Biol Fertil Soils (1994) 17:69–74
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ORIGINAL PAPER

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Enzymic analysis of microbial pattern and process

Received: February 1992

Abstract Enzyme assays, once used primarily to collect descriptive information about soils, have become useful techniques for monitoring microbial activity and uncovering the mechanisms that underlie microbial processes. The simplest paradigm is that decomposition and nutrient cycling are emergent consequences of extracellular enzyme activities that are regulated directly by site-specific factors such as temperature, moisture and nutrient availability, and secondarily by litter chemistry through adsorption, inhibition and stabilization processes. In application, enzyme techniques are employed on three scales of resolution. On the largest scale, assays for ubiquitous enzymes such as phosphatase, esterase, and dehydrogenase are used as general measures of microbial activity. At higher resolution, enzyme specificity is exploited to monitor activity related to specific aspects of macronutrient cycling. At the highest resolution, the enzymatic mechanisms by which microorganisms interact with their environment are addressed.

Key words Enzymes · Microbial activity · Decomposition · Nutrient cycling

Introduction

Enzyme assays belong to the rapidly expanding repertoire of biochemical and molecular techniques with the potential to resolve long-standing questions concerning the structure, function and dynamics of microbial communities. However, unlike most of these methods, which are of recent origin, enzyme assays have been applied to soils since the beginning of the century (Skujiņŝ 1978). Much of this early research was directed toward identifying the sources and nature of soil enzyme activity and attempting to develop protocols for classification and diagnosis of agricultural soils. By the late 1970s, this effort had produced a conceptual foundation (Burns 1983) that I will briefly review before presenting an overview of current applications and future directions.

The fundamental dichotomy in an ecological classification of enzymes is between those confined within the plasma membranes of viable cells and those external to the cell (Fig. 1). The former are sometimes referred to as endoenzymes and the latter variously as exoenzymes, ectoenzymes, or abiontic enzymes (Skujiņŝ 1978; Chróst 1991). From an ecological perspective, extracellular enzymes are the focus of interest because they catalyze the rate limiting steps of decomposition and nutrient cycling: the extracellular degradation of polymeric, insoluble or otherwise complex molecules to yield assimilable macronutrients (e.g., Dighton and Boddy 1989).

Extracellular enzymes can be further classified by their deployment (Fig. 1). Ectoenzymes, defined as extracellular enzymes associated with viable cells (Chróst 1991), include those embedded in or spanning the plasma membrane, those attached to the outer membrane surface through hydrophobic interactions or covalent linkages, and those lying within the periplasmic space or associated with the cell wall. Such enzymes have also been described as biontic (Skujiņ 1978). Enzymes released into the environment by secretion or lysis, and active enzymes associated with dead cells and other non-living soil fractions are described as abiontic. Abiontic enzymes are temporally or spatially displaced from their cell of origin and represent a functional legacy to the extant community (Burns 1983; Nannipieri et al. 1983).

Both autotrophic and heterothrophic microbes deploy a wide range of hydrolases, oxidases, and reductases (Price and Morel 1990). The types of enzymes, their physiological and ecological significance, and the strategies for deployment vary widely among taxa. Although such information is essential for understanding the interactions between microorganisms and their environment, at present much is unknown. The extracellular enzyme system that has received the broadest attention is that responsible for cellulose degradation. This system includes multiple hydrolytic and oxidative enzymes. While

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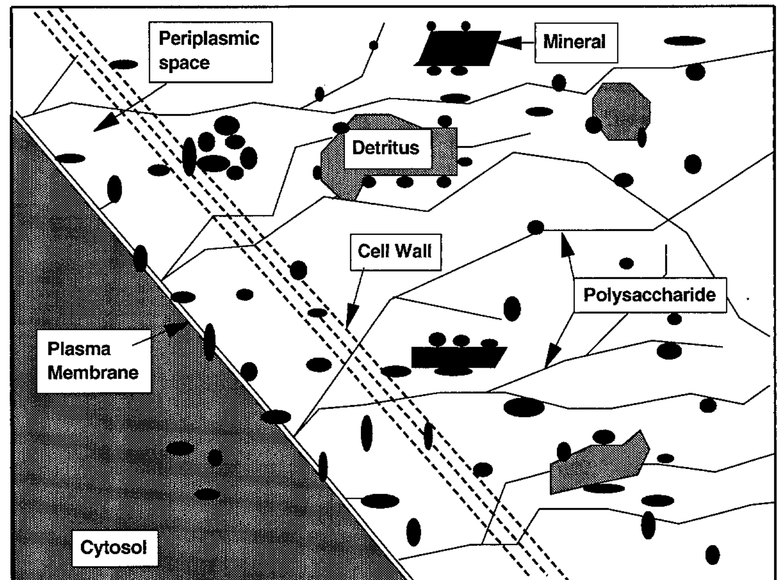
Fig. 1 Conceptual scheme of microbial enzyme deployment. In addition to intracellular enzymes, both heterotrophic and autotrophic microorganisms deploy an array of extracellular hydrolases, oxidases and reductases. These enzymes may be variously associated with the plasma membrane, periplasmic space, cell wall and glycocalyx or may be released into the microenvironment where they become associated with organic and mineral particles



generalities can be made about the types of enzymes involved in cellulolysis, considerable diversity exists among taxa in the number of enzymes synthesized, their substrate affinities, the nature of their interaction, and the strategy of deployment (Eriksson and Wood 1985; Ljungdahl and Eriksson 1985; Marsden and Gray 1986). Although less well studied, such complexity is probably characteristic of most polymer-degrading enzyme systems, e.g. lignin, hemicellulose (Dekker 1985; Kirk and Farrell 1987; Wong et al. 1988).

When this complexity is integrated at the community level, new structure-function relationships emerge. On surfaces, microorganisms from diverse taxa tend to organize into consortia that exhibit a multicellular division of labor (Shapiro 1991). In turn, consortial organization promotes functional synergism in metabolic activities (Costerton et al. 1987; Hamilton 1987; Blenkinsopp and Costerton 1991). This synergism is usually attributed to the proximity of the cells and to an extracellular system of enzmyes and glycocalyx, but this topic has received little investigation (Lock et al. 1984; van Loosedrecht 1990; Sinsabaugh et al. 1991b).

Regulation of extracellular enzyme activities

Like all ecological processes, extracellular enzyme activity in litter and soil is regulated by a suite of environmental variables that interact over a wide range of scale. Though poorly understood, a simplified model has been proposed that has some heuristic value (Sinsabaugh et al. 1991a). In this model, enzyme activity is controlled at two levels (Fig. 2). At the ecosystem level, enzyme production is a function of microbial activity which is regulated by moisture, temperature, and nutrient availability (Insam et al. 1989; Insam 1990). At the microenvironmental level, temperature and moisture continue to influence the activity of released enzymes, but responses are modulated by enzyme-substrate (i.e. substrate in the broad sense: litter, detritus, minerals) interactions such as inhibition, adsorption, stabilization and humification (Sinsabaugh et al. 1991a). These processes alter the apparent pH optima, activation energies, and kinetics of the enzymes and determine their turnover rate (Burns 1983; Boyd and Mortland 1990). In general, the immobilization of abiontic enzymes on organic or mineral particles stabilizes activity, but often at the cost of increases in apparent activa-

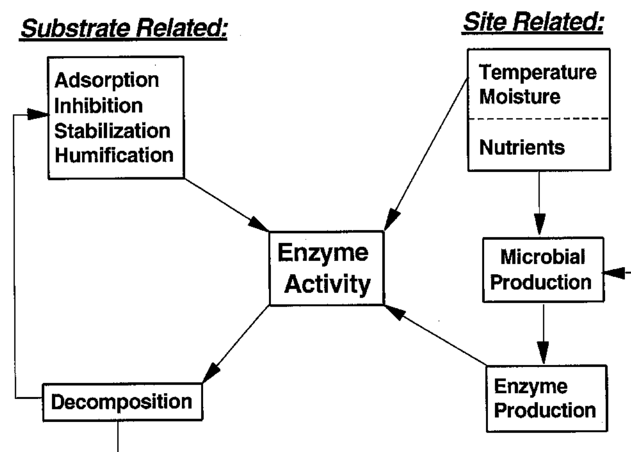


Fig. 2 Two-level model for environmental regulation of extracellular enzyme activity in litter and soils. Site-linked patterns of temperature, moisture and nutrient availability control microbial activity and therefore enzyme production. Once released, enzyme activity is also influenced by substrate characteristics. Temperature and moisture continue to have direct effects, but enzyme interactions with dissolved and particulate substrates control turnover rate and alter apparent enzyme properties such as Michaelis-Menton kinetic constants, activation energies, and pH optima. The activity of these enzymes contributes to decomposition, a process that affects future activity by altering substrate properties and recovering nutrients required to sustain microbial activity

tion energy (E_a), half-saturation constant (K_m), and pH optimum and a decrease in substrate conversion potential (V_{max}) (e.g. Pettit et al. 1976; 1977; Batistic et al. 1980; Sakar and Burns 1984; Eivazi and Tabatabai 1988; McClaugherty and Linkins 1989; Mayer 1990). The magnitude of these effects depends strongly on the characteristics of both the enzyme and the adsorbing substrate (Sinsabaugh and Linkins 1989; Boyd and Mortland 1990).

Application of enzyme assays in ecological research

While the constraints imposed on microbial activity by large-scale spatiotemporal patterns of temperature, moisture, and nutrient availability are generally known, comparatively little is knwon about the small-scale dynamics of microbial communities. Historically, progress in this area has been limited by methodology. The potential of biochemical and molecular techniques is high resolution analysis of both structure and function in microbial communities. The specificity of enzymatic reactions can be exploited as a research tool for discriminant investigation of the spatial and temporal dynamics of microbial communities and the mechanisms that underlie microbial processes.

Which enzymes are of interest depends, of course, on the question and the scale.

Low-resolution applications

At larger scales, enzyme assays are sometimes used as indices of microbial activity in general. At this level, low-specificity assays that detect ubiquitous classes of enzymes such as dehydrogenase, esterase and phosphatase are the most frequently monitored (e.g., Frankenberger and Dick 1983; Siuda 1984; Jones and Lock 1989; Sikora et al. 1990).

At this scale, the principal advantages of enzyme activity indices are ease of measurement and sensitivity. Most often, assays are used as a surrogate measure of microbial activity in studies directed at larger scale questions. For example, Sinsabaugh et al. (1991 c) measured alkaline phosphatase activity along benthic stream transects to describe the patch structure of epilithic biofilms. In soils, Bonmati et al. (1991) examined spatial variation in enzyme activities, organic carbon, and nitrogen. Because statistical analyses of spatiotemporal dynamics often requires many data points, enzyme assays are particularly useful in this context.

On the negative side, it is clear that no single assay can be a surrogate for the spectrum of processes that we call "microbiological activity." In addition, some assays that have been widely used for this purpose have structural limitations that seriously compromise the interpretation of results: dehydrogenase assays, which purportedly measure electron transport potential, are very inefficient; differences in the kinetics of substrate uptake among taxa affect the outcome of esterase assays (Nannipieri et al. 1990). At present, the best approach is to employ multiple assays, then combine the results into a composite index or use multivariate statistics to compare samples.

Medium-resolution applications

At higher resolution, questions may focus on a specific segment of community activity, such as lignocellulose degradation or organic phosphorus mineralization. As alluded to earlier, each of the major insoluble components of plant litter require multicomponent enzyme systems for complete degradation. Presumably because of the energy costs associated with enzyme production, few microorganisms are capable of elaborating a full complement (Marsden and Gray 1986; Saddler 1986; Kirk and Farrell 1987). Thus, litter decomposition is an emergent process of microbial communities.

In principle, decomposition rates should be correlated with the activity of the enzymes responsible for the degradation, but the relationship is confounded by the plethora of enzymes involved and the fact their substrates are chemically linked and physically intercalated (Marsden and Gray 1986). Despite the complexity, several studies have shown an emergent correlation between cellulase activity and rate of mass loss from leaf litter, both within and between litter types (see review by Sinsabaugh et al. 1991 a). Recently, this approach has been extended to wood decomposition.

Sinsabaugh et al. (1992 a) placed arrays of white birch sticks at eight upland, riparian and lotic sites on a forested watershed in northern New York. For 3 years, samples were analyzed for mass loss, protein, nitrogen and phosphorus accumulation, and the potential activity of 11 classes of extracellular enzymes involved in lignocellulose degradation and N & P cycling. Although there was considerable heterogeneity both within and between sites, mass loss was closely correlated ($r^2 = 0.65-0.83$) with the temporally integrated activities of five lignocellulose-degrading enzymes: β-glucosidase, endocellulase, exocellulase, phenol oxidase, β-xylosidase. Because of the high degree of intercorrelation among these five enzymes, a composite variable called integrated lignocellulase activity was generated from them using principal components analysis. A regression model based on integrated lignocellulase activity accounted for 94% of the variance in mass loss rates (Fig. 3). Models of this type could be useful for estimating decomposition rates within detritus compartments or between landscape patches; the principal advantage being that large numbers of sites could be monitored concurrently.

The enzymes involved in the other macronutrient (N, P, S) cycles have received less attention. There are two categories of interest. First, extracellular enzymes that recover N, P and S from organic substrates. In the case of N, these include chitinases, proteases, peptidases, amidases, deaminases, and nucleases; for P, phosphomonoesterases, phosphodiesterases, and phospholipases; and S, sul-

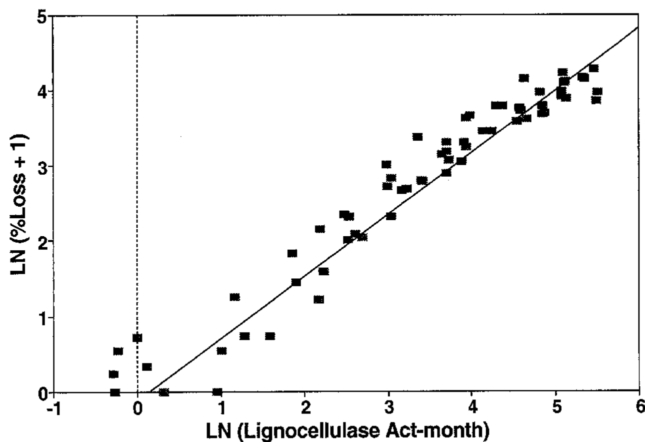


Fig. 3 The relationship between mass loss and temporally integrated lignocellulase activity for white birch sticks at eight upland, riparian and lotic sites over a first-order catchment. Over a 3-year-period, the cumulative activities of five lignocellulose-degrading enzymes (β-glucosidase, endocellulase, exocellulase, β-xylosidase, and phenol oxidase) were found to be strongly linked with mass loss despite pronounced hydrologic and edaphic differences among sites. Integrated lignocellulase activity is a composite variable generated from these five constituent activities using principal components analysis. This relationship suggest that enzyme assays can be used to estimate decomposition rates among landscape units. (From Sinsabaugh et al. 1992a)

fatases. The second category is enzymes that oxidize or reduce inorganic N and S. These enzymes are intracellular and associated with energy-yielding (anaerobic respiration, chemosynthesis) or biosynthetic (assimilatory denitrification, nitrogen fixation) pathway; while of intense interest they fall outside the scope of this overview.

The relationship between extracellular enzyme activities and organic N, P and S pools is receiving increasing attention. The largest body of work has focused on phosphatase activity and phosphorus availability in aquatic systems (e.g., Stewart and Wetzel 1982; Siuda 1984; Berman 1988; Cotner and Wetzel 1991), but several investigations have been conducted in soil systems as well. Tarafdar and Jungk (1987) found that both acid and alkaline phosphatase activities were enhanced in the vicinity of roots while organic P availability declined. Similarly, Häussling and Marshner (1989) who quantified organic and inorganic P, acid phosphatase activity and hyphal lengths in soil fractions under Norway spruce, found that compared with bulk soil, labile organic P concentrations were lower in the rhizosphere and rhizoplane, and phosphatase activity was more than twice as high. Rojo et al. (1990) reported a correlation between organic P mineralization and phosphatase activity for two pasture soils.

The relationships between organic N and extracellular enzyme activities have been less explored. These interactions are more diffuse than those of P because N is associated with nucleic acids, polysaccharides, proteins and humic complexes. Unlike extracellular phosphatases which typically have wide substrate preferences, each of these N pools is accessed by discrete enzyme systems. In aquatic systems, the relationship of protease and aminopeptidase activities to protein degradation has received the most study (e.g., Mayer 1988; Billen 1991). Aminopeptidase activity has also been shown to be induced by low N conditions and noncompetitively inhibited by inorganic N (Chróst 1991). Protease activities have also been studied in soils (e.g. Ladd 1972) but not in the context of their relationship to N acquisition by microorganisms. From this perspective, the best studied relationship in soils is that or urea and urease (Bremner and Mulvaney 1978), a focus of attention because of the use of urea fertilizers in agriculture. Recently, Sinsabaugh et al. (1992b) found an inverse relationship between nitrogen immobilization and chitinase activity for decomposing white birch sticks.

The existence of a common regulatory motif at the cellular level for many ectohydrolases (Chróst 1991), supported at the system level by numerous observations in both aquatic and soil systems of inverse relationships between P availability and phosphatase activity and N availability and N-acquiring enzyme activities, suggest that measurements of specific enzyme activities can be employed as indicators of relative nutrient limitation, a possible alternative to nutrient supplementation trials. This approach has been used in aquatic systems in the case of alkaline phosphatase activity (Wetzel 1981; Gage and Gorham 1985). Recently, Sinsabaugh et al. (1992b) have used it to determine whether N or P was limiting wood decomposition at upland, riparian and lotic study sites. As cited for other applications, the potential advantages of this approach are sensitivity and the capacity for analyzing large numbers of samples. However, this approach may not be applicable to soils (as opposed to litter), at least for short-term assessments, because the bulk of the enzyme activity may be abiontic. Nannipieri et al. (1978) showed that while inorganic P supplemention repressed microbial phosphatase synthesis it did not decrease bulk soil activity.

In the past decade interest in the formation and mineralization of soil organic sulfur has grown, motivated by concerns about deposition effects (David and Mitchell 1987). Because much of the organic sulfur in soils exists as ester sulfates, the activity of extracellular sulfatases have received close attention (Ganeshamurthy and Nielsen 1990 and references therein). The oxidation of carbon-bonded sulfur from the amino acids methionine and cysteine (Hale and Fitzgerald 1990 and references therein), and the generation of methylated sulfur gases, both intracellular processes, have also been actively investigated (Drotar et al. 1987).

High-resolution applications

At higher resolution, the questions become physiological and biochemical. How do microorganisms and extracellular enzymes interact with their environment? These are mechanistic questions. Research effort of this scale involves identifying and characterizing enzymes and evaluating effects of substrate interactions such as inhibition,

stabilization, humification and adsorption on enzyme kinetics and turnover (e.g. Batistic et al. 1980; Nannipieri et al. 1988; Ruggeiro and Radogna 1988; Sarkar et al. 1989; Boyd and Mortland 1990).

This is also the scale where element cycles converge. Price and Morel (1990) have recently emphasized the role of extracellular enzymes as a nexus for macro and micronutrient cycles. They identify three linkage mechanisms: (1) noncompetitive inhibition of ectoenzymes by heavy metal ions, (2) requirement of many ectoenzymes for metal ion cofactors, and (3) use of metal ions as electron acceptors for transmembrane reductases. Examples include carbonic anhydrase, urease, protease and phosphatase, which all require metal ions for activity (Price and Morel 1990).

Conclusion

Enzyme assays offer the advantages of sensitivity, specificity and facility. Because they can be applied over a range of scales, they are attractive techniques for monitoring microbial activity and investigating process mechanisms, scale integration and patch dynamics.

Acknowledgements This paper was originally presented to the Soil Ecology Society on 3 April 1991.

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