Microbial community composition shapes enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia

Jörg Schnecker [a, b, *], Birgit Wild [a, b], Mounir Takriti [a, b], Ricardo J. Eloy Alves [b, c], Norman Gentsch [d], Antje Gittel [e, f], Angelika Hofer [a], Karoline Klaus [a], Anna Knoltsch [a, b], Nikolay Lashchinskiy [g], Robert Mikutta [d], Andreas Richter [a, b]

[a] University of Vienna, Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research, Vienna, Austria
[b] Austrian Polar Research Institute, Vienna, Austria
[c] University of Vienna, Department of Ecogenomics and Systems Biology, Division of Archaea Biology and Ecogenomics, Vienna, Austria
[d] Leibniz Universität Hannover, Institut für Bodenkunde, Hannover, Germany
[e] Aarhus University, Center for Geomicrobiology, Department of Bioscience, Aarhus, Denmark
[f] University of Bergen, Centre for Geobiology, Bergen, Norway
[g] Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

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ABSTRACT

Soil horizons below 30 cm depth contain about 60% of the organic carbon stored in soils. Although insight into the physical and chemical stabilization of soil organic matter (SOM) and into microbial community composition in these horizons is being gained, information on microbial functions of subsoil microbial communities and on associated microbially-mediated processes remains sparse. To identify possible controls on enzyme patterns, we correlated enzyme patterns with biotic and abiotic soil parameters, as well as with microbial community composition, estimated using phospholipid fatty acid profiles. Enzyme patterns (i.e. distance-matrixes calculated from these enzyme activities) were calculated from the activities of six extracellular enzymes (cellobiohydrolase, leucine-amino-peptidase, N-acetylglucosaminidase, chitotriosidase, phosphatase and phenoloxidase), which had been measured in soil samples from organic topsoil horizons, mineral topsoil horizons, and mineral subsoil horizons from seven ecosystems along a 1500 km latitudinal transect in Western Siberia. We found that hydrolytic enzyme activities decreased rapidly with depth, whereas oxidative enzyme activities in mineral horizons were as high as, or higher than in organic topsoil horizons. Enzyme patterns varied more strongly between ecosystems in mineral subsoil horizons than in organic topsoils. The enzyme patterns in topsoil horizons were correlated with SOM content (i.e., C and N content) and microbial community composition. In contrast, the enzyme patterns in mineral subsoil horizons were related to water content, soil pH and microbial community composition. The lack of correlation between enzyme patterns and SOM quantity in the mineral subsoils suggests that SOM chemistry, spatial separation or physical stabilization of SOM rather than SOM content might determine substrate availability for enzymatic breakdown. The correlation of microbial community composition and enzyme patterns in all horizons, suggests that microbial community composition shapes enzyme patterns and might act as a modifier for the usual dependency of decomposition rates on SOM content or C/N ratios.

1. Introduction

Extracellular enzymes break down soil organic matter (SOM) at every depth of the soil profile. Nonetheless most studies on enzyme activities focused on topsoil horizons in the upper 20 cm of the soil profile (e.g. Sinsabaugh et al., 2008; Wallenstein et al., 2009; Kaiser et al., 2010) although up to 60% of the carbon stored in soils are located below 30 cm (Jobbágy and Jackson, 2000). These subsoil

horizons differ from well studied topsoil horizons in a number of physical and chemical conditions that might influence enzyme activities and decomposition in general (Rumpel and Kögel-Knabner, 2011): Temperature decreases from topsoils to subsoils whereas soil moisture increases with depth, either improving conditions for decomposition in arid systems (Rovira and Vallejo, 2002), or impairing them in systems where water logging occurs and O_2 availability is low (Kleber, 2010; Davidson et al., 2012). Soil pH, one of the factors often associated with enzyme activities (Sinsabaugh et al., 2008), also changes with depth (Eilers et al., 2012). In addition to these direct influences on enzyme activities, the availability of substrate for enzymatic breakdown decreases with depth. First, SOM is less abundant in subsoils, which leads to a high probability of a spatial disconnection of enzyme and substrate (Holden and Fierer, 2005). Second, a high proportion of SOM in subsoils is bound to minerals, stabilized by metal ions, or occluded in aggregates and therefore access for microorganism is limited (von Lützow et al., 2006). In addition to physical hurdles for decomposition, SOM in subsoils is chemically different from topsoil SOM. While the main proportion of SOM in topsoils is plant derived material, SOM in subsoils is microbially transformed (Wallander et al., 2003). During this microbial transformation of SOM, carbon is lost, mainly as CO_2, whereas most of the nitrogen (N) is recycled and remains in the system, resulting in lower C/N ratios of subsoil SOM (Rumpel and Kögel-Knabner, 2011).

To fulfill the microbial demand for energy and nutrients, microorganisms need to adapt to the chemical composition of SOM and to the C/N ratio of the available substrate by adjusting their enzyme production (Sinsabaugh et al., 2008). Changes in enzyme production might either be physiological (Stone et al., 2014) or they might result from a shift in microbial community composition (Kaiser et al., 2014). Although the influence of microbial community composition on major microbial processes, such as C mineralization and N mineralization, has been recently challenged (Colman and Schimel, 2013), its influence on enzyme activities has been demonstrated repeatedly (e.g. Strickland et al., 2009; McGuire and Treseder, 2010; Schnecker et al., 2014). Microbial community composition, as another potential control on enzyme activities, has already been shown to change more strongly with soil depth, within ecosystems, than between topsoils of different ecosystems (Eilers et al., 2012; Gittel et al., 2014).

Relations of enzyme activities to key factors such as pH, moisture, SOM content (Keeler et al., 2009), chemical composition of SOM (Grandy et al., 2009; Sinsabaugh and Follstad Shah, 2010), and microbial community composition (Waldrop and Firestone, 2006; Talbot et al., 2013) are well established in topsoil horizons. Whether enzyme activities in the subsoils are related to these key factors is still largely unknown since few studies have addressed changes of enzyme activities and their potential controls with soil depth so far (e.g.: Brockett et al., 2012; Kramer et al., 2013; Turner et al., 2014; Schnecker et al., 2014; Stone et al., 2014).

In this study we investigated enzyme patterns in different soil horizons, including mineral subsoils, from a wide range of ecosystems to identify potential drivers for these enzyme patterns. We measured potential activities of six extracellular enzymes in organic topsoil horizons, mineral topsoil horizons and mineral subsoil horizons in seven ecosystems along a 1500 km-long north–south transect in Western Siberia. In addition to enzyme activities, we analyzed microbial community composition (using phospholipid fatty acid analysis) as well as abiotic soil parameters and related these factors to the enzyme patterns.

We hypothesized: (1) enzyme patterns in topsoil and subsoil horizons are both related to the same key parameters, such as SOM content, pH and microbial community composition. Microbial community composition has been shown to differ more strongly between topsoils and subsoils than between topsoils of different ecosystems (Meyer et al., 2006; Eilers et al., 2012). Since enzyme activities and enzyme patterns are often related to microbial community composition; (2) enzyme activities and enzyme patterns change with depth and differ more strongly between horizons than between ecosystems. The ecosystems along the transect showed large differences in vegetation and presumably in the chemical composition of litter entering the soil; (3) enzyme patterns would be more variable and show greater differences between ecosystems in the topsoil horizons, where the main constituents of SOM are plant-derived, than in mineral subsoil horizons.

2. Material and methods

2.1. Sampling sites

Soil samples were taken from seven ecosystems along a 1500 km latitudinal transect in Western Siberia, in August and September 2012. The ecosystems included tundra, northern taiga, middle taiga, and southern taiga, forest steppe (one forest site and one meadow site), and steppe. All soils were sampled from the active layer in an unfrozen state. Basic soil and climate parameters are provided in Table 1 and Table S1. Climate data are derived from Stolbovoi and McCallum (2002), soil classification follows the World Reference Base for Soil Resources (IUSS Working Group WRB, 2006).

At all sites, we sampled the three dominant soil horizons of five replicate soil pits. We categorized the three horizon types as organic topsoil horizon (uppermost horizon, O), mineral topsoil horizon (second horizon, A), and mineral subsoil horizon (third horizon, M). We removed living plant roots from the samples and sieved them to <2 mm. We did this for samples from all sites, except for the tundra, where samples were manually homogenized because they were too moist for sieving. Before further analyses, soil water content was adjusted to a minimum of 60% for organic topsoils (except steppe), to 15% for mineral topsoils and steppe organic topsoils, and to 10% for mineral subsoils, respectively.

2.2. Soil parameters

Soil pH was determined in 1 M KCl extracts. Samples for determination of organic C, total N content, and $\delta^{13}C$ were dried at 60 °C and ground with a ball mill. Ground samples were analyzed with EA-IRMS (CE Instrument EA 1110 elemental analyzer, coupled to a Finnigan MAT DeltaPlus IRMS with a Finnigan MAT ConFlo II Interface, Thermo Fisher Scientific, Waltham, MA, USA). Mineral topsoils and subsoils at both forest steppe sites, as well as all horizons of the steppe site, contained traces of carbonate. Carbonate was removed from these samples by acidification with HCl before EA-IRMS analysis. Water holding capacity (WHC) was determined as the amount of water that remained in saturated soil, from which water could be lost by drainage but not by evaporation after two days (Reynolds and Topp, 2007).

Microbial C and N were estimated using chloroform-fumigation−extraction (Kaiser et al., 2010 modified after Brookes et al., 1985): Soil samples, fumigated with chloroform, as well as unfumigated samples were extracted with 0.5 M K_2SO_4. Dissolved organic C and total dissolved N were determined in both sets of extracts with a DOC/TN analyzer (Shimadzu TOC-VCPH/CPN/TNM-1, Vienna, Austria). Microbial C and N were calculated as the difference between fumigated and non-fumigated samples, without correction for extraction efficiency. C/N ratios of SOM and microbial biomass were calculated on a mass basis.