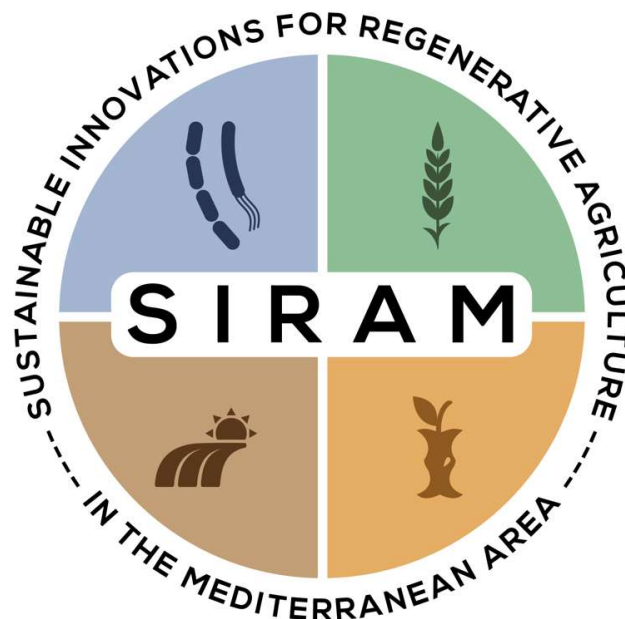




D1.3. REPORT ON THE SELECTED BIOMASSES DETAILING THEIR AVAILABILITY, COSTS, AGRONOMICAL AND MICROBIOLOGICAL CHARACTERISTICS

Lead Beneficiary: Agricultural Research Centre (ARC)

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Date: 07/12/2022



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DOCUMENT INFORMATION

Grant Agreement	
Project title	Sustainable innovations for Regenerative Agriculture in the Mediterranean area
Project acronym	SIRAM
Project coordinator	Edoardo Puglisi, UCSC
Project duration	1 June 2022 – 31 May 2025 (36 months)
Related work packages	WP 1 – Screening and selection of beneficial microorganisms, recyclable biomasses and resistant varieties
Related task(s)	Task 1.2 Biomasses.
Lead organisation	ARC
Contributing partner(s)	ARC, CRRA, UCSC, IRTA
Due date	1 June 2023
Submission date	7 December 2022
Dissemination level	Confidential, only for members of the Consortium including the Commission Services

EXECUTIVE SUMMARY

The present Deliverable (D1.3) contains the results obtained in the context of Task 1.2 (T1.2. Biomasses). In particular, in Table 1 it lists the local resources selected for application in SIRAM case studies with their available volumes and agronomic properties (nutrient loads such as N, P, K and pH). Moreover, details on origin and preparation of biomasses are given, in order to describe their contribution to circular economy concept applied in SIRAM. Methods used for characterization of selected biomasses are listed in Section 2. Finally, further steps necessary in Task 1.2 are detailed.

1. BIOMASSES FOR APPLICATION IN THE PROJECT CASE STUDIES

As described in the project proposal, Case Studies 1, 2, 4, 6, 7 and 8 foresee application of biomasses. CSs 4 and 8 will focus both on potato as target crop. Partners involved in these CSs (ARC and UC) agreed to consider biomasses contribution to sustainable cultivation and biocontrol of disease affecting potatoes only in CS4, while in CS8 beneficial microbes (as described in D1.2) and resistant varieties will be applied. Table 1 reports the organic biomasses chosen for application in these Case Studies, detailing their chemical and agronomical properties.

In particular:

- CS1 will apply (both at the level of pot and field experiment) the solid fraction of manure (after solid-liquid separation) deriving from a dairy cattle farm. The solid fraction of manure will also be tested as co-formulate for application of beneficial microorganisms in CS1 to unravel the most effective and promising approach for maximizing positive interactions between manure and microorganisms.
- CS2 will apply cover crop residues as green manure. A wide range of cover crop species (as monocultures and mixtures) will be tested at the field level to select the most effective and promising approach for enhancing soil carbon and nutrient cycling.
- CS4 will apply compost prepared with equal layers from i) livestock slurries, ii) potato crop residues, iii) citrus and grapes tree pruning wastes, and soil. Layers were laid at the top of the other, with agricultural sulfur in between, and allowed to decompose for three months. The temperature was monitored regularly to check the process of completion of the decomposition. Physico-chemical properties of representative samples of compost, like porosity, moisture content, water holding capacity, Nitrogen, phosphorus and K concentration, Carbon and organic matter content, were checked. 25 Kg /tree of this compost will be applied in citrus farm in An Nubariyah region, Egypt. This compost will also be used as co-formulate for application of beneficial microorganisms described in D1.2.
- In CS6, solid dairy manure, digestate from biogas production and cover crops will be applied or included in field trials. Dairy manure is available from commercial farms; raw dairy manure (not processed) is usually applied. Digestate comes from a biogas plant based on dairy manure and external organic materials from food industry, for biogas production; the effluent from the reactor is separated in solid and liquid fractions; digestates applied correspond to the liquid fraction from the effluent. Cover crops are biomasses incorporated into the soil as green manure after being grown in the field; different species (mainly Gramineae, Cruciferae and Leguminosae) are used.
- CS7 will apply compost (both in pot and field experiments) prepared with equal layers consisting in i) livestock slurries, ii) crop residues. Residues are from tomato and potato and include olive pomace and tree pruning wastes, too. The Compost was prepared in a pilot-plant using the turning-pile system in trapezoidal piles (8 m length,

1.5 m width on the base and 1 m height) containing approximately 5000 kg in each pile. The piles were turned every two weeks during the bio-oxidative phase. Temperature and moisture were used as monitoring parameters to follow the composting progress. Water was added during turning to keep the moisture levels in the range between 40% and 60%. The compost will be used also as co-formulate for the application of beneficial microorganisms (selected in T1.1) to assess the best efficient tool to improve plant resilience to abiotic (salinity and drought) and biotic (fungal diseases) stresses due to the interaction between interacting beneficial microorganisms and solid biomass.

Table 1: Agronomic properties of biomasses used in all case studied

Partner	CS	Biomass	Physico-chemical properties	
UCSC	CS1	Manure (solid fraction after separation-dry matter)	pH	7.5-8.5
			N content (%)	1.5-3.5
			P content (%)	0.5-0.7
			K content (%)	0.8-0.9
			C/N ratio	15-30
			Organic content (%)	82-86
			Humidity (%)	75-85
			Organic carbon (%)	47-50
			Microbial count on agar plate (CFU/g)	Bacteria: 2E10^8 Fungi and yeasts: 2E10^4
	CS2	Cover crops (dry matter)	N content (%)	1-5
C content (%)			37-44	
biomass production			3-6 Mg ha-1	
ARC	CS4	Compost	pH	7.47
			N content (%)	1.8
			P content (%)	0.31
			K content (%)	0.43
			C/N ratio	1:16
			Organic content (%)	14.0
			Humidity (%)	32
			Organic carbon (%)	8.12
			EC (dS/m)	0.64
			biomass production	960 kg
CRR A	CS7	Compost	pH	7.6
			N content (%)	1.6
			P content (%)	0.25
			K content (%)	0.47
			C/N ratio	18.40

IRTA	CS6		Organic content (%)	2.7
			Humidity (%)	15-19
			EC (dS/m)	40-60
		Dairy manure	pH	8.5-9.5
			N content (%)	1.5-3.5
			P content (%)	0.5-1.5
			K content (%)	1.5-3.5
			C/N ratio	9-12
			Organic content (%)	25-50
			Humidity (%)	60-75
			EC (dS/m)	3.5-5.5
		Digestate	pH	8.5-9
			N content (%)	9-11
			P content (%)	2.5-3
			K content (%)	1.5-3
			C/N ratio	6.5-9.5
			Organic content (%)	68-72
			Humidity (%)	4-5
			EC (dS/m)	93-95
		Cover crops (dry matter): Gramineae (ryegrass, oat)	N content (%)	1-2
			P and K content (%)	1-2
			biomass production	1-4 Mg/ha
		Cover crops (dry matter): Cruciferae and Leguminosae (forage rapeseed, mustard, vetch)	N content (%)	2-4
			P and K content (%)	1-3
			biomass production	2-5 Mg/ha

In general, the selected biomasses allow to develop a circular economy system in farms by

- recycling waste products and biomasses available locally and boost their potential as soil amendments (CS1, CS4, CS6, ...)
- minimizing N losses and reducing leaching of N to groundwater thanks to fertilization strategies based on cover crops and adequate rates of manure (CS2, CS6).
- increase N pools available to crops thanks to the introduction of legume cultivation as cover crop monocultures or mixtures.

These are the foreseen costs and availability for the selected biomasses:

Biomass	Case study	Foreseen costs	Availability
Manure	1, 6	No cost. Manure is a by- product of dairy farming system.	Widely available. The dairy sector is one of the main livestock ones in the regions involved.
Digestate	6	No cost itself. Transport cost from the plant to the field, dependant on distance. Digestate is a by-product of biogas plants.	Limited to the presence of biogas plants; there is an increasing number of these manure treatment plants.
Cover crops	2, 6	50-300 € ha ⁻¹ , according to the species monoculture/mixture selected	As the cover crops are incorporated into the soil in the same field/plot where produced, availability is completely linked to the implantation.
Compost	4	300-400 € according to transport cost for manure to the farm	Widely available in the regions involved.

2. METHODS FOR BIOMASS PRODUCTION AND CHARACTERIZATION

Chemical characterization of biomasses has followed standard official methods in each country. The following is a general description of methods.

NITROGEN CONTENT

The total **nitrogen** supplying ability of the compost was determined by distilling compost with an alkaline potassium permanganate solution. During the distillation the amino-N hydrolyzed nitrogen liberated as ammonia was measured. This serves as an index of the nitrogen status of soil. Kjeldahl method was adopted to measure total N content of soil samples (Kjeldahl 1883; Bremner 1996). In 1000 mL flask (Kjeldahl flask), 20 g of compost was taken and added to 20 mL distilled water. Then 0.32% potassium permanganate (100 mL) and 100 mL of 25% NaOH solution were mixed and immediately deposited it to kjeldahl assembly. The contents were distilled in a kjeldahl at a steady rate and liberated ammonia was collected in an Erlenmeyer flask (250 mL), containing 20 mL of 2% boric acid solution with methyl red and bromocresol green indicator. With the absorption of ammonia, the

pinkish colour turns to green. After 30 min, it was titrated with 0.02 N H₂SO₄ till the colour changed from green to original shades (pink). Blank (without compost) was run simultaneously. The carbon and nitrogen ratio of compost mass (C/N) was calculated according to Zhang et al. (2011).

PHOSPHORUS CONTENT

2.5 g of compost were mixed with 20 mL sodium bicarbonate solution (2%) and a spoon of charcoal was shaken for 30 min. Then, samples were filtered and 5 mL of this aliquot was mixed with 1 mL composite reagent (5 mL of 3% ammonium molybdate, 12.5 mL of concentrated sulfuric acid, 5 mL of ascorbic acid and 25 mL of potassium antimony tartrate). For the control, the distilled water was taken in place of aliquot. After 5 min, blue color was developed. The optical density was measured by UV Spectrophotometer at 660 nm wavelength (Olsen et al. 1954).

K CONTENT

Compost available potassium was measured by chemical extraction method. 10 g of compost were shaken on an electrical shaker with 40 mL 2% ammonium acetate for one hour and filtered immediately through dry filter paper. The volume of the filtrate was maintained up to 40 mL by adding distilled water. Potassium concentration in the extract was determined with the help of a flame photometer. After standardization, calibration was done by solutions of different potassium concentrations (10, 15 and 40 ppm, Van Rast et al. 1999).

ORGANIC CONTENT

The compost **organic carbon (OC)** was measured using the Walkley Black method. 0.1 g of compost was mixed with 10 ml 1 N K₂Cr₂O₇ and 20 mL of H₂SO₄. 5 ml of concentrated orthophosphoric acid and 25 mL distilled water was added and allowed to cool. Then two drops of diphenyl amine indicator were added. The contents were titrated with 0.5 N ferrous ammonium sulfate. Finally, the solution turned green represents the percentage of **organic matter** in a compost sample. A blank without compost is run by the same procedure. For calculating organic matter (OM), the Van Bemmelen factor of 1.724 is used because organic matter contains 58% of carbon ($OM = OC \times 1.724$) (Petard 1993).

EC

A 20-g compost sample was mixed with 50 mL distilled water for 20 min. The solution was used for measurement of electrical conductivity (EC). The conductivity of the compost supernatant liquid was determined with the help of salt (KCl) using Equiptronic's digital electrical conductivity bridge (Consort C1010). A 0.01 M KCl solution was used for standardization of the conductivity meter.

COMPOST PRODUCTION (CS4)

The fermented biofertilizer was produced as described by Restrepo Rivera (2007), with modifications. All raw materials originate from organic farming. The raw materials were mixed in water at 25-30 °C in a 220 l food container. After the addition of each raw material, warm water was added, by adding about 15 liters at a time up to a final volume of 200 litres and the suspension was carefully mixed.

The quantity and order in which the raw materials were added are described below.

Starter preparation (about 7 liter)

Order	Raw material	Dose per 5 l of water
1	Sucrose	500 g
2	Brewer's yeast	150 g
3	Fresh horse manure	1 kg
4	Sucrose	500 g

Biofertilizer preparation

Order	Raw material	Dose per 200 l final volume
1	Fresh horse manure	35 kg
2	Grape marc	3 kg
3	Sucrose	1 kg
4	Wood ash	5 kg
5	Zeolite	2 kg
6	Laying of red wine	5 l
7	Brewer's yeast	150 g
8	Unfiltered freshly milked	2 l

	milk	
9	Starter	About 7 l
10	Sucrose	2 kg

After adding and mixing well all the raw materials, the container was closed tightly. A tube was placed on the lid of the container for venting the fermentation gases. The container must be placed outdoors or in a large greenhouse, since the fermentation process involves methane production which can be dangerous in a close environment.

3. CONCLUSIONS AND NEXT STEPS

Compost, manure, dry cover crops will be applied in 5 SIRAM CSs to implement circular economy concept by recycling waste biomasses and reducing nutrient losses in the farm system. These biomasses have already been characterized in terms of chemical and agronomical properties.

Selected biomasses should be characterized also in terms of risk assessment, thus further analyses should be performed to evaluate presence of pesticides residues or detrimental microorganisms, metal content, in compliance with EU regulations.

As explained above, biomasses applied in CSs 1, 4 and 7 will be also tested for their capabilities to adsorb and retain microbial cells as carriers. Vital stain assays will be used to monitor the viability of microbial suspensions both immediately after their incorporation onto the carrier and during their use over several weeks under various storage conditions. These activities will be performed

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