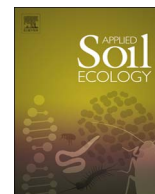




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## Simultaneous determination of multiple soil enzyme activities for soil health-biogeochemical indices

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### ABSTRACT

Enzyme activities (EAs) are soil health indicators of changes in soil biogeochemical cycling potential.  $\beta$ -glucosidase,  $\beta$ -glucosaminidase, acid phosphomonoesterase, and arylsulfatase are commonly assayed as indices of C, C and N, P and S cycling, respectively. These EAs can be measured in air-dried soil with appropriate substrates following similar assay conditions. Although the assays are similar, the current protocol is to measure each single EA independently. Subsequently, different approaches have been used to obtain a single index for all EAs (e.g., geometric mean of the sum of all EAs divided by the number of enzymes) to represent biogeochemical cycling and soil organic matter (SOM) dynamics. However, the burgeoning interest in soil health has created a need for high-throughput (and simpler) assays that are more cost effective compared to measuring multiple enzymes independently. Therefore, we evaluated simultaneous determination of two to three EAs in the same soil sample in the following combined assays: 1)  $\beta$ -glucosidase and acid phosphomonoesterase (C and P cycling), 2)  $\beta$ -glucosaminidase and arylsulfatase (C, N and S cycling), 3)  $\beta$ -glucosidase and  $\beta$ -glucosaminidase (C and N cycling), and 4)  $\beta$ -glucosidase, acid phosphomonoesterase and  $\beta$ -glucosaminidase (biogeochemical potential index). The results from combined EAs showed significant correlations with the sum of EAs calculated from individual assays ( $r > 0.931$ ,  $p < 0.001$ ). The combined EAs also showed positive significant correlations with soil organic C ( $r = 0.765$ – $0.96$ ,  $p < 0.001$ ). We provide four options to assay multiple enzymes simultaneously, which reduces the time, resources, and chemical wastes generated from assaying the four enzyme activities individually.

### 1. Introduction

Enzyme activities (EAs) are considered soil quality/health indicators reflecting changes in biogeochemical cycling and soil organic matter (SOM) dynamics (Dick and Tabatabai, 1992; Karlen et al., 1997; Karlen et al., 2001; Doran and Parkin, 1994; Dick, 2011). Enzyme activities can be more responsive to management practices (e.g., crop rotations, fertilization, tillage, and amendments) compared to other soil properties (Dick, 1994; Deng and Tabatabai, 1997; Ndiaye et al., 2000; Nannipieri et al., 2003; Acosta-Martínez and Klose, 2008; Lehman et al., 2015). Furthermore, EA responses are correlated to other soil properties suggesting they can be used to discriminate how management effects may also affect soil properties like bulk density, soil pH, and distribution of nutrients and SOM (Acosta-Martínez and Klose, 2008; Lehman et al., 2015).

Enzyme activities have been identified as key soil health biological indicators by recent initiatives representing public and private interests such as the Soil Health Institute (<https://soilhealthinstitute.org/>), Soil Health Partnership (<http://soilhealthpartnership.org/>), and the United States Department of Agricultural – Natural Resource Conservation Service's Soil Health Division (<https://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/?cid=stelprdb1237522>). Soil enzymes most commonly assessed are hydrolases such as phosphatases, sulfatases,  $\beta$ -glucosidase, or  $\beta$ -glucosaminidase as indices of P, S, C, and C and N cycling respectively (Table 1). These enzymes hydrolyze various chemical bonds (i.e., ester, glucosyl) in organic matter, releasing plant available inorganic forms of phosphates or sulfates, or carbohydrates used as energy sources by soil organisms. Generally, these hydrolyzing EAs are measured in air-dried soil under appropriate substrates and similar assay conditions including buffer pH, incubation time, and colorimetric determination of

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**Table 1**  
Description of ecological role of enzymes, and typical steps and reagents used for the original vs combined enzyme assays.

Enzyme ecological role in nature	Steps and Reagents for an Enzyme Assay <sup>a</sup>	Original Assay <sup>b</sup> (Single)	Combined Assays <sup>c</sup> (Two to Three Enzymes)
<b>Acid phosphatase (P cycling)</b> Produces plant available phosphates (predominant in acid soils)	Buffer: MUB pH 6.5 Substrate: <i>p</i> -Nitrophenyl phosphate (0.05 M) Solution to stop: 0.5 N NaOH rx: CaCl <sub>2</sub> (0.5 M) Reaction: RNH <sub>2</sub> PO <sub>4</sub> + H <sub>2</sub> O → R-OH + Na <sub>2</sub> HPO <sub>4</sub> Total vol: 5 mL	2 mL 0.5 mL 2 mL 0.5 mL Total vol: 5 mL	<b>Combined assay-1</b> 2 mL 0.5 mL <b>Combined assay-2</b> 0.5 mL <b>Combined assay-3</b> 2 mL 0.5 mL <b>Combined assay-4</b> 0.5 mL
<b>β-Glucosidase (C cycling)</b> Cellulose degradation, produce glucose as an energy source microbial populations in soil	Buffer: MUB pH 6.0 Substrate: <i>p</i> -Nitrophenyl-α-D-glucopyranoside (0.05 M) Solution to stop: THAM pH 12.0 (0.1 M) rx: CaCl <sub>2</sub> (0.5 M) Reaction: Glucoside-R + H <sub>2</sub> O → Glucose + R-OH	2 mL 0.5 mL 2 mL 0.5 mL Total vol: 5 mL	2 mL 0.5 mL 1.5 mL 0.5 mL Total Vol: 5 mL
<b>β-Glucosaminidase (C and N cycling)</b> Chitin degradation, produces amino sugars which represent additional energy sources in soil	Buffer: Acetate buffer pH 5.5 (0.1 M) Substrate: <i>p</i> -nitrophenyl-N-acetyl-β-D-glucosaminide (0.010 M) Solution to stop: 0.5 N NaOH rx: CaCl <sub>2</sub> (0.5 M) Reaction: R-N-acetyl-β-D-glucosaminide → R-OH + N-acetyl-β-D-glucosaminide	2 mL 0.5 mL 2 mL 0.5 mL Total vol: 5 mL	0.5 mL 1.5 mL 0.5 mL Total Vol: 5 mL
<b>Arylsulfatase (S cycling)</b> Produces plant available sulfates (SO <sub>4</sub> )	Buffer: Acetate buffer pH 5.8 (0.5 M) Substrate: <i>p</i> -Nitrophenyl sulfate (0.05 M) Solution to stop: 0.5 N NaOH rx: CaCl <sub>2</sub> (0.5 M) Reaction: ROSO <sub>3</sub> + H <sub>2</sub> O → R-OH + H + SO <sub>4</sub> Total vol: 5 mL	2 mL 0.5 mL 2 mL 0.5 mL Total vol: 5 mL	2 mL 0.5 mL Total Vol: 5 mL

<sup>a</sup> The reaction under an assay is provided, where R corresponds to the *p*-nitrophenyl moiety of the substrate and R-OH represents the released *p*-nitrophenol after incubation, which was determined colorimetrically.

<sup>b</sup> Original (single) assay is for one substrate at a time under controlled conditions as described in [Tabatabai \(1994\)](#) and [Dick \(2011\)](#). We used 0.5 g of soil (rather than 1 g), which reduced all solutions by half, and incubated without toluene. For these soils, we had to use 1.0 M of CaCl<sub>2</sub>.

<sup>c</sup> Two to three substrates are added to evaluate multiple enzyme activities simultaneously using similar conditions of the individual assays.

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