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Enzyme properties down the soil profile - A matter of substrate quality in rhizosphere and detritusphere

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ABSTRACT

The decomposition of soil organic matter depends strongly on its availability to microorganisms and their enzymes. The rhizosphere and detritusphere are microbial hot spots due to additional substrate input, leading to high abundance, specific species diversity and functional diversity of microbial communities. However, rhizosphere and detritusphere differ in substrate quality, localization, and duration of input. We hypothesized that the contrasting substrate availability between rhizosphere and detritusphere affects the activity of microorganisms and associated enzymes. Organic carbon (C) from the rhizosphere and detritusphere decreases with soil depth and, consequently, microbial hot spots become rarer and competition for C and nutrients increases. In deeper soil (>40 cm depth) the amount and quality of substrates is expected to decrease and, therefore, the effect of contrasting substrate input to disappear. Plant N uptake is expected to reduce N availability in the rhizosphere of maize compared to the detritusphere and bare fallow. These hypotheses were tested in a factorial field experiment with 1) maize-planted, 2) maize litter-amended, and 3) bare sites. Enzyme kinetic parameters (V_{max}, K_m, K_a), extractable organic C and microbial biomass C were compared in soil affected by rhizosphere and detritusphere throughout the profile to 70 cm depth, to assess microbial C and nutrient limitations. A decrease in enzyme activity with depth due to resource scarcity and lower substrate quality appeared in planted and litter-amended soil. N limitation in planted soil increased the activity and substrate affinity of proteolytic enzymes to provide for microbial N demand through SOM decomposition. This was in line with lower V_{max} ratios (V_{max} for C-cycling enzymes divided by V_{max} for N-cycling enzymes) in planted relative to litter-amended topsoil. The catalytic efficiency of enzymes decreased 2- to 20-fold from top- (<40 cm) to subsoil (>40 cm), irrespective of the substrate input. Substrate quality in the rhizosphere and detritusphere affected enzyme activities only in the topsoil, whereas a sharp decline of C input with depth led to similar activities in the subsoil. Most of the enzyme indexes reflected shifts in allocation of C and nutrients in the rhizosphere and detritusphere. The presented results underline the role of microorganisms as critical links in the C and nutrient transfers in the rhizosphere and detritusphere.

1. Introduction

Enzymes in soil catalyze nearly all important transformations in the carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) cycles (Aon et al., 2001; Wallenstein and Burns, 2011). Decomposition of organics is strongly dependent on microbes and enzymes, which are especially abundant in the rhizosphere and detritusphere – two main microbial hot spot environments in soil. The rhizosphere is characterized by high density and quality of substrates for microorganisms (Garbeva et al., 2008; Marschner et al., 2012, 2001), and plants provide a variety of C and energy sources from their roots

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(Gregory, 2006; Paterson, 2003; Paterson et al., 2007). The detritusphere contains large amounts of cellulose, hemicelluloses and lignin, as main components of plant residues (Kandeler et al., 1999; Marschner et al., 2012; Nannipieri et al., 2012).

The microbial C:N:P ratios (ecological stoichiometry) are frequently used to indicate how allocation of C and nutrients by microorganisms influence microbial demands on soil pools (Cleveland and Liptzin, 2007; Heuck et al., 2015; Sinsabaugh et al., 2015). Nowadays it is widely accepted that microbial C:N:P stoichiometry affects microbial mineralization of C sources (Mooshammer et al., 2012). Microbial respiration (CO_2) and N_2O production are well known indicators describing microbial activities in soil (Blagodatskaya et al., 2014). In combination with the ratios of commonly measured enzyme activities (Table 1), these indicators provide insights into the microbial community that is investing energy for microbial fitness (Sinsabaugh et al., 2012; 2008; Tapia-Torres et al., 2015). The production of extracellular enzymes is regulated by nutrient availability and energy demand (Sinsabaugh et al., 2009). Thus, enzyme activities are reliable microbial activity indicators and are closely interrelated with soil quality (Bending et al., 2004; Paudel et al., 2011).

Most enzyme studies are restricted to the topsoil, despite the fact that microbial substrate utilization takes place throughout the whole soil profile (Sinsabaugh et al., 1993; Sinsabaugh and Moorhead, 1994; Vranova et al., 2013). Furthermore, only the potential enzyme activity is considered in most studies, whereas rates of enzyme-substrate complex dissociation and enzyme-substrate complex formation are neglected (Koshland, 2002). Therefore, it is of great interest to study how microbial functioning and enzyme systems vary throughout the soil profile.

As interactions between substrate composition, microbial competition, and nutrient availability are complex, we established a factorial field manipulation experiment including maize-planted, maize litter-amended and bare fallow sites. These sites differed (1) in sources of different substrate quality (root-derived vs. litter-derived vs. none) and (2) in the distribution of substrates with depth. Both substrate quantity and quality strongly decrease with soil depth (Fierer et al., 2003a), because most roots are localized in the topsoil, so the rates of C input to subsoil are low (Fierer et al., 2003b). Therefore, the subsoil microbial communities differ in composition and activity from the surface communities (Blume et al., 2002; Fierer et al., 2003a; Fritze et al., 2000).

We combined substrate-induced emission of carbon dioxide (CO_2) and nitrous oxide (N_2O) with kinetics of the enzymes β-glucosidase (BG), β-cellobiohydrolase (CE), β-xylosidase (BX), acid phosphatase (AP), and leucine- (LE) and tyrosine- (TY) aminopeptidases to disentangle the effects of substrate quality and substrate amount on microbial activity along the depth gradient. Several approaches for integrating the various enzyme activities into unified indexes were compared (Table 1) (Hill et al., 2014; Moorhead et al., 2016, 2013; Nannipieri et al., 2012; Sinsabaugh et al., 2008). These activity indexes of multiple enzymes were related to dissolved organic C (DOC) and extractable nitrogen (EN).

We hypothesized that the contrasting substrate availability between planted soil and litter-amended soil, reflecting the rhizosphere and detritusphere, respectively, would affect the activity of microorganisms and associated enzymes. The effect of the contrasting substrate availabilities on microbial substrate utilization was predicted to decline with depth due to the lower amount and quality of substrates in the subsoil (>40 cm depth) compared to the topsoil. Furthermore, we hypothesized that lower N contents in the maize-planted soil, due to plant N uptake, would lead to stronger competition between microbes compared to the fallow control. This, in turn, would increase proteases, because of an inversely proportional relationship to low substrate availability (Olander and Vitousek, 2000; Sims and Wander, 2002; Stursova et al., 2006). To our knowledge this is the first study using a broad range of activity indicators to elucidate the tight interactions between microbial activity and contrasting substrate input down the soil profile.

2. Material and methods

2.1. Study site

The experimental arable field was located in the north-west of Göttingen, Lower-Saxony, Germany (51°33′N, 9°53′E; 158 m NN). The area has a temperate climate with a long-term mean annual precipitation of 645 mm and mean air temperature of 8.7 °C. The dominant soil types are Luvisols occasionally with stagnic properties (Table 2; Kramer et al., 2012; Pausch et al., 2013).

In April 2012 the field was tilled with a chisel plough to a depth of 12 cm and maize was sown at a density of 12 grains m^{-2}. Nitrogen fertilizers (ammonium nitrate urea solution: 110 kg N ha^{-1} and diammonium phosphate: 110 kg N ha^{-1}) were applied to all treatments, shortly before and after sowing the maize. The corn was not irrigated during plant growth.

In September 2012 corncobs were harvested and maize plants were cut at a height of 10 cm above soil surface. The maize above-ground biomass was hackled to a particle size of 1 cm^2 and air-dried to gain litter. In April 2013 the herb layer developed during spring was removed by Glyphosate (4 l ha^{-1}). Three weeks later the soil was tilled to a depth of 12 cm, maize sown at a density of 9 grains m^{-2} and fertilized similarly to 2012. In September 2013 maize plants were harvested and removed from the experimental field site.

2.2. Treatments

In May 2012 a total of 12 experimental plots (size 5 × 5 m) were conducted and arranged in two adjacent rows separated by a 5 m buffer stripe within and 2 m buffer stripes between rows. Three treatments, each replicated four times, were established differing in resource quality: plant (maize as crop), litter (application of maize litter) and fallow. Maize was removed from the eight plots within the first three weeks after seeding to set up the litter and fallow treatments. For the litter-treated soil four plots received 0.8 kg m^{-2} dry maize litter (equivalent to 0.35 kg C m^{-2}, C-content = 44%) approximating the above-ground biomass of maize in June. Litter was grubbed into the first 10 cm of soil on June the 6th 2013. This coincided with the start of the crop growth period to ensure the same conditions for the herbivore and detritivore communities. To accomplish comparable environmental conditions between plots, the litter-amended and the fallow control plots were shaded with blinds (mechanical shading; AGROFLOR Kunststoff GmbH, Wolfurt, Austria). The shading level represented the mean leaf area index of plants during the vegetation period. In addition, plots were regularly weeded to prevent plant carbon input by herbs.

2.3. Soil sampling and preparation

We sampled in each plot soil from 0 to 50 cm in 10 cm increments, and from 60 to 70 cm depth, of each plot in July 2013. Each plot was sampled in one position, using a Riverside auger (inner diameter 5 cm, Eijkelkamp, Giesbeek, The Netherlands). The soil samples were frozen at −18 °C until analysis. Prior to analysis the soil samples were thawed at 4 °C. After thawing the soil samples were sieved (<2 mm) and fine roots and other plant debris were carefully removed with tweezers. The soil was then pre-incubated at 22 °C for 72 h. Soil sub-samples from each plot and