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„Controls on soil organic matter decomposition along a  
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# I. General Introduction

## 1. Global carbon and nitrogen

Since the discovery of the importance of CO<sub>2</sub> for the global temperature by Arrhenius at the end of the 19<sup>th</sup> century, it has been a major subject for scientists all over the world (Falkowski et al. 2000; Schlesinger & Andrews 2000; Pearson & Palmer 2000). Enhanced atmospheric greenhouse gases influence global climate (Kaufmann et al. 2006) and alter biogeochemical cycles of elements, i.e. carbon (C) and nitrogen (N) (Conant et al. 2011). Globally carbon is distributed in different pools with very variable turnover times. Large parts are stored in sediments and rocks which are stable with turnover times of hundreds of millions of years. More active pools, and therefore more relevant in terms of climate change, are found in the terrestrial system (vegetation and soils), the oceans and the atmosphere (Chapin et al. 2002). Estimates for the terrestrial biosphere range from 450 to 650 Pg C for living organic biomass mostly in vegetation, between 1 500 and 2 400 Pg C in litter and soils, plus 300 – 700 Pg C in wetland soils and around 1 700 Pg C in permafrost soils (IPCC 2014). The atmospheric carbon pool mainly consists of carbon dioxide (CO<sub>2</sub>) with 828 Pg C and of methane (CH<sub>4</sub>) with ~3.7 Pg C (IPCC 2014).

Carbon release from the terrestrial system occurs with losses through autotrophic (CO<sub>2</sub> release of primary producers i.e. plants) and heterotrophic (CO<sub>2</sub> release during decomposition) respiration (Falkowski et al. 2000). The part of gross primary production (GPP) that is not respired is known as net ecosystem production and is the amount of carbon which is stored in ecosystems (Chapin et al. 2009; De Deyn et al. 2008). Inputs of organic material into soils are plant and root litter or root exudates which become decomposed by microbial decomposer communities (Chapin et al. 2002). The balance between input of organic material and carbon released defines whether an ecosystem is a carbon sink or source (McKane et al. 1997).

The second major component of organic material is nitrogen (Xu et al. 2013) with a connected element cycle to carbon (Neff et al. 2002; Chapin et al. 2009). Nitrogen is the major component in the earth's atmosphere in form of N<sub>2</sub>, but only a few organisms (i.e. bacteria such as Rhizobia or Frankia) are able to fix and use atmospheric N (Chapin et al. 2002). Biological fixation of N<sub>2</sub> is energetically expensive and can therefore be limited by energy (i.e. carbon) or by other nutrients (Vitousek et al. 2002). Furthermore, N is deposited from the atmosphere as NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> via precipitation, aerosols or lightning or enters the soil as dead organic matter (Chapin et al. 2002). However, compared to carbon only small amounts of existing nitrogen are biologically available in soils, but most recycles within vegetation and soil organic matter (Chapin et al. 2002). Reich &

Oleksyn (2004) postulated an increase in N limitation towards the poles due to temperature restrictions on N mineralization from unavailable organic N. This results in a retarded N cycle compared to other elements (Vitousek et al. 2002) and the limitation by N of many ecosystems (LeBauer & Treseder 2008; Vitousek et al. 2002). Nitrogen decomposition in soil starts with the entry of N containing organic material and the build-up of the soil organic matter pool, in which N is bound as proteins, nucleic acids, chitin or humic substances, which prevents the direct uptake by microbes due to the size of these polymers (Chapin et al. 2002). This organic matter must be depolymerized via extracellular enzymes, and can subsequently be taken up by soil microorganisms and either used for biomass production or get mineralized and excreted as inorganic nitrogen (Ågren & Andersson 2012). Mineralization of organic N compounds has long been considered as the rate limiting step in soil N cycling. However, Schimel & Bennett (2004) suggest that the depolymerization of N-containing compounds is the limiting step in the soil N cycle and plants, as well as microorganisms are in fact able to use organic N-molecules (Näsholm et al. 2009; Farrell et al. 2013), especially under N limiting conditions.

## **2. Soil organic matter decomposition**

Estimates of the amount of carbon, which is stored in high latitude soils range from 33 up to more than 50 percent of the global carbon in soils (Harden et al. 2012; Gorham 1991; Tarnocai et al. 2009; Schuur et al. 2009). Unfavorable climatic conditions which occur in high latitudes constrain decomposition, nutrient availability and plant productivity (Mack et al. 2004; Sturm et al. 2005) and may be the reason for the enormous carbon stock. Decomposition is vulnerable to climate warming with direct and indirect effects of temperature (Sistla et al. 2012; Shaver et al. 2000). Additionally to abiotic controls (i.e., temperature, soil moisture, minerals and pH), litter quality, nutrient availability and the decomposer community structure exert controls over decomposition (Chapin et al. 2002; Mack et al. 2004; Schneckner et al. 2015; Kaiser et al. 2014). For a long time chemical traits (i.e., recalcitrance) of SOM were considered to be a main reason for the persistence of organic matter in soils but more recently SOM persistence due to controlling environmental and biological factors has been described as an ecosystem characteristic (Lützow et al. 2006; Schmidt et al. 2011).

Most studies on SOM decomposition were conducted in the upper 10 – 20 cm of the soil, but specifically in arctic soils a large fraction of organic C is stored in deeper layers, which may also

be vulnerable to altered climatic conditions (Trumbore 2009). Thus future models on impacts of climate change should include C of deeper soil layers (Rumpel & Kögel-Knabner 2011; Trumbore 2009). Distinct controls over decomposition in subsoils are the accessibility of organic matter due to physical disconnection of microorganisms and their substrate, the sorption of SOM to minerals as well as the energy limitation of microbes (Schmidt et al. 2011; Chapin et al. 2002).

The starting point of organic matter deconstruction is depolymerization by microbial extracellular enzymes. In soils, deconstruction of high molecular weight molecules into smaller molecules is crucial for microorganism to assimilate organic matter (Moorhead et al. 2012; Sinsabaugh et al. 2008). Microbes therefore release enzymes into the environment, so called extracellular enzymes or exoenzymes, which can have a narrow (most hydrolytic enzymes) or broad substrate specificity (oxidative enzymes) (Ågren & Andersson, 2012). Exoenzymes themselves can be degraded by proteases (protein degrading enzymes) making a constant production of these enzymes necessary (Chapin et al. 2002). The production of enzymes needs a high investment in nitrogen and is consequently restricted under N limited conditions (Allison & Vitousek 2005). Weintraub & Schimel (2003) postulated that enzyme production can also be carbon limited depending on the “return on investment”. More precisely, if the return of carbon or energy from resulting substrate is higher than the investment into the enzymes, microbes may enhance their growth or activity. Contrary, if the investment exceeds the return of benefits, the microbes will suffer from starvation, even though there are existing carbon resources. The extent of above mentioned controls varies but temperature is crucial for microbial activity and enzyme kinetics (Bai et al. 2013; Rustad et al. 2001).

A further approach to describe limitation of soil organic matter decomposition is ecological stoichiometry. Every organism is build up by different elements in a specific ratio; some organisms are homeostatic with regard to their resource stoichiometry (i.e., their biomass stoichiometry is independent of the stoichiometry of their food), while others show some plasticity (Sternner & Elser 2002). Soil microbes are often confronted with plant substrates with much higher C:N ratios than their own biomass C:N ratio or with soil organic matter which has a similar C:N ratio to the microbial biomass. The average leaf litter C:N is about 71:1 and root litter C:N even higher with 97:1 (Yuan et al. 2011; Yuan & Chen 2009). If carbon is available in excess microbes are thought to mineralize C to CO<sub>2</sub>, also called “overflow metabolism” (Weintraub & Schimel 2003). If N is in excess (and carbon or other elements i.e. phosphor (P) are limiting), nitrogen will be mineralized and excreted as NH<sub>4</sub><sup>+</sup> (Mooshammer, Wanek, Hämmerle, et al. 2014). Substrate C:N ratios decrease with further processing as plant-derived substrates with a wide C:N and C:P ratio become incorporated into microbial biomass with a low C:N and C:P ratio.

Thus, SOM C:N and C:P ratios decrease with soil depth (Rumpel & Kögel-Knabner 2011). The value of substrate C:N where nutrient limitation switches to carbon (=energy) limitation is called threshold elementary ratio (TER) with additional microbial efficiencies to use C or N which can be regulated independently when facing varying substrate stoichiometry (Mooshammer et al. 2014).

Plants can exert control over SOM decomposition by the so-called ‘priming effect’, which was first published by Löhnis (1926) but gained broader recognition only later (Kuzyakov et al. 2000). Blagodatskaya & Kuzyakov (2008) defined priming effects as “changes in the microbial activity as a response to altered amounts and availability of carbon”. In other words changes in microbial activity (exoenzyme production, nutrient mineralization or growth) in response to the input of fresh, easily decomposable organic matter, lead to changes in decomposition of native SOM. Priming effects can be positive and negative (Guenet et al. 2010; Kuzyakov et al. 2000). More precisely, positive priming describes the accelerated decomposition of recalcitrant or more stable SOM as response to the availability of fresh organic matter, whereas negative effects describe the preferred decomposition of the labile fresh organic matter with restricted decomposition of recalcitrant SOM (Fontaine et al. 2003). One explanation for priming is that changes in SOM decomposition result from the competition between microbes of different physiologies (Fontaine et al. 2003). Input of labile substrate (e.g. glucose) enhances growth and activity of fast growing r-strategists, while slow growing K-strategists become outcompeted and subsequently, recalcitrant SOM decomposition by K-strategists decreases. More stable substrate (cellulose and proteins), in turn, may support K-strategists (Fontaine et al. 2003). In addition, real or apparent priming can be distinguished depending on the origin of the mineralized substrates. Apparent priming is the enhanced turnover of microbial carbon due to changes in biomass turnover of microbes without any additional decomposition of SOM. Real priming, in contrast, describes enhanced SOM decomposition through higher microbial activity (Blagodatskaya & Kuzyakov 2008). Another hypothesis for priming is known as “nutrient mining” where microbes use energy out of oxidation of labile organic matter to degrade recalcitrant matter to acquire needed nitrogen (Moorhead & Sinsabaugh 2006). Hence, increased availability of fresh nutrients (i.e., nitrogen) lead to a decrease in decomposition of recalcitrant C (Moorhead & Sinsabaugh 2006; Wang et al. 2004). This leads to the conclusion of enhanced carbon storage in nitrogen limited ecosystems when N becomes more available and microorganisms reduce mining though decomposition of recalcitrant organic matter (Craine et al. 2007; Wang et al. 2004; Berg & Matzner 1997).

In soils, only a small proportion (0.1 – 2%) of microbes are considered to be active at any given time, whereas up to 10 – 40% of all microbes are at least potentially active due to their ability to switch from dormant to active states after environmental conditions change (Blagodatskaya & Kuzyakov 2013). The potentially active microorganisms are able to use fresh substrate within a short time (hours) while dormant microbes need hours to days for switching to an active state (Blagodatskaya & Kuzyakov 2013), both are therefore important for the alterations of microbial activity after input of fresh substrate. Moreover, the active fraction of microorganisms recycles dead or lysed cells which act in this case as a relatively labile substrate pool but is part of the SOM (Blagodatskaya & Kuzyakov 2013; Miltner et al. 2012). Furthermore, changes in decomposer community composition in deeper soils recently gained in recognition (Schnecker et al. 2015; Eilers et al. 2012). In tundra soils the abundance of ectomycorrhizal fungi decrease while actinobacteria increase with depth with accompanied reductions in decomposition and extracellular enzymes (Gittel et al. 2014). Ectomycorrhizal fungi live in symbiosis with plants which provide fresh organic matter for energetically expensive decomposition of recalcitrant SOM (Talbot et al. 2008). The abundance of mycorrhizal fungi is important for decomposition.

### **3. Research gap and study aims**

The great quantities of organic carbon, which are stored either in deeper soil horizons or in the permafrost (Tarnocai et al. 2009) may become unstable in a future climate and a source for additional atmospheric CO<sub>2</sub> and thus for a positive feedback on global warming. The dynamics of destabilization of the carbon pools are difficult to predict and the quantification of the released CO<sub>2</sub> is challenging. The interplay of climate, plant growth and SOM decomposition is still an enigma (De Deyn et al. 2008; Schuur et al. 2015). In accordance with recent global change predictions especially soils at higher latitudes are supposed to be affected by increasing temperatures with increased SOM decomposition (Schuur et al. 2008), higher rates of nutrient mineralization and thereby increasing nutrient availability (Hobbie et al. 2002). Nutrient availability exerts a strong control over SOM decomposition, but it is still unclear whether altered nutrient inputs, e.g. plants roots, root exudates or bioturbation (Rumpel & Kögel-Knabner 2011), specifically in deeper soil horizons will alter the dynamics of SOM decomposition.

The aim of my thesis was to study the influence of altered substrate availability (protein and/or cellulose) in SOM decomposition in different mineral horizons along a 1.500 km latitudinal transect in Western Siberia covering four biomes. This transect was established to compare the SOM decomposition of different biomes, i.e. tundra, different boreal forest types (northern, middle and southern taiga), forest steppe and steppe and between horizons. We expected to find different levels of N-availability along the transect, as higher latitude biomes in general have higher SOM C:N ratios and are thus thought to be stronger N-limited (Xu et al. 2013).

Towards this goal we conducted an incubation experiment with the first and second mineral horizons collected from seven ecosystem types along this latitudinal transect. We added cellulose or a mixture of cellulose and protein to the soils and also incubated an unamended control. Hence, we hypothesized that the addition of cellulose together with protein would lead to a stronger increase of microbial growth and activity (respiration), as well as potential extracellular enzyme activities, in more N-limited soils (in the north) compared to the more southern soils with relatively higher N availability. Moreover, we hypothesized that cellulose amendments to deeper soil horizons would lead to a strong increase in microbial activity, due to lower C:N ratios in deeper soil horizons and more likely limitation of microbial decomposers in carbon and energy. Soil respiration rates were measured weekly using a head space gas sampling method. Carbon and nitrogen containing pools, microbial biomass, nitrogen mineralization rates and potential enzyme activities were measured before substrate addition and again after six weeks incubation time.

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## II. Manuscript

### 1. Abstract

Climate change induced alterations in the global carbon cycle are predicted to influence the input of organic matter into soils and - as a result – alter microbial decomposition of soil organic matter. These alterations may be specifically pronounced in high latitudinal ecosystems where production and decomposition rates are exceedingly limited by nitrogen. Even though it is commonly assumed that nitrogen limitation increases with latitude and available energy (carbon) decreases with soil depth, little is known about changes in decomposition rates of different soil horizons along a latitudinal transect and how this may change due to alterations in organic matter input. I here report a study in which soil samples of the first and second mineral horizon of four biomes along a 1.500 km continental latitudinal transect in Western Siberia were supplemented with cellulose and protein in a laboratory incubation experiment. I measured respiration rates, soil carbon and nitrogen pools, microbial biomass and potential enzyme activities. My results show a significant increase in respiration rates after cellulose plus protein amendments; however, these increases exhibited no clear latitudinal pattern. Similarly, microbial activity estimated as potential enzyme activities was shown to be positively influenced by nitrogen amendments without any effect of latitude or type of enzyme. In contrast, cellulose amendment failed to increase respiration or microbial biomass production in both horizons, corroborating the importance of nitrogen for microbial processes, even in lower mineral horizons, which were thought to be carbon (energy) limited. In conclusion, neither the suggested ameliorating effects of cellulose amendments on decomposer communities in deeper soil nor a latitudinal pattern of the effect of protein addition could be found in our study. However, additional protein amendment increased the respiration and enzyme production of decomposer communities, which lead us to the conclusion that the input of nitrogen containing organic matter has the potential to enhance the activity of decomposers in northern ecosystems.

## 2. Introduction

Plant material in form of litter, root litter or root exudates are the main organic carbon (C) and nitrogen (N) containing components entering the soil and are the basis for the so called soil organic matter (SOM) (Chapin et al. 2002). Plant organic matter is decomposed by microorganisms, processed into microbial synthesis products or build into their biomass and upon death becoming also part of the SOM (Chapin et al. 2002; Cotrufo et al. 2013). Organic matter is decomposed and potentially mineralized by soil microbes and this occurs if the environmental conditions (e.g. temperature, soil moisture or pH) are appropriate (Chapin et al. 2002). Moreover, decomposition is controlled, amongst other factors (i.e. physical conditions), by nutrient availability (Mack et al. 2004). Most ecosystems are limited by N (LeBauer & Treseder 2008; Vitousek et al. 2002) with a postulated increase of N limitation of plant production from temperate regions towards the poles (Reich & Oleksyn 2004). This nutrient limitation of plants leads to low N biomass, which induces a nutrient limitation for soil microbes (De Deyn et al. 2008), which then negatively feeds back on plant available mineralized nutrients. Plant litter enters topsoil horizons and possibly can be further translocated to subsoil horizons via roots (litter or exudates), bioturbation and dissolved organic carbon. This dissolved organic matter is mainly microbial derived and can be further stabilized on mineral compounds (Rumpel & Kögel-Knabner 2011).

SOM decomposition starts with the deconstruction of complex biopolymers, such as proteins, nucleic acids or chitin, into smaller, soluble molecules by extracellular enzymes produced and released by microbes (Chapin et al. 2002). The production of these enzymes needs a high amount of N and energy (Allison et al. 2010). Hence, strongly N limited microorganisms cannot channel nitrogen to enzyme production, which further limits their ability to access soil N. Thus, enzyme production should increase with available N until a certain point, leading to a non-linear response of microbial communities to increased N availability (Allison & Vitousek 2005).

The relationship between C and N in terrestrial ecosystems can be described by the theory of ecological stoichiometry where the ratio of carbon to nutrients is used to characterize pools and infers processes (Sterner & Elser 2002). Plant materials have higher C:N ratios than soil organic matter. In soils, C:N ratios decrease with soil depth caused by microbial processing of organic matter (Xu et al. 2013). Microbes, in turn, have relatively constant C:N ratios (Cleveland & Liptzin 2007; Xu et al. 2013) and are thought to mineralize elements in excess (Mooshammer, Wanek, Zechmeister-Boltenstern, et al. 2014; Schimel & Bennett 2004). The efficiency of nutrient use and the mineralization of excess nutrients can be adjusted by microbes in line with the threshold

elemental ratio (TER) theory (Mooshammer, Wanek, Hämmerle, et al. 2014).  $TER_{C:N}$  reflects the substrate C:N ratio at which a system is co-limited by C and N. Thus, below  $TER_{C:N}$  N is in excess of the demand of the microbial community and the microbial community is C (or energy) limited (Mooshammer, Wanek, Hämmerle, et al. 2014). Hence, the ratio of microbial C:N to soil C:N may be used as proxy to describe N limitation in terrestrial systems.

However, not only the elemental composition, but also substrate quality can affect SOM decomposition (Chapin et al. 2002). Specifically, labile, easily decomposable organic matter can stimulate the decomposition of older, more recalcitrant material, thereby enhancing  $CO_2$  losses from soil. This phenomenon is known as priming effect (Blagodatskaya & Kuzyakov 2008), and is considered to occur primarily in the rhizosphere where plants release root exudates (low molecular substances).

Even small alterations of SOM decomposition by global warming may significantly influence the atmospheric  $CO_2$  concentration and thus lead to a feedback of climate on warming (IPCC 2007). The direction of such an alteration is however uncertain. On the one hand, warming is expected to directly increase microbial activity and accelerate decomposition (Langley et al. 2009; Xie et al. 2005; Hagerty et al. 2014). On the other hand, the resulting increased availability of nutrients could ameliorate the nutrient limitation of plant growth and microbes and lead to enhanced carbon sequestration (Jastrow et al. 2005; Norby et al. 2005). Increased nitrogen availability may also accelerate SOM decomposition by alleviation of microbial nitrogen limitation and therefore lead to further carbon losses (Mack et al. 2004). In organic horizons where fresh organic material (plant litter and root exudates) input is highest and provides energy to soil microbes, enhanced decomposition of more recalcitrant materials can be observed (Kuzyakov 2010). In deep soil horizons fresh organic matter is scarce and microbes are limited in energy (Fontaine et al. 2007). If microbes in deep soil are limited in energy, addition of fresh organic material such as cellulose would provide the missing energy and lead to increase respiration and growth of microbes. Respiration can result from microbial growth but also the maintenance of existing cells leads to  $CO_2$  release (Hoehler & Jørgensen 2013).

Recently it was shown that protein depolymerization (Wild et al. 2015) and enzyme pattern can differ along continental transects (Schnecker et al. 2015), but are even more variable in different soil depths (Schnecker et al. 2015). The extent to which microbial mediated processes in deep soils respond to increased C and N availability, is however still poorly understood.

The objective of this master thesis therefore was to evaluate nitrogen limitation of soil organic matter decomposition in two upper mineral soil horizons along a latitudinal continental transect

in Western Siberia. Possible positive effects of climate change on plant production, such as higher input of plant material into soil were simulated by addition of cellulose. To test whether N is a limiting resource a subset of all samples received additionally protein amendments.

As N limitation has been found to increase in soils at higher latitude (Sistla et al. 2012) we hypothesized that the addition of protein as organic nitrogen source will lead to a stronger increase of microbial growth and respiration in higher latitude soils compared to lower latitudes soils. Moreover, we hypothesized that the addition of organic substrates will increase the overall enzyme activity in strongly nitrogen limited microbial communities due to the need of nitrogen for enzyme production and the need for enzymes for decomposition. Thus under such N limiting conditions, higher enzyme activities are expected after protein addition than after addition of cellulose.

Soil C:N ratios decrease with depth and concomitantly decreases the limitation in nitrogen. The upper mineral horizons are therefore rather nitrogen limited, the lower mineral horizons are more likely carbon or energy limited. We therefore hypothesized that cellulose addition will increase microbial growth/activity in lower horizons that are rather carbon than nitrogen limited, but not in upper horizons.

We incubated soil samples in triplicates for 42 days at constant temperature and moisture content. One replicate of each sample was kept untreated as a control group, the second was incubated with cellulose and the third received cellulose and protein additions. Soil respiration rates were measured weekly, soil parameters such as pH, clay content, microbial biomass, carbon and nitrogen pools and enzyme activities were determined before and after incubation.

### 3. Material and Methods

#### 3.1. Study site and soil sampling

Soils were sampled along a latitudinal transect in Western Siberia in August 2012 (Figure 1). The transect covered ecosystems in the southern tundra (TU), northern taiga (NT), middle taiga (CT), southern taiga (ST), forest-steppe (where forest (FF) and meadow (FS) ecosystems were sampled) and the steppe (SP). The mean annual temperature (MAT) along the transect ranged from -7.6°C in the tundra to +1°C in the steppe, whereas the mean annual precipitation (MAP) was highest at the northern and middle taiga (430 mm - 437.5 mm) and decreased towards north (tundra 391 mm) and south (steppe 309.2 mm). A more detailed overview of sampling sites is shown in table 1.

At each site, soils were sampled from five pits (1 m wide and 1.5 m deep), sieved (2 mm) and roots were manually removed. The soil samples were stored for five months at 4°C until the start of the incubation experiment. For this experiment two mineral horizons were chosen, more precisely the upper mineral horizon with carbon contents between 1.35% and 12.03% and the second mineral horizon with carbon contents between 0.26% – 1.41%. Soil classifications according to the World Reference Base for Soil Resources (IUSS Working Group WRB 2006) are listed in table 1. For simplification the horizons can be abbreviated as upper and lower horizon.

#### 3.2. Incubation Experiment

Before the start of the incubation experiment the water content was adjusted to 60% of the maximum water holding capacity (WHC, defined as the amount of water that a fully saturated soil is able to hold) for all soil samples. Replicates of each soil sample were weighed (30g) into 100mL glass bottles, closed with a parafilm, divided into four groups and pre-incubated at 12°C for two weeks. One set of samples (initial) was harvested before incubation to determine the initial soil status. The remaining three subgroups were incubated for 42 days at 12°C incubation temperature and the WHC was adjusted weekly. One group served as control group (K) without any addition, the second group was amended with cellulose (Z) and the third one was amended with a cellulose-protein mixture (P). Amendments were adjusted to 5% of the soil carbon content for cellulose or cellulose-protein mixture with 5atom% <sup>13</sup>C and a C:N ratio of 10:1.

#### 3.3. Respiration measurements

To determine microbial utilization of the substrates over time, soil respiration was measured weekly. Glass bottles with samples were taken out of the incubator and closed with a plastic plug in a screw-cap lid. Flasks were then evacuated using a suction pump followed by air refilling

with a known CO<sub>2</sub> concentration (91 ppm ± 6.5 standard error); this procedure was repeated four times. Then flasks were put back into the incubator and gas samples of the headspace air were taken after four and six hours for upper and lower mineral horizons, respectively. Gas (20 ml) was sampled with syringes (previously treated with ambient and compressed air to avoid contaminations) and transferred into pre-evacuated glass vials. The procedure ended with adjustment of the water content, and flasks were closed again with parafilm. Gas samples were analyzed for <sup>12</sup>CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub> concentrations using a gas bench (GasBench II system coupled to a DeltaV Advantage IRMS, Thermo Scientific).

### Calculations

$$\text{Respiration rate} = \frac{(dCO_2(\text{sample} - \text{blank}) * t)}{\frac{V_{\text{mol}} * V}{g \text{ DW}}}$$

Respiration rate...nmol CO<sub>2</sub> h<sup>-1</sup> g<sup>-1</sup>DW

t...time in hours

V<sub>mol</sub>...8.314\*285.15/101325\*1000 in L mol<sup>-1</sup>

8.314...molar gas constant; 285.15°K=12°C incubation temperature;

101325 Pa standard atmosphere pressure

V...(116-0.8\*Fresh weight)/1000 in L

116 ml ...Volume of flask; soil volume = 0.8 \*Fresh weight

g DW...Fresh weight \* (dry weight/fresh weight) in g

### *3.4. Soil properties*

The water content of soils was measured gravimetrically by drying 5g of fresh soil at 60°C for 48h. Dried and ground soil was used to determine organic carbon and nitrogen contents by an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) combined with a continuous flow stable isotope ratio mass spectrometer (DeltaPLUS, Thermo Finnigan, Bremen, Germany). Soils with high pH (FF, FS and SP) were previously treated with HCl to remove carbonates (Harris et al. 2001). Clay (<0.002 mm) content was determined via pipetting method.

### Extractable C and N, microbial biomass, N pools and potential enzyme activity

Extracts were made of 2g fresh soil with 15mL 0.5 M  $K_2SO_4$ , put on a shaker (1 hour) then filtered through ashless paper filters (Whatman Ltd., UK) and stored at  $-20^{\circ}C$  for later processing. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were analyzed with a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu). Microbial biomass (microbial carbon and nitrogen) contents were determined with ethanol free chloroform fumigation (Amato 1988) by fumigating 1g of soil, that has previously been frozen to break and lyse microbial cells. Soil was then extracted with 0.5 M  $K_2SO_4$  and analyzed with the TOC/TN analyzer. Microbial C and microbial N is the difference of extractable carbon and nitrogen after fumigation to extractable DOC and TDN without fumigation.

Ammonium ( $NH_4^+$ ) and nitrite ( $NO_3^-$ ) were measured photometrically in microtiter plates following the method modifications of Hood-Nowotny et al. (2010). Total free amino acids (TFAA) were determined using the fluorometric OPAME procedure after Jones et al. (2002).

In fresh samples potential enzyme activities of cellulose ( $\beta$ -glucosidase), exochitinase (NAGase), protease (leucine-aminopeptidase), phenoloxidase and peroxidase were measured fluorimetrically or photometrically as described in Kaiser et al. (2010). 1g soil was suspended in 100 mL of 100 mM sodium acetat buffer (pH 5.5). 200  $\mu$ L were transferred in triplicates into a microtiter plate and substrates (MUF  $\beta$ -glucopyranoside for cellulase, MUF N-acetyl- $\beta$ -glucosaminide for exochitinase, AMC L-Leucin-4-amino-methyl-coumarin for protease) added and incubated at room temperature for 140 min. Cellulase and exochitinase activities were stopped by NaOH addition. Measurements were performed with a fluorimeter (Tecan Infinite M200 fluorimeter, Werfen, Austria) at 356 nm extinction and 450 nm emission wave length. L-3,4-dihydroxyphenylalanin (DOPA) was used as substrate for phenoloxidase and peroxidase activities. Peroxidase assays additionally received 10  $\mu$ L of  $H_2O_2$ . The activity was calculated as difference between immediately photometric measurements and after 20 h (450 nm absorbance).

### *3.5. Gross nitrogen mineralization and immobilization*

For the determination of gross nitrogen mineralization rates fresh soil (2g for upper mineral horizon and 4g for lower mineral horizon) was incubated with 500 $\mu$ L ( $^{15}NH_4$ ) $_2SO_4$  (0.125mM, 10 atom%  $^{15}N$ ) for 4h and 24h and extracted with 15 mL 2M KCl, as described in Wanek et al. (2010) and Kaiser et al. (2011). Extracted  $NH_4^+$  was converted into  $NH_3$  by addition of 100mg MgO to the extractant, which was subsequently captured in acid traps (filter paper with 4  $\mu$ L 2.5M  $KHSO_4$ ). After drying in a desiccator over sulfuric acid, the acid traps were analyzed with an EA-

IRMS system (EA 1110, CE Instruments, Milano, Italy; IRMS, Delta-PLUS, Thermo Finnigan, Bremen, Germany).

### *3.6. Statistics*

Calculations of data were performed in Excel (Microsoft Office Version 2007) and graphic illustrations created in Sigma Plot 12.5 (Systac Software).

Statistical analyses were carried out in R Studio 3.0.2 (R Development Core Team 2013). Data were tested for normal distribution and homoscedasticity. Cumulative respiration was statistically determined in Sigma Plot (Systac Software) with one way ANOVAs and Tukey-HSD tests to identify significant different groups. In case of non-normal distributed data, a ranked test (Kruskal-Wallis with Tukey-HSD post-hoc test) was applied.

Response ratios were calculated by dividing the measured pool size or transformation rate of a treated sample by that of the control sample for each replicate separately. Paired t-tests were performed to evaluate significant differences between the treatment and the control and mean values calculated. A principal component analysis of all response ratios except mineralization and immobilization rates was performed and statistically surveyed in R (R Development Core Team 2013) and illustrated in Sigma Plot (Systac Software). Shapiro-Wilk was used to test for normal distribution and Wilcoxon test was performed for homoscedasticity.

## 4. Results

### 4.1. Initial soil properties

In soils from all sites, organic  $C_{\text{soil}}$ , total  $N_{\text{soil}}$ ,  $C:N_{\text{soil}}$ , total dissolved nitrogen, microbial C and microbial N were consistently higher in upper than lower mineral horizons, while microbial C:N was higher in lower horizons. Similarly, all sites except steppe and forest-steppe meadow showed higher dissolved organic N and ammonium concentrations in the upper horizon. Nitrate increased with depth in the three northern sites and decreased with depth at the other sites. In all but the forest-steppe site total free amino acid concentrations were lower than ammonium concentrations and close to the detection limit in most of the lower mineral horizons. Similar to total C concentration, dissolved organic carbon (DOC) was highest in the middle taiga and decreased with soil depth, except for northern taiga, forest-steppe meadow and steppe, where the lower horizon contained more DOC (Table 2).

Soil pH values were acidic and ranged between 3.06 and 3.86 at the northern sites (tundra and taiga) and increased towards the south to 5.08 and 7.92 in the steppe (upper and lower horizon, respectively). Clay content ranged between 5.72 to 40.0 %, with lowest values in both horizons of tundra and southern taiga (5.72 to 12.45 %), and highest values in the lower horizons of forest-steppe meadow and forest-steppe forest (40.0 and 34.0 %, respectively)(Table 1). Differences between upper and lower horizons of individual sites did not show a consistent pattern.

To assess the stoichiometric imbalance between soil microbial community and its resource the ratio of resource C:N ( $C:N_{\text{soil}}$ ) over microbial biomass C:N ( $C:N_{\text{mic}}$ ) was calculated. The middle taiga showed highest values of 2.95 and 2.39 in the upper and lower horizon. Ratios consistently decreased with depth and lower mineral horizon of tundra, northern taiga and southern taiga showed lowest ratios with 0.47 and 0.55, respectively.

A Principle component analysis (PCA) of all measured soil parameters revealed a clear separation of upper and lower horizons with more variation within upper horizons than within lower horizons. The differentiation of upper and lower horizons along PC1 axis (explaining 52% of the variation), was mainly driven by total dissolved nitrogen, ammonium and total free amino acids. PC2 (explaining 20% of the variation), separated the northern sites (tundra, northern and middle taiga) from the four southern sites, driven by  $C:N_{\text{soil}}$ , DOC content and pH (Figure 2).

#### 4.2. Responses to cellulose and cellulose-protein amendments

##### 4.2.1. Effects of substrate amendments on respiration

Cumulative respiration after the six weeks incubation time of the control group (without amendments) was highest in both middle taiga horizons (52.2 and 6.01  $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ DW}$ , respectively). The respiration-ratio of upper to lower mineral horizons of respiration per unit dry weight varied between two and 29-fold (steppe, tundra and southern taiga), whereas ratios of cumulative respiration between the two horizons calculated per unit C were lower with values between 0.5 and 3.6 (forest-steppe forest and tundra). Highest respiration per unit C was found in upper horizon of tundra and lower horizon of steppe (1 326 and 1 294  $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ C}$ ) (Table 3). Significant differences of cumulative respiration (per g DW) in pairwise comparisons within horizons were found 8 out of 20 possible times in upper mineral horizon and 10 out of 20 in lower mineral horizon (ANOVA with Tukey HSD post hoc  $p < 0.05$  if normal distributed or Kruskal-Wallis with Tukey-HSD post hoc  $p < 0.05$ ) (Figure 3).

Cellulose as well as cellulose-protein additions led to an increase in respiration rates over time compared to the control with few exceptions in the lower horizon, where respiration rates remained unchanged. In the upper horizons especially the cellulose-protein amendments led to an increase in soil respiration. In week four of the incubation process the respiration rates of the cellulose-protein amended soils drop in tundra, northern taiga, middle taiga and southern taiga in both horizons and increased only slightly afterwards. A similar but weaker pattern was observed for the cellulose treatment at these sites. Respiration rates in the forest-steppe forest and forest-steppe meadow decreased at week four in both horizons after cellulose-protein treatment, but were stable in the cellulose treatment. The steppe did not reveal any such pattern in either horizon except for a higher respiration rate at the beginning of the incubation (Figure 4). Cumulative respiration after six weeks incubation time was significantly higher in all sites but tundra upper horizon and middle taiga lower horizon. Cellulose exclusively induced significant increases in respiration in upper horizons of northern taiga, forest-steppe forest and steppe (Figure 5).

##### 4.2.2. Effects of substrate additions on carbon and nitrogen pools, microbial biomass and extracellular enzyme activities

Effects of substrate additions on soil parameters are displayed as response ratios calculated by dividing the pools and rates of the treated samples by the control samples (paired t-test  $p < 0.05$ ) (Figures 6 and 7).

Cellulose additions significantly increased the dissolved organic carbon in southern taiga upper horizon and tundra, middle taiga, forest-steppe forest, forest-steppe meadow and steppe lower horizon. Total dissolved nitrogen concentrations decreased significantly in middle taiga, southern taiga, forest-steppe forest and steppe upper horizons and middle taiga, forest-steppe forest and steppe lower horizon. Furthermore, dissolved organic nitrogen decreased significantly in both horizons of middle taiga and the lower horizon of southern taiga. Ammonium decreased in northern and middle taiga upper horizon and increased in forest-steppe meadow lower horizon. Nitrate decreased in forest-steppe forest upper horizon and both horizons of the steppe. Moreover, total free amino acids increased in southern taiga upper horizon and steppe lower horizon. Nitrogen mineralization decreased after cellulose amendments in both horizons forest-steppe meadow and southern taiga upper horizon.

Microbial C, N and C:N ratios exhibited only a few significance response ratios. Microbial C decreased in tundra both horizons and forest-steppe forest lower horizon while microbial N decreased in northern taiga and forest-steppe meadow upper horizon and forest-steppe forest lower horizon. Microbial C:N significantly decreased in tundra both horizons, but increased in southern taiga upper horizon and forest-steppe forest lower horizon (Figure 6).

The addition of a cellulose-protein mixture, led to more significant changes in the above described parameters compared to cellulose alone. Dissolved organic carbon significantly increased in northern and middle taiga and steppe upper horizon and steppe lower horizon and by contrast decreased in forest-steppe meadow lower horizon. In case of total dissolved nitrogen all but middle and southern taiga upper horizon and tundra lower horizon increased after substrate addition, whereas dissolved organic nitrogen significantly decreased only in forest-steppe forest upper horizon. Ammonium concentrations increased in tundra, forest-steppe forest, forest-steppe meadow and steppe upper horizon and all sites except for tundra measurements in case of the lower horizons. Furthermore, nitrate decreased in middle taiga upper horizon and increased in forest-steppe meadow lower horizon. Total free amino acids increase in northern and middle taiga and decreased in forest-steppe meadow upper horizon and decreased in forest-steppe forest lower horizon. Nitrogen mineralization rates tended to increase, but only significantly in tundra, forest-steppe forest upper horizon and steppe lower horizon, while decreased in forest-steppe meadow upper horizon. In the lower horizons nitrogen mineralization was close to or below detection limit. Nitrogen immobilization rates mostly increased in both horizons; significant differences were found in forest-steppe forest for the upper horizon and middle taiga for the lower horizon.

Microbial C decreased in tundra for both horizons, increased in forest-steppe forest upper

horizon and decreased in lower horizon. Microbial N decreased in northern taiga upper horizon and forest-steppe forest lower horizon. Moreover, microbial C:N decreased in tundra upper horizon and increased in southern taiga upper horizon and northern taiga and forest-steppe forest lower horizon (Figure 7).

Potential activity rates of all measured extracellular enzymes (phenoloxidase, peroxidase,  $\beta$ -glucosidase, NAGase and leucine-aminopeptidase) showed variable and mostly insignificant responses to cellulose additions in both horizons. Leucine-aminopeptidase increased in southern taiga and decreased in forest-steppe forest upper horizons. The other enzymes activity changes in upper horizons were not significant. Phenoloxidase and peroxidase activities increased in tundra and phenoloxidase activity also increased forest-steppe meadow lower horizon. Only in upper horizons of tundra soils  $\beta$ -glucosidase decreased. NAGase decreased in southern taiga and leucine-aminopeptidase increased in steppe lower horizon (Figure 6). However, combined cellulose and protein additions induced more increases in potential enzyme activities. Phenoloxidase increased in northern, middle taiga and forest-steppe meadow upper horizon, as well as in middle, southern taiga and steppe lower horizon. Peroxidase only increased in forest-steppe meadow both horizons and tundra lower horizon. NAGase showed higher activity in forest-steppe meadow and steppe lower horizon and leucine-aminopeptidase increased in all taiga sites upper horizon and tundra, middle taiga, forest-steppe meadow and steppe lower horizon (Figure 7).

#### PCA response ratios

A principle component analysis of the response ratios revealed a significant differentiation along PC1 axis (explaining 22% of variation) between cellulose and cellulose-protein additions of both horizons and all sites, except for two samples (Wilcoxon test,  $p < 0.001$ ). Main driving factors were the enzyme activities of phenoloxidase, peroxidase,  $\beta$ -glucosidase and NAGase. These enzyme rates responded more likely positive to additional protein than to only cellulose additions to the soils. Microbial carbon and nitrogen as well as total free amino acids, dissolved organic carbon, total dissolved nitrogen and ammonium separated the samples along PC axis 2 (explaining 16%) (Figure 8).

## 5. Discussion

### 5.1. Initial soil conditions

Soil properties (i.e., organic soil C, total soil N,  $C:N_{\text{Soil}}$ , DOC, TDN, DON,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , TFAA,  $C_{\text{Mic}}$  and  $N_{\text{Mic}}$ ) neither increased nor decreased consistently with latitude, in fact highest values were mostly found in the middle taiga ecosystem. These properties excluding ammonium, nitrate and total free amino acids consistently decreased with depth in almost every site. Significant decrease of organic C and total N with depth has been reported in Wild et al. (2015). Nitrate concentrations were higher in lower mineral horizon than upper mineral horizon in the three most northern sites (tundra, northern and middle taiga) with maximum values detected in southern sites (forest-steppe forest upper horizon and steppe lower horizon). This is similar to another study, where an increase in inorganic nitrogen compounds compared to the total dissolved nitrogen pool of mineral soil horizons suggested less N limitation of microorganisms due to enhanced mineralization and nitrification (Wild et al. 2015). We found differences in pH and clay content along the transect. The pH was acidic in the north and increased towards south with relatively high values in the steppe, where additionally higher content of carbonates occurred. Lower clay content in tundra and southern taiga may result in less stability for organic matter due to the missing protection by sorption to mineral surfaces (Rumpel & Kögel-Knabner 2011).

Highest cumulative respiration of control soils was found in middle taiga upper mineral horizon (Figure 3) together with highest organic C and DOC concentrations. Despite relatively high soil organic carbon contents of forest-steppe forest upper horizon, cumulative respiration was lowest. In this soil the clay content was high (30.9 %) presumably resulting in stabilization of existing carbon as the stabilization processes proposed by Rumpel & Kögel-Knabner (2011). The ratios of cumulative respiration of upper to lower horizon were particularly high in soils of tundra and southern taiga due to relatively low respiration rates of the lower mineral horizon suggesting C or energy limitation of these lower soil horizons (Table 3). Low organic carbon content (4.13 and 4.79 mg C g<sup>-1</sup> DW, respectively) and C:N imbalances of 0.47 (tundra) and 0.55 (southern taiga) corroborate this suggestion. Less pronounced differences in cumulative respiration between horizons occurred after calculation per unit C indicating similar degradation potential of present soil carbon by microbes of deeper soil horizons.

According to the theory of stoichiometric imbalance and proposed limitations in either energy (C) or nutrients (i.e. N) (Mooshammer, Wanek, Zechmeister-Boltenstern, et al. 2014) the middle taiga showed highest values (2.95 and 2.39) suggesting that microbes in these soils appear to be limited by nitrogen. Aside from stoichiometry other factors such as the chemical composition of organic matter and availability for microbes could control decomposition in these soils (Schnecker et al. 2015; Schmidt et al. 2011; Lützow et al. 2006; Rumpel & Kögel-Knabner 2011). Taken together the initial soil conditions exhibited no consistent latitudinal pattern neither in soil C, N or C:N ratios nor nitrogen containing pools, hence as expected the measured soil parameters mostly decreased with depth.

### *5.2. Responses to amendments*

Our first hypothesis was that protein addition as an organic nitrogen source will enhance microbial growth and respiration more in higher latitude soils because of their stronger N limitation compared to lower latitude soils. Hence, we analyzed cumulative respiration and microbial biomass. In contrast to our hypothesis no consistent latitudinal pattern after cellulose or cellulose plus protein amendment in respiration or microbial biomass was observable. Cumulative respiration increased with cellulose and protein amendment significantly compared to the control group in all upper mineral horizons except for the most northern site, where the increase did not lead to a significant result (ANOVA with Tukey HSD  $p > 0.05$ ). We measured the highest increase with over 800% at forest-steppe forest upper mineral horizon (Figure 5). Respiration of the corresponding control was especially low despite moderate initial soil organic C and high total N concentrations. This disproves our hypothesis that higher latitude soils will react more on N-containing substrate with a latitudinal decrease following the postulated increase of N limitation polewards (Reich & Oleksyn 2004). Although soil C:N ratio of forest-steppe forest upper horizon was relatively low (12.9), cumulative respiration increased most with cellulose and protein addition. Other studies found substrate accessibility or physical controls (i.e., minerals) especially in lower soil horizons are more important than stoichiometry for decomposition of organic matter (Lützow et al. 2006; Schmidt et al. 2011). In accordance with our hypothesis northern taiga upper horizon with highest C:N of 27.4, indicating microbial N limitation, showed second highest cumulative respiration values after substrate supplementation.

Contrary to our expectations, microbial C decreased in the most northern site after addition of cellulose and protein. In both tundra soils, significant decreases in microbial carbon and insignificant lower microbial nitrogen were found, accompanied with decreases in microbial C:N ratios. Except for forest-steppe forest upper horizon where microbial C and N increased, remaining sites and horizons did not change significantly in microbial biomass. With regard to the phenomenon called 'priming effect', first reaction of soil microbes to fresh organic matter supply is often an increase in turnover of carbon of predominantly fast growing r-strategists, leading to an apparent priming effect, i.e., to an enhanced liberation of carbon from soil, which is not due to enhanced soil organic matter decomposition. This apparent priming effect can then be followed by an enhanced SOM turnover by soil microorganisms (e.g., by slow growing K-strategists) which is then called the real priming effect (Kuzyakov 2010). Our findings of decreased or unchanged microbial biomass accompanied by a significantly increased respiration rates and increased  $\delta^{13}\text{C}$  values in  $\text{CO}_2$  (data not shown), suggest that microbes actively decomposed the amended cellulose and protein. Unfortunately, the priming effect could not be analyzed in our study due to problems with the uniform distribution of the label in the soil. Another study of priming in mineral soils revealed enhanced SOM decomposition after substrate amendments without affecting microbial growth or community structure (Wild et al. 2014) indicating that a large proportion of inactive microbes which only become active when substrate is available occur in arctic soils. Kuzyakov (2010) proposed further characteristics of microbial activity, substrate utilization or community structures are critical for the better understanding of the underlying mechanisms.

Our second hypothesis implied the consequences of microbial enzyme production after substrate addition under N limited conditions. We expected higher enzyme activities after cellulose and protein addition compared to solely cellulose amendment, since microbes need N to produce enzymes. The enzyme pattern illustrated in the figures 6 and 7 via heat maps of response ratios proved this hypothesis. Additional protein as N source enhanced potential enzyme activities compared to the supply of carbon only, thus displaying the microbial N demand and utilization.

Exclusive cellulose addition led to only one significant enhanced enzyme activity in upper horizons (leucine-aminopeptidase in southern taiga) and few in the lower mineral horizon (phenoloxidase in tundra and forest-steppe meadow, peroxidase in tundra and leucine-aminopeptidase in steppe). Moreover,  $\beta$ -glucosidase as a specifically cellulose degrading enzyme (Bhat & Bhat 1997) only decreased in lower mineral horizon (tundra) without significant changes in the remaining sites. This is similar to findings by Allison & Vitousek (2005), who detected no

significant increase in specific enzyme production after adding corresponding substrate (i.e., no significant increase in  $\beta$ -glucosidase after cellulose addition). By contrast, enzyme production increased when required simple molecules such as ammonium and phosphate were supplemented in addition. They concluded that microorganisms are only able to enhance their enzyme production if other required nutrients are available. Therefore, we suggest that more nitrogen is bioavailable in lower mineral horizons than in upper mineral horizons, because of the higher potential response of enzyme activities in the latter horizon after cellulose addition.

However, a more pronounced increase in enzymes was found after cellulose and protein addition. This is in line with our hypothesis that enzyme activities increase with an additional N source in N limited ecosystems. Continually excreted enzymes (Allison et al. 2010) may have targeted proteins, and the primarily degradation products provided N for further enzyme production as found by Allison & Vitousek (2005). Most significant increases were found for leucine-aminopeptidase and phenoloxidase (seven and six times, respectively). Leucine-aminopeptidase is commonly used as model for protein degrading enzymes (Sinsabaugh et al. 2009), hence, production can be induced by protein addition. Oxidative enzymes like phenoloxidases are thought to target more recalcitrant SOM and are often utilized by nutrient miners to make N and/or C available (Moorhead et al. 2012). Thus, decomposers may have produced phenoloxidase to degrade complex molecules to aim for nitrogen. In upper mineral horizons leucine-aminopeptidase increased significantly in all taiga sites suggesting that microbes in these soils could be severely limited by N. Moreover, in the same soils ammonium concentrations did not increase significantly suggesting high nitrogen use efficiency and confirming higher N limitation of higher latitudinal soils, excluding tundra. Significantly enhanced mineralization rates and ammonium concentrations were found after C and N substrate addition leading to the assumption of lower nitrogen use efficiency and less limitation of N in the tundra soil. Further constraints than soil organic matter stoichiometry may occur in tundra because initial data of soil C:N and N containing pools would suggest N limitation. Additionally, leucine-aminopeptidase significantly increased in four lower soil horizons, but without latitudinal trend. Especially in lower horizons of tundra and forest-steppe forest an increase in enzymes was accompanied with decreases of microbial biomass. The total microbial community in soils includes microorganisms of various stages, i.e. active, potentially active, dormant or dead microorganisms. While only few are active, a higher proportion has the ability to switch to active state if they receive fresh organic matter (Blagodatskaya & Kuzyakov 2013). Our findings suggest that only a part of the potentially active and dormant microbes were actually able to utilize the amendments and increased respiration and enzyme production.

In summary, enzyme activities improved with additional N to cellulose addition as illustrated in the principal component analysis (Figure 8) and act as main separation factors between both treatments (PCA axis 1) additionally confirming N limitation of the sample soils.

We further hypothesized higher microbial activity after cellulose addition in lower than in upper mineral horizons due to a decrease in nitrogen limitation with depth based on decreasing C:N ratios. This alleviating effect of cellulose addition on microbial activity of lower mineral horizons could not be unequivocally supported with this study. The declining C:N ratios of initial soil parameters strengthened our hypothesis of decreasing limitation in nitrogen with soil depth. Additionally to decreasing C:N ratios, C:N<sub>Soil</sub> to C:N<sub>Mic</sub> imbalances of tundra, northern taiga and southern taiga lower horizon (0.47 and 0.55) supported our hypothesis. However, significant increases in microbial respiration occurred only in the upper horizon of northern taiga, forest-steppe forest and steppe with insignificant increases of residual sites. No significant increases were found for all lower horizons (Figure 5). This suggests better utilization of cellulose in upper mineral horizons although these horizons seem to exhibit a stronger N limitation. Contrary, we found enhanced enzyme activities, which is also an indicator for microbial activity in lower horizons. This was accompanied with reduced dissolved organic carbon concentrations in almost every site, indicating a rigorous consumption of available C. In the upper horizon enzyme activities and dissolved organic carbon concentrations remained mainly unchanged. In summary, microorganisms in upper mineral horizons seem to invest less into enzyme production, but invest C in metabolic activities leading to higher respiration, and had higher dissolved organic carbon contents in their environment. In contrast, microbes of lower mineral horizons appear to invest more likely into enzyme production, thus respiring less and exhaust soil dissolved organic carbon. In comparison to the positive effects of cellulose plus protein amendment in both horizons and the conclusion of microbial nitrogen limitation, SOM quantity and stoichiometry were no accurate predictors of microbial limitations in mineral soil horizons. These findings are supported by other studies (Schnecker et al. 2015; Wild et al. 2015). Moreover, similar results were reported by Sistla et al. (2012) where microbial activities in tussock tundra soils could not be increased with cellulose addition but indeed increased after supplementary N containing amendments.

In conclusion, mineral soil horizons from arctic and subarctic ecosystems have the potential to increase carbon losses as a response to increased labile substrate availability, a scenario that could likely emerge in regard of climate change. Respiration increased significantly in nearly all studied sites and in both soil horizons after the addition of cellulose and protein, but in contrast

to our expectations there was no latitudinal trend detectable. Moreover, supply of exclusive carbon affected respiration less, underpinning the importance of nitrogen for the microbial processes. Microbial biomass was little responsive to both amendments; only when nitrogen was supplied additionally, the production of enzymes increased which is a further evidence for the nitrogen limitation of high latitudinal soils.

## 6. Tables and Figures

Table 1: Sampling site characteristics. MAT (mean annual temperature); MAP (mean annual precipitation); soil types were classified by Norman Gentsch, Institute of Soil Science, Leibniz Universität Hannover, Germany.

| Site                                 | Coordinates <sup>a</sup>       | MAT <sup>a</sup><br>(°C) | MAP <sup>a</sup><br>(mm) | Soil type <sup>b</sup> | Horizon <sup>c</sup> | Horizon<br>type <sup>b</sup> | Depth <sup>b</sup><br>(cm) | Carbon<br>content<br>(%) | Clay<br>(%)    |
|--------------------------------------|--------------------------------|--------------------------|--------------------------|------------------------|----------------------|------------------------------|----------------------------|--------------------------|----------------|
| <b>Tundra</b>                        | 67°16'20.05"N<br>78°50'13.85"E | -7.6                     | 392                      | Turbic Cryosol         | Upp.m.h.             | A                            | 2-13                       | 2.25-3.78                | 12.5 (± 0.84)  |
|                                      |                                |                          |                          |                        | Low.m.h.             | Bg,BCg                       | 6-57                       | 0.26-0.58                | 9.26 (± 1.22)  |
| <b>Northern<br/>Taiga</b>            | 63°17'37.54"N<br>74°32'9.18"E  | -4.6                     | 430                      | Histic Podzol          | Upp.m.h.             | EA,AE                        | 8-30                       | 2.83-4.48                | 29.1 (± 10.13) |
|                                      |                                |                          |                          |                        | Low.m.h.             | Bg                           | 14-47                      | 0.49-1.41                | 22.5 (± 1.14)  |
| <b>Middle<br/>Taiga</b>              | 60°09'27.08"N<br>71°42'57.34"E | -2.2                     | 438                      | Endogleyic Regosol     | Upp.m.h.             | A,EA,AE                      | 6-14                       | 3.12-12.03               | 22.3 (±1.80)   |
|                                      |                                |                          |                          |                        | Low.m.h.             | E,EA                         | 12-55                      | 0.75-2.72                | 27.0 (± 1.03)  |
| <b>Southern<br/>Taiga</b>            | 58°17'58.90"N<br>68°34'53.71"E | -0.5                     | 396                      | Albic Podzol           | Upp.m.h.             | A,AE                         | 4-18                       | 3.34-5.53                | 8.42 (±1.23)   |
|                                      |                                |                          |                          |                        | Low.m.h.             | E,EA                         | 15-59                      | 0.39-0.57                | 5.72 (± 1.04)  |
| <b>Forest-<br/>steppe<br/>forest</b> | 56°14'11.56"N<br>70°42'54.90"E | 0.7                      | 340                      | Haplic Phaeozem        | Upp.m.h.             | A                            | 7-46                       | 3.59-6.24                | 30.9 (± 1.39)  |
|                                      |                                |                          |                          |                        | Low.m.h.             | B                            | 57-109                     | 0.47-0.55                | 34.0 (± 0.66)  |
| <b>Forest-<br/>steppe<br/>meadow</b> | 56°13'54.50"N<br>70°43'28.46"E | 0.7                      | 340                      | Luvic Phaeozem         | Upp.m.h.             | A                            | 4-35                       | 1.98-2.88                | 24.1 (± 1.96)  |
|                                      |                                |                          |                          |                        | Low.m.h.             | Bt                           | 26-84                      | 0.49-0.69                | 40.0 (± 0.45)  |
| <b>Steppe</b>                        | 54°41'41.33"N<br>71°38'45.88"E | 1                        | 309                      | Calcic Kastanozem      | Upp.m.h.             | Ak                           | 8-37                       | 1.35-2.88                | 20.6 (± 1.90)  |
|                                      |                                |                          |                          |                        | Low.m.h.             | Bk                           | 27-109                     | 0.48-0.89                | 27.9 (± 2.47)  |

<sup>a</sup>: Stolbovoi & McCallum (2002). Land resources of Russia (CD). International Institute for Applied Systems Analysis and the Russian Academy of Science, Laxenburg, Austria.

<sup>b</sup>: Data from Wild et al. (2015)

<sup>c</sup>: Upp.m.h.: upper mineral horizon; Low.m.h.: lower mineral horizon

Table 2: Initial soil characteristics. Depicted are mean values ( $n=5$ ,  $\pm$  standard error) of the soil C to N ratio on a mass basis ( $C:N_{\text{Soil}}$ ), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), total free amino acids (TFAA), ratio C to N microbial on mass basis ( $C:N_{\text{Mic}}$ ), ratio of soil C:N over microbial C:N ( $C:N_{\text{Soil}}/C:N_{\text{Mic}}$ ).

Upper mineral horizon (Upp. m. h.), lower mineral horizon (Low. m. h.); not detectable (nd); \*, 1 replicate only.

|                             | Organic C <sub>Soil</sub> | Total N <sub>Soil</sub>   | C:N <sub>Soil</sub> | pH            | Clay           | DOC                       | TDN                       | DON                       | NH <sub>4</sub> <sup>+</sup>                            | NO <sub>3</sub> <sup>-</sup>                            | TFAA                           | C <sub>Mic</sub>        | N <sub>Mic</sub>        | C:N <sub>Mic</sub> | C:N <sub>Soil</sub> :to -<br>C:N <sub>Mic</sub> |
|-----------------------------|---------------------------|---------------------------|---------------------|---------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------------------------------------|---------------------------------------------------------|--------------------------------|-------------------------|-------------------------|--------------------|-------------------------------------------------|
|                             | [mg C g <sup>-1</sup> DW] | [mg N g <sup>-1</sup> DW] |                     | (KCl)         | %              | [μg C g <sup>-1</sup> DW] | [μg N g <sup>-1</sup> DW] | [μg N g <sup>-1</sup> DW] | [μg NH <sub>4</sub> <sup>+</sup> -N g <sup>-1</sup> DW] | [μg NO <sub>3</sub> <sup>-</sup> -N g <sup>-1</sup> DW] | [μg TFAA-N g <sup>-1</sup> DW] | [μg g <sup>-1</sup> DW] | [μg g <sup>-1</sup> DW] |                    |                                                 |
| <b>Tundra</b>               |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 30.4 (± 3.05)             | 1.83 (± 0.12)             | 16.4 (± 0.73)       | 3.70 (± 0.03) | 12.5 (± 0.84)  | 104 (± 25.2)              | 8.76 (± 1.62)             | 6.79 (± 1.33)             | 1.72 (± 0.60)                                           | 0.24 (± 0.05)                                           | 0.88 (± 0.25)                  | 120 (± 32.2)            | 13.9 (± 3.99)           | 8.90 (± 0.63)      | 1.90 (± 0.18)                                   |
| Low. m. h.                  | 4.13 (± 0.51)             | 0.37 (± 0.04)             | 11.1 (± 0.63)       | 3.86 (± 0.05) | 9.26 (± 1.22)  | 34.2 (± 5.31)             | 2.65 (± 0.36)             | 1.54 (± 0.28)             | 0.69 (± 0.25)                                           | 0.41 (± 0.11)                                           | 0.03 (± 0.01)                  | 34.6 (± 3.82)           | 1.34 (± 0.30)           | 31.3 (± 6.63)      | 0.47 (± 0.13)                                   |
| <b>Northern taiga</b>       |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 37.0 (± 3.12)             | 1.36 (± 0.08)             | 27.4 (± 1.99)       | 3.06 (± 0.05) | 29.1 (± 10.13) | 130 (± 13.3)              | 9.91 (± 1.27)             | 7.34 (± 0.38)             | 2.29 (± 0.91)                                           | 0.27 (± 0.03)                                           | 0.59 (± 0.03)                  | 66.5 (± 4.79)           | 5.34 (± 0.80)           | 14.2 (± 3.06)      | 2.35 (± 0.50)                                   |
| Low. m. h.                  | 8.17 (± 1.71)             | 0.50 (± 0.06)             | 15.7 (± 1.53)       | 3.72 (± 0.06) | 22.5 (± 1.14)  | 146 (± 15.1)              | 5.71 (± 0.56)             | 4.13 (± 0.38)             | 0.95 (± 0.26)                                           | 0.64 (± 0.07)                                           | 0.20 (± 0.03)                  | 177 (± 23.0)            | 5.89 (± 0.75)           | 30.7 (± 2.60)      | 0.55 (± 0.12)                                   |
| <b>Middle taiga</b>         |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 75.0 (± 17.26)            | 3.46 (± 0.65)             | 20.8 (± 1.85)       | 3.32 (± 0.08) | 22.3 (± 1.80)  | 186 (± 13.6)              | 24.9 (± 4.32)             | 15.4 (± 1.83)             | 9.19 (± 2.52)                                           | 0.33 (± 0.04)                                           | 1.60 (± 0.19)                  | 365 (± 78.0)            | 54.4 (± 14.8)           | 7.90 (± 1.33)      | 2.95 (± 0.55)                                   |
| Low. m. h.                  | 16.7 (± 3.76)             | 0.97 (± 0.13)             | 16.3 (± 1.70)       | 3.48 (± 0.05) | 27.0 (± 1.03)  | 121 (± 6.11)              | 7.14 (± 0.99)             | 4.50 (± 0.78)             | 1.15 (± 0.32)                                           | 1.48 (± 0.41)                                           | 0.37 (± 0.01)                  | 53.2 (± 10.4)           | 6.37 (± 0.69)           | 8.42 (± 1.38)      | 2.39 (± 0.70)                                   |
| <b>Southern taiga</b>       |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 43.4 (± 3.64)             | 3.11 (± 0.18)             | 14.0 (± 0.80)       | 3.62 (± 0.07) | 8.42 (± 1.23)  | 121 (± 18.9)              | 18.5 (± 3.14)             | 5.54 (± 1.57)             | 6.51 (± 2.38)                                           | 6.39 (± 2.31)                                           | 1.04 (± 0.05)                  | 277 (± 26.6)            | 34.9 (± 4.42)           | 8.09 (± 0.45)      | 1.75 (± 0.16)                                   |
| Low. m. h.                  | 4.79 (± 0.30)             | 0.51 (± 0.03)             | 9.4 (± 0.18)        | 3.76 (± 0.07) | 5.72 (± 1.04)  | 40.9 (± 6.37)             | 2.44 (± 0.38)             | 1.70 (± 0.32)             | 0.32*                                                   | 0.68 (± 0.20)                                           | 0.10 (± 0.02)                  | 74.0 (± 7.32)           | 4.29 (± 0.41)           | 17.3 (± 0.88)      | 0.55 (± 0.03)                                   |
| <b>Forest-steppe forest</b> |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 45.6 (± 4.52)             | 3.57 (± 0.43)             | 12.9 (± 0.25)       | 4.26 (± 0.06) | 30.9 (± 1.39)  | 56.3 (± 4.13)             | 7.01 (± 0.81)             | 2.96 (± 0.35)             | 0.35 (± 0.06)                                           | 3.56 (± 0.97)                                           | 0.46 (± 0.02)                  | 140 (± 11.6)            | 18.0 (± 1.39)           | 7.79 (± 0.14)      | 1.66 (± 0.04)                                   |
| Low. m. h.                  | 5.16 (± 0.15)             | 0.52 (± 0.03)             | 10.1 (± 0.35)       | 4.06 (± 0.04) | 34.0 (± 0.66)  | 35.4 (± 7.15)             | 2.11 (± 0.22)             | 1.43 (± 0.20)             | 0.17 (± 0.05)                                           | 0.50 (± 0.14)                                           | 0.05 (± 0.03)                  | 30.6 (± 7.98)           | 2.51 (± 0.34)           | 11.8 (± 1.98)      | 0.98 (± 0.20)                                   |
| <b>Forest-steppe meadow</b> |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 24.5 (± 1.57)             | 1.87 (± 0.11)             | 13.0 (± 0.13)       | 4.14 (± 0.02) | 24.1 (± 1.96)  | 37.5 (± 3.41)             | 3.43 (± 0.25)             | 2.86 (± 0.22)             | 0.35 (± 0.06)                                           | 0.23 (± 0.03)                                           | 0.39 (± 0.05)                  | 181 (± 21.7)            | 19.3 (± 2.86)           | 9.56 (± 0.42)      | 1.38 (± 0.06)                                   |
| Low. m. h.                  | 5.85 (± 0.35)             | 0.55 (± 0.03)             | 10.7 (± 0.22)       | 4.02 (± 0.07) | 40.0 (± 0.45)  | 41.3 (± 3.27)             | 2.35 (± 0.31)             | 1.67 (± 0.09)             | 0.50 (± 0.27)                                           | 0.18 (± 0.03)                                           | nd                             | 51.5 (± 3.67)           | 3.67 (± 0.29)           | 14.3 (± 1.18)      | 0.76 (± 0.05)                                   |
| <b>Steppe</b>               |                           |                           |                     |               |                |                           |                           |                           |                                                         |                                                         |                                |                         |                         |                    |                                                 |
| Upp. m. h.                  | 20.1 (± 2.73)             | 1.84 (± 0.21)             | 10.8 (± 0.26)       | 5.08 (± 0.32) | 20.6 (± 1.90)  | 66.5 (± 8.23)             | 6.23 (± 0.82)             | 1.80 (± 0.52)             | 0.09 (± 0.03)                                           | 4.55 (± 1.25)                                           | 0.06 (± 0.03)                  | 210 (± 15.6)            | 25.4 (± 2.08)           | 8.30 (± 0.28)      | 1.32 (± 0.07)                                   |
| Low. m. h.                  | 7.16 (± 0.81)             | 0.79 (± 0.10)             | 9.2 (± 0.18)        | 7.92 (± 0.41) | 27.9 (± 2.47)  | 86.0 (± 9.37)             | 5.01 (± 0.26)             | 3.04 (± 0.55)             | nd                                                      | 1.98 (± 0.39)                                           | 0.03 (± 0.01)                  | 78.8 (± 10.5)           | 8.95 (± 1.03)           | 8.72 (± 0.31)      | 1.06 (± 0.05)                                   |

Table 3: Cumulative respiration (week 0-6) of control group per g dry weight and g C content. Ratios are calculated by upper mineral horizon over lower mineral horizon.

|                      | Cumulative respiration<br>$\mu\text{mol CO}_2 \text{ g}^{-1} \text{ DW } (\pm \text{std. error})$ |                       | Ratio<br>Upp/Low | Cumulative respiration<br>$\mu\text{mol CO}_2 \text{ g}^{-1} \text{ C } (\pm \text{std. error})$ |                       | Ratio<br>Upp/Low |
|----------------------|---------------------------------------------------------------------------------------------------|-----------------------|------------------|--------------------------------------------------------------------------------------------------|-----------------------|------------------|
|                      | Upp.m.h. <sup>a</sup>                                                                             | Low.m.h. <sup>a</sup> |                  | Upp.m.h. <sup>a</sup>                                                                            | Low.m.h. <sup>a</sup> |                  |
| Tundra               | 41.9 ( $\pm 12.0$ )                                                                               | 1.72 ( $\pm 0.27$ )   | 29               | 1 326 ( $\pm 344$ )                                                                              | 421 ( $\pm 43$ )      | 3.6              |
| Northern Taiga       | 19.2 ( $\pm 1.69$ )                                                                               | 4.01 ( $\pm 0.75$ )   | 5                | 547 ( $\pm 85$ )                                                                                 | 508 ( $\pm 35$ )      | 1.1              |
| Middle Taiga         | 52.2 ( $\pm 9.26$ )                                                                               | 6.01 ( $\pm 1.71$ )   | 10               | 751 ( $\pm 67$ )                                                                                 | 344 ( $\pm 24$ )      | 2.2              |
| Southern Taiga       | 39.0 ( $\pm 4.73$ )                                                                               | 1.47 ( $\pm 0.17$ )   | 29               | 890 ( $\pm 51$ )                                                                                 | 304 ( $\pm 27$ )      | 3.0              |
| Forest-steppe Forest | 12.0 ( $\pm 1.27$ )                                                                               | 3.17 ( $\pm 0.25$ )   | 4                | 272 ( $\pm 41$ )                                                                                 | 614 ( $\pm 45$ )      | 0.5              |
| Forest-steppe Meadow | 23.2 ( $\pm 3.29$ )                                                                               | 2.62 ( $\pm 0.12$ )   | 9                | 940 ( $\pm 106$ )                                                                                | 452 ( $\pm 22$ )      | 2.1              |
| Steppe               | 15.9 ( $\pm 1.61$ )                                                                               | 8.71 ( $\pm 0.44$ )   | 2                | 849 ( $\pm 139$ )                                                                                | 1 294 ( $\pm 181$ )   | 0.7              |

<sup>a</sup>: Upp.m.h....upper mineral horizon; Low.m.h....lower mineral horizon

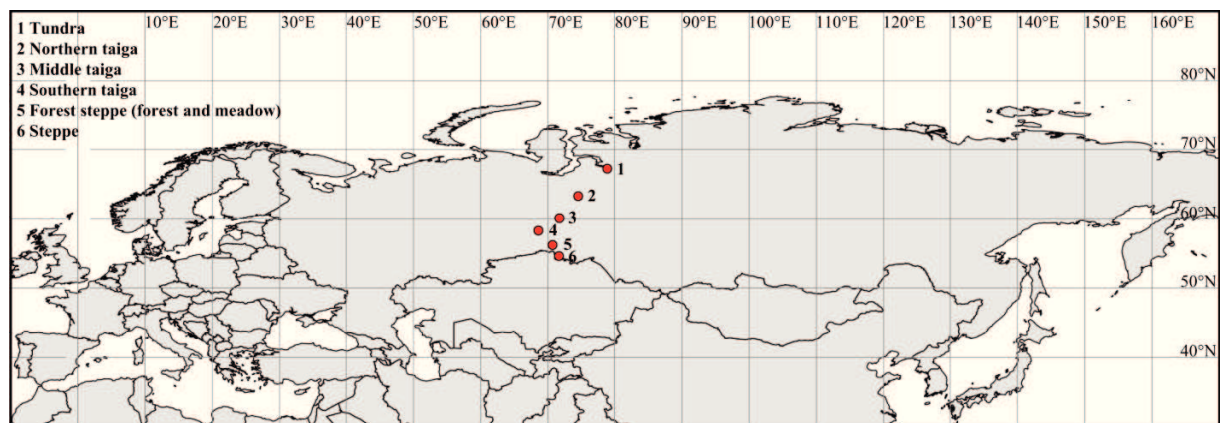


Figure 1: Map of sampling sites along a latitudinal transect in Western Siberia (after Wild et al., 2015).

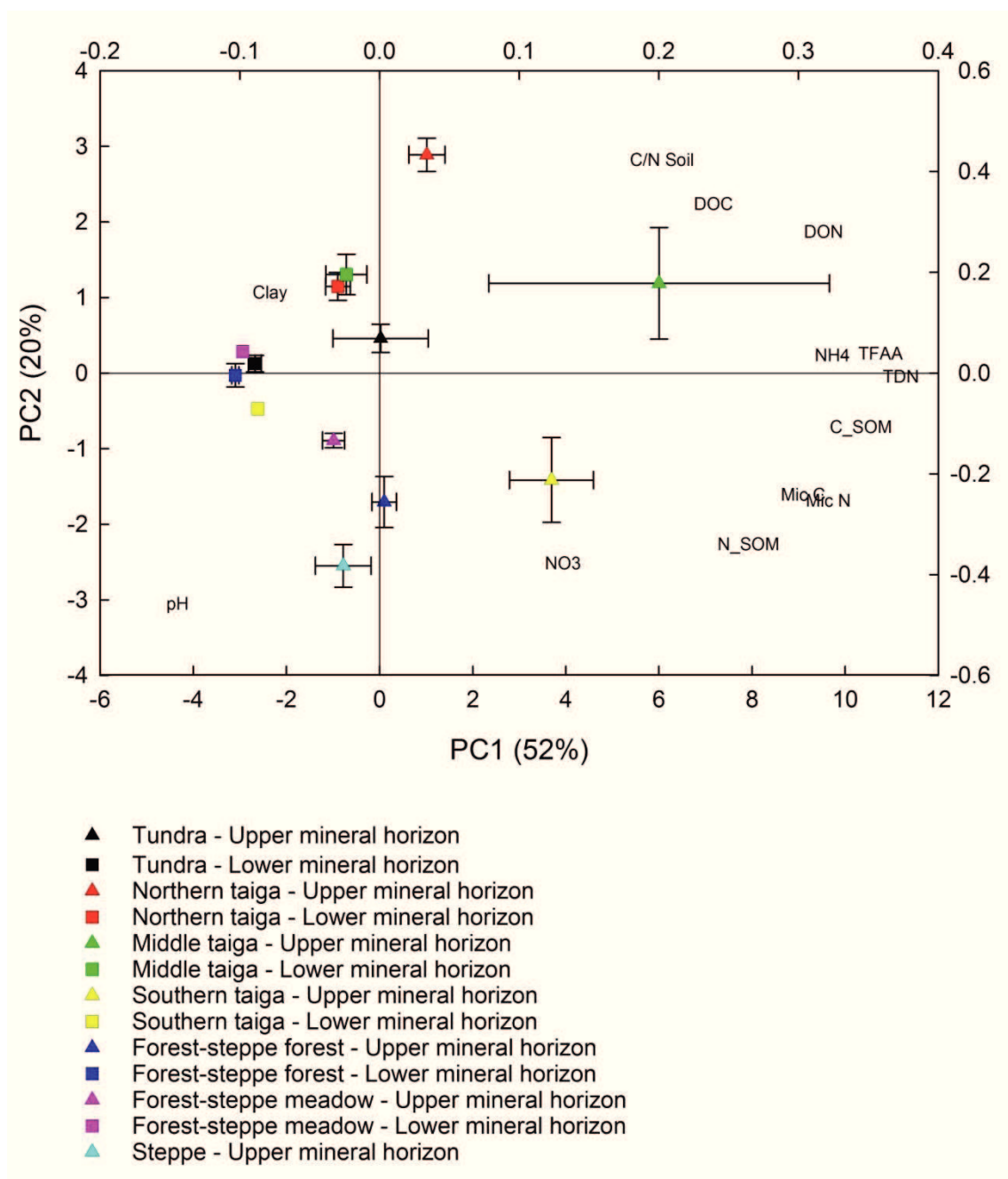


Figure 2: Principal component analysis (PCA) of initial soil parameters. Total organic soil carbon (C\_SOM), total nitrogen (N\_SOM), C:N ratio of soil organic matter (C/N Soil), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>), total free amino acids (TFAA), microbial carbon (Mic C), microbial nitrogen (Mic N). Symbols represent mean values (n=5); error bars indicate standard errors.

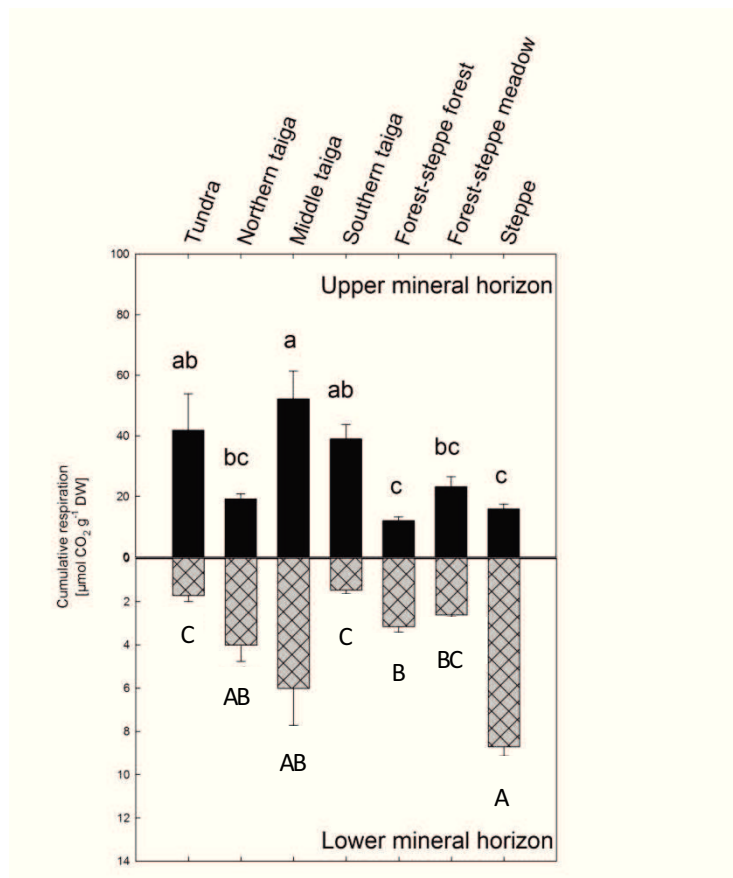


Figure 3: Cumulative respiration of control samples (no amendments) with significant differences between sites indicated by different letters; mean values ( $n=5$ ) and standard error. Upper horizons on top in black; lower horizons below in grey. Note that axis have different scaling. Statistics applying one-way ANOVA and Tukey-HSD ( $p<0.05$ ) if data were normal distributed, Kruskal-Wallis with Tukey-HSD post-hoc test was performed if not normal distributed data were obtained.

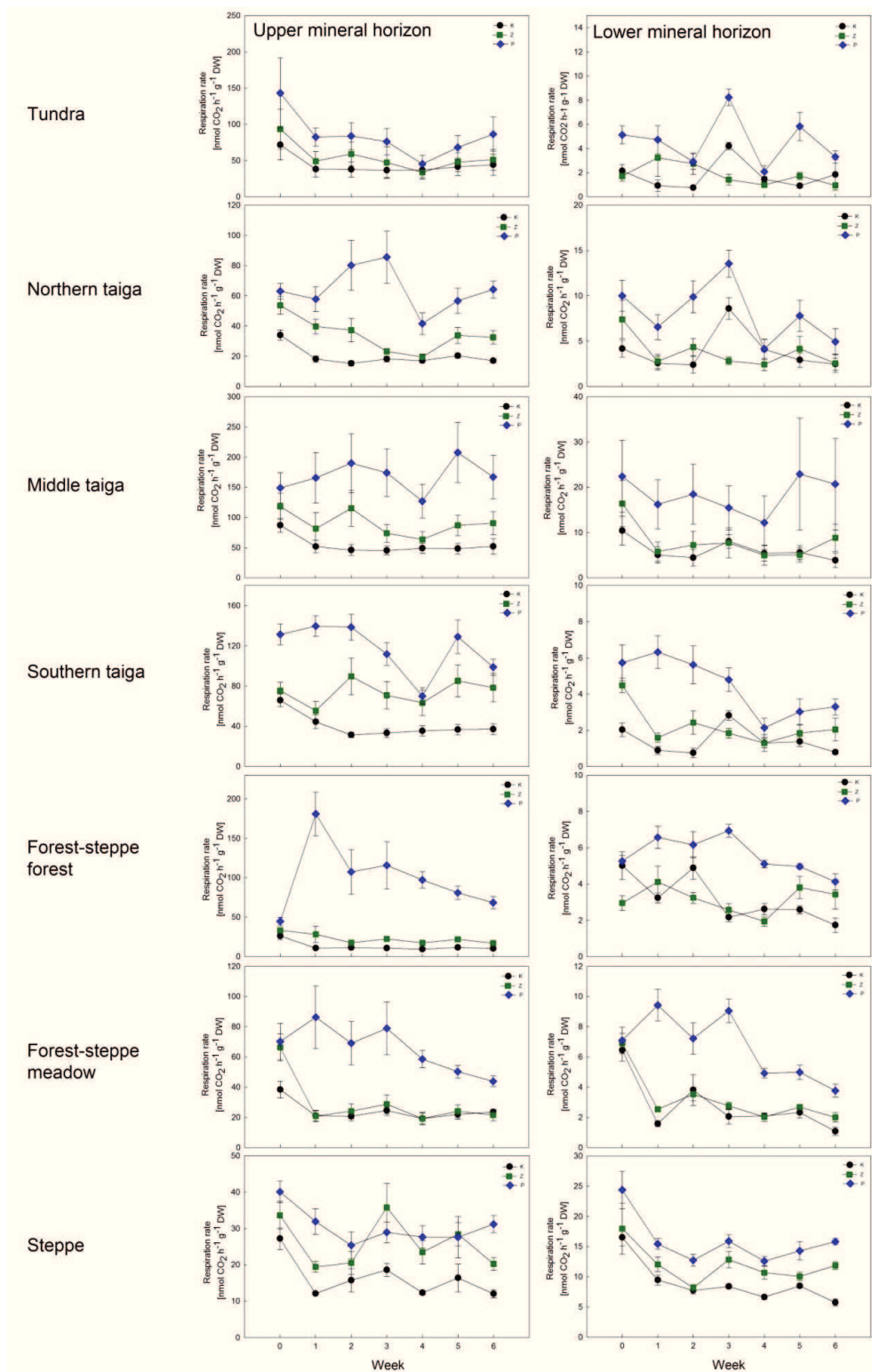


Figure 4: Weekly respiration rates of control group (black; K), cellulose (green; Z) and cellulose/protein (blue; P) treatments (means,  $n=5$ , error bars represent standard errors). Upper mineral horizons are depicted in the left panels, lower mineral horizons in the right panels. Data points are mean values plus/minus standard error. Note that axis have different scaling.

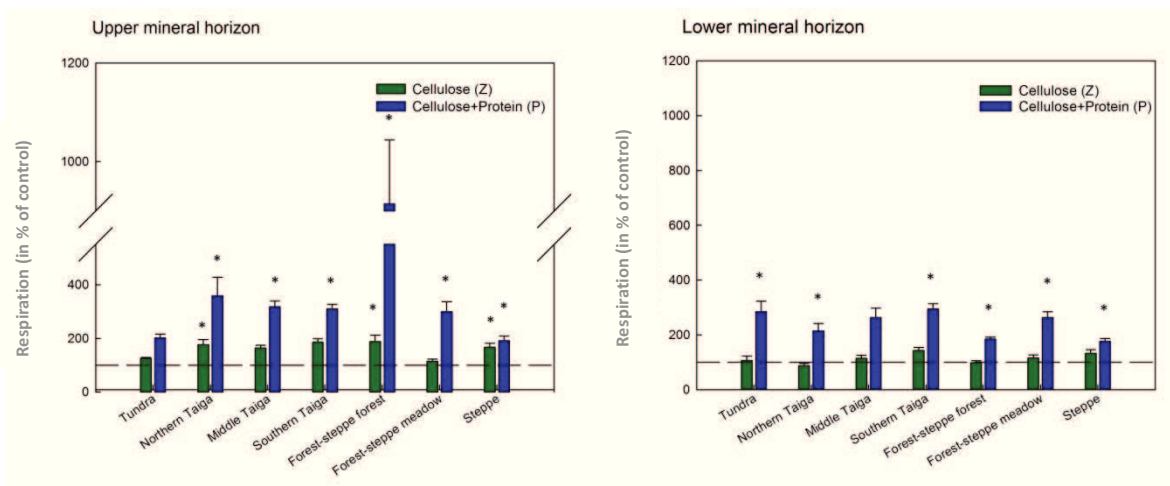


Figure 5: Cumulative respiration of samples from cellulose and cellulose/protein treatments normalized to the respiration of the respective controls (means,  $n=5$  and standard errors). Increases or decreases in percent of control (dashed line); asterisks indicate significances (ANOVA with Post-hoc Tukey HSD test  $p<0.05$ ); data were log transformed to achieve normal distribution and homoscedasticity.

| Cellulose             | Cumulative respiration | Dissolved organic carbon | Total dissolved nitrogen | Dissolved organic nitrogen | Ammonium | Nitrate | Total free amino acids | Mineralization rates | Immobilization rates | Microbial carbon | Microbial nitrogen | Microbial C/N | Phenoloxidase | Peroxidase | $\beta$ -Glucosidase | NAGase | Leucine-Amino-peptidase |
|-----------------------|------------------------|--------------------------|--------------------------|----------------------------|----------|---------|------------------------|----------------------|----------------------|------------------|--------------------|---------------|---------------|------------|----------------------|--------|-------------------------|
| Upper mineral horizon |                        |                          |                          |                            |          |         |                        |                      |                      |                  |                    |               |               |            |                      |        |                         |
| Tundra                | 1.2                    | 1.0                      | 1.1                      | 5.3                        | 0.8      | 1.5     | 1.1                    | 0.9                  | 1.3                  | 0.8              | 0.9                | 0.9           | 1.5           | 1.6        | 2.2                  | 2.1    | 0.9                     |
| Northern taiga        | 1.8                    | 1.0                      | 0.6                      | 0.8                        | 0.3      | 1.3     | 1.3                    | 0.3                  | 3.3                  | 0.9              | 0.7                | 1.2           | 0.9           | 1.0        | 1.0                  | 0.9    | 0.9                     |
| Middle taiga          | 1.6                    | 0.9                      | 0.4                      | 0.5                        | 0.1      | 2.1     | 1.0                    | 1.7                  | 2.5                  | 1.3              | 3.2                | 1.1           | 1.2           | 1.1        | 0.9                  | 0.8    | 1.3                     |
| Southern taiga        | 1.9                    | 1.4                      | 0.5                      | 1.4                        | 0.3      | 0.2     | 1.9                    | 1.0                  | 1.3                  | 1.0              | 0.8                | 1.2           | 1.1           | 1.2        | 0.8                  | 1.0    | 1.3                     |
| Forest-steppe forest  | 1.9                    | 1.0                      | 0.4                      | 0.8                        | 0.5      | 0.1     | 0.9                    | n.a.                 | 0.9                  | 1.0              | 1.0                | 1.0           | 0.7           | 0.6        | 0.5                  | 0.6    | 0.5                     |
| Forest-steppe meadow  | 1.1                    | 1.6                      | 1.0                      | 3.3                        | 0.8      | 0.2     | 1.6                    | 0.3                  | 1.8                  | 0.6              | 0.5                | 1.1           | 1.5           | 1.3        | 0.9                  | 1.3    | 1.2                     |
| Steppe                | 1.7                    | 1.1                      | 0.5                      | 1.2                        | 1.5      | 0.1     | 1.5                    | 0.9                  | 3.0                  | 1.0              | 0.9                | 1.1           | 1.0           | 1.2        | 1.3                  | 1.1    | 1.0                     |
| Lower mineral horizon |                        |                          |                          |                            |          |         |                        |                      |                      |                  |                    |               |               |            |                      |        |                         |
| Tundra                | 1.1                    | 0.7                      | 0.1                      | n.a.                       | n.a.     | 1.8     | 0.3                    | n.a.                 | 1.5                  | 0.6              | 1.3                | 0.6           | 1.6           | 1.3        | 0.7                  | 1.3    | 1.3                     |
| Northern taiga        | 0.9                    | 1.1                      | 0.9                      | 1.9                        | 1.5      | 0.9     | 0.8                    | n.a.                 | 17.1                 | 1.4              | 0.9                | 1.5           | 1.1           | 1.1        | 1.7                  | 1.1    | 0.9                     |
| Middle taiga          | 1.2                    | 0.7                      | 0.4                      | 0.1                        | 0.9      | 0.8     | 1.0                    | n.a.                 | 2.1                  | 1.8              | 2.2                | 1.0           | 1.2           | 1.1        | 1.5                  | 1.1    | 1.0                     |
| Southern taiga        | 1.5                    | 0.8                      | 0.9                      | 0.3                        | 2.2      | 0.5     | 0.8                    | 0.1                  | 1.3                  | 1.2              | 1.0                | 1.6           | 0.9           | 1.1        | 0.8                  | 0.8    | 0.9                     |
| Forest-steppe forest  | 1.0                    | 0.7                      | 0.5                      | n.a.                       | 0.3      | 6.2     | n.a.                   | n.a.                 | 0.7                  | 0.2              | 0.1                | 2.0           | 1.0           | 4.2        | 6.0                  | 1.9    | 1.0                     |
| Forest-steppe meadow  | 1.2                    | 0.8                      | 1.7                      | 1.0                        | 15.2     | n.a.    | 0.4                    | 0.1                  | 0.8                  | 0.7              | 0.4                | 2.4           | 1.6           | 2.2        | 1.1                  | 0.8    | 1.1                     |
| Steppe                | 1.3                    | 1.2                      | 0.8                      | 1.3                        | 1.7      | 0.2     | 0.5                    | 6.6                  | 1.5                  | 0.7              | 0.6                | 1.4           | 1.9           | n.a.       | 1.3                  | 2.0    | 1.9                     |

Figure 6: Heat map of response ratios (i.e., pools or fluxes in cellulose treatment group divided by control group) after six weeks of incubation, illustrated with a color code. Decreases are indicated in blue, increases in red and no changes in white. Bold values indicate significant differences between treatment and control group using paired t-test ( $p < 0.05$ ). Underlines values are not statistically valid.

| Cellulose + Protein   | Cumulative respiration | Dissolved organic carbon | Total dissolved nitrogen | Dissolved organic nitrogen | Ammonium | Nitrate | Total free amino acids | Mineralization rates | Immobilization rates | Microbial carbon | Microbial nitrogen | Microbial C/N | Phenoloxidase | Peroxidase | $\beta$ -Glucosidase | NAGase | Leucine-Aminopeptidase |
|-----------------------|------------------------|--------------------------|--------------------------|----------------------------|----------|---------|------------------------|----------------------|----------------------|------------------|--------------------|---------------|---------------|------------|----------------------|--------|------------------------|
| Upper mineral horizon |                        |                          |                          |                            |          |         |                        |                      |                      |                  |                    |               |               |            |                      |        |                        |
| Tundra                | 2.0                    | 1.4                      | 16.3                     | 7.4                        | 54.6     | 1.9     | 1.1                    | 3.8                  | 5.1                  | 0.6              | 0.9                | 0.9           | 1.5           | 1.5        | 1.7                  | 1.6    | 1.0                    |
| Northern taiga        | 3.6                    | 1.3                      | 2.0                      | 1.7                        | 2.5      | 1.1     | 2.1                    | 1.0                  | 3.0                  | 0.7              | 0.5                | 1.5           | 1.2           | 1.1        | 1.4                  | 1.1    | 1.5                    |
| Middle taiga          | 3.2                    | 1.5                      | 1.7                      | 1.6                        | 2.0      | 0.3     | 3.0                    | 6.8                  | 6.0                  | 1.0              | 2.2                | 1.8           | 1.6           | 1.3        | 1.2                  | 1.0    | 1.7                    |
| Southern taiga        | 3.1                    | 1.1                      | 2.0                      | 1.7                        | 3.1      | 2.1     | 1.1                    | 1.9                  | 5.9                  | 1.3              | 1.0                | 1.4           | 1.0           | 1.0        | 1.2                  | 1.2    | 1.7                    |
| Forest-steppe forest  | 9.1                    | 1.1                      | 3.3                      | 0.4                        | 13.1     | 1.1     | 1.0                    | 4.2                  | 18.1                 | 1.2              | 1.6                | 0.8           | 1.6           | 1.6        | 1.8                  | 2.5    | 1.4                    |
| Forest-steppe meadow  | 3.0                    | 0.9                      | 3.5                      | n.a.                       | 8.9      | 6.0     | 0.4                    | 0.3                  | 1.0                  | 0.7              | 0.7                | 1.0           | 1.6           | 1.5        | 1.0                  | 1.2    | 1.2                    |
| Steppe                | 1.9                    | 1.3                      | 2.6                      | 0.3                        | 37.1     | 1.6     | 1.1                    | 4.5                  | 6.6                  | 1.0              | 0.9                | 1.2           | 1.3           | 1.6        | 1.6                  | 1.8    | 1.2                    |
| Lower mineral horizon |                        |                          |                          |                            |          |         |                        |                      |                      |                  |                    |               |               |            |                      |        |                        |
| Tundra                | 2.8                    | 1.3                      | 10.8                     | n.a.                       | n.a.     | 1.1     | 5.3                    | n.a.                 | 1.8                  | 0.4              | 0.5                | 0.6           | 1.4           | 1.5        | 1.2                  | 1.0    | 1.3                    |
| Northern taiga        | 2.1                    | 1.2                      | 2.9                      | 5.2                        | 7.6      | 0.8     | 1.2                    | 1.3                  | 22.3                 | 1.9              | 0.8                | 2.6           | 1.3           | 1.2        | 2.4                  | 1.4    | 1.0                    |
| Middle taiga          | 2.6                    | 1.0                      | 2.3                      | 1.2                        | 6.7      | 0.8     | 1.1                    | n.a.                 | 7.5                  | 2.1              | 2.0                | 1.3           | 1.2           | 1.2        | 1.7                  | 1.2    | 1.5                    |
| Southern taiga        | 3.1                    | 0.9                      | 3.4                      | 1.4                        | 11.6     | 1.3     | 1.0                    | 0.7                  | 4.5                  | 1.8              | 1.4                | 1.3           | 1.3           | 1.2        | 0.9                  | 1.0    | 1.0                    |
| Forest-steppe forest  | 1.8                    | 0.8                      | 2.5                      | n.a.                       | 8.0      | 0.9     | 0.1                    | 8.2                  | 1.0                  | 0.3              | 0.1                | 4.0           | 1.1           | 3.4        | 1.8                  | 2.4    | 1.1                    |
| Forest-steppe meadow  | 2.6                    | 0.6                      | 3.2                      | 0.7                        | 44.2     | n.a.    | 0.2                    | 0.8                  | 1.7                  | 0.7              | 0.8                | 1.1           | 2.0           | 6.5        | 1.6                  | 2.5    | 1.4                    |
| Steppe                | 1.8                    | 1.2                      | 2.0                      | 1.6                        | 23.3     | 2.2     | 0.8                    | 7.6                  | 6.0                  | 0.8              | 0.7                | 1.4           | 2.5           | n.a.       | 2.2                  | 2.7    | 3.6                    |

Figure 7: Heat map of response ratios (i.e., pools or fluxes in cellulose/protein treatment group divided by control group) after six weeks of incubation, illustrated with a color code. Decreases are indicated in blue, increases in red and no changes in white. Bold values indicate significant differences between treatment and control group using paired t-test ( $p < 0.05$ ). Underlines values are not statistically valid.

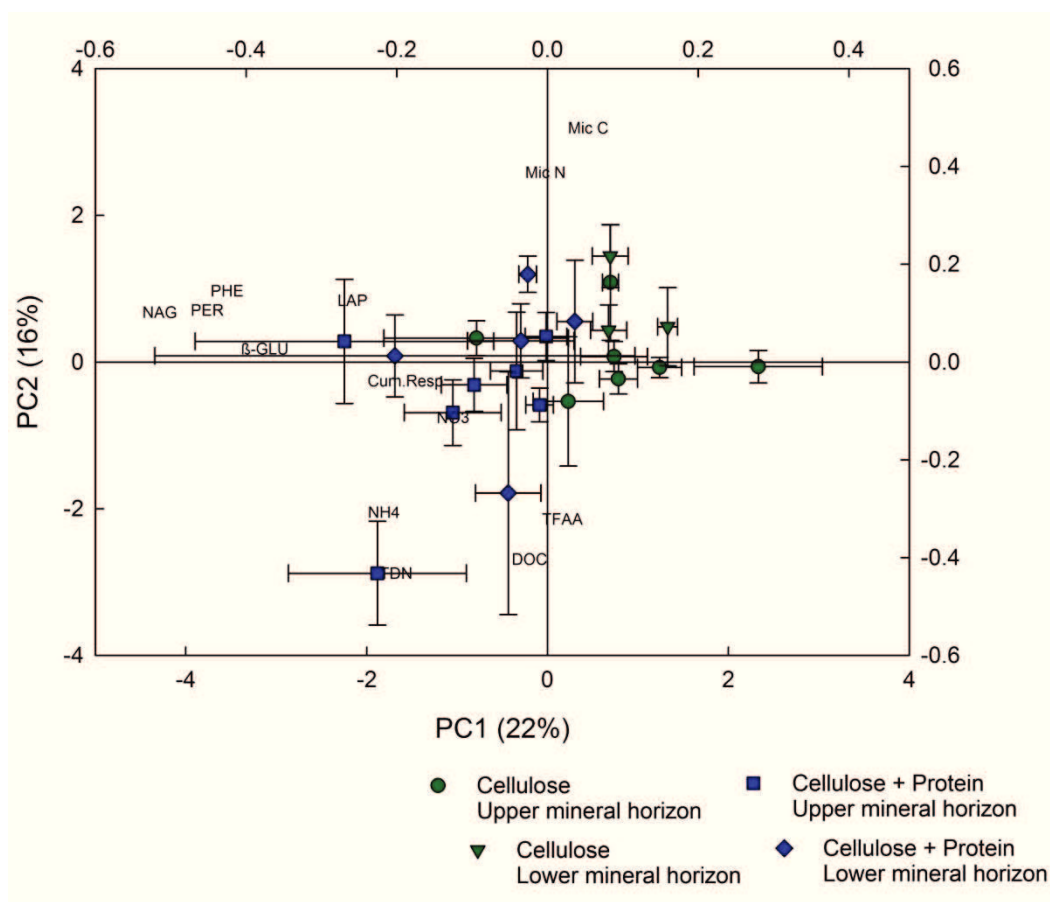


Figure 8: Principal component analysis (PCA) of response ratios of cellulose or cellulose/ protein compared to controls; symbols represent mean values ( $n=5$ ) and standard errors.

Cumulative respiration (Cum.Resp.), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>), total free amino acids (TFAA), Microbial carbon (Mic C), microbial nitrogen (Mic N), phenoloxidase (PHE), peroxidase (PER),  $\beta$ -glucosidase ( $\beta$ -GLU), NAGase (NAG), leucine-aminopeptidase (LAP).

Significant differences between cellulose and cellulose/protein amendments were tested by the Wilcoxon test ( $p < 0.001$ ).

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## Zusammenfassung

Die Veränderungen des globalen Kohlenstoffkreislaufs werden voraussichtlich die Einträge von organischem Material in Böden beeinflussen und dadurch auch den mikrobiellen Abbau von organischem Material modifizieren. Vor allem in arktischen und subarktischen Ökosystemen können die Auswirkungen solcher Veränderungen beachtlich sein, da deren Produktivität und Abbauraten durch die Stickstoffverfügbarkeit stark limitiert sind. Bisherige Studien deuten darauf hin, dass mit der geographischen Breite die Stickstofflimitierung zunimmt und die Energieverfügbarkeit (Kohlenstoff) mit der Bodentiefe stagniert. Jedoch ist bisher noch wenig über die Veränderungen des Abbaus in verschiedenen Bodenschichten entlang eines Breitengradienten bekannt. Ebenso fraglich ist, wie sich diese in Folge von veränderter Nährstoffzufuhr verhalten werden.

Zu diesem Zweck wurden in dieser Studie Bodenproben des ersten und zweiten mineralischen Bodenhorizontes eines 1 500 km langen kontinentalen Transekts durch Westsibirien, welcher sich über vier nördliche Ökosysteme erstreckt, mit zusätzlichen Nährstoffen versetzt. Zellulose und Protein wurden hierzu als Energiequelle bzw. Protein als zusätzliche Stickstoffquelle ausgewählt. Den Teilgruppen wurden einerseits nur Zellulose und andererseits Zellulose mit Protein (C:N Verhältnis von 10:1) hinzugefügt. Es wurden wöchentliche Messungen der Respirationsraten durchgeführt sowie die Kohlenstoff- und Stickstoff-Pools, mikrobielle Biomasse und potentielle Enzymaktivitäten vor und nach der sechswöchigen Inkubationszeit ermittelt. Die Ergebnisse zeigten, dass die Zugabe von Zellulose und Protein die Respirationsraten signifikant steigern konnten, jedoch diese Steigerungen keinem klaren Muster über die Breitengrade hinweg folgten. Gleiches gilt für die mikrobielle Aktivität in Form von Enzymproduktion, die mit zusätzlichem Stickstoff, ohne Einfluss von Breitengraden oder Enzymart, erhöht werden konnte. Entgegen der Erwartungen konnte die Zugabe von Zellulose als Energiequelle weder einen Anstieg der Respiration noch der mikrobiellen Biomasse in beiden Horizonten bedingen. Dies unterstreicht die Wichtigkeit der Stickstoffverfügbarkeit für mikrobielle Prozesse auch in tiefen mineralischen Bodenhorizonten, welche als energie- oder kohlenstofflimitiert vermutet wurden.

Zusammenfassend konnte ich mit meiner Studie weder die verbessernde Wirkung von Zellulose auf die mikrobiellen Zersetzer in tiefen Bodenschichten noch die unterschiedliche Wirkungsweise von Stickstoffzufuhr entlang des Gradienten beweisen. Es konnte allerdings gezeigt werden, dass die zusätzliche Proteingabe die mikrobielle Aktivitäten wie Respiration und Enzymproduktion signifikant steigern kann und die Verfügbarkeit von stickstoffhaltigem Material potentiell die Aktivität von mikrobiellen Zersetzern, in zur Zeit noch limitierten nördlichen Ökosystemen, erhöhen kann.



## Curriculum vitae

Anna Teufl

### Education and Academic degree

|             |                                                                                                                                                                                                                                                       |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2014 -      | Bachelor degree program in Physiotherapy at the University of Applied Science Joanneum, Austria                                                                                                                                                       |
| 2011 - 2015 | Master Studies Ecology at the University of Vienna, specialization on Microbial, Molecular and Chemical Ecology, Austria                                                                                                                              |
| 2012 – 2013 | ERASMUS exchange at the University of Manchester, UK                                                                                                                                                                                                  |
| 2013 – 2015 | Master thesis at the Division of Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, University of Vienna. Thesis title „Controls on soil organic matter decomposition along a latitudinal transect in Western Siberia“ |
| 2007 - 2010 | Bachelor Studies of Biology with specialization on ecology at the University of Vienna. Bachelor thesis: „Nährstofflimitation eines alpinen Ökosystems und die Folgen einer Nährstoffhinzufügung“                                                     |
| 2006 - 2007 | Bachelor studies of Nutrition Science at the University of Vienna, Austria                                                                                                                                                                            |
| 20.06.2006  | Matura at the BORG Scheibbs (University entrance diploma)                                                                                                                                                                                             |
| 2002 - 2006 | Gymnasium BORG Scheibbs, Lower Austria                                                                                                                                                                                                                |
| 1998 - 2002 | Secondary school in Gaming, Lower Austria                                                                                                                                                                                                             |
| 1994 - 1998 | Primary school in Gaming, Lower Austria                                                                                                                                                                                                               |

### Working experience

|                          |                                                                                                                        |
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| July - August 2010, 2011 | Internships at the Wassercluster Lunz, Austria<br>4 – 6 weeks additional assistant in the team of Prof. Dr. Tom Battin |
| March – June 2011        | Laboratory work at the Department of Terrestrial Ecology and Ecosystem Research, University of Vienna                  |