Soil Enzyme Introduction Rev. 1.0

Ward Laboratories Inc.

Soil macro and microbiological activity is responsible for storing, transforming and cycling nutrients in the soil. Soil macrobiology, such as arthropods and worms, create large tunnels in the soil for air and water to enter the soil, mix soil by bringing organic matter to the soil surface and partially decompose organic residues. Microbiology, such as bacteria, fungi, and actinomycetes (collectively referred to as microbes), finish decomposing organic residues, complete nutrient cycles, improve aggregation and build soil organic matter (SOM). In order to transform and release nutrients in the soil, soil microbes depend on an extensive variety of enzymes to complete this process.

Soil Microbiology and Enzymes

Most microbes in the soil exist under starvation conditions. Fierce competition for food, water and space within the soil causes many microbes to live in a dormant state until resources become available. Often, soil microbes will gather around nutrient rich areas that contain ideal conditions (i.e. water, temperature, pH) for nutrient uptake. These locations, known as microsites, are prominent in the rhizosphere where gatherings of soil microbiology, collectively referred to as soil microbial communities, can be found attached to root hairs, on plant residues and on and within aggregates. Soil microbial communities cluster around living roots because of the unique relationship microbial communities have with plant roots.

Plants are dependent on roots to anchor themselves in the soil and allow nutrient and water uptake. In addition to stabilizing the plant, roots also secrete a vast array of unique chemical compounds that attract and repel certain soil microbes, build soil aggregates, change the soil pH and inhibit the growth of competing plants. These secreted chemicals, referred to as root exudates, help cultivate the microbiome that exists around the root and is exclusive to soil type, climate and vegetation of the area. Since the root has limited exposure to necessary nutrients for growth and production, root exudates are released in the rhizosphere to attract microbes to the root area. The release of simple nutrients to support soil microbial communities is exchanged for microbial scavenging of essential plant nutrients. Like plants, soil microbes require macro and micro nutrients to survive, reproduce and perform basic continuous maintenance. The soil nutrients in direct contact with the plant root are limited, requiring soil microbes to rely on various chemical and biological processes to obtain nutrients from the soil environment.

Most nutrients in the soil are locked away in large, complex compounds that soil microbes are unable to pass through their cell wall. In order to breakdown these large, complex compounds, soil microbes produce specific protein catalysts, known as enzymes, to fracture complex compounds into simpler forms of nutrients. Thus, soil enzymes are important tools for soil microbes to create accessible nutrient forms that are small enough for both the plant and the microbe to pass through their cell wall.

The Production of Soil Enzymes

Soil nutrient availability varies over space and time. Often, soil nutrient supply does not align with plant and microbial nutritional demand. If the nutrient supply is sufficient to meet plant and microbe demand, little to no production of nutrient specific enzymes are created. If the nutrient supply cannot be met, enzymes are produced to fill the nutrient gap. The production of enzymes is costly to a microbe's limited resources; thus, enzyme production is mostly carried out when the need is great, and the substrate is available. The abundance, availability and activity of enzymes is not only dependent on production but also on the location of soil enzymes in the soil environment.

Large complex compounds from living and dead plants, animals and microbes are often physically and chemically attached to clays, minerals or other organic compounds in the soil. These soil components shield important sources of nutrients from microbial access; requiring enzymes to not only be present but also mobile. Enzymes exist predominantly in three different forms to access nutrients: intracellular, free extracellular and adsorbed extracellular (See Fig 1).

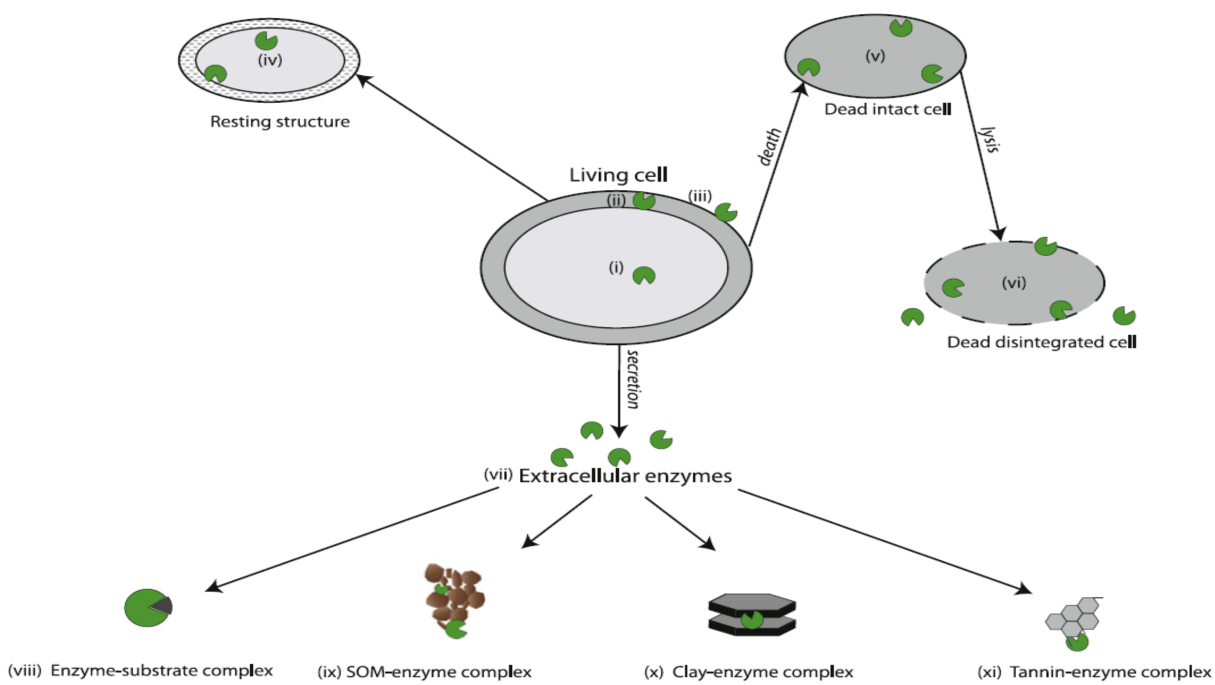

Figure 1: Enzyme locations within the soil as described by Burns 2013

All soil enzymes are created within the microbial cell. Some enzymes, known as intracellular enzymes, exist primarily within the cell or within the cytoplasm of the cell and act on substrates that are small enough to pass through the cell but require additional processing prior to being used by the cell. These enzymes can also prepare other proteins and enzymes for the hostile soil environmental. Intracellular enzymes can exist only within cell and upon death and, can be released into the soil environment through cell lysis (the rupturing of a cell). These enzymes are quickly denatured or attacked by proteases, an enzyme designed to breakdown a wide variety of proteins. Extracellular enzymes, or enzymes designed to exist outside the cell, can become attached to the cell wall. If a cell develops a polysaccharidic coat or biofilm, the cell may create a scaffold-like structure, known as a cellulosome, which protrudes attached soil enzymes into the surrounding soil environment. These enzymes can orient their active sites to stay open and can protect areas of the cell that may be subjected to attack by predators. Other extracellular enzymes are designed to enter the soil environment. By adjusting the structure of the enzymes, these extracellular enzymes are better able to withstand environmental stressors, such as temperature and pH, that cause denaturing and have better resistance to predation than intracellular enzymes. Once exuded from the parent cell, these enzymes, known as free extracellular enzymes, exist within the soil solution and are an important mode of enzyme transportation in the soil environment. Through diffusion, these enzymes spread throughout the soil environment, but often become stabilized by becoming adsorbed (attached to the surface of the material) or absorbed (attached within the material) with clay minerals, soil organic matter or humic material. Once an enzyme becomes stabilized in the soil, these enzymes are referred to as extracellular enzymes. If the active site of an extracellular enzyme is still exposed to the soil environment, the adsorbed enzyme can continue breaking down substrates into plant and microbial available nutrients long after the parent microbial cell has died. Adsorbed enzymes can also be protected from predation and other denaturing situations. Absorbed enzymes are often not exposed to substrates and become ineffective, unless the enzyme becomes exposed to the soil environment. The ability of extracellular enzymes to continue acting on substrates within the soil environment may be an important source of response to changes in substrate availability in soil that acts as an early alert system to microbial communities. This could allow communities to become more efficient in enzyme production or, if enzymes are active enough, the microbe may conserve those resources required to create enzymes by not producing them.

The Shape and Specificity of Soil Enzymes

Each enzyme is constructed by microbes using long chains of amino acids that are distinctively folded to form active sites specific to select substrates. Substrates, or the complex compound that will be acted upon by the enzyme, bind to the enzyme on the active sites to form an enzyme-substrate complex. At this point, depending on the type of enzyme, substrates can either become fused together to create a new product or bonds within the substrate are fractured and separate products will be released. This process is often viewed as a “lock and key” mechanism (See Figure 2) where the shape of the substrate must match the shape of the active site in the enzyme. After this process is completed, the enzyme is ready to accept another substrate. If the active site of an enzyme becomes permanently altered due to extreme changes in environmental conditions (i.e. pH and temperature), the substrate can no longer bind to enzyme and no products will be created. This process is known as denaturing and can not be reversed.

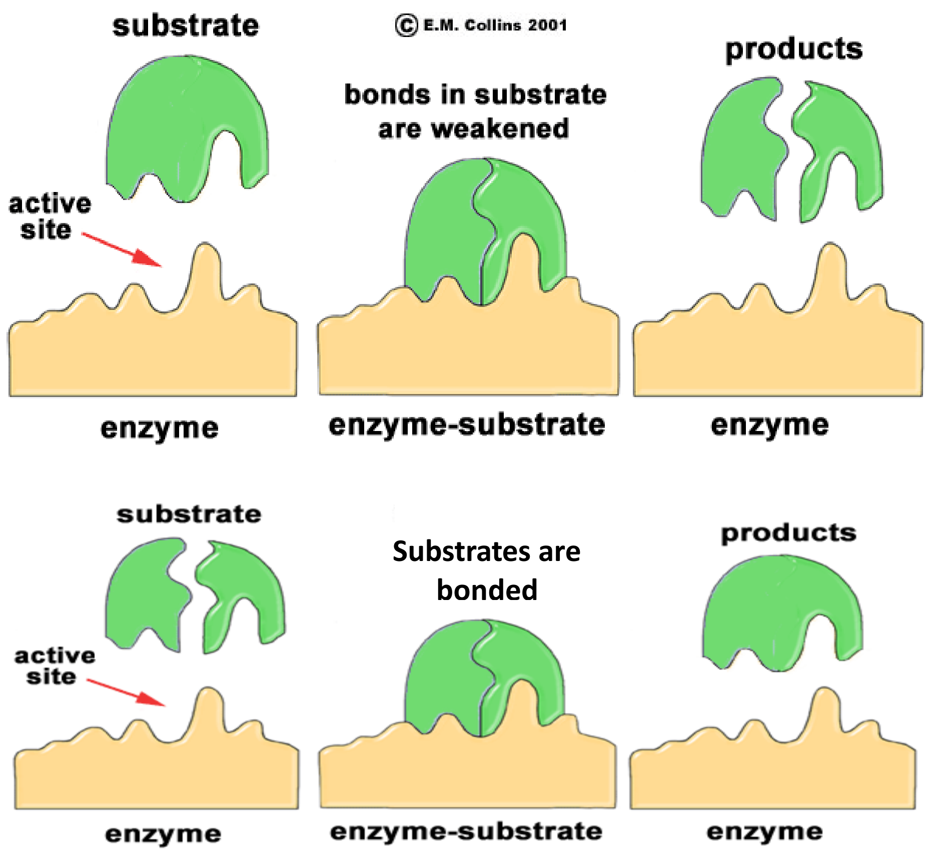

Figure 2: The "Lock and Key" Enzyme Mechanisms
(Image from E.M. Collins 2001)

The particular structure of soil enzymes allows only one kind of substrate to be processed and the activity of many enzymes is required to completely breakdown a complex compound. Soil enzymes target specific complex compounds such as proteins, carbohydrates, amino sugars, organic phosphates, and lignins to release readily available nutrients in the soil. For instance, cellulose is a basic structural component of plant cell walls that is composed of a long change of bonded sugar molecules. These sugar molecules, predominantly made of glucose, are an important source of energy for soil microbes. Soil microbes produce a collective group of soil enzymes, known as cellulases, to disassemble the ridgid structure of cellulose to release glucose molecules. The cellulose structure is fractured by three dominant groups of enzymes: endoglucanases, exoglucanases and β-glucosidases (See Figure 3). Endoglucanases randomly cleave the large chained cellulose into smaller fractions while exoglucanases further reduce the cellulose chain to cellulobiose units (two linked glucose molecules). β-glucosidases cleave the cellulobiose molecules to release glucose as a readily available nutrient for microbe uptake. β-glucosidase activity is a very commonly monitored enzyme because it is not only responsible for the energy flow in the soil system but is only produced when cellulobiose is abundant.

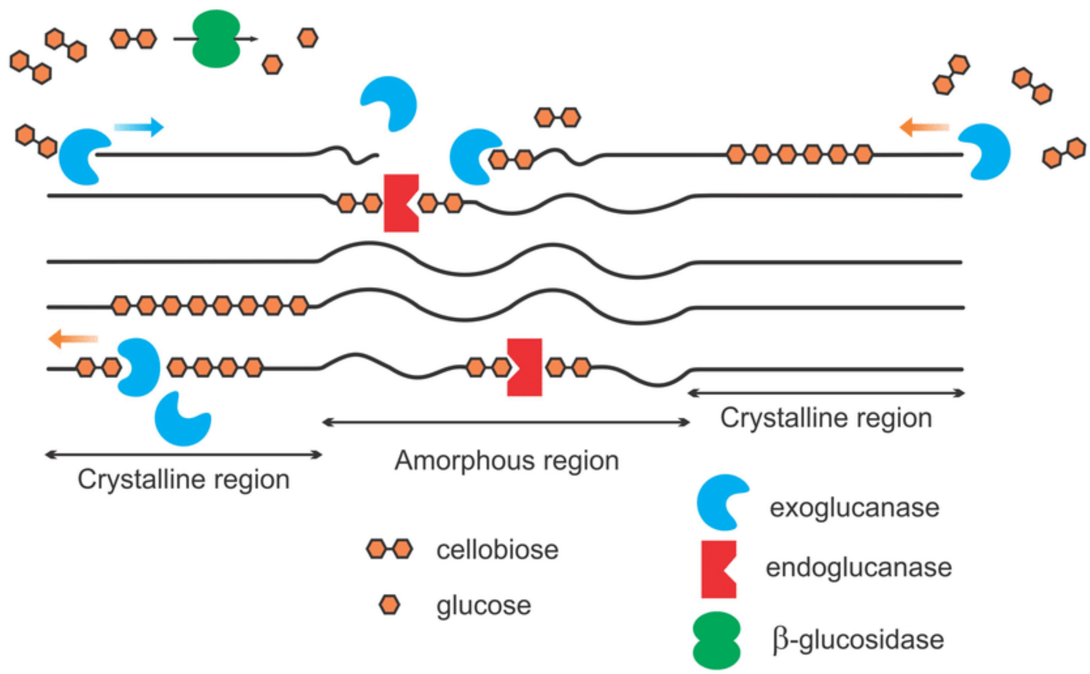

Figure 3: Simplified schematic of cellulase enzymes acting on cellulose

The long-chained cellulose is fractured by the accumulated effort of exoglucanases, endoglucanases and β-glucosidase enzymes to release glucose in the soil environment. (Image from Akhtar 2016)

Understanding the role of soil enzymes and monitoring the activity of specific enzymes provides earlier indication of how soil nutrient cycles are changing or responding to any changes in nutrient or energy supply and demand within the soil enviornment. Thus, soil enzymes are often measured due to their rapid response to soil management practices and are often referred to as “bio-indicators” of soil health.

The creation and distribution of enzymes reflects the availability of substrates in the soil environment. The specificity of soil enzymes provides a unique insight into early changes in the specific cycles in the soil environment. Although several enzymes are involved in nutrient and organic matter cycling, specific enzymes have been identified due to their vital role in soil metabolic processes, sensitivity to changes in soil management and ease of measurement. By monitoring select enzyme activities, the impact of soil management decisions on important cycles in the soil may be detected earlier than many other soil measurements.

Measuring Soil Enzyme Activity

Soil enzyme testing at Ward Laboratories, Inc. is conducted by analyzing the consumption of a substrate and the release of a colored product. The consumption of product is measured over time and results are expressed as a rate of enzyme activity. By using controlled soil conditions (e.g. pH, temperature), the enzyme activity rates can indicate the potential activity for the soil enzymes under ideal conditions. This allows a comparison of potential enzyme activities between different soil management practices. Similarly, the same site can be tracked over time to monitor subtle changes in microbial dynamics and provide an indication of the microbial community response to changing soil environmental conditions and management.

Soil enzyme activity is reported as a rate of product (or color) produced (i.e. β-glucosidase is reported as ppm pNP g^{-1} soil h^{-1}). Interpretation of soil enzyme activity requires an understanding of nutrient or organic matter cycling. Often, healthy, active systems have increased enzyme activity, relating to better cycling of nutrients and organic matter quality in the soil. Nevertheless, sites with recent disturbances may have higher activity levels due to increased substrate availability when compared to non-disturbed sites. A conventional tillage field versus a no-till field in its first year of transition may indicate the tilled field has higher enzyme activity. This is because the act of tilling a field provides aeration and better distribution of substrates to microbes. The increased activity cannot be fully sustained in this system and often causes the enzymes to access the nutrients in organic matter, leading to a loss of organic matter the following year. A list of common soil characteristics and soil management impacts can be found in the interpretation guide.

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.