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Tannins can slow-down but also speed-up soil enzymatic activity in boreal forest

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ABSTRACT

The boreal forest ecosystem stores substantial amounts of soil organic matter (SOM), which may act either as a source or as a sink for atmospheric CO_2 under climate change and that is why enzymatic SOM degradation is gaining increasing attention. The boreal forest ecosystem is rich in plant secondary compounds and in particular tannins which are seen as enzyme inhibitors. We studied changes in enzymatic activity after the addition of tannins. Our experimental design combined direct studies of the tannin effects on enzymes with laboratory soil mesocosm experiments. Our results showed that the addition of tannins directly led to both decreases and increases in the catalytic activity of enzymes, however, some differences between enzymes were observed. Overall, low concentrations of tannins increased the coiled structures of the enzymes boosting their catalytic activity. High concentrations of tannins acted in the opposite way, thereby diminishing the catalytic activity. We observed that tannins caused a similar change in enzymatic activity in soil mesocosm experiments. Tannin-enzyme synergy needs more study as these interactions can potentially play an important role in SOM decomposition of future climate, especially in the tannin-rich ecosystems.

1. Introduction

Soil organic matter (SOM) contains more carbon (C) than global vegetation and the atmosphere combined and therefore SOM is a crucial component in the C cycle (Farrior et al., 2015; Kaiser et al., 2015; Li et al., 2012; Schmidt et al., 2011). The enzymatic release of C from SOM and subsequent conversion into CO_2 or CH_4 can cause significant increase of atmospheric concentrations of these greenhouse gases, which in turn accelerate global warming (Kaiser et al., 2015; Li et al., 2012; Schmidt et al., 2011). Soil organic matter decomposition is gaining increasing attention for this reason (Hunter, 2008; Melillo et al., 2011). Ecosystems with high SOM content, like boreal forest, are of particular interest. The boreal forest soil ecosystem is characterized also by high content of plant secondary compounds of which tannins are the most abundant group (Peñuelas and Estiarte, 1998; Tharayil et al., 2011). Global scale assessments and future projections point to increase in temperature, drought, and levels of greenhouse gases (IPCC, 2013; Schindler and Lee, 2010). These factors affect the production of plant secondary compounds (Jamieson et al., 2015, 2012; Peñuelas and Estiarte, 1998; Väisänen et al., 2013; Zhao et al., 2016). It was recently proven that trees produce more reactive tannins in response to climatic stress (Tharayil et al., 2011).

Tannins are polyphenolic compounds usually divided into hydrolysable tannins (HT), and condensed tannins (CT, proanthocyanidins). The role of tannins in boreal forest soil span numerous functions, like defense against herbivores, metal complexation, influence on C and N cycling and inhibition of microbial activity (Hättenschwiler and Vitousek, 2000; Kraus et al., 2003). Tannins are seen as protein precipitating agents and potential enzyme inhibitors (Adamczyk et al., 2012; Goldstein and Swain, 1963; Hagerman, 2012; Strumeyer and Malin, 1970; Uchida et al., 1987; Upadhyay and Singh, 2011). Numerous direct biochemical studies proved that tannins decrease enzymatic activity (Adamczyk et al., 2009; Goldstein and Swain, 1963; Hagerman, 2012; Uchida et al., 1987; Upadhyay and Singh, 2011), but in some cases only a minor decrease in activity was observed (Juntheikki and Julkunen-Tiitto,

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2000; Strumeyer and Malin, 1970). There is surprisingly little information on the interference of tannins with soil enzymes (Fierer et al., 2001; Joanisse et al., 2007). There is currently no evidence that demonstrates and explains the enhancement of enzymatic activity through reaction with tannins.

Here we wanted to take a first step to explore the possibility that tannins can accelerate or decelerate enzymes responsible for SOM decomposition. We studied changes in enzymatic activity of beta-glucosidase, acid phosphatase, arylsulfatase and chitinase, crucial enzymes in C, P, S, N cycling (Acosta-Martínez et al., 2007). We hypothesized that (i) tannins, through changes in enzyme conformation, can increase or decrease the enzyme activity, (ii) there are differences between enzymes in response to tannins, and (iii) both, increase and decrease of enzymatic activity through reaction with tannins, occur in soil conditions, but inhibitory effect is prevalent.

2. Materials and methods

2.1. Experimental setup

Our experimental design combined direct biochemical studies of the tannin effects on enzymes (no soil included) with laboratory soil mesocosm studies. In direct biochemical part, we studied the effect of tannins in different concentrations on enzyme activity, kinetics of enzyme inhibition by high tannin concentration, enzyme precipitation through very high tannin concentrations and residual activity of precipitated enzymes. We also studied changes in enzyme secondary structure due to reaction with tannins. Biochemical studies took into account condensed tannins (CT) and hydrolysable tannins (HT).

In laboratory soil mesocosm studies we added different amounts of tannins to two soils differing in characteristics including the native amounts of condensed tannins (called later CT-rich and CT-poor soil). Under soil conditions we studied only the effect of CT as they constitute the major source of tannins and soil concentration of HT is very low (Adamczyk et al., 2009). We used CT in 3 doses, 10 mg, 50 mg and 100 mg per soil bottle. One soil bottle contained 1.26 g or 1.46 g SOM for CT-poor and CT-rich soil, respectively. Amount of 10 mg CT was equal to the native amount of CT in CT-rich soil. Incubation lasted for 20 days. This incubation time was chosen on the basis of earlier studies (e.g. Kanerva et al., 2006) in which changes in N and C transformations occurred up to 15 days, with no difference at later time points in comparison to control (i.e. no tannin addition).

Below we outline the methods, but more details can be found in Supplementary Materials.

2.2. Characterization of tannins

As representative of HT we used tannic acid (TA) which was already characterized (Adamczyk et al., 2012) according to Salminen and Karonen (2011). The TA contained simple galloyl glucoses (tri-, tetra- and pentagalloyl glucoses), gallotannins (hexa- to tridecagalloyl glucoses), but also gallic acid and digallic acid. The average molecular mass was approximated as 1000 Da.

Condensed tannins from Norway spruce needles were extracted and fractionated as previously described (Fierer et al., 2001; Kanerva et al., 2006). The CT fraction was analyzed by two different LC-MS methods (Karonen et al., 2011; Engström et al., 2014).

2.3. Biochemical studies of tannin effect on enzymatic activity

Activities of acid phosphatase, beta-glucosidase, arylsulfatase and chitinase were measured as described earlier (de la Mata et al., 1993; Garzillo et al., 1996; Muller and Schmidt, 1986; Parham and Deng, 2000). The temperature during all incubations was 37 °C. The conditions for enzyme measurements (time of incubation with substrate, pH, concentration of substrate) were optimized to obtain enzyme kinetics with the highest velocity. Briefly, acetic buffer was mixed with enzyme and 5 min later tannins were added. After 10 min of incubation a substrate was added and mixtures were incubated for 5 min (acid phosphatase), 10 min (beta-glucosidase and chitinase) or 15 min (arylsulfatase). Following substrates were used: for glucosidase *p*-nitrophenyl b-D-glucopyranoside, for acid phosphatase *p*-nitrophenyl phosphate, for arylsulphatase *p*-nitrophenyl sulfate and for chitinase *p*-nitrophenyl *N*-acetyl-b-D-glucosamidine. After the incubation, 25 μl of incubation mixture was transferred to a new tube containing 600 μl of 0.5 M NaOH. After 10 min, the absorbance was measured at 405 nm. The results are presented as residual activity of the control (100% activity, no tannin addition). All analyses were done in 5 laboratory replicates.

To study kinetics of enzyme inhibition we used the same methods as for the measurements of enzyme activity with following concentrations of substrates: for glucosidase 10–60 mM, for acid phosphatase 5–40 mM, for arylsulphatase and chitinase 1–20 mM. The kinetics of enzyme inhibitions were calculated using Lineweaver-Burk and Eadie–Hofstee equations (Wilson and Walker, 2010).

Studies of enzyme precipitation by very high tannin concentrations were conducted as earlier (Adamczyk et al., 2014). Standard curves were prepared separately for TA and CT, and for each enzyme.

Measurements of residual activity in enzyme-tannin complexes were conducted on enzyme-tannin precipitates. Briefly, buffer (pH range from 3.5 to 7) was added to precipitates and substrate. After incubation at room temperature, (time of incubation as in earlier measurements of enzymatic activity), samples were centrifuged and 0.1 ml of supernatant was moved to the next tube already containing 0.3 ml 0.5 M NaOH and the absorbance was measured at 405 nm. We compared these residual activities to activities of the same amount of enzymes under the same conditions but without precipitation step. Studies were made in 5 laboratory replicates for each enzyme and each pH value.

2.3.1. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of enzymes were recorded with a FTIR spectrometer (Shimadzu IRPrestige-21; Shimadzu Corporation, Japan) with IRsolution 1.40 software (Shimadzu, Kyoto, Japan). We scanned: i) enzymes without tannins, ii) tannin and enzyme ratios in which activity was inhibited and ratios in which activity was increased and iii) tannins without enzymes. Liquid samples were vacuum freeze dried to dry powder and 3 mg of sample were mixed with 300 mg of KBr and pressed into discs. All tests were done in triplicate. FTIR analysis of enzyme conformation was based on Shen et al. (2014). The IR spectra of 1700–1600 cm^{-1} (Amide I), commonly used to study changes in protein conformation, were treated with second derivative to increase resolution (Byler and Susi, 1986; Baker et al., 2014). Band assignments were done on the basis of literature (Byler and Susi, 1986; Choi and Ma, 2005; Jackson and Mantsch, 1995; Kong and Yu, 2007; Lu et al., 2015).

2.4. Soil microcosm experiment

Samples were taken from the soil organic layer of two study sites, one located in Eno (middle-eastern Finland), called CT-poor soil and the other located in Salla (northern Finland), called CT-rich soil. Both sites have three replicate plots. The characteristics of the soils can be found in Table 1. For more information see Adamczyk et al. (2015), Luiro et al. (2010), Smolander et al. (2010).