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Emerging tools for measuring and modeling the *in situ* activity of soil extracellular enzymes

Matthew D. Wallenstein [a,b,*], Michael N. Weintraub [c]

[a] Natural Resource Ecology Laboratory, Colorado State University, Campus Delivery 1499, Fort Collins, CO 80524-1499, USA
[b] Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA
[c] Department of Environmental Sciences, University of Toledo, Toledo, OH 43606-3390, USA

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ABSTRACT

Current soil enzyme methods measure potential enzyme activities, which are indicative of overall enzyme concentrations. However, they do not provide insight in the actual rates of enzymatically catalyzed reactions under natural *in situ* conditions. The objectives of this review are to (1) clarify what is being measured by current standard soil enzymology methods; (2) present an overview of the factors that control *in situ* activities of soil enzymes; and (3) evaluate how emerging technologies and modeling approaches could enhance our understanding of *in situ* extracellular enzyme activity (EEA). Genomic studies targeting functional genes coding for extracellular enzymes can identify the genetic potential of microbial communities to produce enzymes. Microbial regulation of enzyme production can be assessed with transcriptomic studies of mRNA. Emerging proteomic tools could be used assess the pool sizes, diversity, and microbial source of soil enzymes. New mass-spectrometry approaches can be used to quantify the products of enzymatic degradation. The insights gathered from these approaches will foster improved models of decomposition that explicitly include enzymes and microbial species or functional groups. A comprehensive approach to measuring *in situ* activity and elucidating the regulation of enzyme production and stabilization is required to advance our understanding of the biochemistry of decomposition.

1. Introduction

Current soil enzyme methods (e.g. Saiya-Cork et al., 2002) measure potential enzyme activities, which are indicative of overall enzyme concentrations. However, they do not provide insight into the actual rates of enzymatically catalyzed reactions under natural *in situ* conditions. Actual *in situ* enzyme activities are controlled by complex interactions between substrate availability, enzyme concentration, soil physics, and thermodynamics. The concentration of active enzymes at any microsite within the soil matrix results from the balance of enzyme production, stabilization, and degradation rates. New approaches are required to improve our understanding of the drivers of *in situ* enzyme activities.

Extracellular enzymes are the primary means by which microbes degrade the insoluble macromolecules that comprise soil organic matter (SOM) and detritus into smaller, soluble molecules that can be assimilated (Fig. 1; Burns, 1982; Sinsabaugh, 1994; Burns and Dick, 2002). This depolymerization and solubilization is the initial, rate-limiting step of decomposition and nutrient mineralization, thus extracellular enzymes allow microbes to access the otherwise biologically unavailable carbon and nutrients in SOM. Some of the more abundant soil organic compounds that are degraded enzymatically include lignin, cellulose, starch, lipids, chitin, and proteins.

Extracellular enzymes may be associated with a cell's plasma membrane, periplasmic space, cell wall, or glycocalyx, or may be completely released into the cell's microenvironment (Sinsabaugh, 1994). Although extracellular enzymes that are released by cells into their environment may be stabilized, denatured, or degraded, some will survive in solution. Once exposed to the soil environment, extracellular enzymes complex with their target substrate molecules and either hydrolyze or oxidize the substrates into smaller molecules. These soluble, low molecular mass products can then be utilized as carbon and/or nutrient sources by the cell. A great diversity of enzymes exists in soil, and due to the diversity of compounds contained within SOM, the diversity of the soil community, and the diversity of the physical soil matrix, multiple soil

* Corresponding author. Natural Resource Ecology Laboratory, Colorado State University, Campus Delivery 1499, Fort Collins, CO 80524-1499, USA. Tel.: +1 970 491 7056.
E-mail addresses: matthew.wallenstein@colostate.edu (M.D. Wallenstein), michael.weintraub@utoledo.edu (M.N. Weintraub).

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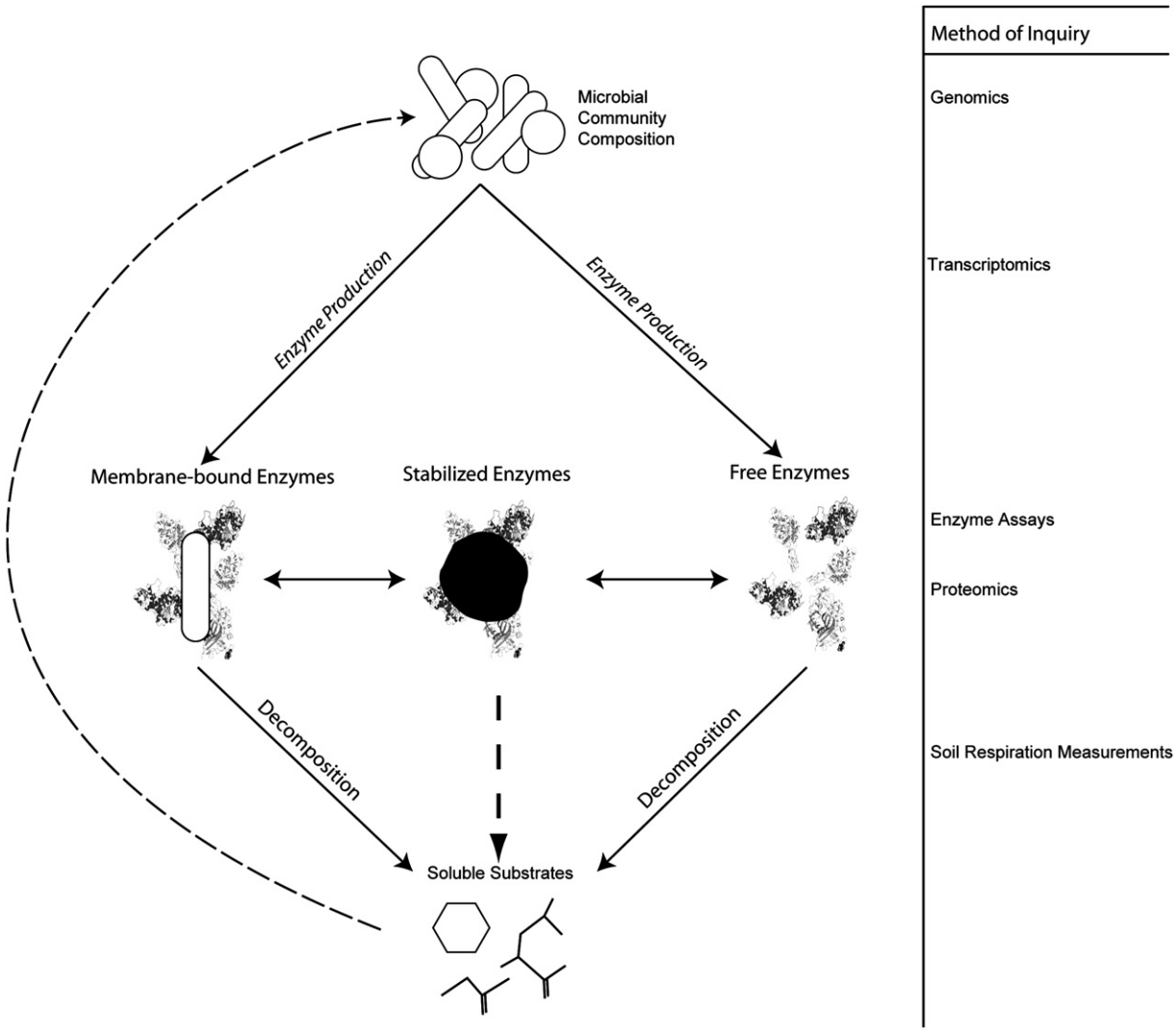

Fig. 1. Linkages between enzyme production by microbes, enzyme stabilization, enzyme activity, and decomposition. Microbial community composition determines the potential pool of enzymes, whose production (transcription and translation) is regulated by environmental conditions and substrate availability. The stabilization of enzymes and thus, potential activity, results from interactions of EE with soil particles. Enzyme activity is controlled by numerous factors including availability of substrates, temperature, and pH. The activity of EE results in the decomposition of organic matter, which releases soluble substrates that are metabolized by soil microbes, feeding back to microbial community composition. The text on the right side of the figure indicates the tools available to study each aspect of soil enzymology.

enzymes are required to efficiently degrade different chemical fractions of SOM.

While current methods continue to enhance our understanding of the roles of soil enzymes in ecosystem functioning, new emerging technologies in combination with modeling will result in a much more comprehensive understanding of controls on and consequences of *in situ* soil enzyme activity. The objectives of this review are to (1) clarify what is being measured by current standard soil enzymology methods, (2) present an overview of the factors that control *in situ* activities of soil enzymes, and (3) evaluate how emerging technologies and modeling approaches could enhance our understanding of *in situ* extracellular enzyme activity (EEA).

2. What are we *really* measuring with current enzyme techniques?

The most common method of measuring soil enzyme activity involves adding either a synthetic substrate linked to a fluorescent molecule (fluorophore) or a substrate that forms a colored compound (chromophore) to a dilute homogenized soil slurry and measuring the increase in fluorescence or absorbance over a fixed incubation time (Saiya-Cork et al., 2002). Soil slurries are used rather than filtered extracts because many of the active extracellular enzymes are bound to soil particles. In recent years, the throughput of these methods has increased through the use of 96-well microplates and microplate readers (Wirth and Wolf, 1992; Kremer, 1994; Marx et al., 2001; Saiya-Cork et al., 2002), and HPLC has been used to measure several enzyme activities simultaneously (Freeman and Nevison, 1999). While current soil extracellular enzyme methods have greatly enhanced our understanding of soil function, there are a number of limitations that provide opportunities for improvement. For example, current soil enzyme methodology is limited in several respects:

1. Only potential enzyme activities are measured under lab conditions with non-limiting amounts of substrates, which does not provide adequate information on *in situ* activities.
2. Only the sizes of enzyme pools (as potential activity) are measured, and there is no information provided on enzyme production or turnover rates.
3. Relatively simple, soluble substrates are used, which may not adequately represent the activities of enzymes that degrade