Manual of Environmental Microbiology

Soil enzymes: linking proteomics and ecological process

Steven D. Allison
Department of Ecology and Evolutionary Biology
University of California, Irvine
Irvine, CA 92697
Phone: (949) 824-9423
Fax: (949) 824-2181
Email: allisons@uci.edu

Tracy B. Gartner
Department of Biology and Environmental Science
Carthage College
Kenosha, WI 53140
Phone: (262) 551-5803
Fax:
Email: tgartner@carthage.edu

Keri Holland
Department of Ecology, Evolution & Marine Biology
University of California, Santa Barbara
Santa Barbara, CA 93106
Phone: (805) 893-7379
Fax: (805) 893-4724
Email: holland@lifesci.ucsb.edu

Michael Weintraub
Department of Earth, Ecological, and Environmental Sciences
University of Toledo
Toledo, OH 43606
Phone: (419) 530-2009
Fax: (419) 530-4421
Email: michael.weintraub@utoledo.edu

Robert L. Sinsabaugh*
Department of Biology
University of New Mexico
Albuquerque, NM 87131

Phone: (505) 277-3407
Fax: (505) 277 0304
Email: rlsinsab@unm.edu

*Corresponding author. Send proofs to:

Robert L. Sinsabaugh
Department of Biology
University of New Mexico
167A Castetter Hall
Albuquerque, NM 87131

1. Introduction

1.1 Overview

"Enzymes are things invented by biologists that explain things which otherwise require harder thinking."

-Jerome Ysroael Lettvin

"*Ecosystems .... are functionally a system of stored, immobilized enzymes*."

-Robert G. Wetzel

Extracellular enzymes are the proximate agents of organic matter transformation in soils. From a physiological perspective, these enzymes catalyze the initial steps in catabolism: the conversion of polymeric compounds into dissolved substrates that microbes can consume for growth. From an ecosystem perspective, extracellular enzymes are the mediators of decomposition, dissolved organic carbon (C) production, and nitrogen (N) and phosphorus (P) mineralization.

The production of extracellular enzymes is controlled at the organismal level by pairing low level constitutive synthesis with induction-repression pathways tied to environmental substrate availability (21). Enzyme production is also coordinated at the population and community level by quorum sensing systems that have not yet been extensively studied in soil systems (6). The existence of these higher-order regulatory systems underscores the role of extracellular enzymes in the integration of microbial community function.

Once expressed, the function and fate of extracellular enzymes are environmentally controlled (Fig. 1). Most commonly, this control is considered in terms of bulk environmental conditions such as moisture, temperature and pH. However, enzyme kinetics and enzyme turnover are also affected by soil structure, which controls enzyme immobilization and substrate accessibility (80). As extracellular enzymes interact with humic molecules, reactive oxidants, proteases and soil particles, their function is progressively compromised.

Traditionally, activities of extracellular enzymes have been difficult to interpret due to technological difficulties and knowledge gaps (16, 38). However, these challenges are being overcome, and measurements of extracellular enzyme activity (EEA) are increasingly used in ecosystem ecology as indices of nutrient limitation, organic matter characteristics, microbial activity, and ecosystem response to environmental perturbations (4, 61, 64, 83). High throughput protocols based on microplate technology can largely automate measurements of many enzyme activities and allow kinetic parameters to be estimated across multiple soil fractions. Data on microbial distributions and diversity are accumulating rapidly, and advances in molecular biology are providing new tools that are applicable to extracellular enzyme studies. In particular, proteomic approaches can be used to identify the extracellular enzymes that link genomic information with ecological process.

1.2 Classification and assay of extracellular enzyme activity

Enzymes are formally classified by the chemical reaction that they catalyze (39). In soils, the extracellular enzymes of interest are those involved in the degradation of plant and microbial cell walls, the generation of humus, and the mineralization of organic N and P. These enzymes can be broadly divided into those that catalyze hydrolytic reactions, typically the cleavage of the

C-O and C-N bonds that link monomers (e.g. glycosidases, peptidases, esterases), and those that catalyze oxidative reactions, typically C-C and C-O-C bonds. The hydrolases can be further resolved into those that release monomers from the ends of polymers (exohydrolases) and those that that cleave interior linkages (endohydrolases). The oxidative enzymes can be roughly divided into oxygenases and peroxidases that use molecular oxygen and hydrogen peroxide, respectively, as electron acceptors.

The vast majority of EEA studies focus on exohydrolase activities, principally because they are the easiest to assay. Soluble substrates, linked to colorigenic or fluorigenic moieties are readily available. Because exohydrolases typically generate monomeric or dimeric products that can be directly consumed by microorganisms, these activities can often be related to other measures of microbial activity (83). Endohydrolase activities are more problematic to study. Their substrates are often insoluble, and it can be difficult to quantify the number of bonds broken (37). Theoretically, these activities are further removed from microbial metabolism and are strongly influenced by organic matter composition and soil structure.

Studies of oxidative EEA are on the increase. Oxidative enzymes are associated with the degradation of lignin, tannin and other secondary aromatic compounds and the formation of humic substances. These enzymes have low substrate specificity, utilize free-radical mechanisms, and catalyze both degradative and polymerization reactions (22). On ecosystem scales, these activities can be linked to dissolved organic C export and soil C sequestration (33, 34, 91).

In the sections that follow, we present brief overviews of recent advances in extracellular enzyme research.

2. Microbial community controls on enzyme production

2.1 Economic framework for extracellular enzyme production

The synthesis of enzymes requires energy and consumes nutrients that are lost to the producer if the enzyme is released into the environment. Moreover, the producer has little control over the function and fate of the enzymes released. In a competitive and resource-limited environment, these factors dictate that microorganisms regulate extracellular enzyme production to limit costs and maximize resource returns. This economic view is supported by the ubiquity of signal pathways that link enzyme expression to environmental substrate availability or population density (20, 84). An economic framework is therefore a good starting point for predicting patterns of enzyme activity at the community level in relation to environmental gradients in substrate composition and nutrient availability.

There is substantial evidence that microbial communities allocate resources to enzyme production in relation to substrate availability and growth requirements (3, 20, 71). In fertilization experiments, there is an inverse relationship between phosphatase activity and high P availability (20, 61). For N, the situation is more complex because N mineralization is closely coupled to C mineralization (55), and organic N is found in a diversity of compounds (e.g. amino sugars, polypeptides, humus) that are degraded by different enzymes. However, there is evidence that increased inorganic N availability suppresses peptidase activities (81), and conversely that addition of organic N can induce aminohydrolase and peptidase activities (74, 95). In some systems, there is also an inverse relationship between inorganic N availability and oxidative enzyme activity (75).

2.2 Distribution of extracellular enzyme activity across groups

If the controls on enzyme expression are similar across prominent taxa, the distribution of EEA should reflect nutrient demand and optimal resource allocation at the microbial community level (57). This assumption is the basis for comparisons of EEA among systems (71, 73, 76). However, the identities of microbes that produce particular enzymes within the soil matrix are largely unknown. Most molecular analyses of community composition focus on rRNA sequences or other taxonomic tags that permit little inference about the degradative capabilities of uncultured organisms. Comparative studies of functional gene diversity within soil communities are increasing (47, 49), but many sequences cannot be matched to specific taxa, and genes that are present may not be expressed. Schulze et al. (70) addressed the latter problem by isolating and sequencing proteins extracted from soil particles, providing direct evidence for the contribution of particular taxa to extracellular function.

Consequently, most information about the taxonomic distribution of extracellular enzyme production comes from culture studies. The literature on cellulases, ligninases and other enzymes involved in the degradation of cell walls is the subject of frequent reviews (7, 14, 42). In general, the ability to produce hydrolases that degrade the polysaccharide components of cell walls is widely distributed across bacteria and fungi (48). The expression of oxygenases and peroxidases appears to be more restricted. Many basidiomycete fungi produce peroxidative enzymes capable of degrading lignin and other recalcitrant aromatic compounds (14, 42), but other fungi and many bacteria also produce oxidative enzymes to degrade aromatic molecules (22).

At the ecosystem level, soil EEA should reflect enzyme production by the active members of the microbial community. Like macroorganisms, microorganisms show r- or K-

selected traits based on the range of substrates they can utilize, their rates of growth, and their ability to prevent interference from competitors (28, 30). Based on the ability of microbes to mineralize C, Fierer et al. (30) suggest that many Alpha-proteobacteria and Bacteroidetes may be more r-selected, whereas many Beta-proteobacteria and Gram positive bacteria may be more K-selected. K-selected microbes are more persistent and degrade recalcitrant substrates that require a large investment in extracellular enzyme production with a lower probable return (28). Furthermore, studies of enzyme evolution suggest that specialist function may be advantageous in specific environments while generalist functions may confer a wider advantage in more heterogeneous environments (29). Therefore, successional patterns in the active microbial community driven by changes in substrate availability can determine EEA distributions (72).

3. Plant controls on extracellular enzyme activity

3.1 Enzyme production by roots

Plant roots produce a variety of extracellular enzymes, including phosphatase, invertase, amylase, and protease (reviewed in 24). Of these, phosphatase is most prevalent. In response to low ambient inorganic phosphate concentrations, phosphatase production in the root zone increases as much as two orders of magnitude (79, 94).

3.2 Enzyme production by mycorrhizal fungi

Root activity may be supplemented by enzyme production from mycorrhizal fungi (reviewed by 66). Arbuscular mycorrhizal fungi (AMF) are primarily involved in P capture via production of phosphatases, accounting for 48-59% of total P uptake when P is supplied in

organic form, but only 22-33% when supplied in inorganic form (82). In contrast, the AMF contribution to N mineralization is much more limited (36, 50).

The ability of ectomycorrhizal fungi (ECM) to obtain nutrients from complex organic sources remains uncertain given that only a fraction of currently known ECM species have been examined for EEA (23). However, genes encoding lignin and manganese peroxidases are present in a number of basidiomycetes (19), and ECM show proteolytic activity in systems where N mineralization is limited (18, 78). Cellulase, phosphatase, and polyphenol oxidase activities have also been observed in ECM mats (8, 35).

Though unable to attack lignin directly with peroxidases (9-11), ericoid mycorrhizal fungi can degrade soil organic matter by producing hydrogen peroxide and hydroxyl radicals, which degrade cellulose (40) and participate in side chain oxidation and demethylation of lignin (15). Other enzymes such as laccase and catechol oxidase target hydrolysable polyphenols (10, 11); polyphenols often link with N- and P-containing substrates in humus, so degrading polyphenols contributes to N and P mineralization.

3.3 Effects of plant inputs on enzyme production

Soil microbes are commonly thought to be C-limited (87); thus the timing of plant C inputs to the soil is one of the principal controls on microbial EEA production (85). Inputs of plant C from root exudates are typically greatest at the height of the plant growing season, when photosynthetic rates and nutrient demand are highest. These exudates contain a complex mixture of organic acid anions, sugars, amino acids, purines, nucleosides, inorganic ions (e.g. HCO_3^-, OH^-, H^+), and enzymes (24) and may be the principal form of C inputs to soil (13).

3.4 Invasive species

There is good evidence that invasive plant species alter nutrient cycling processes, and that these changes are sometimes mediated by EEA (27, 43). On young lava flows on the Island of Hawaii, invasion by the N-fixing tree *Falcataria moluccana* dramatically increased acid phosphatase activity, a change which could alleviate secondary P limitation and facilitate further invasion (2). In a greenhouse experiment with understory plants from temperate deciduous forest, most soil EEAs were greater in pots containing an invasive stiltgrass (*Microstegium vimineum*) than in pots containing native blueberry (*Vaccinium* spp.) (43). In contrast, both P- and N- degrading activities decreased beneath pasture grass (*Cenchrus ciliaris*) compared to native caatinga canopy (*Ziziphus joazeiro* and *Spondias tuberosa*) in Brazil (96). Given that both positive and negative changes occur, more studies are needed before making general statements about enzyme responses to plant invasions, and how these changes may feed back to invasibility.

4. Abiotic controls on extracellular enzyme activity

Interpreting potential EEA measurements as indices of recent plant and microbial enzyme production on an ecosystem scale can be problematic. Soils contain a large stabilized enzyme pool with turnover times of weeks to months, and possibly much longer (16, 25). Soil texture and the size distribution of soil particles largely determine the size of this abiotic pool, which can vary for different enzymes (52). Enzymes are stabilized in soils a number of ways: microencapsulation, adsorption, physical entrapment, cross-linking, copolymer formation, or ion exchange (93). The type of substrate (carbohydrate, lipid, clay) stabilizing the enzyme determines the processes involved and the impact of stabilization on enzyme kinetic parameters

(half-saturation constants, activation energy, reaction rate) (41). For example, many researchers have observed a shift in the pH optima of clay bound enzymes; this is thought to result from changes in enzyme conformation because of electrostatic interactions with mineral surfaces (45).

Generally, the abiotic enzyme pool is thought to be functionally passive because the enzymes or their substrates are physically occluded or kinetically compromised, although this may not always be the case (65, 67). As a result, some researchers find poor correlations between potential EEA and measures of microbial biomass or activity (e.g. 89, 92). Also, significant changes in active EEA as consequence of disturbance or succession within the microbial community tend to be masked when measured in association with a larger passive pool. However, stabilization effects are not necessarily disadvantageous for enzyme producers. Stabilization may extend the functional ranges and turnover times of enzymes, resulting in a greater return on resource investment in spatially and temporally heterogeneous environments.

The kinetics of soil enzymes are also directly affected by water availability, pH, substrate availability, and temperature (54, 59, 65). In some cases, activity optima may be adapted to local conditions. For example, enzymes isolated from extreme thermophiles are stable at high temperatures, while enzymes produced by psychrophiles show high stability at low temperatures (86). Similarly, enzymes derived from halophiles are active at high salinities.

Abiotic variables establish the landscape in which enzyme producers compete. For example, diffusion rates are affected by moisture availability, soil structure, and the chemical properties of enzyme and substrates. High diffusion rates lower the return on investment to the enzyme producer and could result in the down-regulation of enzyme production (1, 28). However, if water availability is too low, the diffusion of substrates, products and enzymes

diminishes rapidly (56), and enzyme function may decline due to increasing solute concentrations and shifts in pH.

For comparative purposes, EEA potentials are generally measured under reference conditions that may be very different from those *in situ*. Depending on the research question, this abstraction can either complicate or facilitate the relation of EEA to *in situ* process rates. The distribution of enzymes throughout the soil matrix reflects the dynamic interaction between soil structure, environmental conditions, and microbial community characteristics (Fig. 1). As a result, soil EEA profiles can integrate microbial activity over timescales that exceed biomass turnover. Thus, bulk soil EEA measurements are most useful for research questions about long-term (i.e. seasonal to annual) responses of microbes to the soil environment. To assess short-term changes in active enzyme pools, i.e. those connected most directly to the extant microbial community, it is usually necessary to isolate selected soil fractions, such as the rhizosphere.

5. Enzymes and soil carbon dynamics

5.1 Decomposition and microbial succession

The decomposition of a cohort of plant material can be described as a successional loop that links substrate, microorganisms and extracellular enzymes: substrate composition selects for microbial populations that produce extracellular enzymes that modify the substrate, leading to changes in microbial community composition (26). Early in decomposition, litter is colonized by fast-growing, opportunistic microorganisms that have a high affinity for soluble substrates. As decomposition proceeds, this group is succeeded by slower growing decomposers specializing in holocellulose degradation. Eventually, all non-lignified polysaccharides in the litter are

consumed, and the decomposition of the remaining material is tied to the oxidative breakdown of lignin and humic condensates by slow-growing decomposers. EEA potentials, linked to both substrate composition and microbial activity through the successional loop, are versatile metrics for monitoring or comparing decomposition processes.

5.2 Enzymes and carbon sequestration

Litter material that does not decompose is sequestered in the soil organic matter pool as humus. The quantity of this material is a function of initial litter composition, nutrient availability, biotic interactions, and environmental conditions. In general, more C tends to be sequestered from litter with high concentrations of lignin, tannin, and other secondary compounds, largely because the oxidative degradation of these aromatics creates reactive intermediates that condense with other molecules to produce humics (12).

Enzymatic responses to increasing N availability have important consequences for soil C pools. In Michigan, USA, N deposition stimulated C-degrading enzymes and soil C losses in forests dominated by labile litter inputs, while suppressing oxidative enzymes and soil C losses in forests receiving more recalcitrant litter inputs (17, 90). Similar results have been identified for soil organic matter: decomposition of light soil fractions typically increases with N addition, but the heavier, more recalcitrant soil fractions become further stabilized, presumably as a result of lower oxidative enzyme activity (60). Such shifts in enzyme activity have been used to explain many negative or small responses of decomposition to N addition, especially in recalcitrant substrates (32, 51).

Some micronutrients, particularly Mn and Ca, can also affect decomposition rates. Mn is essential for Mn peroxidase, a lignin-degrading enzyme produced by many fungi, and is also

involved in the regulation of other lignolytic enzymes, including laccase and lignin peroxidase (5, 63). Ca availability can limit the growth of lignin-degrading white rot fungi (46).

5.3 Mineral-carbon interactions

The storage of organic C (OC) in soil is strongly influenced by soil texture. Because of their surface area and layered structure, clay minerals provide most of the sorptive capacity of soils. Clays stabilize organic matter by shielding molecules from enzymatic attack within small pores or by altering substrate conformations (53, 97). These immobilized organic molecules may not be exposed to decomposition until protective clay minerals are degraded by weathering processes.

Another common mechanism by which clays protect OC from enzymatic attack is through multivalent-cation bridging between negatively charged organic groups and negatively charged clay particles (reviewed in 44). Multivalent cations cause clays to remain flocculated, reducing the exposure of OC adsorbed onto clay particles to attack by microbial enzymes. Second, they can also reduce the efficiency of enzymatic degradation of OC by altering the three dimensional orientation of organic molecules containing carboxyl groups (44).

6. Methodological Considerations

6.1 Measurement principles

The Michaelis-Menton equation describes how the rate of a simple enzymatic reaction, V, changes with substrate concentration, S:

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

V_{max} is the maximum velocity of the reaction when all enzymes are functioning at capacity. K_m is a half saturation constant, i.e. the concentration of substrate at which V is half of V_{max}., and a measure of the binding affinity between the substrate and the enzyme.

In most ecological studies only V_{max} is measured and values are generally interpreted as comparative indicators of effective enzyme concentrations. For soils, V_{max} is typically measured in a homogenized slurry containing a buffer with added substrate. Slurries are necessary to reduce diffusion barriers and ensure that enzymes have adequate access to substrate. Enzyme activity is expressed as the rate of substrate depletion or product formation. Often V_{max} is measured at optimal pH and temperature to maximize assay sensitivity, even if these conditions differ substantially from those *in situ*. However, it is also common to conduct enzyme assays at *in situ* pH and temperature to obtain more realistic comparisons of potential activity across systems.

Enzyme assays are sometimes performed on soil samples that have previously been air-dried or frozen. These steps are likely to alter enzyme activity relative to fresh samples and should be performed only when logistical constraints preclude assaying fresh samples.

The incubation time of the enzyme assay is another consideration. Longer assays (>12 hours) increase the likelihood of substrate depletion, product uptake, and enzyme turnover or synthesis, resulting in reaction rates that are not stable over time. For comparing potential activities among soils, incubations should be held to the minimum time required to measure accumulation of the reaction product to provide the best measure of initial rate of reaction. In

some cases, the linearity of the reaction over time can be verified by repeated measurements of product concentration.

6.2 Recent advances in microplate enzyme assays

Recently, high throughput, high sensitivity assays based on microplate technology have been developed to determine the activities of a wide variety of different soil enzymes (68, 77). Many of these assays use substrates that yield fluorescent reaction products, such as methyl coumarin, which can be detected with high sensitivity (68). These methods have greatly increased the ease of measuring soil enzyme activity.

Microplate technology also enables well-known colorimetric assays to be scaled down for high-throughput analyses. Two main challenges must be overcome to reduce variability to acceptable levels in these assays. First, there must be adequate homogenization of the environmental sample to ensure that the slurry in the microplate wells is representative of the initial material. This issue is particularly relevant for fresh litter or plant samples that do not easily break into small pieces. For these types of samples, a more rigorous homogenization is required, and/or wide-orifice pipette tips can be used to transfer the sample homogenate into microplate wells.

The second challenge is that particles from the sample homogenate scatter the light beam of the microplate reader and make absorbance readings highly variable. This issue can be overcome by running a large number of replicate samples and controls (i.e. ~16). Alternatively, if the particles settle during the incubation period, the assay solution (containing the reaction product) can be carefully pipetted into new plates before determining the product concentration, leaving the particles behind.

The above principles can be applied to microplate procedures using a variety of different substrates and enzymes. For example, many hydrolase assays are based on the cleavage of a p-nitrophenol (or p-nitroaniline) group from compounds analogous to naturally-occurring substrates. Oxidase assays, for enzymes such as polyphenol oxidase and peroxidase, are also readily adaptable to microplate readers. For these enzymes, no separate color development step is required, since the enzymatic reaction results directly in the formation of a colored product. Other enzymes, such as urease, require more complicated chemical procedures to assay the concentration of product formed, but these procedures are also easily adapted to the microplate scale.

7. Models

7.1 Enzyme-driven decomposition models

Over the past few decades there have been successive efforts to generate heuristic models that link soil enzyme activity with decomposition processes. In most models, enzyme activities are tied to substrate degradation through Michaelis-Menton kinetics, in contrast to larger scale models in which decomposition rates are represented as first-order functions of organic matter concentration, which in turn may be modulated by environmental parameters such as temperature, moisture or nutrient availability (62). In enzyme-based models, enzymes act as proximal controls on C and nutrient mineralization, and changes in model parameters affect these processes by altering enzyme production or activity.

Sinsabaugh and Moorhead (76) presented a model in which enzyme activity depended on the availability of nutrients, and decomposition rates depended on the activity of lignocellulose-

degrading enzymes. As N and P availability increased, the microbial community was assumed to allocate more resources toward the production of enzymes that generated assimilable C, i.e. lignocellulases, thereby increasing decomposition rates. Allison (1) adopted a similar conceptual framework for modeling enzyme production but extended it to include the N cost of enzyme synthesis.

Schimel and Weintraub (69) developed a simulation that included dual enzyme-substrate controls on decomposer activity. They recognized that the enzymatic breakdown of organic matter could be limited by the availability of effective enzyme binding sites as well as by enzyme production and kinetics. This model provided a biochemical mechanism that linked two empirical observations: microbial communities can be C-limited even when soil organic matter is abundant, and manipulations that increase enzyme activity do not necessarily increase substrate degradation rates.

Moorhead and Sinsabaugh (58) combined an enzyme-driven decomposition model with a multi-guild representation of microbial community structure. The model included compartments for three guilds of soil microbes (opportunists, decomposers, miners) distinguished by their substrate affinities and growth characteristics. By simulating the successional loop, the model captures the differential effects of N on decomposition and projects the humus input to soil organic matter.

7.2 Soil structure models

The heterogeneous spatial structure of soil imposes constraints on enzyme dynamics and the economics of microbial nutrition. Vetter et al. (88) developed a model to predict the flux of soluble compounds to bacteria foraging within particulate organic matter. Based on model

results, they concluded that foraging with extracellular enzymes would be a viable strategy only under conditions where substrate was abundant within the foraging radius of the bacterium. Therefore, extracellular enzymes should be produced in a spatially heterogeneous pattern corresponding to substrate availability within soils and particulate organic matter. Allison (1) showed that rates of enzyme diffusion controlled growth rates of enzyme-producing microbes, and mediated competitive relationships with cheaters (opportunists) that did not produce enzymes. If rates of enzyme diffusion were high, spatial structure in the model system declined and cheaters drove enzyme producers to extinction, resulting in low enzyme concentrations and system collapse. A model by Ekschmitt et al. (28) also predicts that enzyme-producing bacteria will only survive in environments with restricted diffusion. These models provide a mechanistic explanation for observations that most microorganisms, particularly bacteria, tend to retain extracellular enzymes within periplasmic spaces or attached to outer envelopes.

8. Directions

Innovative approaches based on molecular biology are resolving long-standing questions about the mechanisms of biogeochemical processes and the controls on microbial diversity (e.g. 31). Much of this work focuses on genomic analyses of microbial communities to elucidate the distribution of taxa or genes associated with particular soil processes. Because few organisms have been cultured and fewer still have had their genomes sequenced, there remains a vast gap between taxonomic and functional information on microbial communities. A similar gap isolates ecological genomics from ecological process. Ecological proteomics—the study of ecologically relevant proteins—bridges these gaps.

Recent technological advances make it possible to pursue ecological proteomics at high levels of spatial and taxonomic resolution. Schulze et al. (70) demonstrated the utility of this approach by extracting proteins from a variety of natural habitats including surface water, soil solution, and soil particles. For soils from a beech forest ecosystem, they found that nearly all of the 75 proteins (representing 16 taxa, ~50% bacterial) identified from soil solution were intracellular proteins. However, proteins desorbed directly from soil particles were predominantly extracellular enzymes, mostly cellulases and laccases, including both fungal and bacterial enzymes. In effect, soil particles acted as collectors and stabilizers of lignocellulose-degrading enzymes.

These types of studies demonstrate that technological impediments to ecological proteomics can be overcome to yield ecologically relevant information on microbial processes. As a result, increasing effort can be devoted to linking ecological function with proteomic and genomic information. The cost of partial protein sequencing is now comparable to that of other ecological analyses, and for many enzymes, particularly exohydrolases, kinetic analyses can be largely automated using robotic dispensers and microplate spectrofluorometers. By combining proteomic analyses with kinetic assays, the molecular basis of decomposition processes can be compared across systems and directly linked to microbial community composition and soil structure. Combined with nucleic acid sequencing, the potential exists to resolve the contribution of individual populations of microbes to emergent ecological processes.

References

1. **Allison, S. D.** 2005. Cheaters, diffusion, and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Letters **8**:626-635.
2. **Allison, S. D., C. B. Nielsen, and R. F. Hughes.** in press. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology & Biochemistry.
3. **Allison, S. D., and P. M. Vitousek.** 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology & Biochemistry **37**:937-944.
4. **Aon, M. A., M. N. Cabello, D. E. Sarena, A. C. Colaneri, M. G. Franco, J. L. Burgos, and S. Cortassa.** 2001. I. Spatio-temporal patterns of soil microbial and enzymatic activities in an agricultural soil. Applied Soil Ecology **18**:239-254.
5. **Archibald, F., and B. Roy.** 1992. Production of manganic chelates by laccase from the lignin-degrading fungus *Trametes* (*Coriolus*) *versicolor*. Applied and Environmental Microbiology **58**:1496-1499.
6. **Barras, F., F. Vangijsegem, and A. K. Chatterjee.** 1994. Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. Annual Review of Phytopathology **32**:201-234.
7. **Beguin, P.** 1990. Molecular-biology of cellulose degradation. Annual Review of Microbiology **44**:219-248.
8. **Bending, G. D., and D. J. Read.** 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. The foraging behaviour of ectomycorrhizal mycelium and the translocation of nutrients from exploited organic matter. New Phytologist **130**:401-409.

9. **Bending, G. D., and D. J. Read.** 1996. Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. Soil Biology & Biochemistry **28**:1595-1602.

10. **Bending, G. D., and D. J. Read.** 1996. Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil Biology & Biochemistry **28**:1603-1612.

11. **Bending, G. D., and D. J. Read.** 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycological Research **101**:1348-1354.

12. **Berg, B.** 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management **133**:13-22.

13. **Bertin, C., X. Yang, and L. A. Weston.** 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil **256**:67.

14. **Blanchette, R. A.** 1991. Delignification by wood-decay fungi. Annual Review of Phytopathology **29**:381-398.

15. **Burke, R. M., and J. W. G. Cairney.** 2002. Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza **12**:105-116.

16. **Burns, R. G.** 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology & Biochemistry **14**:423-427.

17. **Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and D. F. Parkhurst.** 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology **81**:2359-2365.

18. **Chalot, M., and A. Brun.** 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. Fems Microbiology Reviews **22**:21-44.

19. **Chen, D. M., A. F. S. Taylor, R. M. Burke, and J. W. G. Cairney.** 2001. Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi. New Phytologist **152**:151-158.

20. **Chróst, R. J.** 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes, p. 29-59. *In* R. J. Chróst (ed.), *Microbial Enzymes in Aquatic Environments*, Springer-Verlag, New York.

21. **Chróst, R. J., and W. Siuda.** 2002. Ecology of microbial enzymes in lake ecosystems, p. 35-72. *In* R. G. Burns and R. P. Dick (eds.), *Enzymes in the Environment*, Marcel Dekker, New York.

22. **Claus, H.** 2004. Laccases: structure, reactions, distribution. Micron **35**:93-96.

23. **Courty, P.-E., K. Pritsch, M. Schloter, A. Hartmann, and J. Garbaye.** 2005. Activity profiling of ectomycorrhizal communities in two forest soils using multiple enzymatic tests. New Phytologist **167**:309-319.

24. **Dakora, F. D., and D. A. Phillips.** 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil **245**:35-47.

25. **De Cesare, F., A. M. V. Garzillo, V. Buonocore, and L. Badalucco.** 2000. Use of sonication for measuring acid phosphatase activity in soil. Soil Biology & Biochemistry **32**:825-832.

26. **Dighton, J.** 2003. Fungi in ecosystem processes. Marcel Dekker, New York.

27. **Ehrenfeld, J. G.** 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems **6**:503-523.

28. **Ekschmitt, K., M. Q. Liu, S. Vetter, O. Fox, and V. Wolters.** 2005. Strategies used by soil biota to overcome soil organic matter stability - why is dead organic matter left over in the soil? Geoderma **128**:167-176.

29. **Elena, S. F., and R. E. Lenski.** 2003. Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. Nature Reviews Genetics **4**:457-469.

30. **Fierer, N., M. A. Bradford, and R. B. Jackson.** in press. Towards an ecological classification of soil bacteria. Ecology.

31. **Fierer, N., and R. B. Jackson.** 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

32. **Fog, K.** 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Review **63**:433-462.

33. **Freeman, C., N. Ostle, and H. Kang.** 2001. An enzymic 'latch' on a global carbon store. Nature **409**:149.

34. **Gallo, M. E., C. L. Lauber, S. E. Cabaniss, M. P. Waldrop, R. L. Sinsabaugh, and D. R. Zak.** 2005. Soil organic matter and litter chemistry response to experimental N deposition in northern temperate deciduous forest ecosystems. Global Change Biology **11**:1514-1521.

35. **Griffiths, R. P., M. A. Castellano, and B. A. Caldwell.** 1991. Ectomycorrhizal mats formed by *Gautieria monticola* and *Hysterangium setchelli* and their association with Douglas-fir seedlings, a case study. Plant and Soil **134**:255-259.

36. **Hodge, A., C. D. Campbell, and A. H. Fitter.** 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature **413**:297-299.

37. **Hoppe, H.-G., C. Arnosti, and G. F. Herndl.** 2002. Ecological significance of bacterial enzymes in the marine environment, *In* R. G. Burns and R. P. Dick (eds.), *Enzymes in the Environment*, Marcel Dekker, New York.

38. **Insam, H.** 2001. Developments in soil microbiology since the mid 1960s. Geoderma **100**:389-402.

39. **IUBMB.** 1992. Enzyme Nomenclature 1992. Academic Press, San Diego.

40. **Joseleau, J.-P., S. Gharibian, J. Comtat, A. Lefebrve, and K. Ruel.** 1994. Indirect involvement of ligninolytic enzyme systems in cell wall degradation. FEMS Microbiology Letters **13**:255-264.

41. **Kelleher, B. P., A. J. Simpson, K. O. Willeford, M. J. Simpson, R. Stout, A. Rafferty, and W. L. Kingery.** 2004. Acid phosphatase interactions with organo-mineral complexes: influence on catalytic activity. Biogeochemistry **71**:285-297.

42. **Kirk, T. K., and R. L. Farrell.** 1987. Enzymatic "combustion": the microbial degradation of lignin. Annual Review of Microbiology **41**:465-505.

43. **Kourtev, P. S., J. G. Ehrenfeld, and M. Häggblom.** 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology & Biochemistry **35**:895-905.

44. **Krull, E., J. Baldock, and J. Skjemstad.** 2001. Soil texture effects on decomposition and soil carbon storage, p. 103-110. *In Net Ecosystem Exchange CRC Workshop Proceedings*.

45. **Leprince, F., and H. Quiquampoix.** 1996. Extracellular enzyme activity in soil: Effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. European Journal of Soil Science **47**:511-522.

46. **Lindeberg, G.** 1944. Über die Physiologie Ligninabbauender Bodenhymenomyzeten. Symbolae Botanicae Upsalienses **8**:1-183.

47. **Luis, P., G. Walther, H. Kellner, F. Martin, and F. Buscot.** 2004. Diversity of laccase genes from basidiomycetes in a forest soil. Soil Biology & Biochemistry **36**:1025-1036.

48. **Lynd, L. R., P. J. Weimer, W. H. van Zyl, and I. S. Pretorius.** 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiology and Molecular Biology Reviews **66**:506-577.

49. **Lyons, J. I., S. Y. Newell, A. Buchan, and M. A. Moran** 2003. Diversity of ascomycete laccase gene sequences in a southeastern US salt marsh. Microbial Ecology **45**:270-281.

50. **Mader, P., H. Vierhailig, R. Streitwolf-Engel, T. Boller, B. Frey, P. Christie, and A. Wiemken.** 2000. Transport of 15N from soil compartments separated by a polytetraflouroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. New Phytologist **146**:155-161.

51. **Magill, A. H., and J. D. Aber.** 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant and Soil **203**:301-311.

52. **Marx, M.-C., E. Kandeler, M. Wood, N. Wermbter, and S. C. Jarvis.** 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biology & Biochemistry **37**:35-48.

53. **Mayer, L. M., L. L. Schick, K. R. Hardy, R. Wagai, and J. McCarthy.** 2004. Organic matter in small mesopores in sediments and soils. Geochimica et Cosmochimica Acta **68**:3863-3872.

54. **McClaugherty, C. A., and A. E. Linkins.** 1990. Temperature responses of enzymes in 2 forest soils. Soil Biology & Biochemistry **22**:29-33.

55. **McGill, W. B., and C. V. Cole.** 1981. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma **26**:267-286.

56. **Moldrup, P., T. Olesen, T. Komatsu, P. Schjonning, and D. E. Rolston.** 2001. Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. Soil Science Society of America Journal **65**:613-623.

57. **Moorhead, D. L., and R. L. Sinsabaugh.** 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. Applied Soil Ecology **14**:71-79.

58. **Moorhead, D. L., and R. L. Sinsabaugh.** in press. A theoretical model of litter decay and microbial interaction. Ecological Monographs.

59. **Nannipieri, P., L. Muccini, and C. Ciardi.** 1983. Microbial biomass and enzyme activities - production and persistence. Soil Biology & Biochemistry **15**:679-685.

60. **Neff, J. C., A. R. Townsend, G. Gleixner, S. J. Lehman, J. Turnbull, and W. D. Bowman.** 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature **419**:915-917.

61. **Olander, L. P., and P. M. Vitousek.** 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry **49**:175-190.

62. **Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima.** 1987. Analysis of factors controlling soil organic-matter levels in great-plains grasslands. Soil Science Society of America Journal **51**:1173-1179.

63. **Perez, J., and T. W. Jeffries.** 1992. Roles of manganese and organic acid chelators in regulating lignin degradation and biosynthesis of peroxidases by *Phanerochaete chrysosporium*. Applied and Environmental Microbiology **58**:2402-2409.

64. **Perucci, P.** 1992. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biology and Fertility of Soils **14**:54-60.

65. **Quiquampoix, H., S. Servagent-Noinville, and M. Baron.** 2002. Enzyme adsorption on soil mineral surfaces and consequences for the catalytic activity, p. 285-306. *In* R. G. Burns and R. P. Dick (eds.), *Enzymes in the Environment*, Marcel Dekker, New York.

66. **Read, D. J., and J. Perez-Moreno.** 2003. Mycorrhizas and nutrient cycling in ecosystems: A journey towards relevance? New Phytologist **157**:475-492.

67. **Renella, G., L. Landi, and P. Nannipieri.** 2002. Hydrolase activities during and after the chloroform fumigation of soil as affected by protease activity. Soil Biology & Biochemistry **34**:51-60.

68. **Saiya-Cork, K. R., R. L. Sinsabaugh, and D. R. Zak.** 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology & Biochemistry **34**:1309-1315.

69. **Schimel, J. P., and M. N. Weintraub.** 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry **35**:549-563.

70. **Schulze, W. X., G. Gleixner, K. Kaiser, G. Guggenberger, M. Mann, and E.-D. Schulze.** 2005. A proteomic fingerprint of dissolved organic carbon and of soil particles. Oecologia **142**:335-343.

71. **Sinsabaugh, R. L., R. K. Antibus, A. E. Linkins, C. A. McClaugherty, L. Rayburn, D. Repert, and T. Weiland.** 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology **74**:1586-1593.

72. **Sinsabaugh, R. L., M. M. Carreiro, and S. Alvarez.** 2002. Enzyme and microbial dynamics during litter decomposition, p. 249-266. *In* R. G. Burns and R. P. Dick (eds.), *Enzymes in the Environment*, Marcel Dekker, New York.

73. **Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert.** 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry **60**:1-24.

74. **Sinsabaugh, R. L., S. Findlay, P. Franchini, and D. Fischer.** 1997. Enzymatic analysis of riverine bacterioplankton production. Limnology and Oceanography **42**:29-38.

75. **Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak.** 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry **75**:201-215.

76. **Sinsabaugh, R. L., and D. L. Moorhead.** 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology & Biochemistry **26**:1305-1311.

77. **Sinsabaugh, R. L., H. Reynolds, and T. M. Long.** 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biology & Biochemistry **32**:2095-2097.

78. **Smith, S. E., and D. J. Read.** 1997. Mycorrhizal Symbiosis, 2nd edition. Academic Press, San Diego.

79. **Smith, V. H.** 2002. Effects of resource supplies on the structure and function of microbial communities. Antonie van Leeuwenhoek **81**:99-106.

80. **Sollins, P., P. Homann, and B. A. Caldwell.** 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma **74**:65-105.

81. **Stursova, M., C. L. Crenshaw, and R. L. Sinsabaugh.** 2006. Microbial responses to long term N deposition in a semi-arid grassland. Microbial Ecology **51**:90-98.

82. **Tarafdar, J. C., and H. Marschner.** 1994. Phosphatase-activity in the rhizosphere and hydrosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biology & Biochemistry:387-395.

83. **Trasar-Cepeda, C., C. Leirós, F. Gil-Sotres, and S. Seoane.** 1998. Towards a biochemical quality index for soils: an expression relating several biological and biochemical properties. Biology and Fertility of Soils **26**:100-106.

84. **Travisano, M., and G. J. Velicer.** 2004. Strategies of microbial cheater control. Trends in Microbiology **12**:72-78.

85. **Tscherko, D., U. Hammesfahr, M.-C. Marx, and E. Kandeler.** 2004. Shifts in rhizosphere microbial communities and enzyme activity of *Poa alpina* across an alpine chronosequence. Soil Biology & Biochemistry **36**:1685-1698.

86. **van den Burg, B.** 2003. Extremophiles as a source for novel enzymes. Current Opinion in Microbiology **6**:213-218.

87. **Vance, E. D., and F. S. Chapin, III.** 2001. Substrate limitations to microbial activity in taiga forest floors. Soil Biology & Biochemistry **33**:173-188.

88. **Vetter, Y. A., J. W. Denning, P. A. Jumars, and B. B. Krieger-Brockett.** 1998. A predictive model of bacterial foraging by means of freely released extracellular enzymes. Microbial Ecology **36**:75-92.

89. **Waldrop, M. P., T. C. Balser, and M. K. Firestone.** 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry **32**:1837-1846.

90. **Waldrop, M. P., D. R. Zak, and R. L. Sinsabaugh.** 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry **36**:1443-1451.

91. **Waldrop, M. P., D. R. Zak, R. L. Sinsabaugh, M. Gallo, and C. Lauber.** 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzyme activity. Ecological Applications **14**:1172-1177.

92. **Watanabe, K., and K. Hayano.** 1995. Seasonal variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus* spp in paddy field soil. Soil Biology & Biochemistry **27**:197-203.

93. **Weetall, H.** 1975. Immobilized enzymes and their application in the food and beverage industry. Process Biochemistry **10**:3-24.

94. **Weintraub, M. N., and J. P. Schimel.** 2005. The seasonal dynamics of amino acids and other nutrients in Alaskan Arctic tundra soils. Biogeochemistry **73**:359-380.

95. **Weintraub, M. N., and J. P. Schimel.** 2005. Seasonal protein dynamics in Alaskan arctic tundra soils. Soil Biology & Biochemistry **37**:1469-1475.

96. **Wick, B., H. Tiessen, and R. S. C. Menezes.** 2000. Land quality changes following the conversion of the natural vegetation into silvo-pastoral systems in semi-arid NE Brazil. Plant and Soil **222**:59-70.

97. **Zimmerman, A. R., J. Chorover, K. W. Goyne, and S. L. Brantley.** 2004. Protection of mesopore-adsorbed organic matter from enzymatic degradation. Environmental Science & Technology **38**:4542-4548.

Figure 1. Extracellular enzyme activity catalyzes organic matter decomposition and nutrient mineralization in soil. Extracellular enzyme production is induced by signal pathways linked to substrate availability (1) and microbial community organization (2). Upon release, the function and fate of extracellular enzymes is determined by environmental conditions and soil structure (3). These abiotic variables determine the economics of microbial enzyme production in relation to microbial growth.