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Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability

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ABSTRACT

In a litter manipulation experiment in a temperate deciduous oak forest in Central Europe, we examined soil carbon contents and density fractions, as well as β-glucosidase and polyphenol oxidase enzyme activities, which play central roles in the degradation of litter and soil organic matter. Our measurements were taken in the DIRT (detritus input and removal treatments) plots, where manipulations include doubling of leaf litter or woody debris inputs, as well as removal of litter, trenching to prevent root inputs, and removal of all litter inputs. After 10 years of manipulation, soil C content did not vary predictably among plots, although the amount of light fraction material was greater in control and litter addition plots compared to litter removal plots. Even after 10 years of litter addition, there were no significant differences in activities of either enzyme in double litter plots compared to control plots, a result consistent with other observed measures of microbial activity. However, removal of roots and litter caused significant decreases in β-glucosidase activities very quickly, and these differences increased over time. However, polyphenol oxidase activities were not significant different among treatments. Enzyme activities were not correlated with total soil carbon contents, but activities of both enzymes were significantly and positively related to the amount of light fraction carbon, suggesting that enzymes respond to increases in labile carbon availability.

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1. Introduction

Bacteria and fungi deploy enzymes extracellular to break down organic matter into assimilable forms, and these enzymes play a central role in the degradation of litter and soil organic matter (McLaren, 1975; Waldrop and Firestone, 2004; Kotroczó et al., 2008). Estimation of extracellular enzyme activity (EEA) directly assesses microbial activity and microbial contribution to nutrient cycling (Sinsabaugh et al., 2002; Kotroczó et al., 2009). Changes in the activity of the extracellular enzymes that degrade the major components of soil organic matter have been linked to shifts in rates of decomposition and soil carbon (C) storage (Carreiro et al., 2000; Waldrop et al., 2004; Sinsabaugh et al., 2005; Jensen et al., 2012). In turn, EEA is influenced by soil physical, chemical, microbiological, and biochemical properties (Makoi and Ndakidemi, 2008). By quantifying the potential activity of these enzymes, it is possible to make inferences about the relative effort directed by microorganisms toward obtaining carbon, nitrogen, or phosphorus from specific sources (Sinsabaugh et al., 1999; Rovira and Vallejo, 2002; Huang et al., 2011).

β-Glucosidase is important to the degradation 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 derived predominantly from soil microbial heterotrophs, in particular members of the mucorales, such as *Actinomucor* and *Mortierella* (Hayano and Tubaki, 1985). Enzyme synthesis in these organisms is induced by the products of cellulose breakdown, including cellobiose, glucose and their metabolites (Stewart and Leatherwood, 1976).

In litter and soil, oxidative enzymes, variously classified as phenol oxidases, polyphenol oxidases, laccases, or peroxidases, mediate the formation and degradation of lignin and humus (Sinsabaugh et al., 1999; Waldrop et al., 2004). Soil oxidative

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enzymes have been shown to be sensitive to management practices as well as soil pH and other soil properties (Sinsabaugh et al., 2008; Tian et al., 2010). Phenol oxidase is produced primarily by white rot fungi, and is specific for highly recalcitrant organic matter, such as lignin (Carlisle and Watkinson, 1994; Moorhead and Sinsabaugh, 2006; Sinsabaugh, 2010). Increases in phenol oxidase activity relative to other enzymes gives another indication of changes in the relative contribution of bacteria and fungi to microbial activity as well as an additional indication of the quality of the organic matter present (Giai and Boerner, 2007).

We examined the activities of two enzymes critical to carbon cycling in soils: polyphenol oxidase (PPO) (EC 1.10.3.2) and β-glucosidase (BG) (EC 3.2.1.21) in litter manipulation plots located in a deciduous temperate oak forest in Hungary. We also measured total carbon content in surface soils (0–15 cm) and carbon content of light density fraction (LF), intermediate, aggregate fraction (AF) and heavy fraction (HF) pools in order to determine if enzyme activities were sensitive to either total soil C or else only to relatively labile, or decomposable pools. Our research site in the Síkfőkút Forest is part of the international DIRT (detritus input and removal treatments) project. The main objective of DIRT is to explore how changes in the quality and quantity of detritus inputs affect soil organic matter composition and content (Nadelhoffer et al., 2004). We hypothesized that increased detrital inputs would provide increased labile carbon substrates to soils, and would increase BG enzyme activities after ten years, which are commonly reported to be highly sensitive to substrate availability (Sinsabaugh et al., 1993). Thus, we also expected BG enzyme activities to decrease in detritus removal plots with decreases in labile carbon inputs. However, we hypothesized that PPO activity would not increase significantly in either litter additions or removal plots, because lignin concentrations likely would not change after only 10 years.

2. Materials and method

2.1. Study site

The 27 ha Síkfőkút Forest experimental site is located in the south part of the Bükk Mountains in North Hungary at an altitude of 320–340 m (N47°55′ E20°26′). This forest has been part of the Bükk National Park since 1976, which protects this territory from development. Mean annual temperature is 10 °C and mean annual precipitation is 553 mm. The average amount of total aboveground dry detritus (including branches, twigs, fruits and buds) was 6572 kg ha^{-1} (2003–2005) (Tóth et al., 2007). Soils are (cambisols) and the vegetation is classified as a *Quercetum petraeae-cerris* community. Leaf litter is comprised of (in decreasing order): sessile oak (*Quercus petraea*), Turkey oak (*Quercus cerris*), Hedge maple (*Acer campestre*), and cornelian cherry (*Cornus mas*) (Kotroczó et al., 2012). Soils are brown forest soils (cambisols) (Świtoniak et al., 2014) with clay illuviation (Fekete et al., 2011a, 2012), with a pH_{H2O} in surface soils (0–15 cm) ranging between 4.85 and 5.50 depending on the detrital treatment (Tóth et al., 2007).

The Síkfőkút DIRT project is part of an international network of litter manipulation experiments that started in forest and grassland plots at the University of Wisconsin in 1957 (Neilson and Hole, 1963). Six treatments were established at our DIRT experimental site in the autumn of 2000. There are two detritus addition plots: double leaf litter (DL) plots and double wood (DW) plots where the amount of wood detritus (branches, twigs and bark) was doubled. Litter exclusion treatments include no litter (NL), no living roots (NR), and no litter or living roots, termed no inputs (NI) (Table 1). Each plot is 7m × 7 m (49 m^2), and plots are replicated in triplicate.

2.2. Soil sampling and analyses

Soil samples for enzyme analysis were collected every three months during the growing season from May 2002 to November 2005 and from March 2010 to November 2012. Five 0–15 cm depth cores were sampled from each plot with a 2 cm diameter Oakfield soil corer (Oakfield Apparatus Company, USA). There were four analytical replicates per sample per assay. The samples were homogenized and stored for one week at 4 °C. Soil samples from early years were sieved, dried, ground, and pretreated with 10% hydrochloric acid to eliminate inorganic carbonate content before organic carbon analysis by dry combustion (Matejovic, 1997) using a Elementar Vario EL CHNS elemental analyzer (ELEMENTAR Analysensysteme GmbH, Germany) (Fekete et al., 2014).

BG 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 12.0 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. BG activities were expressed as μmol pNP g^{-1} dry soil h^{-1}.

The soil PPO assay was based on the release of L-β-3.4-dihidroxi-fenilalanin (L-Dopa). 5 mM substrate solution of L-Dopa was weighed in 0.1 mM acetate buffer (pH 5.0). 2.0 ml of sample homogenate and 2.0 ml of L-Dopa solution were weighed into polypropylene tubes and incubated for 1 h in a water bath at 30 °C. We used 2.0 ml of sample homogenate and 2.0 ml of acetate buffer as a control. The mixture was centrifuged for 10 min at 2500 × *g* and the absorbance measured at 460 nm (Sinsabaugh et al., 1999). PPO activities were expressed as μmol g^{-1} dry soil h^{-1}.

Soils for density fractionation were collected in 2011, composited within each field plot, and fractionated by density using sodium polytungstate following Sollins et al. (2006). Three density fractions were recovered, representing light (1.85 g cm^{-3}),

Table 1
The DIRT (Detritus Input and Removal Treatments) plots at Síkfőkút (Hungary).

Treatment	Method
Control (CO)	Normal litter inputs
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

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