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Spatial variability of enzyme activities and microbial biomass in the upper layers of *Quercus petraea* forest soil

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ABSTRACT

Extracellular lignocellulose-degrading enzymes are responsible for the transformation of organic matter in hardwood forest soils. The spatial variability on a 12 × 12 m plot and vertical distribution (0–8 cm) of the ligninolytic enzymes laccase and Mn-peroxidase, the polysaccharide-specific hydrolytic enzymes endoglucanase, endoxylanase, cellobiohydrolase, 1,4-β-glucosidase, 1,4-β-xylosidase and 1,4-β-*N*-acetylglucosaminidase and the phosphorus-mineralizing acid phosphatase were studied in a *Quercus petraea* forest soil profile. Activities of all tested enzymes exhibited high spatial variability in the L and H horizons. Acid phosphatase and 1,4-β-*N*-acetylglucosaminidase exhibited low variability in both horizons, while the variability of Mn-peroxidase activity in the L horizon, and endoxylanase and cellobiohydrolase activities in the H horizon were very high. The L horizon contained 4× more microbial biomass (based on PLFA) and 7× fungal biomass (based on ergosterol content) than the H horizon. The L horizon also contained relatively more fungi-specific and less actinomycete-specific PLFA. There were no significant correlations between enzyme activities and total microbial biomass. In the L horizon cellulose and hemicellulose-degrading enzymes correlated with each other and also with 1,4-β-*N*-acetylglucosaminidase and acid phosphatase activities. Laccase, Mn-peroxidase and acid phosphatase activities correlated in the H horizon. The soil profile showed a gradient of pH, organic carbon and humic compound content, microbial biomass and enzyme activities, all decreasing with soil depth. Ligninolytic enzymes showed preferential localization in the upper part of the H horizon. Differences in enzyme activities were accompanied by differences in the microbial community composition where the relative amount of fungal biomass decreased and actinomycete biomass increased with soil depth. The results also showed that the vertical gradients occur at a small scale: the upper and lower parts of the H horizon only 1 cm apart were significantly different with respect to seven out of nine activities, microbial biomass content and community composition.

1. Introduction

Plant litter input into broadleaved forest soils was estimated to be 3.5 t ha^{-1} per year (Bray and Gorham, 1964) which, together with the input from plant roots represents the main sources of organic matter in forest soils (Litton et al., 2003). The decomposition of litter is one of the main processes responsible for the formation of temperate forest soils with developed organic (H) horizon characteristics such as a high content of humic compounds. Recent studies have shown that the activities of extracellular enzymes, especially all those participating in lignocellulose degradation, are not only correlated with litter decay but are also used for nutrients acquisition by microorganisms in the deeper soil horizons (Moorhead and Sinsabaugh, 2000; Caldwell, 2005). It is presumed that litter decomposition in temperate forests is driven mainly by the activity of saprotrophic fungi (Hättenschwiler et al., 2005; Baldrian, 2008).

Several studies have focused on the measurement of enzyme activities and microbial biomass in forest litter and soils (Caldwell, 2005). However, these have largely neglected the small-scale spatial variability (i.e. <10 m) of enzyme distribution that might be related to localized differences in microbial biomass and community composition. Furthermore, although some studies described the differences in the activity of extracellular enzymes with soil depth (Trasar-Cepeda et al., 2000; Taylor et al., 2002; Andersson et al., 2004; Wittmann et al., 2004), the usual sampling by soil horizons might underestimate any small-scale differences.

The aims of this study were to characterize the spatial variability and distribution of cellulolytic and ligninolytic enzyme activities in the L and H horizons of oak (*Quercus petraea*) forest soil at a 1-cm scale. Since we detected considerable production of lignocellulose-degrading enzymes during litter decomposition and

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soil colonization by fungi isolated from the studied soil (Valášková et al., 2007; Šnajdr et al., in press), we also investigated the relationship between soil fungi and bacteria biomass and enzyme activities as well as the spatial distribution of individual enzymes.

2. Materials and methods

2.1. Study site and sampling

Soil and litter samples were collected in the Xaverovský Háj Natural Reserve, near Prague, Czech Republic in a sessile oak (*Q. petraea*) forest, a site previously used for the isolation of saprotrophic basidiomycetes (Valášková et al., 2007). The soil was an acidic cambisol with developed L, H, Ah and A horizons: L – thickness 0.5–1.5 cm, pH 4.3; 46.2% C; 1.76% N; H – thickness 1.5–2.5 cm, pH 3.7; 21.5% C; 0.56% N, Ah – thickness 6–8 cm, pH 3.4; 3.0–14.3% C; 0.10–0.39% N.

For the study of the variability of soil enzyme activities and microbial biomass, soil cores (45 mm diameter) were collected in May and August 2006. In a preliminary experiment, soil tubes were collected at various distances in a nested grid design and analyzed for enzyme activities and microbial biomass. Based on these results, semivariograms were calculated using Surfer 7.0 (Golden Software, USA) and tested for spatial dependence. For all measured values, samples located more than 30 cm apart were found to be spatially independent. Therefore, 49 cores in total were collected in a square grid on a 12×12 m plot where a consistent litter layer was present on the forest floor and there was no plant growth. The soil cores were separated into independent samples of the L and H horizon material, homogenized and used for enzyme activity assays and ergosterol analyses, PLFA analysis was performed for samples collected in May 2006. Dry mass was estimated after drying at 85 °C until constant mass.

For the study of the vertical distribution of soil properties, soil cores were collected in September 2006. In total six 2×2 plots with a litter layer on the forest floor (no growth of grasses) were selected and four soil cores were collected on each of these plots. For each soil core, L horizon material (0.5–1.0 cm) was processed as a whole, the rest of the soil core material was separated into 1-cm thick slices to a depth of 8 cm. Slices from the same depth of each plot were combined to yield one sample. In total, 54 samples were prepared (6 sites $\times$ 9 depth segments). Typically, samples from 0–2 cm depth contained H horizon material only and samples from 4–8 cm depth Ah horizon material only. Samples of the L horizon were cut into approximately 0.25 cm^2 pieces, while the samples from the deeper soil horizons were sieved using a 2-mm sieve. The resulting samples were used for enzyme activity assays, ergosterol and PLFA analyses, respiration measurement, analyses of bacterial counts, pH, and quantifying the organic fraction and humic material.

2.2. Enzyme extraction and assays

The highest recovery of all the extractable enzymes was achieved with 100 mM phosphate buffer, pH 7. We did not find differences in extraction yields of enzyme activities originating from different soil horizons (data not shown). Homogenized samples of soil or litter material were extracted at 4 °C for 2 h on an orbital shaker (100 rpm) with 100 mM phosphate buffer, pH 7 (16:1 w/v), filtered through Whatman #5 filter paper and desalted using PD-10 desalting columns (Pharmacia, Sweden) according to the suppliers protocol to remove inhibitory small-molecular-mass compounds. The desalted samples were kept at −18 °C until enzyme activity analysis.

Laccase (EC 1.10.3.2) activity was measured by monitoring the oxidation of ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) in citrate–phosphate (100 mM citrate, 200 mM phosphate) buffer (pH 5.0) at 420 nm as described previously (Šnajdr et al., in press).

Manganese peroxidase (MnP, EC 1.11.1.13) was assayed using succinate–lactate buffer (100 mM, pH 4.5) as described previously (Šnajdr et al., in press). MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3,3-dimethylaminobenzoic acid) were oxidatively coupled by the enzymes, and the resulting purple indamine dye was detected spectrophotometrically at 595 nm. The results were corrected by the activities of the samples without manganese (for MnP) – the addition of manganese sulfate was substituted by an equimolar amount of ethylenediaminetetraacetate (EDTA). One unit of enzyme activity was defined as the amount of enzyme forming 1 μmol of reaction product per min.

Endo-1,4-β-glucanase (EC 3.2.1.4) and endo-1,4-β-xylanase (EC 3.2.1.8), were routinely measured with azo-dyed carbohydrate substrates (carboxymethyl cellulose and birchwood xylan, respectively) using the protocol of the supplier (Megazyme, Ireland). The reaction mixture contained 0.2 mL of 2% dyed substrate in 200 mM sodium acetate buffer (pH 5.0), and 0.2 mL sample. The reaction mixture was incubated at 40 °C for 60 min and the reaction was stopped by adding 1 mL of ethanol followed by 10 s vortexing and 10 min centrifugation (10 000×*g*) (Valášková et al., 2007). The amount of released dye was measured at 595 nm and the enzyme activity was calculated according to standard curves correlating the dye release with the release of reducing sugars. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of reducing sugars per min.

Cellobiohydrolase (EC 3.2.1.91) was assayed in microplates using *p*-nitrophenyl-β-D-cellobioside (PNPC). The reaction mixture contained 0.16 mL of 1.2 mM PNPC in 50 mM sodium acetate buffer (pH 5.0) and 0.04 mL sample. Reaction mixtures were incubated at 40 °C for 90–120 min. The reaction was stopped by adding 0.1 mL of 0.5 M sodium carbonate, and absorbance was read at 400 nm.

1,4-β-glucosidase (EC 3.2.1.21), 1,4-β-xylosidase (EC 3.2.1.37) and 1,4-β-*N*-acetylglucosaminidase (chitinase; EC 3.2.1.52) were assayed using *p*-nitrophenyl-β-D-glucoside, *p*-nitrophenyl-β-D-xyloside and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, respectively, using the same method (Valášková et al., 2007).

Acid phosphatase (EC 3.1.3.1) was assayed using 2 g L^{-1} *p*-nitrophenylphosphate in 50 mM sodium acetate buffer (pH 5.0) as described previously (Šnajdr et al., in press). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per min.

All spectrophotometric measurements were done in a microplate reader (Sunrise, Tecan) or a UV–VIS spectrophotometer (Lambda 11, Perkin–Elmer) and expressed per g dry mass of soil or litter. Statistical analyses were performed by Statistica 7.0 software package (StatSoft, USA). One-way ANOVA followed by the Tukey HSD test was used for testing statistical significance. Coefficient of variance (CV (%) = 100 × average/standard deviation) was used as a measure of variability.

2.3. Quantification of microbial biomass

The samples for phospholipid fatty acid (PLFA) analysis were extracted by a mixture of chloroform–methanol–phosphate buffer (1:2:0.8) according to (Bligh and Dyer, 1959). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck) and the samples were subjected to mild alkaline methanolysis (Šnajdr et al., in press). The free methyl esters of phospholipid fatty acids were analyzed by gas chromatography–mass spectrometry (Varian 3400; ITS-40, Finnigan). Fungal biomass was quantified based on 18:2ω6,9 content ($PLFA_{fung}$), bacterial biomass was quantified as a sum of i14:0, i15:0, a15:0, 16:1ω7t, 16:1ω9, 16:1ω7, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 10Me-18:0