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DISSERTATION

Titel der Dissertation

„Enzyme activities and microbial community composition in soils from northern latitudes with special emphasis on cryoturbated arctic soils“

verfasst von

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“Denn von allen Gedanken schätz ich doch am meisten die interessanten.”

Frank Spilker, Die Sterne, 1997

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Chapter 1

Introduction and Outline

Decomposition of soil organic matter (SOM) is the antagonist of photosynthesis and nitrogen fixation in terrestrial systems. The first step in SOM decomposition is the enzymatic breakdown of organic polymers into smaller molecules that can be taken up by plants and microorganisms. Soil microorganisms are responsible for this first step of decomposition by producing and exuding extracellular enzymes to satisfy their nutrient and energy demand (Allison et al., 2011). Around 100 of the enzymes active in soil have been assayed and partially described (Dick and Burns, 2011). In this thesis, with the goal to identify potential controls on enzyme activities in soils, we considered potential activities of six extracellular enzymes: cellobiohydrolase (CBH), leucine-aminopetidase (LAP), β-1,4-poly-N-acetylglucosaminidase (CHT), N-acetyl-β-D-glucosaminidase (NAG), phosphatase (PHO), and phenoloxidase (POX).

Cellobiohydrolase (CBH)

CBH removes cellobioside from the non-reducing end of the cellulose chain. Together with endocellulase, which randomly attacks cellulose polymers, and β-glucosidase, which releases glucose, CBH is thus involved in the degradation of cellulose, the most abundant carbohydrate on Earth (Deng and Popova, 2011). Since these enzymes are usually highly correlated to each other, activities of individual enzymes, mostly CBH and β-glucosidase, are used to represent the degradation of cellulose and thus a major pathway for microorganism to acquire energy-rich carbohydrates (Sinsabaugh et al., 2008).

Leucine-aminopeptidase (LAP)

The main forms of N in soil are peptides and proteins of either plant or of microbial origin (Nannipieri and Eldor, 2009). The process of protein depolymerisation, which is the first step in the solubilisation of these N forms, has even been suggested to be rate limiting for the whole soil N cycle (Schimel and Bennett, 2004). Enzymes that are able to hydrolyze peptide bonds are seen as the main agents for this first step of microbial N uptake. LAP and alanine-aminopeptidase are often used to estimate this depolymerization of proteins and are used together with NAG as N-acquiring enzymes (Sinsabaugh and Follstad Shah, 2010).

β-1,4-poly-N-acetylglucosaminidase (CHT) and N-acetyl-β-D-glucosaminidase (NAG)

As decomposition in soils progresses, SOM becomes more and more transformed by microorganisms and enriched in microbial remains (Wallander et al., 2003). The amount of microbial products and microbial necromass can be quite substantial (Miltner et al., 2011) and might account for as much as 80% of SOM (Liang and Balser, 2011). The enzymes degrading chitin and microbial cell-walls (chitinases) that are commonly measured include CHT, which randomly cuts 1,4-β linkages in chitin and chitodextrines, and NAG which hydrolyzes chitin from the non-reducing end and releases N-acetylglucosamine units (Dick and Burns, 2011). Chitinases are crucial for microbial carbon (C) acquisition, but since N-acetylglucosamine also contains nitrogen (N), NAG is also commonly used as a N acquiring enzyme when calculating microbial nutrient demand (Sinsabaugh and Follstad Shah, 2010).

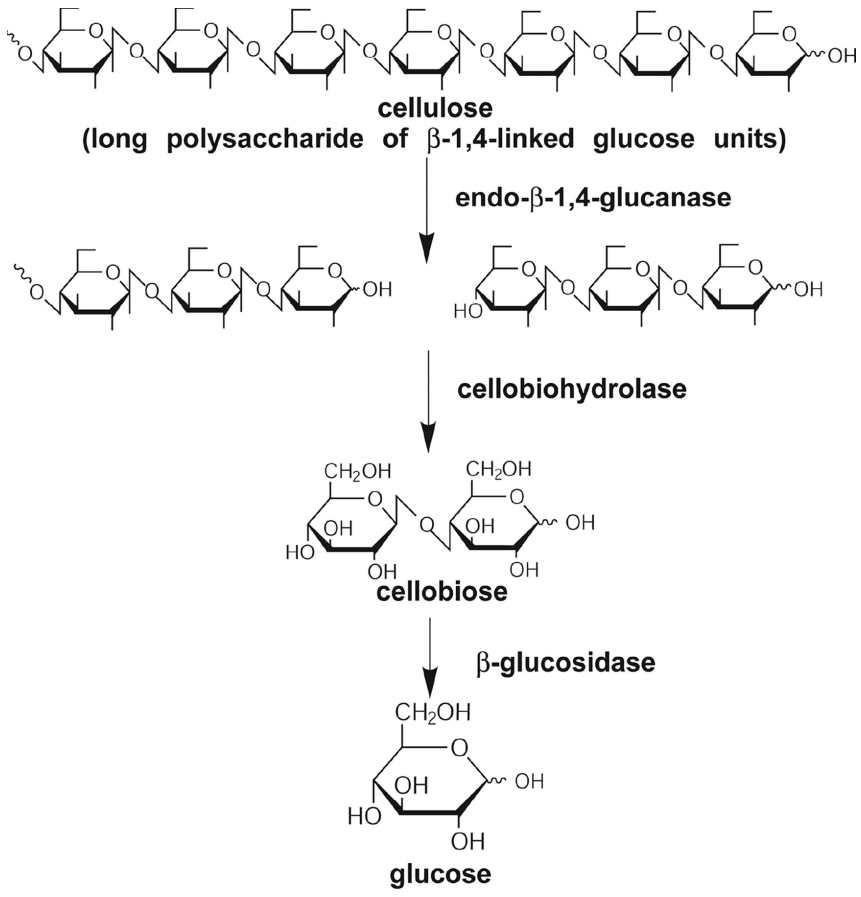

Figure 1. Enzymes involvedin the breakdown of Cellulose. Figure from Xie et al. (2007)

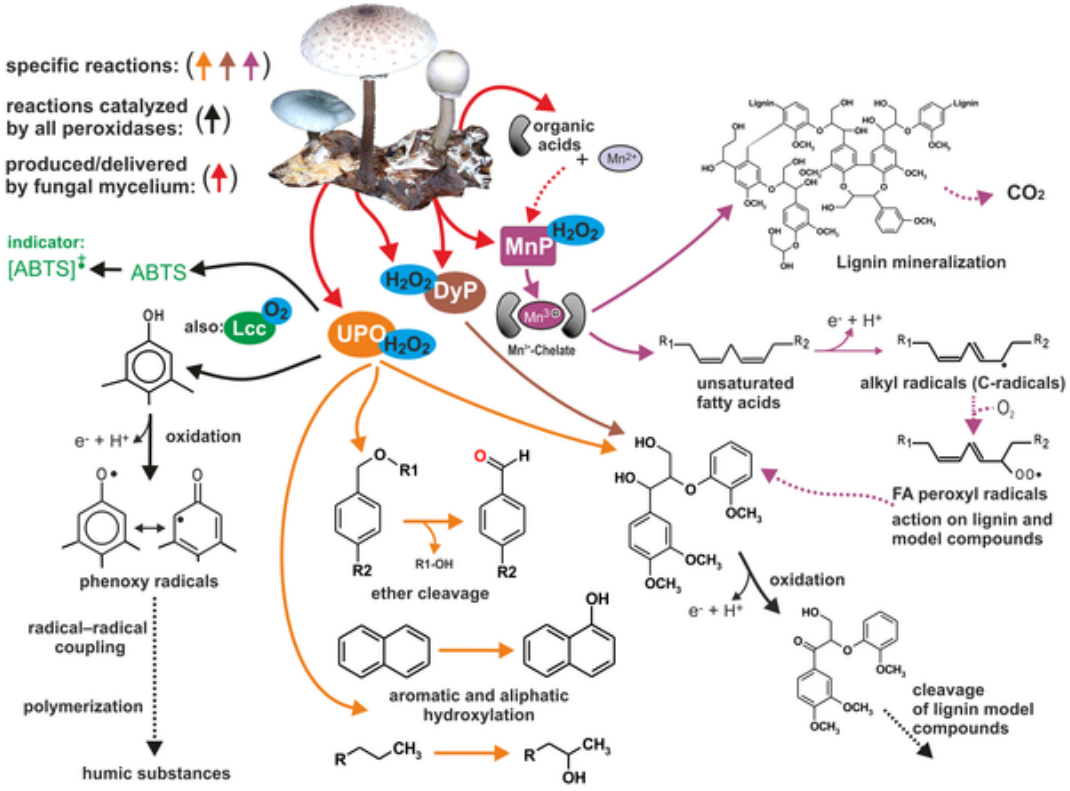

Figure 2. Reactions catalysed by oxidative enzymes. Figure from Kellner et al. (2014). Lcc are laccases, UPO are unidentified peroxygenases, DyP are dye-decolorized peroxidasesand and MnP are Manganese peroxidases.

Phosphatase (PHO)

In addition to C and N, phosphorus can also limit microbial growth and functioning. Organic phosphorus constitutes up to 50% of total soil P (Dalal, 1977). Phosphatase activity is thus needed to transform higher-molecular weight organic P, which cannot be directly taken up by plants and microorganisms. The widely measured phosphatases are categorized, depending on their pH optimum, as alkaline and acid phosphatases, both phosphomonesterases. Phosphomonesterases are regarded as representative for P-acquiring enzymes (Sinsabaugh and Follstad Shah, 2010).

Phenoloxidase (POX)

The second most abundant plant macromolecule in litter is lignin. The branched polymer, composed of substituted phenylpropane units (Baldrian and Snajdr, 2011) can be decomposed by oxidative enzymes such as POX. Similar to POX, which uses O2, peroxidases use H2O2 for the oxidation of their substrate (Sinsabaugh, 2010). In addition to lignin, other complex substrates can be attacked by oxidative enzymes. Many of these substances contain too little energy to maintain microbial metabolism. The main function of oxidative enzymes might thus be the release of N containing substances out of SOM (Moorhead and Sinsabaugh, 2006). This so called nitrogen mining, frees components which can in turn be used by specific hydrolytic enzymes. Additionally, oxidative enzymes can be produced to degrade toxins such as (poly)phenols (Sinsabaugh, 2010). These functions other than lignin degradation are most likely the main ones in for instance deeper soil layers, where most of the plant derived lignin has already been decomposed and decomposition in general has progressed.

Already from the categorization in C, N and P-acquiring enzymes it can be inferred that enzymes are mainly produced to make C, N and P available to organisms in the soil. All soil-dwelling organisms produce enzymes which can at least upon their death be released and add to the pool of extracellular enzymes (Burns et al., 2013). Organisms that produce and actively exude enzymes on purpose are mainly soil microorganisms (Arnosti et al., 2013).

Within soil microorganisms, saprotrophic fungi play an important role in the decomposition of SOM, since they are considered the most efficient degraders of lignocellulose (Baldrian and Snajdr, 2011). They are classically categorized by their ability to degrade lignin (Osono, 2007). While soft rot and brown rot fungi only modify lignin, white rot fungi (predominantly Basidiomycota) are considered the only organisms which can completely degrade lignin (Osono, 2007). In recent years, mycorrhizal fungi have been suggested to also actively decompose SOM (Talbot et al., 2013, 2008). Because mycorrhiza receive an extra supply of carbohydrates by plants, they can undertake the cost-intensive mining for N and P.

Within the domaines of bacteria and archaea, the ability to produce extracellular enzymes, including oxidative enzymes, is wide spread (Ausec et al., 2011a; Freedman and Zak, 2014). Expression of bacterial laccase, a subgroup of phenoloxidases, might even be more efficient than expression of laccases by fungi (Ausec et al., 2011b). Especially actinobacteria seem to be potent SOM decomposers. They cannot only produce laccases, it has even been suggested that they take over the role of fungi in anoxic environments (Boer et al., 2005; DeAngelis et al., 2011; Gittel et al., 2014). Although the ability to produce extracellular enzymes is wide spread, not all microorganisms produce enzymes and some of them take profit from the enzymatic activity of nearby microbes. These so called "cheaters" are thought to be able to quickly take up and grow faster on easily assimilable substrates than enzyme-producing microbes that need to invest energy and nutrients into enzyme production (Allison, 2005). The abundance of cheaters in relation to the enzyme-producing part of the microbial community might strongly influence decomposition in general (Kaiser et al., 2014), and microbial community composition might thus influence the enzymatic potential in soils.

Numerous studies have demonstrated a connection between enzyme activities or enzyme patterns and microbial community composition, in a wide range of ecosystems: Kaiser et al. (2010) for instance showed that enzyme patterns were strongly influenced by the composition of the microbial community over the course of two years in a temperate beech forest. Burke et al. (2011) found that mycorrhiza communities were related to enzymes involved in the N cycle of a northern hardwood forest. Talbot et al. (2013) suggested that ECM and saprotrophic fungi produce different enzyme sets in a hardwood forest in California. Kramer et al. (2013) found a connection of enzymes and community composition in agricultural soil in Germany and Stone et al. (2014) in two different soils in the tropics. Gittel et al. (2014) suggested that a shift in microbial community composition led to a change in enzyme activity caused by a reduction in ectomycorrhizal fungi and an increase in the abundance of actinobacteria with depth in arctic soils. These examples show a strong connection of microbial community composition to enzyme activities and enzyme patterns. Microbial community composition is thus considered a main driver and control over enzyme activities in soils (Wallenstein and Burns, 2011).

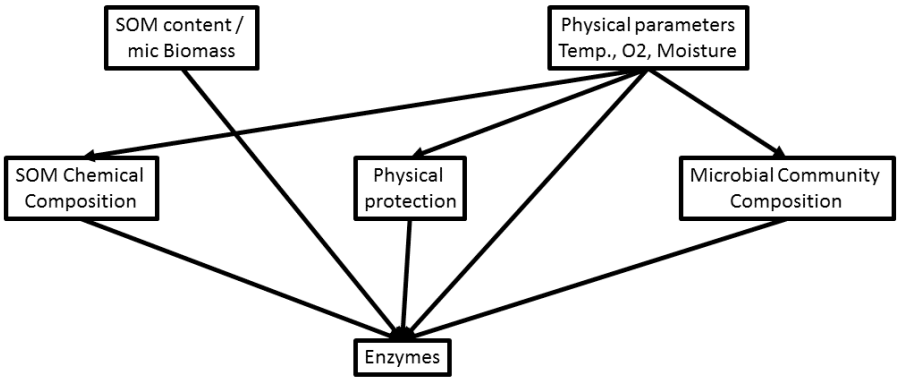

Figure 3. Potential direct and indirect controls on extracellulaer enzyme activities in soils

Controls on enzyme activities

Besides microbial community composition, which determines the enzymatic potential in soils, enzyme activities are often related to the availability of their substrate and are either exuded when specific nutrients are scarce (Sinsabaugh et al., 2009) or when they are plentiful (Sistla and Schimel, 2012). Especially hydrolytic enzyme activities, however, have also been shown to strongly depend on SOM content in general (Sinsabaugh et al., 2008) and on microbial abundance, whereas oxidative enzymes do not follow this trend on a global scale (Sinsabaugh et al., 2008). One reason for this might be that oxidative enzymes are not only produced to acquire nutrients, but are involved in nutrient mining to free substrates for other enzymes. Additionally oxidative enzymes are produced to degrade toxins such as polyphenols (Sinsabaugh, 2010). These triggers for enzyme production are not necessarily related to SOM content or microbial biomass.

Hydrolytic and oxidative enzymes may also be stabilized differently in soils. While hydrolytic enzymes tend to be stabilized at particulate organic matter, oxidative enzymes are preferentially sorbed to mineral surfaces (Allison and Jastrow, 2006). Although sorption to particles or minerals can inhibit the bound enzymes, they can also protected against degradation and enzymes can thus persist longer in the soil (Allison and Jastrow, 2006; Quiquampoix and Burns, 2007). The complexation of enzymes in organo-mineral complexes has even been reported to increase enzyme activities (Marx et al., 2005; Pflug, 1982). Such stabilization mechanisms might lead to large differences in the lifetime of enzymes in general and might specifically lead to differences between hydrolytic and oxidative enzymes in topsoil horizons, with their high amount of particulate organic matter, and mineral subsoil horizons, with their large mineral surfaces. All of these biotic and abiotic controls and enzyme activities themselves are again subjected to major abiotic controls, such as temperature, pH, O_2, and water availability (Burns et al., 2013; Fierer and Jackson, 2006; Lauber et al., 2009; Mikutta et al., 2007; Sinsabaugh et al., 2008; Wallenstein et al., 2011).

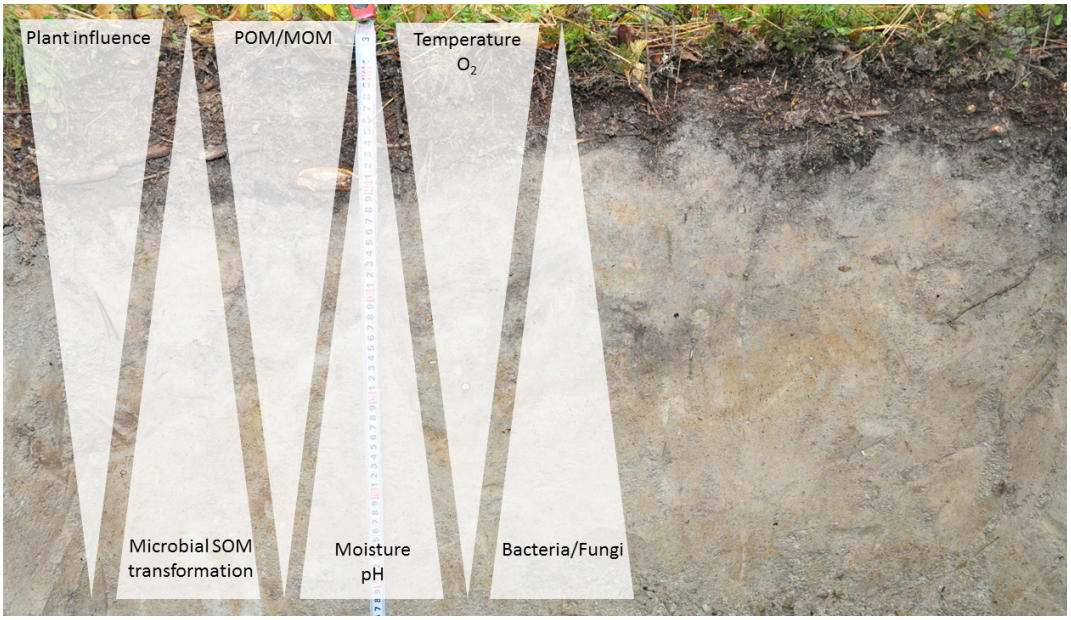

Figure 4. Biotic and abiotic factors that might control enzyme activities change with soil depth.

Enzymes in the subsoil

Abiotic conditions and as a consequence many potential controls on enzyme activities and enzyme patterns change with soil depth (Eilers et al., 2012; Rumpel and Kögel-Knabner, 2011) and thus enzyme activities and enzyme patterns can be expected to shift accordingly. Studies on depth gradients in soil profiles often only state a decrease in all or specific hydrolytic enzymes (Dick, 1984; Speir et al., 1984; Taylor et al., 2002) and only few have tried to find explanations and investigate enzyme activities and patterns in detail. Kramer et al (Kramer et al., 2013) for instance investigated enzyme activities in three different depths of agricultural soils and found huge differences in enzyme patterns between topsoil and subsoil, and a different depth gradient for hydrolytic and oxidative enzymes. The different depth gradients are suggested to be the result of different stabilization mechanisms of enzymes. Additionally, the authors suggest that the higher enzyme activities per unit of microbial biomass in the deeper soil layers are a response of the microbial community to the spatial disconnection of substrate and microbes, and might be the result of a microbial community with less cheaters and a higher proportion of the community producing enzymes. Another recent paper describes a depth gradient in a tropical rainforest ecosystem (Stone et al., 2014). In this study, microbial community composition changed with depth whereas enzyme activities per unit microbial C did not change with depth or only showed a slight increase. Similar to Kramer et al (2013), the authors suggest that the microbial community in the subsoil is more metabolically active than in the topsoil, but regard changes in microbial community structure to be secondary to physiological changes. The two studies differ in that Stone et al. (2014) did not include oxidative enzyme activities, which showed a strong increase with depth especially when related to microbial biomass in the study conducted by Kramer et al. (2013). Although conducted in very different ecosystems, both studies highlight the differences in enzyme patterns between topsoil and subsoil horizons and demonstrate a connection of the microbial community composition and enzyme activities.

The two above mentioned studies demonstrate that enzyme activities are related to microbial community composition and that both change with depth in soil profiles where also physical parameters, SOM chemis-

try and availability of nutrients and C gradually change with depth (Eilers et al., 2012; Rumpel and Kögel-Knabner, 2011). If these relations hold true when some of these factors do not change homogeneously with depth cannot be inferred from these studies. An example for a rupture of this gradual change with soils depth can be found in arctic permafrost soils.

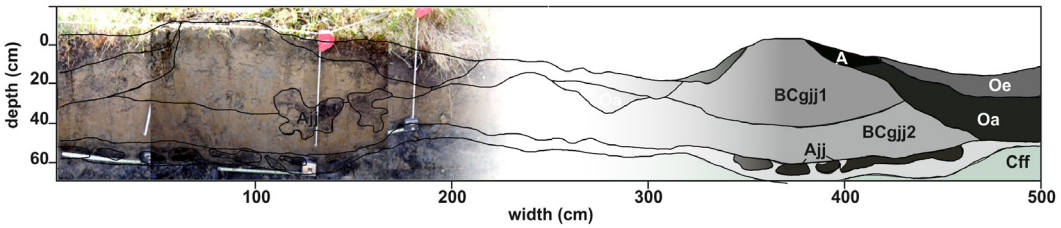

Figure 5. A typical soil profile found in the arctic permafrost region with cryoturbated pockets (Ajj)

Cryoturbated material and the Arctic

Permafrost soils are subjected to repeated freeze-thaw cycles with freezing fronts moving from topsoil to subsoil but also from subsoil to topsoil. This can cause turbation of these cryosols which leads to the subduction of topsoil organic matter, sometimes even living plants, into deeper mineral soil layers (Bockheim, 2007). Cryoturbations are an abundant feature in permafrost soils worldwide and the C stored in these pockets of organic material has been estimated to be around 400 Gt C (Harden et al., 2012). This is half the amount of C in today's atmosphere and in the range of C present in the global forest biomass (Kuhry et al., 2010).

Cryoturbated arctic soils provide a natural disruption of the horizontal layering of soils and thus of the classical continuum of SOM, microbial community composition and enzyme patterns that change with depth in other soils. Pockets of cryoturbated organic material contain usually about 5% to 10% of OC, and thus up to 50 times the amount of C per unit soil mass than the surrounding mineral subsoils (Kaiser et al., 2007; Wild et al., 2013). Soil organic matter chemistry of cryoturbated pockets has been shown to be similar to organic topsoil material (Xu et al., 2009). At the same time, cryoturbated material is subjected to the same water regimes, temperatures, and days in an unfrozen state, as the surrounding mineral horizon. Organic material in cryoturbated pockets is much older than the present topsoil but younger than the adjacent subsoil. The decomposition of cryoturbated OM is slower than of the chemically similar topsoil material even at same temperature and moisture conditions (Kaiser et al., 2007). Also N cycling in cryoturbated pockets has been shown to be slowed down (Wild et al., 2013), and microorganisms in these horizons have been found to be limited in N, despite relatively low SOM C/N ratios (Wild et al., 2014). One reason for this retardation of decomposition and slowed down N cycling might be a mismatch of microbial community and chemical composition of SOM. Although soil organic matter chemistry of cryoturbated pockets is similar to the topsoil, the microbial decomposer community resembles that of mineral subsoil horizons (Gittel et al., 2014). This setup of a microbial community, which might not be adapted to the chemical properties of its substrate, provides the opportunity to investigate drivers for extracellular enzyme activities, which either reflect microbial community composition or SOM properties or both.

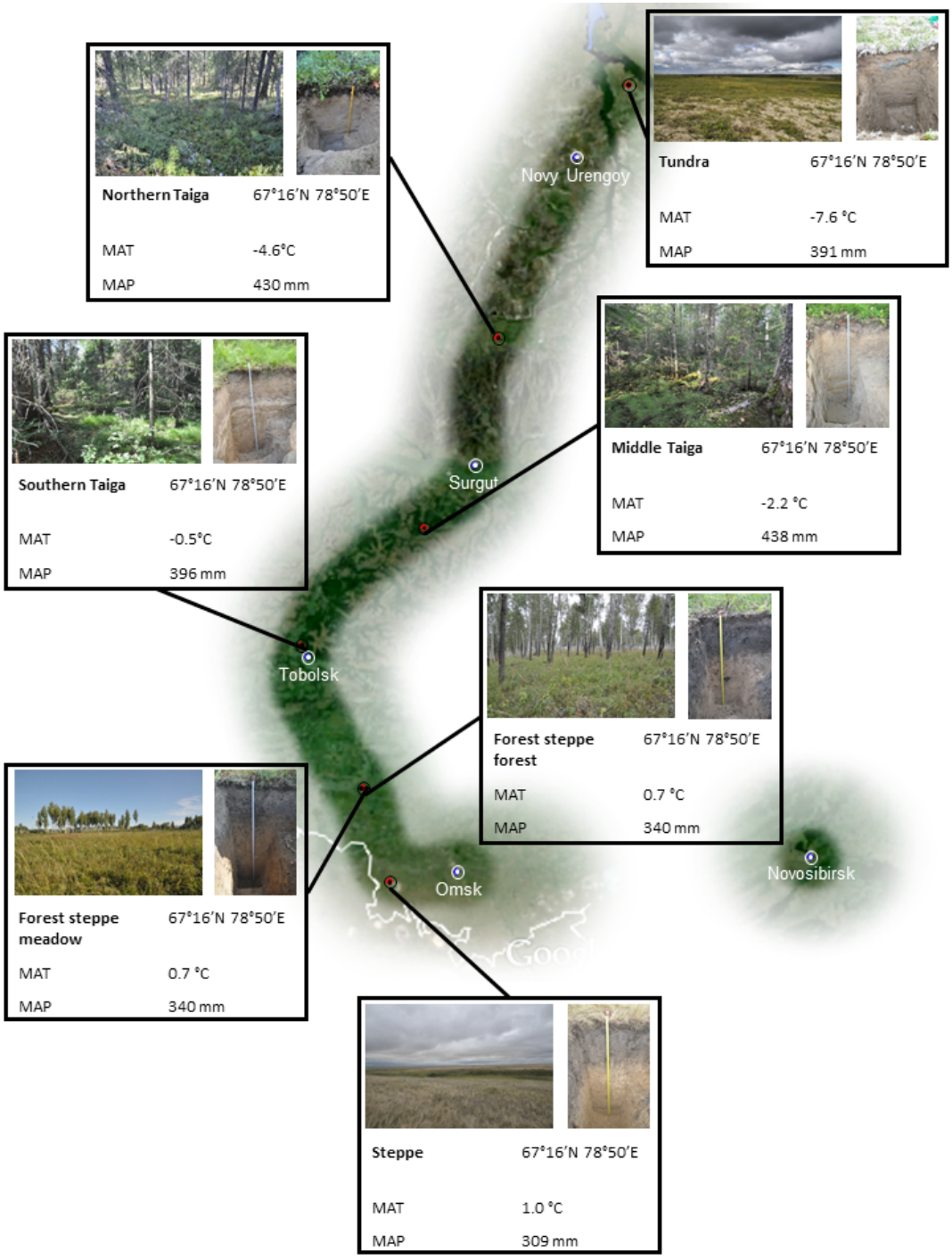

Figure 6. Latitudinal transect in Western Siberia from Tundra to Steppe. Samples from this transect were sampled in Summer 2012

Challenge and Aim

Extracellular enzymes in soil solubilize SOM for microbial uptake and consequently growth and respiration. A detailed knowledge of the regulations and controls of enzyme activities is crucial to better understand decomposition mechanisms and to better predict how changes in physical properties (temperature, moisture) might affect enzyme activities and decomposition in general. Especially subsoils, which contain about 60% of the soil C, are not well understood in terms of the regulation and controls on enzyme activities and patterns.

In the light of global climate change, subsoil horizons in permafrost soils are of special importance for two reasons: First, arctic soils contain huge amounts of C (1690 Gt C; Tarnocai et al., 2009) whereof around 400 Gt can be found in mineral subsoils and additional 400 Gt of C are stored in cryoturbated pockets of organic material in the subsoil (Harden et al., 2012). Second, climate change is predicted to be specifically pronounced in northern ecosystems, where with an expected increase in surface temperature of up to 8°C by 2100, around 25% of the arctic permafrost might thaw (IPCC, 2007).

The main goal of this thesis therefore was to enhance our understanding of enzyme patterns and enzyme activities in topsoil and subsoil horizons with special emphasis on cryoturbated material in permafrost soils. We specifically intended to identify potential controls on enzyme activities and enzyme patterns and to link enzymes (microbial functions) to microbial community structure.

Outline

One main aspect of this study was to link extracellular enzyme activities to microbial community composition, estimated with phospholipid fatty acids (PLFA) analysis. Samples for this thesis were taken at remote places in northern Siberia and it was necessary to transport and store soil samples longer than recommended, prior to analyses. Samples for enzyme activity measurement can be stored, if necessary, in plastic at 4°C for some days without drastic changes in enzyme patterns (Lorenz and Dick, 2011). The suggested storage for soil samples for PLFA analysis is however to keep the samples continuously frozen until analysis (Högberg et al., 2007; Liu et al., 2008). This was not feasible, since at remote places continuous power supply or supply of dry ice or liquid nitrogen is not available.

Chapter two describes a method to store and transport samples for subsequent PLFA analysis. We tested the idea that the use of a commercially available salt solution, which is usually used to preserve DNA and RNA samples in soils and other environmental samples, would also conserve PLFA, as enzymes that usually degrade these substances are salted out under these conditions. The paper in chapter 2 describes this work and shows that storage in RNA-later is comparable to freezing of samples, and is thus a suitable method for storing soil samples for later analysis of PLFA.

Chapter three presents the patterns of six extracellular enzymes in topsoil and subsoil horizons from seven ecosystems along a 1,500 km latitudinal transect in Western Siberia. We found that these patterns differed stronger between horizons than between ecosystems. Additionally, enzyme patterns in the subsoil were more variable than in the topsoil, despite the different inputs from tundra over taiga to steppe ecosystems. While enzyme patterns in the topsoil were related to SOM properties (C, N, $\delta^{13}C$) and microbial community composition, enzyme patterns in the subsoil were mainly related to microbial community composition, but not to SOM properties. Especially the lack of correlation between SOM properties and enzyme patterns in subsoil questions that C, N and C:N ratios are strong predictors for the way SOM is decomposed in subsoil and asks for caution when simplifying these relationships in ecosystem models.

Chapter four focuses on cryoturbated arctic soils, where we specifically investigated the controls on microbial community composition and individual enzyme activities. Based on soil samples from three different sites over continuous permafrost, we found that microbial community composition in cryoturbated material was similar to mineral subsoil, although the SOM chemistry in these pockets was similar to topsoil material. This finding led us to the conclusion that the microbial community in cryoturbated pockets was shaped by the abiotic factors in the subsoil and not by SOM properties. We used structural equation modeling to find direct and indirect controls on individual enzyme activities (CBH, LAP and POX). In "regular" horizons, which included soil layers that showed a continuous decrease in C content with depth, enzyme activities were controlled C and microbial biomass in the case of CBH, and N in the case of LAP and POX. In cryoturbated horizons, enzyme activities were additionally (CBH, LAP) or exclusively (POX) controlled by microbial community composition. We suggest that the microbial community in regular soil horizons is adapted to the physical environment and to SOM. Enzyme activities were thus related to SOM content. In cryoturbated organic matter however, microbial communities were adapted to the subsoil environment but were restricted in their potential to produce adequate enzymes to decompose the organic material present. We thus found a strong dependency of enzyme activities on microbial community composition. The mismatch of topsoil SOM and subsoil microbial community might be the reason for the slowed down decomposition in cryoturbated pockets.

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Chapter 2

A field method to store samples from temperate mountain grassland soils for analysis of phospholipid fatty acids

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Short communication

A field method to store samples from temperate mountain grassland soils for analysis of phospholipid fatty acids

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ABSTRACT

The storage of soil samples for PLFA analysis can lead to shifts in the microbial community composition. We show here that conserving samples in RNAlater, which is already widely used to store samples for DNA and RNA analysis, proved to be as sufficient as freezing at −20 °C and preferable over storage at 4 °C for temperate mountain grassland soil. The total amount of extracted PLFAs was not changed by any storage treatment. Storage at 4 °C led to an alteration of seven out of thirty individual biomarkers, while freezing and storage in RNAlater caused changes in the amount of fungal biomarkers but had no effect on any other microbial group. We therefore suggest that RNAlater could be used to preserve soil samples for PLFA analysis when immediate extraction or freezing of samples is not possible, for example during sampling campaigns in remote areas or during transport and shipping.

In the last decades the use of phospholipid fatty acids (PLFAs) as microbial biomarkers has proven to be a reliable tool to analyse microbial community structure in soils (Ruess and Chamberlain, 2010; Frostegård et al., 2011). Moreover, PLFA analysis together with ^{13}C labelling allows to trace carbon fluxes, e.g. from plants to soil and between microbial groups (Pelz et al., 2005; Paterson et al., 2007). Although a relatively robust method in general, storage before the extraction of the PLFAs tends to alter the resulting microbial community profiles. Several studies have compared the effects of drying and rewetting, freezing or sample storage at different temperatures above 0 °C (Petersen and Klug, 1994; Schutter and Dick, 2000; Trabue et al., 2006; Lee et al., 2007). Most of these studies conclude that it is best to use fresh soil, but if storage is unavoidable the most widely used method is to freeze soils (Högberg et al., 2006; Liu et al., 2008; Schindlbacher et al., 2011). Soils should be frozen continuously and PLFAs should be extracted in a frozen state to avoid nutrient release by freeze-thaw events and associated shifts in microbial community composition (Mannisto et al., 2009). However, when working in remote areas such as alpine or arctic regions it is often neither possible to process samples immediately nor can the samples be kept frozen continuously. We therefore tested the use of a commercial product, RNAlater (Sigma–Aldrich, Austria), to store samples for subsequent PLFA analysis. RNAlater is designed to preserve biological samples for later DNA or RNA analysis. The solution is a mixture of sodium ethylene diamine tetraacetic acid (EDTA), ammonium sulphate and a citrate buffer at pH 5.2 and is designed to readily infiltrate into biological samples and deactivate RNA degrading enzymes (Lader, 2001). The goal of the present study therefore was to test whether storage of soil samples in RNAlater also preserves PLFAs for later analysis. Towards this end we compared PLFA patterns of soil samples stored in RNAlater with those of fresh samples extracted immediately after sampling and samples which were either stored at 4 °C or frozen at −20 °C for 40 days prior to extraction.

Soil samples were taken in October 2010 from a typical temperate mountain grassland near Neustift, Stubai Valley (1900 m a.s.l). The soil (pH 6.8) has been classified as Dystric Cambisol (Bahn et al., 2006, 2008). Samples were taken from the A horizon, sieved (2 mm) and living fine roots were carefully removed to reduce the contribution of plant-derived PLFA markers (Kaiser et al., 2010a). Four independent treatments in five replicates with 1 g soil each were established as follows: Soil samples were transported from the field to the laboratory within 48 h and all treatments were initiated at the same time: Fresh soil was directly extracted (control, C). Samples were either stored at −20 °C (freezing treatment, F) or at 4 °C (cool treatment, S) or treated with RNAlater (R) for 40 days. In the latter case, 1 g of soil was mixed with 2 mL of

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RNAlater in 3-mL polypropylene vials and kept at 4 °C. These samples were then transferred to 30-mL glass vials and the original vials were washed twice with 1 ml RNAlater. After centrifugation (1000 *g* for 10 min) the supernatant was pipetted off and discarded. The remaining pellet was used for extraction. In addition, blanks (RNAlater only) were also routinely processed, but did not show any interfering substances. Frozen samples were extracted in a frozen state to avoid thawing effects.

Regardless of treatment all samples were extracted according to Kaiser et al. (2010b). Total lipids were extracted with a mixture of chloroform, methanol and 0.15 M citric acid buffer, at pH 4 (1:2:0.8, v/v/v). Neutral lipids and phospholipids were separated on silica columns (Supelco, LC-Si SPE, Austria) using chloroform, acetone and methanol as eluents. After addition of methyl-nonadecanoate (19:0) as an internal standard and conversion to fatty acid methyl esters (FAMEs) by alkaline methanolysis, samples were dried and re-dissolved in isooctane and analysed with gas chromatography with flame ionisation detection (Trace GC Ultra, Thermo) on a DB23 column (Agilent 60 m × 0.25 mm × 0.25 μm). Bacterial and fungal FAME mixtures (bacterial acid methyl ester mix and 37 Comp. FAME Mix, Supelco) were used as qualitative standards. The internal standard 19:0 was used to calculate the concentration of FAMEs. The assignment of individual PLFAs to microbial groups is shown in Table 1.

The different storage treatments had no statistically significant effect on the total yield of PLFAs and therefore on the estimates of microbial biomass (Fig. 1). Several other studies, however, found significantly higher total fatty acid methyl esters (FAMEs) content in samples stored at 4 °C or −20 °C compared to controls (Schutter and Dick, 2000; Lee et al., 2007). Freezing as well as the RNAlater treatment led to significantly lower fungal biomarker amounts than the controls, mainly because of the biomarker 18:2ω6,9 (Table 1). The RNAlater treatment also reduced the amount of the 18:3ω3,6,9 fungal marker compared to control and freeze treatment but the contribution of this biomarker was relatively small compared to the other two fungal biomarkers (18:2ω6,9 and 18:1ω9). Lee et al. (2007) also reported changes in the relative amount of fungal biomarkers in one out of three tested soils caused by freezing, but other than in our study the amount of fungi was increased despite a reduction of total FAMEs. Except the fungal PLFAs, no other biomarker that we analysed was significantly different between control and RNAlater treatment. In contrast, storage at 4 °C increased the amount of seven out of the thirty PLFAs significantly, indicating a shift in the microbial community composition (Table 1).

In conclusion, we were able to demonstrate that storage of soils in RNAlater for later PLFA analysis provides an alternative to freezing or storage at low temperatures under the conditions tested. Despite the fact that storage in RNAlater and at −20 °C led to an underestimation of the contribution of fungal biomarkers to the microbial community compared to freshly extracted control soils, they are preferable to storage at low temperatures, which led to

Table 1
Assignment of individual phospholipid fatty acids to microbial groups and mean concentrations and standard errors of individual biomarkers. Actinobacteria (actino), Gram-positive bacteria (gram +), Gram-negative bacteria (gram −), general bacterial (bacteria) and fungi are calculated as the sum of the respective individual biomarkers. The amount of bacteria was calculated as a sum of gram +, gram −, and general bacterial markers. The two sample comparison tests (Student's *t*-tests, Welch's *t*-test or Mann–Whitney-*U* tests) were performed with the statistical software R version 2.12.1 (R Development Core Team, 2010). The statistical significances are presented as following: ***, *p*-value < 0.001; **, *p*-values < 0.01; *, *p*-values < 0.05; n.s., *p*-values > 0.05.

PLFA	Marker	Mean concentration of PLFA markers (nmol g^{-1} dry-weight)				Two sample comparison tests			
		Control (C)	−20 °C (F)	RNAlater (R)	4 °C (S)	C vs F	C vs R	C vs S	F vs R
i14:0	general	6.33 ± 0.94	10.16 ± 1.61	8.87 ± 0.88	10.12 ± 0.87	n.s.	n.s.	*	n.s.
14:0	general	5.11 ± 0.71	7.21 ± 0.85	6.45 ± 0.61	7.40 ± 0.42	n.s.	n.s.	*	n.s.
i15:0	gram +	36.41 ± 3.02	39.77 ± 3.35	40.28 ± 3.15	41.76 ± 1.60	n.s.	n.s.	n.s.	n.s.
a15:0	gram +	32.63 ± 2.69	35.43 ± 2.93	36.17 ± 2.64	40.18 ± 1.71	n.s.	n.s.	*	n.s.
15:0	bacteria	3.97 ± 0.34	4.19 ± 0.28	3.91 ± 0.32	4.30 ± 0.14	n.s.	n.s.	n.s.	n.s.
i16:0	gram +	18.30 ± 0.84	18.85 ± 0.84	18.56 ± 1.52	18.36 ± 0.38	n.s.	n.s.	n.s.	n.s.
16:0	general	85.23 ± 3.26	85.75 ± 2.16	81.95 ± 5.01	83.01 ± 2.17	n.s.	n.s.	n.s.	n.s.
16:1ω11	general	7.95 ± 0.45	8.57 ± 0.48	8.93 ± 0.59	10.49 ± 0.52	n.s.	n.s.	**	n.s.
16:1ω9	gram −	7.58 ± 0.63	7.64 ± 0.43	7.63 ± 0.24	6.28 ± 0.38	n.s.	n.s.	n.s.	n.s.
10Me16:0	actino	32.72 ± 1.00	35.74 ± 1.42	33.07 ± 1.58	36.85 ± 2.11	n.s.	n.s.	n.s.	n.s.
16:1ω7	gram −	42.73 ± 3.22	40.36 ± 2.74	41.04 ± 3.88	50.62 ± 1.33	n.s.	n.s.	n.s.	n.s.
16:1ω6	general	5.04 ± 0.55	5.32 ± 0.49	4.55 ± 0.58	6.45 ± 0.19	n.s.	n.s.	*	n.s.
16:1ω5	gram −	27.48 ± 1.29	28.22 ± 1.15	27.59 ± 1.60	31.50 ± 1.15	n.s.	n.s.	*	n.s.
i17:0	gram +	10.35 ± 0.44	10.13 ± 0.41	9.95 ± 0.85	9.65 ± 0.25	n.s.	n.s.	n.s.	n.s.
a17:0	gram +	9.54 ± 0.39	9.69 ± 0.33	9.37 ± 0.76	9.57 ± 0.31	n.s.	n.s.	n.s.	n.s.
cy18:0	gram −	3.19 ± 0.15	3.36 ± 0.11	3.37 ± 0.21	3.78 ± 0.14	n.s.	n.s.	*	n.s.
17:0	bacteria	2.57 ± 0.12	2.63 ± 0.10	2.43 ± 0.19	2.40 ± 0.07	n.s.	n.s.	n.s.	n.s.
cy17:0	gram −	21.03 ± 0.58	20.87 ± 0.53	20.46 ± 1.17	21.55 ± 0.44	n.s.	n.s.	n.s.	n.s.
17:1ω6	bacteria	2.22 ± 0.10	2.36 ± 0.08	2.30 ± 0.17	2.48 ± 0.11	n.s.	n.s.	n.s.	n.s.
18:0	general	15.86 ± 0.73	15.10 ± 0.89	13.31 ± 0.68	14.48 ± 0.80	n.s.	n.s.	n.s.	n.s.
18:1ω9	fungi	29.23 ± 1.42	28.66 ± 2.44	24.83 ± 2.89	26.98 ± 2.07	n.s.	n.s.	n.s.	n.s.
18:1ω7	gram -	77.73 ± 3.01	87.99 ± 3.82	81.66 ± 4.96	88.19 ± 8.57	n.s.	n.s.	n.s.	n.s.
18:1ω5	bacteria	6.97 ± 0.18	7.43 ± 0.27	6.82 ± 0.33	7.48 ± 0.59	n.s.	n.s.	n.s.	n.s.
18:2ω6,9	fungi	28.95 ± 1.04	13.51 ± 0.58	12.37 ± 0.40	27.57 ± 2.49	***	***	n.s.	n.s.
cy19:0	gram −	3.95 ± 0.16	4.28 ± 0.16	4.11 ± 0.21	3.81 ± 0.19	n.s.	n.s.	n.s.	n.s.
19:1ω8	general	22.24 ± 1.66	27.77 ± 2.12	23.47 ± 1.72	24.57 ± 3.58	n.s.	n.s.	n.s.	n.s.
18:3ω3,6,9	fungi	2.68 ± 0.22	2.32 ± 0.21	1.65 ± 0.10	2.36 ± 0.36	n.s.	**	n.s.	*
20:0	general	3.27 ± 0.11	2.91 ± 0.02	3.25 ± 0.12	3.32 ± 0.16	*	n.s.	n.s.	n.s.
20:1ω9	general	1.72 ± 0.04	1.72 ± 0.04	1.63 ± 0.06	1.88 ± 0.10	n.s.	n.s.	n.s.	n.s.
Fungi		60.86 ± 1.72	44.49 ± 2.20	38.85 ± 3.27	56.90 ± 3.44	***	***	n.s.	n.s.
Bacteria		306.64 ± 13.65	323.2 ± 12.93	315.64 ± 20.76	341.90 ± 14.86	n.s.	n.s.	n.s.	n.s.
Gram + bacteria		107.22 ± 7.28	113.86 ± 7.48	114.33 ± 8.77	119.52 ± 3.97	n.s.	n.s.	n.s.	n.s.
Gram − bacteria		183.70 ± 6.45	192.72 ± 6.34	185.84 ± 11.32	205.73 ± 11.32	n.s.	n.s.	n.s.	n.s.
Actinobacteria		32.72 ± 1.00	35.74 ± 1.42	33.07 ± 1.58	36.85 ± 2.11	n.s.	n.s.	n.s.	n.s.

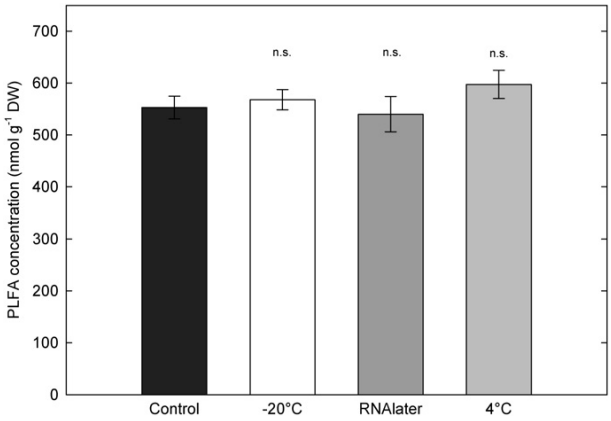

Fig. 1. Effect of sample storage on the total phospholipid fatty acid content. The total amount of PLFAs was calculated as the sum of all biomarkers presented in Table 1. None of the storage treatments were significantly different from controls ($n = 5$; $p < 0.05$, Student's *t*-tests). Control, extracted immediately; −20 °C, stored frozen for 40 days; RNAlater, stored in RNAlater for 40 days; 4 °C, stored at 4 °C for 40 days.

strong alterations of the PLFA pattern. Storage in RNAlater reduces the risk of freeze–thaw effects and eliminates the need of keeping the samples at a constant temperature and may thus be especially applicable as a field method to conserve soils from remote areas during transport and shipping to the laboratory. However, caution is necessary in the interpretation of fungal biomarkers after storage in RNAlater, which should only be directly compared to results from soils stored frozen. As we have tested the method for temperate mountain grassland soil only, we suggest that the suitability of storage in RNAlater shall be tested for other soil types before use.

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Chapter 3

Enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia

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TITLE

Enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia

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ABSTRACT

Soil horizons below 30 cm depth contain about 60% of the organic carbon stored in soils. Although we gain more and more insights into physical and chemical stabilisation of soil organic matter (SOM) and into microbial community composition in these horizons, we still lack information on microbial functions of subsoil microbial communities and consequently on the mediated microbial processes.

We here report on the activities of six extracellular enzymes (cellobiohydrolase, leucine-amino-peptidase, N-acetylglucosaminidase, chitotriosidase, phosphatase and phenoloxidase), and the resulting enzyme patterns (i.e. distance-matrixes calculated from these enzyme activities). We sampled soils from organic topsoil horizons, mineral topsoil horizons, and mineral subsoil horizons from seven ecosystems along a 1,500 km latitudinal transect in Western Siberia. 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.

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. In mineral subsoils, the microbial community might act as a

45 modifier for the usual dependency of decomposition rates on SOM content or C/N ratios. Our
46 results thus ask for caution when explicitly considering subsoils in ecosystem models

47

48 KEYWORDS

49 Extracellular enzymes; enzyme patterns; subsoil; PLFA; tundra; boreal forests; steppe;
50 permafrost; latitudinal transect

51

52 INTRODUCTION

53 Extracellular enzymes break down soil organic matter (SOM) at every depth of the soil
54 profile. Nonetheless most studies on enzyme activities focused on topsoil horizons in the
55 upper 10 cm of the soil profile although up to 60 % of the carbon stored in soils are located
56 below 30 cm, in subsoil horizons (Jobbágy and Jackson, 2000). Subsoil horizons differ from
57 well studied topsoil horizons in a number of physical and chemical conditions that might
58 influence enzyme activities and decomposition in general: Temperature decreases from
59 topsoils to subsoils whereas soil moisture increases with depth, either improving conditions
60 for decomposition in arid systems (Rovira and Vallejo, 2002), or impairing them in systems
61 where water logging occurs and O_2 availability is low (Davidson et al., 2012; Kleber, 2010).
62 Soil pH, one of the factors often associated with enzyme activities (Sinsabaugh et al., 2008),
63 also usually increases with depth (Eilers et al., 2012). In addition to these direct influences on
64 enzyme activities, the availability of substrate for enzymatic breakdown decreases with depth.
65 First, SOM is less abundant in subsoils, which leads to a high probability of a spatial
66 disconnection of enzyme and substrate (Holden and Fierer, 2005). Second, a high proportion
67 of SOM in subsoils is bound to minerals, stabilized by metal ions, or occluded in aggregates
68 and therefore access for microorganism is limited (von Lützow et al., 2006). In addition to
69 physical hurdles for decomposition, SOM in subsoils is chemically different from topsoil
70 SOM. While the main proportion of SOM in topsoils is plant derived material, SOM in
71 subsoils is microbially transformed (Wallander et al., 2003). During this microbial
72 transformation of SOM, carbon is lost, mainly as CO_2, whereas most of the nitrogen (N) is
73 recycled and remains in the system resulting in lower C/N ratios of subsoil SOM (Rumpel and
74 Kögel-Knabner, 2011).

75 To fulfill the microbial demand for energy and nutrients, microorganisms need to adapt to the
76 chemical composition of SOM and to the C/N ratio of the available substrate by adjusting

77 their enzyme production (Sinsabaugh et al., 2008). Changes in enzyme production might
78 either be physiological (Stone et al., 2014) or they might result from a shift in microbial
79 community composition (Kaiser et al., 2014). Although the influence of microbial community
80 composition on major microbial processes, such as C mineralization and N mineralization,
81 has been recently challenged (Colman and Schimel, 2013), its influence on enzyme activities
82 has been demonstrated repeatedly (e.g. McGuire and Treseder, 2010; Schnecker et al., 2014;
83 Strickland et al., 2009). Microbial community composition, as another potential control on
84 enzyme activities, has already been shown to change more strongly with soil depth, within
85 ecosystems, than between topsoils of different ecosystems (Eilers et al., 2012; Gittel et al.,
86 2014).

87 Relations of enzyme activities to key factors such as pH, moisture, SOM content (Keeler et
88 al., 2009), chemical composition of SOM (Grandy et al., 2009; Sinsabaugh and Follstad Shah,
89 2010), and microbial community composition (Talbot et al., 2013; Waldrop and Firestone,
90 2006) are well established in topsoil horizons. Whether enzyme activities in the subsoils are
91 related to these key factors is still largely unknown since only few studies have addressed
92 changes of enzyme activities and their potential controls with soil depth so far (e.g.: Brockett
93 et al., 2012; Kramer et al., 2013; Turner et al., 2013; Schnecker et al., 2014; Stone et al.,
94 2014).

95 In this study we investigated enzyme patterns in different soil horizons, including mineral
96 subsoils, from a wide range of ecosystems to identify potential drivers for these enzyme
97 patterns. We measured potential activities of six extracellular enzymes (cellobiohydrolase,
98 leucine-amino-peptidase, N-acetylglucosaminidase, chitotriosidase, phosphatase and
99 phenoloxidase) in organic topsoil horizons (0-10 cm; measured from the soil surface), mineral
100 topsoil horizons (6-28 cm) and mineral subsoil horizons (23-75 cm) in seven ecosystems
101 along a 1,500 km-long north-south transect in Western Siberia. The transect ranged from

102 tundra in the North (67°16'N 78°50'E) to a continental steppe in the South (54°41'N
103 71°38'E). In addition to enzyme activities, we analyzed microbial community composition
104 (using phospholipid fatty acid analysis) as well as abiotic soil parameters and related these
105 factors to the enzyme patterns.

106 We hypothesized that 1) enzyme patterns in topsoil and subsoil horizons are both related to
107 the same key parameters, such as SOM content, pH and microbial community composition.
108 Microbial community composition has been shown to differ more strongly between topsoils
109 and subsoils than between topsoils of different ecosystems (Eilers et al., 2012; Meyer et al.,
110 2006). Since enzyme activities and enzyme patterns are often related to microbial community
111 composition, we expected that 2) enzyme activities and enzyme patterns change with depth
112 and differ more strongly between horizons than between ecosystems. The ecosystems along
113 the transect showed large differences in vegetation and presumably in the chemical
114 composition of litter entering the soil. We thus additionally expected that 3) enzyme patterns
115 would be more variable and show greater differences between ecosystems in the topsoil
116 horizons, where the main constituents of SOM are plant-derived, than in mineral subsoil
117 horizons.

118

MATERIAL AND METHODS

Sampling Sites

Sites and soil sampling are described in detail in Wild et al (2014 in review). In short, soil samples were taken from seven ecosystems along a 1,500 km latitudinal transect in Western Siberia. The ecosystems included tundra, northern taiga, middle taiga, and southern taiga, forest steppe (one forest site and one meadow site), and steppe. Basic soil and climate parameters are provided in Table 1 and Table S1. Climate data are derived from Stolbovoi and McCallum (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 analysus, 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.

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). Mineral topsoils and subsoils at both forest steppe sites, as well as all horizons of the steppe site, contained traces

143 of carbonate. Carbonate was removed from these samples by acidification with HCl before
144 EA-IRMS analysis. Water holding capacity (WHC) was determined as the amount of water
145 that remained in saturated soil, from which water could be lost by drainage but not by
146 evaporation after two days (Reynolds and Topp, 2007).

147 Microbial C and N were estimated using chloroform-fumigation-extraction (Kaiser et al.,
148 2010 modified after Brookes et al., 1985): Soil samples, fumigated with chloroform, as well
149 as unfumigated samples were extracted with 0.5 M K_2SO_4. Dissolved organic C and total
150 dissolved N were determined in both sets of extracts with a DOC/TN analyzer (Shimadzu
151 TOC-VCPH/CPN/TNM-1). Microbial C and N were calculated as the difference between
152 fumigated and non-fumigated samples. C/N ratios of SOM and microbial biomass were
153 calculated gravimetrically.

154 *Potential extracellular enzyme activities*

155 We measured potential enzyme activities fluorimetrically and photometrically using a
156 microplate assay (Kaiser et al., 2010). For the fluorimetric assay, we used MUF (4-
157 methylumbelliferyl) labeled substrates: β-D-cellobioside for cellobiohydrolase (CBH),
158 triacetylchitotrioside for chititriosidase (CHT), N-acetyl-β-D-glucosaminide for N-acetyl-
159 glucosaminidase (NAG) and phosphate for phosphatase (PHO). L-leucine-7-amido-4-methyl
160 coumarin was used as substrate for leucine-amino-peptidase (LAP). Phenoloxidase (POX)
161 activities were measured using L-3,4-dihydroxiphenylalanine (DOPA) as substrate in a
162 photometric assay. Assays for CBH, CHT, NAG, PHO and LAP were incubated for 140 min
163 at room temperature in a sodium acetate buffer (pH 5.5) and activity was measured
164 fluorimetrically (excitation 365 nm and emission 450 nm). POX activity was measured
165 photometrically (absorbance 450 nm) immediately and after incubation for 20 hours at room
166 temperature.

167

168 *Phospholipid fatty acid (PLFA) analysis*

169 Extraction and measurement of PLFAs followed the procedure described by Frostegård et al.
170 (1991) with the modifications by Kaiser et al. (2010). PLFAs were extracted from 1 g soil
171 with chloroform/methanol/citric acid buffer and purified on silica columns (LC-Si SPE,
172 Supleco, Bellefonte, PA, USA) using chloroform, acetone, and methanol. After addition of
173 the internal standard (methyl-nonadecanoate), PLFAs were converted to fatty acid methyl
174 esters (FAMEs) by alkaline methanolysis. Samples were analyzed on a Thermo Trace GC
175 with FID detection (Thermo Fisher Scientific, Waltham, MA, USA), using a DB-23 column
176 (Agilent, Vienna, Austria). FAMEs were identified using qualitative standard mixes (37
177 Components FAME Mix and Bacterial Acid Methyl Esters CP Mix, Supelco) and quantified
178 using the internal standard. We categorized the fatty acids 18:1ω9, 18:2ω6,9, and 18:3ω3,6,9
179 as markers for fungi; i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0 (9/10), cy19:0 (9/10), 16:1ω7,
180 16:1ω9, 18:1ω7, 15:0, and 17:0 as bacterial markers. We used the above mentioned markers
181 together with 14:0, i14:0, 16:0, 18:0, 20:0, 22:0, 16:1ω11, and 19:1ω8 for the calculation of
182 total PLFA content (Schnecker et al., 2012).

183

184 *Statistics*

185 We calculated enzyme patterns to identify differences between horizons and between sites. To
186 account for the different methods of measuring enzyme activities and the inherent differences
187 in enzyme activities of different horizons, the individual enzyme activities per gram dry soil
188 were log transformed and standardized by calculating the proportion of each enzyme to the
189 sum of all enzymes. With these values, we calculated Euclidean distance matrixes. We used
190 these matrixes to create Nonmetric Multidimensional Scaling (NMDS) plots. To identify

differences between sites and horizons we used Permutational Multivariate Analysis of Variance Using Distance Matrices (ADONIS). This analysis is implemented in the R-package vegan (Oksanen et al., 2013). Additionally we used Mantel tests based on Spearman correlations of the calculated enzyme distance matrices with soil parameters and with microbial community composition (represented as a distance matrix based on relative abundances of individual PLFA biomarkers). We performed these analyses for the whole data set, as well as for data sets of the three horizon classes individually.

To evaluate whether differences between horizons or sites were stronger, we used two-way-ANOVA. To find differences within sites or within horizons, we used one-way ANOVA and Tukey HSD as post-hoc test. We did this for soil parameters, enzyme activities, and fungi:bacteria ratios, as well as for distances between different horizons and within horizons (variability). Before analysis, data were log-transformed or rank-normalized to meet the assumptions for ANOVA. Differences and correlations were assumed to be significant at $p<0.05$. Statistics were performed in R 3.0.2 (R Development Core Team, 2013) using the vegan package (Oksanen et al., 2013).

206 RESULTS

207 *Enzyme activities*

208 All measured hydrolytic enzyme activities - calculated per gram dry soil - differed more
209 strongly between horizons than between sites (Table 2). Hydrolytic enzyme activities were
210 highest in organic topsoil horizons followed by mineral topsoils and mineral subsoil horizons,
211 with the exception of LAP in steppe (Figure 1). The oxidative enzyme POX did not follow
212 this pattern and showed greater differences between sites than between horizons (Table 2,
213 Figure 1). When hydrolytic enzyme activities were calculated on a microbial C basis,
214 differences between horizons were often smaller than differences between sites (Table 2,
215 Figure 2). POX activity, on a microbial C basis, was highest in the mineral subsoils followed
216 by mineral topsoils (except northern taiga). The lowest rates were found in organic topsoil
217 horizons at all sites. Differences between sites were ambiguous and POX activities, on a
218 microbial C basis, in mineral subsoil horizons was the only enzyme activity that showed a
219 clear north-south trend and decreased from the tundra site in the north to the steppe site in the
220 South (Figure 2).

221

222 *Enzyme patterns*

223 To evaluate differences in the way microbes decompose SOM in different horizons, we used
224 log-transformed and standardized enzyme activities to calculate distance matrixes. The
225 resulting enzyme patterns differed more strongly between horizons than between sites
226 (ADONIS: horizons $R^2 = 0.66$, sites $R^2 = 0.14$; Figure 3). The mean distances and thus the
227 variability of enzyme patterns were greatest in the mineral subsoil horizons, followed by
228 mineral topsoil horizons, and organic topsoil horizons (Insert in Figure 3). Enzyme patterns of
229 different horizons in the South clustered closer together than enzyme patterns of different

230 horizons in the North in the NMDS plot (Figure 3). This trend was more pronounced for
231 differences (mean distances) between organic topsoils and mineral subsoils, which were
232 significantly higher in the northern sites (tundra and taiga) than in the southern sites (forest-
233 steppe and steppe; Figure 5A). This trend could also, but weaker, be seen for differences
234 between organic topsoils and mineral topsoils (Figure 5B). The differences between mineral
235 topsoils and mineral subsoils did not show a decrease from North to South (Figure 5C).

236 Correlations (Mantel tests) of enzyme patterns with biotic and abiotic factors varied between
237 horizons (Table 3). The most striking difference between organic topsoils and mineral
238 subsoils is the absence of a correlation between enzyme patterns and SOM properties (C, N,
239 C/N ratio) in the mineral subsoils.

240

241 *Microbial community composition*

242 Microbial community composition differed significantly between horizons and between sites
243 with both factors exerting a similar influence (site $R^2 = 0.26$ and horizon $R^2 = 0.25$).
244 Differences between horizons were mainly caused by a decrease in fungal markers (18:3ω3
245 and 18:2ω6) with soil depth (Figure 4). The differences in microbial community composition
246 between horizons were further reflected in the fungi:bacteria ratios which decreased from
247 organic topsoils, to mineral topsoils and mineral subsoils (Figure 6). Although differences
248 between horizons were not as pronounced for microbial community composition as for
249 enzyme patterns, the variability within the horizons was also highest in mineral subsoils
250 followed by mineral topsoils and organic topsoils (Insert in Figure 4). Microbial community
251 composition in organic topsoils and mineral subsoils differed stronger in the North than in the
252 South, but the distinction between tundra and taiga on the one hand, and forest-steppe and
253 steppe on the other hand was not as clear as for enzyme patterns (Figure 5D). With the

254 exception of tundra, differences between organic topsoils and mineral topsoils also showed a
255 decrease from North to South (Figure 5E). Correlations of microbial community composition
256 with biotic and abiotic factors were similar to those observed for enzyme patterns (Table 3).
257 The correlations of microbial community composition and SOM parameters (C, N, CN ratio,
258 $\delta^{13}C$) were strongest in the organic topsoils, but decreased to mineral topsoils and further to
259 mineral subsoils (Table 3).

DISCUSSION

Enzyme activities and enzyme patterns

Subsoil horizons are usually characterized by lower temperatures, higher water content, and lower O_2 availability compared to topsoil horizons. They also exhibit a reduced influence of plants and a higher proportion of the present SOM is associated with minerals (Rumpel and Kögel-Knabner, 2011). These factors are most likely responsible for the clear separation of enzyme patterns according to horizons ($R^2 = 0.66$), which was stronger than the differences in enzyme patterns between sites ($R^2 = 0.14$; Figure 3), in our study. The main factor responsible for the distinct enzyme patterns in topsoils and subsoils was the independent change of oxidative and hydrolytic enzyme activities with depth. While hydrolytic enzyme activities (on a microbial C basis) did not show a homogeneous change with depth, POX activities (on a microbial C basis) increased with depth (Figure 2). An explanation for this might be that the activity of hydrolytic enzymes is often directly related to SOM content (Schnecker et al., 2014; Sinsabaugh et al., 2008). Since SOM content and the amount of regular polymers, that can be broken down hydrolytically, decrease with depth (Rumpel and Kögel-Knabner, 2011), hydrolytic enzyme activities can be expected to decrease accordingly. Oxidative enzymes, in contrast, are unspecific and are often not produced to directly acquire nutrients (Sinsabaugh, 2010). Instead, oxidative enzymes can degrade humic complexes and thereby free substrates for other enzymes (Hobbie and Horton, 2007; Talbot et al., 2008) or degrade toxic substances such as phenols (Sinsabaugh, 2010). The production of oxidative enzymes might thus be related to the amount of irregular polymers or the amount of toxins, which are independent from SOM content. In addition, and in contrast to hydrolytic enzymes, oxidative enzymes are preferentially stabilized on mineral surfaces and might thus prevail longer in mineral subsoils (Kramer et al., 2013). Overall, the contrasting behavior of hydrolytic and oxidative enzymes

284 presumably led to the more pronounced differences in enzyme pattern between horizons than
285 between sites.

286 Differences between sites and a latitudinal trend could be found for differences between
287 enzyme patterns (expressed as mean distances), in organic topsoil and mineral subsoil
288 horizons (Figure 3 and 5). We found that enzyme patterns in topsoils and subsoils were most
289 similar at the southernmost site, although organic topsoils and mineral subsoils in this steppe
290 ecosystem were up to one meter apart from each other (Table 1). In contrast, at the tundra site
291 enzyme patterns showed the greatest differences of all sites between organic topsoils and
292 mineral subsoils, which are less than 50 cm apart. This trend from North to South was not
293 caused by the variability of enzyme patterns in topsoils, but by the large variability in mineral
294 subsoils (Figure 3). This is in contrast to our hypothesis that greater differences between
295 ecosystems would occur in organic topsoil horizons due to the diverse litter inputs in different
296 ecosystems. Again, these differences between ecosystems in mineral horizons can be
297 connected to POX activity, on a microbial C basis, which was the only enzyme activity that
298 decreased from North to South. Also in this case POX activities might have been controlled
299 by physical parameters, which vary especially in subsoils of different ecosystems. Anoxia and
300 water saturation for instance are common features of subsoil horizons of high latitude
301 ecosystems, whereas they can be neglected as important factors in arid steppe subsoils. The
302 lack of oxygen, influences oxidative enzyme gravely and although it has been proposed that
303 anoxia does not directly influence hydrolytic enzyme enzyme activities (Hall et al., 2014),
304 individual enzymes or their substrates might be differently stabilized on mineral surfaces
305 (Turner et al., 2014). These stabilization mechanisms can be influenced by physical and
306 chemical factors such as O_2 availability and pH (von Lützow et al., 2006).

307

308

309 *Enzyme patterns and microbial community composition*

310 Physical factors in subsoil horizons might also have indirectly influenced enzyme patterns by
311 affecting microbial community composition (Schnecker et al., 2014). In all three soil horizon
312 types, we found significant correlations between enzyme patterns and microbial community
313 composition (Table 3). Microbial community composition itself was significantly different in
314 mineral subsoils and organic topsoils from all seven ecosystems along this 1,500 km long
315 Siberian transect. The differences between horizons even outranked differences between
316 ecosystems in topsoil horizons, with a mean distance between organic topsoils and mineral
317 subsoils of 17.1±0.4 (Figure 5) and mean distance within organic topsoil horizons of 10.9±0.2
318 (Insert in Figure 4). Similar trends were found for North-American forest systems where the
319 variability of the microbial community composition was greater within soil profiles than
320 between 54 topsoil horizons collected from a wide range of ecosystems (Eilers et al., 2012).
321 This clear picture of the stronger influence of depth than of geographical distance on
322 microbial community composition becomes however blurred when subsoil horizons were also
323 considered. When we included mineral topsoils and mineral subsoils in the statistical
324 analyses, we found significant differences in microbial community composition between
325 ecosystems ($R^2 = 0.26$), which were as strong as differences between horizons ($R^2 = 0.25$).

326 These findings might have two implications: First, the consistent correlations of enzyme
327 patterns and microbial community composition point to distinct functional capacities of
328 individual microbial communities in different soil horizons. This also indicates that enzyme
329 patterns are an estimate for a functional community composition. Second, the differences
330 between horizons were more pronounced in enzyme patterns than in the microbial community
331 composition, (Figure 5), which might indicate an additional physiological adaptation of the
332 microbial communities on top of community shifts from topsoils to subsoils.

333

334 *Enzyme patterns and soil organic matter*

335 So far, we have shown relations of enzyme patterns and microbial community composition
336 and argued the potential controls of physical parameters over both. Enzyme patterns may
337 however also reflect the availability of different substrates, as well as microbial energy and
338 nutrient demand, and are often related to C/N ratios of the microbial biomass or of the SOM
339 (Sinsabaugh et al., 2008). While, in this present study, enzyme patterns and microbial
340 community composition were related to C and N content in the organic topsoil horizons, these
341 relations were not found in mineral topsoils and mineral subsoils. In contrast to SOM
342 quantity, SOM chemistry might be more important in subsoil horizons. Here, the distinct
343 microbial communities that are presumably adapted to the different environments along the
344 transect could have led to a diverging chemical composition of SOM, similar to a proposed
345 diverging of litter chemistry with ongoing decomposition (Wickings et al., 2012). This
346 divergence has been found in a litter decomposition study and has not been shown for SOM.
347 Enzyme patterns might, nonetheless, reflect a diverged SOM chemistry, and therefore show
348 greater variability in subsoils, where a high proportion of SOM is microbially transformed,
349 than in topsoils, where a higher amount of plant components is present.

350 In summary our findings show that topsoil horizons and subsoil horizons harbor different
351 microbial communities, which support distinct ways to decompose the available SOM. The
352 differences in enzyme patterns between horizons outranked the differences between
353 ecosystems. In contradiction to our hypothesis, we found a higher variability of enzyme
354 patterns in subsoil horizons, which might have been caused by an interplay of physical
355 conditions, microbial community composition and chemical composition of SOM. Although
356 we were not able to identify and describe the mechanisms that shape the microbial community
357 and control enzyme patterns in subsoil horizons in detail, we found that enzyme patterns and
358 thus the strategy of the microbial community to decompose SOM, were not related to SOM

content and C/N ratios. In subsoil horizons, the microbial community, with its functional ability, might act as a modifier for the usual dependency of decomposition rates on SOM content or C/N ratios. Our result thus ask for caution when subsoils are considered in ecosystem models.

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FIGURES

Figure 1. Extracellular enzyme activities per gram dry soil. Left panel shows activities on a log-scale for each horizon (O are organic topsoils, black; A are mineral topsoils, light grey; M are mineral subsoils, dark grey) at each site (TU=Tundra; NT=northern taiga; MT=middle taiga; ST=southern taiga; FF=forest steppe forest; FM=forest steppe meadow; SP=steppe) individually. Right panel shows the mean of the individual horizons over all sites. Significant differences for horizon means are derived from ANOVA and Tukey HSD tests and are indicated by small letters ($p>0.05$). Results from two-way ANOVAs are given in Table 2.

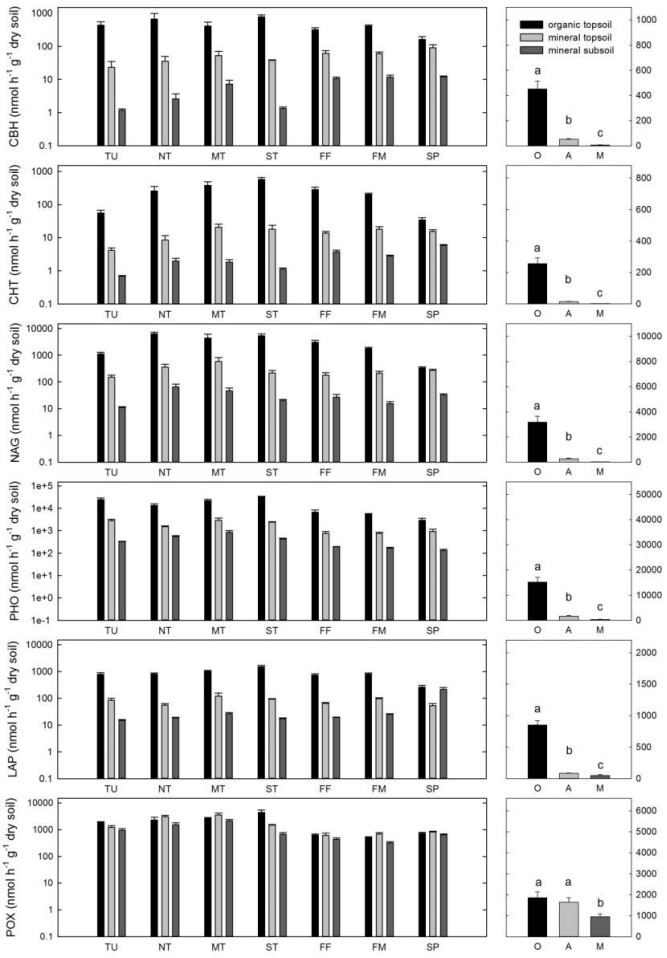
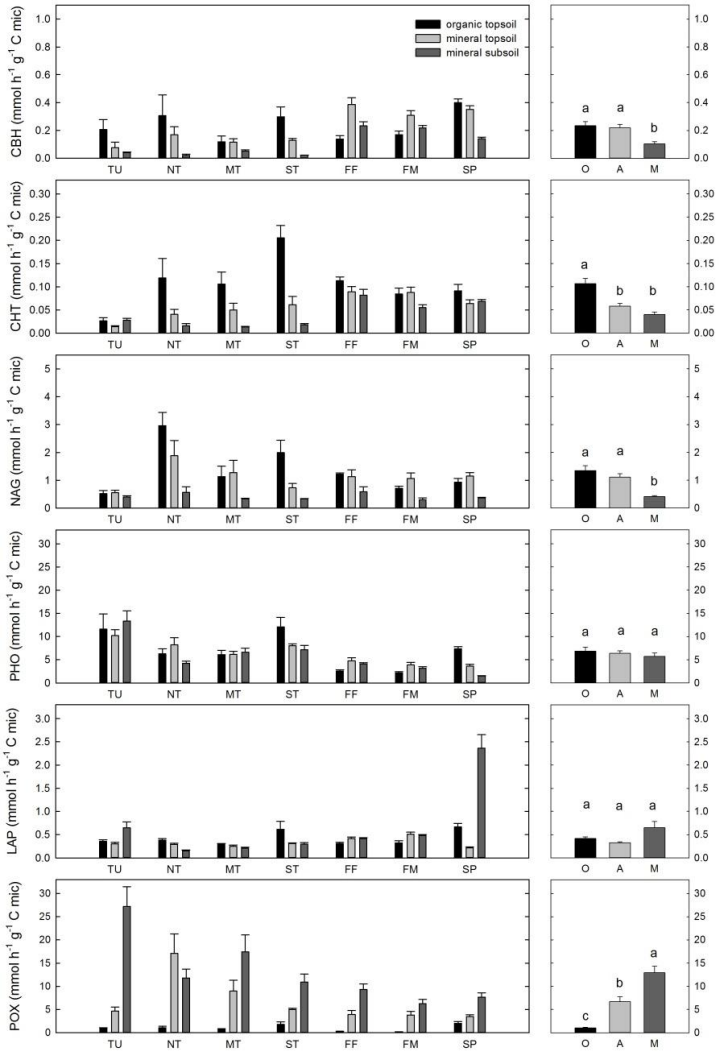
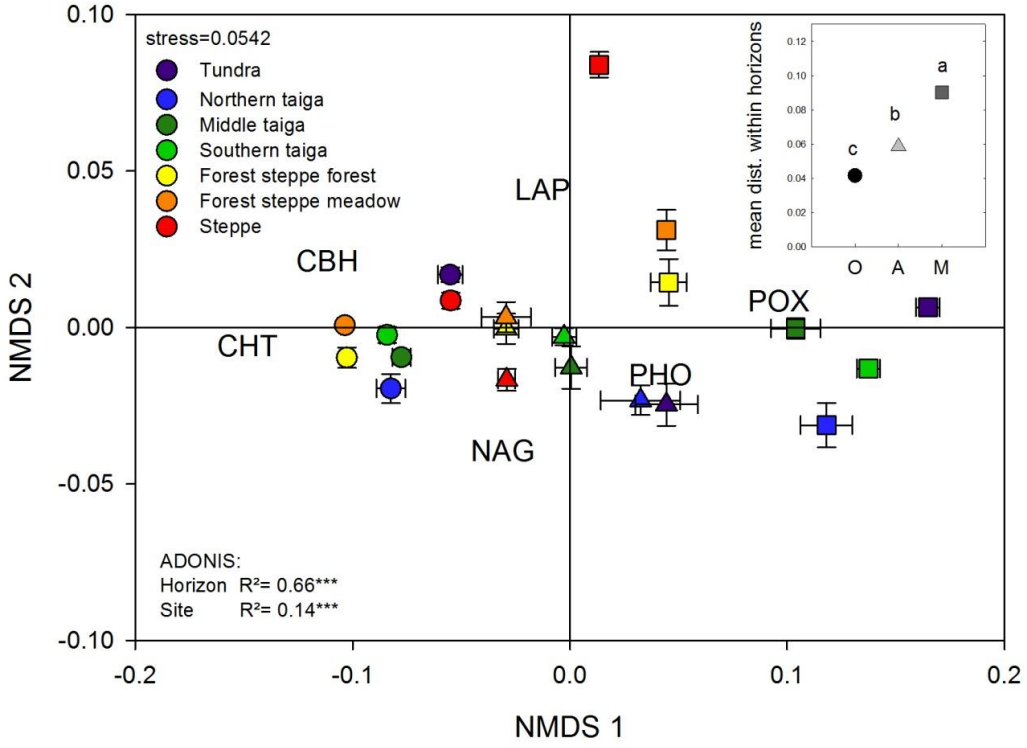


Figure 2. Extracellular enzyme activities per gram microbial C. Left panel shows activities for each horizon (O are organic topsoils, black; A are mineral topsoils, light grey; M are mineral subsoils, dark grey) at each site (TU=Tundra; NT=northern taiga; MT=middle taiga; ST=southern taiga; FF=forest steppe forest; FM=forest steppe meadow; SP=steppe) individually. Right panel shows the mean of the individual horizons over all sites. Significant differences for horizon means are derived from ANOVA and Tukey HSD tests and are indicated by small letters ($p>0.05$). Results from two-way ANOVAs are given in Table 2.



384 **Figure 3. Enzyme patterns.** NMDS plot of enzyme patterns calculated from a distance
385 matrix of standardized enzyme activities. Symbols are the mean values of the replicated
386 individual horizons of each site. Error bars are SE. Sites are indicated by color (Tundra is
387 purple; northern taiga is blue; middle taiga is dark green; southern taiga light green; forest
388 steppe forest is yellow; forest steppe meadow is orange; steppe is red). Horizons are indicated
389 by different symbols (circles are organic topsoils; triangles are mineral topsoils; squares are
390 mineral subsoisl). The insert in the upper right corner shows the distances within the horizons.
391 The results of the ADONIS analysis show that horizon has a stronger effect than site.
392 Asterisks indicate significance (*** mean p>0.001).



393

394

Figure 4. Microbial community composition. NMDS plot of microbial community composition calculated as distance matrix of the relative abundances of all individual PLFA markers. Symbols are the mean values of the replicated individual horizons of each site. Error bars are SE. Sites are indicated by color (Tundra is purple; northern taiga is blue; middle taiga is dark green; southern taiga light green; forest steppe forest is yellow; forest steppe meadow is orange; steppe is red). Horizons are indicated by different symbols (circles are organic topsoils; triangles are mineral topsoils; squares are mineral subsoils). The insert in the lower right corner shows the distances within the horizons. The results of the ADONIS analysis show that horizon and site have equal influence. Asterisks indicate significance (*** mean p>0.001).

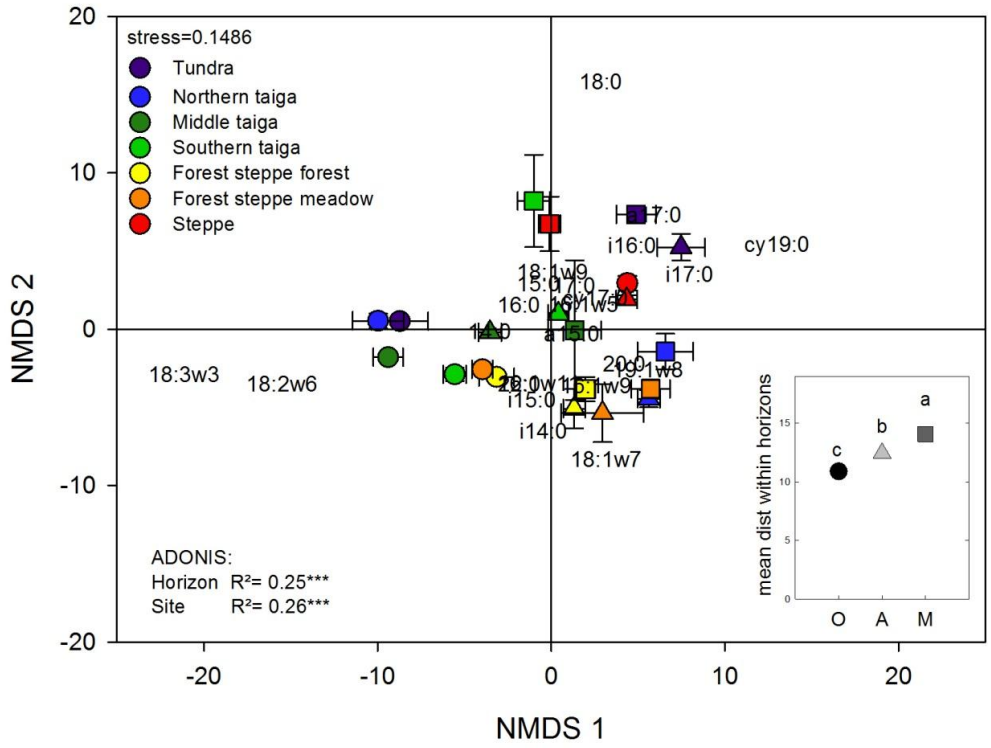


Figure 5. Differences between horizons in enzyme pattern and microbial community composition. Values are the mean distances between the respective shown horizons. Sites are indicated by color (Tundra is purple; northern taiga is blue; middle taiga is dark green; southern taiga light green; forest steppe forest is yellow; forest steppe meadow is orange; steppe is red). Black dots are the mean values over all sites.

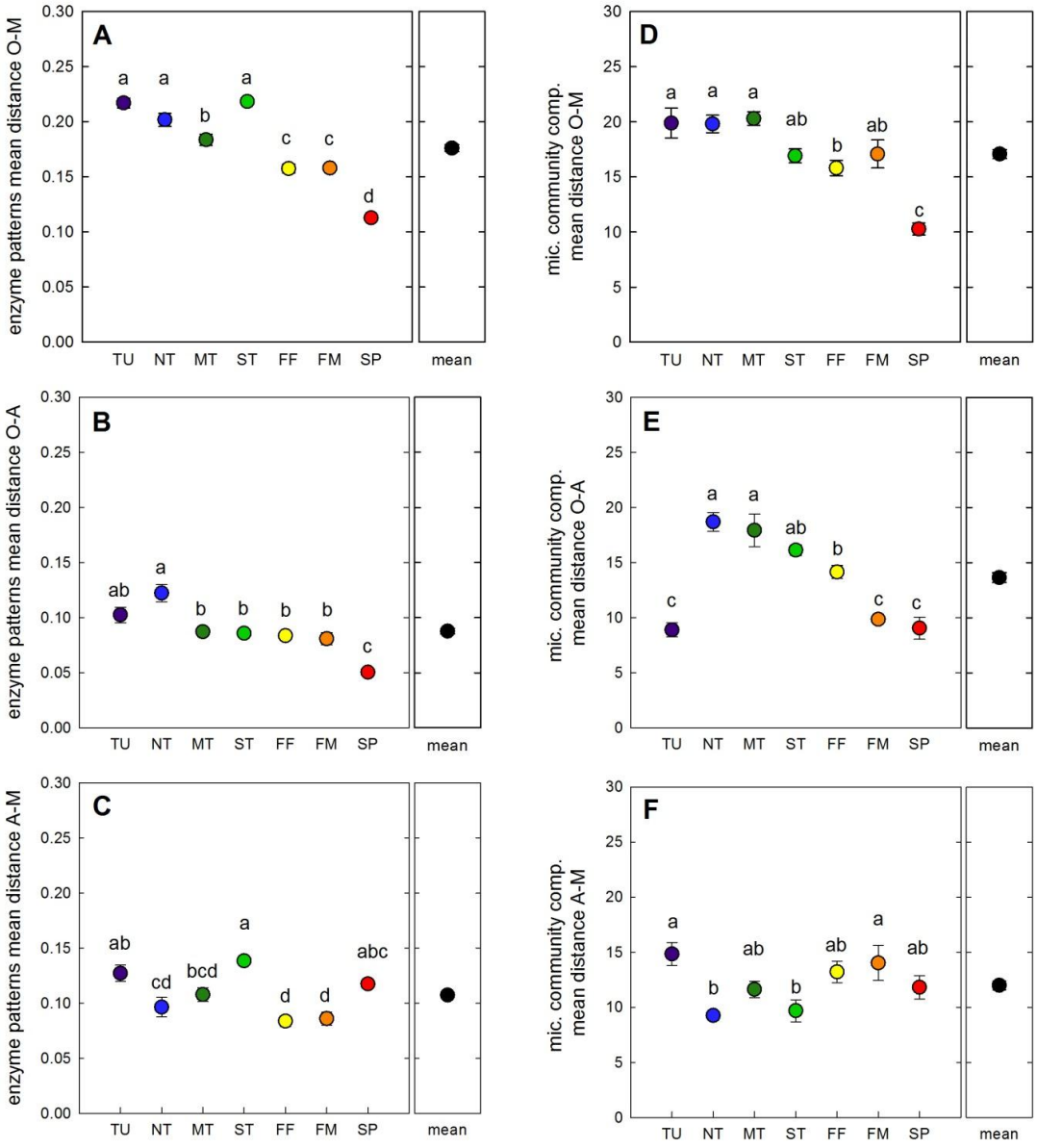
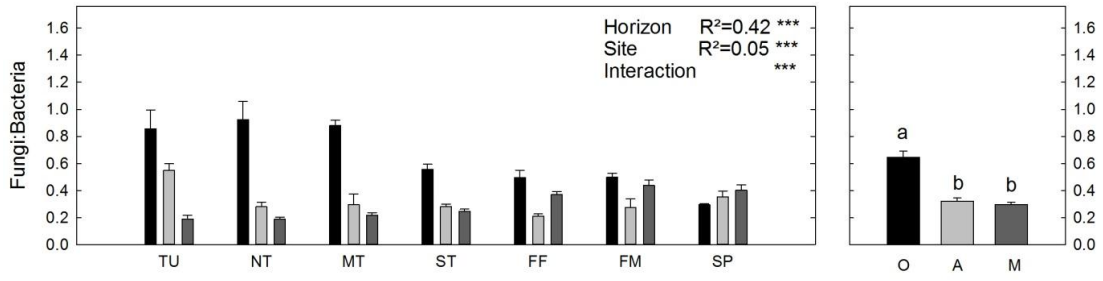


Figure 6. Fungi:bacteria ratios. Left panel shows each horizon (O are organic topsoils, black; A are mineral topsoils, light grey; M are mineral subsoils, dark grey) at each site (TU=Tundra; NT=northern taiga; MT=middle taiga; ST=southern taiga; FF=forest steppe forest; FM=forest steppe meadow; SP=steppe) individually. Right panel shows the mean of the individual horizons over all sites. Results for two-way ANOVA are shown in the left panel. Asterisks indicate significance (*** mean p>0.001). In the right panel significant differences for horizon means are derived from ANOVA and Tukey HSD tests and are indicated by small letters (p>0.05).



426 TABLES

427 Table1

						Organic topsoils		Mineral topsoils		Mineral subsoils	
	Coordinates	MAT	MAP	Aridity index	Soil Type	Horizon	Depth	Horizon	Depth	Horizon	Depth
		°C	mm				cm		cm		cm
Tundra	67°16'N 78°50'E	-7.6	391	1.30	Turbic Cryosol	O	0-6	A	2-13	Bg, BCg	6-57
Northern taiga	63°17'N 74°32'E	-4.6	430	1.06	Histic Podzol	Oi, Oe	0-22	AE, EA	8-30	Bg	14-47
Middle taiga	60°09'N 71°43'E	-2.2	438	0.89	Endogleyic Regosol	Oi	0- 6	A, AE, EA	6-14	E, EA	12-55
Southern taiga	58°18'N 68°35'E	-0.5	396	0.71	Albic Podzol	Oi	0-7	A, AE	4-18	E, EA	15-59
Forest steppe: Forest	56°14'N 70°43'E	0.7	340	0.53	Haplic Phaeozem	O, Oa	0-10	A	7-46	B	57-109
Forest steppe: Meadow	56°14'N 70°43'E	0.7	340	0.53	Luvic Phaeozem	Oa	0-7	A	4-35	Bt	26-84
Steppe	54°41'N 71°38'E	1.0	309	0.44	Calcic Kastanozem	OA	0-12	Ak	8-37	Bk	27-109

428 Basic soil and site caracterization of sites along the latitudinal transect. MAT is mean annual temperature; MAP is mean annual precipitation.

429 Aridity index has a threshold for drylands at 0.65 (Maestre et al., 2012).

430

Table 2

	Activities per g DM			Activities per g Cmic		
	Horizon	Site	Interaction	Horizon	Site	Interaction
cellobiohydrolase (CBH)	**0.80**	0.05	***	0.36	**0.52**	***
chititriosidase (CHT)	**0.82**	0.06	***	0.24	**0.32**	***
N-acetyl-glucosaminidase (NAG)	**0.84**	0.06	***	**0.43**	0.16	**
phosphatase (PHO)	**0.79**	0.16	***	0.03	**0.56**	***
leucine-amino-peptidase (LAP)	**0.83**	0.01	***	0.05	**0.28**	***
phenoloxidase (POX)	0.03	**0.17**	***	**0.73**	0.10	***

Two-way ANOVA R^2 for enzyme activities, based on dry soil and on microbial C basisi; only significant differences are shown. Bold letters indicate higher R^2. Asterisks indicate significance (**mean $p < 0.01$; *** mean $p < 0.001$).

435 Table 3

	Enzyme patterns			Microbial community composition		
	Organic topsoils	Mineral topsoils	Mineral subsoils	Organic topsoils	Mineral topsoils	Mineral subsoils
C content	0.06			0.29	0.03	
N content	0.22			0.30		
SOM C/N	0.05	0.04		0.27	0.04	
SOM $\delta^{13}C$	0.27			0.29	0.12	0.03
Microbial C				0.11	0.03	
Microbial N	0.03		0.02	0.18		
Microbial C/N		0.05		0.24	0.02	
pH	0.02	0.03	0.29	0.05	0.14	0.09
Water holding capacity	0.03		0.20	0.25	0.05	
Fungi:bacteria ratio	0.10	0.03	0.24	0.49	0.08	0.20
Mic. community comp.	0.16	0.04	0.17	-	-	-
Enzyme patterns	-	-	-	0.16	0.04	0.17

436 Results of Mantel tests of enzyme patterns (distance matrix with standardized enzyme activities) and microbial community composition (distance

437 matrix of relative amounts of PLFA) with abiotic and biotic variables. Only significant relations are shown ($p < 0.05$). Values are R^2.

	C content mg g-1 dry soil	N content mg g-1 dry soil	SOM C/N	SOM $\delta^{13}C$ ‰	Microbial C µg g-1 dry soil	Microbial N µg g-1 dry soil	Microbial C/N	pH	WHC g g-1 dry soil
Tundra									
Organic topsoils	308 ± 33.4	8.81 ± 0.59	34.9 ± 3.09	-26.6 ± 0.16	2289 ± 326	328 ± 36.1	6.89 ± 0.30	3.78 ± 0.08	4.70 ± 0.61
Mineral topsoils	30.4 ± 2.73	1.83 ± 0.11	16.4 ± 0.66	-25.7 ± 0.04	290 ± 48.7	30.5 ± 4.89	9.54 ± 0.28	3.70 ± 0.03	0.79 ± 0.08
Mineral subsoils	4.51 ± 0.39	0.39 ± 0.03	11.7 ± 0.27	-24.7 ± 0.07	31.6 ± 6.23	1.89 ± 0.24	19.2 ± 3.69	3.83 ± 0.04	0.25 ± 0.01
Northern taiga									
Organic topsoils	448 ± 6.25	12.5 ± 0.24	35.9 ± 0.64	-28.3 ± 0.13	2133 ± 46.7	332 ± 11.4	6.46 ± 0.21	2.76 ± 0.04	6.61 ± 0.30
Mineral topsoils	37.0 ± 2.79	1.36 ± 0.07	27.4 ± 1.78	-27.1 ± 0.24	201 ± 23.6	13.7 ± 1.50	14.8 ± 1.18	3.06 ± 0.05	0.71 ± 0.02
Mineral subsoils	8.17 ± 1.53	0.50 ± 0.05	15.7 ± 1.37	-25.9 ± 0.16	133 ± 13.7	3.43 ± 0.27	38.7 ± 2.41	3.72 ± 0.05	0.41 ± 0.03
Middle taiga									
Organic topsoils	426 ± 21.9	17.4 ± 0.91	24.5 ± 0.48	-29.0 ± 0.16	3669 ± 342	505 ± 51.5	7.33 ± 0.34	3.66 ± 0.05	4.99 ± 0.59
Mineral topsoils	74.7 ± 15.4	3.46 ± 0.58	20.8 ± 1.65	-26.9 ± 0.21	489 ± 104	47.4 ± 11.7	11.0 ± 0.79	3.32 ± 0.07	0.97 ± 0.10
Mineral subsoils	16.7 ± 3.36	0.97 ± 0.12	16.3 ± 1.53	-26.5 ± 0.23	136 ± 24.5	5.43 ± 0.77	25.2 ± 2.46	3.48 ± 0.04	0.65 ± 0.02
Southern taiga									
Organic topsoils	398 ± 16.4	15.8 ± 0.79	25.4 ± 0.71	-28.6 ± 0.21	3065 ± 583	628 ± 71.0	4.82 ± 0.61	4.26 ± 0.09	5.85 ± 0.83
Mineral topsoils	43.4 ± 3.26	3.11 ± 0.16	14.0 ± 0.72	-26.8 ± 0.03	302 ± 19.6	36.3 ± 2.91	8.42 ± 0.50	3.62 ± 0.07	0.76 ± 0.02
Mineral subsoils	4.56 ± 0.22	0.49 ± 0.03	9.40 ± 0.20	-25.3 ± 0.12	61.4 ± 5.36	3.32 ± 0.14	18.4 ± 0.95	3.83 ± 0.04	0.30 ± 0.02
Forest steppe: Forest									
Organic topsoils	293 ± 21.5	17.7 ± 1.12	16.5 ± 0.27	-27.9 ± 0.10	2504 ± 381	399 ± 59.6	6.31 ± 0.39	6.64 ± 0.33	3.83 ± 0.41
Mineral topsoils	45.6 ± 4.04	3.57 ± 0.39	12.9 ± 0.22	-25.7 ± 0.21	156 ± 8.43	11.5 ± 0.71	13.6 ± 0.28	4.26 ± 0.05	0.78 ± 0.03
Mineral subsoils	5.16 ± 0.13	0.52 ± 0.03	10.1 ± 0.31	-25.4 ± 0.14	46.9 ± 1.72	2.9 ± 0.11	16.3 ± 0.67	4.06 ± 0.04	0.62 ± 0.01
Forest steppe: Meadow									
Organic topsoils	202 ± 20.3	14.0 ± 1.40	14.4 ± 0.14	-27.6 ± 0.06	2585 ± 330	390 ± 27.2	6.53 ± 0.42	5.54 ± 0.23	3.68 ± 0.18
Mineral topsoils	25.0 ± 1.65	1.90 ± 0.12	13.1 ± 0.10	-26.2 ± 0.08	189 ± 20.0	14.0 ± 1.52	13.5 ± 0.42	4.15 ± 0.03	0.66 ± 0.03
Mineral subsoils	5.85 ± 0.31	0.55 ± 0.03	10.7 ± 0.19	-25.9 ± 0.05	53.2 ± 3.61	2.72 ± 0.16	19.6 ± 0.91	4.02 ± 0.07	0.61 ± 0.01
Steppe									
Organic topsoils	36.9 ± 2.65	3.33 ± 0.22	11.1 ± 0.12	-25.5 ± 0.11	401 ± 65.3	36.1 ± 6.58	11.3 ± 0.39	4.62 ± 0.09	0.72 ± 0.02
Mineral topsoils	20.1 ± 2.44	1.84 ± 0.19	10.8 ± 0.23	-25.1 ± 0.06	247 ± 34.0	17.9 ± 2.33	13.9 ± 0.50	5.08 ± 0.28	0.57 ± 0.01
Mineral subsoils	7.16 ± 0.73	0.79 ± 0.09	9.15 ± 0.16	-24.9 ± 0.07	87.9 ± 6.37	5.00 ± 0.72	19.5 ± 2.70	7.92 ± 0.36	0.51 ± 0.02
Mean (CV)									
Organic topsoils	302 (0.47)	12.8 (0.40)	23.2 (0.41)	-27.6 (0.04)	2378 (0.51)	374 (0.52)	7.09 (0.29)	4.47 (0.28)	4.34 (0.48)
Mineral topsoils	39.9 (0.56)	2.45 (0.44)	16.6 (0.35)	-26.2 (0.03)	270 (0.55)	24.8 (0.69)	12.1 (0.22)	3.88 (0.18)	0.75 (0.22)
Mineral subsoils	7.62 (0.68)	0.61 (0.39)	11.9 (0.28)	-25.5 (0.03)	80.5 (0.58)	3.64 (0.42)	22.7 (0.38)	4.44 (0.34)	0.49 (0.30)

Table S1 Soil organic matter properties, microbial biomass properties and soil parameters. Values for horizons at individual sites are mean values of the 5 replicated soil pits (mean ± standard error). Values for overall means are given as mean and the coefficient of variance in parentheses.

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Chapter 4

Effects of Soil Organic Matter Properties and Microbia Community Composition on Enzyme Activities in Cryoturbated Arctic Soils

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Effects of Soil Organic Matter Properties and Microbial Community Composition on Enzyme Activities in Cryoturbated Arctic Soils

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Abstract

Enzyme-mediated decomposition of soil organic matter (SOM) is controlled, amongst other factors, by organic matter properties and by the microbial decomposer community present. Since microbial community composition and SOM properties are often interrelated and both change with soil depth, the drivers of enzymatic decomposition are hard to dissect. We investigated soils from three regions in the Siberian Arctic, where carbon rich topsoil material has been incorporated into the subsoil (cryoturbation). We took advantage of this subduction to test if SOM properties shape microbial community composition, and to identify controls of both on enzyme activities. We found that microbial community composition (estimated by phospholipid fatty acid analysis), was similar in cryoturbated material and in surrounding subsoil, although carbon and nitrogen contents were similar in cryoturbated material and topsoils. This suggests that the microbial community in cryoturbated material was not well adapted to SOM properties. We also measured three potential enzyme activities (cellobiohydrolase, leucine-amino-peptidase and phenoloxidase) and used structural equation models (SEMs) to identify direct and indirect drivers of the three enzyme activities. The models included microbial community composition, carbon and nitrogen contents, clay content, water content, and pH. Models for regular horizons, excluding cryoturbated material, showed that all enzyme activities were mainly controlled by carbon or nitrogen. Microbial community composition had no effect. In contrast, models for cryoturbated material showed that enzyme activities were also related to microbial community composition. The additional control of microbial community composition could have restrained enzyme activities and furthermore decomposition in general. The functional decoupling of SOM properties and microbial community composition might thus be one of the reasons for low decomposition rates and the persistence of 400 Gt carbon stored in cryoturbated material.

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Introduction

Decomposition of soil organic matter (SOM) depends on extracellular enzymes, produced by microorganisms. Microbes exude enzymes to acquire carbon (C) or limiting nutrients [1], and to target the most abundant substrates [2]. Extracellular enzyme activities are therefore often related to the chemical composition of SOM and its C and nitrogen (N) content [3,4].

SOM quality and quantity are, however, not the only controls on microbial enzyme activities. Extracellular enzymes have often been found to be related to microbial diversity or the abundance of individual microbial groups [5,6]. Microbial community composition in turn is shaped by environmental factors such as temperature, moisture, O_2 availability and pH [7]. Changes in these factors can promote specific microbial groups that are better adapted to the new environment, but these microbial groups might also differ in their functional properties. This could in turn alter microbial enzyme activities, microbial processes and ultimately decomposition of SOM [8,9].

In most soils, edaphic factors including pH, nutrient levels, and the quantity and quality of SOM change with depth from horizon to horizon [7,10]. Along this gradient also microbial biomass

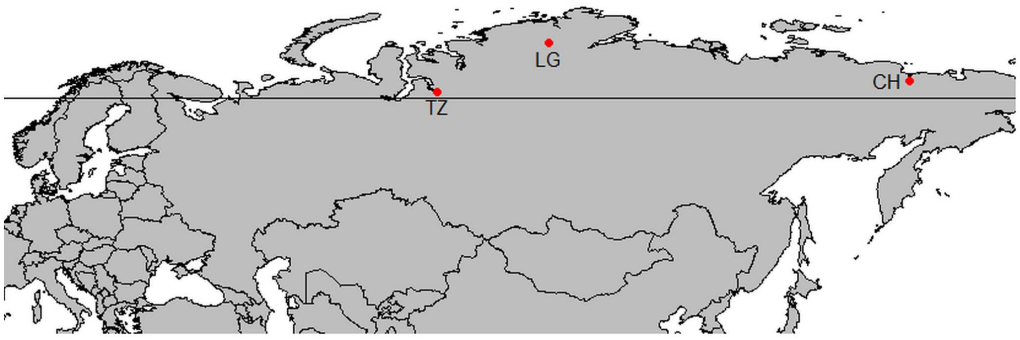

Figure 1. Map showing the three sampling sites in the Siberian Arctic. Tazovsky (TZ; 67°16′N, 78°50′E), Logata (LG; 73°25′N, 98°16′E) and Cherskiy (CH; 68°45′N, 161°20′E). The horizontal line is the polar circle.
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decreases [11–13], and microbial community composition shifts [7,14,15]. These co-correlations and interactions of different factors make it difficult to identify the main drivers of microbial community composition and microbial processes. Attempts to dissect the possible drivers in manipulative experiments have shown that changes of environmental conditions and the inoculation of a specific microbial community onto a non-native substrate can both cause constrained microbial functions [16,17].

One example where SOM is subjected to unfamiliar abiotic environmental conditions can be found in arctic soils. In the arctic permafrost region, freeze-thaw processes cause the subduction of C-rich topsoil material into deeper soil layers [18]. This cryoturbated material is poorly decomposed and similar to topsoil horizons in terms of SOM composition and C and N content [19–21]. Cryoturbated permafrost soils are wide spread in the Arctic and cryoturbated material accounts for up to 400 Gt of carbon [22]. The main reason for the persistence of these large amounts of C is most likely connected to the position of cryoturbated material deep in the soil profile, where abiotic environmental conditions are quite different from those in topsoil horizons. Lower temperatures and O_2 contents, higher water contents, and shorter frost-free periods are related to soil depth [23,24], and are therefore similar in cryoturbated material and in surrounding mineral subsoil horizons. In a previous study, we found that microbial community composition was similar in cryoturbated material and mineral subsoil horizons and different in topsoil horizons, although C and N contents of cryoturbated material were similar to mineral topsoil horizons [25]. The decoupling of SOM properties and microbial community composition could influence the production of extracellular enzyme and thus impair the ability of the microbial community to decompose organic matter.

The objective of this study was to identify the effect of soil properties (C and N content) and microbial community composition on microbial enzyme activities in cryoturbated arctic soils. We constructed structural equation models for three potential enzyme activities: the C-acquiring enzyme cellobiohydrolase (CBH), the N-acquiring enzyme leucine-amino-peptidase (LAP) and the oxidative enzyme phenoloxidase (POX), which is involved in the decomposition of complex organic substances [3]. We also included C and N content, clay content, water content, pH, and microbial biomass, as well as microbial community composition derived from phospholipid fatty acid (PLFA) analysis as potential drivers in the models. All data were obtained from measurements of soil samplesfrom three regions in the Siberian Arctic: Northeast Siberia (Kolyma area), Central Siberia (Taymyr peninsula) and Western Siberia (near Tazovsky). Samples were taken from main horizons and pockets of cryoturbated material. We hypothesized that over all three arctic sites microbial community composition would be similar in cryoturbated material and in the surrounding subsoil. Because of the decoupling of SOM properties (similar in topsoil and cryoturbated material) and microbial community composition in cryoturbated material, we expected the microbial community composition to stronger influence microbial enzyme activities in cryoturbated material than in regular soil horizons.

Material and Methods

Sites and sampling

We collected soil samples from three regions in the Siberian Arctic (Figure 1). Sampling was carried out in northeast Siberia near Cherskiy (68°45′N, 161°20′E), around 180 km north-west of the town Khatanga, at the river Logata (73°25′N, 98°16′E), and near the town of Tazovsky (67°16′N, 78°50′E). Basic climate data and vegetation classes for the three sites can be found in Table 1. Vegetation classes were determined in the field. All climate data are interpolated data of records from 1950–2000 derived from WorldClim [26]. No specific permissions were required for these locations and activities. Our study did not involve endangered or protected species.

In each region, we chose two or three land cover types in which we sampled three 5 m-long soil pits each, down to the permafrost. Soil samples were taken from all distinct horizons of the active layer, including pockets of cryoturbated topsoil material in the mineral subsoil.

Since all of the horizons were affected by cryoturbation processes and varied in size, we accounted for spatial variability in the field by pooling subsamples from several places within each of the horizons of a single pit. We sampled between 300 g and 500 g of fresh soil per horizon.

To avoid any influence of cut-off roots on enzyme activities and phospholipid fatty acids (PLFAs), living roots were carefully removed before further analyses [27]. Soil horizons were grouped in organic topsoil (n = 17), mineral topsoil (n = 18), mineral subsoil (n = 23), and cryoturbated material (n = 43). Organic topsoils included horizons with an organic C content of more than 17% [28]. Mineral topsoils contained topsoil horizons including O/A-horizons and A-horizons with C content below 17%. B, C and BC horizons were combined in the category mineral subsoils. Although all horizons in these arctic soils can be turbated, horizons in the categories organic topsoil, mineral topsoil and mineral subsoil roughly followed a depth-related decrease in C content, and will further on be referred to as regular soil. In contrast to regular soil, subducted, SOM-rich material, which was

Table 1. Climate and Vegetation.

	Coordinates	Year of sampling	Vegetation classes	MAT	Tmax	Tmin	MART	MAP
				°C	°C	°C	°C	mm
Cherskiy (CH)	68°45′N, 161°20′E	2010	shrubby grass tundra	−12.7	14.0	−35.9	49.9	160
			shrubby tussock tundra					
			shrubby lichens tundra					
Logata (LG)	73°25′N, 98°16′E	2011	dryas tundra	−13.5	14.4	−36.6	51.0	270
			grassy moss tundra					
Tazovsky (TZ)	67°16′N, 78°50′E	2012	shrubby lichens tundra	−8.2	17.2	−29.9	47.1	454
			larch woodland with shrubby lichens understory					

Climate data are derived from WorldClim database including mean annual temperature (MAT), maximum temperature of the warmest month (Tmax), minimum temperature of the coldest month (Tmin) mean annual range in temperature (MART) and mean annual precipitation (MAP).
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surrounded by mineral subsoil material with a significantly lower C content, will be further on referred to as cryoturbated material.

Soil properties

Clay content was determined using a pipette method following DIN 66100 (German Industrial Standard). Water content was determined gravimetrically by drying and expressed as percent of fresh mass. Soil pH was determined in a water suspension at a solid to solution ratio of 1:2.5. After acid fumigation for 48 h to remove carbonates [29], samples were analyzed for total organic carbon (C) and nitrogen (N) contents using an EA-IRMS system (Vario ISOTOPE cube, Elementar, Hanau, Germany).

Potential extracellular enzyme activities

We measured potential enzyme activities fluorimetrically and photometrically using a microplate assay [6]. For the fluorimetric assay we used 4-methylumbelliferyl-β-D-cellobioside, and L-leucine-7-amido-4-methyl coumarin as substrates for cellobiohydrolase (CBH) and leucine-amino-peptidase (LAP), respectively. Phenoloxidase (POX) activities were measured using L-3,4-dihydroxiphenylalanine (DOPA) as substrate in a photometric assay. Assays for CBH and LAP were incubated for 140 min at room temperature in a sodium-acetate-buffer (pH 5.5) and measured afterwards fluorimetrically (excitation 365 nm and emission 450 nm). POX assay was measured photometrically (absorbance 450 nm) immediately and after incubation for 20 hours at room temperature.

Phospholipid fatty acid (PLFA) analysis

Samples for analysis of PLFA were stored in RNAlater prior to extraction [30]. Extraction and measurement followed the procedure described by Frostegård et al. [31] with the modifications described by Kaiser et al. [6]. In short, PLFAs were extracted from 1 g of soil with chloroform/methanol/citric acid buffer and purified on silica columns (LC-Si SPE, Supleco, Bellefonte, PA, USA) using chloroform, acetone, and methanol. After addition of the internal standard (methyl-nonadecanoate), PLFAs were converted to fatty acid methyl esters (FAMEs) by alkaline methanolysis. Samples were analyzed on a Thermo Trace GC with FID detection (Thermo Fisher Scientific, Waltham, MA, USA), using a DB-23 column (Agilent, Vienna, Austria). For quantification of the marker 10Me16:0 we additionally used a DB-5 column (Agilent, Vienna, Austria). FAMEs were identified using qualitative standard mixes (37 Components FAME Mix and Bacterial Acid Methyl Esters Mix, Supelco) and quantified using the internal standard. We categorized the fatty acids 16:1ω5, 18:1ω9, 18:2ω6,9, and 18:3ω3,6,9 as markers for fungi; i15:0, a15:0, i16:0, i17:0, and a17:0 as markers for gram positive bacteria; cy17:0 (9/10), cy18:0 (11/12), cy19:0 (9/10), 16:1ω7, 16:1ω9, and 18:1ω7 for gram negative bacteria; 10Me16:0 as marker for actinobacteria; 15:0, 17:0, 17:1ω6, 18:1ω5 as general bacterial markers. All markers for gram positive bacteria, gram negative bacteria, actinobacteria and general bacteria markers were used to calculate bacterial biomass and fungi: bacteria ratios. We used the above mentioned markers together with 14:0, i14:0, 16:0, 18:0, 20:0, 16:1ω11, and 19:1ω8 as a proxy for microbial biomass [6].

Statistics

Before statistical analyses, data were checked for normality, and log-transformed or rank-normalized if necessary. To determine if differences in enzyme activities and microbial community composition were greater between horizons or between sites, we performed two-way-ANOVAs. Differences in soil parameters and enzyme activities between horizons were addressed by ANOVA and Tukey-HSD. To find differences in microbial community composition, we performed principal component analysis (PCA) with the relative abundances of all PLFA markers from all samples, and analyzed the PC-axes with ANOVA and Tukey-HSD test afterwards. When p-values were below 0.05, differences were considered significant.

To find differences in the causal associations in our data, we used structural equation modeling (SEM) following Colman and Schimel [32] and Grace [33]. We constructed a conceptual base model using clay content, water content, pH, C and N contents, microbial biomass, and microbial community composition (PC1, PC2, PC3 from the PLFA-PCA) as direct and indirect factors controlling individual enzyme activities. Clay content in O-horizons was assumed to be zero. The models were run for regular soil (organic topsoil, mineral topsoil and mineral subsoil) and cryoturbated material separately, using PCA axes from separate PCAs run individually for the two groups. Climate parameters were not included in the models, since these parameters represent topsoil conditions and might therefore bias models describing differences between horizons. To achieve a representative model, indicated by a low model chi-squared (χ^2) and a high model p-value ($P>0.05$), individual paths or variables

were removed or added. Model optimization was done by removing not significant paths ($P>0.05$). Alternative models were compared using Akaike's Information Criterion (AIC) to determine the most parsimonious model. All statistical analyses, calculations and maps were performed and created in R version 3.0.0 [34].

Results

Soil properties

In regular soil, both C and N content, and C:N ratios decreased from organic topsoil over mineral topsoil to mineral subsoil (Table 2). In cryoturbated material these parameters were in the range of mineral topsoil, and were always significantly different from organic topsoil and mineral subsoil (Table 2). Water content was highest in organic topsoils followed by mineral topsoil and cryoturbated material and mineral subsoil. pH was significantly higher in subsoil mineral horizons and cryoturbated material than in organic topsoil and mineral topsoil horizons. Interestingly, clay content was highest in cryoturbated material (27.7%) followed by mineral topsoil (22.1%) and mineral subsoil (21.9%) (Table 2).

Microbial enzyme activity

All enzyme activities were significantly different between horizons and sampling sites, but differences between horizons had the greater explanatory power (Table S1). Hydrolytic enzyme activities (CBH and LAP) decreased from organic topsoil over mineral topsoil to mineral subsoil, showing the same patterns as the decrease of C and N content from organic topsoil over mineral topsoil to mineral subsoil (Figure 2). POX activity was significantly higher in mineral subsoil than in organic and mineral topsoil horizons (Figure 2).

Although cryoturbated material and mineral topsoil had similar C and N contents (Table 2), only CBH and POX activites were similar, whereas LAP activity was significantly lower in cryoturbated material (Figure 2). CBH:LAP ratios, as an indicator of the C:N acquisition activity, were highest in cryoturbated material and mineral topsoil followed by organic topsoil and mineral subsoil. The ratios of CBH:POX and LAP:POX were both significantly lower in cryoturbated material than in organic topsoils (Figure 2), and LAP:POX was even significantly lower in than in mineral topsoils.

Microbial community

To assess microbial community composition, we analyzed PLFAs and used the obtained relative abundances of 27 individual biomarkers from all sites in a PCA. The PCA showed a clear separation of topsoil (organic and mineral) on one side and subsoil mineral and cryoturbated material on the other side along the PC1 axis (Figure 3). The effect of horizon ($R^2 = 0.58$) was stronger than that of sampling site ($R^2 = 0.07$; Table S1). Along PC2, horizons were similarly separated, but site effects were stronger ($R^2 = 0.43$) than horizon effects ($R^2 = 0.17$; Table S1). PC3 showed no statistically significant differences between horizon categories but a significant effect of sampling site (Table S1). Fungal biomarkers were strongly represented in PC1, and are most likely responsible for the differences in microbial community composition between topsoil (organic and mineral) and subsoil (mineral subsoil and cryoturbated material). These different fungal abundances are also reflected in the fungi:bacteria ratios, which were two times higher in topsoils than in subsoils (Table 3). The results from the PCA, and the patterns in fungi:bacteria ratios, indicate that the microbial community composition in cryoturbated material is similar to that in mineral subsoil, and significantly different

Table 2. Soil properties of the different horizon categories.

	Samplin depth	Organic carbon (C)	Total nitrogen (N)	C:N ratio	Water content	pH	Clay
	cm	%	%	w/w	% of fresh soil		%
Organic topsoil (n = 17)		**25.2±1.29 (a)**	**0.98±0.06 (a)**	**26.7±1.77 (a)**	**65.5±2.01 (a)**	**5.16±0.11 (a)**	**n.a.**
Cherskiy (n = 9)	0–30	25.5±1.72	1.12±0.06	23.0±1.48	64.9±2.98	5.13±0.08	n.a.
Logata (n = 3)	0–30	25.4±3.39	1.01±0.06	25.1±2.78	70.1±5.19	5.61±0.21	n.a.
Tazovsky (n = 5)	0–20	24.5±2.06	0.72±0.02	34.2±2.73	64.0±2.25	4.97±0.25	n.a.
Mineral topsoil (n = 18)		**8.53±1.33 (b)**	**0.45±0.06 (b)**	**18.0±0.79 (b)**	**40.8±3.49 (b)**	**5.50±0.13 (a)**	**22.1±2.38 (ab)**
Cherskiy (n = 2)	0–40	9.69±4.10	0.43±0.16	21.5±1.73	50.5±14.7	4.80±0.33	7.77±5.50
Logata (n = 11)	0–30	11.0±1.37	0.58±0.06	18.5±0.97	45.6±3.24	5.69±0.15	12.4±3.97
Tazovsky (n = 5)	0–20	2.67±0.61	0.17±0.03	15.6±0.70	26.4±3.70	5.36±0.16	23.9±2.73
Mineral subsoil (n = 23)		**1.10±0.17 (c)**	**0.09±0.01 (c)**	**11.5±0.57 (c)**	**20.7±1.01 (c)**	**6.34±0.11 (b)**	**21.9±1.41 (b)**
Cherskiy (n = 5)	10–90	1.58±0.32	0.13±0.02	11.9±0.84	17.6±0.89	5.81±0.10	18.3±1.04
Logata (n = 7)	5–60	1.85±0.10	0.14±0.00	13.6±0.43	25.2±0.22	6.46±0.13	29.6±1.71
Tazovsky (n = 11)	5–100	0.40±0.06	0.04±0.00	10.0±0.80	19.2±1.59	6.50±0.17	18.5±1.38
Cryoturbated material (n = 43)		**8.39±0.74 (b)**	**0.48±0.04 (b)**	**16.6±0.56 (b)**	**40.0±1.59 (b)**	**6.06±0.09 (b)**	**27.7±1.28 (a)**
Cherskiy (n = 15)	20–80	9.84±1.30	0.61±0.07	15.7±0.43	40.5±2.33	5.74±0.11	29.5±2.21
Logata (n = 19)	15–50	8.37±0.76	0.47±0.03	17.3±0.88	43.6±1.96	6.45±0.10	23.3±2.20
Tazovsky (n = 9)	20–90	6.00±1.98	0.30±0.08	16.7±1.65	31.6±4.32	5.78±0.22	21.6±4.81

Values are mean values (± standard error) over all sites and for each horizon per site. Letters in parentheses indicate significantly different ($P<0.05$) groups between horizons derived from ANOVA and Tukey-HSD tests.

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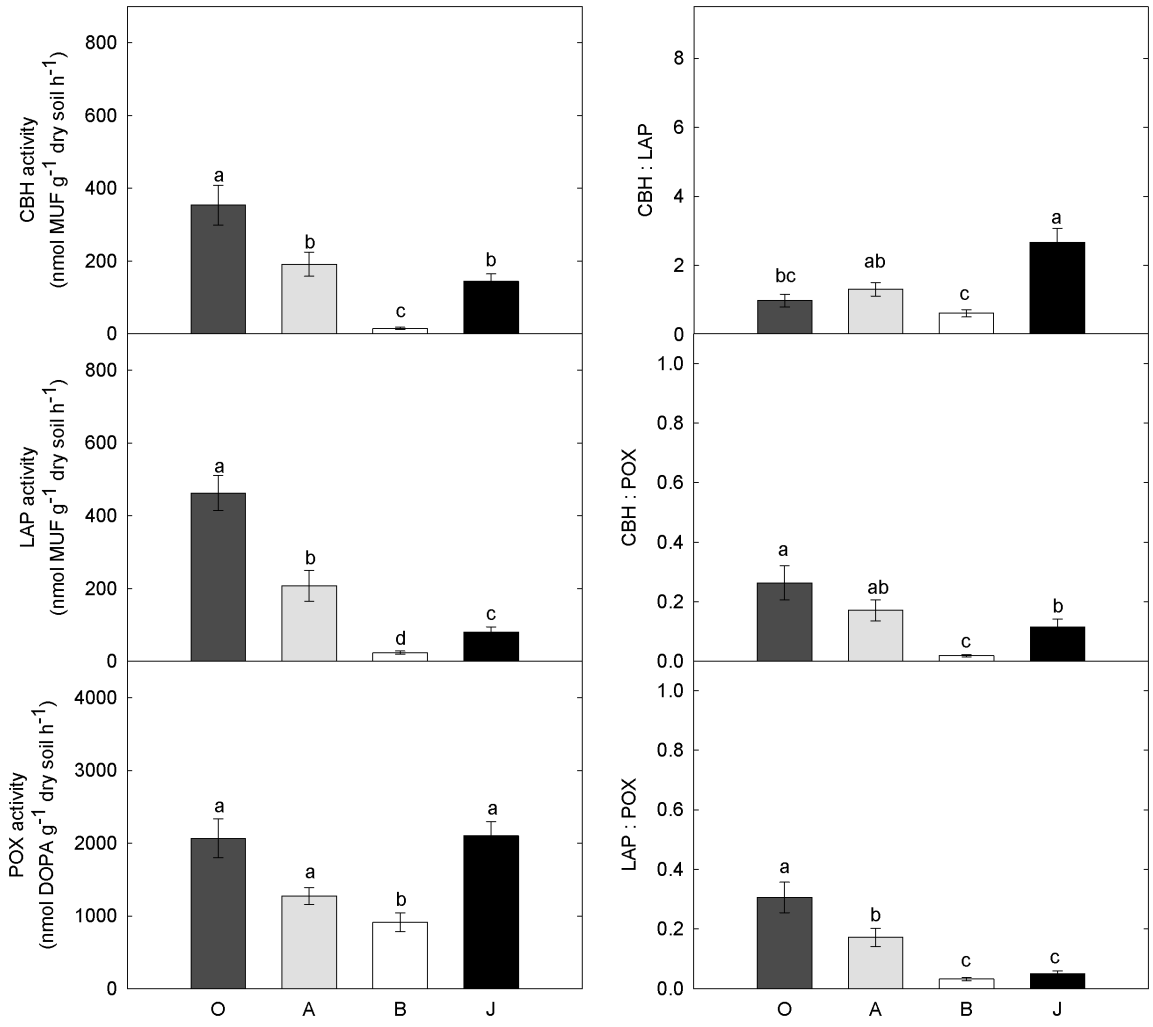

Figure 2. Extracellular enzyme activities and enzyme ratios in different horizons in arctic soils. Left panel: Extracellular enzyme activities for the C-acquiring enzyme cellobiohydrolase (CBH), the N-acquiring enzyme leucine-amino-peptidase (LAP) and the oxidative enzyme phenoloxidase (POX). Right panel: Ratios of the three enzyme activities to each other. Given are the means and standard errors for the individual horizon categories: organic topsoil (O), mineral topsoil (A), mineral subsoil (B), and cryoturbated material (J). Colors indicate different horizon categories: organic topsoil is dark grey, mineral topsoil is light grey, mineral subsoil is white, and cryoturbated material is black. Small letters indicate different statistical groups derived from ANOVA and Tukey-HSD tests.
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($P<0.05$) from the composition of the topsoil microbial community.

The sum of all PLFAs, a proxy for microbial biomass, was five times higher in cryoturbated material than in mineral subsoil, but lower by a factor of two compared to mineral topsoils, although C and N contents were similar in mineral topsoils and cryoturbated material (Tables 2 and 3).

Effects of microbial community composition, carbon and nitrogen contents, and soil parameters on enzyme activity

Since we expected different controls on enzyme activities in regular soil (organic topsoil, mineral topsoil and mineral subsoil) and cryoturbated material, we performed structural equation modeling (SEM) individually for regular soil and cryoturbated material. For the structural equation models we used the first three principal components of PCAs, performed separately for regular soil or for cryoturbated material. A table with the factor loadings is provided in (Table S2).

In regular soil, the PCA showed a strong influence of fungal abundance along the first principal component (PC1). The second component (PC2) and the third component (PC3) did not represent specific groups (Table S2). The structural equation models for the three analyzed enzyme activities (CBH, LAP and POX) showed that in regular soils all activities were related to C or N content, but not to microbial community composition (Figure 4). CBH was directly affected by C and microbial biomass with the model explaining 77% of the variance in enzyme activity ($R^2 = 0.77$, $\chi^2 = 7.04$, $P = 0.13$, Figure 4a). LAP was only directly affected by N ($R^2 = 0.85$, $\chi^2 = 5.31$, $P = 0.07$, Figure 4b). POX was directly affected by N and clay content ($R^2 = 0.53$, $\chi^2 = 0.13$, $P = 0.72$, Figure 4c).

In contrast, enzyme activities in cryoturbated material were influenced by microbial community composition. Microbial community composition was represented by principal component

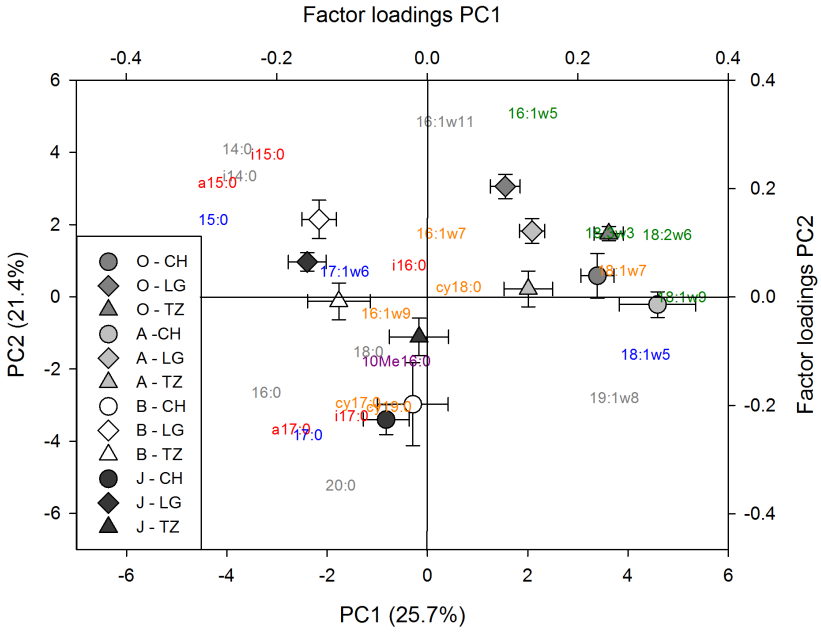

Figure 3. Differences in microbial community composition in different horizons in arctic soils. Principal component analysis (PCA) with relative abundances of all PFLA biomarkers. Colors indicate different horizon categories: organic topsoil (O) is dark grey, mineral topsoil (A) is light grey, mineral subsoil (B) is white, and cryoturbated material (J) is black. Symbols indicate sites: circles Cherskiy, diamonds Logata, and triangles Tazovsky. Symbols are the mean values of the coordinates for the individual categories, derived from the PCA with individual samples (n = 101). Error bars are SE. Colors of PLFA markers indicate general markers (grey), gram-positive markers (red), gram-negative markers (orange), bacterial markers (blue) and fungal markers (green).
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Table 3. Properties of the microbial community.

	Microbial biomass (total PLFAs)	Fungi:bacteria ratio	PLFA PC1	PLFA PC2	PLFA PC3
	nmol g-1 soil				
Organic topsoil (n = 17)	**1833±234 (a)**	**0.55±0.05 (a)**	**a**	**a**	**n.s**
Cherskiy (n = 9)	1117±91.4	0.59±0.08			
Logata (n = 3)	2019±290	0.48±0.09			
Tazovsky (n = 5)	3009±274	0.52±0.03			
Mineral topsoil (n = 18)	**650±103 (b)**	**0.53±0.05 (a)**	**a**	**a**	**n.s**
Cherskiy (n = 2)	624±321	0.91±0.18			
Logata (n = 11)	675±116	0.51±0.05			
Tazovsky (n = 5)	606±215	0.40±0.06			
Mineral subsoil (n = 23)	**59.3±12.9 (d)**	**0.21±0.01 (b)**	**b**	**ab**	**n.s**
Cherskiy (n = 5)	22.3±6.65	0.24±0.03			
Logata (n = 7)	115±27.8	0.21±0.02			
Tazovsky (n = 11)	40.4±10.1	0.19±0.02			
Cryoturbated material (n = 43)	**301±41.3 (c)**	**0.21±0.01 (b)**	**b**	**b**	**n.s**
Cherskiy (n = 15)	175±31.4	0.22±0.01			
Logata (n = 19)	336±48.5	0.19±0.01			
Tazovsky (n = 9)	435±140	0.25±0.04			

Total amount of PLFAs, fungi:bacteria ratios and statistical results for the first three principal components derived from a PCA with relative abundances of all PLFA biomarkers. Values are mean values (± standard error) over all sites and for each horizon per site. Letters in parentheses indicate significantly different ($P<0.05$) groups between horizons derived from ANOVA and Tukey-HSD tests.
doi:10.1371/journal.pone.0094076.t003

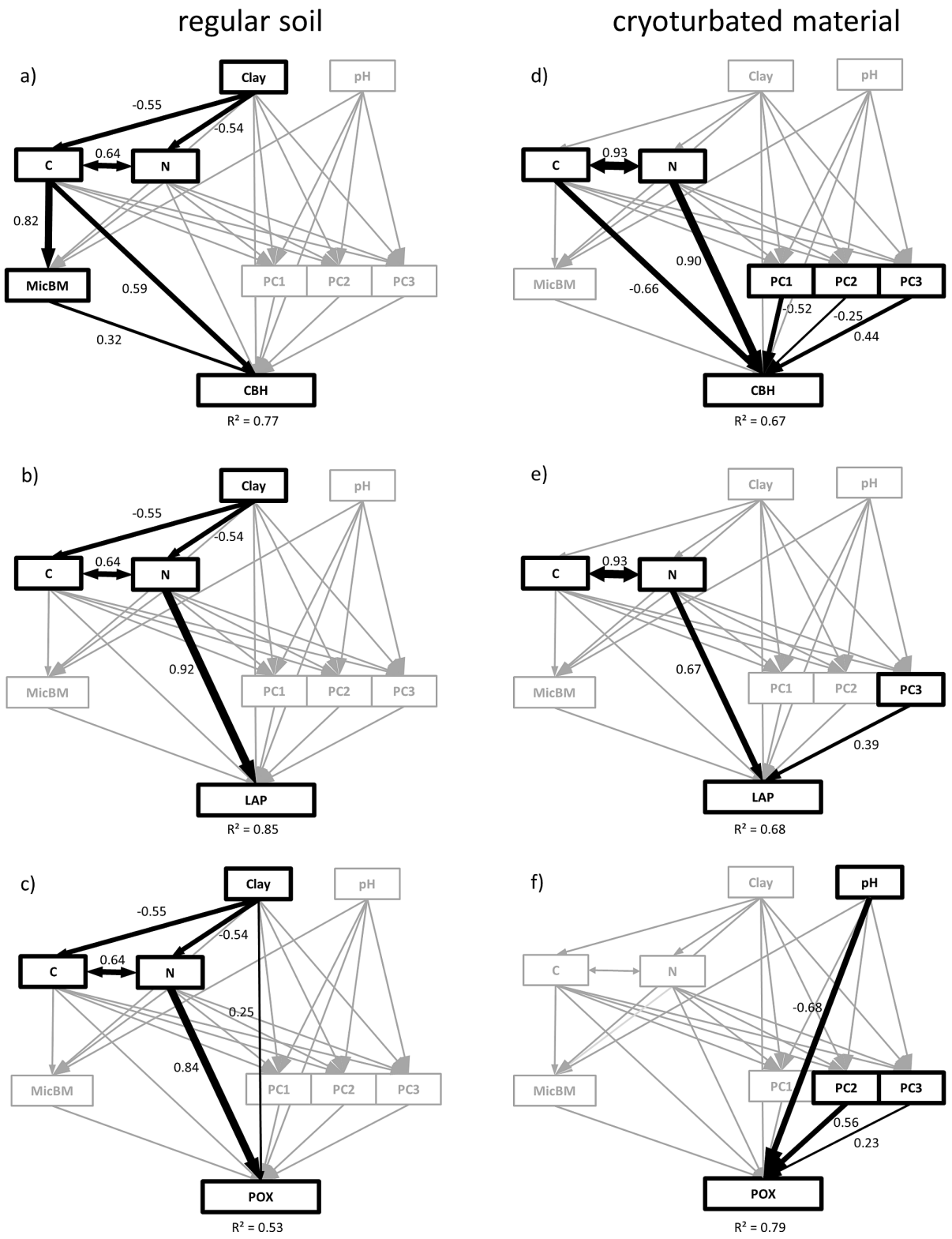

Figure 4. Direct and indirect drivers of extracellular enzyme activities. Structural equation models for extracellular enzyme activities, cellobiohydrolase (CBH; a, d), leucine-amino-peptidase (LAP; b, e) and phenoloxidase (POX; c,f). Graphs on the left show regular soil (organic topsoil, mineral topsoil, mineral subsoil), right panel shows cryoturbated material. Black boxes and arrows indicate significant factors and paths. Boxes and arrows in grey were removed from the model because either the paths (arrows) were not significant, or the factors (boxes) had no direct or indirect effect on the enzyme activity. The boxes with C, N and Clay are the contents of organic carbon, nitrogen, and clay. Microbial biomass (MicBM) was

calculated as total amount of PLFAs. PC1, PC2 and PC3 are the first three axes of PCAs with relative abundances of all PLFAs. PCAs for regular soil and cryoturbated material have been done individually. Arrow width indicates the strength of the effect and reflects the scaled estimates, which are also given as the numbers beside the respective arrows. The numbers below the boxes with the respective enzymes show R^2 and indicate how much of the variance in enzyme activity is explained by the model.
doi:10.1371/journal.pone.0094076.g004

axes from a PCA based on PLFA profiles for cryoturbated material only (Table S2). PC1 was not connected to a specific microbial group. PC2, however, reflected a strong influence of fungal versus bacterial markers. Gram positive markers and the actinobacteria marker had the highest negative factor loadings on PC3, whereas gram negative markers had high positive factor loadings.

In contrast to regular soils, the structural equation models for cryoturbated material showed that clay content did not significantly affect C and N content. CBH was affected by C, N and by all three PCA axis representing microbial community composition ($R^2 = 0.67$, $\chi^2 = 15.29$, $P = 0.08$, Figure 4d), but was not related to microbial biomass. The model for LAP in cryoturbated material explained 68% of the variance in enzyme activity, and showed that only N and PC3-axis significantly affected LAP activity ($R^2 = 0.68$, $\chi^2 = 4.46$, $P = 0.22$, Figure 4e). POX in cryoturbated material was not affected by C or N, but only by microbial community composition (PC2 and PC3), as well as pH ($R^2 = 0.79$, $\chi^2 = 1.16$, $P = 0.76$, Figure 4f).

Models including water content were not representative, or had a higher AIC and where therefore dismissed for the models presented in Figure 4.

Discussion

Despite the differences in SOM quality and quantity, we found similar microbial community composition in cryoturbated material and mineral subsoil. This suggests that the subsoil microbial community, in cryoturbated material, was not fully adapted to its substrate and thus exhibited potentially constraining effects on decomposition by extracellular enzymes.

Effects of SOM properties on microbial community composition

As opposed to non-cryoturbated soils, where the strong depth dependence of SOM quality and quantity prevents understanding of what influences microbial community composition, the rather unique situation in cryoturbated arctic soils allowed us to elucidate the influence of SOM properties on microbial community composition. Since cryoturbations are up to thousands of years old [21,36], the microbial community in cryoturbated organic material should by now be in equilibrium with the physical subsoil conditions and topsoil SOM properties. Alternatively one of the two factors could be more important in shaping microbial community composition. We found that the subduction of organic matter, that was qualitatively [21] and quantitatively (Table 2) similar to topsoil mineral horizons, into the mineral subsoil, resulted in a microbial community composition similar to that in mineral subsoil (Figure 2, Table 3). Although PLFA analysis can only resolve microbial community composition at a coarse level, our findings are corroborated by a recent study in North-East Siberia, where molecular techniques were used to determine microbial community composition [25]. In this study, the authors described a strong decrease in fungi:bacteria ratio derived from bacterial and fungal marker genes (16S rRNA gene for bacteria and the ITS for fungi) from topsoil to mineral subsoil and cryoturbated material similar to our findings (Table 3). Taken together, this suggests that the microbial community in cryoturbated material was not well adapted to SOM properties. Similarly, a high-to-low elevation soil translocation in an alpine ecosystem showed only minor effects of substrate quality on microbial community composition assessed by PLFA analysis [37]. In this study, differences in microbial community composition were attributed to different temperatures. Although we did not measure abiotic parameters such as temperature and O_2 content in situ, these factors might also have shaped the microbial community in cryoturbated material in arctic soils.

Influence of microbial community composition on microbial enzyme activity

The importance of microbial community composition for microbial processes is currently a hot topic and heavily debated [32,38–40]. For instance, Colman and Schimel [32], argue that microbial community composition has only a minor effect on general microbial processes such as microbial respiration and nitrogen mineralization. In their study of North American continental soils, microbial processes were primarily related to C and N content. In line with these observations, we found no effect of microbial community composition on microbial enzyme activities in regular arctic soil horizons (Figure 4 a–c). Microorganisms in regular soil seem to be in equilibrium with environmental (i.e., edaphic and climatic) conditions and SOM properties, with the result, that although the present microbes produce the enzymes, only substrate properties drive extracellular enzyme activities.

In contrast, in cryoturbated material, the activities of all investigated enzymes were related to microbial community composition (Figure 4 d–f). Here the microorganisms were not adapted to the available substrate, which could have resulted in restrained enzyme activities. This might have caused significantly lower activities of the N-related enzyme LAP in cryoturbated material compared to mineral topsoils (Figure 2). Besides LAP, also the ratio of LAP and POX was up to ten times lower in mineral subsoil and in cryoturbated material compared to topsoil horizons (Figure 2). Although oxidative enzymes, such as POX, are rather unspecific and contribute to the acquisition of both C and N [41], some studies describe oxidative enzymes as predominantly N-acquiring [9]. We found POX to be driven only by N in regular arctic soils, which suggests that POX was at least to a certain extent used to acquire N. Although ratios of hydrolytic enzymes to POX are often interpreted as a measure for recalcitrance of the substrate [35], the ratio between LAP and POX could also be seen as a functional property of the microbial community for N-acquisition. The controlling effect of the microbial community, which seems to be especially pronounced in the degradation of N containing SOM, might also be the cause for a previously described deceleration in the N-cycle in cryoturbated material [42].

The control of the microbial community composition over enzyme activities and decomposition processes might not only be of relevance for decomposition of cryoturbated material in arctic soils. Microbial processes could be controlled similarly in other ecosystems, where the usual equilibrium of SOM and microorganisms might be disrupted, such as in bioturbated soils and in areas, where organic material is repeatedly buried by sedimentation or landslides. Investigation of these systems is warranted to

estimate the importance of our findings for C and N cycling on a global level.

Conclusions

The in situ translocation of topsoil material to the subsoil allowed us to demonstrate that the microbial subsoil community that established in cryoturbated material was not well adapted to SOM properties of cryoturbated material and had a potentially restraining effect on extracellular enzyme activities. The proposed control of microbial community composition might not be limited to potential enzyme activities. Main microbial processes such as C and N mineralization have been found to be strongly reduced in cryoturbated material in arctic soils [6]. This low microbial activity has been held responsible for the high ^{14}C age and the low degree of decomposition of this material. A microbial community that is not adapted to the available substrate might be a reason for low microbial activity, and in turn for the current persistence of cryoturbated material in arctic soils.

Supporting Information

Table S1 Differences between horizons and between sampling sites. R^2 calculated from sum of squares derived from a Two-way-ANOVA for the given parameters and horizon and sampling site as factors. Bold values indicate significant differences ($p<0.001$). Results for the first three principal components derived from a PCA with relative abundances of all PLFA biomarkers.
(DOCX)

Table S2 Factor loadings for the first three axes of the Principal Component Analyses used in structural equation models. PCAs have been performed individually for regular soil, including organic topsoil (O), mineral topsoil (A) and mineral subsoil (B), and for cryoturbated material (J). Markers for individual groups are assigned as following: gram positive bacteria (gram +), gram negative bacteria (gram -), actinobacteria (actino), general bacterial markers (bacteria), fungal markers (fungi) and unspecific markers (general).
(DOCX)

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Author Contributions

Conceived and designed the experiments: AR GG HS. Analyzed the data: JS BW FH. Contributed reagents/materials/analysis tools: AR GG. Wrote the paper: JS BW LF FH AR. Collected field data: JS BW RJEA JB PC NG AG GG AK NL RM HS OS MT TU AR. Analyzed samples in the laboratory: JS BW LF NG AH SK GW.

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Table S1 Differences between horizons and between sampling sites

	Horizon R^2	Site R^2
Cellobiohydrolase activity	**0.51**	**0.20**
Leucine-amino-peptidase activity	**0.63**	**0.21**
Phenoloxidase activity	**0.28**	**0.18**
PLFA PC1	**0.58**	**0.07**
PLFA PC2	**0.17**	**0.43**
PLFA PC3	0.05	**0.26**

R^2 calculated from sum of squares derived from a Two-way-ANOVA for the given parameters and horizon and sampling site as factors. Bold values indicate significant differences ($p<0.001$). Results for the first three principal components derived from a PCA with relative abundances of all PLFA biomarkers.

Table S2. Factor loadings for the first three axes of the Principal Component Analyses used in structural equation models.

PLFA	Marker	Regular soil			Cryoturbated		
		PC1 (26.3%)	PC2 (18.3%)	PC3 (14.5%)	PC1 (30.6%)	PC2 (18.5%)	PC3 (11.1%)
i15:0	gram +	0.281	-0.258	-0.020	-0.284	0.010	-0.217
a15:0	gram +	0.315	-0.193	-0.067	-0.326	0.091	0.027
i16:0	gram +	-0.048	-0.197	-0.078	-0.078	0.074	-0.289
i17:0	gram +	0.147	0.049	-0.397	0.219	-0.028	-0.353
a17:0	gram +	0.207	0.152	-0.260	0.200	0.164	-0.250
16:1w9	gram -	0.096	-0.208	-0.203	0.055	0.260	0.252
16:1w7	gram -	-0.019	-0.118	0.024	-0.107	-0.128	0.136
cy18:0	gram -	0.017	-0.260	-0.248	0.030	-0.019	0.273
cy17:0	gram -	0.062	-0.081	-0.428	0.158	0.180	0.221
18:1w7	gram -	-0.188	-0.194	-0.166	0.087	-0.330	0.053
cy19:0	gram -	0.040	0.264	-0.098	0.111	0.119	0.261
10Me16:0	actino	0.036	0.202	0.058	0.149	-0.053	-0.314
15:0	bacteria	0.277	-0.134	-0.023	-0.275	0.233	0.005
17:0	bacteria	0.086	0.079	-0.351	0.087	0.309	0.171
17:1w6	bacteria	0.147	0.173	0.106	-0.092	0.084	0.129
18:1w5	bacteria	-0.245	0.012	-0.239	0.254	-0.174	0.050
16:1w5	fungi	-0.002	-0.376	0.081	-0.187	-0.311	0.098
18:1w9	fungi	-0.327	-0.131	-0.056	0.213	-0.232	0.102
18:2w6	fungi	-0.311	-0.173	0.054	0.081	-0.285	0.265
18:3w3	fungi	-0.230	-0.088	0.132	0.023	-0.222	0.057
i14:0	general	0.315	-0.029	0.107	-0.295	0.000	-0.164
14:0	general	0.296	-0.123	0.205	-0.308	0.054	-0.128
16:0	general	0.119	0.180	0.052	0.042	0.358	0.195
16:1w11	general	0.101	-0.322	0.015	-0.215	-0.271	0.108
18:0	general	0.029	0.240	0.182	0.111	0.043	-0.186
19:1w8	general	-0.255	0.065	-0.214	0.276	-0.087	-0.174
20:0	general	0.049	0.297	-0.281	0.273	0.182	-0.083

PCAs have been performed individually for regular soil, including organic topsoil (O), mineral topsoil (A) and mineral subsoil (B), and for cryoturbated material (J). Markers for individual groups are assigned as following: gram positive bacteria (gram +), gram negative bacteria (gram -), actinobacteria (actino), general bacterial markers (bacteria), fungal markers (fungi) and unspecific markers (general).

Chapter 5

Synthesis

I have demonstrated here a connection of enzymes and microbial community composition in different soil horizons (chapters three and four of this thesis). Regardless of the chemical composition of the soil horizons – from topsoil to cryoturbated organic pockets or mineral subsoil – the fungi:bacteria ratio decreased with depth, in both studies (Figure 7 upper panels). This decrease has already been described by Gittel et al (2014) applying molecular techniques on samples form one of the studied arctic sites. The shift in fungi:bacteria ratio led to the suggestion, that bacteria, and in particular actinobacteria, might have taken over the role of saprotrophic fungi in deeper soil layers where they are the main producers of oxidative enzymes. While it has been shown that actionbacteria can produce oxidative enzymes (Boer, Folman, Summerbell, & Boddy, 2005; DeAngelis et al., 2011) and that their role in the decomposition of SOM, especially under anoxic conditions, should not be neglected (Ausec, van Elsas, & Mandic-Mulec, 2011; Ausec, Zakrzewski, Goesmann, Schlüter, & Mandic-Mulec, 2011), I found yet another shift in the microbial community composition: The decrease in fungi:bacteria ratio, was not at the same rate for all fungal PLFA marker. The fungal marker 18:2ω6,9

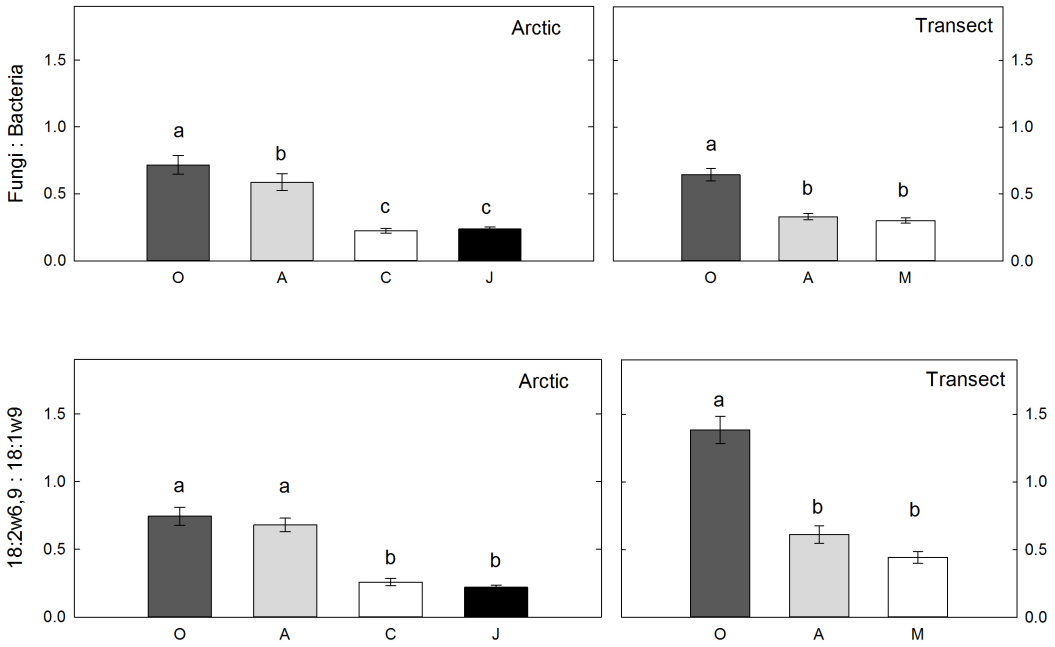
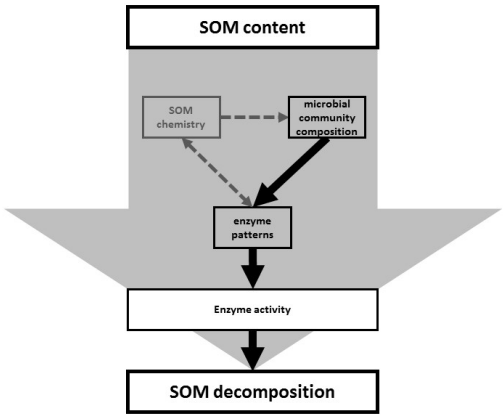


Figure 7. Changes in Fungi:Bacteria ratios and within the fungal community

consistently decreased stronger, than the second main fungal marker 18:1ω9. Therefore, the ratio of these two markers shifted in favor of the marker 18:1ω9 with depth in the arctic soils, as well as in the soils along the transect (Figure 7 lower panels). The stronger decrease of the marker 18:2ω6,9 is especially interesting, since it is, although under discussion, sometimes regarded as a marker for ectomycorrhizal fungi (Olsson, 1999). Since both fungal markers can also be found in plant roots (Högberg et al., 2007; Joergensen and Wichern, 2008), changes in the relative amounts of 18:1ω9 and 18:2ω6,9 might be related to changes in the root biomass. Living roots were however carefully remove, prior to analysis, which has been shown to render the contribution of roots to these biomarkere negligible (Kaiser et al. 2010). The observed changes might thus reflect changes in fungi:bacteria ratios as well as changes in the fungal community with depth. The differences in the fungal community composition in topsoil and subsoil horizons might be the reason for the higher proportion of oxidative enzymes in the subsoil and for their different ways of decomposition, rather than a replacement of fungi by actionobacteria.

The findings of this thesis are the basis for a concept that is proposed and discussed below. A key assumption for this concept is that the different enzyme patterns in topsoil and subsoil horizons are not a measure for how well the microbes decompose SOM. Enzyme pattern rather reflect the composition of microbial communities which are adapted to their physical and chemical environment. The here proposed concept for decomposition which is based on the connections of microbial community composition, enzyme patterns and SOM chemistry is depicted in Figure 8: The left panel shows the situation in "regular" soil horizons. The main driver for decomposition is the amount of SOC and SON. This connection has been shown for a wide range of soils in North America and was described, for example, by Colman and Schimel (2013). Also the connection of SOM content and at least most of the hydrolytic enzymes is well established (Sinsabaugh et al., 2008; Chapter 4). Underlying this connection of SOM content and SOM decomposition is a microbial community that is adapted to its environment. This includes physical and chemical factors such as temperature, water and O2 regimes and pH. In addition, the microbial community is also adapted to the SOM composition and is able to produce enzymes to degrade the provided substrates. Such a connection has been found especially in litter decomposition experiments, where it has been shown, that a microbial community from a certain ecosystem can better decompose litter from that system than from a foreign systems (e.g., Ayres et al., 2009). The home-field advantage of decomposition has since become a highly cited concept in soil microbiology (e.g. (Kowalchuk, 2012; Makkonen et al., 2012; Philippot et al., 2013; Throckmortonet al., 2012). The enzyme-mediated connection of the soil microbial community and SOM chemistry might however also work the other way round. For instance, Wickings et. al. (2012) suggested that the microbial community



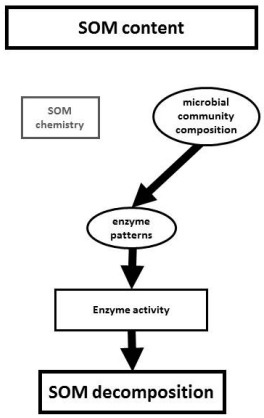


Figure 8. A microorganism based concept for decomposition. Left panel shows the situation in a "regular" soil. right panel shows the diconnection of decomposition rates and SOM content caused by a microbial community which is not adapted to SOM chemistry

shapes the chemical composition of SOM. Either way, an intact triangle of microbial community, enzyme patterns and SOM chemistry causes SOM content to be the main driver for SOM decomposition in an undisturbed system.

In contrast to that, the panel on the right side shows a situation where the microbial community is not adapted to SOM chemistry. This might be for instance the case in cryoturbated pockets in arctic soils (Chapter 4). The environmental factors in the subsoil only allow the establishment of a subsoil community, with a certain enzymatic potential (Chapter 3; Waldrop & Firestone, 2006). Since this enzymatic potential does not fit the chemical composition of SOM, SOM is not decomposed at its full potential and organic matter, in this case in cryoturbated pockets, can persist longer than expected from its carbon content alone. In this case SOM content is only a minor driver for enzyme activities and decomposition. This part of the concept is again in line with the home-field advantage, where the inoculation of a certain litter with its native community lead to higher decomposition rates than when litter was incubated with a foreign community (Ayres et al., 2009; Strickland et al., 2009a; Strickland, Osburn, Lauber, Fierer, & Bradford, 2009b).

The two studies described in Chapter 3 and Chapter 4, highlight the strong link of the microbial community composition and enzyme patterns in soils. They also show that this linkage might even lead to a disconnection of SOM content and SOM decomposition rates under conditions that cause a shift in microbial community composition. This might happen when for instance temperature rises, moisture content or O2 availability changes, i.e. changes predicted by global change scenarios. We have also shown, especially in Chapter 3, that SOM content, but also bulk C/N are only weak predictors for the way SOM is decomposed (enzyme patterns). The findings presented in this thesis highlight the relevance of the composition of the microbial community and should be seen as a warning not to oversimplify controls on microbial processes such as enzyme mediated decomposition, in the complex matrix, soil.

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Appendix A

Summary & Zusammenfassung

SUMMARY

Soil microorganisms exude enzymes to acquire carbon and nutrients, necessary for energy production and microbial growth. Although a crucial step in soil organic matter (SOM) decomposition at all soil depths, little is known about the controls on enzyme activities and enzyme patterns in subsoil horizons. This is of special interest in the arctic permafrost region, where 400 Gt of carbon are currently stored as cryoturbated organic material, which has been subducted into the mineral subsoil by freeze-thaw cycles.

Since a main focus of this thesis is the connection of microbial community composition (MCC) and enzyme activities and enzyme patterns in soils from remote regions, it was necessary to develop a method to store and transport soil samples without keeping them frozen continuously. This method is described in the first part of this thesis.

The second part of this thesis describes enzyme patterns and their controls in topsoils and subsoils. Enzyme patterns differed stronger between horizons, which were at the most 1 m apart, than between seven ecosystems along a 1,500 km North-South transect in Western Siberia. In topsoil horizons, enzyme patterns were correlated to SOM parameters (C, N, C/N) and MCC. In mineral subsoil horizons, enzyme patterns were also correlated to MCC but not to SOM parameters.

Additionally we found that in non-turbated horizons of arctic permafrost soils, enzyme activities were controlled by C or N content, whereas in cryoturbated material all enzyme activities were additionally affected by the MCC (third part of the thesis).

Overall this thesis highlights the different enzyme patterns in topsoil and subsoil horizons and identifies MCC as an important control over enzyme activities and enzyme patterns in subsoils - a control that might even restrain decomposition in general and might be a reason for the persistence of cryoturbated organic material in arctic permafrost soils.

ZUSAMMENFASSUNG

Mikroorganismen im Boden scheiden Enzyme aus um Kohlenstoff und Nährstoffe, für Energie und Wachstum, zu akquirieren. Obwohl dieser Schritt von großer Bedeutung beim Abbau organischen Materials in allen Bodenhorizonten ist, weiß man bis jetzt vergleichsweise wenig über die Kontrollen über Aktivitäten und Muster von Enzymen in Unterböden. Dies ist gerade in arktischen Permafrostregionen von großer Bedeutung, da hier 400 Gt Kohlenstoff in Form von kryoturbiertem Material, welches durch Frier-Tau-Zyklen in den Unterboden eingebracht wurde, vorliegen.

Da die Verbindung zwischen mikrobieller Gemeinschaftszusammensetzung und Enzymaktivitäten und Enzymmustern einen wichtigen Platz in dieser Arbeit einnimmt, war es notwendig eine Methode zum Lagern und Transportieren von Bodenproben, ohne die Notwendigkeit diese konstant gefroren zu halten zu, entwickeln. Diese Methode wird im ersten Teil der Arbeit beschrieben.

Der zweite Teil dieser Arbeit behandelt Enzymmuster und deren Kontrollen in Ober-und Unterböden. Die Enzymmuster unterschieden sich stärker zwischen den Horizonten, die maximal einen Meter auseinander waren, als zwischen den sieben Ökosystemen entlang des 1500 km langen Nord-Süd Transekts in Westsibirien. In den oberen Bodenschichten waren die Enzymaktivitäten mit Bodenparametern (C, N, C/N) aber auch mit der mikrobiellen Gemeinschaftszusammensetzung korreliert. In den mineralischen Unterböden waren die Enzymmuster ebenfalls mit der Zusammensetzung der mikrobiellen Gemeinschaft korreliert, nicht aber mit den Bodenparametern.

Zusätzlich zeigen unsere Untersuchungen von arktischen Permafrostböden, dass Enzymaktivitäten in nicht turbierten Bodenhorizonten vom C- bzw. N-Gehalt kontrolliert werden, während Enzymaktivitäten in kryoturbiertem Material eine zusätzliche Kontrolle durch die mikrobielle Gemeinschaftszusammensetzung erfahren.

Zusammenfassend zeigt diese Arbeit Unterschiede in den Enzymmustern in Ober- und Unterböden auf und unterstreicht die Bedeutung der Zusammensetzung der mikrobiellen Gemeinschaft als Kontrolle über Enzymaktivitäten und Enzymmuster in Unterböden. Dies ist eine Kontrolle, die sogar den Abbau im Allgemeinen reduzieren könnte und die somit eventuell zur Konservierung von kryoturbiertem Material in arktischen Permafrostböden beiträgt.

Appendix B

Acknowledgements

This was supposed to be intelligently written, witty and smart. Since, witty and smart tend to turn into presumptuous and arrogant too easily and this section is too important to me, I decided to keep it simple:

I want to thank first of all Lisi for supporting me whatsoever. Further, I want to thank my family Ruth, Adi, Heidrun, and Simon as well as my late grandfather Alois Wenninger. I also want to thank long-time friends Matthias, Lucia, Philipp, Michael and Martin for their open ears during the last years.

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THANK YOU.

Appendix C

Curriculum Vitae

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Education

2014	PhD	Biology	University of Vienna
2010	Mag. rer. nat (MSc equivalent)	Ecology	University of Vienna

Work experience

2010-2014	**PhD-Thesis**, Dept. of Microbiology and Ecosystem Science, University of Vienna Title: “Enzyme activities and microbial community composition in soils from northern latitudes with special emphasis on cryoturbated arctic soils” Supervisor: Dr. Andreas Richter
December 2011	Method training, Leibniz University Hannover Method: Density Fractionation Advisors: Dr. Georg Guggenberger and Dr. Robert Mikutta
2008-2010	**Diploma Thesis**, Dept. of Chemical Ecology and Ecosystem Research, University of Vienna Title: “Resource limitation of Decomposition: The role of fungi” Supervisor: Dr. Andreas Richter
2009	Method development, Dept. of Chemical Ecology and Ecosystem Research, University of Vienna Method: a pool dilution method for phosphorus mineralization Advisors: Dr. Andreas Richter and Dr. Wolfgang Wanek
2008-2009	Research assistant, Dept. of Chemical Ecology and Ecosystem Research, University of Vienna Advisors: Dr. Andreas Richter and Dr. Wolfgang Wanek
September 2008	Method training, Helmholz Zentrum München Method: Assay for mycorrhizal enzyme activities Advisor: Dr. Karin Pritsch
September 2007	Research assistant, Department of Forest Ecology and Soils, Federal Research and Training Center for Forests, Natural Hazards and Landscape (BFW) Advisors: Dr. Sophie Zechmeister-Boltenster and Dr. Barbara Kitzler

July, August 2007	Research assistant, Dept. of Chemical Ecology and Ecosystem Research, University of Vienna Advisors: Dr. Andreas Richter

Teaching Experience

2014	Co-Instructor, practical course: “Element cycles in terrestrial ecosystems”, University of Vienna
2009-2011	Teaching assistant, practical course: “Functional Ecology”, University of Vienna

Field experience

2010-2012	Sampling campaigns in the Siberian Arctic for 4-6 weeks in Summer
2009	Student field course in Western Siberia
2008-2010	Regular sampling campaigns at a beech-forest study site near Vienna
2008	Student field course in Costa Rica
2007	Student field courses in the European Alps

Work experience outside Academia

2002-2013	Volunteer work as a paramedic at the Austrian Red Cross
2001, 2002, 2005, 2006	Shift worker at Buntmetall Amstetten Ges.m.b.H, during Summer
2002-2003	Alternative Civil Service as a paramedic at the Austrian Red Cross
1999, 2000	Office intern at Buntmetall Amstetten Ges.m.b.H, during Summer

Technical Expertise

Measurements: HPLC, GC-FID, GC-MS, Pyrolysis-GC-MS, GC-IRMS

Instrument maintenance: HPLC, GC-FID, GC-MS and Pyrolysis-GC-MS

Software: MS Office, R, Primer6, Statgraphics, Sigmaplot, Visual Basic, XCalibur, Chromeleon

Peer-reviewed Publications

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8. Mooshammer M, Wanek W, **Schnecker J**, Wild B, Leitner S, Hofhansl F, Blöchl A, Hämmerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S, and Richter A. 2012. Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* 93, 770-82.
9. Koranda M., **Schnecker J**, Kaiser C, Fuchslueger L, Kitzler B, Stange CF, Sessitsch A, Zechmeister-Boltenstern S, and Richter A. 2011. Microbial processes and community composition in the rhizosphere of European beech – The influence of plant C exudates. *Soil Biology & Biochemistry* 43, 551-558.
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