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Soil enzyme activity in response to long-term organic matter manipulation

Zsolt Kotroczó [a], Zsuzsa Veres [a], István Fekete [b], Zsolt Krakomperger [a], János Attila Tóth [a], Kate Lajtha [c], Béla Tóthmérész [d,*]

[a] Debrecen University, Department of Ecology, H-4032 Debrecen, Egyetem tér 1, Hungary
[b] College of Nyíregyháza, Institute of Environmental Sciences, H-4400 Nyíregyháza, Sóstói út 31, Hungary
[c] Oregon State University, Department of Crop and Soil Science, 3017 Agricultural and Life Sciences Building, Corvallis, OR 97331, USA
[d] MTA-DE Biodiversity and Ecosystem Services Research Group, Debrecen H-4010, P.O. Box 71, Hungary

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ABSTRACT

Enzymes are considered to be a key soil component catalysing important transformations related to decomposition and nutrient turnover, and their activity in soil can be used as a measure of soil health. As part of the Síkfőkút DIRT (Detritus Input and Removal Treatments) Project in a temperate deciduous forest in northern Hungary, we examined the extent to which enzyme activity in soil is influenced by both the quality and quantity of plant detrital inputs. DIRT treatments include doubling of leaf litter and woody debris inputs as well as removal of litter and trenching to prevent root inputs. Our objective was to examine seasonal dynamics of soil phosphatase and β-glucosidase activities and to determine the effects of detrital manipulations on these dynamics. We found that the litter additions did not affect enzyme activities, but removal of roots caused significant decreases in enzyme activities. We conclude that plant-induced changes to soil enzyme activities are driven primarily by readily available, labile carbon provided by root turnover and root exudation rather than by aboveground detrital inputs. However, these results could also have been affected by changes in soil chemistry with detrital input removal: after only 6 years of litter removal, soil cation content decreased and soils became more acidic, both of which could inhibit enzyme activity. The soil phosphatase and β-glucosidase enzymes measured showed similar seasonal dynamics. Both enzymes showed the highest activities in spring coincident with high soil moisture and, presumably, high root activity. The minimal response of soil enzyme activity to dramatic litter additions suggests a level of resilience in ecosystem function in this forest, and suggests that aboveground litter is not a significant source of labile carbon to microbes in the mineral soil.

1. Introduction

Soil enzymes play an essential role in nutrient mineralization and the decomposition of organic matter, and their activities are key drivers of nutrient supply to plants. Soil enzyme activities are "sensors" of soil organic matter (SOM) decomposition since they integrate information about microbial status and soil physico-chemical conditions (Aon and Colaneri, 2001; Baum et al., 2003; Sinsabaugh et al., 2008). They have been similarly used in studies on the influence of soil treatments on soil fertility (Chen et al., 2003), and may correlate well with nutrient availability (Asmar et al., 1994; János et al., 2011). Enzyme activities are generally higher in the rhizosphere than in bulk soil as a result of greater microbial activities, sustained by root exudates or due to the release of enzymes from roots (George et al., 2005; Villányi et al., 2006).

There is currently great interest in the use of extracellular enzyme activities as biological indicators of soil quality, because they are relatively simple to measure, are sensitive to environmental stress and respond rapidly to changes in land management (Yakovchenko et al., 1996; Dick, 1997). Extracellular enzyme activities can also be directly affected by factors such as temperature, moisture, pH, nutrient availability and chemical properties of the litter (Sinsabaugh et al., 2008; Baldrian et al., 2012).

Bacteria and fungi secrete extracellular enzymes, such as phosphatase and β-glucosidase which constitute an important part of the soil matrix as abiotic enzymes (Sinsabaugh and Moorhead, 1994). Phosphatases and β-glucosidase play important role in organic matter mineralization in soil. Phosphatases (EC3.1.3) are among the enzymes that transform P from non-available, organically bound forms into phosphate ions that can be absorbed by

* Corresponding author. Tel.: +36 52 512 900; fax: +36 52 431 148.
E-mail address: tothmerb@gmail.com (B. Tóthmérész).

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microorganisms and plants (Eivazi and Tabatabai, 1977). Phosphatase enzymes can be a good indicator of the organic phosphorus mineralization potential and biological activity of soils (Dick and Tabatabai, 1993). Phosphatase activity is related to soil and vegetation conditions (Herbien and Neal, 1990), responds to changes in management, and can be related to seasonal changes in soil temperature and moisture (Speir and Cowling, 1991). Activity is affected by environmental conditions but also reflects and feeds back on community composition (Sinsabaugh et al., 2002; Lellei-Kovács et al., 2011). β-Glucosidase (EC3.2.1.21) is responsible for the terminal hydrolysis (to glucose) of cellobiose fragments from hydrolysis of cellulose, the main component of plant polysaccharides (Turner et al., 2002). It is active in the first phases of degradation of organic compounds that reduce the molecular size of organic structures, thus facilitating future microbe enzyme activity (Sardans et al., 2008). β-Glucosidase is produced by many diverse fungi, including the wood-rotting basidiomycetes (both white- and brown-rot) (Conn and Dighton, 2000).

Soil pH also affects the activity of soil enzymes through its controls on microbial enzyme production, ionization-induced conformational changes of enzymes, and availability of substrates and enzymatic co-factors (Tabatabai, 1994). A global scale analysis using data from more than 40 ecosystems demonstrated that the activities of all measured soil enzymes (included phosphatases and β-glucosidases) are correlated with soil pH (Sinsabaugh et al., 2008).

We monitored the activities of two enzyme groups, phosphatases and β-glucosidases, in a deciduous temperate oak forest in Hungary under different litter manipulation treatments. We measured the activity of acid phosphomonesterase (EC3.1.3.2), referred to from hereon as phosphatase. Our research in the Síkfőkút DIRT Experiment constitutes an important part of a long term project which involves five experimental sites in the USA (Andrews Experimental Forest, Bousson Experimental Forest, Harvard Forest, University of Michigan Biological Station, Santa Rita) (Nadelhoffer et al., 2004) and one in Germany (Universität Bayreuth BITÖK). The goal of this international project is to assess how rates and sources of plant detrital inputs control the accumulation and dynamics of SOM and nutrients in forest soils over decadal time scales, and how ecosystem function is affected by, and affects, these changes in SOM dynamics. The project is derived from a program started in forest and grassland ecosystems at the University of Wisconsin in 1957 (Neilson and Hole, 1963).

We hypothesized that increased detrital inputs would provide increased labile carbon substrates to soils, and would increase enzyme activities, particularly that of β-glucosidase, which is commonly reported to be highly sensitive to substrate availability (Sinsabaugh et al., 1993). We also expected to see decreased enzyme activities in soils with decreases in labile carbon inputs, and hypothesized that reductions in enzyme activities would be greatest in plots without roots, as roots exudation and fine root turnover supply soil with the most significant and accessible amounts of labile carbon that could alleviate carbon limitation of microbial enzyme synthesis (Averill and Finzi, 2011), and the removal of root inputs removes the associated ectomycorrhizal fungi known to be significant producers of both phosphatase and β-glucosidase.

2. Material and methods

2.1. Study site

The experimental site of 27 ha is located in the south part of the Bükk Mountains in North Hungary at 320–340 m altitude (N 47°55′ E 20°46′). This forest has been part of the Bükk National Park since 1976, which protects this territory from development. Annual precipitation is 550 mm. Soils are brown forest Cambisols with clay illuviation, and the vegetation is classified as a *Quercetum petraeae-cerris* community (Jakucs, 1973; Kotroczó et al., 2012). Six treatments were established at the Síkfőkút DIRT experimental site in the autumn of 2000. Each plot is 7 m × 7 m (49 m^2), and plots are replicated in triplicate (Table 1). Annually, 160 kg of aboveground litter is removed from no litter (NL) plots and transferred to double litter (DL) plots, and 17 kg of woody litter is added to double wood (DW) plots. These quantities correspond to the mean annual leaf litter and branch litter input that falls into a 49 m^2 area (Tóth et al., 1985). No roots (NR) and no input (NI) plots were trenched to a depth of 1 m, and reinforced Delta MS 500 plastic sheeting was inserted into the trenches to prevent root ingrowth. The NR and NI plots are kept free of seedling and herbaceous vegetation with herbicide treatment (Medalon, 0.16 g glyphosate–ammonium m^{-2}). Mosses were burned from the plots by open flame heat treatment.

2.2. Soil sampling and analyses

Soil samples were collected every three months during the growing seasons from 2002 to 2006. Five cores were taken from each plot 15 cm depth with a 2 cm diameter Oakfield soil corer (Oakfield Apparatus Company, USA). Four analytical replicates per sample per assay were used. The samples were homogenized and stored for one week at 4 °C. Soil temperature was measured hourly in all 18 plots by Onset StowAway®TidbiT® temperature loggers at a depth of 10 cm. Soil moisture was determined after drying at 105 °C. Soil pH was measured every three months in the A horizon soils. 5 g of fresh soil was combined with 50 mL of DI water and was allowed to sit for 30 min. The soil pH (Cole–Parmer) probe was placed in the soil solution and was allowed to equilibrate for 6 min until a stable pH was measured.

β-Glucosidase activity was assayed by the method of Sinsabaugh et al. (1999), using the substrate analogue para-nitrophenyl-β-d-glucopyranoside (pNPG). The concentrations of buffer and terminator solutions were increased from those used in the original method to account for the greater buffering capacity of our soils. Soil suspensions (1 ml) were weighed into polypropylene test tubes (four replicate samples per plot) and incubated for 3 h in a water bath at 30 °C with 1 ml of 0.1 M Na-acetate buffer (pH 5.0) and 1 ml of 10 mM pNPG dissolved in buffer. The reaction was stopped by adding 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.1 M Tris–hydroxymethyl (aminomethane), adjusted to pH 12 with NaOH. The mixture was centrifuged for 10 min at 2500 × *g* and the absorbance measured at 410 nm. Values were corrected for a blank (substrate added immediately after the addition of $CaCl_2$ and Tris–NaOH) and for adsorption of released para-nitrophenol (pNP) in the soil.

The soil phosphatase assay is similarly based on the release of pNP from p-nitrophenyl-phosphate (pNP-PO_4), and is terminated

Table 1
DIRT treatments and how they are applied (Síkfőkút, Hungary).

Treatment	Method
Control (CO)	Normal litter inputs are allowed.
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was totally removed by rake. This process was repeated continuously during the year.
No Roots (NR)	Roots are excluded with impenetrable barriers extending from the soil surface to the top of the "C" horizon.
No Inputs (NI)	Combination of No Litter and No Roots treatments.
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from No Litter plots.
Double Wood (DW)	Aboveground wood inputs are doubled based on measured input rates of woody debris fall.