Methods of Soil Enzymology

Richard P. Dick, editor

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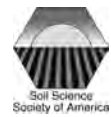

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Preface

This book follows a series of methods books related to soil microbiology published under the auspices of the Soil Science Society of America (SSSA). A number of soil enzyme activity assays were published in Part 2 of the Agronomy Monograph 9, *Methods of Soil Analysis* that had two editions. Subsequently, another methods book devoted solely to soil microbiology and biochemistry was published in the SSSA Book Series (1994) entitled *Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties*, SSSA Book Series 5 (Weaver et al., editors). In this book, M.A. Tabatabai authored a chapter entitled "Soil Enzymes" (p. 801–834) (Tabatabai, 1994), which provided an important foundation for this new book, for which he co-authored three chapters.

The impetus to develop a book solely devoted to soil enzymology came about for several reasons. One is that a comprehensive set of soil enzyme activity assays (Chapters 6 to 12), representing the spectrum of published methods, does not exist in a single publication. Rather, methods are scattered throughout journal articles or are embedded in soil methods books as chapters alongside other soil biological or chemical methods. Additionally for activity measures more methods have been developed since the "Soil Enzyme" chapter was published in the SSSA Book Series 5.

The contents and organization of Chapters 6 to 12 for the enzyme activity assays were based on ecological functions rather than a strict Enzyme Commission, numerical classification scheme (EC number). This was done purposefully to guide operators to suites of enzymes that are related to a given biogeochemical process such as nutrient cycling or other soil functions. However, it should be noted that many enzymes may not have a single process they participate in (e.g. hydrolytic enzymes from the C and N cycles may be involved in both cycles). Therefore it important to be aware of all the potential roles that a given enzyme may play in biochemical processes. Nonetheless, all of these chapters include the EC classification number for each enzyme the first time they are specifically discussed or introduced where the EC number is based on the chemical reactions they catalyze.

Another component of this book is the inclusion of enzymological methods that are not classical activity assays. In this regard, there are chapters on extraction of enzymes from soils (Chapter 16) and methods for stabilizing enzymes on solid supports (Chapter 15) for potential applications in remediation of polluted soils or other environmental applications. Chapter 13, which can be characterized as semi-quantitative, provides important methods for studying fine-scale (mm) spatial distribution of enzyme activities in soil and rhizospheres in undisturbed soil profiles. To accomplish this goal, Chapter 13 presents methods that make in situ imprints of soil enzyme activity profiles and for assaying of individual plant roots tips.

Beyond presenting enzymological methods, this book provides a context for scientists and practitioners to use soil enzymological methods effectively (Chapters 1 to 5). The book reports on the rich history of soil enzymology that stretches back to the very late 19th century (Chapter 1). Building on this, and to put the methods within a research and applications context, Chapter 2 gives important information on the microbial ecology of extracellular enzyme in soils. Kinetics of soil enzymes is reported in Chapter 3, which provides guidance on proper methods and interpretation of the Michaelis–Menten equation. Chapter 4 presents the basics for developing new enzyme activity assays. Chapter 5 gives guidance on sampling and storing soils with specific recommendations for soil sample pretreatment on enzyme assays.

An important criterion for including a given method in this book was whether the method had been rigorously tested and provides quantitative measures of activities under optimal reaction conditions (substrate saturation, pH, required cofactors if needed, and so forth). This was done to allow development of data that can be compared across studies, independent of the operator. Some have proposed that pH should not be buffered to allow results to better reflect activities under in situ conditions. This would be a viable option if the goal is to study pH effects or functional capacity of the enzymes in the natural environment. However, as a standardized method to allow comparison among various soil samples within and among studies, the enzyme assay should be conducted under buffered conditions at optimal pH.

With these criteria in mind some assays that may be found in the literature were not included in the book. One example is that we did not include bench-scale methods that use the methylumbelliferyl (MUF) labeled substrate. This is because there is severe quenching of fluorescence by humic substances. Fluorescence is also sensitive to temperature, pH, and many other environmental factors that impact its detection. For quantitative detection of fluorescence, it is essential to develop a standard curve for every sample and every batch of analyses—making it impractical from a labor and cost perspective. Nonetheless, MUF-based microplate assays are generating increasing interest due, in part, to its high sensitivity and the capacity to simultaneously assay multiple enzymes using a small amount of samples. We included a microplate MUF-based method (Chapter 14) because the use of multichannel pipettes enable a standard curve be conducted for each sample and each batch analysis with adequate replication to reduce variability. There is evidence that the proposed microplate method can pick up treatment effects if run under a very strict and consistent set of protocols. However, it is less clear if data can be compared from one study or another due. The method does not necessarily represent an optimized method, and thus, should be used and interpreted with caution.

This book will be useful to microbiologists, biochemists, and ecologists involved in studying microbial communities and their ability to deliver ecological services, soil biochemical processes (e.g. nutrient transformations and soil organic matter formation), and potential functional capacity of the microbial community and soil ecosystems. These methods can complement or be correlated with other measures of microbial biomass and/or diversity to provide insights into biochemical functions of soils. The overall objective of the book is to provide a set of standardized enzymological methods, enabling meta analysis of data from the literature and enhancing global collaboration and interactions among scientists. The

activity assays could be used by commercial labs with potential applications to assess soil quality or for environmental soil remediation.

I am honored that the leading scientists who have extensive, in-depth experience and expertise in soil enzymology took the time and effort to develop these excellent chapters. This select group of scientists is uniquely suited to write these chapters and have first-hand knowledge of the enzymology methods they have presented. This ensures that the methods presented are current, relevant, and readily applicable. I want to thank all contributing authors for their diligence and patience in bringing this book to fruition with such collegiality.

Richard P. Dick, Ohio State University

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Tabatabai, M.A. 1994. Soil enzymes. p. 801–834. *In* R.W. Weaver et al. (ed.) Methods of soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Ser. 5. SSSA, Madison, WI.

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Abstract. A variety of methods were developed to measure soil biological activity. All these methods are not suited to produce generally accepted results, but they give relative information about the ecological status of soil. Soil enzymatic activity assays is only one way to measure the ecosystem status of soils.Â Soil enzymology is nowadays of practical importance because the influence of agro-chemicals, industrial waste, heavy metals, as well as soil fertility management can be measured. Especially the search for urease inhibitor is of practical interest in order to reduce ammonia losses from soils (Schaller, 2009). Soil Enzymology. Book Â· January 2011 with 77 Reads. How we measure 'reads'.Â Soil enzymes are one of the vital key mediators involved in nutrient recycling and the decomposition of organic matter and thereby in maintaining soil quality and fertility. This Soil Biology volume covers the various facets of soil enzymes, such as their functions, biochemical and microbiological properties and the factors affecting their activities. Enzymes in the rhizosphere, in forest soils, and in volcanic ash-derived soils are described. Soil enzymes covered include phosphohydrolases, lignocellulose-degrading enzymes, phenol oxidases, fungal oxidoreductases, keratinases, pectinases, xyla