Deliverable 3.3: Implementation and assessment of AWM strategies in organic and mixed farming systems (version 1)

Grant Agreement number: 101083589
Start date of the project: 01/05/2023 **End date of the project:** 30/04/2027
Deliverable due date: 15/11/2024 **Date of delivery:** 15/11/2024
Classification: Public

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 AUA

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1. Introduction

1.1. Objective of the deliverable

The objectives of D3.3 (M18), D3.5 (M30) and D3.6 (M42) are to report on the implementation and assessments in organic and mixed farming systems. These deliverables refer to Task 3.2 "*Implementation of AWM strategies under organic and mixed farming systems*." and Task 3.3 "*Assessments of AWM strategies and design of the Best Practices for the transition to AWM in organic and mixed farming systems*".

In particular, D3.3 will report what has been implemented during the first experimental year in the GOOD Living Labs (LLs).

The following Key Performance Indicators (KPIs) are covering both the conventional and the organic-mixed sites of the LLs.

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 Nº of crops that AWM solutions will be tested
	40 Nº of AWM strategies included in the repository

1.2. Connection with other Work Packages and Tasks

The results obtained from the implementation and assessments of cover crops (CC) and Agroecological Weed Management (AWM) practices will:

- Provide a list of Best Practices for AWM in organic and mixed farming systems (reference to D3.7 by M46)
- Provide content for the implementation of the co-creation activities in the LLs (reference to WP1)
- Inform the relevant consortium experts and the LL managers about the best-performed CC to be inoculated in the second experimental year (reference to Task 4.1)
- Give feedback to the digital experts of the consortium about the performance of CC and the effectiveness of AWM practices to support the development of the AWM Toolbox (reference to Task 5.3)
- Be used to complement the socio-economic and environmental assessments, as well as support the formulation of policy recommendations (reference to WP6)
- Provide content for dissemination material (reference to WP7) and the writing of factsheets and practice abstracts

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2. GOOD Living Labs

GOOD has 15 LLs which are currently carrying our research in organic and mixed farming systems. Those are listed in the following Table.

***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_triticale/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			

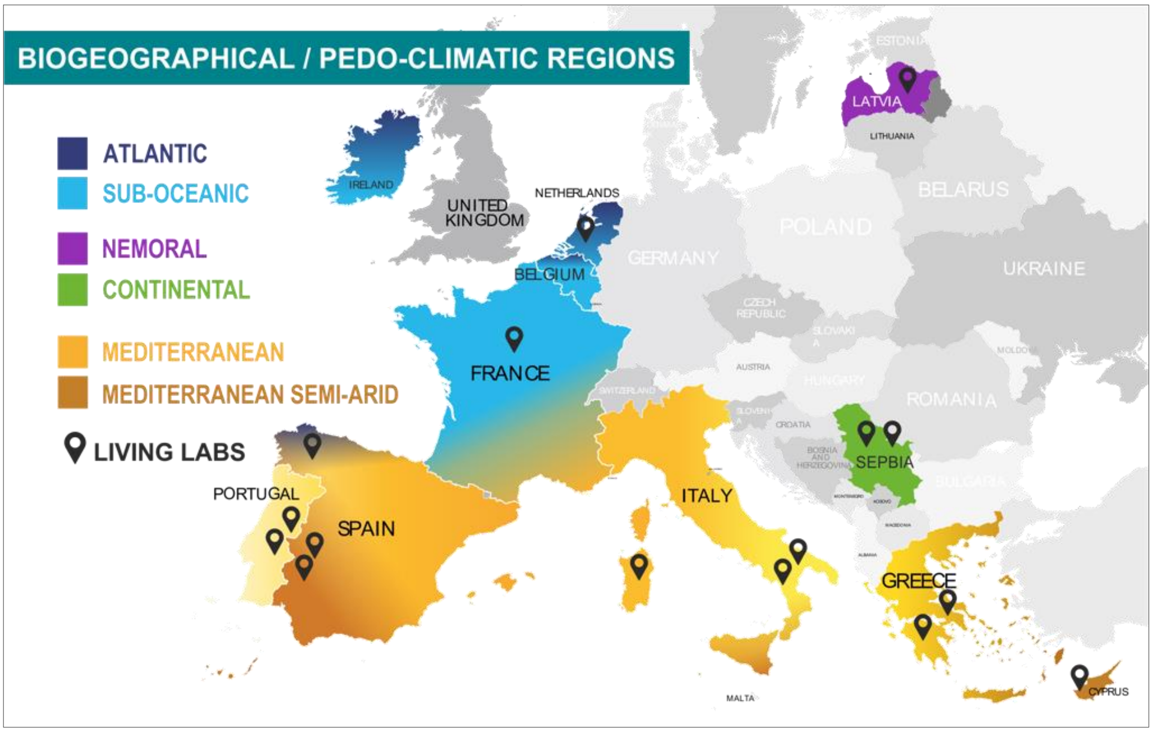

***Figure 1**: Geographical distribution of GOOD Living Labs (excluding the French in the organic and mixed systems). In accordance with the European Union's designation which is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ opinion on the Kosovo declaration of independence.*

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2.1. Annual crops

LV_rye-pea/11

At the site of the organic trial, winter rye (cultivar 'Kaupo') was the main crop studied in a field trial with four replicates (blocks) in a split-plot RCBD. The main plots consisted of two non-chemical agroecological weed management practices and an untreated control. The two weed management practices tested were physical weed control with double harrowing and false seedbed. Five cover crop treatments formed the subplots: CC1 was the untreated control (no cover crop), CC2 was white clover (*Trifolium repens* L.) undersown in spring (seeding rate was 3 kg ha−1), CC3 was red clover (*Trifolium pratense* L.) undersown in spring (sowing rate was 16 kg ha−1), CC4 with Italian clover (*Trifolium incarnatum* L.) undersown in spring (seeding rate was 7 kg ha−1) and CC5 with white clover undersown in fall (seeding rate was 3 kg ha−1). According to the experimental design described above, there were again 60 experimental units (defined).

This field showed a greater diversity of weed species and botanical families compared to the conventional site. The most dominant weed species with a frequency of ≥ 22% at all 135 sampling points (two or three sampling points per plot), were the following*:* *Sonchus arvensis* L. 1753 (perennial sowthistle; botanical family Asteraceae), *Gnaphalium uliginosum* L. 1753 not A. Rich. 1848 (marsh cudweed; botanical family Asteraceae), *V. arvensis* (botanical family Violaceae), *Polygonum arenastrum* Boreau 1857 (equal-leaved knotgrass; botanical family Polygonaceae), *Plantago major* L. (broadleaf plantain; botanical family Plantaginaceae), *Galinsoga parviflora* Cav. 1796 (gallant soldier; botanical family Asteraceae), *Solidago canadensis* L. (Canada goldenrod; botanical family Asteraceae), *M. perforata* (botanical family Asteraceae), *Artemisia vulgaris* L. (common mugwort; botanical family Asteraceae), *Mentha arvensis* L. (wild mint; botanical family Lamiaceae), *Elymus repens* (L.) Gould (couch grass; botanical family Poaceae).

In false seedbed main plots, CC3 produced more than 100 g biomass m^{-2}. This was the highest yielding combination of weed management practice and cover crop in terms of cover crop biomass production. As a result, the **false seedbed × CC3 interaction** reduced total weed density by 64% compared to the untreated control × CC1 interaction. However, when comparing these two interactions, the corresponding reduction in weed biomass was only 24%, which is likely due to the fact that weed biomass production per plant increases when weed density decreases. In addition, the integrated agroecological approach of the **false seedbed × CC2 interaction** reduced total weed density and total weed biomass by 76% and 23%, respectively, compared to the untreated control × CC1 interaction. CC2 and CC3 had higher weed suppression potential in false seedbed main plots compared to the main plots with physical weed control. Crop density and biomass were not significantly affected by weed management, cover crops and their interaction (P-Value ≥ 0.05). The grain yield of rye was 2.77 t ha^{-1} and 2.68 t ha^{-1} in the subplots false seedbed × CC2 and false seedbed × CC3, respectively.

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NL_onion/12

Onion was studied as the main crop in an organic field trial conducted in a Randomized Complete Block Design (RCBD) with three replicates (blocks). The seed rate for onions was 950.000 seeds/ha. Four cover crops were sown previous the onion cultivation: untreated control, *Phacelia tanacetifolia*, Yellow mustard (*Sinapis alba*) and grass (*Lolium spp*.). Three weed management practices were studied as subplots: untreated control, mechanical control (interlines) and manual weed control (in onion lines). A total of 16 experimental units (subplots) were created based on the experimental design. Prior to sowing the cover crops, the field had been cultivated with faba beans, selected for their nitrogen-fixing capabilities. Consequently, no fertilization was applied either before or during the establishment of the cover crops. Cover crops were sown in September 2023 (Phacelia seed rate: 10 kg ha^{-1}; Mustard seed rate: 20Kg/ha; Grass seed rate: 20kg/ha). Cover crops were ploughed under in November 2023 and onion main crop was sown in April 2024.

The predominant weed species in the experimental area were the following: *Matricaria* spp. (botanical family Asteraceae), *Lamium purpureum* L. (red dead-nettle; botanical family Lamiaceae), *Veronica agrestis* L. (green field-speedwell; botanical family Plantaginaceae), *Atriplex* spp. (botanical family Amaranthaceae) and *Senecio* spp. (botanical family Asteraceae). The weather in May and June 2024 was not in our favor, as these are the key months for mechanical weed control. Frequent and heavy rainfall made it nearly impossible to hoe or harrow under suitable conditions. There was considerable damage due to weed competition and onion plants being pulled during manual weed removal in June. Yield measurements have not yet been conducted, as the onion crop requires sufficient drying prior to assessment.

Data about the best CC performance, weed biomass and main weed species are still being processed. Based on the initial assessments of crop biomass, it is expected that the effects of the interactions between cover crops and weed management treatments on marketable onion bulb yield will be statistically significant.

RS_soybean/13

Soybean (cultivar 'Lela') was investigated as the main crop in a field trial conducted in a Randomized Complete Block Design (RCBD) with three replicates (blocks) and a 100kg/ha seed rate. Four cover crops were studied as main plots: untreated control, rye, common vetch and oat. Five weed management practices were studied as subplots: untreated control, false seedbed, mechanical weed control, bio-based herbicide, false seedbed and mulching. The preceding crop was maize. A total of 60 experimental units (subplots) were created based on the experimental design. Cover crops were sown in the fall (seed rate: rye – 90 kg/ha, vetch and oats – 120 kg/ha). Soybean was sown after mechanical termination of the cover crops and incorporation of their residues into the soil.

In the cover crop evaluation, rye and oats had a higher biomass (about 30–50%) than vetch at the beginning of the season, and the differences were even more pronounced before the termination of the cover crop in the second evaluation, where the biomass of rye and oats was about 200 g m^{-2}, while vetch produced only 20 g m^{-2}. According to the LL managers, no weeds were present in any of the cover crops, which is probably due to the extensive crop rotation used in the previous growing seasons on the wider experimental area. Only a few weeds were

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detected in the plots without cover crops, i.e. mainly *Chenopodium album* L. (common lambsquarters; botanical family Amaranthaceae), which occasionally appeared in small patches. The **best performing CC was rye**, which was selected for grow in next two years.
In the main crop, the most abundant annual weeds in the untreated plots were: *C. album* (botanical family Amaranthaceae), *Abutilon theophrasti* Medic. (velvetleaf; botanical family Malvaceae), Amaranthus hybridus L. (smooth pigweed; botanical family Amaranthaceae), *Amaranthus retroflexus* L. (redroot pigweed; botanical family Amaranthaceae), *Chenopodium hybridum* L. (mapleleaf goosefoot; botanical family Amaranthaceae), *Datura stramonium* L. (jimsonweed; botanical family Solanaceae), and *Solanum nigrum* L. (black nightshade; botanical family Solanaceae). The following perennial weeds, which belong to the botanical family Poaceae, were also present in many plots of the soybean trial field: *Sorghum halepense* (L.) Pers. (johnsongrass; botanical family Poaceae), *Cynodon dactylon* (L.) Pers. (bermudagrass; botanical family Poaceae), *Cirsium arvense* (L.) Scop. (Canada thistle; botanical family Poaceae) and *Convolvulus arvensis* L. (field bindweed; botanical family Convolvulaceae).

The best weed control treatment **was mechanical weed control with 50-60% of the efficacy in combination with CC**. Due to low efficacy of other treatments and unfavorable meteorological conditions (soybean was not irrigated in this conditions), there was no yield on any treatments on soybean organic site, so that, soybean production in organic system is very challenging since soybean is not a strong competitor against weeds.

RS_maize/14

On the organic trial site, the field trial was set up in a Randomized Complete Block Design (RCBD) with three replicates (blocks) according to the split-plot arrangement. Maize (hybrid ‘ZP 388’) was the main crop studied (seeding rate: 60.000plants/ha). The cover crops planted in the fall on the main plots of the experimental field were: rye (seeding rate 90 kg ha^{-1}), vetch (seeding rate 120 kg ha^{-1}) and oats (seeding rate 120 kg ha^{-1}). An untreated control (plots without cover crop) was also included. After cover crop termination with a plant cutter in early April, five weed management practices were assigned to the subplots: untreated control, bioherbicide application (pelargonic acid before emergence of the main crop), mulching, false seedbed (twice before sowing the main crop) and mechanical weed control (inter-row cultivation). The field was not fertilized. As a result, 60 experimental units (subplots) were formed.

Regarding the measurements during the active growth phase of the cover crop, rye and oats had higher crop biomass (>30-50%) compared to common vetch. Vetch produced 20% and 45% less biomass than rye and oats respectively at the beginning of the season, while oats had 33% more biomass than rye. However, at the second evaluation, biomass of both rye and vetch were about 95 g/m^2, while oats had biomass of 65 g/m^2. The preceding crop for both LLs was maize. No weeds were present across all cover crops, while some of them were recorded in no cover crop variant (mostly *Chenopodium album*).

In the maize field (during main crop performance), the most abundant annual weeds in the untreated plots were: *C. album* (botanical family Amaranthaceae), *Abutilon theophrasti* Medic. (velvetleaf; botanical family Malvaceae), *Amaranthus hybridus* L. (smooth pigweed; botanical family Amaranthaceae), *Amaranthus retroflexus* L. (redroot pigweed; botanical family Amaranthaceae), *Chenopodium hybridum* L. (mapleleaf goosefoot; botanical family

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Amaranthaceae), *Datura stramonium* L. (jimsonweed; botanical family Solanaceae) and *Solanum nigrum* L. (black nightshade; botanical family Solanaceae). The following perennial weeds, which belong to the botanical family Poaceae, were also present in many plots of the maize trial field: *Sorghum halepense* (L.) Pers. (johnsongrass; botanical family Poaceae), *Cynodon dactylon* (L.) Pers. (bermudagrass; botanical family Poaceae), *Cirsium arvense* (L.) Scop. (Canada thistle; botanical family Poaceae) and *Convolvulus arvensis* L. (field bindweed; botanical family Convolvulaceae).

The best results were obtained with **mechanical weed control (50-60% efficacy) combined with cover crop.** The highest yields were recorded in the false seed bed and mechanical weed control 2.5-3.5 t/ha. In general, maize yields were low due to the difficult meteorological conditions (no rainfall events and drought) that prevailed in the experimental area. Maize growing in organic production system is possible and integrating of CC and mechanical weed control and false seed bed could be a promising tool in organic production.

IT_triticale/15

The trial was set up in a Randomized Complete Block Design (RCBD) with seven weed management treatments repeated three times. Triticale was the crop studied (seeding rate: 190kg/ha). The treatment list included: *Medicago truncatula* Gaertn. cover crop, *Trifolium brachycalycinum* (Katzn. & Morley) Katzn. cover crop, *M. truncatula* + *T. brachycalycinum* cover crop mixture, untreated control, mechanical weed control (localized mowing on interrows and total mowing), cultural weed control (using of pelargonic acid). The cover crops were sown together with the main crop in November and covered the areas between the rows (intercropping). The seeding rate was 10 kg seed ha^{-1} for *M. truncatula* and 15 kg seed ha^{-1} for *T. brachycalycinum*.

The most dominant weed species in the organic experiment were: *Rapistrum rugosum* (L.) All. (fam. Brassicaceae), *Chrysanthemum coronarium* (fam. Asteraceae), *Sulla coronaria*, *Sonchus oleraceus/tenerrimus*, *Fumaria spp*., *Roemeria spp*. (fam. Papaveraceae).

Weed and main crop biomass, plant coverage, diversity and productivity parameters were measured together with additional ones such as straw yield and components of grain. In a general sense, cover crop performances were lower than expected due to competition from triticale itself from the stem elongation phase. Average grain yield was about 6 t ha^{-1} and there was a max total weed density about 192 plants m^{-2}.

The total density of weed species varied between 78.2 plants m^{-2} (*T. brachycalycinum*) and 119 plants m^{-2} (*M.truncatula*) while its biomass was 167.49–134.85 kg/ha (P-Value $\geq$ 0.05).

Total mowing treatment was not applied as there was a very low number of weeds, due to the correct crop rotation adopted by the farmer. **The treatment with pelargonic acid was effective in containing the weeds, but it may reduce the production.** The triticale grain production was higher in the *M.truncatula*, mechanical method and untreated control (7,319 kg/ha, 6,369kg/ha and 7,026 kg/ha respectively). They were also the treatments with the highest straw yield per ha.

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GR_wheat/16

On the conventional site, durum wheat (cultivar 'Maesta') was investigated as the main crop in a field trial conducted in a Randomized Complete Block Design (RCBD) with three replicates (blocks) according to the split-plot design. Four cover crops were studied as main plots: untreated control, *Trifolium alexandrinum*, *Lolium perenne* and the mixture of the two species (*T. alexandrinum* + *L. perenne*). Four weed management practices were studied as subplots: untreated control, false seedbed, stale seedbed and mechanical weed control. On stale seedbed plots, pre-sowing weed control was done with the application of the bioherbicide pelargonic acid. A total of 48 experimental units (subplots) were created based on the experimental design. Cover crops were sown in the fall (seed rate 300 kg ha^{-1}). Durum wheat was sown after mechanical termination of the cover crops and incorporation of their residues into the soil.

The mixture of the two cover crops (*T. alexandrinum* + *L. perenne*) resulted in significantly higher cover crop biomass production and improved weed suppression compared to the cover crop monocultures of *L. perenne* and *T. alexandrinum* (P-Value ≤ 0.001). In the durum wheat field, the most frequent weed species were *Avena sterilis* L. (sterile wild oat; botanical family Poaceae) with an occurrence at 180 of 192 sampling points (94% frequency), *Sinapis arvensis* L. (wild mustard; botanical family Brassicaceae) with an occurrence at 164 of 192 sampling points (85% frequency), *Veronica hederifolia* L. (ivy-leaved speedwell; botanical family Plantaginaceae) with an occurrence at 116 of 240 sampling points (60% frequency) and *Gallium aparine* L. (cleavers; botanical family Rubiaceae) with an occurrence at 144 of 240 sampling points (60% frequency). Weed diversity was affected by the main factors and their interactions (P-Value ≤ 0.01). Specifically, the number of botanical weed families and the number of weed species present were low in the interaction subplots (≤ 6): *T. alexandrinum* + *L. perenne* × false seedbed and *T. alexandrinum* + *L. perenne* × stale seedbed. Untreated, non-cover crop plots without additional weed management were infested with more than 10 different botanical weed families and weed species.

The highest value of weed biomass (358.0 g m^{-2}) corresponded to the interaction *T. alexandrinum* × untreated control. Very high weed biomass values were also observed in the main plots without cover crop where no other weed control method was applied (303.2 g m^{-2}). Weed biomass was also high in the plots where *L. perenne* was grown as cover crop without any other weed control measures (301.2 g m^{-2}). Even in the main plots without cover crop, stale seedbed and false seedbed reduced weed biomass by 65% (125.2.0 g m^{-2}) and 67% (111.8 g m^{-2}), respectively, compared to the *T. alexandrinum* × untreated control interaction. The lowest weed biomass (10.8 g m^{-2}) was observed in the interaction plots ***T. alexandrinum* + *L. perenne* × false seedbed**. The combinations *L. perenne* × false seedbed, *L. perenne* × mechanical weed control and *T. alexandrinum* + *L. perenne* × false seedbed were equally effective.

The highest yield was observed with the combination ***T. alexandrinum* + *L. perenne* × false seedbed** (3,366.2 g m^{-2}). The next highest yields were obtained with the combinations *T. alexandrinum* + *L. perenne* × stale seedbed (3,182.8 kg ha^{-1}) and *T. alexandrinum* + *L. perenne* × mechanical weed control (3,305.3 kg ha^{-1}). The combination *T. alexandrinum* + *L. perenne* × false seedbed increased the grain yield by 17% compared to the combination *L. perenne* × false seedbed. The lowest yields were observed for the plots without cover crop, which also received no subsequent weed control treatment (1,507.0 kg ha^{-1}).

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PT_Cowpea 17

On the organic trial site of cowpea, the field trial was set up in four replicates (blocks) according to the split-plot arrangement. The 36 subplots were 4x33 m and the total area approximately 4752m^2.The cover crops were sown after the mowing of the trial plots with a chain-mower to remove early germinated winter weeds. Cowpea was the main crop studied in spring-summer 2024. Cover crops were sown in December 2023 after a delay caused by heavy rainfall in November which made the access to the field by tractors difficult. Three cover crop options were introduced: rye (*Secale cereale* L.), a three-species mixture of oat (*Avena sativa* L.), lupin (*Lupinus* spp.), and turnip (*Brassica rapa* subsp. rapa), and a six-species mixture of oat, rye, lupin, turnip, mustard (*Sinapis alba* L.), and flax (*Linum usitatissimum* L.). The seeding rate for rye as single cc was 100 kg seed ha^{-1}. The seeding rates in the three-species mixture were 60 kg seed ha^{-1} for oat, 30 kg seed ha^{-1} for lupine and 3 kg seed ha^{-1} for turnip. In the six-species mixture, the seeding rates were the same for oat, lupine and turnip, and 40 kg seed ha^{-1} for rye, 6 kg seed ha^{-1} for flax, and 3 kg seed ha^{-1} for mustard. The cover crops were terminated at the end of May 2024. Three termination methods were applied: Tillage with a disc harrow, Mowing with a chain mower and Roller-crimper. The late establishment of the cover crop in 2023 led to very slow development and therefore weed suppression was not optimal. The termination of the cover crop was also not optimal, because it had not reached the ideal developmental stage, but the results of weed suppression were ideal (especially the plots treated with roller crimper showing >90% weed suppression).

Therefore, the sowing of cover crops in the second year is scheduled to be implemented earlier in the season to give a growth advantage to the cover crops. Due to aggressive growth of annual rye grass and vetch from a previous production, the fields were heavily infested. Among the cover crop treatments, the six-species mixture showed the best ground coverage (P-Value $\leq$ 0.05). Statistical analysis and correlations is ongoing. Cowpea sowing was done in June (seeding rate 100 kg/ha.) with a direct seeder.

Three weed management practices were studied as subplots: untreated control, mowing with a chain mower+tillage and mowing with a manual disc cutter simulating the roller-crimper. There was no fertilization or irrigation in the cover crops and the cowpea. Harvest took place only in the tilled plots, since most of the blocks had crop failure (either due to the thick mulching that prevented germination of the main crop or due to the extreme weather events that occurred and are reported below).

Due to the precipitation in combination with the dry periods, 2024 was a bad year for cowpea production for the entire region of the Living Lab and it was clearly reflected in the performance of the main crop in the LL. Even though this site has some more annual precipation in comparison to the conventional trial site of cowpea, this year, the fields were quite dry at germination and early developmental stage. As we noticed the dry soil condition, we requested the farmer to irrigate several times during the growing season. This improved germination, but continued irrigation by the farmer is likely to have had a negative effect on its further growth, as cowpea does not like overhead irrigation.

The crop itself grew considerably better on the tilled treatment than it did on the shredded or compacted lines. It actually barely germinated on the blocks with the roller-crimper cover crop finishing treatment. At the same time, those blocks were also the ones with the best weed control. Although the crop did develop in some of the blocks, it never formed pods. The most likely problem seems to have been a combination of the general weather conditions together

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with the compacted soil which prevented the cow pea to fully develop. Therefore, before sowing this year´s cover crop the soil will be slightly decompacted with a subsoiler and then direct sowing of the cover crops will be conducted. The most prevalent weed species in the cowpea main crop were three and were observed only in the tilled plots: *Amaranthus retroflexus* L. (redroot pigweed; botanical family Amaranthaceae), *Chenopodium hybridum* L. (mapleleaf goosefoot; botanical family Amaranthaceae), *Datura stramonium* L. (jimsonweed; botanical family Solanaceae). The weed suppression in the main crop was statistically signicantly affected by the treatments of shredder and roller crimper (P-value ≤ 0.001) compared to the tillage and the untreated control.

ES_rice/ 18

On the organic site, the field trial was set up in a Randomized Complete Block Design (RCBD) with four replicates (blocks) according to the split-plot arrangement. Rice (cultivar 'Thaiperla') was the main crop investigated. The cover crops planted in November on the main plots were: *Avena sativa* (seeding rate 173 kg ha^{-1}), *Medicago scutelatta* (seeding rate 30 kg ha^{-1}) and *Hordeum vulgare* (seeding rate 106 kg ha^{-1}). A control plot with no cover crop (spontaneous cover) was also included. After termination of the cover crops by mowing at the end of April, the residues of the cover crop were incorporated into the soil. Dry sowing was applied for rice by mid -May (sowing dose: 250kg/ha) and waterlogged was effective in June, maintaining the water film during all rice cycle. Three weed management practices were assigned to the subplots: weedy (untreated control), False seed bed and mechanical control. In this way, 48 experimental units (subplots) were formed.

In the first evaluation of the cover crops in late winter, *M. scutelatta* produced 90% less biomass than *A. sativa* and *H. vulgare*. In the second assessment before termination, *A. sativa* and *H. vulgare* produced significantly higher amounts of biomass (12,700–10,600kg ha^{-1}) than the corresponding amounts of the legume cover crop *M. scutelatta* (6,357 kg ha^{-1}). The total biomass of winter weeds amounted to 6,913.6 kg ha^{-1} in the main plots without cover crop. Lower values were found for the cover crops *A. sativa* (87%), *M. scutelatta* (54%) and *H. vulgare* (84%). It is noteworthy that although *M. scutelatta* produced less biomass than ***A. sativa*, the legume cover crop achieved a significantly higher ground cover** (P-Value ≤ 0.01) **and thus weed suppression**. This can be attributed to the fact that annual medics (*Medicago* spp.) have great potential as weed-smothering cover crops because of their prostrate growth habit, short life span and good germination, which leads to rapid ground cover. Cover crops have enough biomass to have an impact on weeds. **Avena sativa had the best performance**. **Effects on weeds are not clear, but over the main crop and on soil fertility and biochemical indicators there was an increment when *Avena sativa* was incorporated**. So that, oat will be used as cover crop to be inoculated with AMF for the second and third experimental year. Nevertheless, all CC will be tested again in the 2nd and 3rd years.

The weed flora consisted mainly of noxious weed species that typically infest paddy fields and belong to two botanical families, Poaceae and Cyperaceae, which were present in all experimental units (frequency 100%). The predominant weeds of the botanical family Poaceae were *Echinochloa crus-galli* (L.) P.Beauv. (barnyardgrass) and species of the genus *Leptochloa* spp. (spangletops). The predominant weeds from the botanical family Cyperaceae were the perennial sedges *Cyperus rotundus* L. (purple nutsedge) and *Cyperus esculentus* L. (yellow nutsedge) and the annual sedge *Cyperus difformis* L. (smallflower umbrella-sedge).

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Crop biomass was highest in the *M. scutellata* × weedy plots (4,324 kg ha^{-1}) and *M. scutellata* × mechanical control (5,207 kg ha^{-1}). The lowest weed density corresponded to the non-cover crop x weedy treatment and the *M. scutellata* × weedy. The lowest weed biomass of the first dominant species (*Echinochloa spp*.) was in *Hordeum vulgare* x mechanical control (2,516 kg ha^{-1}). Anyway, the effect of treatments on weeds has been ineffective. The preceding cover crop has not been effective in stopping the emergence of viable weed seeds. There were no significant differences between mechanical control and false seedbed.
Grain yield data is not yet available as grain moisture (21%) is unacceptable at this stage of the growing season. Yield data will be available in late November 2024 and will be interpreted in relation to crop growth and weed parameters.

2.2.Perennial crops

PT_olives/22

The field trial was set up in four replicates (blocks) according to the split-plot arrangement. The cover crops were sown between the tree rows after the mowing of the trial plots with a chain-mower to remove early germinated winter weeds. Cover crops were sown in December 2023 after a delay caused by heavy rainfall in November which made the access to the field by tractors difficult. Three cover crop options were introduced: rye (*Secale cereale* L.), a three-species mixture of oat (*Avena sativa* L.), lupin (*Lupinus* spp.), and turnip (*Brassica rapa* subsp. rapa), and a six-species mixture of oat, rye, lupin, turnip, mustard (*Sinapis alba* L.), and flax (Linum usitatissimum L.). The seeding rate for rye as single cc was 100 kg seed ha^{-1}. The seeding rates in the three-species mixture were 60 kg seed ha-1 for oat, 30 kg seed ha^{-1} for lupine and 3 kg seed ha^{-1} for turnip. In the six-species mixture, the seeding rates were the same for oat, lupine and turnip, and 40 kg seed ha^{-1} for rye, 6 kg seed ha^{-1} for flax, and 3 kg seed ha^{-1} for mustard. One block wasn't sown with cover crops and left untreated. The sowing was conducted with an APV Direct Seeder, which spread the seeds at a controlled rate. However, without irrigation or fertilizer, the late establishment hindered crop development, and weed suppression remained suboptimal. There was no fertilization or irrigation in the cover crops. The cover crops were terminated at the end of May 2024. Mowing with chain mower was the termination method and the residuals were left as mulch on the soil surface.

Additionally to cover crops, two weed management practices were studied as subplots: untreated control and mowing with a chain mower. The late establishment of the cover crop led to very slow development and therefore weed suppression was not optimal. Furthermore, uniquely in the organic olive site, there was a very thick layer of remaining plant residues from previous cultivations, which was very humid due to rain and therefore difficult to shred. There was an attempt to reduce this plant material through mowing but in hindsight it was not enough to reduce the remaining plant residues.

The termination of the cover crop was also not optimal, because it had not reached the ideal developmental stage. Of the 3 cover crop options, the **mixture of 6 species outperformed** the other 2, due to the diversity of species. The weed suppression was significantly higher in the six-species mixture compared to the single species and the three-species mixture in terms of ground coverage by the cover crops (P-value $\leq$0.05). In adverse conditions, some of the crops can still have a relatively good performance. No measurements on the main crop will be done for the first year based on the protocol. During the growing season of the cover crops (winter), the dominant weeds were *Sonchus* spp. (thistles; botanical family Asteraceae), *Avena sterilis* L.

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(sterile wild oat; botanical family Poaceae), *Veronica hederifolia* L. (ivy-leaved speedwell; botanical family Plantaginaceae), *Lolium rigidum* L. (rigid ryegrass; botanical family Poaceae), *Sinapis arvensis* L. (wild mustard; botanical family Brassicaceae).

IT_Citrus/23

A field trial was conducted in a citrus orchard using the Randomized Complete Block Design (RCBD) with five treatments and three replicates (blocks). Three cover crops were established between the tree rows: *Vicia faba* (seeding rate 358 kg ha^{-1}), *Trifolium alexandrinum* (seeding rate 50 kg ha^{-1}) and the mixture *Vicia villosa* + *Avena sativa* (seeding rate 50 + 60 kg ha^{-1}). After cover crop termination, in new plots an additional weed control method were evaluated: mechanical weed control through soil tillage with a spader and mowing. An untreated control was also included in the treatment list (no cover crop and no weed control). All treatments relate to weed suppression in the inter-rows of the citrus orchard. Cover crops have been sown in November and they were terminated in May by mowing with a flail mower.

During the active growth phase of the cover crops, the dominant winter weeds in the experimental area were species of the genus *Sonchus* spp. (thistles; botanical family Asteraceae), *Gallium aparine* L. (cleavers; botanical family Rubiaceae), *Stellaria media* (L.) Vill. (chickweed; botanical family Caryophyllaceae), *Avena sterilis* L. (sterile wild oat; botanical family Poaceae) and *Oxalis pes-caprae* L. (Bermuda buttercup; botanical family Oxalidaceae). An excessive presence of *Oxalis pes-caprae* L. has led to a lack of well establishment of cover crops.

The cover crop mixture ***V. villosa* + *A. sativa*** produced a highest amount of biomass (165.64 g m^{-2}) which was 73% and 62% compared to *T. alexandrinum* and *V. faba*, respectively. *V. villosa* + *A. sativa* reduced total weed biomass by 72% compared to the untreated control, whereas *T. alexandrinum*, *V. faba* reduced 28% and 67% respectively.

During the summer growing season, when all treatments were completed, the predominant weeds in the citrus orchard were species of the genera *Bidens* spp. (botanical family Asteraceae), *Conyza* spp. (fleabanes; botanical family Asteraceae), and the noxious rhizomatous perennial summer grass *Cynodon dactylon* (L.) Pers. (Bermudagrass; botanical family Poaceae). *V. villosa* + *A. sativa* reduced total weed density by 68% compared to the untreated control, respectively. *V. faba*, *T. alexandrinum* and mechanical control reduced around 10%, 9,4% and 78%, respectively in the second sampling. The cover crop ***Vicia villosa* Roth + *Avena sativa* L. performed better** than the other two cover crops and it proved to be the most effective treatment in controlling the weeds.

IT_grapes/24

A field trial was conducted in a vineyard using the Randomized Complete Block Design (RCBD) with five treatments repeated in three replicates (blocks). Three cover crops were established between the vine rows: *Vicia faba* (seeding rate 327 kg ha^{-1}), *Trifolium alexandrinum* (seeding rate 45 kg ha^{-1}) and the two-species mixture *Vicia villosa* + *Avena sativa* (seeding rate 45 + 55 kg ha^{-1}). After cover crop termination, in new plots, an additional weed control method was evaluated: mechanical weed control through soil tillage with a spader and

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mowing. An untreated control was also included in the treatment list. All treatments aimed to suppress weeds in the inter-rows of the vineyard.

During the active cover crop growth, the predominant winter weeds in the experimental area were species of the *Sonchus* spp. (thistles; botanical family Asteraceae), *Gallium aparine* L. (cleavers; botanical family Rubiaceae), *Stellaria media* (L.) Vill. (chickweed; botanical family Caryophyllaceae), *Avena sterilis* L. (sterile wild oat; botanical family Poaceae) and *Oxalis pes-caprae* L. (Bermuda buttercup; botanical family Oxalidaceae). *V. villosa* + *A. sativa* was the most effective cover crop in terms of biomass production (473.8 g m^{-2}). *V. faba* produced 46% less biomass than the mixture while *T. alexandrinum* was the least effective cover crop in terms of biomass production (183.45 g m^{-2}). *V. villosa* + *A. sativa* reduced total weed density by 75%, 82% and 65% compared to *T. alexandrinum*, *V. faba* and the untreated control, respectively. Ther percentage of coverage on the second measurement was about 63 % Vicia villosa Roth + Avena sativa L., 50% *Trifolium alexandrinum* and 36% *Vicia faba*.

During the summer growing season, when all treatments were completed, the predominant weeds in the grapes orchard were species of the genera *Bidens* spp. (botanical family Asteraceae), *Conyza* spp. (fleabanes; botanical family Asteraceae), and the noxious rhizomatous perennial summer grass *Cynodon dactylon* (L.) Pers. (Bermudagrass; botanical family Poaceae). In this season, *V. villosa* + *A. sativa* and mechanical weeding have the best results in reduced the total biomass of weeds (by 97% and 100% respectively in the second sampling) However, in the previous (first) sampling, mechanical control shows similar weed biomass than the untreated control. That could be for several reason: mowing has not been done at this moment or infestation of *C. dactylon* by cutting rhizomes that germinated later in the season, leading to the spread of this particular noxious perennial grass weed species in the field. The cover crop ***Vicia villosa* Roth + *Avena sativa* L. was the most effective treatment** in controlling the weeds.

GR_grapes/25

At the organic site, a field trial was conducted in a vineyard using the Randomized Complete Block Design (RCBD) with six weed management treatments repeated in three replicates (blocks). Specifically, three cover crops were established between the vine rows in late fall: *Vicia sativa* (vetch), *Avena sativa* (oats) and their mixture *V. sativa* + *A. sativa*. All cover crops were sown at a rate of 300 kg ha^{-1}. After termination of the cover crops by mowing in late spring, two additional weed management practices were carried out in early summer: mechanical weed control by mowing and application of the bioherbicide pelargonic acid. An untreated control was also included in the treatment list (no cover crop and no weed control). All treatments relate to weed suppression in the inter-rows of the vineyard.

During the active growth phase of the cover crops, the dominant winter weeds on the experimental area were *Calendula arvensis* (Vaill.) L. (field marigold; botanical family Asteraceae) with an occurrence at 43 out of 48 sampling points (90% frequency), *Euphorbia peplus* L. (petty spurge; botanical family Euphorbiaceae) with an occurrence at 26 out of 48 sampling points (54% frequency), *Avena sterilis* L. (sterile wild oat; botanical family Poaceae) with an occurrence at 22 out of 48 sampling points (56% frequency) and *Fumaria officinalis* L. (common fumitory; botanical family Papaveraceae) with an occurrence at 9 out of 48 sampling points (19% frequency). The cover crop **mixture *V. sativa* + *A. sativa*** had the highest biomass production (5,201.9 kg ha^{-1}), while *V. sativa* was the least productive cover crop (4,022.1 kg

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ha^{-1}) and *A. sativa* had intermediate biomass values (4,491.6 kg ha^{-1}). In addition, the cover crop mixture *V. sativa* + *A. sativa* resulted in 40.6 g weed biomass m^{-2} which was by 70%, 82% and 88% compared to *A. sativa*, *V. sativa* and the untreated control, respectively.

After cover crop termination, the dominant summer weeds on the experimental area were *Convolvulus arvensis* L. (field bindweed; botanical family Convolvulaceae) with an occurrence at 54 out of 84 sampling points (64% frequency) and *Cynanchum acutum* L. (swallow-wort; botanical family Apocynaceae) with an occurrence at 35 out of 84 sampling points (42% frequency). Due to the limited rainfall and the long drought in the Nemea region, the weed density was indeed low compared to previous years. The two species that dominated the field were not affected by the climatic conditions, as both are perennial weed species that typically emerge in the summer months of the growing season after rhizome sprouting in the wider area. Regarding the effects of the treatment, mowing and the application of pelargonic acid reduced the density of *C. arvensis* and *C. acutum* only a short time after treatment. After this knockdown effect, these noxious broadleaf vine-climbing perennial weed species regrew again later in the season (80–85**%). The suppression of *C. arvensis* and *C. acutum* density barely reached 50–60% in the plots with *A. sativa* and *A. sativa* + *V. sativa* cover crop**. **Nevertheless, these cover crops showed some efficacy compared to the untreated control.** In contrast, the *V. sativa* cover crop had no significant effect on weed density.

CY_olives/26

In the olive plantation in Cyprus, four cover crop treatments and four weed management practices were evaluated. The cover crops sown in winter were: *Vicia* spp. (seeding rate 24 kg ha^{-1}), *Pisum sativum* (seeding rate 22 kg ha^{-1}) and the triple species mixture of *Vicia* spp. + *P. sativum* + *Triticum durum* (seeding rate 8 + 8 + 4 kg ha^{-1}). An untreated control without cover crop was also included. In the plots with cover crops, the following four weed management practices were tested: untreated control, mechanical weed control by mowing, mulching (placing 20 cm straw under the experimental trees) and mechanical weed control by hoeing. Planta biomass and plant coverage percentage were assessed as well as soil humidity (dendrometer installation).

Dominant weed families identified included Poaceae and Asteraceae, with *Conyza sp*. and *Sonchus sp*. being the most prevalent species within these families. Additionally, *Oxalis pes-caprae* L. (Bermuda buttercup; botanical family Oxalidaceae) ranked as the third most dominant weed.

Significant weed suppression was observed across all three cover crop species sown in the olive orchard. Among the cover crops, Pea demonstrated the highest biomass accumulation across both sampling dates, with dry matter measurements of 99.85 g at the first sampling and 108.3125 g at the second. All cover crop species maintained a high percentage of plant coverage, effectively competing with weed growth. The most dominant weed families identified in the cover crops assessment were Poaceae and Asteraceae, with *Conyza* sp. and *Sonchus sp*. being the most frequently encountered species within these groups.

Vicia spp. was selected as the preferred cover crop for subsequent experimental periods, although the pea showed a higher biomass. This decision was based on Vicia’s low-height growth vegetation, which minimizes interference with other essential agricultural activities, enabling farmers to implement a broader range of practices without obstruction. Additionally,

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the reduced vegetative height of *Vicia spp.* addresses critical safety and environmental concerns: it lessens fire hazards associated with Cyprus high temperatures and helps mitigate snake habitation risks, enhancing both field safety and crop management efficiency.

Across both sampling dates, mulching and mowing consistently demonstrated substantial weed suppression, across all treatments. The combination of the cover crop ***Vicia* spp. x rotary tillage, *Vicia* spp. x mulching and Pea x mowing** resulted in a low weed biomass (2 g m^{-2}, 9.5 g m^{-2} and 7.5 g m^{-2} respectively). In contrast, *Vicia spp.* × untreated control and *P. sativum* × untreated control had weed biomass values of 85.43 g m^{-2} and 74.46 g m^{-2}, respectively. The reason for this could be the nitrogen supply to the weeds in the *Vicia* spp. plots, which stimulated the emergence of the weeds and favored their growth.

ES-Cherry/27

In a field trial conducted in a cherry orchard in Spain, four different types of cover crops were sown in late fall in the corridors between the rows of trees: *Ornithopus sativus*, *Trifolium subterraneum*, a biodiverse mixture of *O. sativus* + *T. subterraneum* + *Trifolium michelianum* + *Trifolium resupinatum* + *Trifolium vesiculosum* + *Trifolium incarnatum* + *Lolium multiflorum* and a grass mixture of *Festuca arudinacea* + *L. multiflorum* + *Lolium perenne* + *Dactylis glomerata*. In all plots, the sowing rate of the cover crops was 25 kg ha^{-1}. An untreated control with spontaneous vegetation was also maintained. Two terminations methods were applied: mowing and rolling.

Rainfall was very abundant during the sowing period and soil preparation was carried out immediately before sowing. The abundant presence of the noxious perennial weed *Cynodon dactylon* (L.) Pers. (Bermudagrass; botanical family Poaceae) prior to the establishment of the trial caused a strong allelopathic effect on the germination of the cover crop seeds. This species was incorporated into the soil during tillage and there was no time to wash out the allelopathic substances as the cover crop was sown immediately afterwards to take advantage of the favorable weather conditions. The effects on seed emergence were not the same for all species (P-Value ≤ 0.05). The most severely affected cover crop was *T. subterraneum*. Due to the high rainfall in the previous season, the species used in the different covers also showed a high plasticity in their development cycles, resulting in excessively long growth stages, which in turn caused delays in the maintenance of the covers. The intensity of the fall rains prevented earlier seeding. The cover crops that produced sufficient biomass to have an impact on the surface of the inter-rows, although it should be noted that this biomass does not fully correspond to the proposed cover for the 2nd and year: Ornithopus sativus (8,700 kg ha^{-1}), biodiverse mixture (4,969 kg ha^{-1}) and grass mixture (5,604 kg ha^{-1}). The predominant winter weed species belonged to the *Lolium* spp. weeds and *Cynodon*, spp. (botanical family Poaceae). Other genus present to a lesser degree were: *Conyza*, spp and *Agrostis* spp.

Since the goal of cover crops is the ability to self-seed, it is necessary that they complete their cycle and allow their seeds to mature before they are mowed. However, cover crop growth was very slow, with a very long winter break, which delayed the late spring mowing. In addition, thanks to a very rainy year, biomass development was very high and dense, which could hinder the cherry harvest (which starts in mid-May). This was particularly observed in the grass mixture and *O. sativus* plots. Prior control of *C. dactylon* is necessary to avoid allelopathic effects. It is interesting to look for short-cycle cover crops that can be incorporated before the

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cherry harvest. Although the grass mixture and ***O. sativus* have shown good results in terms of biomass and weed reduction** on the plots where they have done well, **a short-cycle *T. subterraneum* cover crop will be sown next year** as it is smaller and the cover crop can be completed before the cherry harvest.

Furthermore, the following weed management treatments are planned to be assessed on the tree lines during the second and third experimental years: Mechanical weed control by mowing and mulching with textile nets. An untreated control will also be included.

ES_apple_grapes/28

At the organic site of this Living Lab, two field trials are set up in a Randomized Complete Block Design (RCBD) with four treatments and three replicates (blocks). The first experiment is conducted in an apple orchard and the second experiment in a vineyard. In 2024, five treatments have been applied in the vineyards: 3 cover crops were sowed (*Brassica spp.* mixture; clover mixture and raygrass mixture); manual weeding and mechanical weeding. The vineyard production was higher in the *Brassica* spp. mixture treatment (> 3,500 kg/ha) but with no significant differences with the other two cover crops. However, ***Brassica* spp. mixture has significant better performance than the mechanical weeding**. For 2024-2025 season the treatment list is the same for both field experiments and includes the following four treatments: untreated control, mulching, mowing and cover-cropping in the inter-rows of the orchards/vineyards. A mixture of subterranean clover + white clover + red clover + Italian ryegrass + English ryegrass was used as a cover crop (the sowing rate was 10 + 15 + 15 + 7.5 + 7.5 kg ha^{-1}). The measurements are not yet available, as the cover crops were planted in November 2024. Mowing is always carried out when the vegetation reaches 25 cm and mulching is done by applying mulch before weeds germinate.

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