History and Interpretation of Soil Enzyme Activity

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Introduction

Microbial communities play a key role in ecosystem-level processes such as decomposition of organic matter, nutrient cycling (Wright and Reddy, 2001), and processes affecting the efficiency of nutrient cycling and ecosystem function (Yao et al., 2000). These microbial processes include the release of extracellular enzymes, which function to convert complex organic molecules to simple organic constituents during decomposition of organic material (Prenger, J. P. and K. R. Reddy, 2004). Soil enzymes are protein structured molecules that increase the reaction rate by catalyzing them without any permanent transformation (Dick and Kandeler, 2004). The substance acted upon by a soil enzyme is called a substrate (Fig. 1).

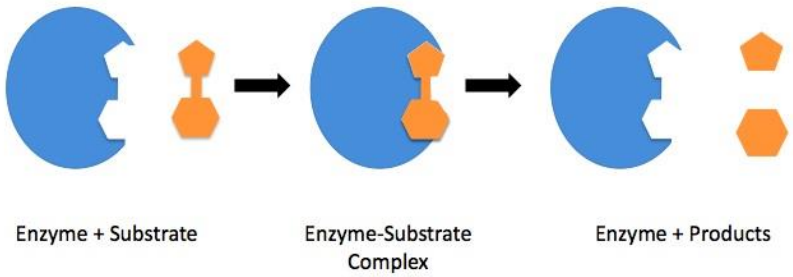

Figure 1. Enzyme acting as a catalyst to breakdown complex substrates to bioavailable products that are more easily accessible to microorganisms.

The enzymatic reaction cleaves the substrate and releases a product, which can be a nutrient contained in the substrate. Enzyme production is a function of microbial activity which is regulated in part by nutrient availability (Sinsabaugh, 1994), where microbes produce enzymes to mobilize resources from compound sources when nutrients are limited (Harder and Dijikhuizen, 1983). These soil enzymes play an important role in biochemical process of organic matter recycling, soil physical properties, and microbial activity and/or biomass (Table 1) (Cherukumalli et al, 2017). The study of soil enzymology has provided insight to the function of enzymatic activity as an indicator of ecosystem biogeochemical

processes, nutrient availability, rates of nutrient and carbon cycling, and even response to climate change. The objective of this publication is to provide a history of soil enzymology and interpretation of enzyme activity.

History of Soil Enzymology

Over 100 years ago, the first known report of soil enzymes by Albert Woods (1899) recorded the activity of oxidizing enzymes, such as peroxidases, and concluded that these extracellular enzymes arose from plant material like decaying roots. This quickly sparked a widespread acceptance of the presence of soil enzymatic activity, which lead to further investigation regarding locations, functions, and significance of this activity (Dick and Burns, 2011). Herbert Conn (1901) further developed the observations of Woods by highlighting the important role of higher plant and microbial enzymatic activity in agricultural processes concluding: “Without their agency in breaking up organic compounds the soil would rapidly become unfit for supporting life”.

In the early 19th century, catalase was the dominant enzyme studied largely due to technological limitations that excluded measurements for assaying other enzymes (Dick and Burns, 2011). During this time, mechanistic studies incorrectly suggested that the reaction was solely inorganic (Osugi 1922), which were partly due to the poor correlations between biological measurements and enzyme activity, and the idea that catalase activity was a soil fertility indicator (Waksman and Dubos, 1926) was swiftly disregarded as too simplistic to reflect the complexity of soils (Waksman, 1927). Nevertheless, research focused on catalase as a biological indicator of soil fertility (Kurtyakov, 1931; Radu, 1931; Rotini, 1931, Galetti, 1932; Matsuno and Ichikava, 1934; Scharrer, 1927, 1928a,b, 1936), and the first paper published on the kinetics of soil catalase (Scharrer, 1933) shifted the prevalent consensus that catalase activity was driven by microorganisms.

In 1957, Mclaren was the first to use irradiation techniques to conclusively establish the occurrence of catalytic enzymes that originate biologically, but are no longer controlled or associated with viable cells, which was later termed “abiontic” activity (Skujiņš,1978). The irradiation techniques demonstrated that at the right irradiation intensity, soils could not culture microorganisms but yet had high measurable urease and phosphatase activities. This supported the idea that although microorganisms were not present, enzymes were capable of being extracellular and catalytic without viable cells. In 1975, Ladd and Butler further substantiated the concept of abiontic enzymes by indicating that enzymes could be bound to on clay minerals, humic substances, or organo-mineral complexes but remain catalytic.

In 1982, Burns described the importance of the role of abiontic enzymes in soil microbial ecology. The quantity of abiontic (extracellular) enzyme activity may be representative not only of the biological capacity of soil for enzymatic conversion of substrate, which is independent of the extent of microbial activity, but may also have an important and unexplored role in the ecology of microorganisms in soil (Burns, 1982). Burns regarded soil as a multi-celled organism that responded to substrate based on highly integrated components that included numerous microbial species, abiotic factors, and extracellular biological catalysts with different properties depending on location within the soil matrix.

Abiontic enzymes were found in various locations within the soil such as within soil solution (Burns 1982). In 1990, Boyd and Mortland discovered that extracellular enzymes were primary stabilized to inorganic surfaces (mainly clay and iron oxides and hydroxides) and complexed with organic colloids through adsorption yet still remain catalytic. Boyd and Mortland (1990) suggested that these organo-mineral systems may provide living environments for viruses and microorganisms, a model for enzyme interactions with natural soil and organic matter, and important regulator for

activities and stabilities of enzymes associated with organic matter and clay-organic matter complexes in soils.

In 2002, Metcalfe and others detected that enzyme-coding genes could be found in specific microorganisms and was first to attempt to relate these genes to relative enzyme activity. The enzyme-coding genes sequenced included chitinase (Metcalfe et al., 2002; LeCleir et al., 2004; Xiao et al., 2005), laccase (Luis et al., 2004) and proteases (Fuka et al., 2008). It was later found that enzyme-coding genes does not strongly correlate to enzyme activity, which suggested that gene detection do not necessarily indicate that is expressed in the environment (Hassett et al, 2009). Genes regulating enzyme production can be present in the DNA, but the presence of mRNA transcript is what informs the gene to be expressed and thus activate enzyme production. However, other studies contradicted this finding, suggesting that gene expression does not explain differences in enzyme activities (Edwards et al, 2011). Nonetheless, this indicated that other factors, such as enzyme prolongation and turnover must be considered. Further research regarding metatranscriptomics using mRNA and cDNA may offer further understanding of microbial enzyme expression in soil (Damon et al., 2012; de Menezes et al., 2012; Haichar et al., 2012). Metatranscriptomics studies RNA, which provides an opportunity to gain insight into the functionality of microbial communities. It is often assumed that gene expression (transcription of DNA into RNA (Fig. 2)) is representative of microbial activity and reflects the response of microorganisms to environmental cues (Myrold et al, 2014). Various studies have discovered that the composition of microbial communities based on RNA differs from that based on DNA and concluded that the active microbial community is simply a subset of the potentially active microbial community (Anderson and Parkin, 2007; Baldrian et al., 2012; Griffiths et al., 2000). The practicality of soil metatranscriptomics has yet to be fully developed but has provided insights about microbial functions in other complex microbial systems (Poretsky et al., 2010).

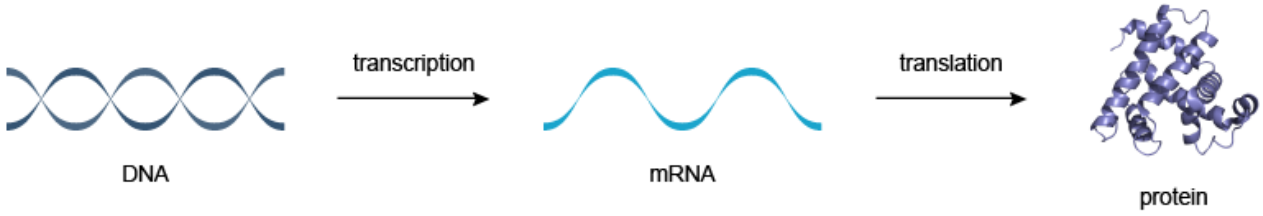

Figure 2. This figure illustrates the process by which DNA is copied to RNA (transcription), and that by which RNA is used to produce proteins (translation).

In regard to aquatic systems, the earliest enzyme studies were proposed by ecologists who recorded the presence of extracellular enzymes during studies of periphytic bacterial cultures in the early 1970s (Corpe and Winters, 1972; Daatselaar and Harder, 1974). Later, various extracellular enzyme activities were being linked to degradation of organic matter in diverse ecosystems such as marine environments (Kobori and Taga, 1979b; Meyer-Reil, 1981, 1983; Hoppe, 1983; Hollibaugh and Azam, 1983; Lancelot and Billen, 1984; Ammerman and Azam, 1985; Hoppe et al., 1988), lakes (Halemejko and Chróst, 1984; Chróst and Overbeck, 1987; Chróst, 1989; Chróst et al., 1989), and rivers (Admiraal and Tubbing, 1991; Münster et al., 1992), which lead to a pioneering series of studies estimating litter decomposition rates based on extracellular enzyme activities (Sinsabaugh and Linkins, 1990; Sinsabaugh et al., 1992, 1994, 2009; Sinsabaugh and Findlay, 1995). During this time, enzyme studies were beginning to focus on wetland ecosystem and the link between enzyme activities and the biogeochemical properties of these systems (Pind et al., 1994; Freeman et al., 1995, 1996, 1997, 1998).

Researchers were beginning to utilize enzymatic analysis to determine rates of soil organic matter decomposition and nutrient mineralization (McLatchey and Reddy, 1998; Kang and Freeman, 1999, 2009; Kominkova et al., 2000; Shackle et al., 2000; Freeman et al., 2004b; Francoeur et al., 2006; Jackson and Vallaire, 2007), which lead to an interest in environmental degradation and the utilization of enzyme activity as a tool to investigate environmental controls on key decomposition processes.

Additional studies have examined the effects of an array of environmental conditions on enzymatic regulated carbon and nutrient cycling, such as; water level drawdown by climate change or human intervention (Freeman et al., 1996, 1997, 1998; Williams et al., 2000; Burns and Ryder, 2001; Corstanje and Reddy, 2004; Mentzer et al., 2006; Song et al., 2007); elevated CO_2, O_3 (Williamson et al., 2010), ultraviolet radiation (Thomas et al., 2009), temperature (Fenner et al., 2005, 2006), land use changes (Ye et al., 2009; Gao et al., 2010), and metals (Siciliano and Lean, 2002; Duarte et al., 2008).

Enzymatic analyses were adopted in wetland studies with diverse objectives, for example, research on constructed wetlands have considered the effects of wastewater, organic matter, or manure additions on enzyme activities (Szogi et al., 2004; Bruland et al., 2009; Dao and Schwartz, 2010; Finocchiaro and Kremer, 2010; Yan and Pan, 2010). Enzyme activities are used extensively as a soil quality indicator (Rokosch et al., 2009) and have also been evaluated to connect microbial ecology and biogeochemical processes (Freeman et al. 1997, Freeman et al. 1998, Kang et al. 1998, Gutknecht et al. 2006, Drenovsky et al. 2008).

Interpretation of Soil Enzymes

Enzyme activities reflect changes in microbial activities and can be measured and utilized as an index of microbiological functional diversity that can include a variety of metabolic processes (i.e. nutrient cycling and decomposition). Because there are a variety of different metabolic process, a representative set of enzyme activities that control the key metabolic pathways are required. A commonly used and efficient method to measure enzyme activity is the fluorometric technique that is based on 4-methylumbelliferone (MUF) substrates that fluoresce upon enzymatic cleavage allowing the amount of product to be measured. Since this method was developed, substrates releasing MUF or other fluorescent products (i.e. 7-amino-4-methyl coumarin, 7-AMC) have been extensively used to measure the activity of many enzymes, including measuring concurrent activity determination of multiple soil enzymes on microplates, mainly developed by Freeman et al. (1995).

In aquatic and terrestrial systems, enzymes have been utilized as indicators of nutrient cycling, most important for carbon cycle being glucosidases, amylase, cellulose, lipase, xylanase, and invertase; for the nitrogen cycle, proteases, amidases, urease, and deaminases; for phosphorus -phosphatase; and for sulfur – arylsulfatase (Karaca et al., 2010; Riah et al., 2014; Trasar-Cepeda, 2012 Nieder et al, 2008). Studies have shown inverse trends with soil depth, as soil depth increases enzyme activity decreases, and this has largely due to the correlation between enzyme activity and microbial activity and the carbon and organic nitrogen contents in the soil (Lal et al, 2010; Li, 2015). Generally, as soil depth increase microbial activity decreases due to limiting conditions such as available nutrients, electron acceptors, temperature, etc. Additional studies have utilized enzyme assays to indicate shifts in microbial processing between major types of resources within a specific nutrient cycle and therefore indicate nutrient limitation (Sinsabaugh and Moorhead, 1994; Schimel and Weintraub, 2003). For example, in phosphorus-limited constructed wetlands, bioavailability of P may be regulated by microbial mineralization of organic P, through the production of monoesterase (alkaline phosphatase) and diesterase (bis-phosphatase) enzymes (Reddy, 2008). As a result, this activity of phosphatase enzymes can be utilized as a direct measure of the relative phosphorus mineralization occurring in aquatic ecosystems (Sinsabaugh et al. 1993).

Soil enzymes have frequently been evaluated based on ratio differences within major carbon, nitrogen, and phosphorus processing enzyme to provide a better understanding of the microbial community response to changing nutrient resources and the relative importance of different nutrients (Caldwell, 2005). Various studies have utilized enzyme assays to indicate shifts in microbial processing between major types of resources within a specific nutrient cycle (Garcia et al., 1994, Sparling et al., 1986). Production of enzymes is determined by energy and nutrient resources, thus the production of enzymes is determined by the availability of nutrients (Allison et al, 2005). Microbes maintain a relatively fixed stoichiometry of cellular components (Cleveland and Liptzin, 2007), and microbes

produce enzymes targeting specific compounds rich in carbon, nitrogen, and phosphorus in order to maintain this internal stoichiometric balance (Sinsabaugh et al, 2008). Studies have shown that there is a relationship between stoichiometry of microbial biomass and available nutrients, as these ratios increase and critical nutrients like phosphorus and nitrogen decrease, enzyme production will increase to acquire more nutrients and vice versa (German et al., 2011; Steinweg, 2013). In addition, multivariate techniques have increasingly been utilized to relate soil enzyme activities to microbial community structure and physiology (Nannipieri et al, 2002). The interpretation of soil enzymes has been central for the development of conceptual models that provide a more inclusive understanding of those key processes linking microbial populations and nutrient dynamics (Sinsabaugh and Moorhead, 1994; Schimel and Weintraub, 2003). For example, Schimel and Weintraub (2003) built a simple theoretical model to incorporate enzyme activity as a function of decomposition rates under carbon and nitrogen limited soil conditions and found that microbial growth may be limited by nitrogen.

Conclusion

Soil enzymes are key players of biochemical processes in both terrestrial and aquatic ecosystems. The processes include decomposition, nutrient cycling, and microbial activity. These soil enzyme activities are soil function indicators that that provide insight to reaction rates for important soil processes, soil productivity, microbial activity, and inhibiting effects of pollutants, etc. (Srinivasroa, 2017). These soil enzymes are sensitive early warning indicators of soil management changes, and are very important for measuring soil quality and thus maintaining ecology integrity in both terrestrial and aquatic systems. Future determination of how to measure and interpret soil enzyme functional diversity (i.e. specific substrates to explore diversity between and within nutrient cycle) will depend on the nature of questions on topics like the linkages between resource availability, microbial community structure and function, and ecosystem processes that require further exploration and research.

Table 1. Role of soil enzymes

Enzymes	Substrate	Significance	Predictor of Soil Function
Phosphatase	Phosphorus	Plant available P	Nutrient cycling
Beta glucosidase	Carbon compounds	Energy for microorganisms	Organic matter decomposition
FDA Hydrolysis	Organic Matter	Carbon and various nutrients	Organic matter decomposition and nutrient cycling
Amidase	Carbon and nitrogen compounds	Plant available NH_4^+	Nutrient cycling
Urease	Nitrogen (urea)	Plant available NH_4^+	Nutrient cycling
Sulfatase	Sulfur	Plant available S	Nutrient cycling

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