WORKFLOW PLAN FOR SOIL RESEARCH UNDER ECHO PROJECT

ECHO PROJECT GENERAL INFO

ECHO is a European CS project in which citizens participate in a Europe-wide soil sampling initiative and contribute to the systematic mapping of soils. Participants receive free sampling kits from us, and with the support of an app and accompanying information brochures (available on the our website, but the process is also shown on our Youtube Channel), they independently carry out a soil analysis and send two soil samples for lab analysis. The collected data are then made publicly available through our ECHO repository. To date, more than 3,500 samples have already been collected in the first 9 participating countries, and from **February through November 2026**, we will continue collecting samples in other countries, including **300 soil samples in Slovenia.**

ECHO is a Research and Innovation Action co-funded by the European Union under the Horizon Europe programme, and UK Research and Innovation (UKRI). It will last 4 years – from June 2023 to May 2027.

The project aims to engage citizens in protecting and restoring soils by building their skills and enhancing their knowledge of soils. Citizens will actively contribute to the project's data collection, promote soil stewardship, and foster behavioural change across the EU.

ECHOREPO, a long-term open access repository with a direct link to the EU Soil Observatory, will enable the citizen science data collected during ECHO to be available for use not only by scientists, but also by the general public, policy makers, farmers, landowners and other end-users, providing added value to existing data and other relevant soil monitoring initiatives. ECHOREPO will provide valuable information about the state of soil health across EU Member States, helping citizens make informed decisions about land use and conservation.

With 16 partners from Europe and Scotland, including 10 leading universities and research organisations, 4 SMEs and 2 Foundations, under the coordination of the Free University of Bolzano-Bozen, ECHO aims to collect data from up to 16,500 sites across Europe and Scotland in different climate and biogeographic regions.

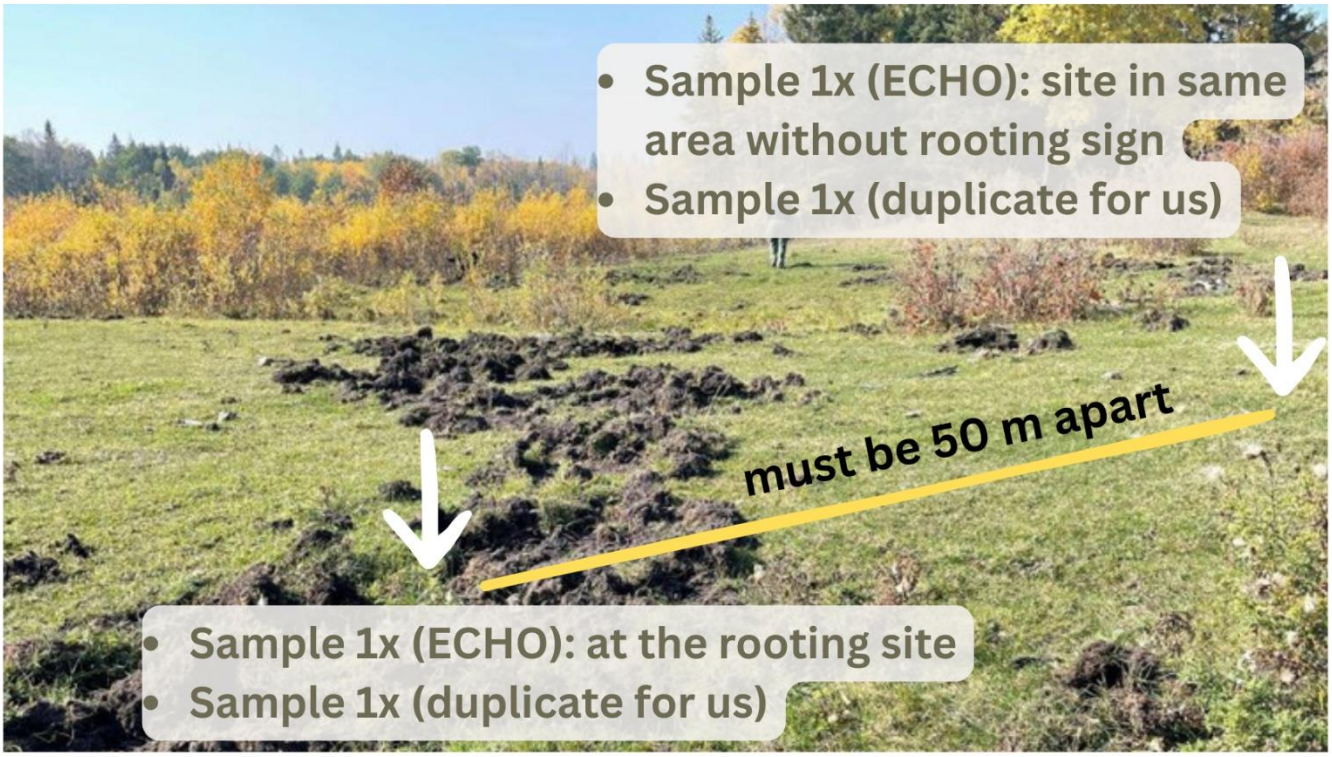
OUR INVOLVEMENT IN THE PROJECT and PROPOSED PLAN

Engage in the role of ECHO Ambassadors, who would act as local facilitators, support the involvement of citizen science groups, receive sampling kits (n = 50, meaning for 50 soil samples, and collect the lab samples to send to Italy for analysis (covered by the project).

Receiving soil sampling kits (n = 50) for laboratory analysis of their quality and microbial diversity, and combining this data with wild boar diet research (parallel information about soil quality).

To combine the information on the quality of soil with wild boar research, you need to sample in a way:

- **i) Choose a bigger area with present rooting sites;**
- **ii) Sample 1 sample for ECHO + duplicate of sample (for us) on the rooting site**
- **iii) In the same area, at least 50 m away (condition for ECHO), a soil sample where there is no rooting present (1 sample for ECHO, duplicate for us).**



Meaning:

for PRIMORSKA HUNTING AREAS: 6 samples per area (2 for ECHO, 4 for us, covering rooting sites vs non-rooting sites per one locality) ➔ see the figure below

Aim: checking the difference in soil features (texture, structure, chemical compounds, metals, etc.) at rooting sites, vs non-rooting sites – even if it is the same area, wild boar tend to root on specific spots in the area (due to possible triggers: differences in soil features, searching for target groups for feeding, such as fungi, invertebrates, parts of plants, etc.).

Proposed sampling plan per hunting areas:

Sample	RIŽANA-ECHO	RIŽANA-for us	OBROV-ECHO	OBROV-for us
At rooting sites	**5**	Duplicates from the same site	**5**	Duplicates from the same site
Near rooting sites	**5**	Duplicates from the same site	**5**	Duplicates from the same site

In the table and the created maps, you can find the exact sampling spots with coordinates.

For Rižana and Žabnik-Obrov, we proposed locations based on camera trap locations, where rooting sites were seen in the field. In the table, there are 10 proposed locations where the rooting sites should be. Take into consideration that for each location, **you also need to sample at least 50 m away**, where the rooting is not present (at the same location). Do not forget also to sample triplicates (all Primorska LUO).

site_id	lovisce	lokacija	lat_2	long_2
SOIL_011	Rižana	Kubed	45.517377	13.8813206
SOIL_012	Rižana	Kubed	45.517254	13.8576509
SOIL_013	Rižana	Kubed	45.537972	13.8962953
SOIL_014	Rižana	Kubed	45.53313	13.8820596
SOIL_015	Rižana	Kubed	45.49439	13.9218494
SOIL_021	Žabnik-Obrov	Brezovo Brdo ob gozdu pri njivi	45.571228	14.0857071
SOIL_022	Žabnik-Obrov	Brezovo Brdo	45.575318	14.0915026

SOIL_023	Žabnik-Obrov	Brezovo Brdo	45.536028	14.0859483
SOIL_024	Žabnik-Obrov	Brezovo Brdo	45.516269	14.0633891
SOIL_025	Žabnik-Obrov	Javorje	45.554712	14.0949369

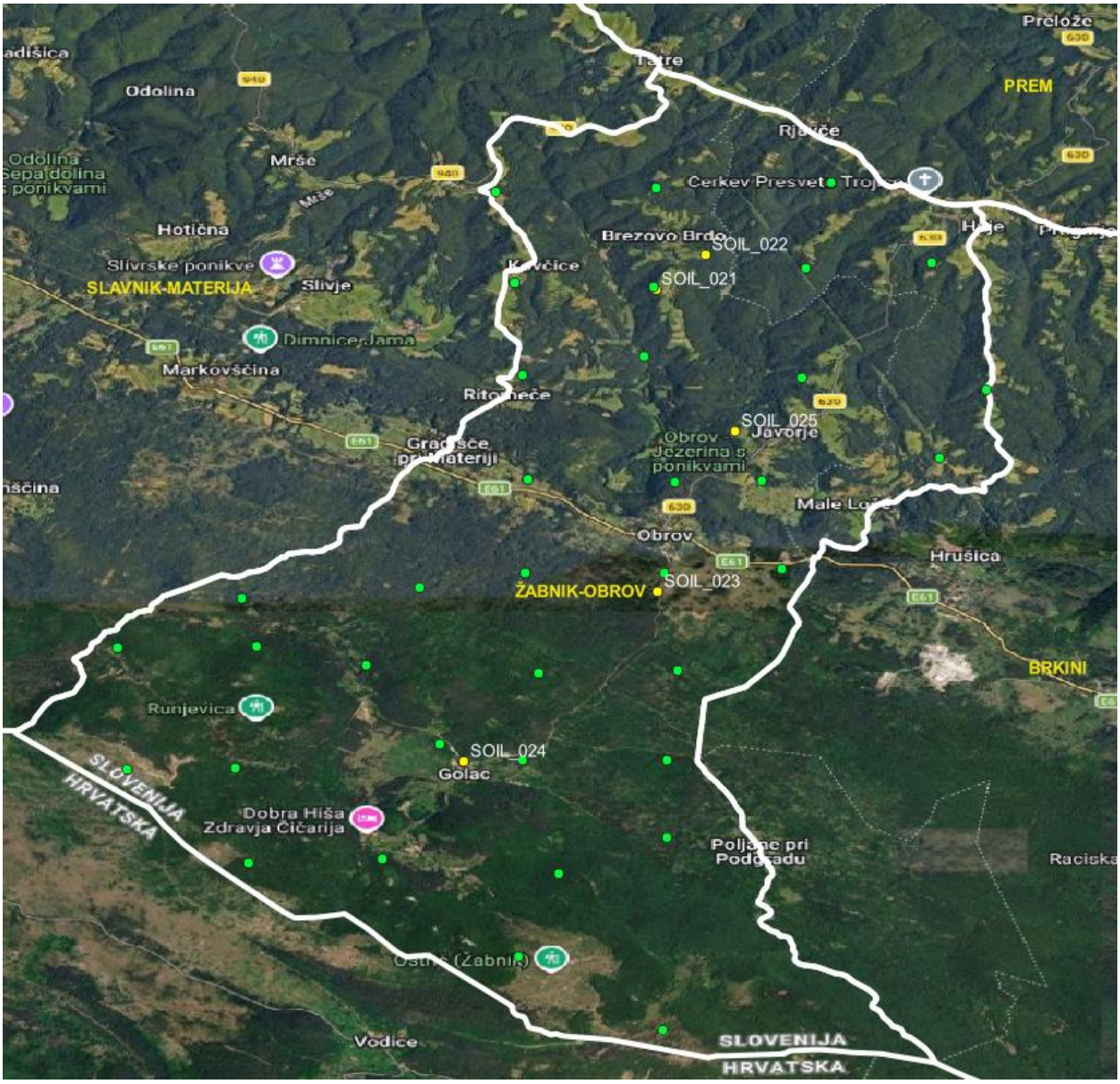
SAMPLE NAMES (for all areas):

FOUR SUBSAMPLES (ECHO + TRIPLICATES FOR famnit):

1. *SOIL_01: sample name on rooting site, ECHO*
2. *SOIL_01_non: sample name on non-rooting near site, ECHO*
3. *SOIL_01_UP: sample name on rooting site, for UP FAMNIT (2x)*
4. *SOIL_01_UP_non: sample name on non-rooting near site, UP FAMNIT (2x)*



Sampling locations for the Rižana hunting area.



Sampling locations for Žabnik-Obrov.

ECHO Laboratory analysis of the soil samples will include:
As

Ca
Cd
Cu
Fe
K
Mg
Mn
Ni
P
Pb
S
Zn

ECHO: Regarding microbial diversity, the analysis includes bacteria and fungi.

PLAN FOR SAMPLING- ECHO:

1. Sampling soil samples for the ECHO project (provided in their instructions)

- step-by-step sampling protocol (urgently go through before the fieldwork!!): file:///C:/Users/ajaAdmin/Downloads/FieldProtocolsENG.pdf
- YouTube channel for sampling guidance:

https://www.youtube.com/watch?v=59fEtIOQ9Xs&list=PLfnsWacMrzczcEqd3l4O69AYbYx78vYBR

- Youtube channel for additional workflows (https://www.youtube.com/@ECHOsoilproject/videos)
- ECHO app, where we need to submit data about soil samples:



During fieldwork, carefully do step by step fieldwork protocol, submit all the data to the ECHO app, and pack **two important soil samples for ECHO** (for DNA biodiversity analysis – 5ml falcon tube + bag of soil) in their soil sampling bag (do not mix samples), and save them in the same ECHO bag that you got.

Warning: Each student should submit 2 samples into ECHO app as Citizen Scientist!

2. Sampling for bridging soil data with wild boar diet research

= Taking the same duplicate soil samples for metabarcoding analysis as for soil sampling (=2 samples/area – rooting site vs non-rooting site).

- Combining the laboratory analysis of soil samples with metabarcoding data of soil samples near rooting sites.

FIELDWORK MATERIAL:

new gloves (for each sample) + ZIP bags (for soil samples; one zip bag is for rooting, one is for non-rooting site) + field sheet for sample data + styrofoam box with a penguin to put the samples on ice, immediately after the sampling.

SAMPLING NAMES:

FOUR SUBSAMPLES (ECHO + DUPLICATES FOR famnit):

1. *SOIL_01: sample name on rooting site, ECHO*
2. *SOIL_01_non: sample name on non-rooting near site, ECHO*
3. *SOIL_01_UP: sample name on rooting site, for UP FAMNIT (2x)*
4. *SOIL_01_UP_non: sample name on non-rooting near site, UP FAMNIT (2x)*

Use same equipment, that you use for ECHO (same shovel, etc.), must avoid contamination.

1. From the same site as you sampled ECHO samples, you need to take duplicates for UP FAMNIT (1 ECHO + 2X rooting UP, + 2x non-rooting UP).
2. With new gloves (for each new soil sample), take with the same shovel (for each site, new kit) a soil sample.
3. Put soil sample into **corresponding ZIP BAG-SOIL BAG** with the **right sample ID** (see map with samples above). Make sure that you put the soil from rooting sites in one bag, and from non-rooting sites in other bag names as "non-rooting").
4. **Do not mix rooting vs non rooting UP FAMNIT samples!!**
5. Put sample + roots zip bags into a larger ZIP bag with corresponding Sample ID for UP FAMNIT. (not for echo)
6. **Immediately insert sample data into the field sample sheet** (see last page of protocol) – especially you need to write Sample ECHO id that was sampled in the same location, so we can see the connection between samples.
7. Be careful to avoid contamination between the samples! New gloves; sterilisation with ethanol in the field if needed.
8. After sampling, put samples on -20*C (in the field), and then on -80*C if possible.



SAMPLEFIELD SOIL UP FAMNIT SHEET

site_id	lovisce	lokacija	lat_2	long_2	Date of sampling	Sample provider	DEPTH (max)	ECHO ID of sample that u take in same location – written on ECHO bag
SOIL_011	Rižana	Kubed						
SOIL_011_non								
SOIL_012	Rižana	Kubed						
SOIL_012_non								
SOIL_013	Rižana	Kubed						
SOIL_013_non								
SOIL_014	Rižana	Kubed						
SOIL_014_non								
SOIL_015	Rižana	Kubed						
SOIL_015_non								
SOIL_021	Žabnik-Obrov	Brezovo Brdo ob gozdu pri njivi						
SOIL_021_non								
SOIL_022	Žabnik-Obrov	Brezovo Brdo						
SOIL_022_non								
SOIL_023	Žabnik-Obrov	Brezovo Brdo						
SOIL_023_non								
SOIL_024	Žabnik-Obrov	Brezovo Brdo						
SOIL_024_non								
SOIL_025	Žabnik-Obrov	Javorje						
SOIL_025_non								