



D4.1. INITIAL DESCRIPTION OF ALL CASE STUDIES LAYOUT

Lead Beneficiary: Institut de Recerca i Tecnologia Agrària (IRTA)

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DOCUMENT INFORMATION

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EXECUTIVE SUMMARY

The present deliverable (D4.1) contains the description of the case studies that will be studied, developed and partaken on the SIRAM project. This Deliverable provides context on the different case studies, including the description of the pedoclimatic conditions and the main agricultural system characteristics in each area, the regenerative agriculture pillars involved for each case study and the trials (field, pot, greenhouse, ...) involved.

1. INTRODUCTION

Eight case studies will take place under the SIRAM project. Several demonstration sites are included in these case studies. The aim of this document is to provide the necessary context for each one of the case studies, which are located in six different countries around the Mediterranean basin: Italy, Morocco, Egypt, Tunisia, Spain and Portugal.

The aim of this deliverable is to store and broadcast preliminary information on each one of the case studies. The information will be able to be used by any member of the consortium to consult when needed and also by the general public.

2. GENERAL DESCRIPTION OF THE CASE STUDIES

The case studies included in the SIRAM project are described in the following document. Each description provides: the climate description and classification, a brief contextualization on the soils and a general description of the agricultural system of the area where the case study takes place.

The SIRAM project is based on four pillars in the context of regenerative agriculture.



- **Beneficial microorganisms** for plant growth and pest control.
- **Agronomical** practices to safeguard soil **quality** and **fertility**.
- **Crop varieties** with resistance towards **abiotic** and **biotic** stresses.
- **Bioeconomy** approaches to **recycle** and **valorize** waste biomasses that restore soil organic matter and have bio stimulant properties.

The description of each case study details which of the four pillars will be developed and a brief explanation of the tasks where they will be.

Finally, each description contains a brief description of the trials, demonstration sites, experiments, etc. included in the case study, providing context for each one.

2.1 EMILIA-ROMAGNA, ITALY. REGENERATIVE FORAGE FARMING AND SLURRY USE

Emilia-Romagna is an administrative region located in the Po valley (Figure 1), Northern Italy, with a 22,453 km² area of and about 4.4 million inhabitants. The agricultural sector is one of the most advanced in the country (>6.5 billion €, around 12% of the overall Italian value) with a great variety of products: milk, pork and poultry meat, cereals, fruits and wine. Although the region is characterized by a continental climate, extreme events such as long dry periods and concentrated heavy rains are increasing due to climate change. Moreover, the indiscriminate use of conventional practices has resulted in lower content of C in the soil, increase of GHGs emission and high nitrate levels in groundwater.



Figure 1. Location of the plots of the case study in Emilia-Romagna, Italy.

The plots involved in this case study are located at the CERZOO research farm in Piacenza (Figure 2) (45°00'18.0"N, 9°42'12.7"E; 68 m above sea level). This site is located on a flat landscape and the soil is a fine, mixed, mesic Udertic Haplustalfs, with a silty-clay texture.

The site is characterized by a temperate climate (Cfa as Köppen classification), with an average annual temperature of 13.2 °C and annual rainfall of 839 mm, based on a 30-year average.

The main arable and forage crops in the area are irrigated summer maize, soybean, and processing tomato, as well as rainfed winter arable crops (e.g. wheat and barley). Dairy farms of this area also use, in some cases, a two-crops per year rotation: summer forage maize + winter forage crop (e.g. barley). Rainfed vineyard is another common crop of the area, which can be mainly found in the southern hill part of the Piacenza province.



Figure 2. Conventional tillage and no-till + cover crop plots, as seen in November 2022.

This case study focuses on three of the four pillars of innovation that form part of the SIRAM:



- **Beneficial microorganisms for plant growth and pest control.** Microorganisms isolated from corn rhizosphere and with plant growth promoting properties (i.e. nitrogen fixation, phosphorus solubilization, phytohormones and siderophore production) will boost and complement regenerative agriculture practices applied to the field to assure nutrient cycling and good yields.

- **Agronomical practices to safeguard soil quality and fertility.** The combination of conservation agriculture practices (e.g. no-till, cover crops) with manure application will steer C and N cycles, increasing nutrient use efficiency and soil fertility.
- **Bioeconomy approaches to recycle and valorize waste biomasses that restore soil organic matter and have bio stimulant properties.** The higher nutrient use efficiency, achieved by the combination of manure, beneficial microorganisms, and conservation practices, will reduce emissions and the use of mineral fertilizers.

2.1.1 No-till plus cover crops and beneficial microorganisms to enhance soil quality and soil C sequestration.

In the last decades, a general trend of soil C reduction in the Po Valley has been observed; several agro-ecosystems are also experiencing an increase of pollution and emissions of GHGs. Reducing tillage and introducing cover crops are increasingly recommended as strategies to reduce losses while increasing soil fertility. In fact, these practices may lead to considerable benefits, including soil C increase, nutrient cycling enhancement, water and soil conservation, increased biodiversity and competition with weeds.

A long-term (>10 yr) field experiment is being continued to contribute to the aims in this challenge. The experimental design is a randomized complete block design with four blocks and four treatments. The experimental treatments include: conventional tillage (based on moldboard plowing and rotary harrowing, without cover crops); no-till + rye as cover crop; no-till + hairy vetch as cover crop; and no-till + an eight species mixtures of cover crops (grasses, legumes, and brassicas). In this case study, the combined effect of novel microbial biostimulants and conservation practices on soil quality and C sequestration will be tested. Mechanisms will be tested in pot experiments (by using undisturbed soil cores taken from this field as basic research).

2.1.2 Fertilization strategies based on manure and beneficial microorganisms to increase N-use efficiency and minimize N losses including N₂O emissions.

Livestock manure is a major source of N for plants, but also of ammonia (NH₃) and nitrous oxide (N₂O) emissions to the atmosphere, and nitrate (NO₃⁻) leaching to groundwater. The main objective here is then to enhance the value of slurry as a fertilizer, thus (i) improving N-use efficiency (closing cycling), (ii) reducing as much as possible the application of mineral fertilizers, (iii) and ensuring minimal risk of agricultural emissions.

The same field experiment of the previous challenge will be used to assess the effect of manure applied alone and as co-formulant for the delivery of beneficial microorganisms on N-use efficiency and N losses into the environment. Similarly to the previous challenge, mechanisms will be tested in pot experiments (by using undisturbed soil cores taken from this field as basic research).

2.2. LOMBARDIA, ITALY. BOOSTING ORGANIC FARMING VIA REGENERATIVE AGRICULTURE

Lombardia is an administrative region located in the North-West of Italy (Figure 3); it has an area of 23,864 km² and about 10 million inhabitants. Since producing 37% of Italian milk, 42% of Italian rice and 40% of Italian pig products, the regional agricultural production is the highest in the country. In the past few years, Lombardia's government, through EU Rural Development Policy, has made investments to promote organic farming systems. However, farmers still need a farming system model to sustainably support their income and a common development view. In fact, current organic farming systems cause a reduction of soil quality and nutrient availability for plants, as well as a difficult weeds control, thus leading to unsatisfactory yield and farmers' income.



Figure 3. Location of the plots of the case study in Lombardia, Italy.

The plots involved in this case study are located at a commercial farm located in Gabbioneta-Bianuova (45°12'41.4"N 10°12'17.0"E; altitude 38 m asl), near Cremona. The site is characterized by a temperate climate, with an average annual temperature of 13.3 °C and annual rainfall of 851 mm (30-year average). The soil has a loam texture (sand 410 g kg⁻¹, silt 430 g kg⁻¹, clay 160 g kg⁻¹).

Since the high presence of dairy farms in the area, crops are mainly grown for forage, including irrigated summer maize and italian ryegrass (as annual crops), and alfalfa (as meadow). In addition, rainfed winter arable crops for cereal production (e.g. wheat and barley) are widespread in the area.

This case study focuses on three of the four pillars of innovation that form part of the SIRAM:



- **Crop varieties with resistance towards abiotic and biotic stresses.** The performances of novel crop varieties (e.g. Quinoa cultivars), in terms of decreased dependence from external inputs (e.g. irrigation water), will be tested.
- **Agronomical practices to safeguard soil quality and fertility.** Using cover crops, especially legumes, will enhance nutrient cycling and soil fertility.
- **Bioeconomy approaches to recycle and valorize waste biomasses that restore soil organic matter and have bio stimulant properties.** Legume cover crops will provide nutrient supply to main crops, lowering dependence of farmers from mineral fertilizers.

2.2.1 Regenerative practices to enhance nutrient cycling under organic farming.

The adoption of intensive farming practices and inefficient organic farming systems has caused a reduction in C and nutrients content in soils of northern Italy, also leading to an increase in GHGs emissions and environment pollution. Promoting plant-soil interactions, by reducing tillage and using cover crops (especially legumes) will enhance soil quality and internal nutrient cycling.

A medium-term (>3 yr) field experiment is being continued (Figure 4) to contribute to the aims in this challenge. The experimental design is a randomized complete block design with four blocks and nine treatments, including a control (traditional management without cover crops) and eight cover crop treatments. The cover crop treatments include rye, hairy vetch, and tillage radish as monocultures and mixtures. In this case study, experimental activities aim to assess the increased soil fertility and crop productivity under cover crop treatments (especially for legumes as cover crops), as well as the lower N₂O emissions and weed presence (especially for grasses as cover crops). Novel indicators of soil microbiological quality, based on the analyses of functional genes involved in nutrient cycling and in plant-growth promoting traits, will be developed.



Figure 4. Cover crops field experiment, as seen in December 2022.

2.2.2 Novel organic farm management to increase smallholder farmers' income.

The regenerative practices introduced with the previous challenge will: (i) increase the content of organic matter in the soil, promoting its productivity potential, (ii) enhance the water use efficiency, reducing evaporative and percolation losses and increasing the water retention capacity of soil, with an improved preservation of precipitation water over time, (iii) ensure a better control of weeds using cover crops, due to greater competition and allelopathic effects.

In addition, novel crop varieties (different Quinoa cultivars) resistant to abiotic stresses (e.g. water stress) will be tested in this challenge with a randomized complete block design, to evaluate the increased economic income of farmers and the decreased dependence from external inputs (i.e. mineral fertilizers and irrigation water).

2.3. FES-MEKNES, MOROCCO. RESILIENT AND CLIMATE-SMART OLIVE GROWING

The Moroccan Region of Fès-Meknes is made up of two prefectures (Fès and Meknes) and seven provinces Boulemane, El Hajeb, Ifrane, Moulay Yaâcoub, Séfrou, Taounate and Taza (Figure 5, left). This region covers an area of 40,075 km² (5.7% of the national territory) and has 4.236.892 inhabitants of which 60.52% are urban. This region is in 4th position in terms of contribution to the national wealth, i.e. 9.3% of national GDP in 2017. It was always well-known for arboriculture, in particular oliviculture (Figure 5, right), which was intensified with the green Moroccan Plan. In Morocco, the olive tree is the first perennial crop in the country, covering 60% of arboriculture area with approximately 784.000 ha and average production of 1.500.000 tons of olives and a contribution of around 5% to agricultural GDP.

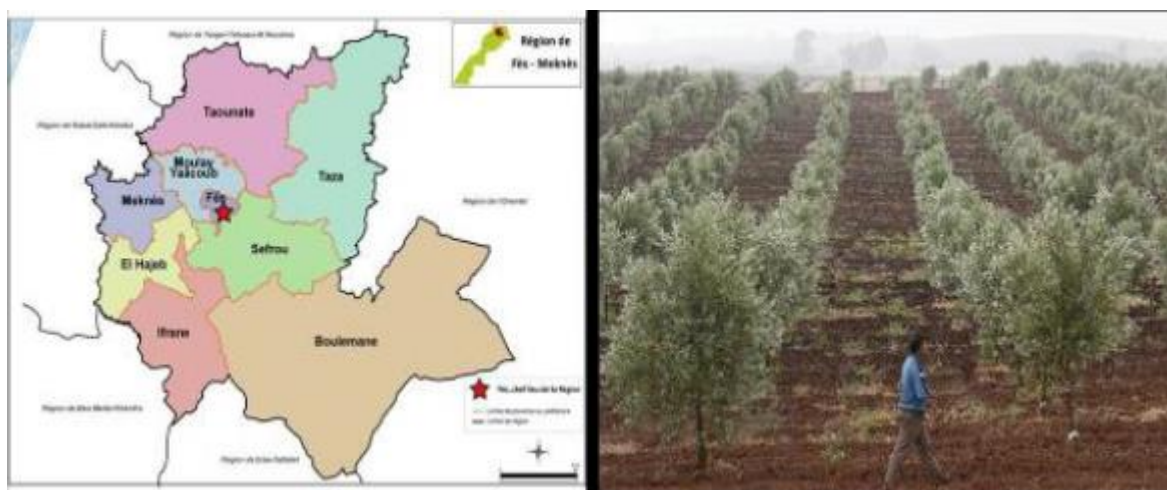


Figure 5. Fès-Meknes region (left) and an olive field (right), the main arboriculture crop in the area.

However, recently olive trees are suffering from different constraints, in particular those related to diseases (Figure 6). Oomycetes pathogens like *Pythium schmitthenneri* were emerged as a major threat to young olive trees grown in nurseries.

Therefore, In this study, we hypothesized that treating olive trees (*Olea europaea* L.) with antagonistic agents could be an effective way for the biocontrol of root rot caused by this pathogen even under non-sterile conditions. Therefore, this case study focuses on preventive measures to raise the robustness and resilience of olive production under climate change. Sustainable disease control strategies for resilient olive production are highly required and have to implement in organic Olive cultivation.



Figure 6. Symptoms of diseases affecting olive trees.

This case study has three main objectives.

1. Microbial characterization of olive soils and screening of potential antagonists to restore soil fertility and mitigate olive diseases

In this case study, new screened PGPR bacteria from different olive fields around the region of Fes-Meknes will be tested for their abilities to suppress *P. schmitthenneri*. The screening experiments will be done in in vitro conditions on agar medium using dual culture bioassay; selected potential microorganisms will be tested on 1-year olive seedlings in pot experiments under greenhouse conditions. The mechanisms of action of effective PGPR bacteria will be investigated in order to enhance their formulation as biological control agents (BCAs). Until now, a collection of beneficial screened bacteria was established and identified.

2. Efficacy of already existing bacterial antagonists from the consortium

Beneficial microorganisms already characterized in our laboratory were used for this experiment. Two bacterial isolates ACBC1 and SF14 and fungal isolate *Trichoderma sp.* (T2) were tested for their ability to suppress *P. schmitthenneri*. The experiment was carried out in the greenhouse. The soil used in this experiment was collected from the pedagogic farm of ENA and had previously held olive plants. In the glasshouse, the non-sterile soil (to characterize the impact on soil microbial community) was homogenized and poured into plastic pots of 5 kg cleaned with 2% sodium hypochlorite (2 kg of soil per pot). Subsequently, one-year-old olive plants (*Olea europaea* L. var. Arbequina) were gently removed from their substrate, cleaned of soil debris, and washed with SDW. Then, the plants were

placed in the pots; therefore, 10 g of the fungal inoculum was spread around the root system of the olive trees and covered with soil. Following that, the bacterial isolates were poured onto the plants by drenching them in 200 mL of bacterial suspension (2×10^8 CFU/g). Regarding the treatment using *Trichoderma sp.*, the suspension was prepared at the rate of 10 g of *Trichoderma* mass in 2 L of SDW. Following that, the pots were soaked in 200 mL of *Trichoderma sp.* conidial suspension. The olive trees were maintained in the flooded soil for 24 hours. Plants were irrigated two to three times per week. The seedlings were kept in the greenhouse under ambient conditions. The in vivo experiment included the treatments described in Table 1. The trial contained eight replicates per treatment, and each pot was arranged in a randomized block. This experiment was repeated twice over time.

Table 1. Treatments used in the bioassay.

Treatment	Composition	Replicates	Period
ACBC1	NSS + pathogen + ACBC1	8	2-3 months
SF14	NSS + pathogen + SF14		
<i>Trichoderma sp.</i>	NSS + pathogen + <i>Trichoderma</i> sp.		
C+	NSS + pathogen		
C-	NSS only		

NSS: non-sterilized soil.

After two to three months, the efficacy of the used treatments was assessed. The disease severity was evaluated by visual observation of plants root system using the five-level scale described by Legrifi et al. (2022): 1 = healthy white roots/no disease observed; 2 = 25% root rot or seemingly healthy roots + onset of root rots, 3 = 50% root rot and early browning, 4 = 75% root rot (browning of root system), and 5 = 100% dead roots. In addition, the effects of the treatments on plant morphology was determined. To do this, four pots of each treatment were randomly selected. Plant/root lengths and plant/root fresh/dry weights were measured.

3. The use of resistant olive varieties and resistant mechanisms

The use of resistant cultivars remains among the best strategies for the control of plant diseases. However, no data on root rot resistant olive varieties have been published. In this context, the resistance of six olive varieties to *P. schmitttheneri*, the causal agent of root rot disease, was assessed. Six different varieties of 1-year-old olive trees have been chosen for this trial (Picholine marocaine, Menara, Haouziya, Arbequina, Arbosana, and Koroneiki). In the greenhouse, 5 kg plastic pots were

washed with 2% sodium hypochlorite before being filled with homogenized sterile soil (1 w soil: 2 w sand) (2 kg of soil per pot). The seedlings were then carefully removed from their substrate, cleaned of soil particles, and washed with SDW. After that, the plants were put into the pots, and 10 g of the fungal inoculum was spread around the olive trees' roots before being covered with soil substrate. The negative control of each cultivar did not receive the inoculum. For a full day, the olive trees were left in the waterlogged soil. Two to three times every week, the plants were irrigated. The plants were kept in the greenhouse at 25 °C. Eight replicas of each variety were used in the trial, and the arrangement of each pot was randomized. After three months, the inoculated plants were evaluated. The parameters to be evaluated in this experiment are the same as those described above.

This case study focuses on two of the four pillars of innovation that form part of the SIRAM:



- **Beneficial microorganisms** for plant growth and pest control.

- **Crop varieties with resistance towards abiotic and biotic stresses.**

2.4. AL MINYA, AN NUBARIYAH AND SALHEYA REGIONS, EGYPT. ORGANIC AGRICULTURE FARM

Modern agricultural practices are being introduced to cultivate new reclaimed lands; such practices include modern irrigation systems, organic farming and integrated pest management. Due to the shortage of fertilizers and other agricultural chemicals, farmers usually produce agricultural commodities organically which are exported. Water resources such as ground and surface water are being used for irrigation purposes in the new lands in Egypt An Nubariyah region. An Nubariyah is a region in El Beheira, a coastal governorate, located in the northern part of the country in the Nile Delta (Figure 7). This region have a good potential to produce field crops such as wheat, maize, citrus, potato, sugar beet, cotton, soybean, sunflower, alfalfa, peach, olive and watermelon under irrigation with providing the water requirements but they are also affected by climatic factors as well as both biotic (pathogens) and abiotic (drought, salinity) stresses. The plots involved in this case study are located in the EL Hanna farm An Nubariyah region (Figure 7).



Figure 7. Location of the plots of the case study in An Nubariyah, Egypt.

There is no doubt that climate change nowadays is considered one of the hottest spots worldwide. These changes in climate penetrated all fields in our life including agriculture, soil, water, air, etc. and climate has a deciding role in all agricultural systems. In Egypt, climate changes have three serious

dimensions, represented in the threat of the sinking of the Nile Delta, water crisis and the decrease in crop production. Soil acts an important role in the global cycles (i.e. carbon (C) and nitrogen (N)) and both are linked and related not only to climate change mitigation but also sustaining agricultural productivity and improving soil quality. Soil C and N sequestration. Moreover, using good and novel long-term management practices are the most important methods for increasing their stocks and mitigating greenhouse gasses (GHGs) emission, from soil to the atmosphere as well for improving soil quality and increasing economic crop production. ARC case study will be carried on a newly reclaimed area of An Nubariyah where major of agricultural project for development is underway West of the Nile Delta, Egypt ,most of the soils have gravelly sand to gravelly loamy sand texture except the soils of low Alluvial plain terraces that have clay loam texture. The analytical data revealed that the soils are slightly to moderately alkaline, mostly non-saline and haven't sodicity effect. The soils are slightly to moderately calcareous having Low gypsum and Organic matter contents. Most of the soils haven't any diagnostic horizons, therefore they were classified under Entisols. Physical, chemical and morphological properties of soils of El-Nubaria were determined for the soil profiles of a marine-lacustrine silty clay soil of shallow to medium depth. The soils can be improved by better management practices through careful addition of organic manures, better balanced fertilization, rotation with green manures and/ or compost and legumes and avoiding irrigation with low quality water as well as construction of an efficient drainage system.

An Nubariyah case study focuses on two of the four pillars of innovation that form part of the SIRAM:



- **Crop varieties with resistance towards abiotic and biotic stresses.** Development of biopesticides using beneficial microorganisms for biological pest control as biological control agents (BCAs). The proposed solutions developed and their nature will make them potentially suitable for integrated and organic systems.
- **Bioeconomy approaches to recycle and valorize waste biomasses that restore soil organic matter and have bio stimulant properties.** This study aims to improve nutrient use efficiency from compost with the objective of reducing emissions and contributing to reduce the use of mineral fertilizers.

An Nubariyah case study will be divided into two specific challenges:

2.4.1. Ecofriendly approaches for underground plant parasitic nematodes diseases management

Citrus are one of the most important crops grown in Egypt for local consumption, export and processing. In 2019, citrus fruit production in Egypt was 4.64 million tonnes. Citrus are ones of the fundamental crops in the Mediterranean Basin, which is one of the regions affected by water scarcity, pests and phytopathogens.

It is known that development of bio pesticides using beneficial microorganisms for bio-pest control is essential for agricultural production. The proposed solutions will be developed under organic agriculture conditions in citrus (An Nubariya farm). Isolation and molecular characterization of isolates, nematodes response to microbial infection, mycopesticide lethal effects, and effect of isolate enzymes on hydrolyzing nematodes' cuticles will be assessed in field experiments.

2.4.2. Novel biopesticides to enhance beneficial microorganism communities and minimize losses:

Biological control is considered by many applied ecologists and pest management specialists to be among the safest, most environmentally benign, and most cost-effective methods of pest control available. Yet the practical implementation of approaches, especially for classical biocontrol, is replete with challenges such as: 1) correct and fast pathogen/pest diagnostics, 2) environment traits (soil type, microbiota and plant reaction), 3) weather forecast, and 4) exact characterization of biocontrol agent in terms of specificity, activity, survival and accumulation in environment, positive/negative effect on citrus crop. Strain selection and formulation, application techniques for microbial agents, droplet size spectra of oil-based formulation sprayers, effects of storage duration and environment on fungal spore and bacteria survival, and mass production of microbial control agents as developed in WP2 and WP4 will be assessed for effective, safe and low-cost novel biopesticides production and application as described in Figure 8.

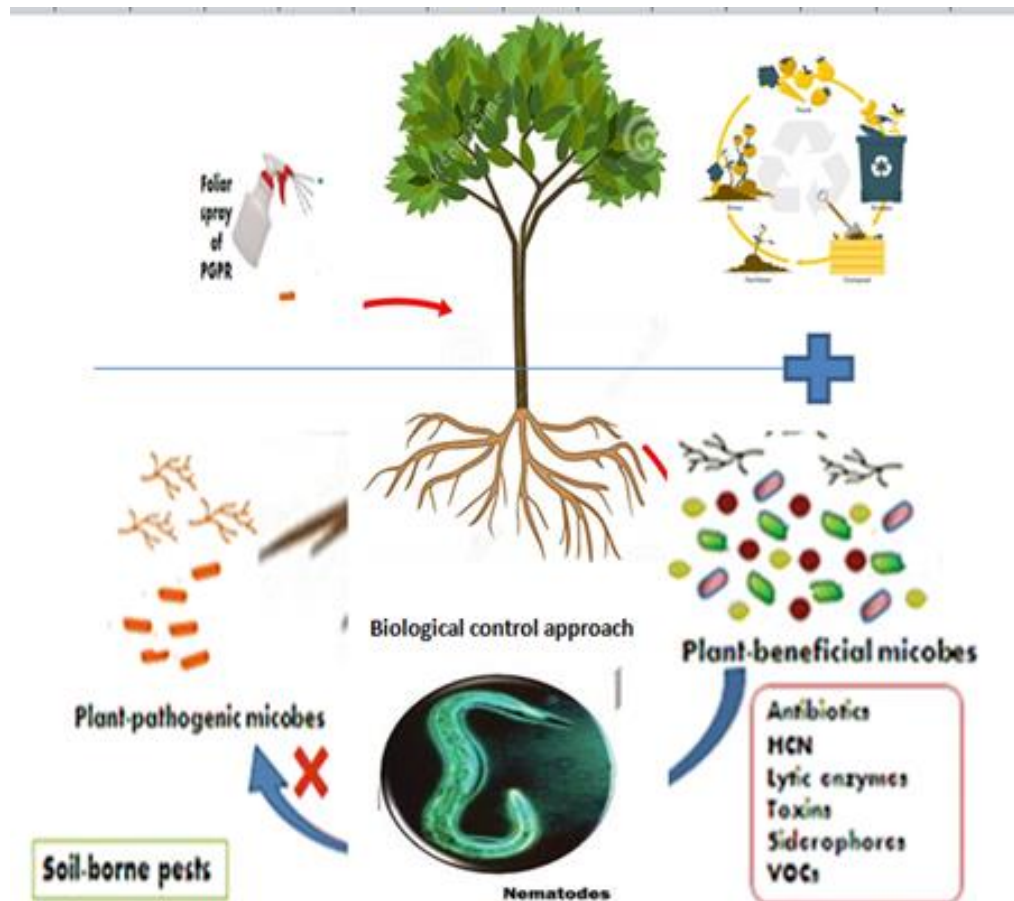


Figure 8. Combination of compost and novel formulation of bio-control agents on management of plant parasitic nematodes

2.5. BERKANE, MOROCCO. EXPERIMENTAL FARM FOR *CITRUS* ROOT ROT AND CANKERS DISEASES AND *PHYTOPHTORA* ROOT ROT.

Berkane is a province in the Eastern Moroccan region. It is located in the Northeast of Morocco, in the region of the Eastern Rif at the north of the BeniSnassen mountains, with a population of 205,000 inhabitants. It is a region which extends from the Algerian border (Oued Kiss) to the Moulouya river in the West (border with the tribe of Ayt Stoute), it dominates a vast region of plains (Figure 9, left): Triffa in the North and Angad in the South. The abundance of water in these mountains has in the past favored the settlement of populations in the villages.

In Morocco, the total surface area of citrus orchards was more than 126,600 ha, of which 32% is located in Souss-Massa, 20% in Gharb, 16% in Berkane (Moulouya), and 13% in Tadla. In Berkane, Citrus (Figure 9, right) covers an area of 22.000 ha with approximate production of 400.000 tons divided into 64% clementines and 35 % oranges. This sector contributes substantially to the national economy, through the generation of income from diverse activities along its value chain and produces an average of 2.6 million tons of fruits per annum.



Figure 9. Berkane region (left) and a citrus orchard (right).

However, with the intensification of cultivation several phytosanitary constraints have emerged. Among them, dry root rot, *Phytophthora* rot root, and citrus cankers (Figure 10).



Figure 10. Symptoms of disease in citrus trees

This case study focuses on two of the four pillars of innovation that form part of the SIRAM:



- **Beneficial microorganisms** for plant growth and pest control.

- **Crop varieties with resistance towards abiotic and biotic stresses.**

This case study will develop in Berkane citrus orchards and will focus on three main specific challenges:

2.5.1. Characterizing the fungal pathogens responsible for citrus root rot

Soil samples and diseases citrus trees were collected and analyzed in the laboratory by using dilution methods to look for fungal pathogens and by using bait traps to isolate *Phytophthora* species (Figure 11). Indeed, isolation was also done from disease plant organs based on surface-disinfection technique. Molecular based technique was applied to identify *Phytophthora* species using both specific ITS genes primers and COX primers. To ensure the aggressiveness of fungal isolates, the pathogenicity was performed during preliminary trials on citrus seedlings under greenhouse conditions. For this trial, pot experiments were arranged in completely randomized design.

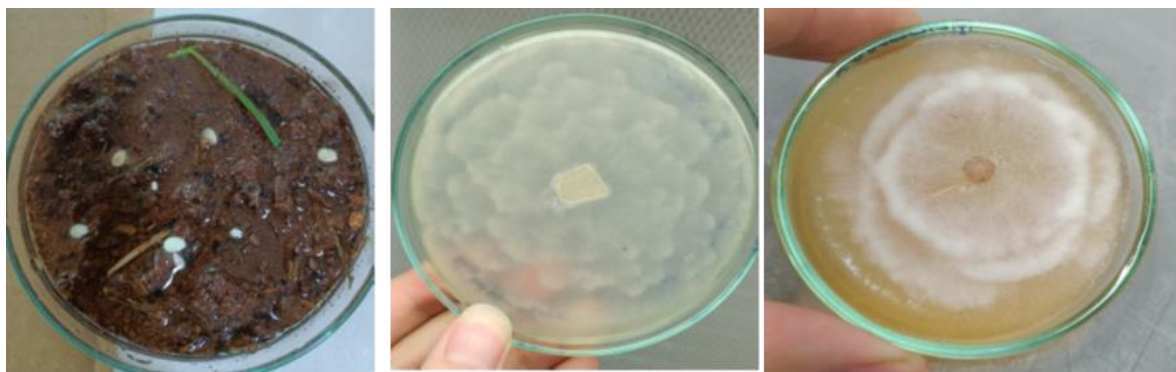


Figure 11. Isolation of *Phytophthora* species

2.5.2. Beneficial bacteria to enhance citrus soil fertility and control main pathogens

The potential of bacteria to minimize the severity of citrus root rot disease was tested on one-year plants under greenhouse conditions. A pure 24-hour culture of each isolate was used to prepare the bacterial inoculum. For three days, the cultures were placed, under shaking at 100 rpm, in flasks containing PBS. The resulting suspension concentration was adjusted to 1×10^9 CFU/mL (OD 600 = 0.8–1) using sterile distilled water. The citrus seedlings were inoculated with the oomycete pathogen. The inoculum consisted of a culture of the pathogen aged 21 days grown in the dark at 25 ± 1 °C in pots filled with a sterilized medium made of 50 mL of V8 juice with 50 g of wheat seeds. Once the inoculum was prepared, the plants were removed carefully from their substrate, cleaned from soil debris, and washed with sterile distilled water (SDW). Subsequently, 10 g of the fungal inoculum was distributed around the root system of the citrus seedlings and then covered with sterile soil. Afterwards, the bacterial isolates were poured by watering the plants with 200 mL of bacterial suspension (2×10^8 CFU/g). Citrus plants were kept in flooded soil for 24 h. The infected seedlings were maintained in a greenhouse at 25 °C. Plants were irrigated 2–3 times per week. The in vivo assay was done as described in Table 1. The trial was repeated twice over time, and plants were organized in a randomized block with 6 repetitions per bacteria. After two months, the efficacy of bacterial treatment on disease symptom reduction was evaluated. A 1–5 scale was then adopted to visually estimate the severity on plant roots: 1 = healthy white roots/no disease observed; 2 = 25% root rot or seemingly healthy roots + onset of root rots, 3 = 50% root rot and early browning, 4 = 75% root rot (browning of root system), and 5 = 100% dead roots.

2.5.3. Varietal resistance: 4 rootstocks vs *Phytophthora* “3C5”

Varietal resistance is the ability of a plant variety to resist infection or damage from a specific pathogen or pest. In the case of citrus root rot caused by *Phytophthora*, some citrus varieties may have a higher degree of resistance than others. Research studies have shown that some citrus rootstock varieties such as *Citrus macrophylla* and *Citrus maxima* have a higher level of resistance to *Phytophthora* root rot than others such as *Citrus sinensis* and *Poncirus trifoliata*. It is important to note that resistance to *Phytophthora* root rot can vary depending on the specific pathogen strain, the environmental conditions, and the cultural practices. Therefore, varietal resistance should be considered as one aspect of an overall management strategy that also includes cultural practices, chemical control, and biological control. In this part of study, five rootstocks were tested for their tolerance to *Phytophthora* diseases, in particular C35, Carrizo, Sour orange (*C. aurantium*) and Macrophylla. The inoculation by

“3C5” was carried out on the trunk of the tree, at a height of 15 cm, after disinfection of the area with 90° alcohol, the bark is then removed with the punch and the pathogen is deposited where the wound is made. This is sealed with a strip of Para film and wrapped in black plastic and tape to prevent drying out. The reading of the results is done after 15 days. The bark of the trunk has been scraped in order to reveal the lesion [6]. The lesion diameters were evaluated 15 days post-inoculation. In addition, other morphological and physiological parameters

2.6. CATALONIA, SPAIN. REGENERATIVE PRACTICES ON RAINFED AND IRRIGATED ARABLE AGRICULTURE.

Catalonia occupies the North-eastern part of the Iberian Peninsula (Figure 12). It has a population of about 7,7 million inhabitants and occupies an area of 31.985 km². Agricultural land represents about 750,000 ha. Catalonia has many different climates, but the most prevalent ones in agricultural areas are Cfb, Csa and Cfa (Köppen classification). These climates are characterized by its low rainfall, and irrigation is needed if willing to achieve potential yield of crops. About one third of agricultural land is nowadays irrigated.



Figure 12. Location of the plots of the case study in Catalonia.

More than half of the agricultural area is devoted to arable (44%) and forage (12%) crop production. Wheat and barley, either rainfed or irrigated, are the predominant winter crops and maize is the predominant summer crop, irrigated. The main use of these crops is for animal feeding. Intensive livestock production (pig, dairy and poultry, mainly) is an important economic sector. Soil is intensively used, especially in livestock farms, and manure or derived products are used as fertilizers. This intensification has resulted in the designation of large agricultural areas as Vulnerable Zones to Nitrate pollution, soil pollution with excess nutrients and loss of soil quality.

The plots involved in this case study are located in the Empordà shire (Figure 12), which has a prevalent Csa climate, the typical temperate Mediterranean climate with hot and dry summers, with a clear minimum of precipitation in summer.

The soils of this region are formed from alluvial deposits in the valley bottom and river terraces in areas that present a xeric soil moisture regime. The soils are deep, moderately well drained, with medium or moderately coarse textures and a variable content of stones. They present little edaphic

development, as in most of the soils the fluventic characteristics can still be observed. Some of the soils can present stains and concretions formed due to redox processes. The chemical characteristics of the soil are very variable in function of the original materials. The pH is generally slightly alkaline, and the carbonate content ranges from low to very high. The dominant soil types in the case study area can be classified as Typic Xerofluvent, calcareous, messic with sandy loam textures.

The main arable and forage crops in the area are irrigated summer maize (sometimes replaced by sorghum or sunflower, instead), in the fields that can be irrigated, and rainfed winter arable crops (wheat, barley and oilseed rape are the most common ones), even if the plot is suitable for irrigation. In some cases, especially those linked to dairy farms, a two-crops per year rotation is performed (summer forage maize + winter forage crop). Farms performing different mixtures of the described rotations can also be identified.

Other crops that can be found in the area are apple, in irrigated land, and vineyard and olive and almond trees in rainfed land.

This case study focuses on three of the four pillars of innovation that form part of the SIRAM:



- **Agronomical practices to safeguard soil quality and fertility.** The efficient use of manure may increase soil organic C, contributing to the mitigation of climate change and enhancing soil quality.

- **Crop varieties with resistance towards abiotic and biotic stresses.** The use of resistant varieties will improve agronomic and economic crop performance.

- **Bioeconomy approaches to recycle and valorize waste biomasses that restore soil organic matter and have bio stimulant properties.** This study aims to improve

nutrient use efficiency from manure with the objective of reducing emissions and contributing to reducing the use of mineral fertilizers.

Two main specific challenges will be addressed in the Catalonia case study, on arable crop production.

2.6.1. Reduced tillage and dairy manure to increase soil organic carbon and rainfed crop performance.

Rainfed agriculture needs to keep as much water as possible during crop growth. Increasing soil organic C improves soil structure and, thus, water infiltration and the amount of water that a soil may keep. Manure contributes to increased C sequestration. Reducing soil tillage reduces soil organic C mineralization and water evaporation. Both contributing to the stated aim.

A long-term (> 20 yr) field experiment on rainfed winter arable crops is being continued (Figure 13), with some modifications, to contribute to the aims in this challenge. In this field experiment, dairy manure or mineral fertilizer have been long applied to different rainfed winter crop rotations. Soil tillage has been introduced as a new factor in the trial. Influence of both factors and its interaction will be measured in crops and soil characteristics: crop yield and quality, soil C sequestration and other soil quality characteristics linked to water dynamics.



Figure 13. Dairy manure and reduced tillage field experiment as seen in June 2022, drone shot.

The use of zero tillage cropping is expected to favor the increase of organic matter in the soil, improve the soil structure and favor the formation of a communicating porosity, improve infiltration and water storage and decrease the risk of erosion. Also, this method consumes less fuel, energy, and labor. But the zero tillage cropping also has its downsides as the elimination of the adventitious plants requires the use of herbicides of systemic types and the elimination of the pests that inhabit the remains of previous crops require the use of pesticides.

To mitigate some of the negative effects of zero tillage cropping, selected varieties, adapted to most incident pests and pathogens and to local climate, will be used.

2.6.2. Cover crops and reduced tillage to increase use efficiency of nutrients and enhance soil quality in irrigated arable systems.

Manure treatment (digestion to obtain biogas; solid-liquid separation; ...) is increasingly being introduced in livestock farms. Digestate and other types of manure may be further used as fertilizer, especially on arable and forage crops. Nevertheless, during the period between the main crops soil is not covered and N leaching or soil erosion may occur. Cover crops may reduce nitrate leaching and contribute to C sequestration.

One long-term (> 8 yr) field experiment on irrigated maize fertilized with different kinds of manure - and derived products- has been resized and focused on the use of different species of cover crops after maize and on reducing soil tillage (Figure 14).



Figure 14. Cover crops and reduced tillage field experiment as seen in November 2022, drone shot.

In this field experiment, three different types of fertilization on maize are tested: dairy slurry digestate and pig slurry and mineral fertilizers as standard managements. Also, three different types of cover crops, with different distributions, are being tested: Mustard, Common vetch, Ryegrass; a treatment without any cover crop is also included. The main aim of using cover crops is to test the efficacy of the different cover crops at reducing nitrate leaching and contribution to C sequestration. It has also introduced a third factor, soil tillage, with two levels: no tillage and conventional soil management. Nutrient balances, soil C sequestration and soil characteristics evolution, linked to water and nutrient dynamics, will be assessed.

2.7. SIDI BOUZID, TUNISIA. EXPERIMENTAL FARM OF OLIVE TREES AND INTENSIVE CROP PRODUCTION AREAS

Sidi Bouzid Region is located in the Center of Tunisia (Figure 15, left). The region is classified as arid with cold and humid winters and hot and dry summers. The average annual evaporation is 1.470 mm. Therefore, agriculture is primarily based on irrigation using groundwater. Soils are deep with an organic matter content ranging from 0.5 to 1.5%, being suited for the majority of the crops. Annual cultivated crops in the region include: vegetables, fodder and cereals. Tree crops mostly include olive, pistachios and almond trees.



Figure 15. Location of the plots of the Tunisia case study in Sidi Bouzid, Tunisia (left) and Experimental field of olive crop in Sidi Bouzid experimental farm (right), containing different local (*cv. chetoui*) and introduced (*cv. Arbosana* and *Arbequina*) olive varieties, as seen in January 2023.

Olive cultivation (Figure 15, right) plays a vital role in the social and economic life of Tunisia. The olive sector (olive growing plus the olive oil industry) provides a direct and indirect livelihood. Olives and olive oil constitute a significant portion of the agricultural production and exports of Tunisia. In addition, Tomato and Potato are among the most cultivated vegetable crops worldwide and in Tunisia. They are strategic crops with national economic importance.

The deficit of hydric resources, the increase in their salinity, as well as the infections by several fungal diseases (vascular wilt, Downy mildew and gray mold) are currently threatening the production of olive and vegetable crops (tomato and potato). In addition, climate change is worsening the impact of these biotic and abiotic stresses. Despite significant actions to conserve agricultural land, the pressure exerted on land and destructive farming practices result in significant erosion that affects three million hectares. Arid areas have salinity problems, and forest areas and rangelands have overexploitation of resources.

Consequently, the urgent strategic objectives in Tunisia are to improve agricultural practices, restore soil fertility, and improve access to water and rational management of water resources, and a sustainable improvement in agricultural productivity. Therefore, a new agricultural development model adapted to climate change must be promoted.

The experiments carried out within the SIRAM project contribute to innovate sustainable agricultural approaches adapted with Tunisian agriculture context. The Tunisian case study is based on the four SIRAM pillars in the context of innovative regenerative agriculture:



- **Beneficial microorganisms for plant growth and disease control.** The use of PGPR and BCAs to improve tomato, potato and olive performance and resilience to fungal diseases.

- **Agronomical practices to safeguard soil quality and fertility.** The reduced or no-tillage associated with the compost on olive field can improve soil quality, soil productivity and surface water quality.

- **Crop varieties with resistance towards abiotic and biotic stresses.** The performance of local and introduced cultivars of (tomato, potato and olive) will be tested in pot experiments towards abiotic stresses (hydric deficit and salinity) and the tested innovative agricultural approaches.

- **Bioeconomy approaches to recycle and valorize waste biomasses that restore soil organic matter and have bio stimulant properties.** The compost composition is based on crop waste residues (as olive pruning product, crop residue for vegetables) in order to valorize the waste biomasses. The effect of each compost composition will be tested on plant growth promotion impact and restoring soil properties.

Most of the agriculture parcels and activities are located at the centre of Tunisia where Sidi Bouzid occupied the important production areas. The Tunisian case study is located in the experimental farm of Sidi Bouzid followed by some planned experiments of the best-selected strategies in private farms. For this, the experimental station of the CRRA contained a pluvial parcel of (24 ha, cv Chemlali) and irrigated intensive and hyper-intensive conduct modes with a varietal collection of local and introduced olive cultivars (Figure 15). Besides, 2 ha are devoted to seasonal crop production (reserved for field experiments of tomato and potato).

The Tunisian study will focus on 2 main challenges involving the 4 SIRAM pillars.

2.7.1. The use of microbial biostimulants to enhance plant resilience towards biotic and abiotic stress

For this, field surveys to Olive and vegetable crops (tomato and potato) in several agricultural parcels in Tunisia are planned in order to collect and identify the most promising microbes (AMF, PGPB and BCAs) species/isolates in T1.1. Laboratory assays are performed to screen the most efficient and with the PGP traits. Field and greenhouse experiments are planned in T4.2. to test the effect of microbes alone or consortia to improve plant resistance, the fitness, yield and product quality of plant genotypes under abiotic stress conditions (drought, salt stress) and biotic stress (Fusarium wilt for tomato, Downy mildew and gray mold for potato and *Verticillium* wilt for Olive).

Regarding this challenge, 4 experiments are designed as follows:

- * Olive Pot experiment: **crop varieties** behavior towards **microbial biostimulants** and/or **compost** under **biotic stress** (fungal disease: *Verticillium* wilt)
- * Olive Pot experiment: **crop varieties** behavior towards **microbial biostimulants** and/or **compost** under **abiotic stress** (salinity stress)
- *Tomato and potato pot experiments: to assess the effect of **microbial biostimulants** against fungal diseases (**biotic stress**) on **different cultivars/varieties**.

2.7.2. The impact of using microbial consortia associated with some regenerative approaches

The reduction of chemical inputs, restoration and regeneration of soil health through an integrated approach based on beneficial microorganisms, organic fertilizers, biostimulants, organic waste biomasses, resistant local crops and agronomical practices based on no-tillage and cover crops is planned. For this sake, compost will be produced from the waste of each designed crop: pruning product, manure and olive pomace (Figure 16).

Some organic bio-stimulants will be tested such as olive leaf extract (OLE) will be tested, as well as orchard irrigation with olive mill waste-water. The impact of the olive mill waste-water on soil quality as well as olive tree production and olive oil quality will be assessed (T4.2). In addition, some grass strips and other no-tillage systems will be installed from April to mid-august in the olive orchards. These plants could be used as a refuge to insects, reducing the use of insecticides in olive orchards. The combination of some regenerative approaches with microbial consortia will be tested for their

impact on soil health, on olive and olive oil quality, finally tree and fruit infection by fungal diseases will be evaluated.



Figure 16. Olive pruning produces one of compost composition (left) and compost process (right) at Experimental farm of CRRRA Sidi Bouzid, Tunisia.

Field experiments are designed for this challenge:

- *Olive open field experiment during all the period of the project (3 years) to assess the long-term effect of adopted regenerative practices (**compost based on olive waste**), **microbial biostimulants**, **resistant cultivars** and **no or reduced tillage**.

- *Tomato open field experiment (February-March 2023 during 4 months): assess the effect of **microbial biostimulants** (*Trichoderma longibrachiatum*) and bacteria (*Pseudomonas sp.*) on two tomato cultivars associated with compost (**based on tomato crop residues**) under **hydric stress** (50%, 75% 100% water need).

- *Potato open field experiment (September 2023 during 4 months): the same challenges as tomato experiment.

2.8. NORTH, CENTER, AND CENTER SOUTH OF PORTUGAL, SURVEILLANCE ON POTATO-GROWING SOILS

Potato (*Solanum tuberosum*) is an important staple crop worldwide and thousands of ha of potatoes are grown every year. Potato has great social and economic importance in Portugal since it is grown throughout the country. The most representative production regions are the North and West Regions, with a total potato growing area of approximately 20,000 hectares and a total production of 430,000 tons. Potatoes can be planted/seeded at any time of the year in Portugal, weather permitting and choosing the variety best suited to the environmental conditions, but in general, four sowings are generally possible. They can be planted from February, after the frosts, in the Center and South and from March to the end of April and from mid-April to the end of May, in the North. The sowing depth is 8-15 cm, depending on the type of soil, climate and type of irrigation. Portugal is facing an increasing lower potato yield average comparatively to European Union and one of the limiting factors may be attributed to plant-parasitic nematodes (PPN), such as potato cyst nematodes (PCN), *Globodera* spp., and root knot nematodes (RKN), *Meloidogyne* spp., a serious problem in potato growing areas. These PPN are among the most economically important nematodes in agriculture, being potato the main host for PCN while RKN can cause significant losses in potato and many other crops in both tropical and temperate climates, depending upon the species. To control PPN, responsible for low yields and poor quality of crops, new efficient bio-tools are needed, as alternatives to synthetic products. The microbiome of potato fields will be studied to identify the core microbiome of healthy soils (without PPN) and diseased soils (with PPN), and predict their functionality to a healthy soil.

The evaluation of microbiomes and the identification of nematodes was carried out before and after potato cultivations. In total, 45 fields from North, Center and South Portugal from the representing areas producing potato were evaluated (Figure 17). Four hundred and fifty soil samples (45 fields x 5 composed samples by 5 independent samples x 2 time points) were collected, before and after potato in fields. The soil was chemically characterized, the microbiome is being assessed, and nematodes were identified (summarized in Table 2).

The total area of the fields sampled was 43 ha. The most common potato varieties used are Agria and Picasso. There are also some farmers that use Kennebec and Stemster but the rest depends on many factors like if it is production for Industry or only to consume, etc. Many farmers do crop rotation, mainly with corn in the North and Center and with cabbage in the South. Some use fallow between potato crops. All use fertilizer, mainly organic, insecticides due to the potato beetle, fungicides because of the mildew during the rainy Winters and herbicides because the climate is warm and it is quite difficult to control weeds without chemicals.



Figure 17. Sampling sites (A) and potato fields (B, C) sampled in Portugal.

Although Portugal has a coastline that faces the Atlantic Ocean, its climate is very much like that of most Mediterranean countries. This means it's hot in the summer and temperate in the winter. In the north, the climate is cool and rainy, while moving south it becomes gradually warmer and sunnier; in the far south, the region of Algarve has a dry and sunny microclimate. In summer, Portugal is protected by the Azores High, so it's usually sunny everywhere, however, a few weather fronts can still affect the northern part of the country. In the rest of the year, and especially from November to March, rainfall can occur, especially in the north where it is more frequent and abundant. Therefore, the landscape in the north is definitely green, while it gradually becomes more arid towards the south, down to Algarve, which has a fairly dry climate. The rainiest season is winter although there are periods of good weather when the Azores Anticyclone settles in the country, but there are also waves of bad weather, with rain and wind. The position of the country, overlooking the ocean, provides good shelter from cold winds and night frosts, which, in fact, are very rare and not intense, especially on the coast, where the records are a few degrees below freezing in the north and around 0 °C in the south. Summer, from June to mid-September, is sunny, and it's mild or even cool on the northern coast and warm in the center and south. South/Center. Heat is more intense in the interior, especially in the south/central, in the plains and in valleys at low altitudes. In the interior, on the border with Spain, the climate is a bit more continental.

Considering the soil characteristics, the pH varied from 4.8 till 7.9 reflecting the fertilization added. Most of the soils had a pH around 6.5. The content in organic matter varied from very low to high. The type of soil is being analysed and the rest of the information like, for example, nitrate content were also determined (Table 2).

From the 45 fields sampled, in 7 of them it was detected PCN. Five populations were identified as *G. rostochiensis* and two as *G. pallida* by PCR-RFLPs. There was also the presence of *Heterodera* spp. Mainly in the fields where the rotation was done with cabbage, probably *H. cruciferae*. There was also the presence of *Pratylenchus* spp. and *Helicotylenchus* spp. that were not identified to the species level. Only one *Meloidogyne* population was detected and was identified, by the esterase phenotypes, as *M. hapla*.

The bacteria were identified and its characterization was reported in deliverables D1.1 and D1.2. Moreover, these bacteria are being tested against *M. incognita* and *M. hapla*, two of the most important RKN in the world, in *in vitro* assays to assess their effects on the nematodes' mortality. The best will be chosen to do the greenhouse pot experiments.

One of the challenges of the SIRAM is to involve soil management, pest control, crop selection and plant-microbe interactions. All these 3 challenges are represented in CS8. The relation between soil type, presence/absence of PPN, resistant potato varieties and the use of microbiome as bio-tools are included in two of the SIRAM pillars:

This case study focuses on two of the four pillars of innovation that form part of the SIRAM:



· **Beneficial microorganisms for plant growth and pest control**

Development of bio-tools using bacteria for biological nematode control as biological control agents (BCAs). The proposed solutions developed will make them potentially suitable to be used for integrated pest management.

· **Crop varieties with resistance towards biotic stresses.** The use of resistant varieties will improve agronomic and economic crop performance.

As proposed, the assays will compare the effects of the resistant cultivars; the bio-tools alone and the cultivars together with the bio-tools. It is expected that the effects of the resistant cultivars together with the bio-tools will become a new methodology to control nematodes that is economically and environmentally friendly.

Finally, biological control by bacteria and resistant potato varieties in soil contaminated with PPN will be tested in pot assays.

There are two specific challenges in CS8.

2.8.1. Bio-tools against PPN

To find bacteria (bio-tools) that can be used in potato crops against a wide range of PPN that causes huge damage to this crop worldwide.

- * Sampling and bacteria isolation.
- * Bacteria identification.
- * Evaluation of the isolated bacteria effects on nematode mortality.

2.8.2. Resistant cultivars against PPN

To improve the characteristics of potato cultivars, through the use of bio-tools, that are already resistant to PPN in order to give more powerful tools that will improve economical inputs and more satisfaction for all stakeholders involved.

- * Pot assays with the bacteria x resistant cultivars against PPN.

Table 2. Summary of potato fields analyzed in Portugal. Bacterial and fungal microbiomes were analyzed in all fields.

	Field					Microbes	Nematodes			Biochemical characterization of soil									
Potato field	GPS coordinates	Potato Variety	Previous year crop	Management (conventional, organic, biodynamic or biodynamic+ organic)	Organic management	Bacterial isolates	Identification	Other nematodes	Fine sand (%)	Organic Matter (%)	pH	Conductivity (mS cm ⁻¹)	Extractable phosphorus (mg kg ⁻¹)	Extractable Potassium (mg kg ⁻¹)	CaO (mg kg ⁻¹)	MgO (mg kg ⁻¹)	Nitric nitrogen (mg kg ⁻¹)	Ammoniacal nitrogen (mg kg ⁻¹)	
field_1	40.305465,-8.470960	Agria, Stemster	Corn	Organic	Manure	no	_____	<i>Pratylenchus</i> spp.- 2 Q	78.77	2.7	6.4	0.16	19	61	93	11	24.45	69.6	
field_2	40.304988,-8.453558	Desiree	Turnip	Organic	Manure	yes	<i>G. rostochiensis</i>	<i>Pratylenchus</i> spp.- 1 Q	78.51	4.61	5.6	0.75	30	191	396	50	125.9	70.93	
field_3	40.621806,-8.660591	Picasso, Stemster	Turnip	Conventional	No	no	<i>Heterodera</i> spp.	<i>Pratylenchus</i> spp.- 7Q and 1J2	94.18	1.57	6	0.1	21	24	64	9	13.16	36.73	
field_4	40.437051,-8.566699	Picasso, Agria, Stemster, Bellarosa	Corn, potato, pumpkin	Organic	Organic fertilizer	no	_____	<i>s</i> spp. 3Q and 1 male; <i>Helicotylenchus</i>	80.1	5.19	5.2	0.76	9	216	334	52	194.84	289.36	
field_5	40.436422,-8.566661	Picasso, Agria, Stemster, Bellarosa	Corn, potato, pumpkin	Organic	Organic fertilizer	no	_____	ND	76.08	3.8	6.3	0.15	46	107	79	9	22.02	47.46	
field_6	40.305537,-8.472508	Agria	NA	Conventional	no	no	<i>Heterodera</i> spp.	ND	91.33	2.14	5.9	1.05	45	195	705	58	196.27	64.64	
field_7	41.321870,-8.700680	Red light, Agria, Yona	Corn	Conventional	no	no	_____	<i>Pratylenchus</i> spp.- 4Q	90.69	4.59	5.2	1.04	1	188	250	29	132.39	532.25	
field_8	41.322030,-8.698454	Jelly	Corn	Conventional	no	yes	<i>G. pallida</i>	<i>tylenchus</i> spp.-8Q, 3 males and	82.74	3.26	5.2	0.58	10	155	93	10	74.9	932.87	
field_9	41.346891,-8.629142	Stemster, Agria	NA	Conventional	no	no	_____	ND	81.11	4.4	6	0.22	13	56	67	14	47.13	46.48	
field_10	41.508228,-8.674528	Agria	Corn, cereals	Organic	Manure	no	_____	<i>chus</i> spp. 3Q and <i>Helicotylenchus</i>	73.26	3.29	5.8	0.29	1	46	144	23	81.53	42.68	
field_11	41.493272,-8.771551	Donata	Carrot, garlic, others	Conventional	no	no	_____	<i>chus</i> spp. 3Q and <i>Helicotylenchus</i>	96.4	0.93	7.4	0.97	16	49	135	29	121.72	36.66	
field_12	41.420487,-8.555869	Picasso, Agria	potato	Organic	Slurry	no	_____	<i>s</i> 3Q, 2J2 and 5 males; <i>Xiphinema</i>	66.26	4.62	4.8	0.41	1	150	124	35	108.13	361.95	
field_13	41.441413,-8.593741	Cara (olho de perdiz), kennebec	water melon, onions	Organic	Manure	no	_____	1 J2 and 2 males; <i>Pratylenchus</i> s	78.39	3	5.1	0.26	7	69	89	12	49.57	135.99	
field_14	41.437518,-8.604059	Kennebec	horticultures	Organic	Manure	no	<i>G. rostochiensis</i>	<i>s</i> spp.- 5Q and 4J2 and <i>Tylenchus</i>	79.61	5.26	6.3	0.37	24	56	131	18	73.57	62.4	
field_15	40.998343,-8.474410	Kennebec	horticultures	Organic	Manure	no	_____	12 J2 and 30 males; <i>Helicotylenchus</i>	83.34	3.12	5.2	0.38	3	107	162	17	58.89	827.24	
field_16	40.991503,-8.465817	Kennebec	bean	Organic	Manure	no	<i>ra</i> spp. <i>Heterodera</i>	pp. 3Q, 3 J2 and 2 males; <i>Rotylenchus</i>	77.28	4.79	6.3	0.19	1	46	88	8	38.34	44.25	
field_17	40.157119,-8.203679	Monalisa, Picasso, Cara	Corn, bean, peper	Organic	Manure	no	_____	2; <i>Rotylenchus</i> spp. - 1Q and 3J2	80.54	4.1	6.3	0.21	79	143	103	19	36.08	70.94	
field_18	40.151947,-8.245734	Kennebec, Spunta, Desire, Stemster	fruit trees	Conventional	no	no	_____	<i>tylenchus</i> spp. 2 J2; <i>Tylencaorynchu</i>	74.01	3	6	0.1	13	44	53	6	11.07	47.05	
field_19	40.134559,-8.259500	Agria	turnips and cabbages	Conventional	no	no	_____	3 males and 4J2; <i>Rotylenchus</i> spp.	81.77	2.28	5.2	0.61	3	149	132	18	83.37	1847.68	
field_20	39.265315,-9.171722	Picasso	Cabbage	Organic	Organic fertilizer	no	_____	<i>tylenchus</i> spp. 8 Q, 1 male and 1	85.75	0.51	7.6	0.57	10	125	303	31	62.64	46.7	
field_21	39.275148,-9.172316	Picasso	Cabbage	Organic	Organic fertilizer	yes	<i>G. rostochiensis</i>	<i>Pratylenchus</i> spp.- 12 Q and 7 J2	100	0.2	5.7	0.47	12	113	142	48	64.87	255.21	
field_22	39.286521,-9.208292	Picasso	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	100	0.76	7.5	0.26	6	35	188	18	16.23	50.76	
field_23	39.286072,-9.209905	Bricata, Mozart	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	<i>Pratylenchus</i> spp. - 2 Q	100	0.26	7.6	0.36	9	30	121	17	36.68	40.53	
field_24	39.268122,-9.221676	Agria, Donata	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	88.16	0.61	6.5	0.21	15	43	124	15	43.5	54.26	
field_25	39.275133,-9.230064	Agria	Cabbage	Organic	Organic fertilizer	no	<i>G.rostochiensis</i>	<i>Pratylenchus</i> spp. - 1Q	93.61	0.4	6.4	0.44	29	55	188	18	67.09	51.85	
field_26	39.277256,-9.251873	Agria	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	92.38	0.58	7.5	0.47	24	48	212	22	53.61	33.44	
field_27	39.272188,-9.236129	Agria	Cabbage	Organic	Organic fertilizer	yes	<i>G. rostochiensis</i>	ND	91.73	0.65	6.5	0.32	24	64	145	20	46.15	99.89	
field_28	39.274232,-9.250132	Rosi	Cabbage	Organic	Organic fertilizer	no	_____	<i>Pratylenchus</i> spp. 7Q and 3 J2	92.12	1.08	6.6	0.29	16	93	152	20	75.64	55.44	
field_29	39.271719,-9.259709	Picasso	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	100	1	7	0.14	21	51	88	16	5.95	37.39	
field_30	39.286097,-9.281706	Agria	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	88.3	0.76	7.8	0.14	15	36	108	60	6.1	25.81	
field_31	39.289538,-9.281702	Bricata	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	78.4	1.17	7.9	0.65	12	101	259	56	23.99	83.46	
field_32	39.294761,-9.280535	Bricata	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	<i>s</i> spp. (females, males and J2 no	100	0.12	7.4	0.38	35	87	162	5	24.87	33.74	
field_33	39.303218,-9.270547	Agria	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	<i>Pratylenchus</i> spp. 14Q and 9 J2	84.79	1	7.8	0.14	22	98	119	31	9	35.11	
field_34	39.216957,-9.317382	Picasso, Jelly	Cabbage	Organic	Organic fertilizer	no	<i>Heterodera</i> spp.	ND	84.78	0.04	7.9	0.38	7	20	119	33	53.27	35.99	
field_35	41.703792,-8.739828	Paradiso, Agria	None	Conventional	Synthetic	no	<i>G. rostochiensis</i>	<i>tylenchus</i> spp. 24Q, 1 male and	77.85	3.9	5.4	0.54	5	122	139	3	53.01	25.92	
field_36	41.475981,-8.621115	Picasso	Ryegrass	Organic	Organic fertilizer	no	_____	Many rabditide	73.15	3.96	5.9	2.42	8	600	802	133	305.53	59.22	
field_37	41.389564,-8.491395	Dayfla	None	Organic	Chorume	no	_____	<i>tylenchus</i> spp. 25Q, 7 males and	88.65	3.61	5.2	0.24	15	91	57	1	26.25	14.43	
field_38	41.470272,-8.503837	Picasso, Paradiso	vegetables	Organic	Manure	no	<i>Heterodera</i> spp.	Many rabditide	57.7	5.15	5.6	1.29	3	240	362	30	205.34	35.32	
field_39	41.389564,-8.491395	Stemster	None	Organic	Chorume	no	<i>Glabodera</i> spp.	spp. 2I2 and <i>Pratylenchus</i> spp. 5	85.46	2.9	5.1	0.38	10	103	71	2	44.47	11.16	
field_40	40.217786,-8.513862	Taurus	Corn, cabbage	Organic	Organic Fertilizer	no	_____	pp. and <i>Pratylenchus</i> spp. 14Q, 1	79.95	1.01	6.8	0.44	<1	40	208	2	23.86	46.41	
field_41	40.219840,-8.506177	Picasso	Corn, cabbage	Organic	Organic Fertilizer	no	_____	(many) and <i>Pratylenchus</i> spp. 2	81.31	1.15	5.5	0.43	1	61	116	2	23.77	14.01	
field_42	40.236008,-8.471329	Taurus	Corn, broccoli	Conventional	Synthetic	no	_____	<i>Pratylenchus</i> spp. 5 Q and 4 J2	78.05	3.21	7.4	0.93	7	50	370	3	108.72	11.48	
field_43	40.235134,-8.453890	x	None	Conventional	Synthetic	yes	<i>G. pallida</i>	<i>s</i> spp. and <i>Pratylenchus</i> spp. 13	76.35	1.5	6.4	0.27	6	55	99	2	20.78	14.22	
field_44	40.223321,-8.475313	Agria	spinach	Conventional	Synthetic	no	_____	ND	86.27	1.89	7.5	0.88	5	124	295	2	42.37	17.87	
field_45	40.222997,-8.477104	Jelly, Donata	broccoli	Conventional	Synthetic	no	_____	ND	84.43	2.28	6.6	1.01	2	240	256	4	63.92	18.72	

3. FINAL REMARKS

The case studies (CSs) in the SIRAM project involve research and demonstration in different Mediterranean soil, climate, crops and agricultural systems conditions. As geographically spread along the Mediterranean basin, different climate and agricultural soils are represented in the CSs. They also involve a wide variety of crops: arable and forage crops, olive and citrus orchards and potato and tomato horticultural crops.

Different aspects of agricultural management are also studied in the eight CSs. In general management, conventional, organic and biodynamic systems are considered. Reducing soil labour, types of manure or biomasses added, cover crops when main crops are not in the field and use of resistant varieties, to biotic and abiotic stresses, are also taken into account. Different of these practices are also focused in increasing soil organic carbon that would help to prevent erosion events, increase soil water holding capacity and related soil characteristics.

There is an important charge of work planned on selecting microbial species able to overcome different problems presently affecting different crops. Biological control of root and aboveground diseases, improving tolerance of crops to drought or reducing water needs are studied.

The CSs sites, fields and trials will be used in practical parts in the formation and divulgation workshops and courses planned in WP6. They will also be used as living-labs and lighthouses in these divulgation activities.

