

WP2. SOIL DIVERSITY AND FERTILITY SERVICES FROM SOWN DIVERSITY IN GRAZED SYSTEMS

Soil sampling t1: autumn 2022

Sampling Design: 27 selected plots x 2 grazing treatments = 54 samples

- Prepare the shipment arrangements and contact the two different laboratories receiving soils samples: HU (Liliane Ruess) and CREAM (Angela Ribas), to arrange shipment prior to sampling.
- **Note that in the autumn 2022, we do not send samples to France for DNA analysis,** only prepare soils for nematodes (HU, Germany), PLFAs (HU, Germany), soil fertility and activity (CREAM, Spain).
- Plan the **soil sampling before the harvest and after the sites have seen some rain ahead of sampling. This is especially relevant for the nematodes.**
- Prepare a cooler with ice cubes or equivalent to store the soils sampled cooled IN THE FIELD AND DURING TRANSPORT TO YOUR LABORATORY.
- It is recommended to send the parcels on Monday to avoid unknown conditions at the carrier during the weekend.
- Soil sampling will be only performed in a selection of **27 selected compositions** that include monocultures of species 1 and 2, four species mixtures, and 6 species mixtures (Table 1).

Table 1. Selected compositions.

Composition	Mixture	Diversity	G1	G2	G3	L1	L2	L3	F1	F2	F3
1	Monoculture	Monoculture	1	0	0	0	0	0	0	0	0
2	Monoculture	Monoculture	0	1	0	0	0	0	0	0	0
4	Monoculture	Monoculture	0	0	0	1	0	0	0	0	0
5	Monoculture	Monoculture	0	0	0	0	1	0	0	0	0
7	Monoculture	Monoculture	0	0	0	0	0	0	1	0	0
8	Monoculture	Monoculture	0	0	0	0	0	0	0	1	0
18	4-sp mixture	Dominance	0.7	0.1	0	0.1	0.1	0	0	0	0
19	4-sp mixture	Dominance	0.1	0.7	0	0.1	0.1	0	0	0	0
20	4-sp mixture	Dominance	0.1	0.1	0	0.7	0.1	0	0	0	0
21	4-sp mixture	Dominance	0.1	0.1	0	0.1	0.7	0	0	0	0
22	4-sp mixture	Dominance	0.7	0.1	0	0	0	0	0.1	0.1	0
23	4-sp mixture	Dominance	0.1	0.7	0	0	0	0	0.1	0.1	0
24	4-sp mixture	Dominance	0.1	0.1	0	0	0	0	0.7	0.1	0
25	4-sp mixture	Dominance	0.1	0.1	0	0	0	0	0.1	0.7	0
26	4-sp mixture	Co-dominance	0.4	0.1	0	0.4	0.1	0	0	0	0
27	4-sp mixture	Co-dominance	0.1	0.4	0	0.1	0.4	0	0	0	0
28	4-sp mixture	Co-dominance	0	0	0	0.4	0.1	0	0.4	0.1	0
29	4-sp mixture	Co-dominance	0	0	0	0.1	0.4	0	0.1	0.4	0
30	4-sp mixture	Co-dominance	0.4	0	0	0.1	0	0	0.4	0.1	0
31	4-sp mixture	Co-dominance	0	0.4	0	0	0.1	0	0.1	0.4	0
32	4-sp mixture	Co-dominance	0.1	0	0	0.4	0	0	0.4	0.1	0
33	4-sp mixture	Co-dominance	0	0.1	0	0	0.4	0	0.1	0.4	0
34	4-sp mixture	Centroid	0.25	0.25	0	0.25	0.25	0	0	0	0
35	4-sp mixture	Centroid	0.25	0.25	0	0	0	0	0.25	0.25	0
36	6-sp mixture	Centroid	0.167	0.167	0	0.167	0.167	0	0.167	0.167	0
37	6-sp mixture	Centroid	0.167	0	0.167	0.167	0	0.167	0.167	0	0.167
38	6-sp mixture	Centroid	0	0.167	0.167	0	0.167	0.167	0	0.167	0.167

- **IMPORTANT NOTE:** Double check to which Plot corresponds the selected compositions in your experimental site (Table 2).

Table 2. Plot – composition randomization per experimental site.

Composition	Plot				
	Spain	France	Slovenia	Lebanon	Jordan
1	11	4	8	29	22
2	2	21	20	35	9
4	26	23	3	37	24
5	39	26	11	26	25
7	22	14	4	6	16
8	9	31	30	3	17
18	35	18	2	27	15
19	4	29	13	14	29
20	28	2	27	38	34
21	8	5	21	28	14
22	1	30	23	39	35
23	25	36	25	1	27
24	27	22	32	20	28
25	40	8	1	33	23
26	5	37	29	36	20
27	19	9	24	7	32
28	10	3	26	19	3
29	31	7	9	34	5
30	17	17	22	9	11
31	3	24	10	22	12
32	15	25	14	16	40
33	14	27	40	8	19
34	16	20	12	12	18
35	29	28	36	13	30
36	34	38	38	32	31
37	13	13	19	40	37
38	37	19	15	18	4

- **In the field:** at each sampling plot and grazed and ungrazed treatments (27 * 2 = 54 samples) take 3 soil cores (0-10 cm depth, Ø 5 cm, Figure 1). Even though there has not been grazing before this soil sampling (autumn 2022) we keep the structure to have 2 replicates per plot.
- Place the soil cores randomly in the middle of the plot (Figure 1).
- Put the 3 cores in one plastic bag. Always manipulate the soils with globes.
- Label the samples as follows: experimental site code (ES, FR, SI, LB, JO) – date (yyyymmdd) - plot ID (1:40) – grazing (yes, no). Example ES-20220622-40-yes.
- Store the soils under refrigerated conditions during the field.

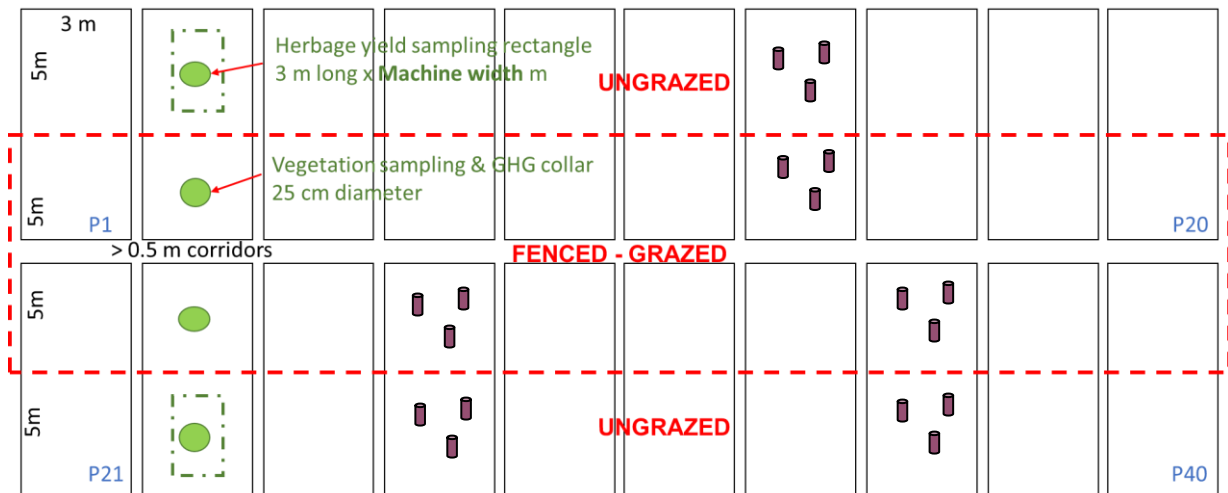


Figure 1. Soil sampling scheme in each plot during soil sampling, autumn 2022. Take 3 cores of soil on the selected plots and in both treatments, grazed and ungrazed.

- **In the laboratory**, homogenise the soil sample inside the bag and prepare the corresponding samples to be sent to the labs (Table 3).
- If you need to store the soils in the lab for a few days before sending them, store them refrigerated or frozen according to the type of analysis (Table 3).
- In the case of soil samples for **nematodes** analysis, keep them in a fridge (4-8 °C, inside plastic bags slightly open for air) during the weekend if needed. Then ship them directly next Monday to Berlin. The **extraction procedure needs living, active animals**. Also, when preparing the parcel for the nematodes analysis (refrigerated conditions), **wrap the samples in bubbles plastic or similar to isolate the sample and avoid the direct contact with the ice packs**.

Table 3. Soil analysis, pre-treatment, temperature of storage and shipment, fresh weight of each sample and shipping address.

SOIL ANALYSIS	PRE-TREATMENT	TEMP.	FESH SOIL WEIGHT PER SAMPLE	SHIPPING ADRESS
Nematodes	None	8 °C	80 -100 g	Prof. Dr. Liliane Rueß Humboldt Universität zu Berlin Institut für Biologie AG Ökologie Philippstraße 13, Haus 22 10115 Berlin, Germany Tel.: 030-2093-49722
PLFAs	Sieve (2 mm)	-20 °C	10 g	Dr. Angela Ribas Centre for Research on Ecology and Forestry Applications (CREAF) Building C, Campus de Bellaterra (UAB) 08193 Cerdanyola del Vallès, Barcelona, Spain. Tel: +34 935811312
Fertility and activity	Sieve (2 mm)	4 °C	150 g	

- Add to the parcels the corresponding letter, <https://susforage.ctfc.cat/logistics/>
 - Letter_shipment_CREAF_fresh_soil
 - Letter_shipment_HU_fresh soil
 - Letter_shipment_HU_frozen soil

- **Parcels coming from not UE countries, please ask the receiving partners for the details.**