES	Cyprus/CUT	CY olives/26
WHEAT	Greece/AUA	GR wheat/16	CHERRY	Spain/CICYTEX	ES cherry/27
COWPEA	Portugal/LSSV	PT cowpea/17	APPLE/GRAPES	Spain/USC	ES apple-grapes/28
RICE	Spain/CICYTEX	ES rice/18			

1.2. Connection with other Work Packages

The overall activities developed in WP3 and in task T3.1 in particular, will foster several AWM strategies that will be defined by the co-creation processes of the LLs and will be complemented with the information, protocols and knowledge gathered in other WPs. Likewise, the activities developed in the pilot sites will serve to provide inputs and evidences to other WPs in order to assess the soil health indicators, Life cycle assessment, weed mapping and feeding the AWM Toolbox.

In detail, WP3 and Task 3.1 will feed and be fed by other WPs:

1.2.1. Establishment of LLs and monitoring protocols (WP1)

WP1 → WP3

- Generate typologies and methodologies for the experimental research to be conducted throughout the project in the organic & mixed farming sites of the LLs, based on farmers' decision-making and perception of AWM and barriers and needs for the agroecological transition of agricultural systems.
- Knowledge on needs, barriers, gaps, and opportunities for AWM that will be used for the establishment of the LLs and the experimental design

- List of weed management innovations and strategies based on the combinations of preventive, cultural, biological, digital and mechanical non-chemical weed control method
- Templates and Protocols (T1.1, T1.2) about key timelines, cover crop species, AWM strategies and termination techniques.
- Templates and Protocols for establishing LLs and LLs boards as well as for experimental results reporting.
- The LL boards established (T1.1) will monitor, update and provide feedback for improving the guidelines and protocols of the pilot farms annually.

WP3 → WP1

- Data and content from the R&I activities to be used in the co-creation activities
- Needs, barriers, gaps and opportunities for AWM implementation and adoption to be discussed in the cooperation meetings with other projects

1.2.2. Arbuscular mycorrhizal fungi (AMF) analyses and soil health indicators (WP4).

Seed inoculation with beneficial microorganism (AMF) will be a strategy to guarantee cover crop establishment and crop productivity and favor their competitive ability against weed species. Additionally, effects of the strategies tested on WP3 on crop productivity, weed diversity and soil health (including chemical, physical and biological parameters) will be assessed in WP4.

Beyond the proposed soil parameters for all LLs, each partner may conduct additional studies that are of interest to them (*nutrients and water availability, endo and meso-fauna, etc...*)

WP4 → WP3

- Protocols for soil sampling (AMF identification, soil health indicators)
- Protocols for seed inoculation of cover crops
- Native AMF identification, reproduction and delivering the inocula for seed inoculation in the second and third year

WP3 → WP4

- Send pooled soils samples (AMF identification, soil health indicators)
- Send to UNIPI root samples to assess mycorrhizal colonization of inoculated and non-inoculated cover crop plants and to evaluate the symbiotic competence of native AMF in the second and third year.

1.2.3. Weed identification and mapping with drone flights. (WP5)

In all the LLs, UAVs will be used annually to acquire photographs of the weed flora. This imagery will enable the production of weed prescription maps to prioritize the dominant and invasive plant species (using AI – T5.1) and proceed to termination activities. Protocols and assessment about weed mapping will be developed by WP5.

All the LLs will deploy UAVs equipped with high-resolution cameras to perform flights once or twice per season, based on the cropping type (once for annuals and if possible twice for perennials).

WP5 → WP3

- Protocols for technical implementation and troubleshooting through sessions and training
- Development a software interface to exchange information with LLs' weed experts.
- AI algorithms for weed identification and mapping

- Provision of actionable information of UAV data and AI models for weed management in the field.

WP3 → WP5

- Information about areas/facilities of UAV flights, the annual calendar of field operations, plantation properties and field characteristics in each site for the successful pilot operation.
- LLs' weed experts will annotate all relevant data through a user-friendly software interface, which EDEN will develop.
- Evidence-based data coming from the experimentation within the LLs to feed the Agroecological Weed Management Toolbox.

1.2.4. Life Cycle Assessment. Societal, economic and environmental impact (WP6)

Several assessments will be done in WP6 regarding the social, economic, and environmental impacts of Agroecological Weed Management, as well as the Life Cycle Assessment (LCA) of these strategies at farm level. The LCA will allow the evaluation of the impacts generated by the different crops, treatments, and approaches used within the LLs and consequently build an articulated picture of the impacts of cover crop-based rotations in different environments

WP6 → WP3

- LCA Common protocol to all partner countries, with a focus on soil health and the impact of agroecological solutions on wildlife (e.g., earthworms) and soil properties

WP3 → WP6

- Relevant evidence-based data from LLs to feed the LCA assessment.

1.2.5. Dissemination, communication and demonstration

All relevant information from WP3 will be communicated and disseminated to stakeholders in participating countries.

WP7 → WP3

- Guidelines to provide useful information from LLs activities to feed the Communication and Dissemination plan.

WP3 → WP7

- Demo-farmers of the LLs will share their experiences with other practitioners through different field events.
- Reporting of demo activities
- Dissemination of the adapted materials through different channels, depending on the characteristics of their LL.

1.2.6. Agroecological performance evaluations: TAPE and OASIS tools

Two existing frameworks will be used for GOOD R&I activities to assess the agro-ecological performance of solutions. The first is the Tool for Agroecology Performance Evaluation (TAPE) developed by FAO and the second is the Original Agroecological Survey and Indicator System (OASIS) developed by the Agroecology Europe initiative.

The guidelines to use these tools will be communicated in M18 (D3.2).

1.3. Technical and environmental context

Weed control is essential for maintaining the productivity, the profitability and the sustainability of plant productions, and also to ensure the sanitary quality of crop products.

The harmfulness of weeds species is defined in relation to a given crop. It can operate at several levels: competition for water, nutrients and light (direct primary harmfulness), impact on crop pests, on cultural or post-harvest operations, risk of toxicity in harvested products (indirect primary harmfulness), or at the farm or territorial scale through the dispersal of seeds and invasive species (secondary harmfulness) (CORDEAU, 2018).

The challenge represented by weed management has been indicated as one of the mayor constraints of farms conversion into organic management (BOND & GRUNDY, 1998).

In organic farming, weed management methods must comply with the conditions set out in article 1.10.1 of Part I of Annex II to Regulation (EU) No 2018/848, which defines the means of preventing “damage caused by pests and weeds” (i.e., “natural enemies”, “choice of species, varieties and heterogeneous material”, “crop rotation”, “cultivation techniques such as biofumigation, mechanical and physical methods”, and “thermal processes such as solarisation and, in the case of protected crops, shallow steam treatment of the soil to a maximum depth of 10 cm”). While for pest and disease control, when these methods are insufficient, the use of a choice of nature-based products is authorized in organic farming (Regulation (EU) 2021/1165), the use of herbicides, even bio-based ones, is totally prohibited.

In practice, today, weed control in organic farming relies heavily on mechanical methods. But these ones are neither fully satisfactory from an environmental point of view. Tillage may be detrimental for soil physical, chemical and biological fertility: increased risk of runoff and soil erosion, accelerated organic matter mineralization and risk of nutrients loss through leaching; disturbance of earthworm activity and antagonistic soil fauna (ARCHAMBEAUD, 2015; WEYERS, 2008). On perennial crops, tillage may also affect the soil bearing capacity and cause injuries to the trees. Moreover, depending on the tillage equipment employed, this practice may even contribute to the multiplication and dissemination of certain perennial weeds (BONIN, 2009).

The introduction of more agroecological and nature-based principles in organic weed management strategies could therefore be a positive way forward for organic and mixed farming, to better preserve functional biodiversity while maintaining crops profitability.

2. Agroecological weed management strategies

Agroecology is a holistic approach that relies on and maximizes the use of natural ecosystem functionalities to support agricultural production. Applied to weed control, it will consist in preventive or curative means of breaking the development cycles of weeds, and thus preventing their harmfulness to the main crop. These means will rely on natural regulatory mechanisms between plant species, among each other, or with the soil microbiome, or even by inducing a temporal shift in weeds emergence. Other alternative methods (mechanical, physical, digital) can also be combined with these natural levers, which, alone, are likely to be only partially effective.

2.1. Agroecological methods

2.1.1. Cover-crops

2.1.1.1. Principles

The use of intercropping cover crops is one of the cornerstones of conservation agriculture applied to arable crops. First developed in response to the European Nitrates Directive, their role has gradually

evolved to exploit the diversity of ecosystem services they can provide: crucifers for their "nitrate trap" effect, legumes for their "green manure" effect, grasses for their ability to restructure the soil, etc. (JUSTES, 2017). The idea of using them for weed management is more recent, and the way to use them for this purpose has yet to be perfected.

The use of ground covers for weed management is based on the dominance relationships that exist between plant species, based mainly on two types of mechanism: **competition** and, probably to a lesser extent, **allelopathy**.

Competition for resources, and especially for light, is the main mechanism involved in the regulation of weeds by cover crops. Competition will give the advantage to species with a high biomass, sown densely and able to grow very quickly. This will smother weed emergence, thus eliminating weeds in the cover crop, but also with an expected long-lasting effect to limit weeds emergence on the following main crop. Allelopathy is a mechanism by which a plant (a living plant or its residues in decomposition) affects the growth of neighboring plants through the production of chemical compounds released into the environment. Root exudates in particular can be released, inhibiting the germination of other species. Under natural conditions, with no human intervention, this is particularly evident in the development of some large monospecific lawns, even consisting of very small species (*Hieracium pilosella*, wild strawberries, etc.). This is also confirmed by many laboratory studies. But the possible use of species with allelopathic properties to control weeds in the field has yet to be demonstrated and developed.

In practice, several approaches can be considered for using cover crops for weed management:

- **On annual crops**, cover crops are used as intercropping crops:
 - 1) The cover crop (monospecific or mixture of species) is sown before the main crop (or unless otherwise possible at the same time as the main crop).
It is recommended to sow the cover crop in early autumn in the case of winter main crops (wheat, triticale, etc.) or in late autumn or winter in the case of spring main crops (soybean, onion, maize, rice, etc.)
 - 2) This cover crop is destroyed at the end of its cycle (either naturally by frost, or by other methods), and then the main crop is sown and cultivated.

In this case, both the cover crop and the main crop are managed as annual crops.
- **On perennial crops (fruit trees or vine)**, the cover crops can be used on the inter-rows of the orchard or vineyard (as annual or perennial crops), or directly on the tree- (or vine) rows.
 - On the inter-rows, in addition to improving weed management, the functions to be targeted are the improvement of the soil's bearing capacity and its physical and biological properties.
 - On the rows, the aim is to help controlling the weeds, while limiting the competition between the cover crop and the trees or vines. Therefore, the objective will rather be to keep the cover crop to a minimum height, either by choosing short species or by applying additional management methods to the cover crop (such as mowing).
 - In all cases (interrows or rows), a "green manure" function can also be considered, by using legume species (which can be with high biomass, or at the contrary, dwarf cultivars). This can be particularly interesting in the case of organic production systems.

2.1.1.2. Soil enrichment with AMF (Arbuscular Mycorrhizal Fungi)

Arbuscular mycorrhizal fungi (AMF) are obligate root endophytes which rely on getting carbohydrates from plants. In return, they can provide multiple ecosystem services to their host plant: enhancement of soil nutrients uptake (especially phosphorus, zinc and copper), help to resist to drought, protection against root pathogens, etc. However, AMF are not only beneficial, and interactions between plants and AMF can range from highly mutualistic to antagonistic (SÅLE, 2022; RINAUDO, 2010). This could be exploited to contribute to control the weeds in the agroecosystems: thus, AMF may have a direct suppressive effect on the growth of many weeds belonging to families that are not usual hosts for AMF (non-mycorrhizal species); they also could act indirectly by enhancing the competitive ability of crop species to the detriment of some mycorrhizal or non-mycorrhizal weed species (EL OMARI, 2021).

In these recent years, many research studies have been conducted on this subject, mainly on arable crops. They conclude to varying levels of effectiveness depending on the crop species, the weed species and the taxonomic diversity of the AMF applied. They also encourage further studies in field conditions, especially under agroecological production systems.

In practice, the simplest way to enrich the soil with AMF is to inoculate them to the seeds before sowing. This can therefore be applied to the cover crops, with two types of expected positive effects on weed control:

- Help to suppress the growth of most undesirable weeds in the cover crop, and thus, also later on the main crop.
- Give the advantage to the cover crop to the detriment of the weeds, favoring their rapid soil coverage and growth, to allow them to smother the weeds, through competition for light.

AMF are naturally present in the soils, but their abundance or taxonomic diversity may differ depending on a multitude of factors (pedological, climatic, agricultural practices intensity, etc.).

In GOOD project, AMF will be isolated from soil samples taken from the LL's experimental sites, to be sure of their adaptation to local conditions. These samples should come from both the conventional and organic fields, to maximize the diversity of taxa collected. They are then multiplied in laboratory conditions, and later inoculated to the seeds of the selected cover crops.

2.1.2. False seed bed practice

The false seed bed technique is a cultural practice that can be implemented before the main crop is sown, with the objective of reducing the weed seeds stock already present in the soil.

After the previous crop has been destroyed, it consists in a succession of shallow tillage operations (less than 10 cm) performed to favor the germination of the weed seeds already present in the soil, and then to destroy the weeds plantlets after their emergence. Several series of false seedings are necessary to reduce the seed stock present in the superficial layers of the soil. The success of the technique relies on a good choice of shallow tillage equipment (harrow, tine or vibrashank cultivator, etc.) and on suitable climatic conditions (soils wet enough for the emergence of weeds).

This technique only concerns the case of annual main crops, and is nowadays quite commonly used in biological farming systems.

2.1.3. Crop rotation

Crop rotation is probably one of the oldest techniques (beside manual weeding) for breaking the development cycle of the weeds. With farming intensification, the diversity of crop rotations has been impoverished, to the detriment of this essential function.

Extending crop rotation and introducing intermediate crops is an option which should be re-examined to facilitate weed management in annual crops.

2.1.4. Vegetal mulch

Vegetal mulching is an ecological technique that can be applied on perennial crops. It consists in hindering weed emergence by depriving them of access to light, by covering the soil with a thick layer of plant fragments.

This mulch can be obtained from woody plants present on the plot (e.g., chipped pruning wood in orchards), or from hedge pruning along field or road edges, or even from crops grown outside the field for this purpose (e.g., straw, miscanthus, etc.).

The mulch layer must be thick enough and able to last long enough on the soil. As it is naturally biodegradable, it must be renewed to maintain its effectiveness. Other materials of animal origin, such as wool, are used for mulching.

2.1.5. Grazing

Integrated (mixed) crop-livestock systems are encouraged in organic farming because they lead to greater resilience and food self-sufficiency, while having a positive impact on a number of ecosystem services (Sanderson et al. 2013; Stark et al. 2018; Schleich et al. 2019; Serakan et al. 2021).

The use of harvest residues of extensive crops (cereal-legume) by animals before the new sowing is very frequent in many regions. This has a double advantage: to feed livestock with local resources and to improve fertilization and soil properties thanks to the manure provided by the animals. This contributes to closing cycles at the local level and reducing the environmental impact of livestock feeding.

In perennial fruit crops, the use of grazing for weed control is usually done with small ruminants or pigs rather than cattle (i.e. sheep in olive/vineyard orchards) or even with fowls (i.e. geese). Depending on the size, height and type of tree pruning, it is important to choose animals that have good weed control ability but do not damage tree trunks or productive shoots. Animals should enter the fields at times of major weed control interest but without hindering management crop tasks.

2.1.6. Biological agents

All plant species, including all agricultural weeds, are attacked by a diversity of other organisms, including fungal and bacterial diseases, insects, mites and mammals. Examples of a natural enemy consistently controlling a weed species are few. (Mohler et al., 2021). Weed control by biocontrol agents is a developing field of research with a long way to go. Experiments have been carried out with the application of microorganisms to the soil and some insects that act as natural enemies. The objective is to interfere with the germination of weed seeds by causing damage or consuming them. i.e: seed coats can be attacked by fungi. Fungi also kill many weed seedlings in the white thread growth stage. Incorporation of green plant materials into the soil reduces weed seedling emergence, and the effect is associated with attack by soil fungi (Mohler et al., 2021). An example: some pathogenic fungi were identified as potential to control bindweed (*Convolvulus arvensis* L.) and some of them could be used as mycoherbicide components. *Colletotrichum linicola* seems potentially effective and field tests alone or in combination with *Stagonospora convolvuli* (Tunali et al. 2009).

2.2. Mechanical and physical techniques

Mechanical means include tillage and mowing.

- **Tillage** is the very basis of the definition of arable crops, and a very wide choice of equipment is therefore available for annual crops. Various working tools can be combined on the same equipment or used alone at separate times.

For perennial crops, a range of offset tools has been developed over the years for working the soil at very shallow depth along the rows of organic orchards and vineyards. They are equipped with feelers that allow the work assembly to be retracted to avoid obstacles and preserve the trunks (intercept tools).

A wide range of tools are available today for on-the-row mechanical weeding, with different modes of action (weeds pulling, or weeds root system cutting): intercept hoeing blades, rotary tillers, harrows, discs, metallic brushes, finger weeders, etc. Various tools can be used together on the same tool carrier, or separately to adapt to soil conditions.

This kind of equipment is still rarely used in conventional systems.

- **Mowing** is commonly used on the inter-rows of grass-covered orchards (either in conventional or organic farming in some countries), and it can also be applied along the planting rows, either to manage cover crops, or the spontaneous flora.

This practice has developed in some organic fruit crops but is rarely used in conventional orchards. It is also based on the use of various mowing tools (shredders, flail mowers, nylon cords brushes) specifically adapted to orchards or vineyards, capable of cutting the grass along the planting rows and between the trees, thanks to offset equipment and avoidance systems.

Other equipment, based on physical means, has also be developed to control the weeds without herbicides:

- **Thermal weeding** using gas, hot water or water steam. Some equipment is available on the market for weeding orchards along the tree rows, and some tools are still at the prototype stage. They must be used on very young weeds (plantlets stage).
- **Electric weeding; laser weeding:** these techniques are still under development, and their interest has still to be confirmed.
- **Physical barriers** can also be used to avoid the emergence of weeds on perennial crops: **synthetic mulch** (plastic or biodegradable materials) or **prefabricated natural mulch** (hemp or coconut fiber canvas, etc.).

2.3. Precision weeding

The development of **tools for detecting and even identifying weeds** opens up an innovative avenue for reducing weeding operations (chemical or even mechanical) and better targeting the weed control interventions. Based on optical means, imagery and artificial intelligence, these tools are still at a very early stage of study, but some applications already exist in the form of field-usable tools (mostly at the prototype stage). Coupled with precision sprayers, they can target only the weeds present, and thus considerably reduce the quantities of herbicides sprayed per hectare.

Their use is being particularly studied on annual crops, to target weed outbreaks when they appear sparsely in the field, and in particular certain perennial weeds that are particularly harmful or difficult to eliminate.

On perennial crops, they could be used to control perennial weeds (e.g., to reduce the amount of glyphosate applied), but compatibility with national regulations concerning restrictions in the authorized number of spraying interventions has to be verified.

Beyond precision spraying, these detection tools can be useful to farmers (both on annual or perennial crops) to have an overview of the weeds present in their field at a given time, and thus better reason their weeding interventions (better choice of active ingredients, positioning of mechanical operations, etc.).

Several of the levers listed above can also be combined to form integrated weed management strategies.

☛ *However, for a better harmonization of experiments in GOOD project, a common base of levers to be tested has been proposed (see below), in compliance with the Grant Agreement. If other options can be tested (depending on local conditions, on crops, or even on knowledge advances from previous works) replacing the proposed treatments they will yet be considered and discussed in the Project Steering Group (PSG).*

2.4. Table with cover crops and AWM strategies for all Living Labs

The main strategies to be used in GOOD in organic and mixed sites will be completed from all LLs inputs in M18. (Version 2 - D3.1)

3. Experimental designs

A minimum of measurements must be done in a common way in all experimental sites (both annual and perennials). For each **LL's main crop, three cover crops and one control plot** (without cover crop) will be established **in the first year**. In the case of the annual crops, these cover crops will rotate with the main crop and will performed at the same time as perennial crops.

In the second and third years, the best performing **cover crop will be inoculated with AMF** provided by WP4. The selected cover crop will be sown twice: inoculated and non-inoculated. A control management with no cover crop (reference) will be also tested.

The cover crops that will be selected are known to be adapted in the six pedo-climatic conditions of GOOD and will be tested in a broad spectrum of conditions, alone or in mixtures (*Avena sativa*, *Secale cereale*, *Lolium spp.*, *Festuca spp.*, *Sorghum spp.*, *Hedysarum coronarium*, *Lupinus spp.*, *Medicago sativa*, *Medicago truncatula*, *Trifolium alexandrinum*, *T. subterraneum*, *T. repens*, *Vicia spp.*, *Brassica oleracea*, *B. rapa*, *Raphanus sativus*, *Sinapis alba*, *Linum usitatissimum*, *Phacelia tanacetifolia*, *Hordeum vulgare*, and *Thymus serpyllum*).

Notice that **all species are not useful for seed inoculation with AMF**. Being a non-host means that the symbiosis will not occur. This is the case of *Brassica spp* or *Lupinus spp*. These species will **not be able to be selected for inoculation** in years 2 and 3.

The use of cover crops will **be combined with other weed control strategies** in the main crop. Treatments in the main crop for each pilot site will include, at least, **one cultural, one mechanical and one weedy** treatment following some of the strategies described in section 2.

The proposed experimental designs in this protocol comply with the minimum requirements established in the GA. Other designs or additional treatments, more complex or more appropriate for each situation, can be developed by partners as long as the minimum requirements of the GA are met.

It is recommended that conventional (WP2) and organic trials (WP3) be carried out on nearby plots to ensure that the soils are similar.

3.1. Annual crops.

3.1.1. First year

Three cover crops or mixtures have to be sown in the first year + Reference management without cover crop.

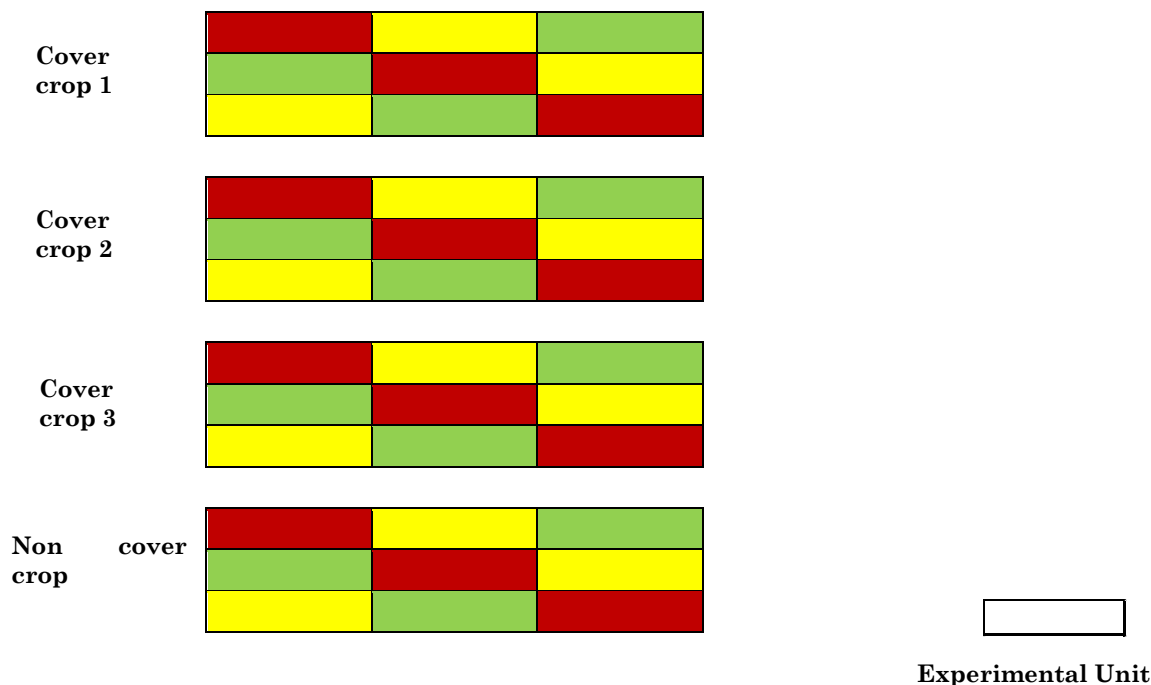
Sowing of the main crop after the termination of the cover crops and applying weed management treatments on the main crop.


The minimum weed management treatments (3) will be: **1 mechanical** weeding; **1 cultural** practice (false seedbed, grazing, intercropping, roller crimper...), **1 untreated control** (weedy)

Each experimental unit has to be repeated at least **three times** in a randomized block design.

DESIGN EXAMPLE:

ORGANIC/MIXED SYSTEMS (36 subplots)



	Treatment 1 (weedy)
	Treatment 2 (mechanical)
	Treatment 3 (cultural)

3.1.2. Second and third year

The best cover crop has to be sown **twice** (inoculated + non inoculated) + Reference management without cover crop.

Sowing of the main crop after the termination of the cover crops and applying weed management treatments on the main crop.

The minimum weed management treatments (3) will be: **1 mechanical** weeding; **1 cultural** practice (false seedbed, grazing, intercropping, roller crimper...), **1 untreated** control (weedy)

DESIGN EXAMPLE:

ORGANIC/MIXED SYSTEMS (27 subplots)



	Treatment 1 (weedy)
	Treatment 2 (mechanical)
	Treatment 3 (cultural)

3.2. Perennial crops.

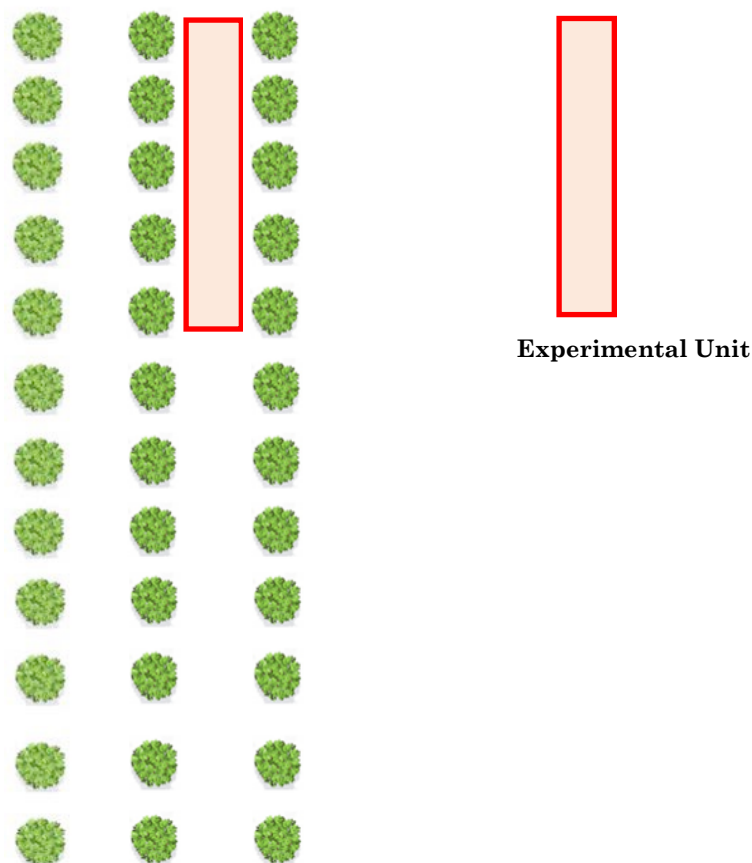
3.2.1. First year

Corridors (**interrows**) will be sown with at least three cover crops or mixtures in the first year

Two reference treatments will also be assessed during the first year: Reference management without cover crop (mechanical weeding) + weedy treatment (untreated)

There will be **5** treatments in total for the organic pilot sites with 3 replications per experimental unit (15 subplots)

No mandatory measurements on the main crop will be done for the first year. But partners can add other treatments of their interest (i.e. testing additional cover crops, starting with AWM practices on crop lines, etc.).



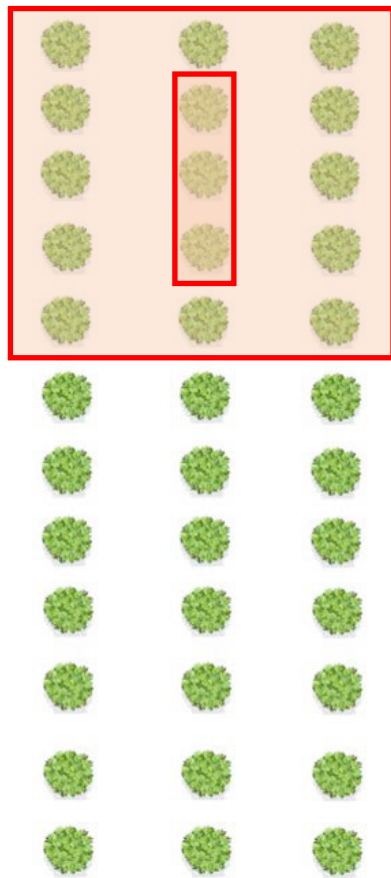
3.2.2. Second and third year

A **single cover crop** is chosen (as a minimum) according to the results obtained in the first year. From year two, this will be used **as a companion crop on the interrows** of the perennial crop, in a new experimental design, which will **combine this cover crop with various AWM practices applied on the tree- or vine-rows**.

The best cover crop have to be sown in the interrows **twice (inoculated +non inoculated) + Reference management** without cover crop. There will be **3 different managements** for the corridors in total.

At least, three AWM practices will be applied in the tree rows: **Weedy+ Cultural practice** (intercropping, grazing, mulching...) + **Mechanical practice** (mowing, manual weeding, mechanical weeding)

EXPERIMENTAL UNIT EXAMPLE:

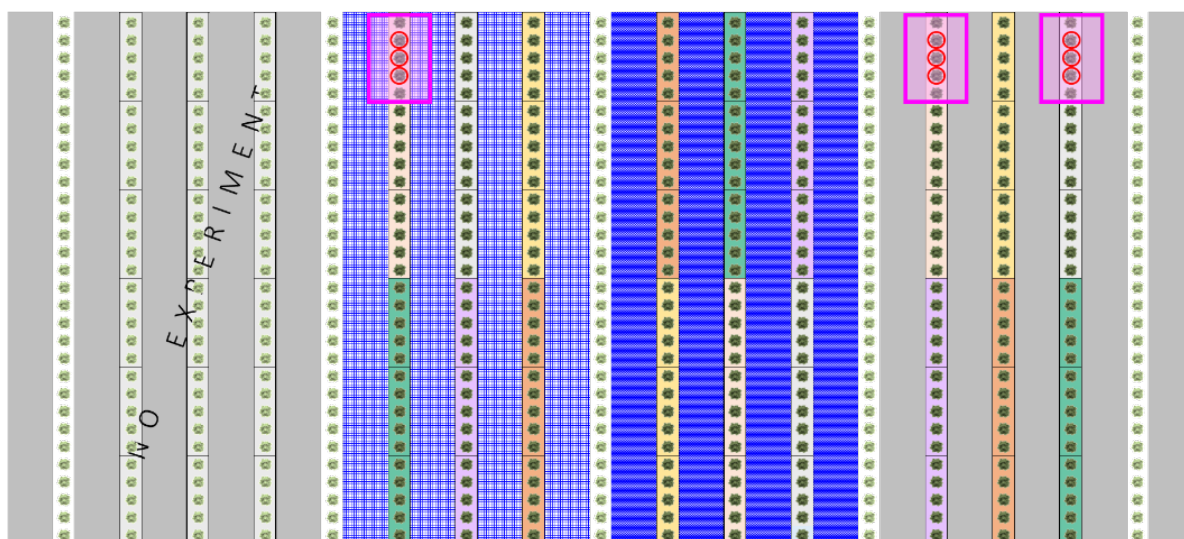


Experimental Unit
(including 3 useful trees)

- **Unit plot minimal size:** the best compromise between:
 - At least 3 “useful” trees (or vines): i.e., trees (or vines) that will be harvested (for yield quantification); they can be surrounded by 2 border trees (or vines)
 - At least a portion of “weeded strip along the row” of approximately 9 m² (+ the related portion on the interrow).
- **Number of replicates:**
 - Each experimental unit plot must be replicated at least 3 times.
 - This can be multiplied by n blocks (2 for instance), if possible (and even more so if there's a good reason for it: for example, two different soil profiles well identified in the orchard).
 - If needed (to minimize the size of the experimental field, or to simplify the protocol implementation), a split-plot design can also be used.

There will be **27 subplots in total**= 3 treatment in corridors x 3 treatments in the tree rows x 3 replications

EXAMPLE FOR A COMPLETE DESIGN (with 6 weed management treatments)




Weed management treatments on the rows:

- Treatment 1
- Treatment 2
- Treatment 3
- Treatment 4
- Treatment 5
- STANDARD PRACTICE ON THE ROWS

(One of these treatments can be a no-weeding reference)



Experimental unit plot for combined weed management strategies (rows & interrows)

Each of these unit plots must include at least 3 "harvestable" trees or vines: 

Cover-crops on the interrows:

- Selected cover-crop without AMF (sown)
(blue is an example)
- Selected cover crop + AMF (inoculated seeds)
- REFERENCE (standard practice on the interrows)

One cover-crop, with or without AMF
 6 weed management treatments on the rows, including the standard practice
 --> **18 weed management strategies in comparison**
 3 replicates per WM strategy ; 5 trees (vines) per repetition
 No block

 --> **54 experimental unit plots**
 (this could be repeated in a second block --> 108 unit experimental plots)

3.3. Designs of organic and mixed pilot sites in each LL.

The experimental designs per LLs in organic and mixed systems will be complete by the second version of Deliverable 3.1 (M18)

4. Observations to be made in the Living Labs

Experiments undertaken in the Living Labs should enable to assess and demonstrate the feasibility of various AWM strategies in context-specific conditions, their effectiveness, and their sustainability (economic and environmental).

A set of observations and monitoring will be realized to measure the ability of each strategy to:

- Control the weeds
- Maintain the productivity and growth of the main crop
- Limit competition for soil water and nutrients
- Maintain or improve the biological and physical soil properties
- Maintain or improve the economic and environmental sustainability

The following indicators will be the minimum to be done in common in order to compare LLs results but others could be proposed and measured by each partner according to its interests and conditions (*Indicators measured in each LLs will be collected in the Sharepoint and included in the second version of Deliverable 3.1*).

4.1. Floristic indicators

4.1.1. Plant biomass *(for cover crops and weeds on annual and perennial crops, and for main crop in annuals)*

Plant biomass is measured by cutting the vegetation at ground level from selected quadrats using cutting equipment (one-hand hedge trimmers, mowers etc). Weeds and cover crops (or main annual crop) are then separated into two samples, and the plants are placed into a drying oven at 60°C for 24 hours. The biomass value is expressed in kg of dry matter per m² (or per ha).

For each date, three samples must be taken per experimental unit plot (using square frames 50 x 50cm).

Measurement frequency:

- On perennial crops: at least one sampling at the beginning of the spring, and another at the beginning of the summer (and additional samples before mowing or mechanical weeding interventions)
- On annual crops: at least one sample in winter and another just before the cover crop termination, and the main crop harvesting.

4.1.2. Plant coverage percentage and Diversity *(for weeds on perennial and annual crops / for cover crops on annual crops and on the interrows of perennial crops)*

In perennial crops (orchard or vineyard), the plant coverage percentage is the best indicator to either measure weeds extension and cover crops' ability to establish and maintain over time, and to allow the comparison between AWM strategies by simple statistical analysis methods.

To enable follow-up over time, the plant coverage rate must be quantified at the same locations throughout the project.

Measures are done within a frame-quadrat, with a minimal size of 50 cm x 50 cm, to 1 m x 1 m. This quadrat is positioned on the row, between two trees (or vines), but, if possible, avoiding the zone directly under a water dripper. The position of the quadrat is chosen (and repaired) at the first observation date, and it will not be changed further.

For measurements on the interrows, the quadrat is positioned between the middle of the interrow (which is the limit of the experimental unit plot) and the border of the “weeded strip” but avoiding the zone where tractor wheels use to pass (Figure 1). When rows and interrows are concerned, two quadrats must be observed per unit plot (“Experimental design N°1”, which is the most common case in GOOD project, in accordance with the basic experimental design described in § 3.). Whereas a single quadrat is observed per unit plot in case of “Experimental design N°2” (standard practice on the interrows / no additional cover crop tested on the interrows). This must be done in **3 different sites per experimental unit**

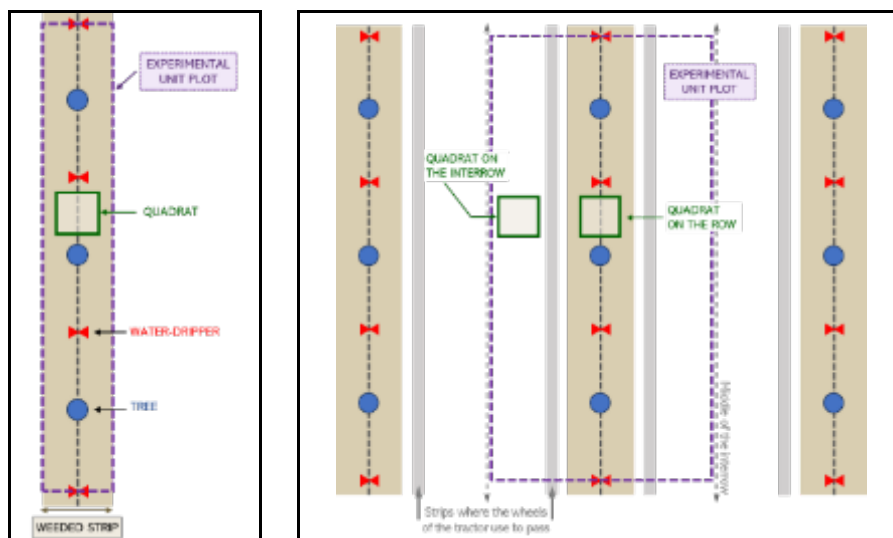


Figure 1: Quadrat position in case of Experimental design N°2 (left) or Experimental design N°1 (right).

The plant coverage percentage is a visual estimation of the percentage of ground covered by vegetation, including weeds, cover crops (where relevant) and mosses. BRAUN-BLANQUET diagrams can be used to help approximate these measurements (Figure 2).

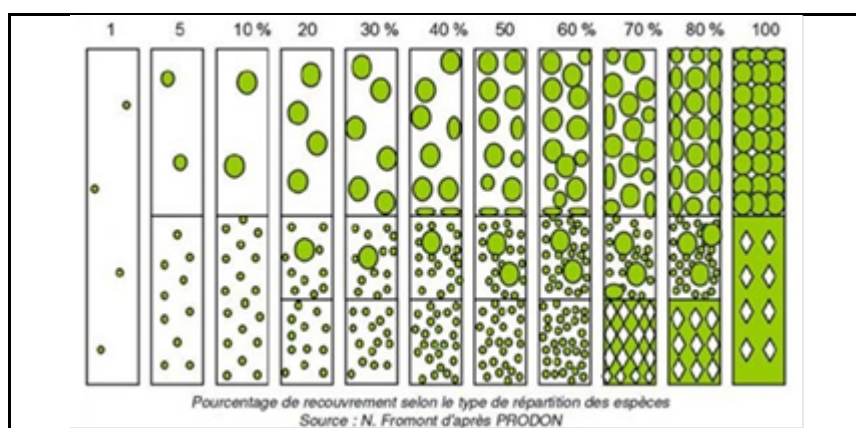


Figure 2: Diagrams to help visual estimation of plant coverage percentage (inspired by the Braun-Blanquet method).

The **coverage percentage** must be measured **for weeds and cover crops**.

At the same time, **Diversity of species/families** should be determined in the same quadrat observations. Plant diversity is the percentage represented by each family (grasses, legumes, etc.) or species of the total number of plants present:

- **Weeds:** at the species level as much as possible. When species recognition is too difficult for the observer, identification to gender or even family level is possible (i.e., “Rumex sp.”, “Poaceae”).
- **Cover crops:** at the species level if relevant.

- **Bryophytes** (mosses), as a single group (no distinction at species level is required).

The percentage of **bare soil** (which can include stones or pebbles in the case of gravelly soils) is deducted by subtracting the sum of all these categories from the maximum rate of 100%.

Identifying the angiosperm flora at species level will enable to deduce another important indicator: **the floristic richness**, which is defined as the number of species present per observation unit.

The estimation of plant coverage percentages by vegetation groups can be done in the field, or later, based on pictures of the quadrats (in this case, a prior inventory of the species present in the field is recommended, to avoid misidentification on the photos). **In all cases, it is highly recommended to take pictures of each quadrat, properly referenced, to allow visualization of the evolution of the flora over time.**

These observations are recommended at a **minimal frequency** of at least once during winter rest and several times between April and October:

- **Perennials:** At least 3 times. 1 sampling in early winter + 1 sampling at the beginning of the spring+ 1 sampling at the beginning of the summer (just before or at the same time of mowing if applicable). Another additional sampling could be done in LLs where climatic conditions allow cover crops to survive during summer (humid)
- **Annuals:** At least 2 times. 1 sampling in winter+ 1 sampling just before the cover crop termination

When weed control measures are applied to certain plots, measurements of plant coverage percentage must be done systematically shortly before weeding, and this on all the plots (even non-weeded ones), to allow comparisons.

Measurements could be done at the same time of the biomass sampling (see below)

4.1.3. Plant density *(for weeds on annual crops)*

Plant density is defined as the **number of plants per surface unit** (commonly per m²).

This indicator is not well suited to weed management studies on perennial crops, where the plant coverage percentage must be preferred.

However, on annual crops, which cover large areas and where the weeds often emerge sparsely, plant density is the indicator most commonly used. Partners may use this indicator **on annual crops instead of plant coverage percentage** if it fits better with their trials.

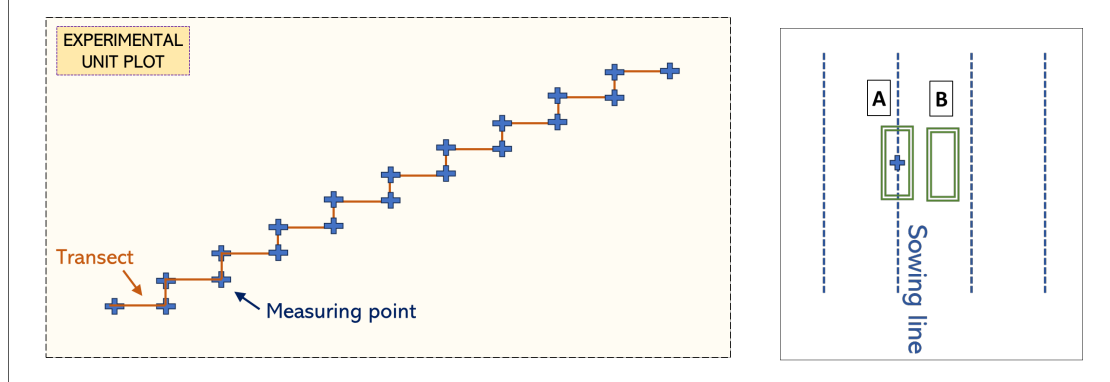
At each observation date, measurements are taken along a walked path, priorly defined inside each experimental unit plot ([Figure 3](#)). Along this transect, 20 measuring points, evenly distributed, are repaired with stakes (when the crop is in place, these markers are placed on the sowing line to not be crushed by the tractors).

At each point, measurements are taken within a 20 cm x 50 cm frame-quadrat (0,1 m²). Weed plants are either counted (when numbers are low), or their abundance is estimated, using the BARRALIS density scale ([Table 1a](#)). These 20 points measurements (corresponding to 2 m²) are added together, after conversion according to [Table 1b](#), to obtain the plant density per m², for the given elementary plot.

These counts must be done **at species level**, each time when possible; identification to genus or species is allowed, when taxa are too complex for the observer to distinguish. By this method, the **floristic richness** can also be deduced.

Note that the plant density indicator can also be used in case of perennial crops, if the pre-planting stage is studied. In this specific case, the orchard has not yet been planted, and the plot is still an open field.

Figure 3: Plant density assessment on field crops.



Left: An example of walked path with 20 measuring points per experimental unit plot. *Right:* In case of row-seeded crops, it can be relevant to double the observations for each point (one quadrat on the sowing line (A), and another on the interline (B)).

Table 1a: Plant density classes
(modified Barralis scale)

CLASS	PLANT DENSITY
1	$0,1 < D \leq 1 \text{ pl/m}^2$
2	$1 < D \leq 3 \text{ pl/m}^2$
3	$3 < D \leq 10 \text{ pl/m}^2$
4	$10 < D \leq 20 \text{ pl/m}^2$
5	$20 < D \leq 50 \text{ pl/m}^2$
6	$50 < D \leq 100 \text{ pl/m}^2$
7	$100 < D \leq 250 \text{ pl/m}^2$
8	$D > 250 \text{ pl/m}^2$

Table 1b: Plant coverage estimation
from Barralis density classes (*)

Class density	Estimated plant coverage
1	$pc < 0,5\%$
2	$0,5 < pc \leq 2\%$
3	$2 < pc \leq 5\%$
4	$5 < pc \leq 20\%$
5	$20 < pc \leq 50\%$
6	$50 < pc \leq 70\%$
7	$70 < pc \leq 90\%$
8	$pc > 90\%$

(*) Depending on the shape of the species, this estimation may be quite imperfect.

4.1.4. Others (optional)

Weed height measurement could be an option on perennial crops, when observations are done on the tree-rows. This indicator is not mandatory.

4.2. Impact on the main crops

4.2.1. Crop productivity

Total production has to be harvested on each experimental plot, and per replicate, to allow reliable statistic comparisons.

- Total grain yield per ha for annual crops
- Total fruits or grapes production per ha for perennial crops.

Other relevant parameters can also be measured according to the crops.

4.2.2. Others (optional)

- Main crop growth (trunk cross section area for perennial crops / biomass for annual crops: see above)
- Grain quality
- Fruit quality and size

- *Root development*
- *Injuries and mortality (in case of perennial crops)*
- *Phytosanitary aspects*

4.3. Impact on the soil (optional except for those related to WP4)

- **Nutrients and water availability**
- *Soil physical properties (water infiltration rate)*
- *Beneficial endo- and meso-fauna*
- **Soil microbiome** (cf. WP4, if the LL is involved in WP4)

4.4. Economic and environmental sustainability

- **Cost and profits of the weed control strategies** (cf. WP6, T6.4)
- **Carbon impact** (cf. WP6, T6.2)
- *Impact on water pollution (optional)*

4.5. Table of indicators measured per LL and methodologies

To be completed in version 2 (M18)

All LLs will keep records on an excel file that will be used for the LCA.

5. Field days and demo events.

Each LL will organize at least 2 field demonstration events to show the results of AWM in practice between M31 and M48. 60 participants are expected per demo event

These events will be adapted to each LL's culture and they will allow to show the results of the field research after the first and second year of experimentation. It could be open field days, practical demonstrations or any other format.

They will be addressed to LL and non-LL stakeholders in order to expand the use of AWM practices. These events will contribute to knowledge input in the co-creation activities of LLs.

A calendar of demo events per LL will be complete by the second version of D3.1 (M18).

6. Reporting of R&I results.

The LL manager will be responsible for collecting all required info and data, and monitor the LLs. The research results obtained will be included in Deliverable 3.3. "Implementation and assessments in organic and mixed farming systems" and updated tree times throughout the project.

This information will also be used to feed the activities developed in WP1, WP4, WP5, WP6 and WP7. A wide dissemination will be made to stakeholders through the different tools, platforms and protocols established in WP7.

Concerning all the activities carried out in the LLs related to organic and mixed sites (meetings, workshops, demo events, dissemination activities...) a reporting protocol and calendar is described in D1.2. The LL manager should use the template in Annex 6 of this document.

The LL manager will also collect, monitor, retain, analyze and report the data, results, outcomes, impacts of the Research and Innovation activities conducted in the organic and mixed farming sites of the LL

A summary of these R&I results will be reported to the Task leader (CICYTEX, María Ramos) at the end of every growing season following the template that will be provided to partners.

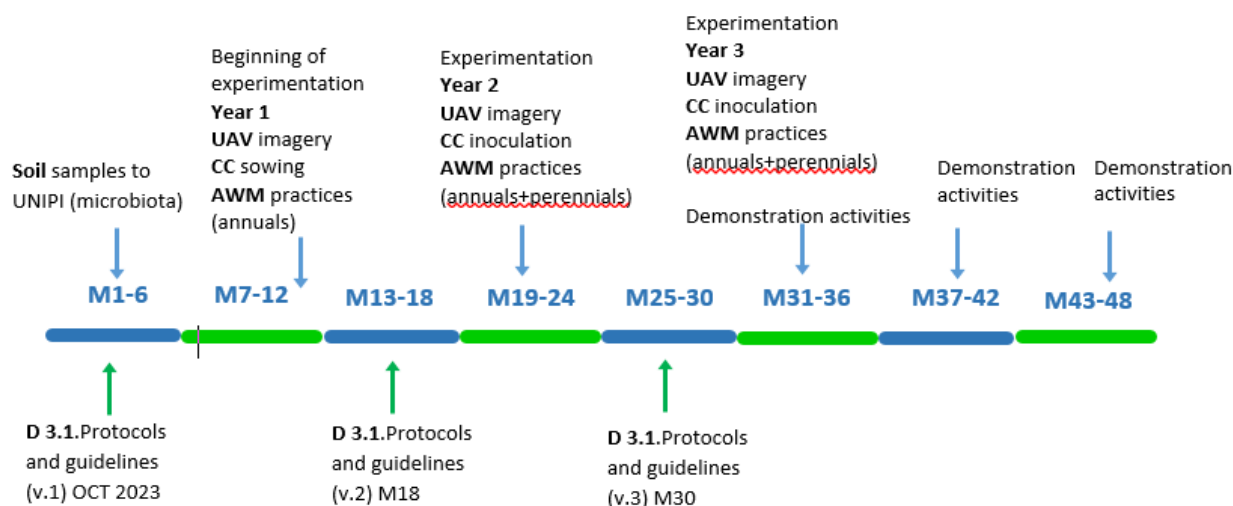
Reporting dates:

- October 2024
- October 2025
- October 2026

7. Calendar of LLs activities throughout the project

7.1. Key timelines

The general timeline for WP3 and Task 3.1 is the following:



Task 3.1 has one deliverable with three (3) updates. Specifically:

- Living Lab methodology for organic and mixed farming systems (version 1) [due to **M6** – October 2023]
- Living Lab methodology for organic and mixed farming systems (version 2) [due to **M18** – October 2024]
- Living Lab methodology for organic and mixed farming systems (version 3) [due to **M30** – October 2025]

7.2. Calendar of LLs activities

A specific calendar per LL regarding field activities (sowing, cc termination, drone flights, measurements etc.) will be published in VERSION 2, M18 (D3.2).

8. Possible Risks and mitigation measures.

There are some critical risks which have been identified and are associated with the LLs establishment and functioning. A risk mitigation plan has been already designed for them and is presented below:

Table 1: Critical risks and risk mitigation measures associated with WP3 and Task 3.1

Risk No. (from GA)	Description	Proposed Mitigation Measures
1	Delays in establishing the LL boards, the LLs and the AWMN	A strict time schedule will be organized at the beginning of the project to ensure early adaptations in case any delays are foreseen. Each LL will appoint a responsible person
2	Climatological risks due to climate change to crops and cover crops establishment	The Knowledge Base interviews & questionnaires, and the literature review will help the LL boards to choose the most optimal time windows for all operations. In case of failure of establishment, alternatives will be also proposed by LL boards
8	Low interest and feedback from stakeholders to participate in LLs	Scheduling of demonstration and dissemination activities based of farmers' calendar. Early contact with more stakeholders than needed will be conducted to ensure engagement
9	Proposed solutions do not meet farmer needs and ambitions	The information-in stage (WP1, WP6, WP7), the development of knowledge base and the demonstration activities and workshops will allow early communication with stakeholders, identify their needs and ambitions, and design tailor-made solutions
14	Failures to follow the experimental design in the LLs	In case of failure of following the experimental design (described in the excellence section), then the PSG and the relevant LL boards will decide on the experimental design that is scientifically sound and allows the implementation of demonstration activities.

If a LL identifies a risk, then it should contact the WP leader, the PSG and Project Coordinator at least 2 months before the start date of the risk, to co-design mitigation measures. If needed, a communication with the GOOD Project Officer could be carried out in cases of deviations from the GA.

A template letter, to address to GOOD Project Officer, is given in Appendix I.

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— APPENDIX I —

Template letter to be used to inform and argue about deviations to Grant Agreement

To be sent to GOOD Project Officer by the Project coordinator after its co-preparation with the respective Living Lab

For individual participants in the Living Lab boards in the Agroecology for weeds – GOOD project (GA: 101083589), funded by the European Union's Horizon Europe research and innovation programme.

Living Lab

Annual crops			Permanent crops		
Country	Code number		Country	Code number	
Latvia	LV_rye-pea/11	<input type="checkbox"/>	France	FR_apple-plum/21	<input type="checkbox"/>
Netherlands	NL_onion/12	<input type="checkbox"/>	Portugal	PT_olives/22	<input type="checkbox"/>
Serbia	RS_soybean/13	<input type="checkbox"/>	Italy	IT_citrus/23	<input type="checkbox"/>
Serbia	RS_maize/14	<input type="checkbox"/>	Italy	IT_grapes/24	<input type="checkbox"/>
Italy	IT_triticale/15	<input type="checkbox"/>	Greece	GR_grapes/25	<input type="checkbox"/>
Greece	GR_wheat/16	<input type="checkbox"/>	Cyprus	CY_olives/26	<input type="checkbox"/>
Portugal	PT_cowpea/17	<input type="checkbox"/>	Spain	ES_cherry/27	<input type="checkbox"/>
Spain	ES_rice/18	<input type="checkbox"/>	Spain	ES_apple-grapes/28	<input type="checkbox"/>

Dear [PROJECT OFFICER]

[NAME OF THE PARTNER] is participating in the Agroecology for weeds- GOOD project through the establishment of a Living Lab (LL) where various research and innovation activities will be carried out with different stakeholders.

The LL activities include the implementation of pilot sites where some Agroecological Weed Management strategies will be evaluated, such as the use of cover crops in combination with other cultural, digital and mechanical practices for weed control.

Common protocols have been proposed for the establishment of the LLs, the experimental designs and the indicators to be measured in each LL according to the Grant Agreement (GA). However, some obstacles have been detected that could lead to a deviation of the GA from the LL performance.

Therefore, a modification with respect to what is established in the GA is requested for the detected issue and only for this LL based on the following arguments,

[DETECTED DEVIATION] _____

[ARGUMENTS JUSTIFYING THE DEVIATION AND ALTERNATIVE PROPOSALS]

Project coordinator

Signature

Date

Name of LL manager

Signature

Date