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Deliverable D.2.4

Selection of the most sustainable WEP and legume combinations for each country conditions.

Document Information

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1. Evaluation of WEPs under innovative farming systems in Spain

The experiments were carried out in the CEBAS – CSIC Experimental Farm (Santomera, Murcia, Spain 38° 06' 14'' N 1° 01' 59'' W) under organic conditions in two consecutive years (January – August 2022 and 2023) corresponding to a semi-arid Mediterranean climate with an annual temperature of 19.2 °C, 300 mm of annual rainfall and a high potential evapotranspiration of 1000 mm y^{-1}. The field experiments were conducted in a soil previously used in a lemon tree orchard cut down in 2020, classified as lithic xeric haploxeroll with a clay-loam texture (clay fraction: 41% illite, 17% smectite and 30% palygorskite). The chemical characteristics of the soil were: total N 5.7 g kg^{-1}, available P 6.0 mg kg^{-1}, available K 33.9 mg kg^{-1}, organic matter 12.6 g kg^{-1}, CaCO3 3 g kg^{-1}, pH 7.14 and EC 0.10 dS m^{-1}.

At the beginning of each experiment, weeds were mechanically removed, and the plot was covered with anti-weed mulching to prevent the emergence of weeds, while an automatic irrigation system was installed using filtered water from the experimental farm, along with a hose system with built-in drippers of 2 litres per hour (Figure 1.1). The between drippers was 25 cm (one plant per dripper; 16 plants per m^{-2}).

Figure 1.1: Field prepared with anti-weed mesh and hoses installed.

Peas and cowpea seeds were purchased from a local distributor in Spain. Purslane seeds were provided by the Department of Agriculture, Crop Production, and Rural Environment (University of Thessaly, Greece). All seeds were sown in peat substrate in greenhouse conditions, and at the stage of the first true leaf, irrigation with Hoagland's solution with nitrogen reduced to 50% started. Plants were transplanted to the field 25 days after sowing (Fig. 1.2).

Figure 1.2: Cowpea and purslane plants right before transplanting

The pea plants were cultivated from January without the use of fertilizers until their full maturation and senescence (120 days). Then, the entire aerial part was air-dried at 60 °C and ground into a fine powder (Fig. 1.3), which was then equally applied to each plant in the respective treatments of crop rotation. Cowpea and purslane plants were cultivated from June to August without the use of fertilizers until the complete maturation of the cowpea plants.

Figure 1.3: Dried and grounded peas and its application on the soil before purslane plants in R (rotation) and IR (intercropping and rotation) treatments.

Experimental studies:

Two experiments have been designed. The first one (January 2022 – August 2022) has been already analysed. The second one (January 2023 – August 2023) is currently ongoing. All soil samples were stored at -20 °C until DNA extraction. Plant tissue were frozen at -80°C and lyophilized for the nutrient determination.

Several measurements were taken:

- Purslane fresh and dry weight.
- Total mineral composition in the aerial tissues (Total N, P, K, P, Fe, S).
- Nutritional composition of purslane (Proteins, sugars, antioxidants, fatty acids).
- Soil pH and electrical conductivity.
- Soil mineral composition (Total N, P, K, P, Fe, S).
- Soil available P, Total carbon and organic carbon content.
- Soil enzymatic activities: β-glucosidase activity, dehydrogenase activity, alkaline phosphomonoesterase activity and urease activity.
- Soil DNA extraction, Illumina sequencing (bacterial 16S V3-V4 and fungal ITS2 region), bioinformatics and biostatistical analyses.

1.1 Effect intercropping and rotation crop systems on purslane growth and rhizosphere microbial taxonomic and functional diversity

The first experiment aimed in the evaluation of the effect of four different treatments: (*i*) Purslane (*Verdolaga oleracea*) monocrop in summer (Control); (*ii*) Purslane – Cowpea (*Vigna unguiculata*) intercropping system, with purslane and cowpea planted in alternative rows in summer (I); (*iii*) Purslane – Pea (*Pisum sativum*) rotation system, where peas were planted in winter and purslane in summer (R); and (i*v*) Purslane – Pea – Cowpea mixed systems intercropping and rotation, where peas were planted in winter and purslane and cowpeas planted in summer (IR). The experiment was a completely randomized design with three plots per treatment. Each plot included 4 irrigation hoses separated by 25 cm each with 7 built-in drippers separated by 25 cm each, with a density of 16 plants m^{-2} and a total of 28 plants per plot (Control and R had 24 purslane plants; I and IR had 12 purslane plants and 12 cowpea plants). Each plot consisted of two samples, corresponding to the 2 central hoses where all the plants in each hose were collected simultaneously and used prepared a batch sample. The two outer hoses were discarded (Fig. 1.4 and 1.5) as outliers. Four weeks after plantation, purslane plants were harvested three times (every two weeks) at 8 cm high in order to let the plant regrowth (Fig. 1.6A). At the third harvest, purslane plants were pulled out to obtain the rhizospheric soil (Fig. 1.6B).

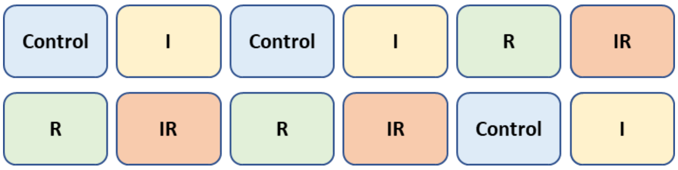

Figure 1.4: First cropping systems experimental design.

Figure 1.5: First cropping experiment 6 weeks after plantation.

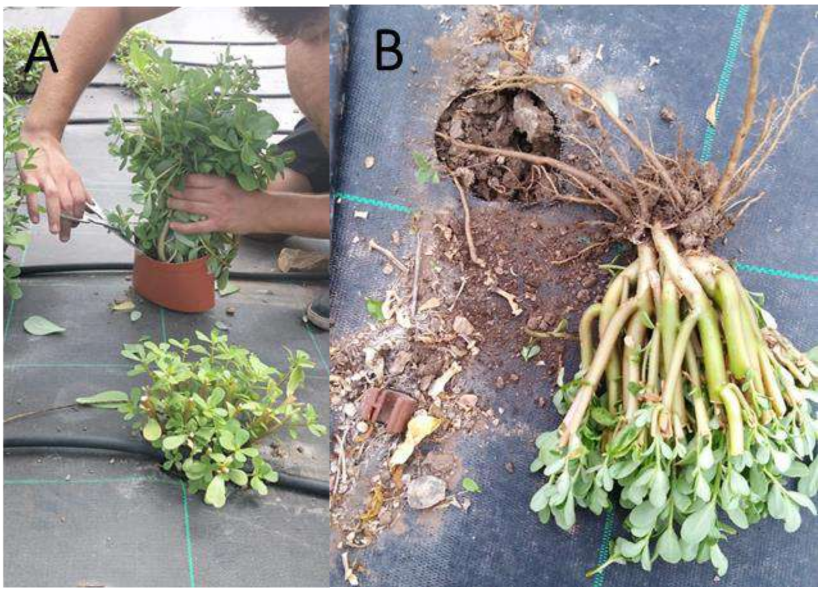

Figure 1.6: (A) First cut of purslane plants at a 8 cm height. (B) Purslane plants pulled out to obtain rhizospheric soil.

Concluding remarks: All cropping systems increased purslane growth, especially the IR treatment. Although only the rotation treatments (R and IR) had effects on the physical, chemical, and microbial properties of the soil. It was found that the improvements in the growth of purslane were mainly caused by the direct interaction with cowpea during purslane development, but the effects on the rhizosphere were caused by the previous cultivation and the application of the green manure from peas.

1.2 Impact of different cropping systems of legumes and purslane on rhizosphere microbial communities over (along) time

The second experiment currently ongoing aims in the evaluation of the different cropping systems over time on purslane and cowpea rhizosphere communities. The treatments studied were: (*i*) Purslane (*Verdolaga oleracea*) monocrop in summer (CP); (*ii*) Cowpea (*Vigna unguiculata*) monocrop in summer (CC); (*iii*) Purslane - cowpea intercropping in summer (I); (*iv)* Purslane – Peas (*Pisum sativum*) rotation, where peas were previously planted in winter (RP); (*v*) Cowpea – Peas rotation rotation, where peas were previously planted in winter (RC); (*vi*) Purslane – Peas – Cowpeas mixed intercropping and rotation, where peas were previously planted in winter (IR). The experiment was a completely randomized design with three plots per treatment. Control and rotation treatments consisted in 4 hoses. Intercropping treatments consisted in 6 hoses (Fig. 1.7 and 1.8). All irrigation hoses were separated by 25 cm each with 7 built-in drippers separated by 25 cm each with a density of 16 plants m^{-2}. Each plot consisted of two samples, corresponding to the 2 central irrigation hoses where all the plants in each hose were collected simultaneously. The two outer hoses were discarded.

CC CCCC	**RC** CCCC	**I** CPCPCP	**RP** PPPP	**IR** CPCPCP	**CP** PPPP
I CPCPCP	**CP** PPPP	**RP** PPPP	**CP** PPPP	**RC** CCCC	**CC** CCCC
IR CPCPCP	**CC** CCCC	**IR** CPCPCP	**RC** CCCC	**I** CPCPCP	**RP** PPPP

Figure 1.7: The experimental design of the second cropping systems. Letter corresponds to the species planted in each hose.

Figure 1.8: The experimental layout of the second cropping system 4 weeks after plantation.

To assess the evolution of soil quality parameters and microbial community composition, several harvests were being conducted: (h0) random soil sampling in each plot prior to pea cultivation; (h1) sampling of rhizospheric soil at the end of spring after pea harvest; (h2) destructive harvest of 2 plants per row 4 weeks after planting; (h3) destructive harvest of 2 plants per row 2 weeks after the previous harvest (Fig. 9B); (h4) final destructive harvest of 2 plants per row 2 weeks after the previous one. In h2, h3 and h4, all purslane plants were harvested as in the first experiment before pulling out 2 samples for rhizospheric soil (Fig. 1.9).

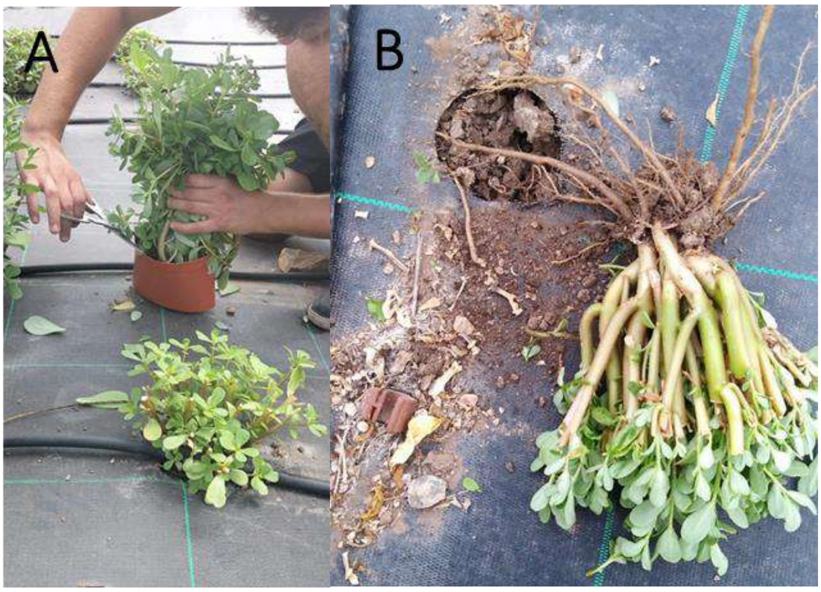

Figure 1.9: (A) First cut of purslane plants at 8 cm height. (B) Purslane plants pulled out to obtain rhizospheric soil.

This experiment is still ongoing. The sampling is expected in July and August.

2. Evaluation of WEPs under innovative farming systems in Greece

The following experiments were carried out at the experimental farm of the University of Thessaly in Velestino Greece.

a) Field experiments regarding the use of *Cichorium spinosum, Crithmum maritimum, Sonchus oleraceus* and *Portulaca oleracea*, in crop rotation systems, following the cultivation of *Phaseolus vulgaris.*
b) Field experiments with *Cichorium spinosum, Crithmum maritimum, Sonchus oleraceus* and *Portulaca oleracea* where the effect of crop rotation with peas was tested in comparison to sole cropping systems.
c) Field experiments with *Portulaca oleracea* where the effect of intercropping with common bean and crop rotation was tested in comparison to sole cropping systems.

Description of experiments

A) The effect of crop rotation on the crop performance of *Cichorium spinosum*

Young seedlings of *Cichorium spinosum* were transplanted to the field in 30th of March 2021 when they reached the 3-4 true leaves stage. They were used in total two treatments, namely the first treatment where the field was previously cultivated with *Phaseolus vulgaris* and the second treatment where field was not cultivated.

Concluding remarks: Crop rotation increased the weight of leaves/plant without affecting the number of leaves/plant.

B) The effect of crop rotation on the crop performance of *Cichorium spinosum*

A field trial was implemented during the growing period September 2022 and March 2023, where the effect of crop rotation with two legumes *Phaseolus vulgaris* L and *Vigna unguiculata* L. on *C. spinosum* crop performance was tested. Three treatments in which the two treatments in the previous growing periods were cultivated *Phaseolus vulgaris* L and *Vigna unguiculata* L. respectively and the control treatment which was uncultivated at the former growing period.

Concluding remarks: crop rotation increased all crop performance parameters tested (rosette diameter, weight and number of leaves, weight of plant compared to the control treatment, whereas no significant differences were recorded between the crop rotation systems. Moreover, chlorophyll content was not affected by crop rotation, while leaf are increased with crop rotation, especially for the system where *V. unguiculata* was rotated with *C. spinosum.*

Figure 2.1. The effect of crop rotation on the crop performance of *C. spinosum* (From Left to right the treatments namely None Crop rotation, Crop rotation with *V. unguiculata* L. and Crop rotation with *P. vulgaris* L.

C) The effect of water deficit stress and crop rotation on the crop performance of *Cichorium spinosum*

In the current study, there were 3 irrigation treatments, namely deficit irrigation (50% of field capacity), full irrigation (100% of field capacity) and the control treatment where the plants were rain-fed, during the growing period of September 2021-May 2022. Moreover, the effect of crop rotation was also tested by applying two treatments, namely the crop rotation with plants of *Phaseolus vulgaris* and no crop rotation.

Concluding remarks: Deficit and full irrigation resulted in higher weight of leaves/plant in plants grown under no crop rotation regime, while deficit irrigation resulted in lower number of leaves under crop rotation conditions. Moreover, crop rotation increased the number of leaves/plant only in the case of full irrigation.

Figure 2.2. The effect of water deficit stress and crop rotation on the crop performance of *C. spinosum*.

D) The effect of manure on the crop performance of *Scolymus hispanicus* grown in the field

The current trial was carried out at the experimental farm of the University of Thessaly during the period March 2022 and June 2022. The experimental setup was similar to experiment D.

Concluding remarks: the application of manure increased all the parameters related to crop performance, except for dry matter of leaves which remained unaffected. A similar trend was recorded for chlorophyll content, the weight of roots and leaf area, whereas dry matter of roots remained unaffected. On the other hand, specific leaf decreased for manure application. Therefore, it could be concluded that manure application has a positive effect on biomass yield.

E) The effect of crop rotation on the crop performance of *Scolymus hispanicus*

The current trial was conducted at the experimental farm of the University of Thessaly during the growing period September 2022 and March 2023. The experimental layout is similar to experiment B.

Concluding remarks: crop rotation improved crop performance parameters over the control treatment, regardless of the legume species, except for dry matter of leaves where no differences were recorded. The same trend was recorded for chlorophyll content and roots weight, while no differences were recorded for dry matter of roots and the leaf area. Finally, specific leaf area was higher for the control treatment, being significantly different only for crop rotation with *P. vulgaris*.

Figure 2.5. The effect of crop rotation on the crop performance of *S. hispanicus* (From left to right the treatments namely None Crop rotation, Crop rotation with *V. unguiculata* L. and Crop rotation with *P. vulgaris* L.).

F) The effect of crop rotation on the crop performance of *Sonchus oleraceus*

The experimental setup was similar to experiment B. The experiment was conducted during the growing period of March 2021-July 2021.

Concluding remarks: no differences recorded between the studied treatments for any of the parameters tested.

Figure 2.7. The effect of crop rotation on the crop performance of *S. oleraceus*.

G) The effect of crop rotation on the crop performance of *Sonchus oleraceus*

The experimental setup was similar to experiment A. The experiment was conducted during the growing period of September 2022-December 2022.

Concluding remarks: crop rotation increased all the growth parameters e.g. rosette diameter, weight of plants, number and weight of leaves per plant, and leaf area regardless of the legume species used in the crop rotation programme. On the other hand, chlorophyll content differed between the two legumes without significant differences between the control treatment and the use of *V. unguiculata*. Finally, no differences were recorded for the dry matter of leaves and specific leaf area.

Figure 2.8. The effect of crop rotation on the crop performance of *S. oleraceus* (From left to right the treatments namely None Crop rotation, Crop rotation with *V. unguiculata* L. and Crop rotation with *P. vulgaris* L.).

General conclusion: the tested wild edible plants responded positive to crop rotation with various legumes, especially during the second growing period of WEPs cultivation were the effects were more profound. Therefore, it could be suggested that the selected species could be integrated in sustainable farming systems and the use of crop rotation could be beneficial for both yield and quality (these results are part of WP4) of the edible products of WEPs, as well as to soil quality (these results are part of WP3).