



RhizeBio In-Field Rhizomicrobiome Sampling Protocol for Grasses and Cereals

Purpose: To collect plant rhizosphere samples from grass and cereal crops for assessing rhizomicrobiome health and functionality.

Sampling Criteria:

- 1) One plant tissue bag should be filled at least ~1/2 full of roots for each area and crop type sampled. Depending on the age of the plants, this could be as few as three large roots (for plants 'in boot' or heading) or as many as 20-25 small roots (for shoots and tillering plants).
- 2) Plants (or bunches of grass) should be randomly selected from different areas across the target field to create a composite sample that is representative of the conditions in the field
- 3) Use gloves when sampling, and rinse off digging tools between sampling in treated and untreated fields or rows.

Root Microbiome Sampling Methodology:

- 1) Randomly select plants across the desired field. Try to select plants that are representative of the conditions in the field as a whole (i.e., slope, soil texture, soil moisture), and ensure that the plants selected are spaced relatively evenly across the field. If plants are too close together to separate, dig up all plants within a small radius (~3-6") and collect the roots of all plants that are dug up.
- 2) Use a spade or shovel to carefully uproot selected plants, ensuring to dig deep enough to pull up the roots (collect as much of the root system as possible).
- 3) Shake soil off the root ball until the majority of soil has been separated.
- 4) Separate the roots from the rest of the plant at the base of the stalk, and place the roots into a plant tissue bag.
- 5) If necessary, use a spade to collect extra soil from the target field, to ensure that at least 2 cups of soil are collected for each condition.
- 6) Label the bags indicating the sample location (Name, Field ID) and whether the soil, roots, and leaves are from a treated or untreated area.

Documentation:

Fill out the RhizeBio Submittal Form, including the field ID, sample reference (indicating whether from treated or untreated area), collection date, coordinates, and additional information. The more information you can provide here, the better we will be able to help you interpret the results.

At harvest, please record the yield in both treated and untreated areas.



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Sample Storage and Shipping Guidelines:

- 1) Ideally, samples should be shipped as soon as possible following collection and spend no more than 5 cumulative days in transit.
- 2) If samples must be stored prior to shipping, samples should be refrigerated at 0-6°C (32-48°F), but not frozen to avoid damaging the microbiology. Samples should be shipped no more than three days after sampling.
- 3) Please freeze the included icepacks overnight before shipping, and place in the bottom of the cooler when shipping to keep the samples cool.

Shipping Instructions:

- 1) Ship the samples to the following address:

RhizeBio, Inc
235 Tuckaseegee Road, Suite A,
Mount Holly, NC 28120

- 2) For any questions, please contact:

Keenan Gerhart
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