universität wien

MASTERARBEIT

Titel der Masterarbeit

"Microbial nitrogen use efficiency along a latitudinal gradient in Western Siberia, Russia"

verfasst von

Anna Knoltsch, BA BSc

angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2013

Content

I General Introduction

1 Soil stoichiometry, community and competition

Stoichiometric relationships structure all biological systems as primary producers and their consumers depend on certain nutrient ratios to maintain essential functions (Sistla and Schimel, 2012; Kuzyakov and Xu, 2013). Soil is known to control microbial and plant growth due to its' limiting potential in nutrients, mainly phosphorus (P) and nitrogen (N), but also carbon (C) is considered as constraint of microbial cell growth in especially deeper soil layers (Berg and McClaugherty, 2003; Turner et al., 2003; Sistla et al, 2012; Kuzyakov and Xu, 2013). While P is mainly limiting in subtropical and tropical latitudes (Vitousek and Farrington, 1997), arctic and boreal regions are usually poor in N (Hunt et al, 1988; Booth et al, 2005; LeBauer and Treseder, 2008; Sistla et al, 2012). Thus, strong resource competition can be observed among plants and microorganisms (Jones et al, 2013; Kuzyakov and Xu, 2013) but also within the microbial community (Vitousek and Howarth, 1991; Geisseler et al, 2010). Stoichiometric flexibility of biological systems, notably at the organismal scale, is of high advantage (Sistla and Schimel, 2012). Plants may shift their elemental composition (Sterner and Elser, 2002) by, first, changing in physiological state, such as adjustment of growth (Elser et al, 2000) or changes of nutrient ratios in plant tissues e.g. by increasing the carbon to nitrogen ratio (C:N) in woody biomass relative to other tissues (Mack et al, 2004; Sistla and Schimel, 2012) or the foliar nitrogen to phosphorus (N:P) ratios (Elser et al, 2000). Second, biotic re-structuring of plant communities may lead to a shifting dominance among organisms that differ in their average stoichiometry, i.e. shrub expansion in sedge-dominated tussock tundra (Chapin et al, 1986) or grasslands (Sistla and Schimel, 2012).

In general, primary producers show high stoichiometric flexibility because of their capacity to accumulate and store C in different plant tissues. Microbial organisms are much more constrained in their stoichiometry than the soil environment they inhabit (Hessen et al., 2004; Sardans et al, 2011) as prokaryotic microbes lack in nutrient allocation (Sistla and Schimel, 2012). Hence, nutrient alterations in autotroph biomass (Hessen et al., 2004) and soils (Mack et al, 2004) influence easily microbial community composition by shifting the community's C:N (Sistla et al, 2014). N enrichment may select for species with high growth rates and low biomass C:N ratios (Elser et al, 2000). Bacteria are characterized by lower microbial biomass C:N ratio compared to the higher C:N ratio showing fungi (Strickland and Rousk, 2010; Sistla et al., 2014). Hence, increasing N availability in soils may favor bacterial growth, which may lead to greater

bacterial to fungal dominance being accompanied by much faster decomposition rates and rapid nutrient cycling (Weintraub and Schimel, 2005; Sistla and Schimel, 2012). Significant ecosystem C and N loss may select for other species and may occur by e.g. fires - presenting an important component in boreal and grassland ecosystems - because of the relatively low volatilization temperatures of C and N, whilst P is mainly converted into available mineral forms through ash deposition (Eisele et al, 1989). Lower N-availability tends to increase fungal abundances in general, but may also stimulate terrestrial cyanobacteria (Eisele et al, 1989). Fungal dominance may lead to an increase in oxidative enzyme production that causes an elevated decomposition of protected soil organic compounds, being rich in both C and N. Fungal-based food webs are further much more resistant to drought and other climate perturbations than bacterial dominated ecosystems due to their life strategy (Sistla and Schimel, 2012).

Microorganisms developed a wide range of mechanisms to take up inorganic but also small soluble organic molecules (Merrick and Edwards, 1995; Xu et al., 2008; Geisseler et al, 2010) by the use of cell membrane proteins (Geisseler et al, 2010). These proteins may either act as energy consuming transporters that are actively transporting specific molecules from the outside in the cytoplasm of the cell or as passive channels, e.g. linking the transport of ammonia (NH_3) with the symport of H^+. Ammonium (NH_4^+) and nitrate (NO_3^-) were long time considered as main N sources for both bacteria and fungi (Merrick and Edwards, 1995; Geisseler et al, 2010; Kuzyakov and Xu, 2013). Latest research, actually, shows a high preference of microorganisms for amino acids (Kuzyakov and Xu, 2013) and there is evidence that they may also take up small peptides (< 31 amino acids in length) directly (Walker and Altman, 2005; Geisseler et al., 2010; Jones and Kielland, 2012). The transport of peptides and amino acids is performed by transport systems that are bound to the cytoplasmatic membrane (Geisseler et al, 2010). Microbial transport systems for both peptides and amino acids are energy consuming (Anraku 1980) and transport amino acids possessing similar chemical properties.

Plants tend to favour amino acids and peptides as N source (Geisseler et al, 2010) but also nitrate (NO_3^-) due to its greater mobility in soils and its negative charge (Xu et al., 2008; Jones et al., 2013). Their discrimination for either organic or inorganic components as N source is influenced by temperature and plant species (Xu et al., 2008). Although different plants within a community may have different preferences and needs for organic and inorganic nitrogen forms, similar mechanisms are regulating the N uptake and transport across most species (Näsholm et al., 1998; Persson and Näsholm 2001). However, compared to their microbial antagonists, plants appear to be inferior competitors in inorganic but also organic N uptake (Kuzyakov and Xu, 2013). NO_3^- is immobilized twice as fast by microorganisms as by plants, while NH_4^+ is even

fivefold faster taken up by microorganisms than by the plant community (Jones et al., 2005). Nonetheless, in long term plants do much better due to slower turnover times of roots (Schimel and Bennett, 2004; Jones et al., 2005) and reallocation of N from microorganisms to plants (Kuzyakov and Xu, 2013).

2 Soil inorganic nitrogen

Inorganic nitrogen is only present in small concentrations in soils and turned over very fast. NH_4^+ is only available in very low concentrations in soils (Jones and Kielland, 2002) and shows especially high depletion in the rhizosphere due to strong root and microbial uptake but very low mobility in the soil solution (Kuzyakov and Xu, 2013). NH_4^+ is either rapidly taken up and assimilated as microbial (microbial NH_4^+ immobilization) or plant biomass, or converted to nitrate (NO_3^-) during the process of nitrification under aerobic conditions (Booth et al, 2005). In this process, NH_4^+ may either be directly reduced to nitrite (NO_2^-) or NO_3^- via heterotrophic nitrification (Focht and Verstraete, 1977) or go through the indirect pathway of autotrophic nitrification. During autotrophic nitrification, NH_4^+ is first converted to hydroxyl amine (NH_2OH) by the enzymatic activity of the ammonium monooxygenase (AMO) (Wood, 1986) and further to NO_2^- via enzymatic reduction of the hydroxylamine oxidoreductase (HAO) (Yamanaka and Sakano, 1980). A broad range of microorganisms, including archaea and fungi besides bacteria, possess the capability of producing NO_3^- through the pathway of nitrification (Focht and Verstraete, 1977). As the produced NO_3^- is highly mobile in the soil medium, it serves much more as inorganic N source in soils as NH_4^+ (Xu et al., 2008). Under anaerobic conditions, NO_3^- and NO_2^- may be further reduced to atmospheric nitrogen (N_2) through the intermediates nitric oxide (NO) and/or nitrous oxide (N_2O, commonly known as laughing gas) by denitrifying bacteria (Bedmar et al, 2005). In soils, only a small fraction of the soil microbial community is able to denitrify. It is assumed that only five percent of the soil inhabiting bacteria possess denitrifying enzymes, while the denitrifying capabilities of fungi or archaea are still unknown (Henry et al., 2006).

Soil properties such as moisture, pH, temperature and substrate quality are controlling factors of all transformation rates (Nadelhoffer et al, 1992; Chapin, 1996; Cookson et al, 2007). Nitrification rates are especially controlled by ammonium concentrations, while rates of denitrification are additionally controlled by nitrate concentrations (Chapin, 1996). Besides, nitrification rates strongly depend on mineralization rates being proportionally high at decreasing N mineralization

rates. High soil C:N ratios may suppress NO_3^- production but promote NO_3^- assimilation (Booth et al, 2005).

3   Soil organic nitrogen

The N pool in soils is dominated by organic N forms such as humus, proteins, peptides and amino acids. While amino acids and small peptides may be taken up directly, proteins have to be broken down biologically or abiotically first before microbes are capable of assimilation (Jones and Hodge, 1999; Jan et al., 2009). Most likely the depolymerization of proteins is dominated by extracellular proteolytic enzyme activity instead of the intracellular breakdown by i.e. protozoa (Jan et al., 2009). These exo-enzymes are released by microbial decomposers, mainly mycorrhizal and saprotrophic fungi (Talbot et al., 2008) but also bacteria. With a few exceptions of ericaceous mycorrhizae, saprotrophic fungi generally appear to be the best competitors in the breakdown of proteins (Booth et al, 2005).

Extracellular proteases catalyze the conversion of proteins to peptides and further to free amino acids by hydrolyzing peptide bonds (James et al., 1977) and, thus, accelerate depolymerization processes. As these exo-enzymes are N-rich compounds, the acquisition of extracellular N requires an investment of intracellular N beforehand (Sistla et al, 2012). Thereby, N uptake is inherently linked to nutrient loss for extracellular enzyme production (Mooshammer et al., 2012). Extracellular proteases possess wide substrate-specificities (Gupta et al., 2002; Geisseler et al., 2010) and there is no even distribution of protease expression within the soil microbial community (Fuka et al., 2008; Jan et al., 2009).

Protein depolymerization appears to be significantly slower than the degradation of amino acids themselves (Schimel and Bennett, 2004). Amino acids underlie a very fast turnover with turnover times of 3-6 hours in soils (Kielland et al, 2007) and only 0.3-1.7 hours in leaf litter (Wanek et al., 2010). Thus, amino acids represent a highly dynamic N pool and account for only 5-6% of soil nitrogen, compared to high concentrations of proteinaceous substances that represent around 40% of the soil N (Schulten and Schnitzer, 1997). There are several factors which may influence protein and amino acid turnover besides size and the activity and physiology of the microbial soil community. A broad variety of biotic factors such as temperature, moisture and pH are considered as major controls on these transformation processes (Kang and Lee, 2005). Changes in temperature may lead to limitations in microbial activity at especially very high and low temperatures (Hoyle et al., 2006). Rises in soil moisture cause increases in protein and amino acid degradation processes, but little influence can be observed at high moisture contents

(Tietema et al., 1992). Soil pH and reducing conditions are considered to further highly influence transformation rates. Especially, amino acid turnover correlates negatively with these soil parameters (Marrs et al., 1988). Furthermore, chemical stabilization by soil organic matter (SOM) is of great importance during protein depolymerisation processes. For instance, there are high amounts of humic substances and tannins (polyphenolic substances) in taiga soils, emerging from coniferous wood and foliage. These compounds are able to bind proteins into recalcitrant complexes causing a decreased bioavailability as they are chemically protected against degradation (Schulten and Schnitzer, 1997; Jones and Kielland, 2002; Kraus et al., 2003; Jones and Kielland, 2012). Contrary, it was also observed that humic acids rather stimulated both growth and activity of nitrifying bacteria (Vallini et al., 1997). This was attributed to an increase of microbial membrane permeability due to the surfactant characteristics of humates, allowing a better utilization of nutrients and energy-yielding substrates of the soil einvironment. Thus, humic substances may also act as stimulator for microbial growth and activity in soils (Ganjegunte et al., 2006), although highly depending on the source and amount of present polyphenolic substances (Vallini et al., 1997; Kraus et al., 2003).

4 Study aim and working hypothesis

Understanding the factors that regulate nutrient availability and cycling in soils is essential for generating predictions of consequences of ecosystem alterations including atmospheric carbon dioxide (CO_2) and reactive nitrogen (N) enrichments and phosphorus (P) depletions (Kang and Lee, 2005; Craine et al, 2007; Sistla and Schimel, 2012). This study aimed to determine the influence of soil nitrogen availability on microbial nitrogen transformation rates. We expected to find different soil nitrogen availability along major ecosystems as they differ highly in chemical, physical and biological composition. Therefore, we conducted soil analyses along a north-south gradient in Western Siberia, Russia, ranging from arctic tundra to mid-latitude steppe. We specifically focused on protein depolymerization and nitrogen mineralization rates as dominant N transformation rates, on the one hand, and on microbial immobilization rates of amino acids and ammonium on the other one, which were all based on ^{15}N pool dilution techniques.

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II Manuscript

1 Abstract

Ecosystems show differences in climatic conditions, vegetation and soil organic matter (SOM) content, especially differing in soil N availability along latitudinal gradients. These circumstances require high physiological adaptation of the soil microbial community to compete successfully for nutrients with plants but also with other soil microbial organisms. In this study we aimed at determining the influence of soil N availability on soil microbial transformation rates, focusing on protein depolymerization and N mineralization rates, all based on ^{15}N pool dilution techniques. Organic and mineral soil samples were taken along a 1,400 km latitudinal transect in Western Siberia, Russia, covering all major ecosystems of tundra, boreal forest, deciduous forest and steppe. N transformation rates seemed to be highly influenced by soil moisture and soil C and N concentrations. Highest protein depolymerization and N mineralization rates occurred in the boreal forest, being accompanied by peaking soil C and N concentrations and high water content, whereas lowest transformation rates were found in tundra and steppe soil. Reduced plant microbial competition for N in deep soil layers was considered to stimulate N mineralization and lower protein depolymerization rates. Highest microbial nitrogen use efficiency (NUE) was found in the southern steppe environment, while values were respectively low at all taiga sites. Unfortunately, NUE could not be calculated for the southern tundra as mineralization rates were under detection limit. High NUE suggested microbial adaptation to high litter C:N ratios, whereas lowest NUE occurred where intermediate litter C:N could be observed. We suggest that initial litter chemistry highly defines microbial NUE, but certainly, there are numerous other factors influencing and changing NUE, e.g. limitations of other nutrients, that should be reconsidered.

Key words: soil microbial transformation rates; N limitation; latitudinal transect; West Siberia

2 Introduction

A new paradigm emerged during the last decade, which characterizes depolymerization of N-containing polymers as main regulator of the overall N cycling (Jones and Kielland, 2002; Schimel and Bennett, 2004; Jan et al, 2009; Fig 1B), while the classical scientific view puts nitrogen mineralization in the center of the terrestrial N cycle (Fig 1A) (Schimel and Benett, 2004). While free amino acids can be taken up directly by the microbial soil community via several membrane transport systems (Jones and Hodge, 1999), polymers are not immediately bioavailable and have to be broken down first to smaller oligomers or monomers (Chapin et al., 2002; Jan et al., 2009). This cleavage is processed by extracellular enzymes (Sistla et al., 2012a) being produced by the soil microbial community. N-containing polymers can be degraded by a range of microbial enzymes, including oxidative enzymes, that are assumed as predominantly N-acquiring enzymes (Talbot et al., 2013; Schnecker et al., 2014) and hydrolytic enzymes, hydrolyzing for instance proteins and peptides (leucine aminopeptidase (LAP)) or chitin (β-1,4-N-acetylglucosaminidase (NAG)) (Sinsabaugh and Shah, 2011). Once the products
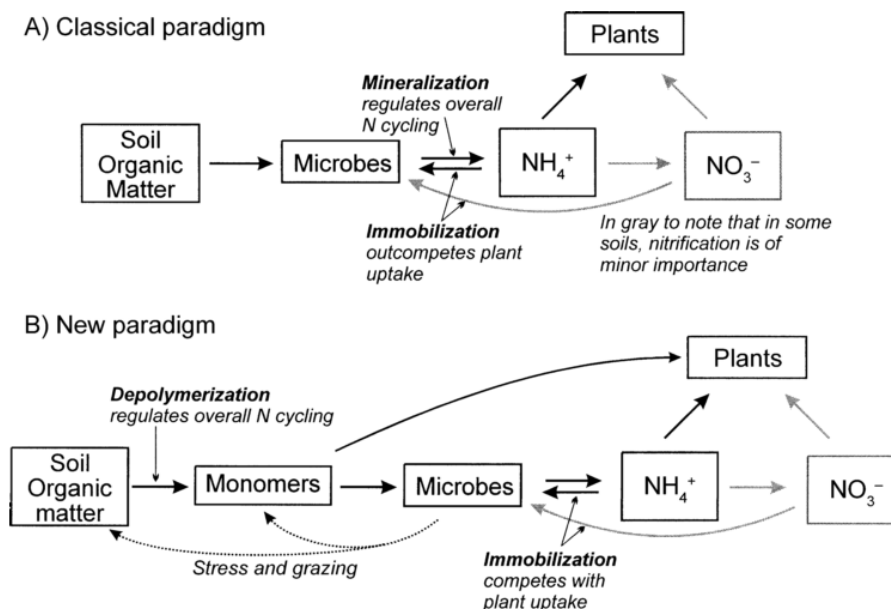

Figure 1: The changing paradigm of the soil N-cycle. (A) The classical paradigm of N-cycling. (B) The new paradigm with depolymerization of N-containing polymers regulating the overall N-cycle. Source: Schimel and Benett, 2004: 594.

of depolymerization enter the dissolved organic nitrogen (DON) pool, they may be taken up and

channeled into microbial metabolism (Fierer et al., 2001), used at higher trophic levels (e.g., micro- and mesofaunal grazing; Elliott et al., 1980) and recycled within the "microbial loop" (Coleman, 1994) or after microbial death (Schimel and Clein, 1996). Depending on the available N sources, which may vary dramatically across soil gradients, the dominating transformation process and main form of N taken up are likely to change (Schimel and Bennett, 2004). N transformation rates and cycling appear to be highly ecosystem specific (Joanisse et al., 2008; Jan et al., 2009), as soils possess great chemical, physical and biological diversity (Jones et al., 2009). Ecosystems along latitudinal gradients show high biological diversity giving rise to variation in litter quality and quantity in a wide range (Xu et al., 2013). Together with different climatic and edaphic conditions, this results in variation in stoichiometry among major ecosystems (Xu et al., 2013), leading to a broad range of soil microbial adaptation (Owen and Jones, 2001; Jones et al., 2009). Xu et al (2013) found strong correlation between latitudinal gradients and soil microbial C:N, suggesting crucial adaptation for microbial N uptake to their soil environment. For example, grassland soils are known to represent N-sufficient conditions (Jan et al., 2009), often even showing inorganic N concentrations comparable to soluble organic ones (Owen and Jones, 2001; Bardgett et al., 2003). Microorganisms living under these circumstances are characterized by high N mineralization rates (Bardgett et al., 2003; Wilkinson et al., 2014) compared to relatively low protein depolymerization rates (Manzoni et al., 2008; Jan et al., 2009), due to N immobilization in the microbial biomass being less important at high bulk N concentrations. Hence, high fractions of N are released back in case of nutrient rich environments through ammonification. Contrary, N-poor soils, as in arctic and boreal ecosystems (Schimel and Bennett, 2004; LeBauer and Treseder, 2008), are considered to be dominated by soluble organic N due to low and slow decomposition and N cycling. As microbial communities are most likely N-limited under such conditions, they retain absorbed N in their biomass (Xu et al., 2013) and only rarely mineralize (Giblin et al., 1991; Schimel and Bennett, 2004). This results in an intense competition for N between plant and microbial community, especially in these low N systems (Jones and Hodge, 1999; Schimel and Bennett, 2004; Xu and Kuzyakov, 2013). Therefore, it is of high advantage to use organic nitrogen forms to decrease dependency on N mineralization and the production of NH_4^+. Although proteinaceous material such as proteins, peptides and amino acids is dominating the soil nitrogen pool (Schulten and Schnitzer, 1997), it may be protected from microbial attack by physical stabilization or chemical protection reacting with polyphenols e.g. tannins and humic substances (Talbot and Finzi, 2008). Microorganisms, but also plants, may use organic N as N source effectively and compete strongly with soil microbial communities (Nashölm et al., 1998; Schimel and Bennett, 2004; Xu et

al., 2006). Thus, soil microsites may highly differ in N availability. Available N, in organic and inorganic form, can diffuse between soil microsites, which may be dominated by either N mineralization or N immobilization (Schimel and Bennett, 2004). Due to microsites that differ strongly in their N availbaility, organisms have to either adapt their physiology or develop mutualistic interactions. Ectomycorrhizal (ECM) fungi may be of importance as they support the depolymerization process of proteins and take up nutrients, especially N, very effectively (Talbot et al., 2013). ECM mycelium in boreal forests even contributes almost 40% to total soil microbial biomass C during summer months (Högberg et al, 2010). Microbial organisms may adapt their physiology by changing their nitrogen use efficiency to sustain their elemental needs (NUE). NUE denotes microbial partitioning of organic N taken up being either incorporated into microbial biomass or released as inorganic N into the environment (Mooshammer et al., 2014a). At high NUE, only small parts of the N taken up, are mineralized and released as ammonium whereas the majority is incorporated into microbial biomass. In contrast, at low NUE only a small fraction of organic N taken up is used for growth while the greater part is mineralized and set free (Mooshammer et al., 2014a).

This project aimed at determining the influence of soil N availability on N transformation rates of the soil microbial community. We hypothesised that higher protein depolymerization rates and amino acid immobilization rates but low nitrogen mineralization rates are found in soils at higher latitudes, having a lower N availability, compared to low-latitude ecosystems. Further, we hypothesized that arctic and boreal soils would exhibit a higher NUE than lower-latitude soils. In contrast to previous studies, which were conducted under laboratory conditions (e.g. Jones and Kielland, 2002; Jan et al., 2009; Jones and Kielland, 2012; Wilkinson et al., 2014), we aimed at characterizing microbial N transformation rates and NUE under field conditions. To achieve this, we sampled soils along a latitudinal gradient from the arctic tundra to the mid-latitude steppe covering all major ecosystems of Western Siberia, Russia.

3 Material and methods

3.1 Sampling sites

We collected soils from seven different sites in Western Siberia, which were located along a 1,400 km latitudinal gradient from the arctic tundra to the mid-latitude steppe (Fig. 1), corresponding to an 8.7°C change in mean annual temperature (Table 1).

Figure 2: Satellite pictures of the latitudinal transect in Western Siberia, © Google earth. Red pins mark the position of the seven different sampling sites, yellow pins the two biggest nearby cities Tazovskiy and Novosibirsk.

The shrubby lichen tundra site (TU) was located approximately 30 km south of the town of Tazovskiy in the southern tundra subzone. The Siberian taiga (boreal forest) was represented by three sites: a *Picea obovata*-forest with high abundances of *Pinus sibirica* and *Vaccinium vitis-idaea* (northern taiga, NT), a boreal coniferous forest with *Sorbus sibirica* and *Pinus sibirica* as most dominant plant species (middle taiga, MT) and a *Picea obovata* forest mixed with *Betula pubescens* and *Abies sibirica* (southern taiga, ST). The forest steppe was characterized by

Table 1: Sampling sites coordinates, elevation, mean annual and August temperature (MAT and Aug_MT), mean annual and August precipitation (MAP and Aug_MP), potential evapotranspiration (PET) and predominant plant species for each site. Climate data: Stolbovoi V and McCallum I (2002), IIASA.

Site	Latitude	Longitude	Elevation (m)	MAT (°C)	MAP (mm)	PET (mm)	Aug_MT (°C)	Aug_MP (mm)	Dominant plant species* (abundancy in descending order)
TU	67°16'20.05"N	78°50'13.85"E	30	-7.6	391	301	9	60	*Arctous erythrocarpa, Empetrum nigrum, Betula nana, Cetraria aculeata, Cladonia rangiferina*
NT	63°17'37.54"N	74°32'9.18"E	131	-4.6	430	405	12	74	*Picea obovata, Pinus sibirica, Vaccinium vitis-idaea, Pleurozium schreberi, Hylocomium splendens*
MT	60° 9'27.08"N	71°42'57.34"E	85	-2.2	438	490	14	74	*Sorbus sibirica, Pinus sibirica, Abies sibirica, Linnaea borealis, Hylocomium splendens*
ST	58°17'58.90"N	68°34'53.71"E	87	-0.5	396	561	14	68	*Picea obovata, Betula pubescens, Abies sibirica, Carex macroura, Rubus saxatilis*
FF	56°14'11.56"N	70°42'54.90"E	106	0.7	340	641	16	59	*Populus tremula, Inula salicina, Calamagrostis arundinacea, Brachypodium pinnatum, Rubus saxatilis*
FM	56°13'54.50 N	70°43'28.46"E	102	0.7	340	641	16	59	*Artemisia macrantha, Calamagrostis epigejos, Vicia cracca, Thalictrum simplex, Rubus saxatilis*
SP	54°41'41.33"N	71°38'45.88"E	72	1	309	700	16	53	*Stipa capillata, Festuca valesiaca, Artemisia austriaca, Potentilla bifurca, Artemisia glauca*

* Plant species and abundancy determined by Nikolay Lashchinskiy, Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

patches of broad leaf hemi-boreal forest (FF) *with Populus tremula* dominance and dry forest meadow (FM) being dominated by grasses such as *Artemisia macrantha* and *Calamagrostis epigejos*. The steppe site (SP) was situated in the mid-latitude Siberian steppe and characterized by the species *Stipa capillata, Festuca valesiaca* and *Artemisia austriaca*.

3.2 Sampling design

The sampling was undertaken in August 2012. At each of the seven sites, five (approximately 1m wide) pits were dug to a depth of 100 cm. Samples were taken from the organic soil horizon (O or OA), the mineral topsoil (A or E) and the mineral subsoil horizon (B or E) beneath. In total, 105 soil samples were collected. Soils were classified according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2006). For basic characterization see Table 2.

Table 2: Physical and chemical parameters of the sampled soils. Values represent means (± standard error).

Site	Horizon	pH (KCl)	Water content (% FW)	C (mg g^{-1} DW)	N (mg g^{-1} DW)	C/N (g g^{-1})	CaCO$_3$ (%)	Soil type according to WRB*
TU	O	3.8 (0.1)	63.94 (4.70)	307.9 (37.4)	8.8 (0.7)	34.9 (3.5)		Turbic Cryosol (thixotropic, reductaquic)
	A	3.7 (0.0)	28.37 (0.97)	30.4 (3.0)	1.8 (0.1)	16.4 (0.7)	-	
	B(C)g	3.9 (0.1)	17.10 (0.50)	4.1 (0.5)	0.4 (0.0)	11.1 (0.8)		
NT	Oi	2.8 (0.0)	68.93 (1.85)	448.4 (7.0)	12.5 (0.3)	35.9 (0.7)		Histic Podzol (oxyaquic)
	AE	3.1 (0.1)	26.27 (1.57)	37.0 (3.1)	1.4 (0.1)	27.4 (2.0)	-	
	Bg	3.7 (0.1)	19.55 (0.83)	8.2 (1.7)	0.5 (0.1)	15.7 (1.5)		
MT	Oi	3.7 (0.1)	57.48 (3.33)	426.1 (24.5)	17.4 (1.0)	24.5 (0.5)		Endogleyic Regosol
	A	3.3 (0.1)	17.33 (1.15)	74.7 (17.3)	3.5 (0.6)	20.8 (1.8)	-	
	CB	3.5 (0.0)	15.86 (2.35)	16.7 (3.8)	1.0 (0.1)	16.3 (1.7)		
ST	Oi	4.3 (0.1)	59.63 (2.16)	398.2 (18.3)	15.8 (0.9)	25.4 (0.8)	-	Albic Podzol
	A(E)	3.6 (0.1)	18.09 (0.70)	43.4 (3.6)	3.1 (0.2)	14.0 (0.8)	0.85 (0.00)	
	E(A)	3.8 (0.1)	7.97 (0.69)	4.8 (0.3)	0.5 (0.0)	9.4 (0.2)	1.28 (0.00)	
FF	Oa	6.6 (0.4)	43.76 (2.32)	292.9 (24.1)	17.7 (1.3)	16.5 (0.3)	-	Haplic Phaeozeme
	A	4.3 (0.1)	32.59 (0.89)	45.6 (4.5)	3.6 (0.4)	12.9 (0.2)	0.55 (0.14)	
	B	4.1 (0.0)	32.49 (4.20)	5.2 (0.1)	0.5 (0.0)	10.1 (0.4)	-	
FM	Oa	5.5 (0.3)	35.02 (6.24)	202.1 (22.7)	14.0 (1.6)	14.4 (0.2)	-	Luvic Phaeozeme
	A	4.1 (0.0)	16.03 (8.04)	24.5 (1.6)	1.9 (0.1)	13.0 (0.1)	1.05 (0.12)	
	Bt	4.0 (0.1)	16.82 (8.00)	5.8 (0.3)	0.5 (0.0)	10.7 (0.2)	0.84 (0.00)	
SP	AO	4.6 (0.1)	19.01 (0.73)	35.3 (5.4)	3.2 (0.5)	11.0 (0.2)	-	Calcic Kastanozem
	Ak	5.1 (0.3)	6.34 (0.38)	20.1 (2.7)	1.8 (0.2)	10.8 (0.3)	1.27 (0.17)	
	Bk	7.9 (0.4)	6.38 (0.91)	7.2 (0.8)	0.8 (0.1)	9.2 (0.2)	7.92 (2.00)	

FW, fresh weight; DW, dry weight. * Soil types classified by Norman Gentsch, Institute of Soil Science, Leibniz Universität Hannover, Germany

3.3   Soil analysis

Soil samples were sieved to 2 mm. After removing roots, the soil was used to determine pH, soil water content, water holding capacity (WHC), total carbon (C) and nitrogen concentrations (N), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON), total free amino acids (TFAA), ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, gross protein depolymerization and gross amino acid immobilization rates, gross nitrogen mineralization and gross NH_4^+-immobilization rates.

Soil water content was determined gravimetrically after drying at 60°C. *pH-values* were measured in 1M KCl-extracts.

3.3.1   Carbon and nitrogen pools

Total organic carbon (C) and *nitrogen (N)* were measured with an elemental analyzer (EA 1110, CE Instruments) coupled with an isotope-ratio mass spectrometer (Deltaplus, Finnigan MAT, Thermo Fisher) using ground, oven-dried soil samples. *Dissolved organic carbon* (*DOC)* and *total dissolved nitrogen (TDN)* were measured in 1M KCl extracts with a TOC/TN analyzer (TOC-V CPH E200V/TNM-1, Shimadzu). *DON* was calculated by subtracting NO_3^- and NH_4^+ from TDN.

NH_4^+ - concentrations were measured colorimetrically by indophenol dye formed by reacting with dichloroisocyanuric acid and salicylate, following a modified protocol after Kandeler and Gerber (1988). NO_3^-- *concentrations* were measured photometrically via VCl_3-reduction of nitrate to nitrite and subsequent nitrite detection by dye formation (Miranda KM, Espey MG, Wink DA, 2001). *Total free amino acids (TFAA)* were determined fluorimetrically after reaction with o-phthaldialdehyde and 3-mercaptopropionic acid (OPAME) procedure.

3.3.2   Nitrogen fluxes

For determining gross protein depolymerization and amino acid immobilization, gross N mineralization and ammonium immobilization, ^{15}N-pool dilution assays were performed, as described elsewhere (Wanek et al., 2010; Wild et al., 2013). This technique allows quantification of fluxes by labeling the respective pools with ^{15}N (i.e., using ^{15}N amino acids for protein depolymerization and amino acid immobilization, and $^{15}NH_4^+$ for N mineralization and ammonium immobilization) and measuring the change in concentration and isotopic enrichment over time (Di et al, 2000).

Gross N mineralization rate (ammonification) was measured by adding 500 µL of 0.125 mM ($^{15}NH_4)_2SO_4$ to soil duplicates (2 g of organic and mineral topsoil, 4 g of mineral subsoil), which

were then incubated for four and 24 hours at 15°C, extracted with 13 mL 2 M KCl, and filtered through ash-free filter paper (Myrold and Tiedje, 1986, modified by Kaiser et al., 2011). NH_4^+ was diffused into acid traps made of Teflon tape enclosing one disc of Whatman filter paper soaked with 10 µL 2.5 M $KHSO_4$ (Wanek et al., 2010). Total N and at% ^{15}N were determined by an elemental analyzer (EA 1110, CE Instruments) coupled with an isotope-ratio mass spectrometer (Deltaplus, Finnigan MAT, Thermo Fisher).

*Gross protein depolymerizati*on was measured according to Wanek et al. (2010) with slight modifications to account for the low amino acid concentrations (Wild et al., 2013). 20 µl of ^{15}N-amino acids (mixture of 20 amino acids; concentration of 62.5ng/µl in dest. H_2O), mixed with 0.5 ml (for mineral horizons) to 1 ml (for organic horizons) 10 mM $CaSO_4$, were added to duplicates of 1 g organic soil or 4 g mineral soil. After incubation for ten or thirty minutes at 15°C, activities were stopped using 20 ml 10 mM $CaSO_4$ with 3.7% formaldehyde. After centrifuging for five minutes at 10,845 g, samples were filtered through synthetic wool and GF/C filters (Whatman) and transferred to cation exchange cartridges (Dionex OnGuard II H cation exchange cartridges, 057085, Thermo Scientific). Before use, cartridges were rinsed with dest. H_2O, activated with 3 M NH_3 and 1 M HCl and rinsed again with distilled water. Amino acids were eluted with 10 mL 3 M NH_3 and 4 mL dest. H_2O from cartridges. 10 µl internal standard (mixture of 0.01% norvaline, norleucine, para-chloro-phenylalanine in 0.1 M HCl) were added to the eluate, which was further dried under N_2 (RapidVap N_2 Dry Evaporation System, LABCONCO). Dried samples were taken up in 1.5 ml 20% ethanol and dried in a SpeedVac (SC110 Vacuum Concentrator, Savant). With each batch of samples, amino acid standards and blanks were processed to account for losses due to ion exchange and drying. Dry samples were taken up in 120 µl 0.1 M HCl, 360 µl dest. H_2O and 320 µl ethanol/pyridine (4:1) and derivatised with 40 µl ethyl chloroformate (ECF) and 800 µl of a 1% ECF in chloroform solution. The organic phase was transferred to GC-vials and dried again in a SpeedVac (SPD131DDA SpeedVac Concentrator, Savant, Thermo Scientific) before being re-dissolved in 50 µl toluol and analyzed with GC-MS (Thermo TriPlus Autosampler, Trace GC Ultra coupled to an ISQ Mass Spectrometer, Thermo Scientific). 2µl of sample were injected in splitless mode at a temperature of 270°C on a PTV-injector, separated on an Agilent DB-5 column with 1 ml/min Helium as carrier gas (GC method: 60°C for 1.5min, ramp 5°C/min to 200°C, ramp 15°C/min to 300°C, 300°C for 4min) and detected in the SIM (Selected Ion Monitoring) mode. Concentrations of alanine, valine, leucine, isoleucine, proline, aspartic acid, glutamic acid and phenylalanine were calculated against external standards. ^{15}N isotopic compositions were based on peak areas of amino acid fragments as described by Wanek et al., 2010.

3.4 Calculation

Gross protein depolymerization and *gross nitrogen mineralization rates* were calculated using equation (1), as described by Wanek et al, 2010:

(1)

$$f = \frac{C_2 - C_1}{t} * \frac{\ln \frac{AP_1 - AP_C}{AP_2 - AP_C}}{\ln \frac{C_2}{C_1}}$$

f ... Gross flux rate in µg g^{-1} dry weight (DW) hours (h)$^{-1}$
t ... Period of incubation in hours (h)
C_1, C_2 ... Concentration of either amino acids or NH_4^+ at time 1 and 2 in µg g^{-1} DW
AP_1, AP_2 ... ^{15}N-atom percent of either amino acid or NH_4^+- pools at time 1 and 2
AP_c ... ^{15}N-atom percent of the unlabeled amino acid or NH_4^+- pool (natural abundance)

Gross immobilization rates by microorganisms were determined by subsequent equation

(2)

$$i = \frac{C_1 - C_2}{t} * 1 + \frac{\ln \frac{AP_2 - AP_C}{AP_1 - AP_C}}{\ln \frac{C_2}{C_1}}$$

i ... Gross amino acid immobilization rate in µg g^{-1} dry weight (DW) hours (h)$^{-1}$
t ... Period of incubation in hours (h)
C_1, C_2 ... Concentration either amino acids or ammonium at time 1 and 2 in µg g^{-1} DW
AP_1, AP_2 ... ^{15}N-atom percent of either amino acids or NH_4^+-Pools at time 1 and 2
AP_c ... ^{15}N-atom percent of the unlabeled amino acid or NH_4^+- pool (natural abundance)

As an indicator for soil microbial nitrogen limitation, *nitrogen use efficiency (NUE)* was calculated based on Wild et al., 2013 and Mooshammer et al., 2014:

(3)

$$NUE = \frac{i_{AAs} - f_m}{i_{AAs}}$$

NUE ... Nitrogen use efficiency
i_{AAs} ... Gross amino acid immobilization rate in µg g^{-1} dry weight (DW) hours (h)$^{-1}$
f_m ... Gross-mineralization rate in µg g^{-1} dry weight (DW) hours (h)$^{-1}$

3.5   Statistical analysis

Statistics were performed in Statgraphics Centurion XVI.I. Data were tested for normal distribution and variance homogeneity. If data did not fit into a normal distributed system, they were $\log_{10}$ or square-root transformed. Significant effects of site or horizon were tested via one-way analysis of variance (One-way ANOVA). Homogenous groups were determined with post hoc Tukey HSD test. Kruskal-Wallis and Mood's Median Tests were performed instead of one-way ANOVA if data didn't show normal distribution and variance homogeneity. Correlation coefficient and its significance were generated via Spearman Rank Correlations since data were not normally distributed. Levels of significance were defined as follows: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

4   Results

4.1   Carbon and nitrogen pools

Dissolved organic carbon (DOC) concentrations were significantly different between sites ($p < 0.001$) and horizon classes ($p < 0.001$). DOC was the highest in boreal forest sites in organic and mineral soil (Figures 1a-c). In the steppe, DOC increased relatively to N with depth. Tundra and forest steppe sites showed low concentrations of DOC in all soil layers.

Total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON) concentrations showed highly significant differences between soil horizons and sampling site ($p < 0.001$, respectively). TDN and DON were again highest in boreal sites in all three soil layers (Figures 1a-c). Lowest concentrations were measured in the tundra site, but also in the steppe for the organic and mineral topsoil. Also forest steppe sites showed relatively low concentrations in TDN and DON.

Total free amino acids (TFAA), ammonium (NH_4^+) and nitrate (NO_3^-) concentrations differed significantly between horizon classes (TFAA $p < 0.001$; NH_4^+ $p < 0.01$; NO_3^- $p < 0.05$) and sites (TFAA $p < 0.001$; NH_4^+ $p < 0.05$; NO_3^- $p < 0.001$), exceeding about tenfold in organic horizons compared to mineral soil on a dry matter basis (Figures 1d-f). Highest C and N concentrations in organic horizons were again measured in the forest sites. The TFAA pool reached overall highest concentrations in the boreal forest, while tundra and steppe ecosystems were characterized by much lower concentrations. NH_4^+ reached generally much higher concentrations than NO_3^- and concentrations even exceeding TFAA concentrations in the southern taiga sampling site. The steppe was characterized by a very high inorganic N pool (especially in NO_3^-), which even surpassed the organic TFAA pool.

In mineral topsoil, NO_3^- concentrations were again very low at all sites, except of the steppe site showing very high concentrations. TFAA were again the most abundant N form. Highest N pools were found in the forest sites, worth noting the middle and southern taiga sites. Again, the steppe showed much higher concentrations in inorganic N forms instead of organic TFAAs. Mineral subsoil showed a relatively low N pool at all sampling sites. The smallest pool was identified once more in the tundra followed by quite low concentrations at the forest steppe sites. Contrary to the upper soil, highest concentrations occurred in inorganic N forms, notably NH_4^+. The steppe was again dominated by inorganic NO_3^- being followed by NH_4^+. TFAA-concentrations in the mineral subsoil were in all soils very low and equal to or even beyond inorganic N concentrations.
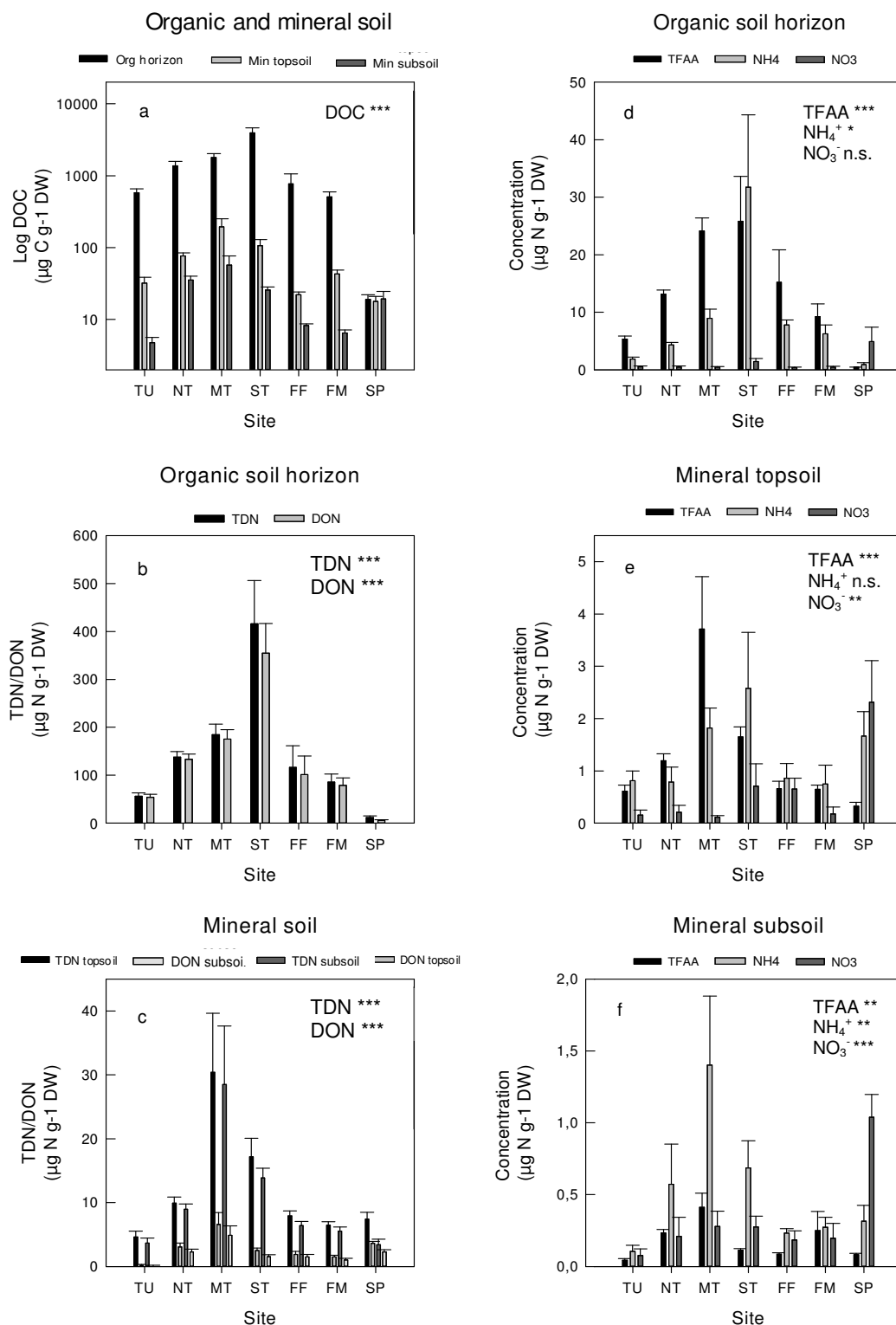
Figure 3: Concentrations of (a) dissolved organic carbon (DOC) for organic and mineral soil, (b) total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON) for organic soil horizon, and (c) for mineral soil; Concentrations of total free amino acids (TFAA), ammonium (NH_4^+) and nitrate (NO_3^-) for (d) organic soil horizon, (e) mineral topsoil and mineral subsoil (f). All bars represent ± standard error. Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant (one-way ANOVA or Kruskal-Wallis test).

4.2 Soil microbial transformation rates

An ANOVA showed the main effect on gross protein depolymerization to be horizon class not sampling site, whereas the main effect on gross N-mineralization was determined to be the sampling site (see Table 3). Nevertheless, statistical analysis was calculated separated for the three horizon classes organic horizon (O), mineral topsoil (A) and mineral subsoil (M).

Table 3: Analysis of Variance for gross protein depolymerization and gross N mineralization.

	Gross protein depolymerization		N mineralization	
	F-Ratio	P-Value	F-Ratio	P-Value
Main effects				
Site	2.7	0.0196	9.2	0.0000
Horizon class	10.1	0.0000	0.2	0.9288

All F-ratios are based on the residual mean square error.

Sampling site differences in gross protein depolymerization, calculated per gram N, could be stated for organic horizon ($p < 0.05$) and mineral topsoil ($p < 0.05$), whereas gross N mineralization differed significantly between the sampling sites in all three soil layers ($p < 0.01$; all transformation rates per g N). Gross protein depolymerization of organic horizon was highest in boreal forest sites (MT, ST, FF), while the lowest rates occurred in the tundra and grassland sites (TU, FS, SP). Mineral topsoil showed no clear pattern in transformation. In mineral subsoil a very high protein depolymerization occurred in the northern taiga (NT), while the other sampling sites showed similar low transformation rates (Figure 2).

Gross N mineralization showed a different pattern in all three soil classes horizons (Figure 2). In the organic horizon, the highest rates were observed in the southern taiga (ST), while the mineralization rates increased from north to south and diminished again from the ST southwards. In mineral topsoil, the N mineralization peaked in the northern taiga (NT) and decreased successively with decreasing latitude, showing higher rates in the boreal sites (CT, ST) than in the hemi-boreal site (FF) and grasslands (FM, SP). In mineral subsoil, a very high transformation occurred in the northern sampling sites compared to the ones in the south. The highest rates were stated in the southern tundra (TU), followed by high rates in the northern (NT) and middle taiga (MT).
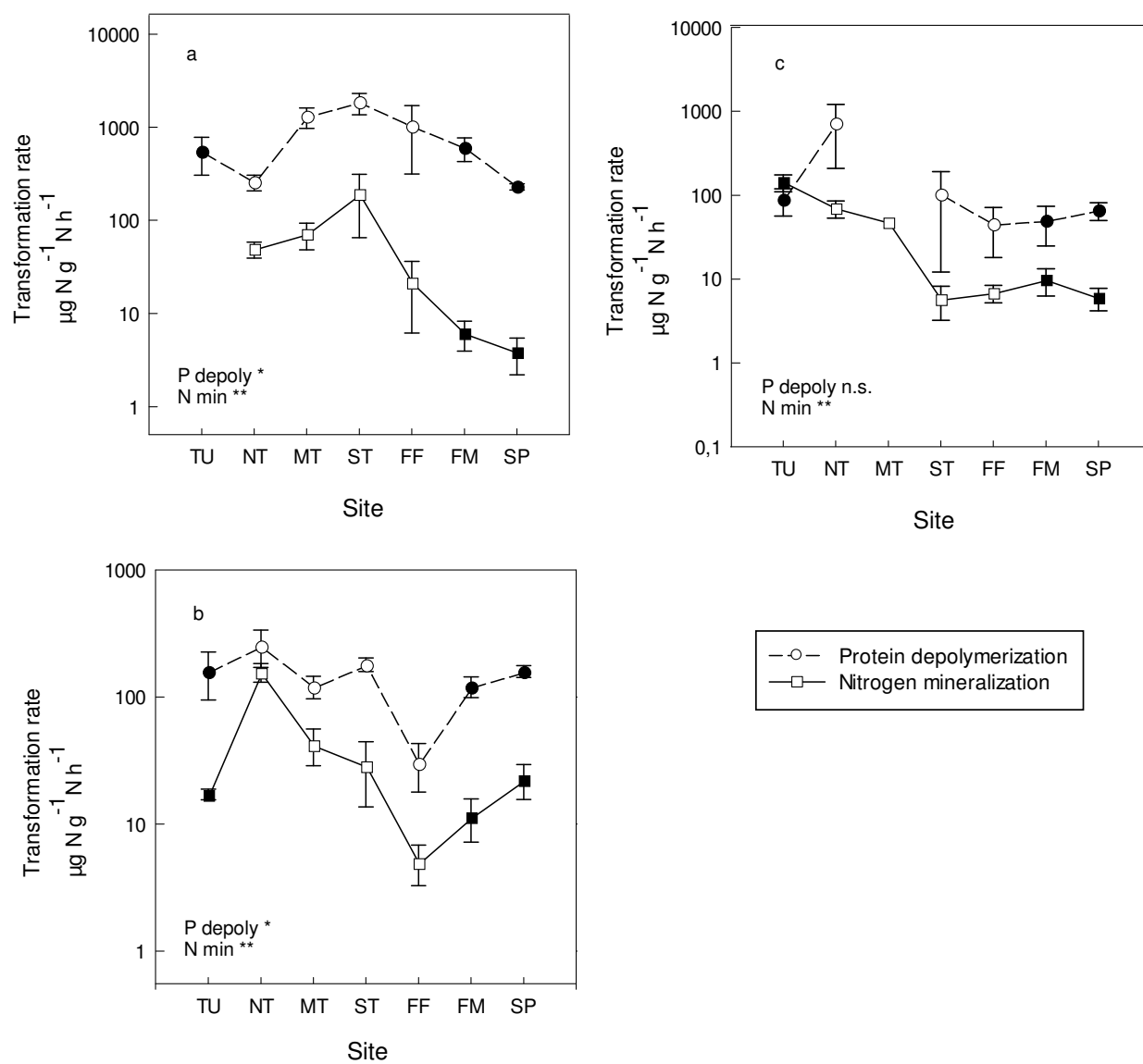
Figure 4: Transformation rates of protein depolymerization (circles) and nitrogen mineralization (squares) at the different sampling sites (black, grassland sites; white, forest sites) for the three soil horizons; organic horizon (a), mineral topsoil (b) and mineral subsoil (c). Note the logarithmic scale. All bars represent ± standard error. Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant (one-way ANOVA or Kruskal-Wallis test).

4.3 Nitrogen use efficiency

Nitrogen use efficiency (NUE) differed between different sites and horizons (Figure 3). Highest NUE was found in organic horizons and within the organic horizons in the Southern sites. NUE in mineral horizons showed no significant differences, neither in topsoil nor in subsoil. Especially in the subsoil high variation among the replicates of the same sites occurred and made it quite challenging to observe any pattern. Contrary, standard error in the topsoil within each site was quite small, but rates varied highly among the sampling sites. Lowest NUE occurred in the southern taiga, while all other sites showed similar high nitrogen use efficiencies.
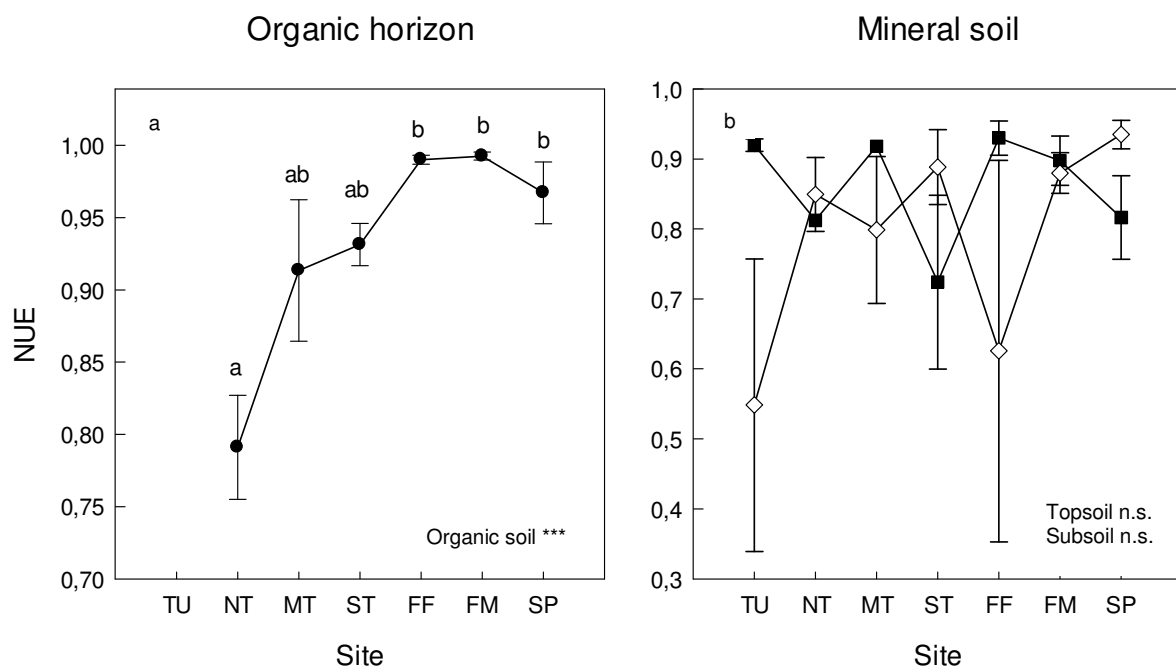

Figure 5: Nitrogen use efficiency (NUE) for (a) organic horizon and (b) mineral topsoil (black squares) and subsoil (white squares) at the seven sampling sites. All bars represent ± standard error. Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant (one-way ANOVA or Kruskal-Wallis test). Homogenous groups were determined with post hoc Tukey HSD test.

4.4 Effects of soil parameters on microbial transformation rates

Gross protein depolymerization and gross amino acid uptake were correlated significantly to different pools in the organic horizon (Table 2). Generally, all forms of organic (TFAA, DON) and inorganic nitrogen (N, TDN, NH_4^+) were correlated with both transformation rates. Furthermore, net N mineralization correlated with gross protein depolymerization and amino acid uptake rates. Gross protein depolymerization of mineral soil did not significantly correlate with any soil parameter. Thus, gross amino acid uptake correlated with net protein depolymerization in the

organic horizon, mineral topsoil and mineral subsoil. Gross N mineralization correlated with almost all parameters in the organic horizon and mineral subsoil, whereas in mineral topsoil no correlations could be stated at all. The strongest correlations of gross N mineralization in the organic horizon were found with different soil C and N pools and the soil C/N ratio. Besides, a significantly strong correlation could be found with net N mineralization in this soil horizon. Gross NH_4^+-uptake correlated similarly with the same parameters but showed also a significant correlation with gross amino acid uptake. In mineral subsoil, gross N mineralization correlated with total soil N, soil C/N ratio, pH-value and WHC. Gross NH_4^+-uptake correlated negatively with total soil N, WHC, TDN and DON. No other significant correlations with C and N pools could be stated in this horizon.

To investigate soil characteristics and soil microbial transformation rates across sites in the three different soil depths, a Principal Component analysis was performed (Figure 4). In the organic horizon, principal component 1 (PC I) accounted for 45% of variation among soil samples and was positively linked to C and N pools, soil C/N ratio and WHC, while variation in PC II could be explained by differing gross transformation rates.
PC I in mineral topsoil accounted for 36% of variation being positively linked to all C and N pools and WHC, but negatively to NO_3^- and pH. Transformation rates did mainly contribute to variation in component II in mineral topsoil. In mineral subsoil, PC I explained variation for 31% being positively linked to DOC, N pools and pH, but showing a negative link to most gross and net fluxes. PC II accounted for 25% of variation between soil samples and may be explained by depolymerization rates, DOC and NH_4^+.

Table 2: Spearman Rank Correlation for organic horizon (O), mineral topsoil (A) and mineral subsoil (M) for all sampling sites. The first value represents the correlation coefficient (r); the second value stands for the significance level (p). Black numbers show significant correlations ($p < 0.05$); grey numbers show not significant correlations ($p > 0.05$).

	Gross protein depolymerization			Gross amino acid uptake			Net protein depolymerization			Gross nitrogen mineralization			Gross NH4+ uptake			Net nitrogen mineralization		
	O	A	M	O	A	M	O	A	M	O	A	M	O	A	M	O	A	M
C	0.2311	-0.0383	0.2027	0.2285	-0.2458	0.0677	-0.1586	0.1007	0.2271	0.7730 ***	-0.0137	-0.0827	0.7592 ***	-0.0013	-0.3622	-0.3523	-0.0170	-0.1315
N	0.5608 **	-0.1415	0.1702	0.6303 ***	-0.1875	-0.0369	-0.2556	0.0782	0.1499	0.4229 *	-0.4160 *	-0.4053 *	0.4654 *	-0.2810	-0.4524 *	-0.5538 **	-0.4797 **	-0.4485 *
C/N	0.0265	0.1444	0.0457	-0.0794	0.0549	0.0887	-0.0523	-0.0012	0.1083	0.7053 ***	0.5037 **	0.5621 **	0.7185 ***	0.3329	0.2868	-0.2462	0.4652 **	0.5064 **
pH	0.0969	-0.2221	-0.0057	0.1807	0.0233	0.0358	-0.2043	-0.0654	-0.1219	-0.5281 **	-0.5838 ***	-0.3866 *	-0.4725 *	-0.4515	-0.2620	0.0578	-0.3487 *	-0.3958 *
WHC	0.1920	-0.0410	-0.2028	0.1640	-0.0685	-0.4275	-0.2236	0.0061	0.1269	0.6417 **	-0.0313	-0.4435 *	0.5746 **	-0.2026	-0.5144 **	-0.1438	0.1099	-0.2332
DOC	0.5831 **	0.2538	0.0062	0.5987 ***	-0.0659	0.0406	-0.2316	0.1691	0.3068	0.9070 ***	0.3971	-0.1262	0.9038 ***	0.4509	-0.1725	-0.5862 **	-0.0882	-0.2236
TDN	0.6009 **	0.2387	0.1316	0.6294 ***	-0.2833	0.1308	-0.2205	0.2589	0.2000	0.8831 ***	0.2978	-0.3278	0.8669 ***	0.5180	-0.3913 *	-0.6085 **	-0.3743 *	-0.4251 *
DON	0.5880 **	0.1907	0.1480	0.6076 **	-0.2344	0.0466	-0.2191	0.1276	0.2211	0.8981 ***	0.2403	-0.3233	0.8808 ***	0.4084	-0.4863 *	-0.5977 **	-0.1925	-0.2890
TFAA	0.5057 **	0.1938	0.1575	0.5751 **	-0.1301	-0.0136	-0.0763	0.1231	0.2942	0.7115 ***	0.3424	-0.0529	0.8277 ***	0.4615	-0.2759	-0.6031 **	-0.1617	-0.0202
NH4+	0.6810 ***	0.1827	-0.0819	0.7962 ***	-0.0910	-0.0060	-0.3468	0.3196	0.0865	0.6130 **	0.0405	-0.2953	0.6238 **	0.0776	-0.2756	-0.5915 **	-0.4169	-0.3292
NO3-	-0.0325	0.1205	0.0820	0.0136	-0.1726	0.1685	-0.0372	0.4319	0.1798	-0.0523	-0.2478	-0.3391	0.0000	-0.1101	-0.2795	-0.2038	-0.2275	-0.4538 *
Gross p-depoly				0.8492 ***	0.5177 **	0.5609 *	-0.0719	0.3364	0.0767	0.4002	0.1943	0.1666	0.4116	0.1932	-0.2437	-0.4704 *	-0.0230	0.2602
Gross AA uptake	0.8492 ***	0.5177 **	0.5609 *				-0.4060 *	-0.5116 **	-0.5534 *	0.3982	-0.0300	0.3970	0.4421 *	-0.2262	0.2561	-0.5110 *	0.0623	0.1723
Net p-depoly.	-0.0719	0.3364	0.0767	-0.4060 *	-0.5116 **	-0.5534 *				-0.0346	0.1362	-0.1564	-0.0073	0.2200	-0.2406	-0.0254	-0.2138	0.0093
Gross N min.	0.4002	0.1943	0.1666	0.3982	-0.0300	0.3970	-0.0346	0.1362	-0.1564				0.8992 ***	0.7991 ***	0.5973 **	-0.4585 *	0.1925	0.7621 ***
Gross NH4+ uptake	0.4116	0.1932	-0.2437	0.4421 *	-0.2262	0.2561	-0.0073	0.2200	-0.2406	0.8992 ***	0.7991 ***	0.5973 **				-0.7223 ***	-0.1922	0.1552
Net N min.	-0.4704 *	-0.0230	0.2602	-0.5110 *	0.0623	0.1723	-0.0254	-0.2138	0.0093	-0.4585 *	0.1925	0.7621 ***	-0.7223 ***	-0.1922	0.1552			

Levels of significance: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$
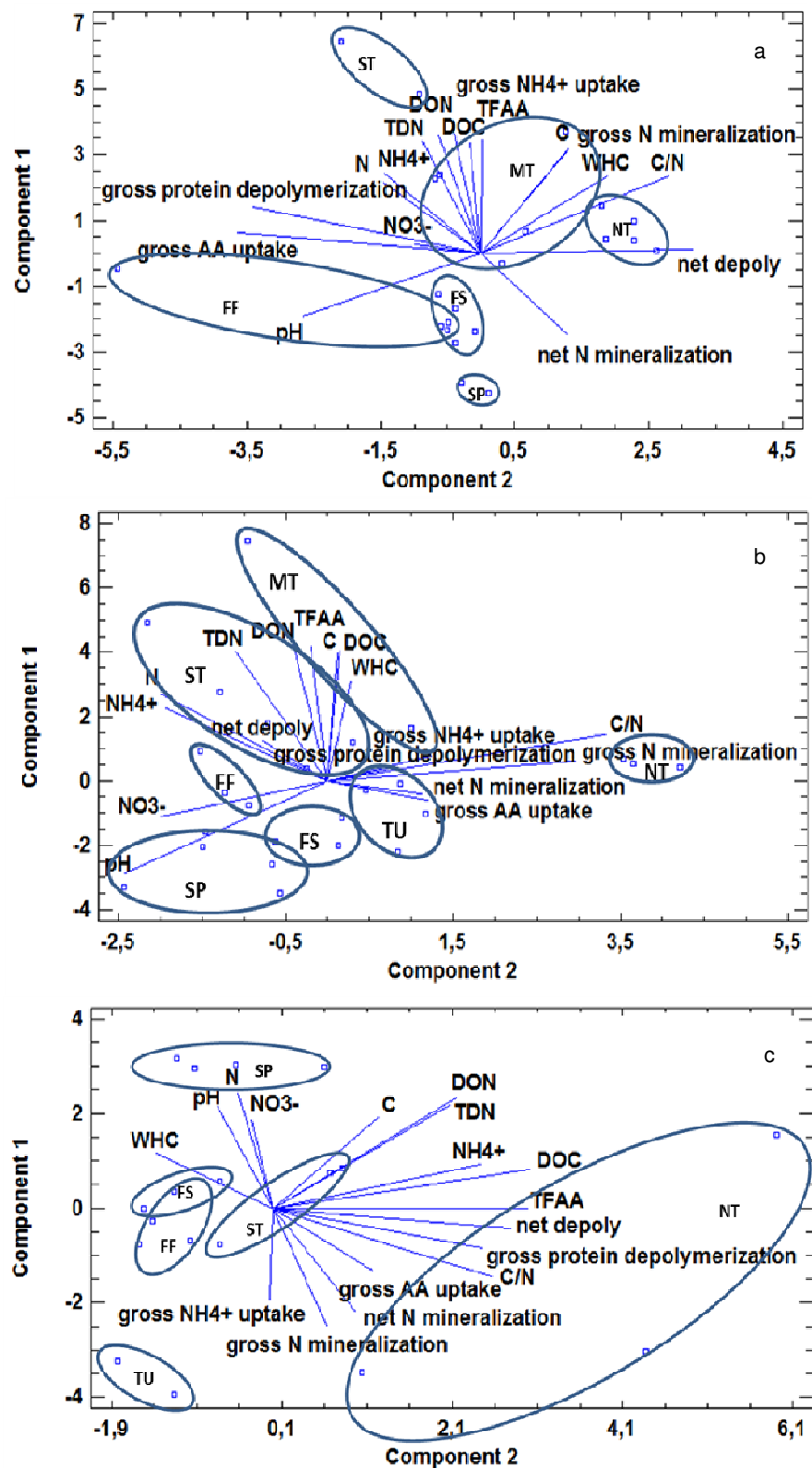
Figure 6: Principal Component Analysis (PCA) for organic horizon (a), mineral topsoil (b) and subsoil (c). Data include all measured pools and fluxes, pH and WHC. TU in organic horizon and MT in mineral subsoil not included due to incomplete data set.

5   Discussion

5.1   N transformation rates across latitudinal gradients

Overall highest protein depolymerisation and N mineralization rates were found in the taiga forest, whereas tundra and steppe were characterized by low transformation rates. These low transformation rates were accompanied by low soil water content. Highest rates occurred in the middle and southern taiga, which were characterized by high soil moisture due to highest annual precipitation along the entire latitudinal transect. These findings suggest that transformation processes highly depend on soil water availability, which may provoke a less active soil microbial community under limited water regimes (Schimel et al., 1996; Booth et al, 2005; Schnecker et al., 2014). Furthermore, high rates in the boreal forest may be due to respectively high vegetation density and high SOM content of soil (Cookson et al., 2007). Several studies already suggested vegetation type (Meyer et al., 2006; Cookson et al., 2007) and SOM content (Ross and Speir, 1979; Booth et al, 2005; Kielland et al, 2007) as important regulators of gross N fluxes and microbial community composition. Other studies argue that major microbial processes are primarily related to C and N availability in soils (Colman and Schimel, 2013). We indeed found highest C and N concentrations in taiga soils and respectively low concentrations in tundra and steppe soils. As a function of arising C and N concentrations, productivity and decomposition processes are stimulated (Christiansen et al., 2012) and may explain the high N fluxes in taiga soils (Ross and Speir, 1979), which may easily exceed those of warmer and more productive ecosystems in the more southern latitudes (Kielland et al, 2007). Boreal ecosystems are further characterized by low pH values which may favour proteolysis in a higher extent than N mineralization (Kielland et al, 2007). This may accelerate protein depolymerization in especially these ecosystems compared to the more southern sampling sites and could explain the observed high fluxes in these soils. The lower protein depolymerization rates in the arctic and the north of the boreal forest may be due to lower temperatures which are considered to reduce the protein turnover (Kielland et al, 2007).

5.2   N transformation rates across depth gradients

Transformation rates were high for both protein depolymerization and N mineralization in the upper organic soil layer. Gross protein depolymerization decreased along the depth gradient which goes in line with former studies stating high turnover rates of amino acids in especially leaf litter (Wanek et al., 2010) and upper soil layers (Kielland et al., 2007; Jones et al., 2009) compared to lower rates in deep soil. This may be due to a decline in microbial biomass with depth (Blume et al, 2002), accompanied by a shift in microbial community composition (Eilers et al., 2012). Hence, microbial soil communities in deep soil may be less active by producing less proteases than microbial communities in upper soil layers (Schnecker et al, 2014), provoking the observed reduction in protein depolymerization rates. Furthermore, deep soils show low abundances in plant biomass being represented only rarely in form of roots. This provides a less competitive environment for microbes in respect of N in deep soil layers and enables a retarded protein depolymerisation. Contrary, in upper soil high microbial and plant biomass leads to respectively high competition and thus nutrient limitation.

While protein depolymerization rates were observed to diminish with increasing soil depth, N mineralization rates increased absolutely but also relatively compared to protein depolymerization rates. As already mentioned, N limitation is assumed to decrease because of reduced competition between microbes and plants and relatively high available amounts of N. Hence, the N demand of microorganisms sinks along depth gradients. Instead of depolymerising the present proteins as N source, they use available compounds just to gain enough C, which is limiting in these soil depths (Nadelhoffer et al., 1991). Hence, N mineralisation occurs where N is more available than needed and not the limiting factor of microbial productivity (Schimel and Bennett, 2004; Booth et al, 2005; Wild et al, 2013). Microbes just take up any source of organic material to compensate for C limitation, mineralizing partly the left over N (Nadelhoffer et al., 1991; Jones and Kielland, 2012). Therefore, amino acids are also taken up and used as energy source. The left over N is mineralized and released as NH_4^+. This may explain the observed rise of N mineralization rates and the decrease in protein depolymerisation rates with increasing soil depth. High fluctuation in N mineralization with depth occurred especially in the boreal sites, where high rates in the organic horizon diminished rapidly with depth, whereas rates in tundra and meadow soil stayed more constant.

Another reason for the high N mineralization rates in deep soil layers may be the fact that consumption rates may be stimulated by substrate addition (Davidson et al, 1991; Booth et al, 2005). Especially deep soil layers are limited in soil organic matter, and soil microbial activity may be enhanced easily if substrate, here in form of amino acids, is added.

5.3 Nitrogen use efficiency

In contrast to our expectations, nitrogen use efficiency of the soil microbial community decreased with increasing latitude in the organic soil horizon. Rates were of same magnitude in the Southern ecosystems and decreased significantly with intermediate values in the southern and middle taiga and showed lowest values in the northern taiga. In the southern tundra, mineralization rates were that low that they fell below the detection limit. Therefore, NUE could not be calculated for this sampling point, but is expected to be one of the highest along the whole sampling transect as arctic systems are known to represent highly N limited systems (LeBauer and Treseder, 2008; Schimel and Bennett, 2004), wherein microbes have to highly compete for the low available N amounts. Therefore, low N mineralization occurs as only small amounts of N are released back in the nutrient limited environment (Manzoni et al., 2008; Mooshammer et al., 2014). Instead, protein depolymerization is high and the major fraction of available N is incorporated into microbial biomass (Sistla et al, 2012), as could be observed in the organic horizon of the tundra site.

Compared to most studies that state increasing N limitation in soils with decreasing latitudes (e.g. Schimel and Bennett, 2004), our results show a converse pattern. Dry grassland sites were characterized by the highest NUE along this latitudinal gradient showing values of almost 1.0 and indicating a high N limitation within these soils. Plants of temperate grasslands are considered as being even more N constrained than in tundra ecosystems (LeBauer and Treseder, 2008), because of the strong co-limitation between N and water availability (Harpole et al., 2007). Plant productivity in temperate grasslands increases highly in response to both N fertilization and water availability. The soil microbial community may experience the same co-limitation in N and water availability because of the generally high temperatures but respectively low precipitation in the grassland ecosystems (see Table 1, p. 23). These circumstances may cause reduced microbial activity to save energy costs and result in high microbial N immobilization but low N mineralization to use all available N to form new biomass. Thus, microbial organisms living within these ecosystems exert a need for high NUE (Sterner and Elser, 2002). Furthermore, average senesced leaf C:N ratios within grassland ecosystems are respectively high with C:N ratios of 60.9 compared to the average C:N ratio of 52.9 across all biomes (Yuan and Chen, 2009). Highest leaf C:N in this study was stated for tundra ecosystems with a C:N ratio of 72.8, which may provoke high microbial N immobilization and low mineralization rates as we could observe in the southern tundra. This proposes high microbial NUE within these low C:N tundra ecosystems.

In contrast, sites showing more N-sufficient conditions, generated by low substrate C:N ratios, establish soil microbial communities which mineralize excess N and lower their N immobilization rates. This results in a low microbial NUE of at least 0.68 in organic soil horizons (Mooshammer et al., 2014) in C:N low ecosystems such as tropical forests, showing senesced leaf C:N ratios of 41.9. Boreal forests are also characterized by relatively low senesced leaf C:N ratios of 48.2 (Yuan and Chen, 2009). Organisms living within these conditions are expected to adapt to their environment (Mooshammer et al., 2014), resulting in an intermediate NUE which was observed in our study. Microbial soil community in the boreal forest ecosystem, especially the northern taiga, showed the lowest NUE along the latitudinal gradient, with an NUE of 0.8 ±0.05. Thus, N immobilization and mineralization rates in organic soil horizons seem to depend mostly on initial litter chemistry and not on climatic regimes (Manzoni et al., 2008).

Mineral topsoil did not show any significant differences in NUE across the latitudinal gradient. Our results are supported by other studies which proposed equal N limitation across different ecosystems (Elser et al, 2007; Jones et al., 2009). This may be due to the fact that organic horizons are mostly influenced by the given environment, climate regime and vegetation, while mineral subsoil is much more isolated and thereby enables more constant conditions. Physical and chemical parameters seem to be more important and major controls on nutrient cycling in deeper soil layers. Especially N mineralization in deep soil horizons suggests high dependency on different N pools, pH and WHC.

6   Conclusion

N transformation rates change with latitude and seem to be influenced not only by climate variables such as temperature and soil moisture, but also by soil C and N concentration, SOM content and pH. Highest protein depolymerization and N mineralization rates occurred in the boreal forest, being accompanied by peaking soil C and N concentrations. Tundra and grassland sites were characterized by much lower transformation rates. Transformation rates were high for both protein depolymerization and N mineralization in the upper organic soil layer. Reduced plant microbial competition for N in deep soil layers may stimulate N mineralization and lower protein depolymerization rates. Contrary to our expectations, soil microbial NUE at temperate grassland sites was characterized by highest values reaching almost 1.0, while values were respectively low at all taiga sites. Unfortunately, NUE could not be calculated for the southern tundra as mineralization rates were under detection limit. High NUE suggested microbial adaptation to high litter C:N ratios, whereas lowest NUE occurred where intermediate litter C:N was observed. Thus, NUE seems to depend mostly on initial litter chemistry. Certainly, there are numerous other factors influencing and changing NUE e.g. the limitation of other nutrients besides N itself which may lead to a decrease in NUE (Mooshammer et al., 2014). Furthermore, enzymes production involves mainly N investment and may have a strong impact on NUE (Schimel and Weintraub, 2003). So definitely, there are much more relevant factors that differentially effect soil community physiology and the microbial N cycle.

7   Acknowledgements

First of all, I thank my family for supporting me all over the last twenty five years. Without my parents and grandmothers' encouragement it wouldn't have been possible to achieve the entire education that I was lucky to experience.

Furthermore, I want to thank all my colleagues from the Division Terrestrial Ecosystem Research, who helped me in any way during my Master thesis. Here, I especially thank Mag. Birgit Wild, who incessantly had time to help me with any matter of concern and who was the best scientific support I can think of. I also like to thank Mag. Florian Hofhansl for having always an open ear and a solution for any technical and statistical concern. Great thanks to Univ.-Prof. Dr. Andreas Richter who enabled this master thesis and all affiliated experiences and acquaintances.

Last but not least, I thank the members of the CRYOCARB - team for their cooperation during field work, especially Mag. Norman Gentsch, who classified the soil types after the World Reference Base.

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III Zusammenfassung

Ökosysteme weißen nicht nur Unterschiede in ihren klimatischen Bedingungen und ihrer Vegetation auf sondern auch in ihrer Bodenzusammensetzung in Form von organischen und mineralischen Bodensubstanzen. Hohe Variation ist vor allem in der Verfügbarkeit diverser Nährstoffe, wie beispielsweise Kohlenstoff (C) und Stickstoff (N), zu beobachten. Vor allem variiert die Nährstoff-Verfügbarkeit in Böden stark entlang latitudinaler Gradiente. Dies setzt eine hohe physiologische Anpassungsfähigkeit der bodenbewohnenden Mikroorganismen voraus, um eine hohe Konkurrenzstärke gegenüber Pflanzen aber auch anderen Bodenorganismen zu gewährleisten. Hierbei spielen vor allem Prozessraten eine grundlegende Rolle. Mikroorganismen können Aminosäuren direkt aufnehmen, um ihren Stickstoffbedarf zu decken. Liegen Aminosäuren allerdings als längere Peptide, Proteine oder anderwärtige Polymere vor, so geht eine obligate Protein-Depolymerisierung der mikrobiellen Aufnahme voraus. Ist die Stickstoff-Verfügbarkeit im Boden gering, werden die depolymerisierten Aminosäuren von der mikrobielle Bodengemeinschaft augenblicklich aufgenommen und als Biomasse assimiliert (mikrobielle Aminosäuren-Immobilisierung). Aufgenommene Aminosäuren können auch wieder ausgeschieden und in Form von Ammonium (NH_4^+) in den Boden freigesetzt werden (Stickstoff-Mineralisierung). Dieser freigesetzte Stickstoff kann folglich wieder aufgenommen, assimiliert (mikrobielle NH_4^+ Immobilisierung) und wieder freigesetzt werden. All diese Transformationsraten variieren in ihrer Höhe je nach dominanter Stickstoff Quelle sowie genereller Stickstoff Verfügbarkeit des jeweiligen Bodens. Die daraus resultierende mikrobielle Anpassungsfähigkeit kann mittels Stickstoff Nutzungseffizienz (NUE) angegeben werden. Die NUE drückt aus wieviel des aufgenommen Stickstoffs in die mikrobielle Biomasse eingebaut beziehungsweise als NH_4^+ wieder freigesetzt wird. Je höher sie ist, umso mehr wird der N von der mikrobiellen Gemeinschaft genützt und als Biomasse assimiliert, d.h. die mikrobielle Aminosäuren-Immobilisierung überwiegt gegenüber der Stickstoff-Mineralisierung.

Ziel dieser Arbeit war die Analyse des Einflusses der Stickstoff-Verfügbarkeit auf mikrobielle Prozessraten in Böden. Hierbei standen Raten der Protein-Depolymerisierung, der Stickstoff-Mineralisierung sowie der mikrobiellen Immobilisierung von Aminosäuren und Ammonium (NH_4^+) im Fokus des Forschungsinteresses. Alle Raten wurden mittels „^{15}N pool dilution" analysiert. Diese Methode erlaubt die Quantifizierung von Flüssen indem betreffende Pools mit dem schwereren Stickstoff Isotop ^{15}N markiert werden und Konzentrationsveränderungen sowie Veränderungen in der Isotopen Anreicherung über die Zeit gemessen werden. So werden

beispielsweise ^{15}N-Aminosäuren zur Bestimmung der Protein-Depolymerisierung und Immobilisierung von Aminosäuren, beziehungsweise $^{15}NH_4^+$ zur Bestimmung der Stickstoff-Mineralisierung und Immobilisierung von NH_4^+ eingesetzt. Hierfür wurden organische und mineralische Bodenproben entlang eines 1400km langen latitudinalen Transekts in West Sibirien, Russland, gezogen. Dadurch wurde sichergestellt, dass alle wesentlichen Ökosysteme abgebildet wurden: die von Permafrost beherrschte Tundra, der boreale Nadelwald der Taiga, der laubwerfende Mischwald und die Steppe.

Zusammenfassend lässt sich feststellen, dass N-Transformationsraten von Klimavariablen wie Temperatur und Bodenfeuchtigkeit, aber auch von C und N Konzentrationen in Böden sowie der Menge organischer Bodensubstanzen abhängig sind. Die höchsten Protein Depolymerisierungsraten und N Mineralisierungsraten wurden im borealen Nadelwald gemessen, begleitet von den höchsten C und N Konzentrationen in diesen Böden. Tundra und Steppe waren hingegen durch niedrigere Transformationsraten gekennzeichnet. Transformationsraten waren generell in allen organischen Horizonten hoch. Eine Abnahme in der Protein Depolymerisierung aber eine Zunahme in der N Mineralisierung mit steigender Bodentiefe lässt auf eine reduzierte Konkurrenz zwischen Pflanzen und Mikroorganismen in tieferen Bodenschichten schließen.

Im Gegensatz zu unserer Annahme, wurde die höchste Stickstoff Nutzungs Effizienz (NUE) in den südlichen Ökosystemen der Steppe und Waldsteppe gemessen, während eine signifikante Abnahme in der mikrobiellen NUE mit zunehmendem geographischen Breitengrad beobachtet werden konnte. Die NUE in der Tundra konnte aufgrund der geringen Mineralisierungsraten, welche unter das Detektionslimit fielen, nicht kalkuliert werden. Die vorgefundene hohe NUE im Süden weißt auf eine starke mikrobielle Adaptierung an das hohe C:N Verhältnis von Streu- und Laubfall hin, während Ökosysteme mit mittleren C:N Verhältnissen durch niedrige NUE Werte gekennzeichnet waren. Die ursprüngliche chemische Zusammensetzung des Streueintrags scheint demnach der bestimmende Faktor der mikrobiellen NUE zu sein. Dennoch sind unzählige weitere Faktoren, wie beispielsweise die Limitierung anderer Nährstoffe, als wesentliche Faktoren in der Entwicklung und Veränderung der mikrobiellen NUE zu berücksichtigen.

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IV Curriculum vitae

Personal Information

Name	Knoltsch, Anna
Nationality	Austria

Education

1995 – 1999	Elementary school Theodor–Körnerschule, Klagenfurt
1999 – 2007	Secondary school Mössingerstraße, Klagenfurt
Jan – June 2006	Pupil exchange at the Johann Goethe Schule, St.Petersburg, Russian Federation
June 2007	Higher education entrance qualification
2007 – 2010	Bachelor of Political Sciences, University of Vienna
2007 – 2011	Bachelor of Biology with specification in Ecology, University of Vienna
Feb 2011 - July 2011	ERASMUS-exchange at the Université Montpellier II, Montpellier, France
2010-2013	Master of Ecology, specification in "Ecosystem Sciences", University of Vienna Master thesis: "Microbial nitrogen use efficiency along a transect in Western Siberia", Department of Microbiology and Ecosystem Science, Division of Terrestrial Ecosystem Research

Working experience

April 2010 – June 2010	Voluntary service in soil field mapping BFW – Federal Office of Wood, Vienna
August 2010	Internship in herbarising Botanical Garden, Klagenfurt
September 2010	Internship in limnology KIS – Carinthian Institute for lake research, Klagenfurt
April - June 2011	Determination of the macrozoobenthos in the lagoon of Thau Université Montpellier II, Montpellier, France
2011 – 2013	Tutor of the practical course "Pflanzenanatomie " Department of Molecular System Biology, University of Vienna
Summer term 2012 - 2014	Tutor of the field course "Kenntnis mitteleuropäischer Lebensgemeinschaften" Faculty of Life Sciences, University of Vienna
Winter term 2012	Tutor of the course "Chemische Methoden der Ökologie" Department for microbiology and ecosystem research, Division of Terrestrial Ecosystem Research, University of Vienna

Scientific publications, posters and talks

Mooshammer et al. (2014): Adjustment of microbial N use efficiency to C:N imbalances regulates soil N cycling. Nature Communications, 5. doi:10.1038/ncomms4694.

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