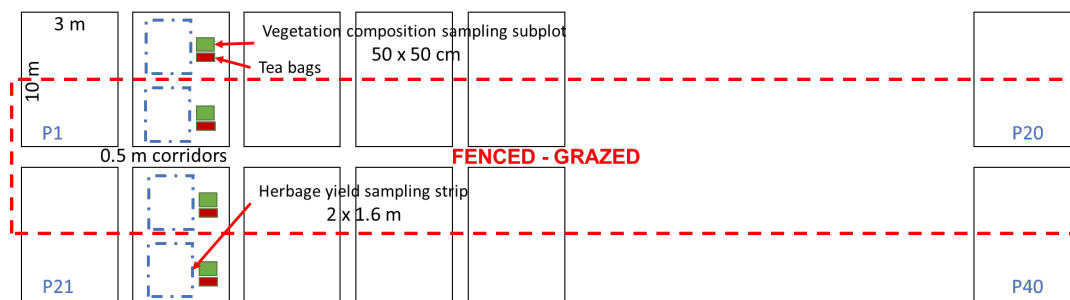


EXPERIMENTAL DESIGN

FIELD ESTABLISHMENT

Establishment protocol

- Sowing will take place in the fall 2021 (spring 2022 if previous fall is impossible)
- Establish 40 plots in two rows: minimum size 3 x 10 m (Figs. 1 and 2)
- Total $3(.5) \times 20 = 70$ m // $10(.5) \times 2 = 21$ m CORRIDORS INCLUDED!
- Recommended field size: 90 x 30 m minimum
- **BEFORE YOU START:** Apply the sampling protocol for soils at T0 (see below; Figs. 3 & 4)
- Sow 0.5 x 0.5 m corridors with the same species as in the adjacent plots
- Mow the 0.5 m corridors to maintain them and facilitate movement between plots
- RANDOMIZE the order of the design (composition) plots (to be provided by CTFC)
- Split each plot into two 5 x 3 m plots by fencing the internal area of the two rows of plots (see figures 1 and 2)



- On one side of each plot a 50 x 50 cm subplot will be established to record species composition and weeds (Fig. 2); be sure you leave at least 0.5 m border around all sampling areas: strip and subplots, except where both (strip & subplot) intersect
- The 40 plots displayed in the field (field plot: PlotID) will have different compositions in the 5 different locations (Table 1) according to the community composition design determined after randomization (Table 2)
- Seed weight at each plot determined in Excel file provided by CTFC, after sowing rates for each species in monoculture, per location, have been determined

- Sow by hand or with experimental sowing machine, being sure you do not contaminate the seed composition of a plot with that of another plot
- Amendment fertilization if soil nutrient deficit

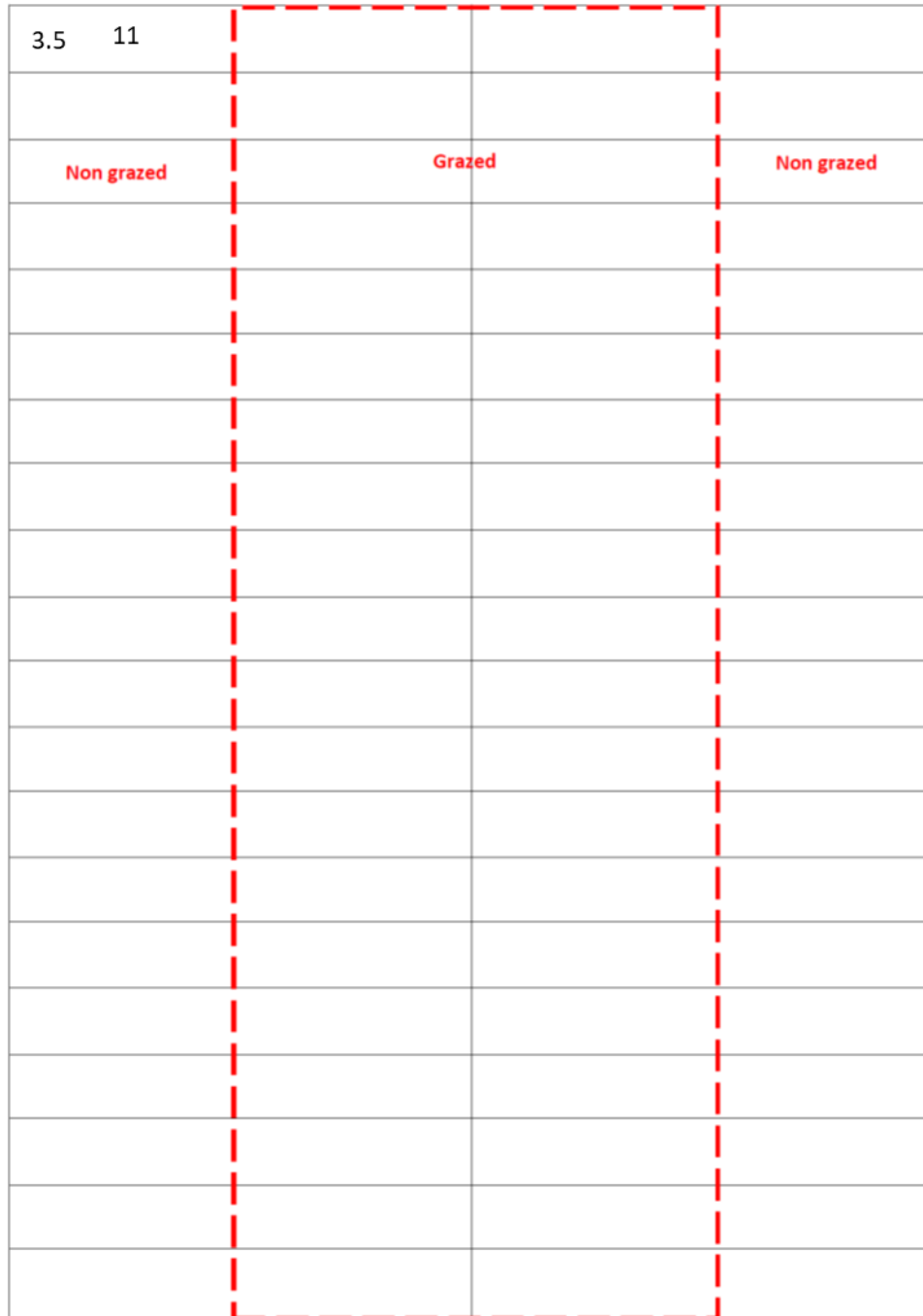


Figure 1. Plot display in the field.

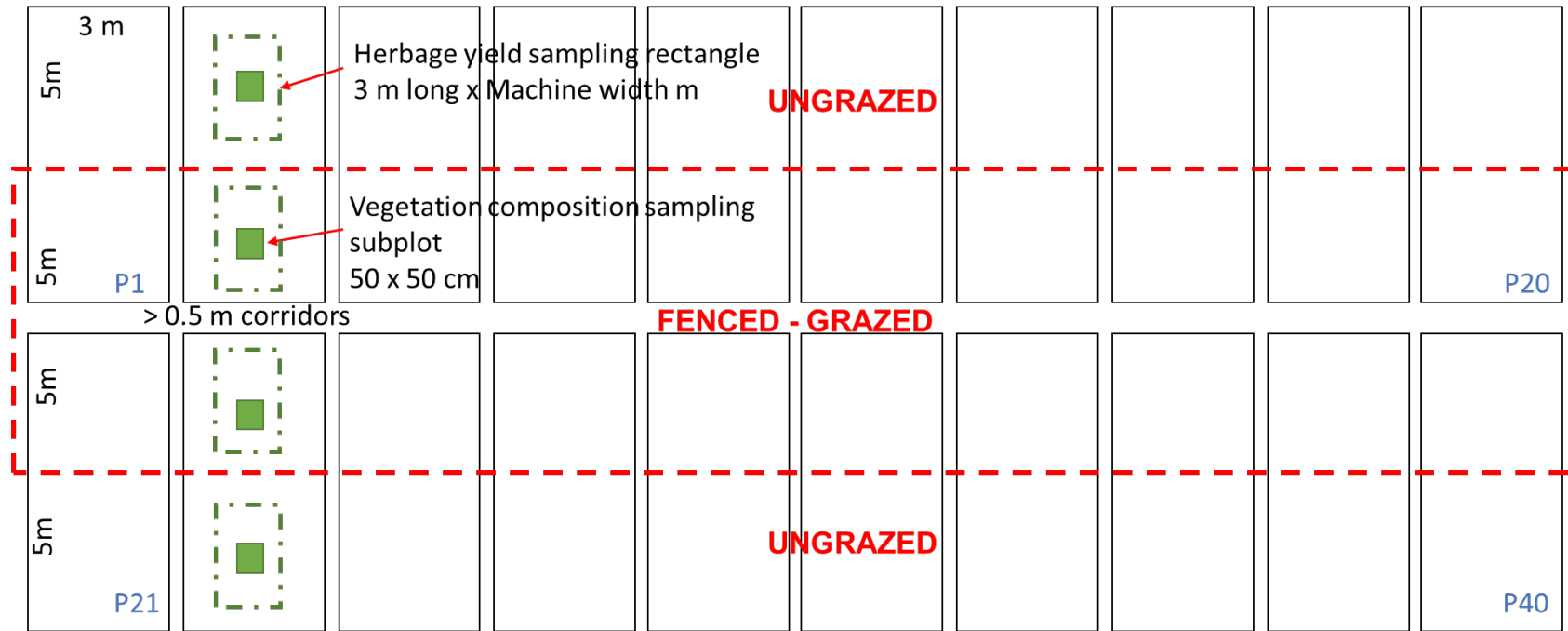


Figure 2. Scheme showing plots dimensions and plot ID (1 to 40; corridors between plots; strip for yield measurement; and subplot for vegetation analysis.

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

Table 1. Randomization of vegetation compositions per location.

Composition	Vegetation	Diversity	SR	E_sp	PFT	G1	G2	G3	L1	L2	L3	F1	F2	F3
1	Monoculture	Monoculture	1	0.00	1	1	0	0	0	0	0	0	0	0
2	Monoculture	Monoculture	1	0.00	1	0	1	0	0	0	0	0	0	0
3	Monoculture	Monoculture	1	0.00	1	0	0	1	0	0	0	0	0	0
4	Monoculture	Monoculture	1	0.00	1	0	0	0	1	0	0	0	0	0
5	Monoculture	Monoculture	1	0.00	1	0	0	0	0	1	0	0	0	0
6	Monoculture	Monoculture	1	0.00	1	0	0	0	0	0	1	0	0	0
7	Monoculture	Monoculture	1	0.00	1	0	0	0	0	0	0	1	0	0
8	Monoculture	Monoculture	1	0.00	1	0	0	0	0	0	0	0	1	0
9	Monoculture	Monoculture	1	0.00	1	0	0	0	0	0	0	0	0	1
10	3-sp mixture	Dominance	3	0.51	3	0.8	0	0	0.1	0	0	0.1	0	0
11	3-sp mixture	Dominance	3	0.51	3	0.1	0	0	0.8	0	0	0.1	0	0
12	3-sp mixture	Dominance	3	0.51	3	0.1	0	0	0.1	0	0	0.8	0	0
13	3-sp mixture	Dominance	3	0.51	3	0	0.8	0	0	0.1	0	0	0.1	0
14	3-sp mixture	Dominance	3	0.51	3	0	0.1	0	0	0.8	0	0	0.1	0
15	3-sp mixture	Dominance	3	0.51	3	0	0.1	0	0	0.1	0	0	0.8	0
16	3-sp mixture	Centroid	3	1.00	3	0.3333	0	0	0.3333	0	0	0.3333	0	0
17	3-sp mixture	Centroid	3	1.00	3	0	0.3333	0	0	0.3333	0	0	0.3333	0
18	4-sp mixture	Dominance	4	0.64	2	0.7	0.1	0	0.1	0.1	0	0	0	0
19	4-sp mixture	Dominance	4	0.64	2	0.1	0.7	0	0.1	0.1	0	0	0	0
20	4-sp mixture	Dominance	4	0.64	2	0.1	0.1	0	0.7	0.1	0	0	0	0
21	4-sp mixture	Dominance	4	0.64	2	0.1	0.1	0	0.1	0.7	0	0	0	0
22	4-sp mixture	Dominance	4	0.64	2	0.7	0.1	0	0	0	0	0.1	0.1	0
23	4-sp mixture	Dominance	4	0.64	2	0.1	0.7	0	0	0	0	0.1	0.1	0
24	4-sp mixture	Dominance	4	0.64	2	0.1	0.1	0	0	0	0	0.7	0.1	0
25	4-sp mixture	Dominance	4	0.64	2	0.1	0.1	0	0	0	0	0.1	0.7	0
26	4-sp mixture	Co-dominance	4	0.88	2	0.4	0.1	0	0.4	0.1	0	0	0	0
27	4-sp mixture	Co-dominance	4	0.88	2	0.1	0.4	0	0	0	0	0.1	0.4	0
28	4-sp mixture	Co-dominance	4	0.88	2	0	0	0	0.4	0.1	0	0.4	0.1	0
29	4-sp mixture	Co-dominance	4	0.88	2	0	0	0	0.1	0.4	0	0.1	0.4	0
30	4-sp mixture	Co-dominance	4	0.88	3	0.4	0	0	0.1	0	0	0.4	0.1	0
31	4-sp mixture	Co-dominance	4	0.88	3	0	0.4	0	0	0.1	0	0.1	0.4	0
32	4-sp mixture	Co-dominance	4	0.88	3	0.1	0	0	0.4	0	0	0.4	0.1	0
33	4-sp mixture	Co-dominance	4	0.88	3	0	0.1	0	0	0.4	0	0.1	0.4	0
34	4-sp mixture	Centroid	4	1.00	2	0.25	0.25	0	0.25	0.25	0	0	0	0
35	4-sp mixture	Centroid	4	1.00	2	0.25	0.25	0	0	0	0	0.25	0.25	0
36	6-sp mixture	Centroid	6	1.00	3	0.1666	0.1666	0	0.1666	0.1666	0	0.1666	0.1666	0
37	6-sp mixture	Centroid	6	1.00	3	0.1666	0	0.1666	0.1666	0	0.1666	0.1666	0	0.1666
38	6-sp mixture	Centroid	6	1.00	3	0	0.1666	0.1666	0	0.1666	0.1666	0	0.1666	0.1666
39	9-sp mixture	Centroid	9	0.98	3	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
40	9-sp mixture	Centroid	9	0.98	3	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11

Table 2. Experimental design and species proportions in the different design plots (composition).

SPECIES COMPOSITION PER LOCATION

Moist Mediterranean

CATALONIA		
Grasses	Legumes	Forbs
<i>Lolium perenne</i> <i>Dactylis glomerata</i> <i>Festuca arundinacea</i>	<i>Medicago sativa</i> <i>Onobrychis viciifolia</i> <i>Trifolium pratense</i>	<i>Cichorium intybus</i> <i>Plantago lanceolata</i> <i>Achillea millefolium</i>
FRANCE		
Grasses	Legumes	Forbs
<i>Lolium perenne</i> <i>Dactylis glomerata</i> <i>Festuca rubra</i>	<i>Vicia sativa</i> <i>Trifolium repens</i> <i>Trifolium pratense</i>	<i>Cichorium intybus</i> <i>Plantago lanceolata</i> <i>Achillea millefolium</i>
SLOVENIA		
Grasses	Legumes	Forbs
<i>Lolium perenne</i> <i>Dactylis glomerata</i> <i>Festuca arundinacea</i>	<i>Medicago sativa</i> <i>Onobrychis viciifolia</i> <i>Trifolium pratense</i>	<i>Cichorium intybus</i> <i>Plantago lanceolata</i> <i>Achillea millefolium</i>

Dry Mediterranean

JORDAN		
Grasses	Legumes	Forbs
<i>Lolium rigidum</i> <i>Hordeum vulgare</i> <i>Avena sativa</i>	<i>Medicago sativa</i> <i>Trifolium incarnatus</i> <i>Vicia villosa</i>	<i>Chicorium intybus</i> <i>Plantago lanceolata</i> <i>Brassica napus</i>
LEBANON		
Grasses	Legumes	Forbs
<i>Lolium rigidum</i> <i>Hordeum vulgare</i> <i>Avena sativa</i>	<i>Medicago sativa</i> <i>Trifolium incarnatus</i> <i>Vicia villosa</i>	<i>Chicorium intybus</i> <i>Plantago lanceolata</i> <i>Brassica napus</i>

Table 3. Species composition used in the experiment in the different locations. Below, some possible species in the Dry Mediterranean experiments (three of each group to be selected).

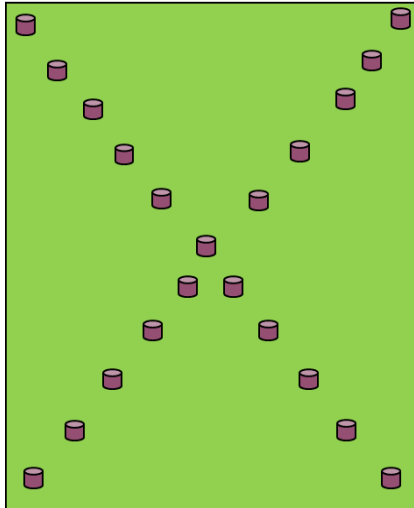
SOIL SAMPLING PROTOCOL AT T0

In the field, **BEFORE you sow!**

- Prepare the shipment arrangements and contact the three different laboratories receiving soils samples (HU, CREAM and INRAE-DIJON) to **arrange shipment prior to sampling**
- Prepare a cooler with ice cubes or equivalent to store the soils sampled **cooled IN THE FIELD AND DURING TRANSPORT TO YOUR LABORATORY**
- Sample soils according to the scheme in Fig. 3, **across the entire experimental field**
- Mix the soil collected in the field according to Fig. 3; **mix gently with gloves** the extracted samples!
- After mixing, weight the fresh soil and separate the soil into 4 subsamples to be shipped to the different laboratories for different measurements, according to Table 4
- Ship the soil samples as soon as possible after sampling
- Store the soil samples in your laboratory according to the requirements in Fig. 4 and Table 4
- Shipp the soil samples to the three laboratories according to Fig. 4 and Table 4)

THIS COMPANY TRANSPORTS FROZEN SAMPLES: WorldCourier

Check Figs. 3 and 4 and Table 4 for details on soil sampling, management, storage and shipment.



T0 Sampling scheme:

- 2 transects per **field** site
- Each 10 meters long
- 10 soil samples per transect

Soil sampling:

- Use soil core about 5 cm diameter
- Take upper soil layer 0-10 cm
- Collect soil in a bucket to get **one composite soil sample per field**
- Mix gently by hand (use gloves)
- Take (4) subsamples for the different analyses (see below)

Figure 3. Soil sampling scheme and soil samples distribution during soil sampling T0. Do this **before** you start sowing.

Summary of the samples distribution

- 800 g soil, fresh weight, for nematodes (**cool 8°C, no sieving, no freezing**) ► HU (Liliane)
- 100 g soil, fresh weight, for PLFAs (**freeze and store at -20°C**) ► HU (Liliane)
- 900 g soil, fresh weight, for fertility/activity (**cooling 4°C**) ► CREAM (Angela)
- 200 g soil, fresh weight, for DNA (**cooling 4°C**) ► Plateforme GenoSol (Katja)

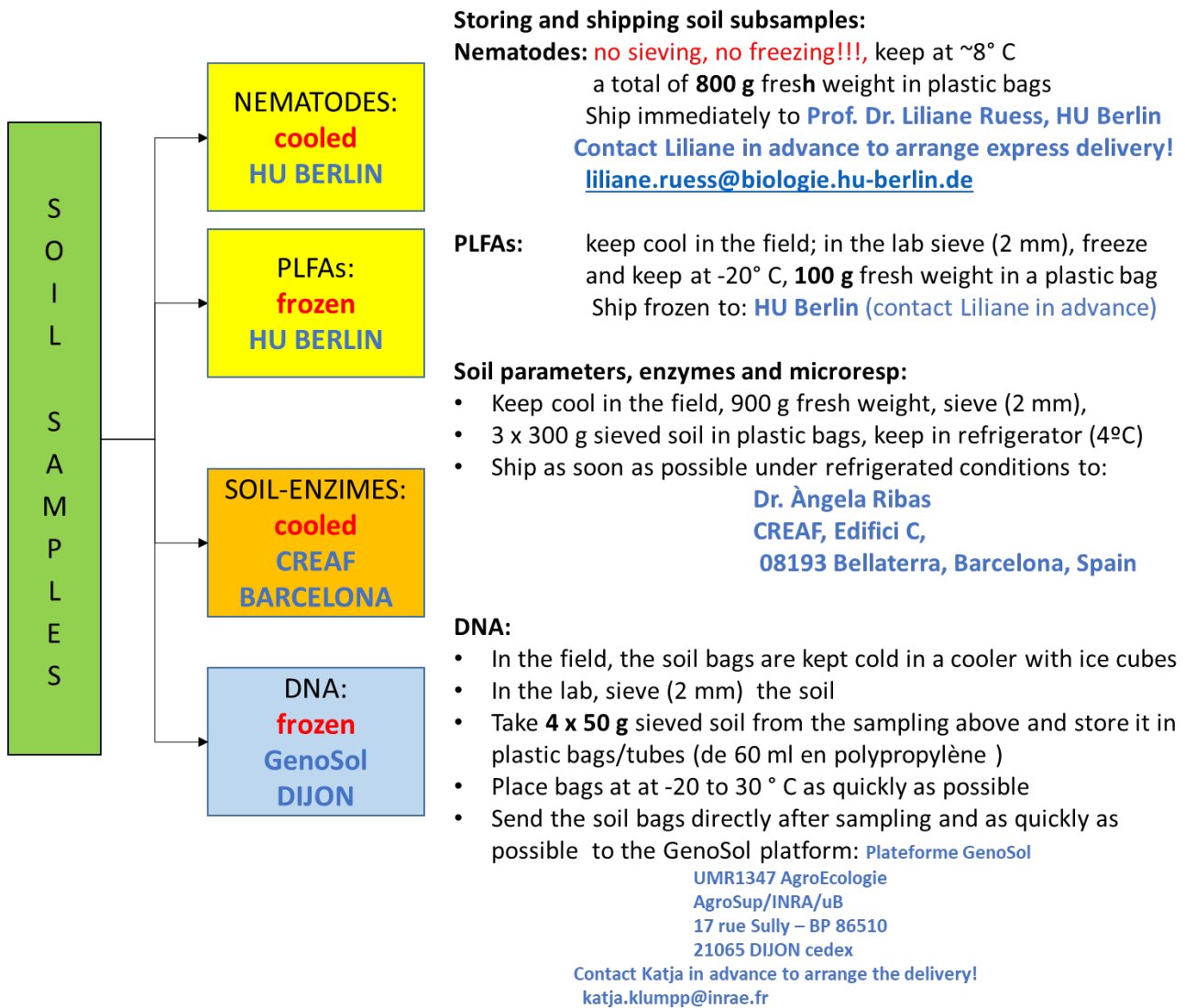


Figure 4. Soil sample management and shipment for soil sampling T0.

	BEFORE SAMPLING	DURING SAMPLING	FRESH SOIL WEIGHT	PRE-TREATMENT	STORAGE IN YOUR LAB	CONTACT PERSON	SHIPPING ADDRESS
Nematodes	Cooler/Prove/Bucket	Mixed - Cooled	800 g	None	8°C	Liliane Ruess	Contact Liliane
PLFAs	Cooler/Prove/Bucket	Mixed - Cooled	100 g	Sieve (2 mm)	-20°C	Liliane Ruess	Liliane Rueß <Liliane.Ruess@hu-berlin.de>
Fertility/Activity	Cooler/Prove/Bucket	Mixed - Cooled	3 x 300 g	Sieve (2 mm)	4°C	Angela Ribas	Dr. Àngela Ribas CREAF, Edifici C, 08193 Bellaterra, Spain
DNA	Cooler/Prove/Bucket	Mixed - Cooled	4 x 50 g	Sieve (2 mm)	-20/30°C	Katja Klumpp	Contact Katja: katja.klumpp@inrae.fr Plateforme GenoSol UMR1347 AgroEcologie AgroSup/INRA/uB 17 rue Sully – BP 86510 21065 DIJON cedex

Table 4. Summary of soil sampling, storage and shipping protocol at T0.