EARTHFORT
635 SW WESTERN BLVD
CORVALLIS, OR 97333

Test Descriptions

Testing Overview:

We utilize a variety of testing methodologies to measure the abundance of life in Soils and Soil Amendments. Except where noted these tests do not identify specific organisms. Rather we measure the Biomass of Total Populations in general categories of the functional groups. The totals of these groups represent a snapshot of the biological profile at the time of testing. While each of these tests can be performed singularly, we have found that together they represent a comprehensive picture of the health and utility of the material tested. After the individual Assays are described, our most common packages will also be described.

Dry Weight:

This is a measure of moisture. Used for soils and solid amendments, such as compost, we determine how much of the material is dry matter. A higher number indicates low moisture, while a lower number indicates higher moisture content. The ideal range for this number is climate and crop specific. For all liquid amendments, this number is blank or reads one (1).

Bacteria – Active (AB) and Total (TB):

Measuring the Biomass of bacteria in a sample is the first step in understanding the health of a soil and the potential benefit of an inoculum or amendment. Total population of bacteria provides us with an indicator of abundance of food for predators, nutrient cycling capacity and general diversity of the bacterial population. We report this number as µg/g or µg/ml of biomass. The Active population is the component of the Total Biomass that is currently metabolizing oxygen; i.e., the functional fraction of the bacteria. The relative range of these two numbers varies based on crop type and season. When looking at inoculants the balance between Active and Total is important for two different reasons: In compost this balance needs to be below 10%, indicating a mature and stable material. In liquid inoculums, higher ratios are better for a foliar application. This high activity assists the organisms stick to the plant surface. For soil application of a liquid, this balance may not be as critical as they will become active in the soil environment.

Fungi – Active (AF) and Total (TF):

Fungi in the soil play an important role, nutrient retention and transportation, soil structure and its relationship to pH. Plant system succession is directly linked to the ratio of Fungi to Bacteria and is the first area we address when we approach remediation steps. Like bacteria, we report Biomass of Fungi in µg/g or µg/ml. Instead of counting individual populations, we measure length and width of fungi

present. Reporting this as biomass we can do direct comparisons of Fungi and Bacteria. When we observe and measure fungi we are looking at 3 primary things, total population, activity level (same basic method as Bacteria) and we look at the average diameter. Diameter not only helps us determine biomass, but in general terms, identifies whether the overall population is beneficial. On average diameter greater than 2.5µm is ideal, and helps in reporting relative diversity, the more diverse the fungi, the better.

Protozoa (PROTS) – Flagellates (F), Amoebae (A), Ciliates(C):

Measuring Protozoa is a bit different from measuring Bacteria and Fungi. Our method involves creating several dilutions of the sample and then correlating presence and absence of each group to create a Most Probable Number #/g or #/ml. Unlike bacteria and fungi, it can take up to 5 days to complete this test. Protozoa are typically single cell organisms that feed upon bacteria. Flagellates and Amoebae are true aerobes, meaning they must have adequate oxygen to survive, while Ciliates are Facultative Anaerobes, meaning they can survive in low oxygen conditions. Numbers of protozoa in are very important as an indicator of potential nutrient cycling, if there are sufficient levels of Flagellates and Amoebae then aerobic nutrient cycling can occur. However, high levels of Ciliates can be an indicator that anaerobic nutrient cycling is occurring. We use Ciliates to help identify potential anaerobic conditions in the sample.

Nematodes (NEM):

The process for identifying and quantifying Nematodes is relatively simple in function, but the results are often a very useful indicator of the health of soil. Nematodes are very important for the nutrient cycling they provide, similar to Protozoa. We report total number of nematodes per gram or ml in the sample, we then breakdown this total population to Genus and Functional Group. The Functional Groups are:

Bacterial Feeders: This group of Beneficial Nematodes feeds on bacteria, they help to keep the bacterial populations in balance and in the process of consumption cycle soluble nutrients in the root zone of the plants.

Fungal Feeders: As the name would suggest this group of Nematodes feeds on fungi, again, keeping these populations in balance and cycling nutrients in the root zone. Many of these types of Nematodes also feed on fungi that can cause disease in plants. Having a good population and variety of these organisms can be a valuable asset for the soil which we grow plants that are more susceptible to some types of fungal diseases.

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Predatory Nematodes: These Nematodes are specialized in eating other Nematodes; typically they prey on Root Feeding Nematodes and can help minimize the damage from them. This group will also consume Protozoa and some types of micro-arthropods. Again, this becomes an excellent source of nutrients for plants.

Fungal/Root Feeders: This is an interesting group of Nematodes, they typically act as Fungal Feeders, but if the population of Fungi is low, or if the right combination of plant and Nematode are present they will eat the roots of the plants. We use this group as an indicator for both healthy fungal populations and, at the same time, for potential disease issues in the plants.

Root Feeders: This is the group of Nematodes that is truly parasitic to plants, there is a wide variety of these types, and depending on the Genus and the plant being grown can be a real problem for production and health of the plant. As few as 1 root feeder per gram of soil can hinder productivity. As an indicator of soil health, this is a group to watch.

By looking at the total population, examining levels of functional groups, and cross-referencing to the plant being grown we can get a fairly good picture of productivity.

In Soil Amendment products, we also look for Nematodes, in liquids we typically find very few; they do not like pure liquid environments. In solid amendments, such as compost, we can find very high numbers of Nematodes, but usually very low diversity, despite the low diversity, compost is one of the best sources for Nematode Inoculants.

Nitrogen Cycling Potential #/acre

This is a value directly correlated to the Protozoa and Nematode levels in the sample. Assuming these organisms survive and there is an adequate level of nitrogen, oxygen and prey, this test result is the potential nitrogen, in pounds per acre that is cycled by these organisms in a 3-6 month period. This value can be very useful in determining efficient application levels of nitrogen fertilizer. This is NOT a measurement of the chemical level ,of nitrogen in the sample.

Mycorrhizal Colonization (MC), ENDO and ECTO:

Mycorrhizal Fungi are an important type of fungi that crates a symbiotic relationship with the root system of most plants, allowing for more nutrient and water uptake into the plant. We test for percentage (%) of colonization of two general types of these fungi, ENDO (inside the root) and ECTO (outside the root). Most plants require just one type, but a few types require both. It is important for us to know the type of plant roots we are testing to be sure we look for the correct type of colonization.

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There are other types of this fungi, most notably Ericoid, found on blueberries and rhododendrons, however this type we are unable to test for.

Escherichia Coli Bacteria (e. coli):

This is currently the only test we perform for finding and enumerating a specific organism. We use a plate count method and report in colony forming units (CFU) per gram or ml of the sample. This test is typically performed on soil amendments to identify potential health risks. Each State has regulations regarding safe levels of this organism for use on food crops and for material handler safety. We utilize an EPA approved method for testing e. Coli.

Packages and Reports:

All of our testing methods are scientifically validated and considered very important indicators of soil health and function. We have consolidated these methods into a cohesive package of testing to make the selection process easier. The Essential Biology Package includes AB, TB, AF, TF and Protozoa. The Advanced Package includes all of these tests plus NEM. Any additional testing, such as MC or e. Coli can be added as needed.

After testing we will provide a report that indicates the levels of each test found and a Desired Range based on the plant indicated by you. These Desired levels are considered optimal and seasonality, agronomic practices environmental factors can adjust these levels. After receiving your Report, we offer Consulting to help determine the best approach to remediate potential problems.

With over 10 years of working directly with clients, both in the field and through the lab, we have developed an expertise in the practical application of the science behind soil biology. No matter your circumstance, we can help you achieve your goals of a healthier more productive soil.

Thank you for your continued efforts!

Matthew Slaughter, Lab Director

Earthfort

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Active Bacteria References

Samples are prepared and stained with fluorescein diacetate (FDA is a substrate that binds and fluoresces to the metabolically active bacteria and fungi) and quantified using direct microscopy.

Ingham, E.R., and D.A. Klein. 1984. Soil fungi: relationships between hyphal activity and staining with fluorescein diacetate. *Soil Biology and Biochemistry.* 16: 273-278.

Ingham, E.R. and D.A. Klein. 1984. Soil fungi: measurement of hyphal length. *Soil Biology and Biochemistry.* 16: 279-280.

Schnürer J., and T. Roswell. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied Environmental Microbiology* 43: 1256-126.

Soderstrom, B.E. 1977. Vital staining of fungi in pure cultures and in soil with fluorescein diacetate. *Soil Biology and Biochemistry.* 9: 59-63.

Stamatiadis, S., J.S. Doran and E.R. Ingham. 1990. Use of staining and inhibitors to separate fungal and bacterial activity in soil. *Soil Biology and Biochemistry.* 22: 81-88.

Total Bacteria References

Direct enumeration of prepared samples is done using microscopy. Samples are prepared by using a FITC (fluorescein isothiocyanate) method.

Babiuk, L.A., and E.A. Paul. 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of a grassland soil. *Canadian Journal of Microbiology*. 16: 57-62.

Ingham, E.R. and K.A. Horton. 1987. Bacterial, fungal and protozoan responses to chloroform fumigation in stored prairie soil. *Soil Biology and Biochemistry.* 19: 545-550.

Ingham, E.R. 1994. Standard Operating Procedure for Total Bacteria. USEPA Global Climate Change Program. Corvallis Environmental Research Lab.

Van Veen, J.A. and E.A. Paul. 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. *Applied and Environmental Microbiology*. 37: 686-692.

Active Fungi References

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Schnürer J., and T. Roswell. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied Environmental Microbiology* 43: 1256-126.

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Stamatiadis, S., J.S. Doran and E.R. Ingham. 1990. Use of staining and inhibitors to separate fungal and bacterial activity in soil. *Soil Biology and Biochemistry.* 22: 81-88.

Total Fungi References

Direct enumeration of prepared samples is done using microscopy. The width and length is measured and converted to biomass.

Ingham, E.R. 1995. Standard Operating Procedure for Microbial Population Dynamics. USEPA Global Climate Change Program. Corvallis Environmental Research Lab.

Lodge, D.J. and E.R. Ingham. 1991. A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. Methods in Soil Ecology. Elsevier, The Netherlands.

Van Veen, J.A. and E.A. Paul. 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. *Applied and Environmental Microbiology*. 37: 686-692.

Protozoa References

Ciliates, flagellates and amoeba are enumerated by direct counting of serial dilutions of the sample using microscopy. Estimates of total protozoa are calculated using the most probable number approach.

Darbyshire, J.F., R.E. Wheatley, M.P. Greaves, and R.H.E. Inkson. 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. *Ecology* 61: 764-771.

Ekelund, F. 1998. Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. *Soil Biology and Biochemistry* 30:1343-1347.

Ingham, E.R. 1993. In Bottomley, P. (ed) *Methods in Soil Agronomy*. Agronomy Society of America, Madison, WI.

Ingham, E.R. 1995. Standard Operating Procedure for Protozoan Population and Community Structure. USEPA Global Climate Change Program. Corvallis Environmental Research Lab.

Lee, J.J., S.H. Hutner, and E.D. Bovee. 1985. *An Illustrated Guide to the Protozoa.* Society of Protozoologists, Lawrence, Kansas. 629pp.

Singh, B.N., 1955. Culturing soil protozoa and estimating their numbers in soil. In D.K. Kevan, Ed. *Soil Zoology.* Butterworths, London, UK, pp. 104-111.

Stevik, K., J. F. Hanssen, and P. D. Jenssen. 1998. A comparison between DAPI direct count (DDC) and most probable number (MPN) to quantify protozoa in infiltration systems. *Journal of Microbiological Methods* 33:13-21.

Nematode References

Nematodes are extracted from the sample using an enhanced Baermann funnel technique. The nematodes are then identified to genus and counted using direct microscopy.

Anderson, R.V. and D.C. Coleman. 1977. The use of glass microbeads in ecological experiments with bacteriophagic nematodes. Journal of Nematology. 9: 319-322.

Baermann, G. 1917. Eine einfache methode zur Auffindung von *Ankylostomum* (Nematoden) Larven in Erdproben. *Mededelingen uit het Geneeskundig Laboratorium te Weltevreden* 41-47.

Bongers, T. 1988. De nematoden van Nederland. Pirola Schoorl. Natuurhist. Biblioth. KNNV nr. 46. Wageningen Agricultural University, The Netherlands.

Goodey, T. 1963. Soil and freshwater nematodes. John Wiley and Sons, New York, NY. Second edition revised by J.B. Goodey.

Ingham, E.R. 1995. Standard Operating Procedure for Nematode Population and Community Structure. USEPA Global Climate Change Program. Corvallis Environmental Research Lab.

Mai, W.F. and H.H. Lyon. 1975. Pictorial key to genera of plant-parasitic nematodes. Fourth edition, revised. Comstock Publishing Associates, Ithaca and London. 219 pp.

Mycorrhizal Colonization References

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Roots are collected from soil samples, cleared in KOH and stained with trypan blue. Using direct microscopy, prepared roots are examined for presence of any mycorrhizal structures i.e. hyphae, arbuscules, vesicles or internal spores in each centimeter and the percent colonization is calculated.

Allen, M.F., et al. 1992. Mycorrhizae and the integration of scales: From molecules to ecosystems. Pp. 488-515. Mycorrhizal Functioning. Chapman and Hall, New York, NY.

Biermann, B. and Linderman, R.G. 1981. Quantifying vesicular-arbuscular mycorrhizae: a proposed method towards standardization. New Phytologist 87:63-67.

McGonigle, T.P., et al. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytology.* 115: 495-501.

Newman, E.I. 1988. Mycorrhizal links between plants: their functioning and ecological significance. *Advances in Ecological Research.* 18: 243-270.

Rajapakse, S. and Miller, JCJ. 1992. Methods for studying vesicular-arbuscular mycorrhizal root colonization and related root physical properties. In: Norris JR, Read DJ a Varma AK, [eds.] *Methods in microbiology. Volume 24: Techniques for the study of mycorrhiza*. Academic Press, London, pp.301-316