SOIL MICROBIAL BIOMASS SERVICE

SOIL SAMPLE SUBMISSION

Collecting soil samples:

1. Define the area you are interested in. Define the specific question you want to answer.

a. Define the area, for example, the tomato part of the garden, a field of ryegrass, an orchard, a forest stand. Be clear in your mind where the boundaries are.

b. In most cases, you will want to compare one kind of management in one place with another management in a second area. For example, does kelp meal or ammonium nitrate grow better tomatoes in your garden? You would want to sample the kelp meal treated part and compare it to the ammonium nitrate fertilizer portion. Consider whether the area was uniform before you started. If you put kelp on a sandy soil, and the inorganic fertilizer on a clay soil, the answers you get might show differences, but won't help you determine which is better for the sandy soil, or a third soil type.

2. Consider the types of variation within the area(s) you are sampling.

a. There will be changes in soil organism numbers when moving away from the stem of a plant to areas with no plants, or areas with different plant species. Bare soil will have different soil organisms than areas with litter on the surface. Areas under old logs will have different organisms than areas under one- or two-year-old logs. Samples need to be collected with an awareness of what will cause more or less variation.

b. Choose the type of place to sample based on the question you want to answer. For example, if you want to know if soil in your tomato patch contains a typical set of organisms for tomato, you might want to randomly sample throughout the tomato area. This would tell you about the whole tomato area. On the other hand, if you want to know whether soil around the plants contains normal numbers of organisms, you might want to sample a constant distance from the tomato plants. A sample taken from near several sick plants needs to be compared to soil from near several healthy plants. Soil taken only from near plants will have less variation than when soil from between rows is included as well. You need to consider what you really want to know.

Another example: Agricultural systems are much more "homogeneous" (uniform) than other kinds of ecosystems. Forests have great heterogeneity, because much less mixing occurs in them than in most grasslands or agricultural areas. In forests, consider that tree distribution is not uniform, that understory plants impose more variation, that the litter, woody debris like branches, twigs, and logs cause a great deal of variability. When sampling in a forest, some care should be taken to reduce the inherent variability within the system. In general, people compare one kind of management with another, so they define a clearcut area and compare it to a thinned area, for example, or an area treated with herbicide with an area not treated with herbicide, or a skid trail compared to a not-compacted area. Random samples taken within these defined areas will result in information with wide variability because samples will come from under logs, next to fir trees, next to maple trees, next to weeds, next to

flowers, from bare patches, etc. This type of variability may mask the effects of management. Thus, taking samples only from within three feet of the dominant tree, only in bare patches, only in places where the soil is covered by litter, only under N-fixing shrubs, or only within an inch of thistle, would reduce variability, and perhaps allow the effect of fertilizer application to be seen. You have to choose what sampling is best to answer the question being asked.

3. In general, numbers of organisms are greatest at the surface of the soil and decrease in a known fashion with depth. There will be organisms in the soil all the way down to bedrock, and even into cracks and crevices in bedrock. However, since the depth distribution is already fairly well known, we suggest the number of samples could be reduced by taking samples only from the surface 0-5 cm (0 to 2.5 inch) depth. If litter is lying on all surfaces, then the litter layer could be sampled instead. But, if any area has no surface litter, then that area couldn't be included. Thus, we recommend sampling from the surface of the soil (remove the recognizable plant debris layers, and sample starting there). The main thing is to be consistent, however.

4. Bulk or lump-together many small (half to one inch diameter) cores.

   a. Mentally divide the area, given that it has been defined (see #1 above), into three equal sections. From the first third of the area, decide whether samples will be taken from specific types of places, or randomly without regard to surface factors that could affect soil organism numbers. Decide where samples will be taken, and then as samples are taken, group all the small soil cores from similar areas into the same plastic, zip-lock baggie. Generally, send enough soil in a plastic baggie to fill a good size coffee mug. Label the outside of the baggie with a permanent marker (black marker works best) with information about where these soil samples came from.

   b. Repeat in the second third, and the last third of your area, putting each in a separate baggie. From each area, you should now have three samples so the general variability within that area can be assessed. If you want to know about specific variability within the area, keep each soil core separate in its own plastic baggie. However, it could be expensive to analyze all the cores individually.

5. Shipping

   a. Keep samples cool. Do not freeze or allow to sit in the sun or in high temperature. The organisms in the soil are like people in many ways - high or low temperature, lack of air, compaction, and other stress will alter the their numbers and activity. Once soil is taken from the field, the numbers and activity of the organisms start to change. That's why the samples need to be sent within 24 hours by overnight, express mail if activities, protozoa or nematodes information are desired. If total bacteria, total fungi or mycorrhizal colonization of roots are the only assays being performed, second day mail, or surface mail (with blue ice to keep samples cool) is acceptable.

6. Fill out the following form. You can call Merline Olson or Nadine Wade at the lab (541) 737-5253 for help or technical advice.

Information for the Soil Microbial Biomass Service:

Date Collected _____

Name of Collector _____

Address _____

_____

_____

Phone:_____ FAX: _____

Please indicate to whom the bill or data should be sent, if different from above.

Ecosystem: (answer each to the best of your ability)

Type of plants: _____
Type of soil (circle) Sand Silt Clay
Slope? _____ Hilltop Mid-slope Foot Swale?
Soil series name (if known)? _____
High or low organic matter? Give % if known _____
Plant litter present? _____ Depth of litter? _____

History (over the last year or longer - attach separate page if necessary)
Typical crop rotation?
Fertilizer use?
Pesticides used?
Herbicides?
Cover crops? Compost?
Irrigation? Other management?

ASSAYS TO BE PERFORMED? On all samples?

Active Bacteria ($5/SAMPLE) _____ Total Bacteria ($10/SAMPLE) _____

Active Fungi ($5/SAMPLE) _____ Total Fungi ($10/SAMPLE) _____

Protozoa ($30/SAMPLE) _____ Nematodes ($15.SAMPLE) _____

VAM ROOT colonization ($25 PER ROOT SYSTEM) _____

VAM spores ($25/SAMPLE) _____

Interpretation of data _____ (requires at least three or more replicate samples from any area) and a list of what the samples are.

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Address: Dr. E.R. Ingham, Soil Microbial Biomass Service
Cordley 2082, Department of Botany and Plant Pathology
Oregon State University, Corvallis, OR 97331-2902

CALL AHEAD TO LET US KNOW YOUR SAMPLES ARE COMING! IF YOU DON'T CALL, YOUR SAMPLES MAY BE RELEGATED TO THE "BOTTOM OF THE PILE"!

Soil Microbial Biomass Service: Interpretation of Microbial Information

The Biomass Service provides information on total and active bacterial biomass, total and active fungal biomass, protozoan numbers, nematode numbers and community structure and vesicular arbuscular mycorrhizal (VAM) spore numbers per gram of soil, and percent VAM colonization of roots.

Soil sampling should result in three samples from any particular area (field or forest stand for example), such that an understanding of the variability of that area can be assessed. We suggest that the area to be sampled be mentally split into three areas. From each of the three areas, between three and ten small soil cores (0-5 cm depth, about 2.5 cm or 1 inch diameter core) should be mixed together in a plastic bag, and about 50 grams of soil (the amount of soil a typical coffee cup would hold) removed from this mixture and mailed by express, overnight mail, to the Soil Microbial Biomass Service. We would appreciate receiving information on the field (soil type, vegetation type, past history of use of the field, recent pesticide use, cropping practices) so this information can be put into the database we are developing on soil foodweb structure in soils and vegetation types from all over the world.

The measures outlined below can be performed on any kind of material, from lake sediment, to rumen material from cattle. However, our expertise in interpreting the information is in soil-related material, including logs, litter, surfaces of plants, and sewage-sludge.

Ratio of total fungal to total bacterial biomass
In work with the structure of the soil foodweb in many different soils, we have discovered that all grassland soil and most agricultural soils have ratios of total fungal biomass to total bacterial biomass of less than one. In other words, the bacterial biomass is greater than the fungal biomass in these soils.

However, in instances where agricultural production is high, the ratio of total fungal to total bacterial biomass is about one, or the biomass of fungi and bacteria is about equal. When agricultural soils become fungal-dominated, productivity is reduced, and in most cases, mixing of the soil (plowing) is needed to return the system to a bacterial-dominated soil.

All conifer forest soils are fungal dominated, and the ratio in all forest soils in which seedling regeneration occurs is above 10. In general, productive forest soils have ratios of fungi to bacteria of greater than 100. This means that fungal biomass greatly outweighs that of bacteria in forest soils. In the case where forest soils lose this fungal-dominance, it is not possible to re-establish seedlings in the soil.

However, in the few studies of riparian soils that have been performed, some deciduous riparian forest soils are bacterial dominated. In the case of riparian aspen and beech soils, the soils are bacterial-dominated, although poplar, oak and maple systems, the soil has more fungal biomass, although not to the extent as in conifer systems. No studies on establishment of seedlings in these systems have been performed.

However, the ratio of total fungal to total bacterial biomass is not the only thing that needs to be examined. The numbers or length of active and total bacteria and fungi are also indicative of the health of soil, although these values must be determined relative to the vegetation present in the system, just as the optimal ratios of total fungal to total bacterial biomass are different for different vegetative regimes.

Biomass of total fungi
Fungal biomass is extremely important in all soils as a means of retaining nutrients that plants need in the upper layers of the soil, i.e., in the root-zone. Without these organisms to take-up nutrients, and either retain (sequester) those nutrients

in their biomass or in soil organic matter, nutrients would wash (leach) through the soil and into ground or surface water. Thus, nutrients need to be first immobilized in the soil through the action of fungi, bacteria and soil arthropods.

Fungi sequester most of the nutrients in forest soils, although significant portions are immobilized by bacteria as well. In soil in which only fungi are present, a few studies indicate that the soil will become more acidic, as fungi produce secondary metabolites. In addition, soil aggregates will be larger than in bacterial-dominated soils, and the major form of soil N will be ammonium. Bacteria are more important in grassland and agricultural soils than fungi and thus bacteria are the more important processors and "retainers" of nutrients in grassland systems.

Total fungal biomass varies depending on exactly which soil type is being considered, as well as the vegetation type, organic matter levels, recent pesticide use, soil disturbance and a variety of other factors, many of which have not been researched completely. However, for normal grassland soils, total fungal biomass levels are usually around 50 to 500 meters per gram of soil. For agricultural soils, fungal biomass is around 1 to 50 meters per gram soil, while for forest soils, fungal biomass is between 500 to 60 km per gram of soil. Quite a bit more work is necessary to establish what the optimal fungal biomass value should be for each type of crop, soil, organic matter, climate, etc. Very little information is available for tropical systems, but that small amount of data indicates that temperate systems work very differently from tropical soils.

The average diameter of hyphae in most soils is about 2.5 micrometers, indicating a typical mixture of zygomycetes, ascomycete and basidiomycetes species present in the soil. On occasion the average diameter may be greater than 2.5 micrometers, indicating a greater component of basidiomycetes hyphae, when the average diameter of hyphae is less than 2.5 micrometers, this indicates a change in species composition to a greater proportion of lower fungi. In most cases, actinomycetes are not differentiated from fungi, since they are hyphal in morphology and are rarely represent a significant proportion of the biomass. However, in some agricultural soils, these narrow diameter "hyphae" are of considerable importance, as demonstrated by Dr. Van Bruggan following cover cropping in some soil types.

Biomass of active fungi
There is a typical seasonal fluctuation of active fungal biomass in all systems. This cycle seems to be related to optimal temperature and moisture, such that a peak in activity usually occurs in the spring as temperature and moisture become optimal after the winter freeze. In systems where moisture becomes limiting in the summer, activity may reach levels the lowest levels of the whole year, even lower than in frozen soil. When temperatures remain warm in the fall and rain begins after the summer drought, a second peak of activity may be observed. In systems where snow insulates the soil, fungal activity be at the highest levels of the entire year, if adequate organic matter is available. Decomposition may have the highest rates through the winter under the snow. If these peaks of activity are not observed, this suggests inadequate organic matter in the soil.

Numbers of total bacteria
Just as fungi are the important players in retaining nutrients in forest soil, bacteria are the important players in agricultural and grassland soils. Bacteria retain nutrients first in their biomass, and second, in their metabolic by-products. In soil in which only bacteria are inoculated, the soil will become more alkaline, will have small aggregates, and generally will have nitrate/nitrite as the dominant form of N. These conditions are beneficial for grasses and row crop plants.

Numbers of total bacteria generally remain the same regardless of soil type or vegetation. Total bacterial numbers range between 10 million and 100 million per gram soil. Bacterial numbers can drop below 1 million in semi-arid agricultural soils. Bacterial numbers can be above 100 million in decomposing logs, in anaerobic soils, and in soil amended with sewage sludge or with high amounts of composted material. In some instances following pesticide treatment, bacterial numbers can

fall to extremely low levels, below 100,000 per gram of soil. In this situation, crop productivity can be quite low and can even show signs of nitrogen deficiency.

Biomass of active bacteria
As with active fungal biomass, bacterial activity usually peaks in the spring, and decreases during the summer with drought. If the temperature remains warm in the fall, and fall rains begin, a second peak of activity usually occurs. The ratio of active fungal to active bacterial biomass, even in forests, shows that bacterial biomass is usually more active than fungal biomass.

Protozoan numbers
Protozoa feed on bacteria, and as they feed on their prey, N is released. It's unclear just how much N is released per individual feeding event, since it undoubtedly depends on whether the bacterium was actively growing, thus containing more N, or whether the bacterium is in stationary phase, or starving and containing much less N. Several studies have shown that a major portion of the nitrogen that cycles through in certain agricultural soils is cycled by protozoa. Without these organisms in soil, plants may suffer a significant reduction in available N. However the optimal relationship between the number of bacteria and the number of protozoa has not been quantified.

There appears to be a great range in protozoan numbers from soil to soil, and even from field to field. Some of the observations that have been made, when dealing with agricultural soil (i.e., bacterial-dominated) is that when protozoan numbers are high, bacterial-feeding nematode numbers will be low, and vice versa. Thus there appears to be significant competition between bacterial-feeding predators for the bacterial prey. Whether this is indicative of the type of bacteria present in the soil, and whether this has any relationship to productivity in agricultural situations is not known.

Testate amoebae are only found in significant and constant numbers in forest soils, and are never found in temperate agricultural soils. Why this is the case is not known, but continues to be observed.

John Cairns (VPI) suggested protozoa as indicators of ecosystem health, based on the rapid return of certain "opportunistic" species of protozoa following certain types of disturbance, while other species of protozoa are typical of less disturbed systems. While most of Cairns work has been performed in aquatic systems, the same concepts have been suggested for soil.

Nematode numbers, community structure
There are four major types of nematodes, which includes bacterial-feeding, fungal-feeding, root-feeding and predatory nematodes. All nematodes are predators, and thus reflect to some extent the availability of their prey groups. However, other organisms prey upon these nematodes as well, and thus nematode numbers can also reflect the balance between the availability of nematode prey, as well as feeding by nematode predators.

Both bacterial-feeding and fungal-feeding nematodes mineralize N from their prey groups. Thus, bacterial-feeding nematodes are more important in bacterial-dominated soils (agriculture and grassland systems), while fungal-feeding nematodes are more important in fungal-dominated soils (conifer and most deciduous forests). Between 40 and 80% of the nitrogen in rapidly-growing crop plants has been shown to come from interactions between bacteria or fungi and their nematode predators. Thus, the presence and numbers of bacterial- and fungal-feeding nematodes is extremely important for productive soils.

Root-feeding nematode numbers can be affected by competitors for roots, including VAM fungi which may prevent root-feeding nematodes from reaching the roots through a variety of mechanisms, nematode-trapping fungi, and other fungi and bacteria that may be active inhibitors of nematode presence in the rhizosphere.

Dr. T. Bongers, from the Netherlands, suggested the use of a Maturity Index for nematodes in soil. Certain species of nematodes are more commonly found following disturbance, while other species are more typical inhabitants of less-disturbed soils. Thus, these organisms may be excellent indicators of soil "health".

VAM spore numbers

Vesicular-arbuscular mycorrhizal fungi are critically important for all crop plants, except a few species of the brassica family (e.g., mustards). A number of researchers have shown that the lack of VAM inoculum, or the lack of the appropriate inoculum can result in poor plant growth, in poor competition with other plants or inability to reproduce or survive under certain extreme conditions. However, most crop fields have adequate VAM spores present, especially if crop residue is turned back into the field. Only under a few situations, of intensive pesticide use, fumigation, or intense fertilizer amendment, will VAM spore inoculum become so low that plant growth will be in jeopardy. In restoration studies, the lack of appropriate inoculum is more likely to be a problem than in other situations where sources of appropriate VAM spores are near-by. Thus, the presence of at least 1 to 5 spores per gram of soil is more than adequate for most crop fields. When the number of spores falls below one per gram, then addition of compost containing high numbers of VAM spores (for example from an alfalfa field, or other legume), or inoculation of VAM spores from a commercial source generally results in positive effects. Recent work shows that the correct VAM species for the particular plant species must be present to attain the best plant yields.

Percent VAM colonization

At least 12% of the root system of grasses, including most crop plants, should be colonized by VAM in order to obtain the minimum required benefits from this symbiotic relationship. In most cases, colonization upwards of 40% is usually seen. In these cases, VAM colonization can limit root-feeding nematode attack of root systems, if the nematode burden is not high. More knowledge of the relationship between plant species, VAM species and soil type, including fertility, is needed to fully predict the optimal relationship between crop plant, VAM species and soil.