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Distribution of microbial biomass and activity of extracellular enzymes in a hardwood forest soil reflect soil moisture content

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ABSTRACT

Forest soils show a considerable level of spatial heterogeneity. It was previously reported that soil moisture content affects microbial biomass and respiration in the soil as well as enzyme-catalyzed processes; as such it can be one of the potential drivers of heterogeneity of microbial distribution and decomposition in forest soils. The moisture mass content in samples of *Quercus petraea* forest soil varied substantially and ranged between 0.35–0.70 g g^{-1} soil wet mass in the litter horizon and 0.10–0.60 g g^{-1} in the humus horizon within a single sampling of a 144 m^2 area as well as of a 0.11 m^2 area. Soil moisture content positively and significantly correlated with microbial (bacterial and fungal) biomass, explained up to 60% of the total variability in microbial biomass and a significant difference in the ratio of fungal to bacterial biomass was found among the samples with high and low moisture content. Also the activity of several extracellular enzymes involved in decomposition (i.e., laccase, Mn-peroxidase, endo-1,4-β-glucanase, endo-1,4-β-xylanase, cellobiohydrolase, β-glucosidase, β-xylosidase, chitinase and acid phosphatase) correlated with soil moisture, although the response varied among soil horizons and sampling dates. The effect of moisture on enzyme activities was probably partially mediated by the changes in biomass content. Forest soil was demonstrated to be a mosaic of small dry and moist patches where the differences in microbial biomass content or enzyme activities vary by tens of percents due to differential moisture content.

1. Introduction

Spatial heterogeneity is one of the characteristic features of the soil environment. This is especially true for forest soils, which are usually not subject to human manipulation over long periods of time and in which heterogeneity easily develops by uneven litter decomposition, the tree effect and other factors. While the vertical stratification of forest soils is widely recognized, the extent of horizontal heterogeneity has scarcely been addressed (Ettema and Wardle, 2002). In our previous study, we described a high level of spatial variability of extracellular enzyme activities and microbial biomass content in the soil of a hardwood forest (Šnajdr et al., 2008b). The effects of soil moisture on soil microorganisms and microbial processes are currently a subject of interest due to the fact that most scenarios for global climate change include changes in precipitation. It was previously reported that soil moisture content affects microbial biomass and respiration in the soil, as well as enzyme-catalyzed processes (Orchard and Cook, 1983; Criquet, 2002). Most of the previous studies, however, focused on the effects of temporary drying or seasonal variation of soil moisture content. There are some indications that soil moisture content in forest soils might be spatially highly variable (Morris, 1999; Möttönen et al., 1999; Stoyan et al., 2000; Gömöryová et al., 2006). None of the previous studies addressed both the litter (L) and humic (H) horizons of soil, although these exhibit profound differences with respect to enzyme activities (Baldrian et al., 2008; Šnajdr et al., 2008b).

It is generally accepted that soil is a spatially heterogeneous environment which also likely applies for soil moisture content, the information on the extent of this heterogeneity at a small scale and its consequences for microbial abundance and decomposition are scarce. Particularly, the previous reports are almost exclusively constrained to the soil organic horizon or deeper horizon and omitted the litter horizon where most of the organic matter decomposition is performed and/or only describe the moisture effect on a very few selected soil properties. The aims of this paper were to describe in detail the level of spatial variability of soil moisture content in the L and H horizons of *Quercus petraea* forest soil and to evaluate whether and to what extent the spatial differences of soil moisture content affect soil microbial biomass expressed as PLFA content and the activity of all the major extracellular enzymes relevant for organic matter decomposition processes. Due to the importance of fungi in decomposition processes in forest

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ecosystems, fungal biomass was also independently quantified by ergosterol content analysis.

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 (*Quercus petraea*) forest, a site previously used for the study of the extracellular enzyme production by saprotrophic fungi (Valášková et al., 2007; Baldrian et al., in press) and for the analysis of the vertical distribution of enzyme activities (Šnajdr et al., 2008b). 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.

The study was conducted on a single 144 m^2 plot, where a total of 49 soil cores (4.5 cm diameter) were collected in a rectangular grid of 7 × 7 samples (closest distance between samples = 2 m). Preliminary experiments demonstrated the spatial independence of samples located at the grid nodes. A homogeneous plot covered with litter was selected for the study to exclude the effect of grass and herbal roots. To evaluate the level of spatial variability in soil moisture content, a small subplot (33 cm × 33 cm, 0.11 m^2) of the study site was analyzed with samples collected in a rectangular grid of 7 × 7 with a distance of 5.5 cm between adjacent samples. The amount of material depended upon the thickness of the sampled horizons and moisture content but generally it was around 5 g wet mass of litter and 12–20 g wet mass of H horizon material.

The periods where relative humidity were expected to be similar but the amount of fungal biomass in soil was expected to differ (May and August) were preliminarily selected based on the results obtained during a previous, independent sampling performed at the same site in the preceding year (Šnajdr et al., 2008b). The temperatures at the soil surface were monitored using a Testo 175 H (Testo, Germany) data logger with six reads per hour to select a time period well representative of the means and circadian temperature changes typical of either spring and late summer and to avoid the effect of climatic extremes.

The material of the soil cores was separated into independent samples of L and H horizon material, homogenized and used for enzyme activity assays and microbial biomass quantification. Organic matter content was estimated by combustion in an oven at 550 °C until constant mass. Soil pH was measured in soil water extract (1 g of soil and 10 ml of water left to stand overnight at room temperature). Two independent aliquots of material from each horizon were used for gravimetric soil moisture content determination, after drying at 85 °C until constant mass. Differences between the two replicates of the same sample were negligible (*t*-value for paired *t*-test >0.9).

2.2. Enzyme extraction and assays

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 supplier's 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 a citrate–phosphate buffer (100 mM citrate, 200 mM phosphate, pH 5.0) at 420 nm (Bourbonnais and Paice, 1990). Manganese peroxidase (MnP, EC 1.11.1.13) was assayed using a succinate–lactate buffer (100 mM, pH 4.5) according to the protocol of Ngo and Lenhoff (1980). 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.

The activities of endo-1,4-β-glucanase (EC 3.2.1.4) and endo-1,4-β-xylanase (EC 3.2.1.8) were 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 a 200 mM sodium acetate buffer (pH 5.0) and 0.2 ml sample. The reaction mixture was incubated at 40 °C for 60 min. The reaction was stopped by adding 1 ml of ethanol followed by 10 s of vortexing and 10 min of 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 of sample. Reaction mixtures were incubated at 40 °C for 90–120 min. Reactions were stopped by adding 0.1 ml of 0.5 M sodium carbonate, and the absorbance at 400 nm was measured. 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 as above (Valášková et al., 2007). Acid phosphatase (EC 3.1.3.1) was assayed using 2 g l^{-1} *p*-nitrophenylphosphate in a 50 mM sodium acetate buffer (pH 5.0), as described previously (Šnajdr et al., 2008a). 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 gram of dry mass of the soil or litter material.

2.3. Quantification of the microbial biomass

The samples used in the phospholipid fatty acid (PLFA) analysis were extracted using a chloroform–methanol–phosphate buffer (1:2:0.8). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck), after which the samples were subjected to mild alkaline methanolysis (Oravecz et al., 2004). The free methyl esters of the phospholipid fatty acids were analyzed using gas chromatography–mass spectrometry (Varian 3400; ITS-40, Finnigan). The GC instrument was equipped with split/splitless injector and a DB-5MS column was used for separation (60 m, 0.25 mm i.d., 0.25 μm film thickness). The temperature program started at 60 °C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 160 °C at a rate of 25 °C min^{-1}. The second temperature ramp was up to 280 °C at a rate of 2.5 °C min^{-1}, this temperature being maintained for 10 min. The solvent delay time was set to 8 min. The transfer line temperature was set to 280 °C. Mass spectra were recorded at 1 scan s^{-1} under electron impact at 70 eV, mass range 50–350 amu. Methylated fatty acids were identified according to their mass