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Enzyme activities as a component of soil biodiversity: A review

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Summary

Soil enzyme activities are the direct expression of the soil community to metabolic requirements and available nutrients. While the diversity of soil organisms is important, the capacity of soil microbial communities to maintain functional diversity of those critical soil processes through disturbance, stress or succession could ultimately be more important to ecosystem productivity and stability than taxonomic diversity. This review examines selected papers containing soil enzyme data that could be used to distinguish enzyme sources and substrate specificity, at scales within and between major nutrient cycles. Developing approaches to assess soil enzyme functional diversity will increase our understanding of the linkages between resource availability, microbial community structure and function, and ecosystem processes.

Introduction

Understanding and maintaining biodiversity has become an increasingly important field of research, as well as a resource management goal. In soil microbial communities, maintaining critical functions may ultimately be more important than maintaining taxonomic diversity. One essential microbial function in soils is the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter. This often requires the activity of extracellular enzymes to process complex organic compounds into assimilable subunits (sugars, amino acids, NH_4^+, PO_4^{-3}). The field of soil enzymology, including numerous methods and applications, has been extensively reviewed (Burns, 1978; Burns and Dick, 2002).

Soil enzyme activities have been related to soil physio-chemical characters (Amador et al., 1997), microbial community structure (Waldrop et al., 2000; Kourtev et al., 2002), vegetation (Waldrop et al., 2000; Sinsabaugh et al., 2002), disturbance (Bolton et al., 1993; Eivazi and Bayan, 1996; Garcia and Hernandez, 1997; Boerner et al., 2000), and succession (Tscherko et al., 2003). Scales of resolution have ranged from the landscape (Bonmati et al., 1991; Decker et al., 1999; Amador et al., 1997) to soil particle size fractions

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(Kandeler et al., 1999). Equations to assess soil quality have included various enzyme activities (Halvorson et al., 1996; Pankhurst et al., 1997; Trasar-Cepeda et al., 1998; Saviozzi et al., 2001; Killham and Staddon, 2002; Speir and Ross, 2002). Soil enzyme data have been the foundation for the development of conceptual models that provide a more comprehensive understanding of those key processes linking microbial populations and nutrient dynamics (Sinsabaugh and Moorhead, 1994; Schimel and Weintraub, 2003). While these studies have typically dealt with differences in soil enzyme activities, it is also possible with these assays to develop specific measures of functional diversity.

Distinct from the physiological or genetic diversity of the soil microbial biomass (Zak et al., 1994; Kennedy and Grewin, 1997; Emmerling et al., 2002; Wellington et al., 2003) which assess *potential*, functional diversity of soil enzymes is related to the actual *activities* resulting from that potential. Functional enzyme diversity can be determined from several interacting sets of information, either independently or interactively. These include the measurements of activities against target substrates from the major nutrient resources, distinguishing different reaction mechanisms to activities within a given enzyme function (e.g., proteolysis), and the possible determination of enzyme sources. The objectives of this paper are to briefly review previous applications of soil enzyme activities and suggest possible approaches that could be used to assess soil enzyme functional diversity between and within major nutrient cycles.

Substrate specificity

Substrate specificity, as either an independent measure of enzyme diversity or as means to distinguish different reaction mechanisms, could resolve those enzyme activities that attack specific detrital components either between or within major nutrient pools (Table 1). Within each type of nutrient, there are specific chemical forms based on structure and bonding. The major forms of carbon are polysaccharide, aromatic (lignin) and aliphatic (polymethylene). The bulk of organic nitrogen is thought to be the in amide form (Knicker et al., 1997), either as peptide or non-peptide C–N bonds. Most organic phosphorus occurs in either a mono- or di-ester form (Dalal, 1977).

Within each of these major nutrient groups, there are specific compounds against which major classes of soil enzymes are active. Keystone to the breakdown of litter are the various cellulolytic activities requiring endo-cellulases, cellobiohydrolases and β-glucosidases (Sinsabaugh et al., 1992), and ligninolytic activities requiring a variety of polyphenol oxidases and peroxidases (Kirk and Ferrell, 1987). Within the nitrogen cycle, substrate diversity for proteins and peptides can be based on hydrolysis of different amino acid groups (Ladd and Butler, 1972; Tabatabai et al., 2002). Release of ammonium from various non-peptide C–N bonds can also be measured for a variety of different substrates, including the frequently measured urease activity. Mineralization of phosphate from organic esters can be resolved into phosphodiesterase and phosphomonoesterase activities, reflecting the use of tissue-based and soil organic phosphates pools, respectively (Dalal, 1977).

Reaction mechanisms

Since enzyme activities are catalyzed at specific reactive sites, another component of enzyme functional diversity could be based on using specific inhibitors or substrates.

The most common use of inhibitors has been with proteolytic enzymes where four major groups of proteases can be distinguished (Morihara, 1974). While broad generalizations about enzyme source can be made for aspartic- (fungal), thiol- (general), metallo- (bacterial) and serine- (general) proteases, separating proteolytic activity into these four classes also represents a component of functional diversity in itself. Different reaction mechanisms are also found among peptidases, where removal of terminal amino acids is by the selective enzyme binding to either the free amino- or carboxy- end of the peptide. Soil peptidase activities have been measured using either aminopeptidase (Saiya-Cork et al., 2002; Sinsabaugh et al., 2002) or carboxypeptidase substrates (Ladd and Butler, 1972; Kamimura and Hayano, 2000), but not both together in a single study.

Sources of soil enzyme activities

Knowing the sources of specific soil enzyme activities would greatly enhance our understanding of which group(s) of organisms are directly accessing a given nutrient resource, thus providing greater insight into the pathways by which energy and nutrients flow through the soil food web.

Molecular methods are now at the stage where specific functional genes and their expression by

Table 1. Examples of specific soil enzyme activities and assays available to assess functional diversity between and within nutrient cycles

Nutrient	Form	Compound	Enzyme	Examples
Carbon	Polysaccharide	Cellulose	Endo-cellulase	Deng and Tabatabai (1994)
			β-Glucosidase	Eivazi and Tabatabai (1988)
		β(1-3) glucan	β(1-3) glucanase	Lethbridge et al. (1978)
		Hemicellulose	Xylanase	Speir et al. (1984)
		Chitin	Endo-chitinase	Rodriguez-Kabana et al. (1983)
			N-acetylglucosaminidase	Parham and Deng (2000)
		Starch	Amylase	Pancholy and Rice (1973)
	Aromatic	Lignin	Phenoloxidase	Sinsabaugh et al. (1992)
			Peroxidase	Sinsabaugh et al. (1992)
			Mn-peroxidase	Criquet et al. (2000)
	Aliphatic	Fatty acid esters	Lipase	Morgan and Cooper (1981)
Nitrogen	Peptide	Protein	Endo-protease	Ladd and Butler (1972), García et al. (1994)
		Peptides	Aminopeptidase	Saiya-Cork et al. (2002)
			Carboxypeptidase	Ladd and Butler (1972)
	Non-peptide	Primary amine	Amidase	Frankenberger and Tabatabai (1980), Dodor and Tabatabai (2003)
			Urease	Sinsabaugh et al. (2000)
			(Adenosine) deaminase	Sato et al. (1986)
			(Aryl) deaminase	Killham and Rashid (1986)
Phosphorus	Diester		Phosphodiesterase	Sparling et al. (1986)
			DNAase, RNAase	Frankenberger et al. (1986)
	Monoester		Phosphomonoesterase	Eivazi and Tabatabai (1977), Sparling et al. (1986)
			Phytase	Svenson (1986)

the soil microbial biomass can be determined (Kelly, 2003; Wellington et al., 2003). Using mass spectrometer-based proteomics, Schulze et al. (2005) have identified the type and biological origin of soil proteins, including enzymes. While these approaches provide valuable information on enzyme potential and expression, more conventional methods may also be able to relate specific activities to source across broader taxonomic categories; i.e., bacteria and fungi.

Acid and neutral-alkaline pH optima have been reported for soil phosphomonoesterases (Eivazi and Tabatabai, 1977; Nakas et al., 1987), lipases (Morgan and Cooper, 1981) and proteases (Kamimura and Hayano, 2000). Whether extracellular enzymes from bacterial sources generally tend to have neutral-alkaline optima while fungal (and plant) extracellular enzymes have acidic optima (e.g., phosphatase; Nakas et al., 1987) must be more extensively tested before pH optima can be reliable used to distinginguish enzyme sources. It should be noted that this approach would be limited to certain enzymes (phosphatases, proteases), because many polysaccharide-hydrolyzing enzymes from bacteria and fungi have acidic pH optima.

On the assumption that extracellular eucaryotic enzymes are glycosylated, Rhee et al. (1987) estimated that fungi contributed approximately 86% of soil cellulase activity, based on the selective binding of extracted soil enzymes to the lectin concanavalin-A. Although certain proteins secreted by bacteria are known to be glycosylated, these are non-enzyme proteins that play various roles in cell

adhesion to surfaces. As with pH optima, broad application of distinguishing enzyme source by glycosylation would require more extensive development and testing, possibly in microcosm studies using general metabolic inhibitors of bacteria and fungi (e.g., Bailey et al., 2003) to shift the population structure.

Among the proteolytic enzymes, selective inhibitors have been used to show that bacteria can be a major source of soil proteolytic activity (Mayaudon et al., 1975; Bach and Munch, 2000; Kamimura and Hayano, 2000).

Approaches to interpreting soil enzyme functional diversity

Soil enzyme functional diversity can be analyzed and interpreted in a variety of ways, depending on the specific research questions. Functional diversity *between* nutrient resources could be based on specific enzyme activities against major C (cellulose), N (protein) and P constituents. Functional diversity *within* a nutrient group can be estimated by measuring cellulase and/or phenoloxidase for carbon, protease and amidase for nitrogen or phosphomono- and diesterases for phosphorus. Greater resolution of within group functional diversity could be gained by focusing within a given enzyme activity; e.g., proteolytic activities separated by inhibitor class.

At the simplest level, soil enzyme diversity has frequently been evaluated as differences in activity. Ratios between and within major C-, N- and P-processing enzymes can provide insight into the microbial community response to changing nutrient resources and the relative importance of different nutrients. Caldwell et al. (1999) found that the relationship between major C- and P-processing enzymes changed under different soil and vegetation regimes. Data from García et al. (1994) show a substantial range in the relationships between major nutrient processing enzymes across 12 Spanish soils. The phosphatase to β-glucosidase ratio ranged from 0.46 to 8.74, the protease to β-glucosidase ratio ranged from 0.01 to 0.27, and the protease to phosphatase ratio ranged from 0.01 to 0.15. Data from a 4-million-year soil chronosequence in Hawaii (Olander and Vitousek, 2000) can be further analyzed to show shifts in the ratios of the major soil enzymes. In modern, 300-year-old soil, the phosphatase to *N*-acetylglucosaminidase ratios in the organic and mineral soil horizons were 2.06 and 2.85, respectively. For 20,000-year-old soil, the ratios increased to 11.4 and 18.6, respectively, suggest an increasing importance of organic phosphorus, relative to organic nitrogen, with soil development. Ratios between energy- and nutrient-acquiring enzymes have been related to litter mass loss (Sinsabaugh and Moorhead, 1994). Sinsabaugh et al. (2002) plotted the distribution of three plant communities on axes of P-acquiring to N-acquiring enzyme activities and cellulase to (phenol)oxidase activities to show different responses to different levels of fertilization.

Several studies have included enzyme assays that could be used to indicate shifts in microbial processing between major types of resources *within* a specific nutrient cycle. Within the nitrogen cycle, data from Garcia et al. (1994) shows shifts in urease to protease ratio from 0.05 to 3.25, suggesting major differences in the relative importance or availability of protein-N versus urea-N. Within the phosphorus cycle, data from Sparling et al. (1986) show phosphodiesterase to phosphomonoesterase ratios ranging from 0.19 to 0.57, suggesting major differences in the organic phosphate pools being accessed across 20 New Zealand grassland soils.

Multiple soil enzyme activities can been mathematically condensed to a single number, such as the "lignocellulase" index, which expresses a hypothetical activity based on real lignin- and polysaccharide-degrading enzymes (Sinsabaugh et al., 1992).

Multivariate techniques have also been used increasingly to relate soil enzyme activities to microbial community structure and physiology (Nannipieri et al., 2002). Waldrop et al. (2000) calculated correlations between major soil enzyme activities and the first principle components axis of soil phospholipid fatty acid profiles (microbial community structure) across various Hawaiian vegetation types. Kourtev et al. (2002) examined the changing relationship between soil enzyme activities and microbial community level physiological profiles resulting from the invasion by exotic plants.

Various visual approaches have also been used. Plotting of various enzyme activities through time allows changing patterns among multiple enzyme activities to be examined (Sinsabaugh et al., 2002). Carreiro et al. (2000) used three-dimensional figures to show the differential effects of nitrogen fertilization on the decomposition rate and cellulase or phenoloxidase activities associated with three tree litters of differing quality.

One particularly useful visual presentation has been the use "star ray" diagrams (Nannipieri et al., 2002), where different enzyme activities are plotted along different radial axes. Sinsabaugh

and co-workers have used such diagrams to show the differential effects of nitrogen fertilization under three tree species on key enzymes responsible for major C, N, and P transformations (Carreiro et al., 2000; Saiya-Cork et al., 2002; Sinsabaugh et al., 2002). Although visually intuitive, showing fertilizer stimulates certain cellulolytic enzymes while depressing phenoloxidase and peptidase activities, such plots do not readily lend themselves to rigorous statistical analysis.

Surprisingly, conventional biodiversity measures (Pankhurst, 1997) have not been widely used in evaluating soil enzyme functional diversity. Tscherko et al. (2003) calculated Shannon diversity and eveness indices to show changes in enzyme diversity across primary successional chronosequences following receding glaciers. This approach could be readily applied to the kind of data expressed in "star ray" diagrams, converting a visually intuitive pattern to a statistically testable number. Although information is lost in the calculation of such indices (Pankhurst, 1997), direct comparison with similar indices of microbial or vegetation community structure would be possible, addressing such questions as how closely soil enzyme functional diversity is related to community structure.

Methodological considerations

Although there are widely used assays for many soil enzymes (Table 1; Burns, 1978; Tabatabai, 1994; Tabatabai and Dick, 2002), several specific considerations should be addressed to optimize such methods. The vast majority of current soil enzyme assays use bulk soils, which include enzymes recently released from active soil organisms in response to nutrient stress and availability as well as a significant amount of enzymes that have been stabilized into the organomineral matrix through time (Nannipieri et al., 2002). Distinguishing the fraction of soil enzyme activity most closely associated with the living biomass from residual immobilized activities should significantly improve our ability to link microbial function (expressed enzyme activities) with microbial physiology (nutrient stress) and resource availability.

The widespread use of artificial colorimetric (Tabatabai, 1994) and fluorometric substrates (Marx et al., 2001) along with multi-well plate reader technology (Wirth and Wolf, 1992; Marx et al., 2001) allows the rapid and inexpensive development of large data sets. However, certain aspects of enzyme functional diversity could be improved using more natural substrates. While artificial β-glucosidase susbstrates are frequently used to estimate cellulolytic activity, there are at least two distinct β-glucans common to soil; β(1-4) cellulose in litter and β(1-3) glucans common in soil polysaccharide (Cheshire, 1979). Use of more natural substrates, e.g., cellulose (Deng and Tabatabai, 1994) and laminarin (Lethbridge et al., 1978) could distinguish which forms are being used. Probably the most frequently used soil enzyme assay is based on the artificial phosphatase substrate, *p*-nitrophenylphosphate. Use of compounds known to occur in soil, such as phytates (Svenson, 1986) or nucleic acids (Frankenberger et al., 1986), would greatly expand our understanding of organic phosphorus turnover.

Conclusions

Adding soil enzyme functional diversity to our growing repertoire of diversity techniques could significantly increase our understanding of the linkages between resource availability, microbial community structure and function, and ecosystem processes. Determining how to measure and interpret soil enzyme functional diversity will largely be determined by the nature of the questions being asked. Possible components of soil enzyme functional diversity include using specific substrates to explore diversity between and within nutrient cycles, as well as specific inhibitors to distinguish different reaction mechanisms. Methods to distinguish broad taxonomic sources of specific soil enzyme activities by pH optima, glycosylation and/or selective inhibitors should be further explored. Ratios of various nutrient-processing enzyme activities can provide insight into how the soil community is responding physiologically to changes in the nutritional environment. In addition to multivariate analyses, use of traditional diversity indices would allow direct comparison of enzyme functional diversity with the taxonomic and physiological diversity of the soil microbial and vegetation communities and the soil foodweb.

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