| Question | Answer |
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| **Sampling Questions** | |
| When is the best time to take a BeCrop sample? | Like most soil tests, the timing depends on the purpose of sampling. To inform in-season management practices, BeCrop samples can be taken before planting when the field is fallow to get an early soil health status snapshot. To evaluate the impacts of a management practice or biological program, BeCrop Tests taken in-season using a standard trial design will provide the strongest insights. |
| Can I take BeCrop samples in the winter or when temperatures are below freezing? | Samples can be taken during colder times of the year. Microbes reduce their metabolic activities and many go dormant in the cold, but are still present in the soil and can be detected by BeCrop. **However, sampling when soil temperatures are below 32 F (0 C) is not recommended.** |
| How often should I test? | The frequency of testing depends on your objective. Typically our customers will test 1-3 times per year to monitor soil health and make necessary amendments throughout the growing season, as needed.<br><br>You can start testing your soil as early as prior to planting until the end of the growing season, or post-harvest to prepare for the following year.<br><br>If you're unsure, you can reach out to our experts for advice on a testing cadence that works for your needs. |
| How many samples are needed in a field? | The number of samples needed within a field varies depending on sampling objectives. For a soil health snapshot or baseline as few as one sample can be taken by compiling composite samples across a field of uniform soil type and management practice history. If a field is split with **multiple soil types or management practices,** then each section should be sampled **separately** to account for these differences. **To gain deeper insights to inform management decisions, understand yield responses to soil biology, or track soilborne disease risk, at least three samples per field/management zone is required.**<br><br>To answer higher level research questions (i.e. how effectively |

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| | does a biological product improve nutrient cycling, do cover crops boost soil biology, how does compost application affect native microbes?) **It is most effective to take replicated samples across zones that are treated and untreated in a BeCrop Trial. This typically requires at least 12-18 total samples.** |
| Why do you need more samples for BeCrop Trials than BeCrop Test? | Multiple replicates are needed in order to conduct robust statistical analysis to confidently determine if changes in the soil microbiome occurred due to treatments or simply due to natural variability and other factors. **Three replicates are required at minimum, with additional replicates providing greater confidence in the conclusions of BeCrop Trial results.** |
| How long does it take to receive results? | Result delivery takes approximately 3 weeks. Lead times vary depending on the sample quality, number of samples, location, and shipping times. |
| How close to the rhizosphere should samples be taken? | When samples are taken in-season, pulling a soil core **as close to the rhizosphere (root zone)** as possible is recommended. That is because this is the most biologically active region of the soil and is where most plant-microbe interactions occur. |
| What are the storage requirements for samples? | Samples are recommended to be shipped as soon as possible after sampling with one-day shipping. They do not require any ice packs or cold storage if expected to arrive at the lab within 5 days. If needed, they can be stored at room temperature for several hours prior to shipping, but should not be stored in an excessively hot environment, such as a truck or car. Samples can also be stored up to 3 days at 0-6 °C (32 - 48 °F), and long term at -20 °C (-4 °F). |
| **How much soil is needed per sample?** | We require 10 grams of soil per sample (about ¼ cup). For BeCrop+ samples we require a minimum of 500 grams (2 cups). For a product sample, we require 25g or 25mL. |
| **How deep should I sample?** | We recommend sampling at a depth of **2-6 inches (5-15cm).** Most pathogens and microbes involved with nutrient cycling and the supply of plant growth promoters are found in the |

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| | rhizosphere, near the topsoil. For some perennial crops or to answer specific research questions, sampling at deeper depths may be appropriate. |
| **Do BeCrop metrics change throughout the season?** | **Yes,** as environmental variables change certain microbial populations may be promoted or may decline |
| When do you recommend pulling trial samples to evaluate biological product performance? | In trials evaluating the impacts of biological products, a baseline (T0) sample is initially taken before product application. We recommend taking the post-application set of samples at **10-20 days** after application. Any additional sampling time points can fall anywhere from **30-100+** days, depending on the cropping system, objectives of biological product evaluation, and what insights other field trials may have provided about the time frame of product impacts. |
| What conditions shall I avoid for the sample's collection? Waterlogged soils? Sampling after an ag input application? | Avoiding sampling when soil is waterlogged is recommended and as for the time after/before ag inputs, will depend on the client's interest and questions they want to answer in the test. |
| **Technical Inquiries** | |
| Can you identify specific species? | **Yes,** we can identify specific species of bacteria, archaea, and fungi. |
| Can you identify nematodes? | No, we cannot identify nematodes with our genomic sequencing process. However, we can measure levels of microbes that act against nematodes by functioning as nematicide agents. |
| Why don't you use a p-value of 0.05 in Trials? | In studies of microbiological systems, it is common practice to assess statistical significance at p-values or q-values ranging from **0.10 to 0.30, as we do in our BeCrop Trial reports.** |
| What's the difference between Amplicon and metagenomics sequencing techniques? | Amplicon sequencing is an effective technique for identifying microbes within a highly biologically active and diverse substance like soil. It is essentially a **biological "barcoding"** |

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| | technique that identifies microbes by **matching up genes that reliably code for specific species.** But amplicon sequencing does not provide deeper functional microbial data itself. That is why Biome Makers pairs it with our **functional database and ecological modeling to generate robust conclusions** on how microbes are functioning and interacting in the soil.<br><br>Meanwhile, metagenomics focuses on decoding the entire genome of microbes. This provides deeper data on the **functionality of specific microbes**, but is **not as effective at analyzing a wide scope of different microbes and their ecological interactions.** |
| How does the BMK pipeline work? | The Biome Makers pipeline consists of **two main steps**. The first step is the wet lab process in which DNA is extracted from soil and sequenced to identify microbial species. The second step is the dry lab process in which **BMK's proprietary functional database and ecological models generate data** that profiles the functionality of the species present in the sample, or in other words, **makes sense of what roles microbes play in the soil relevant to agronomics.** |
| Are BMK databases based on public or proprietary information? | The BMK microbial taxa database is curated through two publicly available databases: SILVA 138.1 for bacteria, and UNITE 8.3 for Fungi. |
| How are scores calculated in reports? | The ratings on BeCrop reports reflects how each sample compares to the BMK database of soil samples. The score is compared across samples in the database from only that specific crop type. **This allows us to provide more accurate and relevant conclusions.** |
| How can I use a report to make an agronomic recommendation? | Similarly to a chemical fertility soil report, BeCrop Tests can be used to identify areas of deficiency in the soil that can be addressed via **the use of various soil health promoting practices.** For example, if a BeCrop Test reveals low levels of potassium solubilization (supply), then a biological product that contains microbes that specialize in the solubilization and release of potassium **may help address this deficiency.** |
| What is functionality data and how do I use it? | Functionality data describes the specific roles that microbes play in the soil. **It describes the ecological roles through which microbes support plant growth, boost yield, and** |

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| | **promote nutrient retention in the soil, among many other benefits.** |
| Can you provide lists of the microbial species detected in each sample? | **Yes, by request** we can provide microbial species identity and abundance data in spreadsheet format. **We also offer free online tools to all clients via the BeCrop Portal that allow the specific microbial species to be explored and compared across samples.** |
| Do you offer chemical fertility testing as well? | We have agreements with several partnership labs in the US to offer chemical fertility tests in conjunction with BeCrop Tests. Services offered through our partners include **the Haney Test, Mehlich I/III, and total soil digestion.** |
| Do you do your own sequencing or outsource? | Our sequencing is done in house. |
| Can you detect soilborne pathogens? | **Yes,** we can detect most major bacterial and fungal soilborne pathogens **and we provide a risk level based on the pathogen abundance** and other factors that influence pathogen impact. |
| Do you provide any resources for result interpretation? | **Yes, we offer our BeCrop Guide**, which is a document providing layman definitions and basic guidelines for interpretation of the BeCrop report. **Our agronomy staff is also available for a short virtual consultation call to review sample results and address any technical questions**. Our BeCrop Advisor Program and online webinars, case studies, and blog articles also provide additional opportunities to learn how to interpret and leverage BeCrop results in agronomic practice. |
| How do you determine microbial functions? | Our microbial function metrics are determined by **a combination of published peer reviewed research findings and microbiome modeling based on machine learning** that assesses and predicts how certain microbes interact in the soil. |
| **Can we distinguish dead from alive DNA?** | We have validated in our lab a protocol that uses propidium monoazide (PMA) to capture extracellular DNA, which we can perform previous to the DNA isolation step. The taxonomic annotation of species in low relative abundance is |

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| | the most variable. Functional annotation, as well as ecological interactions among taxa that are also present in the BeCrop report, are less affected by the removal of extracellular DNA.<br><br>**However, for biomarker discovery/diagnostics applications we are of the philosophy that all signals present in the soil, from dead and alive microorganisms, are relevant.** Thus, our BeCrop reports are based on total DNA extraction and amplification. Samples are then compared to other samples derived from the same crop to normalize each of the markers into quintiles. So, we do not recommend removing extracellular DNA for BeCrop samples, given that interpretation is performed in the context of other samples for which we have performed total DNA extraction.<br><br>**For self-contained R&D projects where samples are compared to one another, we can definitely implement the PMA protocol.** Also, for our BeCrop Product report (absolute bacterial quantification of Ag-inputs) some of our clients request the removal of extracellular DNA for evaluating the viability of their products as well as their shelf life. |
| **What are the practical applications of the results?** | BeCrop Tests serve a wide variety of practical applications. **They can be used to identify and address problems involving soilborne pathogens, microbial nutrient mobilization, and crop stress tolerance. BeCrop Tests can also inform soil health focused management practices, evaluate biological agriculture inputs, and identify areas of potential improvement to bolster yield and reduce input costs**. |
| **Is BeCrop a quantitative or qualitative analysis?** | The BeCrop analysis is quantitative, and it is transformed later into a qualitative report. |

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| **Taxons can strongly differ according to the geographic location. How could you hold that comparing samples within your global database is a reliable indicator of the specific functions of the soil microbiome?** | Soil microbial taxons have a clear spatial distribution which is unique due to evolutionary factors, however **if based on the functions and ecological services we can compute functional metrics, these are the same for all taxons on the planet. What's the power behind this approach?** Some algorithms to predict purchasing behaviors might not know your name, but based on your network and historical purchase is able to predict the type of shoes you've been dreaming about for the last months. |
| **What does the F/B ratio tell us about? How can this index be interpreted?** | Bacteria, which have a lower C:N ratio than fungi, need food rich in nitrogen (e.g. green manure, legume residues). **A fertilizer with a low C:N ratio therefore favors the bacterial community in a soil, whereas a substrate with a relatively high C:N ratio enables growth of the fungal population.**<br><br>Due to their structure and C:N ratio between 7:1 and 25:1, fungi need a greater amount of carbon to grow and reproduce and will therefore 'collect' the required amount of carbon available for this from the soil organic matter. Bacteria, however, have a lower C:N ratio (between 5:1 and 7:1) and a higher nitrogen requirement and take more nitrogen from the soil for their own requirements. |
| **What's the main difference between arbuscular mycorrhizal fungi and ectomycorrhizal fungi?** | Arbuscular mycorrhizal fungi (the phylum Glomeromycota) first appeared early in the history of land plants (Remy et al., 1994) and hence they associate with plant species in diverse plant taxa (Schüβler et al., |

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| | 2001). They are obligate mutualistic symbionts and hence rely entirely on carbon supply from host plants (Smith and Read, 2008). While they are abundant in root systems of herbaceous plants (Hiiesalu et al., 2014), they are also hosted by diverse tree species (Liu et al., 2015).<br><br>Ectomycorrhizal fungi, which consist mainly of the phyla Ascomycota and Basidiomycota, appeared in the era of seed plant diversification (Hibbett and Matheny, 2009). In contrast to arbuscular mycorrhizal fungi, some of them may obtain carbon not only from plants but also from soil by decomposing dead organic matter. **Ectomycorrhizae are fungi that are only externally associated with the plant root, whereas endomycorrhizae (arbuscular) form their associations within the cells of the host.** |
| **Can you compare relative/absolute abundance with CFU/gr ?** | **No**, due to the differences in nature of genomic analysis compared to cultured based plating. |
| **Can you compare the BeCrop F:B ratio to that of the PLFA?** | **No, not at this time.** |
| **What type of correlation exists between BeCrop indexes and performance metrics like germination rate, root growth, nutrient use efficiency, etc?** | It would be imprecise to give an answer for specific nutrient results to predict germination rates or rates of growth for a specific crop as there are many other external factors that can affect this, like location and soil type, etc, but our technology can serve as an informative tool to predict yield or other rates if used in a trial manner, |

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| | as can be seen in this study: https://journals.asm.org/doi/10.1128/mSphere.00130-21 |
| Is there a direct relationship with pathogen load and soil moisture/temperature? | Depending on the organism, it is probable as these conditions encourage microbial growth, but it depends on their vital cycle. |
| How is biodiversity calculated at Biome Makers? | Phylogenetic entropy index, which **takes into account the phylogenetic relationship of all microorganisms in the sample.** |
| Q: At what level of resolution are you able to detect bacteria and fungi ? | A: We have a threshold we use to filter microbes in order to ensure they are coming from your soil sample and not any background contamination. As long as sample population levels are high enough to distinguish from potential ambient microbial loads, we detect them. |