Soil Enzyme Interpretation Guide Rev. 1.0

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In all soils, soil microbiology is responsible for creating and releasing soil enzymes to cycle nutrients and to form and decompose organic matter in the soil. The continued promotion of soil health practices (i.e. no-till, diverse cropping systems, organic amendments, etc.) has created an interest in earlier detection of soil management effects through changes in soil microbiological activity. Microbial activity can be reflected in enzyme production, activity and stabilization within the soil. In many cases, soil enzyme activity can respond quickly (i.e. within 2 years) to changes in soil managements when compared to other physical and chemical properties, thus supporting the use of enzyme activity as a "bioindicator" of soil health.

Interpretation of soil enzyme activity requires an understanding of nutrient and organic matter cycling. Often, healthy, active systems have higher enzyme activity, relating to better cycling of nutrients and organic matter quality compared to degraded soils. Below is a list of common soil environmental and soil management characteristics that are often studied. The overlapping impact the following soil factors and soil management strategies have on soil microbial activity causes interpretations to be tied to a singular factor. Thus, this interpretation guide is presented with generalized trends of soil enzyme activity responses to a few soil physical and chemical characteristics as well as soil management practices. This is by no means an exhaustive list as the impact of any desired goal in soil management can be tested.

Soil Environmental Factors

The soil is often a hostile environment from a microbial perspective. Although enzyme production begins within the cell, extracellular enzymes are exposed to denaturing, predation and adsorption in the soil environment that can inhibit enzyme activity. In addition, the influences of temperature, moisture and pH affects the rate, concentration and structure of an enzyme while the presence of clay and organic matter can provide more adsorption sites and food sources for continued activity for years to come. Increasing water, temperature and soil organic matter will change microbial numbers and community composition while accelerating the activity of enzymes. A few soil physical and chemical characteristics, outlined below, are important to note when understanding and interpreting soil enzyme activity.

Soil Organic Matter

Soil organic matter (SOM) is the portion of soil that contains the decomposed products of plants, fauna and microbial biomass. This combination of organic compounds is synthesized by microbial activity and serves as an important source of nutrients and erosion prevention while increasing water holding capacity and soil aggregation. It is also an important habitat for soil microbiology. It is considered a stable portion of soil with only a small portion being mineralized (≈5%) under natural conditions. Soil organic matter mineralization and immobilization is strongly impacted by soil microbial activity. Soil microbial communities can decompose a wide range of plant and animal compounds, allowing accumulation of microbial residues to contribute to SOM formation and stabilization. Exposure of starved microbial communities to newly available food sources in the soil (e.g. incorporating crop residues through tillage) can temporarily increase enzyme activity and continued practice can cause decreases in SOM. This frenzy of activity can be easily noted by the earthy smell of tilled soil. In addition, fluctuation in SOM content due to changes in moisture, temperature and oxygen are also impacted by soil management decisions. In general, higher percentages of SOM support larger microbial populations, and thus contain higher enzyme activity. Enzymes can also become adsorbed on soil humus, the stable portion of SOM, supporting continued activity for several years.

Clay Particles

Clay particles consist primarily of microscopic plates that are stacked in a somewhat random manner in the soil. This plate-like structure has a high surface area and net negative charge that greatly influences the physical and chemical properties of soil such as the water and nutrient holding capacity while playing an important role in the adsorption of extracellular enzymes. Once adsorbed on the clay particle surface, the enzyme becomes protected from microbial degradation and environmental stressors. The shape of an enzyme allows for only one active site, or the site where a substrate is acted upon, and depending on how the enzyme is adsorbed to the clay particle, can determine whether the enzyme can act on any further substrates. Although the orientation of the adsorbed enzyme can cause the enzyme to become temporarily inactivated, many of the enzymes will continue contributing to the cycling of soil nutrients for years. The continued activity of adsorbed enzymes supports the use of enzymes as better indicators of long term impacts of soil management decisions. Often, soils with a higher clay content can support more stabilized enzymes and may have higher enzymatic activity.

Soil pH

Soil pH typically reported on a soil test represents the average concentration of hydrogen (H^+) ions within the soil solution. This measurement, sometimes referred to as bulk pH, can provide valuable information for predicting potential microbial reactions and enzyme activities in soil. Although this measurement is an important overall indicator of the soil pH, localized microsites within the soil can be drastically different than bulk pH readings. Shifts in pH can occur near soil colloids, roots and organic matter in response to biological activity and chemical reactions. Plants and soil microbes can alter the soil environment to free soil nutrients or as a defense mechanism against potential predators or competitors. These pH changes, known as microscale pH, can occur within a few micrometers of these sites but drastically change SOM decomposition, pesticide performance and the solubility and availability of plant nutrients within the surrounding area.

Soil pH is considered a universal regulator of soil enzyme activity. Each soil enzyme has an optimal activity over specific pH values because pH impacts the shape and structure of the enzyme and influences substrate availability. Within this range, the enzyme can effectively act upon the substrate and release nutrients. Beyond this range, changes to the enzyme structure begin to impact productivity. Extreme changes in pH can permanently alter an enzyme's structure, known as denaturing, and prevents an enzyme from further acting upon any substrates within the soil environment. Soil enzymes within the soil solution are at a higher risk of denaturing from temperature and pH shifts than adsorbed enzymes because adsorbed enzymes adopt a structural adaptation that prevents denaturing. This allows continued enzyme activity even during changes in microscale pH. The pH optimums of adsorbed enzymes can be 1-2 pH units higher than enzymes in soil solution. For example, absorbed soil urease enzymes have a pH optimum of 8.5-9.0, which is about 1-1.5 pH units higher than urease activity in the soil solution. In the laboratory, soil enzyme assays are carried out under pH optimums that produce the highest potential activity for the selected enzyme. The optimum pH for β-glucosidase (BG), urease (UR), phosphodiesterase (PHD) are 6.0, 7.5-8.8, and 8.0 respectively. These optimums are based on microsite pH and should not be confused with bulk pH, although drastic changes in bulk pH can strongly impact microsite pH. For ideal conditions, bulk pH should still be in the desired range of pH of 6.0- 7.2 for most crops.

Soil Temperature

As soil temperatures rise, chemical and enzymatic reaction rates within the soil increase, causing a rapid rate of microbial growth and enzyme production. The production and distribution of enzymes contributes to faster residue decomposition and cycling of nutrients within the soil. Changes in soil temperature are dependent on factors such as climate, season, soil type, plant and residue cover, soil water content and soil organic matter content. Increases in temperature, in addition to moisture, can lead to an increase in soil enzyme production and excretion into the soil environment promoting decomposition and the release of plant available nutrients. Soil temperatures can experience drastic changes seasonally, and even daily. Fortunately, fluctuations in temperatures have caused native soil microbiology to create unique adaptations to survive under harsh conditions. One such adaptation is the ability of microbes to modify the structure of extracellular enzymes to withstand the natural temperature extremes of the ecosystem. Although some soil enzymes may have greater flexibility in the optimal temperature ranges, most soil enzymes still have similar optimal temperature for maximum effective activity. Enzymes active in decomposition (β-glucosidase) and nutrient cycling (urease, phosphodiesterase) are measured in the lab at 98.6°F (37°C).

Seasonal changes in temperature can alter relative rates of decomposition and cause a variation in enzyme activity. Thus, sampling times for soil enzyme activity should be taken when temperature extremes are at a minimum. For most areas, this would be in the fall or spring. Outside these stable temperature periods, shifts in temperatures, moisture availability and substrate availability can impact enzyme activity and cause improper interpretation of enzyme results.

Soil Depth

Changes is soil management strategies, soil use, or disturbance are often reflected in the root zone because of the interaction of plant roots and soil microbiology. Plants release readily available carbon-based nutrients to encourage soil microbiology to become established in and around the root zone. The close proximity of plant roots to soil microbiology, and the activity of soil enzymes, allows the release of mobile nutrients to supplement both soil microbiology and the plant via the root. The symbiotic relationship between the plant and microbes creates an active zone within the roots that are sensitive to changes in the soil environment. Enzyme activity tends to decrease with soil depth because root and soil microbe interactions are minimized beyond the root zone. When measuring soil enzyme activity, considerations for tillage depth are important. In soils that experience heavy tillage, soil enzyme samples should be measured to the plow layer depth due to the even distribution of soil nutrients throughout the soil profile. In minimally tilled and no tilled soils, sampling the top 6 inches of soil provides an indication of soil biological activity because residue and nutrient inputs occur near the surface of soil in these systems. When comparing activities between management sites with varying degrees of tillage, make sure all enzyme sampling occurs at the same depth to ensure soil enzyme activities are properly interpreted. Unless there is a specific project goal, we suggest a sampling depth of 6 inches.

Soil Moisture

Extracellular soil enzymes are dependent on soil moisture to diffuse substrates through the soil environment and become adsorbed to soil surfaces. In a moisture limited environment, soil enzymes can only access substrates that are within a very close vicinity. Depending on the location and concentration of substrates relative to an enzyme, activity of soil enzymes can be strongly impacted. Soils saturated with water for a long period of time may lead to low microbial biomass, mostly attributed to the low oxygen conditions that are unfavorable for aerobic microbes.

Soil Management Factors

Soil management decisions such as tillage management strategies, crop selection and diversity, seed treatments, cropping rotations, and fertilizer managements have compounding and cascading impacts on all aspects of soil. These decisions shape unique soil microbial communities which influence soil properties such as soil structure, porosity, and nutrient availability for plants. The use of soil enzyme activity to compare different soil management strategies can indicate earlier shifts in soil microbial community dynamics and help address management changes before many other soil testing methods. The impacts of commonly used soil management strategies on soil enzyme activities are given below.

Tillage

Soil structure is formed through physical, chemical and biological activities that create glues to hold soil particles together, called soil aggregation. Different arrangements of aggregates result in different sized pores, creating channels for air and water movement in the soil which ultimately influence bulk density, aeration, permeability and water holding capacity of the soil. Soil aggregation can create areas of high and low oxygen, shield SOM from microbes, and allow diffusion of nutrients and gases through the soil. The mechanical act of tilling a soil damages the aggregate channels and initially flushes the soil with an abundance of air and water, while distributing the once shielded SOM to microorganisms. Most soil microbes are aerobic, or oxygen requiring, organisms that become hyper active in the presence of renewed oxygen and substrates. Invigorated microbial activity leads to greater microbial mineralization and leaching of nutrients from the soil system. Continuous tillage results in poor soil structure, decreased water holding capacity, and lower infiltration rates. In addition, under saturated conditions, microbial activity can consume the limited oxygen causing the areas to become anoxic, or low in oxygen. Lower oxygen levels can inhibit beneficial microbes, allowing pathogenic microbes to thrive. These soil characteristics caused by tillage negatively impact microbial community composition and activity. Furthermore, prolonged tillage practices lead to a loss of aggregate channels and lower substrate availability causing microbes to scavenge nutrients from SOM, resulting in a gradual loss of SOM over time.

In general, soil enzyme activity is found to steadily increase under reduced and no-till fields in comparison to conventional tillage methods. Under no-till systems, application of fertilizers and liming agents are often surface applied and plant residues stay at the surface leading to increases in organic C, N, and P concentrations at the surface soil layer. The presence of increased nutrient concentrations in the soil surface layer promotes increased microbial diversity and activity within the top few inches of soil. In addition, no-till systems or reduced tillage systems allow fungi to thrive. Increased diversity in fungi and bacteria promotes greater activity leading to enhanced aggregation, improved soil structure, stabilized SOM, and increased surfaces for stabilized soil enzymes.

Cover Crops and Crop Rotations

Plants and soil microbes have unique symbiotic relationships in which the plant supplies simple compounds, known collectively as root exudates, to support the colonization of the root by microbial communities. In exchange for the simple compounds, microbes release soil enzymes to mineralize nutrients from the soil for use by the plant and microbe. Individual plants release distinct exudates, encouraging relatively unique microbial communities to exist around the root. Root exudates can shape the soil environment around the root by releasing chemicals that can assist the plant in obtaining nutrients (i.e. iron), avoid pathogens (i.e. allelopathy), and encourage the colonization of beneficial microbes.

Plant diversity, established through a variety of plant types (e.g. perennials, annuals, grasses, broadleaves, legumes, etc.) and root structures (e.g. fibrous, taproot) can present a variety of microhabitats that supports a wider range of microbes. The increased diversity supports greater nutrient competition, prevents the dominance of one or a few microbial species, and generally improves the complexity of the soil microbial community. This soil microbial diversity can be accomplished using cover crops, cropping rotations or a mixture of both. Allowing continuous and diverse vegetative cover mimics a natural soil environment, supporting better nutrient cycling, soil aggregation, soil microbial activity, and water infiltration while suppressing weeds, pathogens and soil erosion. In comparison, a monoculture often reduces microbial and enzymatic diversity because a single crop species creates similar microbial food (e.g. root exudates), habitats (root structures), and chemical signals leading to a dominance of a few organisms in the microbial community. Thus, lower enzyme activity is often found under monoculture systems.

Generally, soil enzyme activity is higher in undisturbed areas than cultivated fields while including cover crops in cultivated fields often increases enzyme activity. Enzyme activity can respond differently to cover crops, indicating the unique soil environment each plant can create. Successful comparisons between cover crop mixes and cropping rotations can assist in future soil management choices.

Soil State	OC (%)	BG and PHD Activity No-Till	Till
Covered	<1	50	60
	<2	145	110
	<3	170	150
	<4	220	170
	>4	300+	220+
No Cover	<1	40	60
	<2	110	100
	<3	130	140
	<4	200	160
	>4	250+	180+

Table 1: A generalized guideline to soil enzyme activity under covered (e.g. natural vegetation, cover crops or diverse cropping rotations) and not covered (e.g. no cover crop, monoculture systems) in different tillage systems and organic carbon (OC) percentages. These are generalized values from literature. Soil enzyme activity is influenced by factors such as those discussed in this interpretation guide and can cause fluctuations in values. Soil enzyme activity should always be used on a comparative basis. Enzyme activity is reported as ppm p-nitrophenyl (pNP) g^{-1} dw soil h^{-1}.

Fertilizers

Crop production often requires the supplemental input of nutrients as fertilizers for proper growth and desirable yields due to nutrient removal from previous crops. Fertilizers can be subdivided into organic (i.e. manure, compost) and inorganic (i.e. chemical fertilizer) categories. Fertilizer type, composition and concentration can impact soil community dynamics and enzyme activity. Generally, organic amendments contain carbon-based complex compounds that require the activity of soil microbiology mineralization to convert the nutrients into plant available forms. Inorganic fertilizers primarily exist in plant available forms, requiring little to no microbial conversion. Synthetic fertilizers may also be consumed by the microbes, leading to a temporary increase in soil microbial community dynamics and activity. However, some inorganic fertilizers can inhibit enzyme production. For example, ammonia-based N fertilizers are often treated with a urease inhibitor that can decrease urease activity with increasing application.

The application of fertilizers (especially N based fertilizers) can have varied impacts on enzymes dependent on application rate, amount, vegetation, and other management decisions. Generally, higher enzyme activity is found under organic fertilization as opposed to inorganic fertilization. Soil microbial communities respond quickly to fertilizer inputs which may skew data if sampled shortly after soil fertilizer application. Sample at least 2-3 months after soil amendments such as manure or fertilizer application.

Linking Enzyme Testing with PLFA and Haney Tests

The Haney, phospholipid fatty acids (PLFA), and enzyme tests depict a holistic understanding of nutrient availability and dynamics, microbial community abundance and diversity, and enzymatic response to changing soil environmental conditions.

The Haney test utilizes a combination of soil testing methods: soil respiration, water extract and H3A extract. Soil respiration indicates a measurement of the soil's microbial biomass and potential for activity under optimal conditions. This test quantifies the amount of CO_2-C a soil can produce over a 24-hour incubation period following a significant drying and rewetting event. The water extract measures the amount of soluble organic carbon and nitrogen readily available to microbes in the soil. The H3A extract contains organic acids that mimic root exudates and provides a measurement of the nutrients the plant will be able to use during the growing season.

The Phospholipid Fatty Acid (PLFA) test provides an analysis of the total living microbial biomass in soil. This analysis depicts the abundance of bacteria, actinomycetes, rhizobia, arbuscular mycorrhizal fungi, saprophytic fungi and protozoa by quantifying specific fatty acids known as biomarkers. Also included are the fungi:bacteria ratio, predator: prey ratio, and gram positive: gram negative bacteria ratio. The PLFA test is a useful tool to monitor microbial community response over a number of years or across different land management practices.

The Haney Test, PLFA and enzyme tests can all be combined to display a unique look a soil from a microbial perspective. Soil microbes produce enzymes based on the availability of substrates or lack of nutrients within the soil environment and is strongly correlated with many of the organic nutrients in the soil. Changes in soil enzyme activity can provide early indications of shifts in microbial nutrients in the soil, as measured by the Haney test, and shifts in the microbial community structure and abundance, as measured by the PLFA. For instance, β-glucosidase activity strongly correlates with organic carbon content in soil and can indicate early changes in SOM. The use of all three tests can provide a wealth of information on the soil microbial environment.

Summary

Soil microbiology produce and exude soil enzymes to cycle nutrients (e.g. urease, phosphodiesterase) and energy (e.g. β-glucosidase) in the soil. Because enzymes are nutrient specific, monitoring fluctuations in potential enzyme activity can be a useful tool in gauging the microbial response to changes in soil managements. Soil enzyme activity comparisons between land management strategies or over successive years may provide earlier indications of SOM generation and destruction and nutrient availability. Changes in key enzyme activities can signal a higher nutrient input and cycling in the soil that can lead to building healthier soils. Healthier soils can aid in weed suppression, lower dependence on fertilizers and store greater amounts of nutrients. This soil enzyme interpretation guide is intended as a resource to aid producers in understanding soil enzyme activity and the role it plays in building healthier soils. To learn more about the role soil enzymes play in the soil or about specific soil enzymes, please visit our website. This interpretation is by no means limited to the above soil environment and management factors as soil enzyme activity may be used to detect the impact of any desired soil management strategy.

Additional information will be added to the website as new information becomes available. Any questions regarding soil enzyme testing may be directed to biotesting@wardlab.com.