

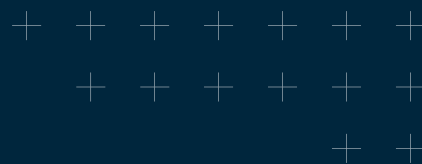
SOIL SAMPLING GUIDE





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INTRODUCING **TRACE GENOMICS**

At Trace Genomics, our goal is to provide high-quality, actionable insights to farmers, agronomists and ag retailers about the biological component of agricultural soils. We have developed the first analytics engine that learns as it maps the living soil, giving growers the tools to incorporate biology into soil management. Combining this with soil chemistry, physical properties and carbon allows customers to make comprehensive informed decisions. By providing a complete picture of the soil environment, Trace is a one-stop resource for informed soil management. This sampling guide aims to help you understand what our soil sample journey includes and how to collect a representative sample.



SOIL BIOLOGY: THE SCIENCE BEHIND IT

Soil Is a Diverse Environment

A teaspoon of healthy soil contains upwards of 1 billion microorganisms (aka microbes). Most of these are similar in size to silt soil particles: 0.002 - 0.05 mm. When Trace uses metagenomics to identify what microbes are in a field, how can you be confident that the soil samples you collect will show a complete picture? Independent academic research as well as investigations by Trace R&D have shown that agricultural soils with a homogeneous treatment across a field do not vary much in the factors that influence the microbiome.^{1,2,3} We also know that composite samples made up of multiple soil cores are sufficient for comparison between fields,⁴ which is why each soil sample is composed of 8-10 soil cores.

How Many Samples per Field?

While collecting soil samples in a high density across a field would provide the most biodiverse data, at Trace we recognize that this is neither economically feasible nor necessary for soil management decisions. Ideally, growers should collect three samples: one sample each in areas of high, medium and low productivity or within established, homogeneous management zones. This prevents missing a pathogen due to uneven pathogen spread and provides biological data that could explain variation in yield.

Does Field Size Matter?

For fields less than 40 acres, fewer samples may be required to capture the in-field variation. Conversely, larger (>160 acre) fields or those with variable management zones may require more than three samples. The Trace agronomist for your region is available to provide recommendations for your specific fields.

EXPLAINING THE PROCESS

A TraceVIEW account is needed in order to begin the ordering process. If you do not have an account, please contact support@tracegenomics.com

- 1. OBTAIN**—Request the sampling supplies you need from Trace Genomics and provide the address you want to ship them to.
- 2. ORDER**—Set up an account and create an order for the samples in TraceVIEW.
- 3. SAMPLE**—Collect your samples. See best practices and FAQ below.
- 4. SHIP**—Prepare samples for shipping and send to the Trace Genomics lab.
- 5. SEQUENCE**—Trace Genomics runs chemistry and sequences the DNA to understand what is in your soil.
- 6. ANALYZE**—The DNA we collect gets compared to our comprehensive database of soil borne organism genomes to identify and quantify the organisms in the sample.
- 7. REPORT**—Results are posted into your account and our agronomists will help you understand the data.

METAGENOMICS:

Sequencing all the DNA in an environmental sample.

MICROBIOME:

All the microorganisms living in a defined environment.

BEST PRACTICES FOR SOIL SAMPLE COLLECTION

NOTE: As samples are collected, keep in mind that the following information is required to start an order in TraceVIEW:

- Sample Name
- Latitude/Longitude
- Sampling Date
- Minimum/Maximum Depth (with Units)
- Current and Previous Crop
- Core Inner Diameter (with Units)

Labeling Sample Bags

Sample bags should be labeled with the sample name and field written on the order form.

For example:

Sample name on order form: #1

Write on bag: Soil #1, [Field Name]

Selecting Sample Locations

ANNUALS

- Follow grid, zone or composite approach.
- Collect samples from within rows and between plants.
- Collect at the same depth as for nutrient analysis when soil is not wet or frozen.

PERENNIALS

- Sample within the plant row, not in between rows. The goal is to sample around the roots and root hairs.
- Cores should be collected from the irrigation wetting zone, but we advise not to sample directly below an emitter and aim ideally between the trunk or vine and emitter.

Minimum Amount of Soil

500-700 grams

1 lb or 1.5-2 cups or $\frac{3}{4}$ of our sampling bag.

Size of Probe

Inner diameter: $\frac{3}{4}$ inch or 1 inch

Depth and Quantity of Cores

Collect and place cores directly into the sample bag until the bag is $\frac{3}{4}$ full.

If sampling at 0-6"

This is about 8-10 cores per sample.

If sampling at 0-8"

This is about 6-8 cores per sample.

If sampling at 0-12"

This is about 4-6 cores per sample (this depth not recommended for TraceCOMPLETE samples).

Area

Select at least 2 locations to sample per field. Record GPS coordinates for each sample.

GRID—go to the center point and go 30 ft radius and sample in a circle around center point.

ZONE—zigzag through the zone and collect cores quantity needed.

Soil Moisture

Moisture levels of 4-25% are ideal. Samples that are too dry can be difficult for analysis. Also avoid sampling soils that are waterlogged or above field capacity; wet samples are difficult for the lab to process.

Cleaning Probe:

We recommend cleaning your equipment with an alcohol solution after each field if possible.

Probe Lubricants:

Please avoid lubricants. Lubricants can impact the microbial communities and biological analysis.

Shipping Instructions

- Domestic prepaid FedEx labels are pre-filled with our shipping address: **615 S Bell Ave, Ames, IA 50010**
- Include your TraceVIEW submittal form with your order number in your package.*
- Include filler material (packing paper, etc.) to prevent sample bags breaking open.

***Failure to include order number in your package may result in a delay of results delivery.**

BULK DENSITY SAMPLING

IMPORTANT: Using the correct sampling method is critical to preserve the integrity of the soil qualities for measurement.

- Need undisturbed soil, not compacted soil!
- It is important to note the ACTUAL length of the core



What Is a Bulk Density (BD) Sample?

- BD is the weight of dry soil in a known volume.
- BD increases with compaction (is more dense).
- BD provides information on the suitability for root growth and soil permeability.

Why Is It Important to Sample Correctly?

- Incorrect sampling methods can cause compaction, which makes the BD appear higher than it is.
- Proper sampling is needed in order to get accurate measurements for core depth and diameter, or BD result will be incorrect.

Where to Collect?

- Between plants or near enough plants that there hasn't been compaction.
- NOT where a tractor has driven.
- Avoid waterlogged or over-dried areas.

How Should You Collect It?

Do NOT use:

- Standard sampling probe—this will not work! It causes soil compaction.

Ideal tools to use:

- Bulk density sampler like one from AMS.
- If you do not have access to bulk density samplers, please contact us for acceptable alternatives.

Characteristics of a Good Sample

- Not compressed

Typical parameters:

- 1.5-2" diameter
- 30cm depth

Approximate weight of cores (30cm depth):

- 1.5" diameter: 400-800g
- 1.75" diameter: 500-1100g
- 2" diameter: 700-1400g

Information We Need from You:

- Inner diameter of probe
- Actual depth
- Core depth

Additional Information



Kellogg Biological Station



Ohio State University Extension



Kansas State Extension



Video from Soil Health Institute

NEMATODE AND INVERTEBRATE SAMPLING PRACTICES

This information was adapted from SCN egg count samples from Iowa State University Extension & Outreach

When?

IN FALL—After harvest and before the soil freezes.

IN SPRING—Before planting, after the ground has thawed and drained. During growing season: from near stunted and/or yellow soybeans.

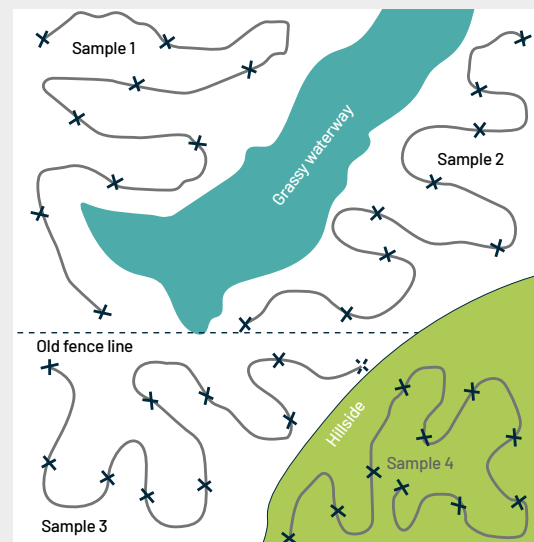
How?

Collect a soil sample according to the detailed instructions in our Soil Sampling Guide. Define the sampling areas within a field by agronomic, cropping history or other logical features (see figure) or divide the field to be sampled into evenly sized areas if conditions are similar throughout the field. Take care not to sample only from “hot spots” or areas of severely damaged plants. Collect soil from the top 8 inches directly in the root zone (if in season and soybeans are being grown).

Important Notes

- Soil samples should NOT be collected when the soil is wet or frozen.
- Samples should be collected from the root zone.

Nematode soil sampling patterns for crop fields with unique features.



SAMPLING EXAMPLE

Here is the recommended sampling process for an example operation of 2,500 acres containing 2 crops with 15 fields/crop:

- 8 total fields sampled
- 2 “good” fields Crop A
- 2 “bad” fields Crop A
- 2 “good” fields Crop B
- 2 “bad” fields Crop B
- (3 samples per field) × (8 fields) = **24 total samples**



STORING SOIL SAMPLES PRIOR TO BIOLOGICAL TESTING

Why Does It Matter?

At Trace Genomics, we provide actionable insights based on your soil microbiome. In order to provide the best guidance possible, we need to get the most accurate “snapshot” of the microorganisms in your soil. The problem with living organisms is that they grow. From the time that you take the soil out of the field, the microbes are exposed to a different environment, and they have the potential to grow (and die) in ways that are different from how they were living in the field. If we aren’t mindful about how soil is stored prior to arrival at the Trace lab, the microbial community can change, which can influence the biological analysis.

Trace scientists have studied the effect of storage time and temperature on the soil microbial communities, and their results align with the larger scientific community: the impact of short-term storage conditions on microbial communities is minimal. While care should be taken, if a soil sample will reach the Trace lab within 3 weeks of the collection date, it is not necessary to treat them differently. (Data available upon request.)



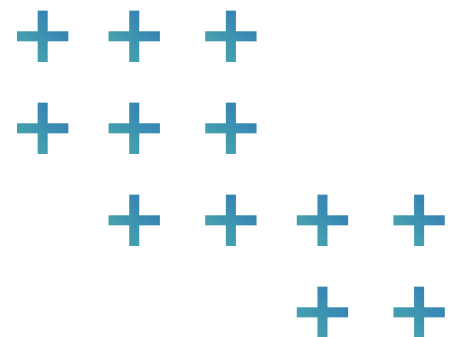
Common Concerns

- 1. TOO HOT**—If the soil sits in a bag in a hot warehouse for an extended period of time, certain species of microbes that may not have been abundant in the field have time to grow. When the DNA is sequenced, it could look like those species were more abundant in the field than they actually were.
- 2. FREEZE/THAW CYCLES**—When microorganisms are exposed to freeze/thaw cycles, this can break open their cell membranes and cause their DNA to break down. Not all microbes withstand freeze/thaw cycles equally, so it changes the structure of the microbial community and is reflected in Trace’s analytics.

Best Practices

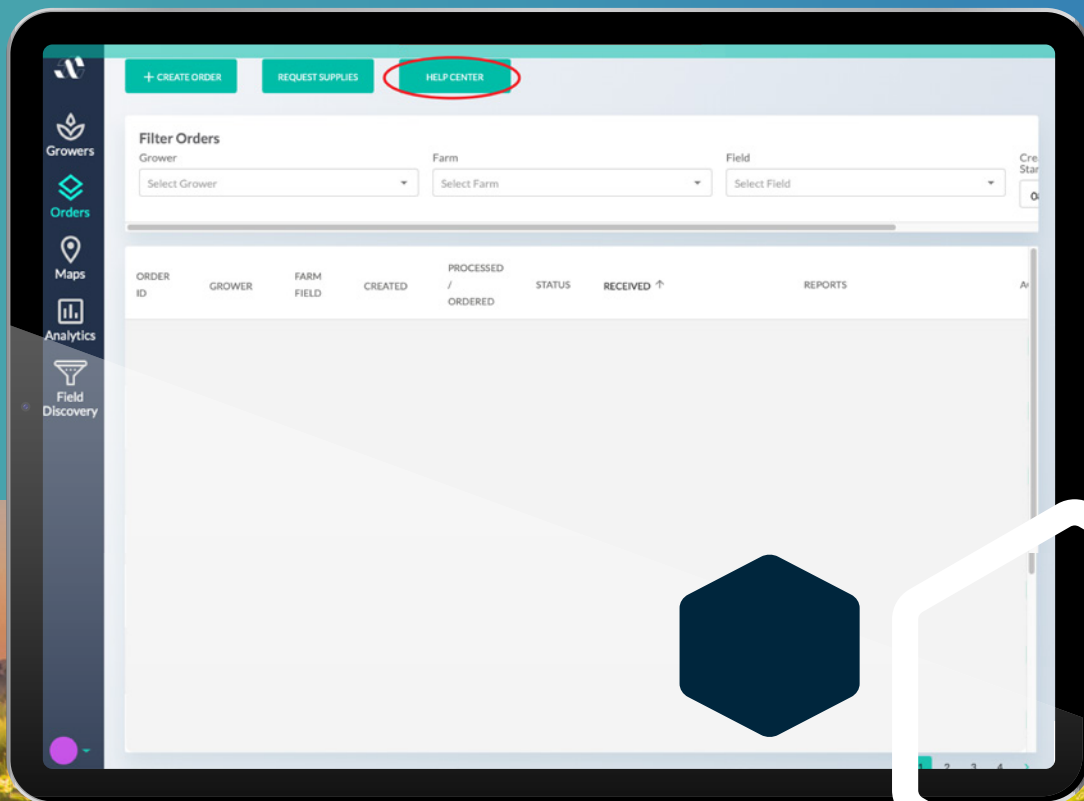
To maintain confidence in soil microbiome integrity, we recommend:

- Ship fresh soil samples at room temperature as soon as possible after collection.
- If it is necessary to store samples for an extended period (>3 weeks) prior to shipping, place samples in refrigerated conditions (not freezing).
- Avoid freeze/thaw cycles whenever possible.
- If you must ship frozen samples, include a data logger to track the shipment temperature.



Do you have a question that wasn't answered in this guide? We're here to help!

Additional supporting documentation regarding sampling practices can be found on TraceVIEW's Help Center, which is accessible through your TraceVIEW account.



To learn more about Trace Genomics and our data-driven solutions, visit our [website](#) or email us at info@tracegenomics.com.



Disclaimers: (a) As with most genomics-based technologies, these maps include detections of DNA evidence from other, closely related organisms. (b) The presence of the pathogen in high concentrations does not necessarily correlate with incidences of disease.