BIODIVERSITY GENOMICS EUROPE WP4

Ecological Restoration - Soil sampling

SOP

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Introduction

The Biodiversity Genomics Europe (BGE) Consortium has the overriding aim of accelerating the use of genomic science to enhance understanding of biodiversity, monitor biodiversity change, and guide interventions to address its decline. The objective is to establish functioning biodiversity genomics networks, data generation and pipelines to characterize biodiversity, and to improve management intervention and biomonitoring programs by practical application of genomic tools.

Soils are one of the main global reservoirs of biodiversity, and a key determinant of ecosystems functioning and performance (Wagg et al., 2014). Despite soil's biodiversity exceeding that of other terrestrial systems, it remains highly understudied (FAO et al. 2020), particularly regarding microorganisms community diversity and interactions in wild vs cultivated lands. In collaboration with the wIldE project, that searches for climate-smart rewilding as tools against the threats of climate change and biodiversity loss, the *Ecological Restoration - Soil* case study is designed to characterize soil biodiversity in Europe using genomic tools, and to evaluate how soil's biodiversity changes across ecological gradients of land abandonment and/or post-disturbance vegetation stages. The case study will inform if changes in diversity, species composition and functional composition of soil communities share similarities across Europe, or in alternative, if they are largely idiosyncratic and site-specific.

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Sampling Design

To evaluate changes in soil biodiversity during the process of ecological succession following land abandonment (or a major disturbance), metabarcoding techniques will be used to assess soil biodiversity, targeting fungi, arthropods, and microbiome. For each local case study, the sampling design involves a chronosequence of replicated plots at different stages of recovery since land abandonment or the last major disturbance (Figure 1). The chronosequence should start with plots representative of early successional stages, mostly old fields (i.e., at some time after land abandonment) and end in late successional old-growth or mature forests, including also the intermediate recovery stages. Other chronosequences representing ecological restoration/rewilding are also considered.

For each case study, four restoration stages will be sampled (early successional, two intermediate stages, and late successional, with no fixed distance between each other), with six independent replicates per restoration stage (i.e., six different plots per each stage, at a minimum of 1 km from each other); this can be adjusted depending on the particular conditions of each case study.

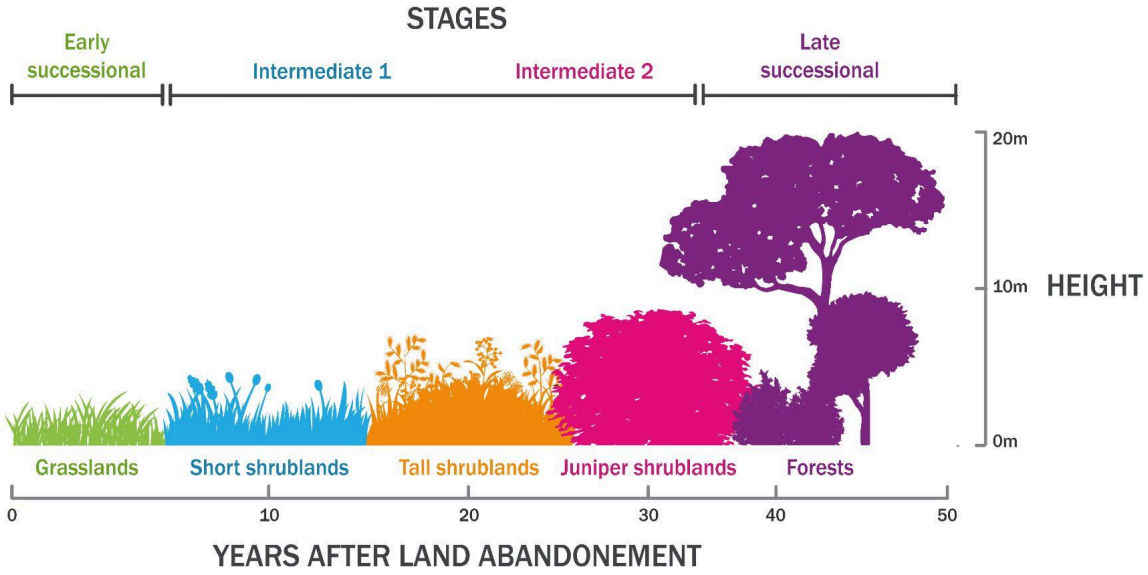

Figure 1. Example of a chronosequence (space for time substitution) representing the stages of vegetation recovery after land abandonment.

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At each replicate and sampling occasion, five sample cores should be sampled within each replicate, collecting two soils samples/depths per core, one at a 10-20 cm depth, and another one at a 20-30 cm depth (Figure 2). As a reference, cores within each replicate can be taken in a square-like manner, with no fixed distance between them (100 mt distance is suggested when possible, but there is no fixed distance required for this study). In total, there will be 240 samples (60 samples per stage, per four stages). Individual labeled tubes will be sent to BIOPOLIS-CIBIO for pooling and downstream analyses.

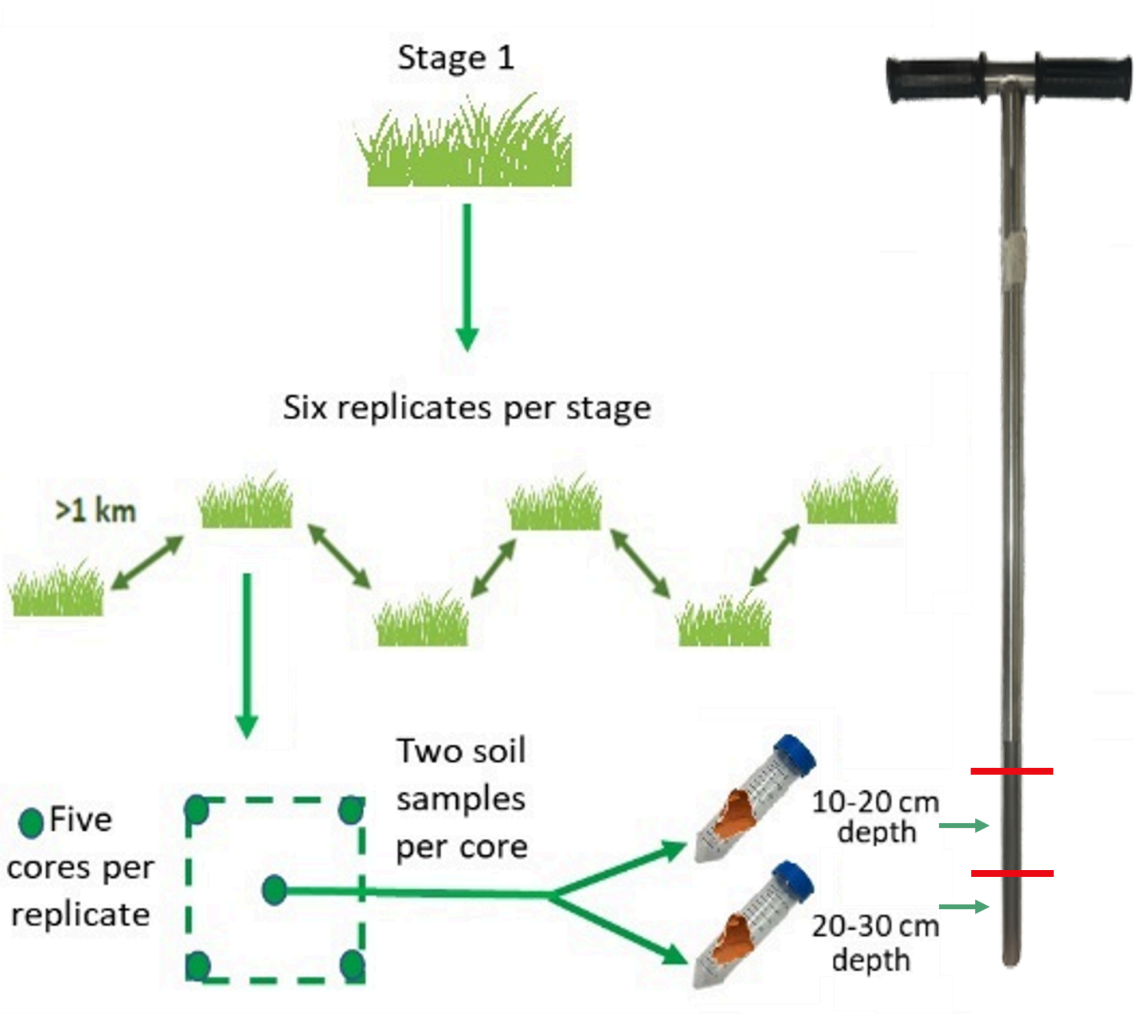

Figure 2. Example of the sampling scheme per successional stage, per replicate. The soil probe (right end in the image) samples one core that can include two depths (below the red lines), if the terrain is deep enough.

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Materials

List of materials needed for soil sampling (Figure 3)

A. Soil probe (a length of more than 1m is recommended to facilitate manipulation)
B. Scaled shovel (e.g. gardening kit)
C. Bulb planter
D. Soil tester (temperature, pH)[1]
E. Gloves, (e.g. latex, nitrile)
F. Sterile 50ml conical tubes
G. Spatulas/spoons
H. 10% bleach solution
I. Dry ice[2] (important), or exceptionally in normal ice (min. Temp -2ºC) in sufficient quantity to cover the expected tubes and some extras
J. Cool boxes or hermetic containers for dry ice (calculate space for 240 tubes plus dry ice)
K. Labels and/or predefined QR codes for samples[3]
L. Tape
M. Hermetic plastic bags (e.g. zip bags -preferable with hard lock)

Figure 3. Materials needed for soil sampling. Letters refer to the materials list.

[1] Optional
[2] Once collected, the soil samples must be promptly stored at negative temperatures. Dry or carbonic ice is the quickest and stablest way of reducing temperature. The quick reduction of temperature inactivates microorganisms and stops enzymatic degradation of its DNA. Once frozen, the samples cannot be thawed but for the genomic DNA extraction process.
[3] Be sure that the labels support negative storing temperatures. Use tape for extra help to fix it to the tube.

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Before starting

Make sure you have all sampling materials (Figure 3) organized and cleaned (10% bleach, NaOCl), to have sorted fieldwork logistics (e.g., vehicle, budget, personnel, etc.), to consider soil characteristics (type, depth, size of grain, etc.), sampling location, storing equipment, permits (if needed), area accessibility, etc.

Taking the soil sample is a demanding physical activity. It is strongly advised to use protective gloves when manipulating the soil probe, and if possible, a team of three or four persons for the sampling would be the best option for a faster and more organized sampling (e.g. one person taking the samples, one recording metadata in PlutoF, two persons rotating to dig the soil).

Bring enough cool boxes to store all the material collected (approximately four boxes of 40 x 40 cm), calculate how much space is needed for the tubes and the dry ice in each box. Take precautions about dry ice supply if the sampling locations are isolated and with no possibility to acquire more when needed. It is advisable to consider at least 30 kg of dry ice for a sampling mission of four days, in properly isolated boxes. The recommendation is to open the boxes the least possible, and to tape the edges to insure isolation, if needed.

Particular attention shall be given to the sampling spot. Avoid sampling at the very border of big rocks, near water streams or within flooding areas, within the tree root perimeter (avoid big trees), and take a photo from the landscape caring to have the sampling point at the center of the photo. If possible, do some previous research on the sampling area looking for any recent events that might have caused a significant perturbation of the soil (e.g., fire, flood, construction, animal parking, clear cut forest, historical site, etc.). Although that sort of event does not preclude the sampling, it would be good to have that information further downstream when interpreting the data.

For recording sample data, the *PlutoF* plattform (Kessy et al. 2010) will be used as a workbench for soil records. It includes a mobile app, *PlutoF Go*, that will record sample data during fieldwork. A set of stickers with unique QR codes will be supplied for adding them to each sample. To optimize time of sampling and storing, the QR codes stickers should be placed on the sterile tubes and reinforced over with tape prior to sampling. A collector must be registered in the *PlutoF* platform (Register → fill in details) prior to sampling in order to appear as collector in the *PlutoF Go* app. Alternatively, project leaders can add “persons” into the platform (Menu “Persons” → Add → fill in details). Make sure you have downloaded the app and enter your personal data correctly. Further information on *PlutoF* is given on page 10. An on-line training session on using *PlutoF* is available upon request, and support will be provided wherever needed before, during and after the field work. QR codes will be read by the *PlutoF Go* app when entering the data. Taking time to open, explore, and practice taking metadata with the app is ***strongly recommended***, as is meant to standardize and optimize time while doing fieldwork.

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During sampling

During all the process, it is important to be careful of not contaminating samples at any stage. The use of gloves when manipulating samples is mandatory, taking particular care when placing the soil sample in each tube, ensuring that whatever touches the sampled soil has not been used before/cleaned to prevent any cross-contamination. For more efficient work, at least two persons should be taking the soil sample: one using the sampling materials, and other one using new sterile gloves for collecting the soil to the sterile tubes. Store the tubes to belong to one particular replicate in separated labeled plastic bags (e.g. zip bags); this will help further arrangement of samples (see Figure 4). Is recommended to have the zip bag labeled with the collection date, collector name(s) and stage/replicate number, you can put it inside the cool box and directly store the tubes with samples inside their correspondent zip bag.

In most types of soil:

1. Before taking the samples, make sure QR codes are well placed in the collection tubes
2. Scan both QR codes tubes prior to sampling the soil in the *PlutoF Go* app[4] (Add material sample → Choose form → Soil → Sample ID code icon). This will optimize time of storage
3. Remove the organic layer in the selected area, and then introduce the soil probe, going at 30 cm in depth[5]
    3.1. If there is not enough soil depth, use the bulb planter to get the sample
4. Carefully remove the probe/bulb planter to prevent the sampled soil to fall
5. Introduce the probe tip inside the collection tube, be careful of not touching the collected soil and the inside part of the tube without gloves
    5.1. When using the bulb planter, just place the collection tube in the center of the soil sampled an carefully release it, untouched soil will fall into the tube
6. Using gloves and a sterilized spatule, slide the soil sample out of the probe to the 50 ml sterile collection tube (the probe might have already incorporated its own device for depositing the soil). If the soil is rather thin or shallow, be sure to remove the top 4-5 cm layer and collect the soil beneath at its maximum depth. Whenever the soil is deep enough (>30 cm), the core samples can be collected from two distinct layers [20-30 cm and 10-20 cm]
    6.1. Fill up 2⁄3 of the tube with soil collected at a depth between 20-30 cm
    6.2. Take another tube and do the same but this time for a depth between 10-20 cm
    6.3. In case of shallow soils use a single 50ml tube and register the depth interval

[4] The *PlutoF Go* app can be found for Android, and for iOS systems. Instructions and other information can be found in the link provided. A quick guide for PlutoF Go is provided in this document.
[5] For shallow soils (<20cm depth) the sample shall be collected at its maximum depth and the depth interval information should be registered.

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7. Close the tube and immediately store it in its correspondent zip bag in dry ice or, exceptionally, on ice and keep it (at least) at -2ºC during transportation[6]
8. Measure the humidity, PH and temperature by inserting the soil tester probe in the hole left by the collection of soil (>5 minuter).This is needed only for one core per replicate.
9. Record the rest of the material collection information in *PlutoF Go* app (Add material sample → Choose form → Soil → fill in the correspondent details)
10. Store samples properly labeled at -20ºC at its final destination

In sandy or unstructured soils:

1. Scratch the top layer of the sand with the shovel, digging approximately 4-5 cm
2. Scan both QR codes tubes prior to sampling, and record the material collection information in *PlutoF Go* app (Add material sample → Choose form → Soil → fill in the correspondent details)
3. From the cleared surface, take a sample with a scaled gardening spoon or with the bulb planter
4. Place the sample in a 50 ml sterile collection tube with the QR labels (prevent contamination of sample using gloves and/or a sterilized spatule)
5. Immediately store the tube in dry ice or, exceptionally, on ice and keep it (at least) at -2ºC during transportation. Do not forget to store all the collected core samples in separate labeled hermetic bags.
6. Measure the humidity, PH and temperature by inserting the soil tester probe in the hole left by the collection of soil (>5 minuter).This is needed only for one core per replicate.
7. Store samples properly labeled at -20ºC at its final destination

IMPORTANT: Clean all the collecting materials before and after sampling using a solution of 10% bleach, (i.e., between different sampling sites), and replace the hand gloves when manipulating a new sample

[6] For the purpose of this study, each depth per core sampled was kept in the original 50 mL tube, closed and directly put in dry ice. Another alternative, for more experienced workers and to save space when transporting, would be to pool the same five depths per core, per stage replicate, on field. In this way, each stage will result with two pools, one for depth *a* (10-20 cm), and another one for depth *b* (20-30 cm). For more information about this sampling scheme, please contact L. Nájera-Cortazar (Useful contact).

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After sampling

Remember: immediately after collecting each sample in the tube, make sure it is properly closed, scanned, and temporarily store it in dry ice (or regular ice if not possible otherwise), to prevent contamination and overgrowth of any microorganism contained in the sample. When returning from fieldwork, **organize samples in plastic zip bags** (if you have not done it during sampling) **placing the 10 cores of each replicate within a zip bag, correctly labeled** (i.e. stage number, replicate number, collector, site, date, etc.), and **store the samples at -20ºC or more as soon as possible** (Figure 4).

Before shipping out the samples to the lab, make sure that the lab confirms the availability to receive the samples and ask the carrier for the time the parcel will spend in transit. Make sure to add sufficient dry ice to last for the entire journey. And lastly, add any permit/document that could be needed to depart from your country.

Figure 4. Space occupation of 240 tubes in -20ºC freezer drawers. Total core's samples of each replicate are contained in separated zip hermetic bags (10 tubes per bag).

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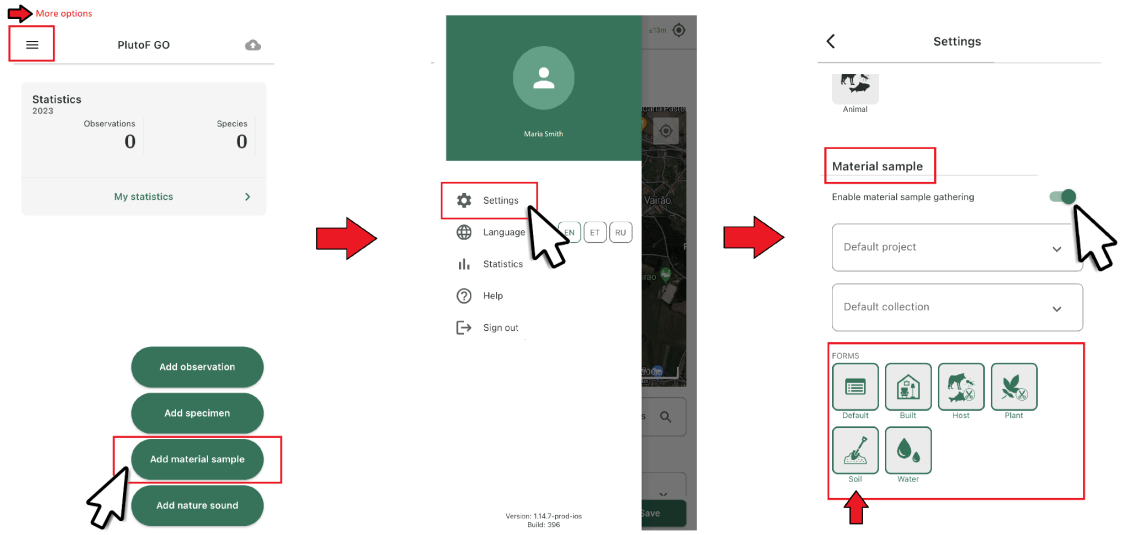
Registering samples in *PlutoF Go* app

PlutoF is an online data management and computing service provider for biological data. *PlutoF Go* is an app that will be used to record samples directly from fieldwork. Before using the app, the data collector should be registered on the *PlutoF* website. This can be done by the user in the option "Become a user", or to be added by the BGE project manager directly on her/his workbench[7]. You can fill all the available information within the soil option, but it is required at least to have the data detailed below. Images below the following list will correspond with the numbers in this list:

1. Open the ***PlutoF Go*** app

2. Go to **Add material sample** box (If this option is not visible in the main page, go to Settings, scroll down to Material sample, activate Enable material sample gathering and make sure the Soil form is highlighted in green as well)



3. The **Location** button will show your position in the map. You should record all the necessary information at the moment of the sample collection, therefore it should capture the coordinates detected by your device's GPS. If there is no internet signal you can still get the coordinates by pressing the compass icon (top right inside the map box). To georeference your site with an external GPS, go to the search icon on the right (magnifying glass icon) and place the complete coordinates in the top box that says

[7] The *PlutoF* project manager will be the only one authorized to add any person to the working project. Any team member will be automatically notified by email when added to any project.

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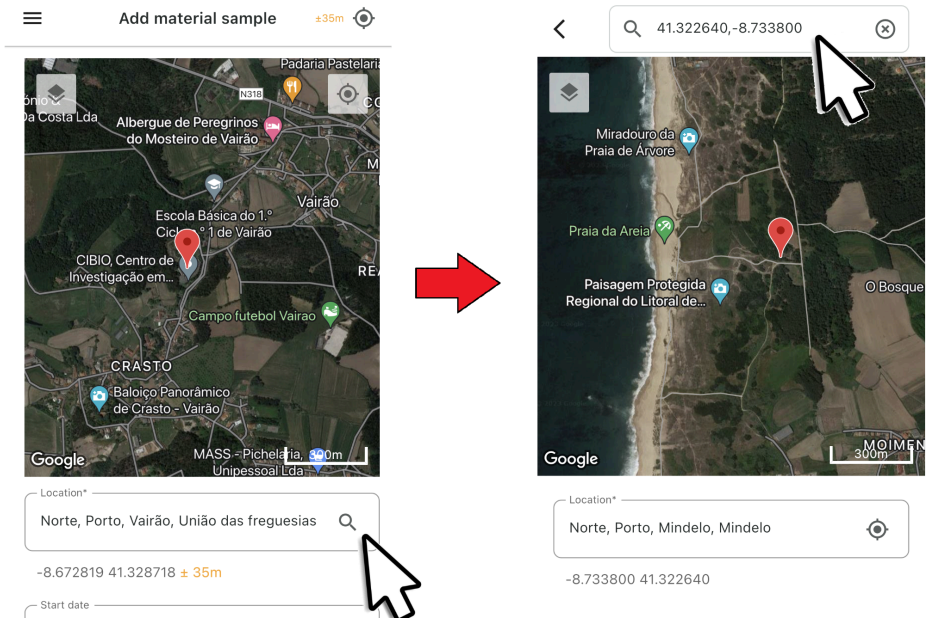
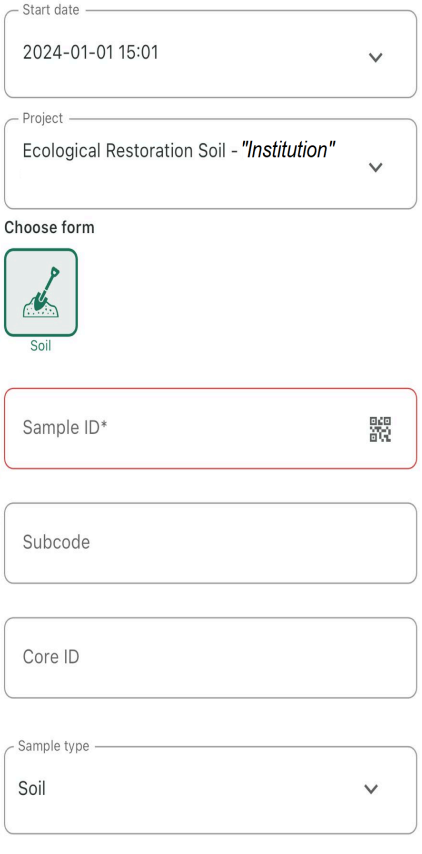
Location, address or… and click search. Once that is set, click the back option on the top left corner and continue with the data entry.





4. **Start date:** the date of the sample collection

5. **Project:** choose the “Ecological Restoration Soil - *Institution*” option. Choose the already created project according to your institution acronym

6. **Choose form:** select “Soil”, highlights

7. **Sample ID:** click on the code icon and point your device camera to the corresponding QR code provided for the soil sampling. QR codes are unique.

8. **Subcode:** Country initials (separator point) + Stage & number + Replicate & number + depth option (*a* = 10-20 cm, *b* = 20-30 cm) **XX.S1R1a - XX.S4R6a**

9. **Core ID**[8]**:** Add the core number (**C1-5**) and depth (**a**: 10-20 cm; **b**: 20-30 cm) that you are sampling (i.e. **C1a**). The first sample taken will be the depth ***b*** (20-30 cm), as is the one in the bottom part of the probe.

10. **Sample type:** select “Soil”

[8] In the previous versions, Core ID was requested to be added in Description, before added to the app.

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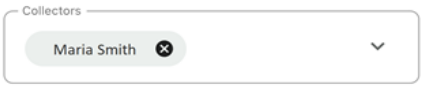


11. **Collectors:** Any person has to be previously registered/added in the *PlutoF* platform

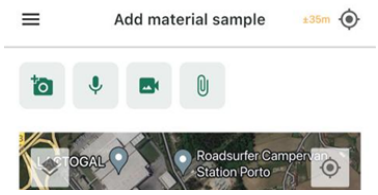
12. **Habitat description**: Fill in details of the **dominant vegetation** of the area, or edit in platform. Complement this information by adding a picture (*see point 18*).

13. **Soil texture:** this *optional*, but is strongly recommended. Using the app **+camera icon**, take a picture of one core per replica (if there is time, a photo of each core would be better), to further assess other soil characteristics for future individual projects.

14. **PH:** This *optional*, recommended for future individual projects.

15. **Temperature:** This *optional*, recommended for future individual projects.

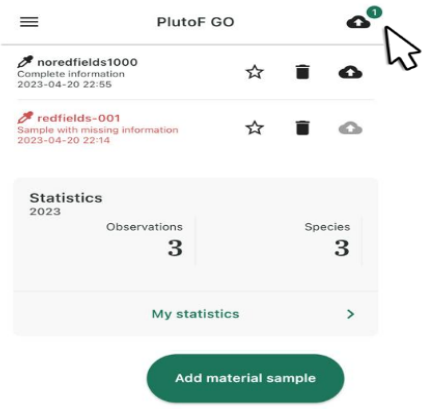
16. **Horizon description:** When there is not enough soil or structured soil it can be specified here.



17. **Photo:** Add a picture of the sampled habitat using the panel that is in the top of the Material sample menu. Use the **+camera icon** (top left) to generate a picture. One set of habitat pictures per replica is enough. Additionally (*optional*), you can add any extra information/metadata you think convenient.

18. Review all the information submitted is accurate and click "**Save**" (bottom of the screen).

19. In the main screen, your entries will be waiting in the queue to be synchronized. Click on the **cloud icon** on the top right to do it.



In the image, there is one good sample entry (dark letters, intense color) that can be synchronized, and another entry with missing information (red letters, faded color) that will not be able to sync until the missing information is filled. This can be done by clicking in the entry and revising the info submitted. Make sure you have a good internet connection to sync your entries, and try to sync often to ensure the data is saved. *You are ready for the next sampling collection.*

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