Soil Biology & Biochemistry 56 (2013) 60–68

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change

Petr Baldrian*, Jaroslav Šnajdr, Věra Merhautová, Petra Dobiášová, Tomáš Cajthaml, Vendula Valášková

Institute of Microbiology of the ASCR, v.v.i., Vídeňská 1083, 14220 Praha 4, Czech Republic

ARTICLE INFO

Article history:
Received 30 August 2011
Received in revised form
20 January 2012
Accepted 23 January 2012
Available online 13 February 2012

Keywords:
Extracellular enzymes
Forest soil
Lignocellulose
Litter
Microbial ecology
Quercus petraea
Seasonality
Climate change

ABSTRACT

The activities of extracellular enzymes that participate in the decomposition of litter and organic matter in forest soils depend on, among other factors, temperature and soil moisture content and also reflect the quality of litter, which changes dramatically after a short litterfall period. Here, we explored the effects of soil temperature and seasonality on the sizes of extracellular enzyme pools and activities in a temperate hardwood forest soil with dominant *Quercus petraea* (cambisol, mean annual temperature 9.3 °C). We hypothesized that the most significant variation of enzyme activity would occur in the litter, which faces greater variations in temperature, moisture content and chemical quality during the season, which decrease with soil depth. The site exhibited relatively large seasonal temperature differences and moderate changes in soil moisture content. Enzyme activity, microbial biomass, soil moisture content, temperature and pH were monitored for three years in the litter (*L*), organic horizon (*O*) and upper mineral horizon (Ah). Enzyme activity *in vitro* strongly increased with temperature until 20–25 °C, the highest temperatures recorded *in situ*. While no significant differences in the pools of most extracellular enzymes and in the content of microbial biomass were found among the seasons, enzyme activity typically increased during the warm period of the year, especially in the *O* and Ah horizons. Approximately 63%, 64%, and 69% of total annual activity was recorded during the warm period of the year in the *L*, *O*, and Ah horizons, respectively. Significant positive correlations were observed between soil moisture content and fungal biomass, but not bacterial biomass, indicating a decrease of the fungal/bacterial biomass ratio under dry conditions. The effect of moisture on enzyme activities was not significant except for endoxylanase in the litter. If soil temperature rises as predicted due to global climate change, enzyme activity is predicted to rise substantially in this ecosystem, especially in winter, when decomposition is not limited by drought and fresh litter that can decompose rapidly is present.

1. Introduction

Substantial variations in temperature or moisture during the year influence considerably the soil processes of biomes with such climatic characteristics, including the decomposition of organic matter. Thus, climatic factors have previously been identified as major causes of the observed seasonal differences in decomposition rates in such environments due to alterations in the pools of various extracellular enzymes, including laccase, polysaccharide hydrolases, phosphatase, urease, protease and others (Bastida et al., 2008; Criquet et al., 2002; Prietzel, 2001; Wittmann et al., 2004). The effects of temperature on respiration or on the activity of selected enzymes has been repeatedly demonstrated (Ise and Moorcroft, 2006; Moore, 1986; Wallenstein et al., 2009). However, in some environments such as the Mediterranean zone, where periods of high temperature are accompanied by temporary droughts, the positive effect of temperature in the warm period of the year is counteracted by the decrease of enzyme pools due to soil or litter desiccation (Criquet et al., 2000, 2004; Sardans and Peñuelas, 2005). Even in the temperate zone, soil moisture content was identified as one of the most important factors affecting the spatial distribution of microbial biomass and extracellular enzymes (Baldrian et al., 2010b).

In temperate forests, temperature variation during the year can be considerable, whereas the effects of drought are usually less pronounced than in the warmer zones. However, the seasonality of the decomposition processes may be seriously affected by the seasonal differences in belowground C flux via plant roots (Högberg et al., 2010; Kaiser et al., 2010) and the fact that the quality of the litter material on the soil surface changes abruptly during the litterfall season, typically restricted to autumn, when fresh litter with a higher content of easily available nutrients and high C/N ratio accumulates on the forest floor (Dilly and Munch, 1996; Fioretto

* Corresponding author. Tel.: +420723770570; fax: +420241062384.
E-mail address: baldrian@biomed.cas.cz (P. Baldrian).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2012.01.020

et al., 2000; Šnajdr et al., 2011). As a result of these phenomena, spring and summer are characterized by a decrease of litter quality due to its ongoing decomposition with the concomitant increase of photosynthetic carbon allocation underground via the mycelia of mycorrhizal fungi. This carbon flow ceases in autumn along with leaf abscission, when it is replaced by the seasonal litterfall. Consequently, the relative proportion of decomposer to symbiotic life strategies of fungi is predicted to increase during the cold period of the year. In addition to changes in microbial community composition, the production of several hydrolases by symbiotic ectomycorrhizal fungi is also increased (Mosca et al., 2007).

Although this theoretical model seems to reasonably predict the behavior of the temperate forest soils, the extent of seasonal differences in enzyme activities and the abundance of their microbial producers have not been addressed sufficiently with the majority of studies obtained in the boreal and tundra ecosystems. Interestingly, these studies showed that considerable decomposition rates can also be achieved during the cold period of the year and that the warmest periods do not necessarily have the highest decomposition rates (Kahkonen et al., 2001; Wallenstein et al., 2009; Wittmann et al., 2004). Data derived from similar studies make it possible to predict the direction and the potential extent of changes in decomposition rates if temperatures increase as a consequence of global climate change.

The aim of this work was to describe the seasonal variations in enzyme pools (i.e., the amount of enzyme molecules) and the biomass of soil microorganisms in hardwood forest soils with dominant *Quercus petraea* and to quantify the seasonal variation of enzyme activities calculated as enzyme pools multiplied by the relative activity of the enzyme at the *in situ* temperature recorded. Litter, organic soil horizon and mineral soil were separately analyzed because they were previously demonstrated to differ substantially in chemical quality, microbial biomass content and community composition (Šnajdr et al., 2008). The enzyme activity and climatic data were collected monthly for three years. We hypothesized that the most significant variation of enzyme activity would occur in the litter, which faces greater variations in temperature, moisture content and chemical quality during the season, which decrease with soil depth. The results obtained in this study were also used to predict the potential increase of enzyme activity under the model scenarios of the future climate change HadAM3H and ECHAM4/OPYC3 (Jacob et al., 2007).

2. Materials and methods

2.1. Study site and sampling

Soil and litter samples were collected in a sessile oak (*Q. petraea*) forest in the Xaverovský Háj Natural Reserve near Prague, Czech Republic, a site where previous studies targeted the spatial variability of extracellular enzyme distribution (Šnajdr et al., 2008), the description of environmental and microbial factors affecting enzyme production (Baldrian et al., 2010a,b; Šnajdr et al., 2011) and the decomposition abilities of saprotrophic fungi (Baldrian et al., 2011; Šnajdr et al., 2010; Valášková et al., 2007). The soil was an acidic cambisol with litter (*L*), organic horizon (*O*), and the mineral horizons Ah and A Litter thickness was 0.5–1.5 cm, with average pH 4.3, 46.2% C, 1.76% N; *O* horizon thickness was 1.5–2.5 cm, average pH 3.7, 21.5% C, 0.56% N; Ah horizon thickness was 6–8 cm, average pH 3.4, 3.0–14.3% C, 0.10–0.39% N.

For the study of seasonal variation of soil enzyme activities and microbial biomass content, soil cores (45 mm in diameter) were collected monthly from September 2005 to August 2008. At each sampling date, a total of six cores were collected from the same 16 m^2 sampling plot with a litter layer on the forest floor (no growth of grasses). For each soil core, *L* horizon material (0.5–1.0 cm), *O* horizon material and Ah horizon material were separated, and the materials from all cores were combined to yield a composite sample of each horizon. Samples of the *L* horizon were cut into approximately 0.25-cm^2 pieces, and the samples from the deeper soil horizons were sieved using a 2-mm sieve. The resulting samples were used for the enzyme assays and the ergosterol and PLFA analyses. For the analysis of temperature effects on enzyme activity, extracellular enzymes were extracted from twelve cores sampled in late summer 2007. The composite sample combined the *L*, *O*, and Ah material of all cores.

Soil pH was measured in soil water extract (1 g soil and 10 mL deionized water were mixed and left to stand overnight at room temperature), and the soil moisture content was assessed by drying the soil at 85 °C until a constant mass was reached.

The temperature was recorded hourly during the sampling period at the soil surface and at interfaces between the *L* and *O*, *O* and Ah, and Ah and *A* horizons. From these data, temperatures in soil horizons were calculated as the averages of temperature recorded immediately above and below the respective horizon.

2.2. Enzyme extraction and assays

Enzymes were extracted from samples on the day of sample collection as previously described (Šnajdr et al., 2008), and at least two independent extractions were performed from each sample. 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 immediately used for enzyme activity analysis. Enzymes for the determination of temperature–activity relationships were extracted from combined samples of the whole *L*, *O* and Ah horizons with a total mass >100 g. Three independent extractions were performed. Extracts were concentrated by ultrafiltration through a 10-kDa nitrocellulose membrane (Amicon, Millipore) before desalting.

Laccase (EC 1.10.3.2) activity was measured by monitoring the oxidation of 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in citrate-phosphate buffer (100 mM citrate and 200 mM phosphate; pH 5.0) at 420 nm (Bourbonnais and Paice, 1990). Manganese peroxidase (MnP, EC 1.11.1.13) activity was assayed using a succinate-lactate buffer (100 mM, pH 4.5) according to Bourbonnais and Paice (1990). 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 3,3-dimethylaminobenzoic acid (DMAB) 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.

Endocellulase (EC 3.2.1.4) and endoxylanase (EC 3.2.1.8) activities 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 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, vortexing for 10 s and centrifuging at 10,000 × g for 10 min (Baldrian, 2009). The amount of released dye was measured at 595 nm, and the enzyme activity was calculated according to standard curves correlating the dye release