The crop microbiome survey

Quantitative assessment of the microbiome associated with croplands worldwide




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Crop Microbiome and Sustainable Agriculture

1. Main objectives:

This research proposal aims to establish a global collaborative network – the CROP MICROBIOME survey – to collect soils from natural and cropland ecosystems worldwide. We aim to identify (1) a list of species of bacteria, fungi and micro-fauna; and (2) functional attributes characterizing the soil crop microbiome worldwide. We will focus on five functionally important and globally distributes crops: (1) rice, (2) wheat, (3) cotton, (4) corn and (5) potato.

2. Sampling design:

2.1. Plot establishment

We are looking for sites with well-established cropping areas across contrasting environmental conditions (e.g. soil types). For each crop type (e.g., rice field), seven locations will be selected for each collaborator: five crop location and two nearby natural ecosystems (wider geographical coverage is encouraged- for example each crop location should be 100 km apart, if possible). A collaborator is expected to contribute at least one crop, which includes seven locations. At each site, a 30 m x 30 m plot should be established. Each collaborator should establish their own plots. Location (i.e. latitude and longitude) and other characteristics of each plot should be recorded as per the sampling template in Appendix 1. In the case of croplands, the crop type (e.g., wheat) and main variety for each species (i.e., *Triticum aestivum*) should be recorded. In the case of natural areas, the ecosystem type (e.g., forest) and the three dominant plant species should be recorded. A representative picture of each plot is required.

2.2. Soil microbiome

Croplands:

A total of ten samples per plot should be collected: five rhizosphere and five bulk soil samples.

Collecting rhizosphere soil samples:

Within each plot, select 5 plant individuals (Fig. 1). These individuals must be separated at least 5 m from each other, and at a similar phenological stage (ideally, flowering). First, dig up the entire plant within a 10-cm radius around the plant, and down to 20-cm soil depth. Take out the soil monolith with the plant individual in the middle of it. Make sure all the bulk soil has been shaken off the plant roots by vigorous shaking. Only a few g of soil should remain attached to the roots. Then, you can detach the soil that it is intimately attached to the plant fine roots with a scalpel. This soil is the rhizosphere soil. Collect fresh rhizosphere

soil by detaching soil with the scalpel from different parts of the plant fine root system. For each plot, combine the rhizosphere soils from each plant individual to achieve a composite rhizosphere soil sample (~5-10 g of soil). Keep the fresh rhizosphere soil in a zipped plastic bag, and label the bag with a permanent marker. Preserve the rhizosphere soils samples in ice during the field work and provide to freeze each composite rhizosphere soil sample at -20°C as soon as possible. Half of the rhizosphere soil should be kept in your laboratory as a back-up, and half shipped to our processing center.

Collecting bulk soil samples:
For bulk soils, please use a corer to sample the top 10 cm of soil in patches of bare soil between plants. A total of five bulk soil cores should be collected and combined to form one composite sample per plot. Sieve the bulk soil at 2-mm and keep the fresh bulk soil in a zipped plastic bag, labelling the bag with a permanent marker. Preserve the samples in ice during the field work. As soon as possible, ~25g of each composite soil sample should be frozen at -20°C; the rest (~250g) should be air-dried. Please keep~250 g of the air-dried bulk soil in your laboratory as a back-up.

Natural ecosystems:
For natural systems, only bulk soil is requested (top 10 cm). Bulk soil should be collected and processed as per above. Half of the bulk soil should be kept in your laboratory as a back-up, and half shipped to our processing center.

2.3. Plant microbiome

Croplands:
Plant material for microbiome analysis will be collected in cropland ecosystems only. From each of the selected five plant individuals, collect the top three leaves from the top of the plant (Fig. 1). Preserve them in a labelled bag. Similarly, collect a portion of the stem of each plant individual (Fig. 1). Finally, after removing the rhizosphere from the roots (see 2.2), collect the roots and include them in labelled bag (Fig. 1). Roots and leaves need to be preserved in ice during the field work, and frozen (-20°C) as soon as possible. Half of the plant tissue should be kept in your laboratory as a back-up, and half shipped to our processing center. Plant material should be shipped frozen.

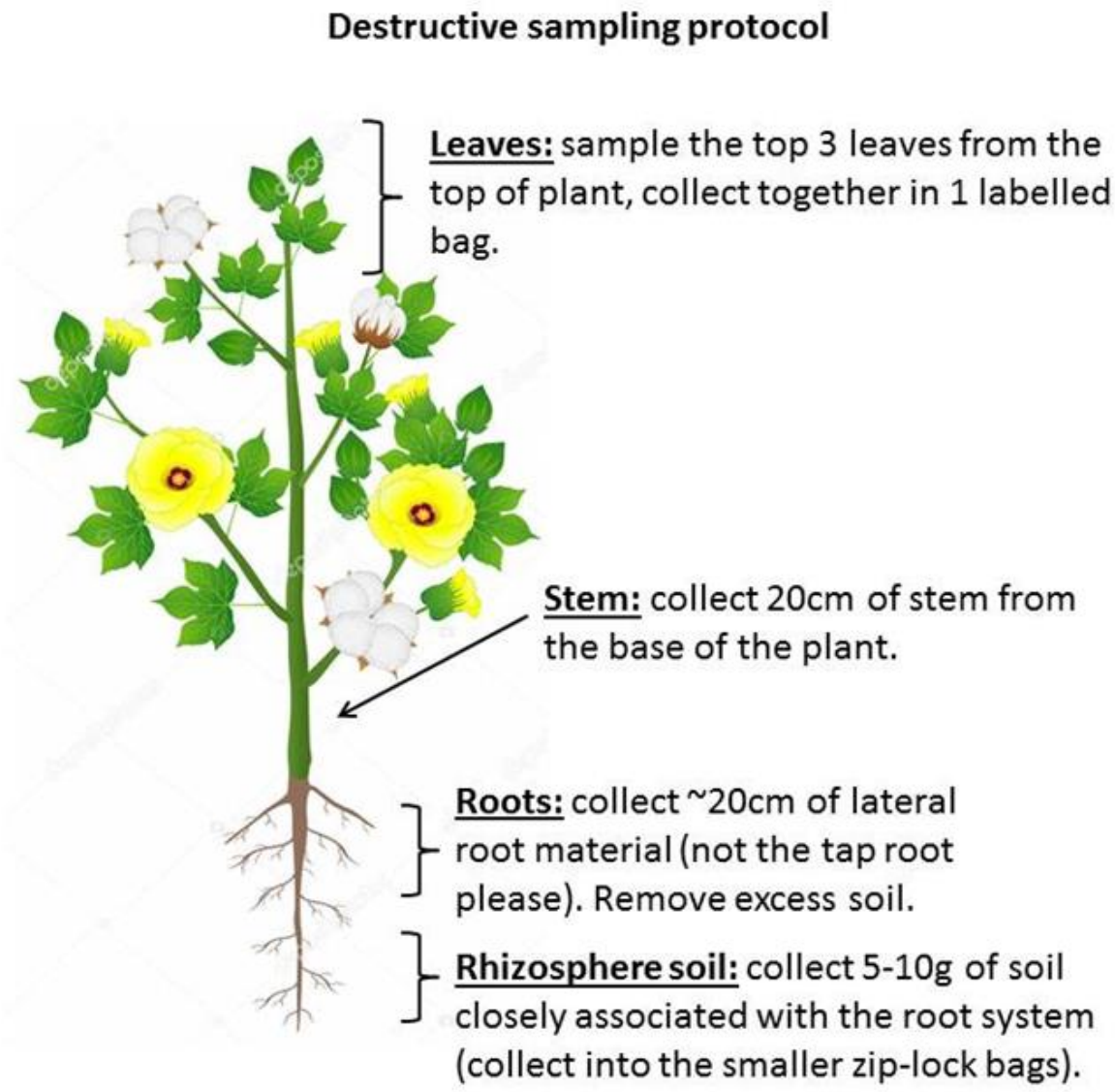

Figure 1. Plant and soil microbiome sampling.

2.4 Soil and plant sample checklist for shipping:

The following soil samples should be shipped to our processing facility:

Crop type (e.g., corn)				
Site	# of locations	Notes	weight per sample	use
Crop	5	one plot per location	n/a	n/a
Natural	2	one plot per location	n/a	n/a
TOTAL	**7 locations**			
For each crop:				
Soil source	# of samples	Notes	weight per sample	use
bulk	7	1 composite sample per location; Frozen	25 g	sequencing
bulk	7	1 composite sample per location; Air-dried	250 g	soil biochemistry
rhizosphere*	5	1 composite sample per location; Frozen	5-10 g	sequencing
TOTAL	**19 soil samples**			
Plant tissue*	# of samples	Notes	weight per sample	
leaves	5	1 composite sample per location; Frozen	n/a	sequencing
stem	5	1 composite sample per location; Frozen	n/a	sequencing
root	5	1 composite sample per location; Frozen	n/a	sequencing
TOTAL	**15 plant samples**			
*crop only				

2.5. Leaf trait measurement

In the field:
Material needed: zip-lock plastic bags, water, storage box (cooling box if possible), permanent marker (to label)
Leaf traits for 5 mature plant individuals in each site should be measured. For each plant individual, choose non-damaged and non-senescent mature leaves (2-3 leaves). Record plant height. Harvest small leaf pieces (max 3-4cm^2) and put them in a plastic bag with a bit of water, to avoid plant tissue drying. Label the plastic bag as follows: Researcher/ Country / Site number / Species / Individual number, and store in ice.

In the lab:
Material needed: picture template (provided under Research Protocols - Leaf Picture Template), data sheet, a glass window (or Plexiglas), a camera (a phone camera is OK), a precision weighing scale, paper bags, pen (to label), an oven.
Print the provided picture template in A4 format (21 x 29.7cm). Label the template (Researcher/ Country/ Site number/ Species). Put the pieces of leaves of each individual plant in their respective quadrats, avoiding overlaps. Add the glass on top, ensuring that all leaves are flat. Take a high-quality picture, carefully ensuring enough light, no reflectance of the glass and no shadow around the leaves. Measure and record the leaf fresh mass of each individual plant on a weighing scale and put the biological material in a paper bag (one bag for each individual plant). Label each paper bag as follows: Researcher/ Country / Site number / Species / Individual number. Incubate all paper bags at 60°C for 48h. Measure and record the leaf dry mass of each individual plant. Put the biological material back in the paper bag and store it. Report fresh and dry masses on the data sheet.

2.6. Shipping soil and leaf samples to Spain, EU

Note that a special permit is needed to send soils to Spain nowadays, so please, contact Dr Manuel Delgado-Baquerizo (m.delgadobaquerizo@gmail.com) before sending soil to Spain.

3. Publication and co-authorship of manuscripts
1. By contributing to the CROP MICROBIOME survey according to this protocol, you will be offered the co-authorship of any primary publication resulting from the samples you have provided, as long as you stay actively engaged (following deadlines and rules for data submission) and in communication with the coordinator. Providing data and material from at least one crop (=5+2 plots) is required to be a co-author.

2. Finally, any participant is free to use the CROP MICROBIOME data obtained from their plots for publication purposes, presentations, courses and other non-published venues (e.g. blog posts).

Appendix 1

Example of a Compiled Sampling Template:

	Information	Details / Intensity
Plot Name:	Spain_Sevilla_Wheat_p1	
Latitude:	37.359947°	
Longitude:	-5.935199°	
Ecosystem type:	Cropland	Wheat
Crop variety	*Triticum aestivum*	
Irrigated?	Yes	Twice per week
Fertilized?	Yes	10 kg N ha-1 yr-1
Mowed?	No	
Tillaged?	Yes	
Pesticide application?	No	
Crop rotation?	Yes	Soybean
Three dominant plant species (in order):	*Triticum aestivum*	
Visual plant cover:	75% plant cover (30x30m plot)	
Picture	Attached	