Carbon and nitrogen mineralization in temperate forest soils at low temperatures

Dissertation

zur Erlangung des akademischen Grades
Doktor der Naturwissenschaften (Dr. rer. nat.)

vorgelegt der
Fakultät für Biologie / Chemie / Geowissenschaften
der Universität Bayreuth

von
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geboren am 18.12.1984 in Lübeck

Bayreuth, im April 2014

Die vorliegende Arbeit wurde in der Zeit von 12/2010 bis 04/2014 in Bayreuth am Lehrstuhl für Bodenökologie unter Betreuung von Herrn Professor Dr. Egbert Matzner angefertigt.

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.).

Dissertation eingereicht am: 22.04.2014

Zulassung durch die Promotionskommission: 30.04.2014

Wissenschaftliches Kolloquium: 19.12.2014

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Zusammenfassung

Jüngere Befunde deuten auf eine erhebliche Bedeutung von C- und N-Umsätzen bei niedrigen Bodentemperaturen in Waldböden der temperierten Zone hin. Bisher wurden solche Umsätze vielfach vernachlässigt. Zur Temperaturabhängigkeit der C- und N-Umsätze bei tiefen Temperaturen ist bisher wenig bekannt, Temperaturanstiege sind während der Wintermonate allerdings als besonders kritisch zu betrachten, da die Temperaturabhängigkeit biologischer Prozesse bei tiefen Temperaturen erhöht ist. Die Temperaturabhängigkeit der C- und N-Umsätze wird vor allem durch Substratqualität und Substratverfügbarkeit beeinflusst. Während der Wintermonate kann auch langanhaltender Bodenfrost als physiologischer Stressfaktor die mikrobielle Gemeinschaft in Waldböden beeinflussen und die Stoffumsätze in der folgenden (trockenen) Vegetationsperiode verändern.

Diese Arbeit hatte zum Ziel, den Anteil der winterlichen Netto-N-Mineralisation an der gesamtjährlichen Netto-N-Mineralisation an zwei Waldstandorten zu quantifizieren. Weiterhin sollten Brutto-Ammonifikation, -Nitrifikation, Netto-N- und C-Mineralisation bei tiefen Temperaturen ermittelt werden sowie deren Temperaturabhängigkeit (Q_{10}-Wert) berechnet werden. Der Einfluss von Substratmenge und -qualität auf die Temperaturabhängigkeit wurde untersucht. Ferner wurden die Auswirkungen von Bodenfrost auf die C-Mineralisation und mikrobielle Biomasse in einer nachfolgenden Trockenperiode untersucht.

Die winterliche Netto-N-Mineralisation wurde in einem *in situ* Experiment mit der *sequential coring* Methode über einen Zeitraum von sechs Monaten bestimmt. Für die Laborexperimente wurde homogenisiertes Material der L/Of-, Oh-(Fichte) und Ah-(Buche)-Horizonte bei Temperaturen von -4, -1, +2, +5 und +8°C inkubiert, die Bestimmung der Brutto-Raten erfolgte mittels der ^{15}N pool dilution technique und die Berechnung der Temperaturabhängigkeiten über die Arrhenius-Funktion (Laborexperiment 1). Eine Erhöhung der Substratmengen im Laborexperiment 2 wurden im Falle der Brutto-Ammonifikation durch die Zugabe von Glycin erreicht, im Falle der Brutto-Nitrifikation durch die Zugabe von Ammonium. Im Laborexperiment 3, das insgesamt über 161 Tage durchgeführt wurde, wurde nach einem Bodenfrost wöchentlich die C-Mineralisation und an 2 Terminen die mikrobielle Biomasse mittels GC-Messungen bzw. Substrat-induzierter Respiration gemessen.

Die winterliche *in situ* Netto-N-Mineralisation betrug unter Buche 44 kg N ha^{-1} 6 $Monate^{-1}$ und unter Fichte 11 kg N ha^{-1} 6 $Monate^{-1}$. Insgesamt wurden 65% des im jährlichen Streufall enthaltenen N unter Buche und 26% des im jährlichen Streufall enthaltenen N unter Fichte

während der Wintermonate mineralisiert. Brutto-Ammonifikation, -Nitrifikation und C-Mineralisation aus dem Inkubationsversuch deuteten ebenfalls auf erhebliche Mineralisierungsraten bei Temperaturen um den Gefrierpunkt hin, wobei Brutto-Ammonifikation und C-Mineralisation in Buche L/Of deutlich höher als in Fichte L/Of waren. Der herbstliche Streufall in Laubwäldern liefert eine große Menge leicht verfügbarer Substrate direkt vor der Winterperiode, welche bei winterlichen Temperaturen mineralisiert werden. Die Q_{10}-Werte der C- und Brutto-N-Mineralisation lagen zwischen 2.4 und 11 und waren höher in Substraten besserer Qualität. Dies deutet darauf hin, dass neben der Substratqualität die Substratverfügbarkeit die Temperaturabhängigkeit in unseren Böden maßgeblich beeinflusst. Dies wurde durch das Experiment zur Substratverfügbarkeit bestätigt, hierin erhöhten Glycinzugaben den Q_{10}-Wert der Brutto-Ammonifikation um Faktor 2, allerdings waren die Raten so hoch, dass ein Anwachsen der mikrobiellen Biomasse vermutet wurde, und die Q_{10}-Werte somit nicht mehr als reine Temperaturabhängigkeit interpretiert werden konnten. Im Gegensatz zu ungestörten Proben wiesen homogenisierte Proben höhere Q_{10}-Werte der Netto-N-Mineralisation auf, was auf eine erhöhte Substratverfügbarkeit durch das Homogenisieren zurückzuführen ist. Das Verhältnis von C-Mineralisation zu Brutto-Ammonifikation war bei tiefen Temperaturen eng (~1) und gibt Hinweise auf einen schnellen Umsatz des mikrobiellen N-Pools oder eine präferenzielle Mineralisierung N-reicher organischer Substanz. Brutto- und Netto-Nitrifikation wiesen bei tiefen Temperaturen in beiden Böden geringe Raten auf, unter Fichte war die Nitrifikation etwas höher als unter Buche, was hier ein moderates Risiko der Nitratauswaschung birgt. Nach einem strengen Bodenfrost erholten sich C-Mineralisation und mikrobielle Biomasse in temperierten Waldböden innerhalb weniger Tage (ca. 1-7 Tage) nach dem Auftauen und nach 90 Tagen waren keine Frosteffekte mehr messbar. Auch haben Frost-Tau-Zyklen keinen Einfluss auf die Bodenrespiration während einer moderaten Austrocknungsphase. Bei optimaler Bodenfeuchte hingegen können verspätete Frosteffekte eintreten (nach 90 Tagen), die die mikrobielle Biomasse reduzieren und zu einem Absinken der C-Mineralisierung in vormals gefrorenen Böden führen können.

Zusammenfassend zeigt diese Arbeit die große Relevanz winterlicher C- und N-Umsätze sowie deren erhöhte Temperaturabhängigkeit bei tiefen Temperaturen. Ein Anstieg der Wintertemperaturen wird substanzielle Effekte auf die C- und N-Umsätze in Waldböden haben, wobei die Effekte mit zunehmender Bodentiefe abnehmen aufgrund der niedrigeren Substratqualität. Änderungen der winterlichen Temperaturen werden v. a. bei Laubwäldern, in

denen der Streufall direkt vor der winterlichen Periode eine große Menge an leicht verfügbarem organischen Material liefert, den C- und N-Haushalt beeinflussen.

Summary

In the past, carbon (C) and nitrogen (N) mineralization in temperate forest soils was often considered negligible at low temperatures during the dormant season, which is questioned by recent findings. Climate models predict warmer winters but little is known about the temperature sensitivity of C and N mineralization at low temperatures. Temperature rises in the lower range are of critical importance as the temperature dependency of microbial processes is enhanced at low temperatures. Besides microbial parameters, substrate availability and quality are known to influence the temperature dependency of decomposition. Around freezing point, soil frost may also affect the soil microbial biomass and reduce their activity, especially during a subsequent (dry) vegetation period.

This study aimed at quantifying the contribution of overwinter net N mineralization to the annual net N mineralization in a temperate European beech and Norway spruce forest soil. Gross ammonification, gross nitrification, net N and C mineralization as well as the temperature response of these processes (Q_{10} value) were determined at low temperatures. The influence of substrate quality and availability on the temperature dependence was investigated. Furthermore, the effects of soil frost on C mineralization and microbial biomass during a subsequent desiccation period were investigated.

Overwinter net N mineralization was determined in a *in situ* study using the *sequential coring* method over six months. Homogenized soil samples of Oi/Oe, Oa (spruce) and A (beech) horizons were incubated at -4, -1, +2, +5 and +8°C during laboratory incubations, gross ammonification and nitrification was determined with the ^{15}N pool dilution technique and the Arrhenius equation was used to calculate temperature dependencies (laboratory experiment 1). Addition of glycine (in case of gross ammonification) and ammonium (in case of gross nitrification) enhanced the substrate availability in homogenized soil samples in laboratory experiment 2. The quantification of C mineralization and microbial biomass after soil samples were exposed to soil frost was conducted by GC measurements twice per week (C mineralization) and the substrate-induced respiration method (microbial biomass) on two time points in the 3rd laboratory experiment.

During the dormant season, 44 kg N ha^{-1} 6 months^{-1} were mineralized under beech and 11 kg N ha^{-1} 6 months^{-1} under spruce, thereby contributing 30% (beech) and 15% (spruce) to the annual net N mineralization. Results from the laboratory incubations confirmed that considerable gross ammonification, nitrification and net N and C mineralization take place at low temperatures. Gross ammonification and C mineralization in beech Oi/Oe exceeded that

of spruce Oi/Oe by a factor of 9 and 5. In deciduous forests, the leaf fall in autumn provides a huge amount of easily decomposable organic matter directly before the winter period, which is mineralized at winter temperatures. Apparent Q_{10} values of C and gross N mineralization were in the range of 2.4 to 11 and higher in substrates of high quality. This gives evidence that, besides substrate quality, substrate availability largely determines the temperature response of decomposition in our soils. The substrate availability experiment could affirm this assumption, as the addition of glycine raised the Q_{10} values of gross ammonification by a factor of 2. However, after glycine addition gross rates were erratically high. Likely, the glycine addition induced microbial growth which biased Q_{10} values and thus, they do not reflect "pure" temperature responses. Likewise, homogenization of soil samples increased substrate availability and Q_{10} values of net N mineralization were higher in homogenized than in undisturbed soil samples. The ratio of C mineralization to gross ammonification was narrow at low temperatures (~1), suggesting preferential mineralization of N rich organic substrates or rapid turnover of the N pool in microbial biomass. Gross and net nitrification were low at low temperatures and rates under spruce slightly exceeded rates under beech, suggesting a moderate risk of nitrate leaching in the spruce site. Microbial biomass and C mineralization quickly recover from soil frost (within 1-7 days) and all frost-related effects disappeared until day 90. Freeze-thaw cycles have no effects on C mineralization during a subsequent moderate desiccation phase. However - under optimal soil moisture conditions - frost-related effects may occur belated (after 90 days), impacting certain microbial groups and leading to a reduction of CO_2 emissions in previously frozen soils.

Generally, this work underlines the great importance of overwinter C and N mineralization as well as their large temperature sensitivity at low temperatures. Increasing winter temperatures are expected to have a huge effect on the C and N cycle in temperate forest soils but effects will decrease with soil depth, likely due to the decreasing substrate quality of the organic matter. Projected temperature changes in winter will particularly affect the C and N cycle in deciduous forests, in which the leaf fall in autumn provides a huge amount of easily decomposable organic matter directly before the dormant season.

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1. Introduction

1.1 Soil processes and temperature sensitivity at low temperatures

Biological processes in soils are known to continue at low temperatures in winter and even below freezing point (Clein and Schimel, 1995; Schimel et al., 2004; Miller et al., 2007; Ueda et al., 2013). Microorganisms remain active in unfrozen water films (Coxson and Parkinson, 1987; Mikan et al., 2002) which exist in soils even below -17°C (Sparrman et al., 2004). In high-altitude and high-latitude ecosystems, overwinter N mineralization is far from being negligible and can account for up to 60% of the annual net N mineralization (Kielland et al., 2006; Campbell et al. 2005, Vestgarden et al., 2003). Likewise, soil microbial activity throughout the winter can account for a large proportion of annual C mineralization (21 - 50%) in Northern subalpine forests (Monson et al., 2006; Zimov et al., 1996).

While overwinter C and N cycling in arctic and tundra soils has been intensively studied in the last decade (reviewed in Campbell et al., 2005), only a few studies have addressed C and gross and net N mineralization during the dormant season in temperate forests (Schindlbacher et al., 2007; Hentschel et al., 2008 & 2009; Goldberg et al., 2008). In temperate forests, topsoils rarely freeze and are often subjected to temperatures >0°C because of the insulating snow cover. Microbial processes are not restricted by water shortage as it may likely occur during the growing season in these ecosystems. In a laboratory incubation with soil columns of the forest floor (Oi+Oe+Oa horizon) from a temperate Norway spruce stand, a total amount of 86 kg N ha^{-1} was leached in 170 d at a temperature of 5°C (Hentschel et al., 2008), emphasizing the magnitude of net N mineralization at relatively low soil temperatures. Large mineral N pools in forest floors in spring have been reported for temperate forests (Ueda et al., 2013) giving evidence for net N mineralization rates in the dormant season. CO_2 production during the dormant season ranged from 12 - 15% in cold-temperate deciduous forests (Mariko et al., 2000; Schindlbacher et al., 2007). Several studies investigated the effects of freezing/thawing with focus on soil respiration and net N mineralization during thawing (reviewed by Matzner and Borken, 2008). However, studies focusing on the temperature sensitivity of C and N mineralization are up to now restricted to the growing season (Ellert and Bettany, 1992; MacDonald et al., 1995; Robinson, 2002; Dalias et al., 2002; Cabrera et al., 2005) and knowledge is scarce when it comes to gross N turnover at low

temperatures. Table 1 summarizes laboratory studies that addressed the temperature responses of N mineralization at higher temperatures. In the temperature range between 10°C to 20°C, the Q_{10} value of net N mineralization is around 2 (Dalias et al., 2002; MacDuff and White, 1985) and slightly higher for coniferous than for grassland soils. However, when temperatures decrease the Q_{10} values increase, e.g. Andersen and Jensen (2001) calculated a Q_{10} of 9.9 for gross ammonification between 3°C and 9°C in a temperate arable soil (Table 1).

Table 1 Q_{10} values of gross and net N mineralization determined in different laboratory studies.

Reference	Ecosystem	T_{min}	T_{max}	Q_{10}	Process
Ellert & Bettany, 1992	boreal aspen forest	5	15	3.4*	net N min.
Emmer & Tietema, 1990	temperate deciduous forest	0	30	1.4	net N min.
Koch et al., 2007	alpine grassland	0	30	1.7	net N min.
Dalias et al., 2002	boreal coniferous forest	10	20	1.7	net N min.
Dalias et al., 2002	temperate spruce forest	10	20	2.3	net N min.
Dalias et al., 2002	Mediterranean conif. forest	10	20	2.5	net N min.
MacDuff & White, 1985	temperate grassland	10	20	1.4	net N min.
MacDuff & White, 1985	temperate grassland	10	20	1.3	net nitrification
Andersen & Jensen, 2001	temperate arable land	3	9	9.9	gross N min.
Andersen & Jensen, 2001	temperate arable land	9	15	1.4	gross N min.
Grenon et al., 2004	subalpine spruce-fir forest	11	23	3.2	gross N min.
Grenon et al., 2004	coastal hemlock forest	11	23	2.0	gross N min.

*Q_{10} value calculated with quadratic function (equation (5), see below)

In response to global warming the frequency and intensity of soil frost and snow covers likely change in temperate forests (Campbell et al., 2005; IPCC, 2013). A rise of soil temperatures in the lower range is of special importance as the temperature sensitivity of microbial processes is enhanced at low temperatures (Dalias et al., 2002; Davidson and Janssens, 2006; Wetterstedt et al., 2010). At temperatures around 20°C, biological processes tend to double (triple) for every 10K rise in temperature (that is, Q_{10} values are in the order of 2-3) (Davidson and Janssens, 2006). At 0°C, Kirschbaum (1995) indicated a Q_{10} value of almost 8 for soil respiration in a relationship fitted to literature data. Hamdi et al. (2013) reported Q_{10}s for soil respiration between 2 and 6 in a temperature range of 0 to +8°C in a broad range of ecosystems (cultivated, forest, grassland, peat and tundra soils).

1.2 Calculating temperature sensitivity: theory and models

Temperature is an important factor for modeling the N cycle in soils and various functions are available to describe the relationship between temperature dependency and turnover rates. The *van´t Hoff* law, developed by van´t Hoff in 1884 (equation 1, cited by Dessureault-Rompé et al., 2010), is commonly used to describe the relationship between temperature and turnover rates:

$$Q_{10} = \frac{R_2}{R_1}^{\frac{10}{T_2 - T_1}} \quad (1)$$

Herein, R_1 is the reaction rate, measured at temperature T_1 and R_2 is the reaction rate measured at temperature T_2. The Q_{10} value is the factor by which the reaction rate increases when the temperature is raised by 10 K (usually around 2-3, see above). The *van´t Hoff* law is less accurate at wide temperature ranges. Based on the *van´t Hoff* law, Arrhenius (1889) developed the Arrhenius equation (2) which is as well commonly used to describe the relationship between temperature and the reaction rate:

$$k = A\, e^{-\frac{E_a}{R*T}} \quad (2)$$

Herein, A is the fitted Arrhenius constant (no dimension), E_a (kJ mol^{-1}) is the fitted activation energy, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the absolute temperature in K and k is the reaction rate. The natural logarithm of the reaction rate is plotted versus the reciprocal of soil temperatures ($1/T$ in K) in Arrhenius plots. E_a can be obtained from the slope of the regression line. The Q_{10} value can be deduced from equation (2) via the following equation (3):

$$\ln Q_{10} = \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T+10}\right) \quad (3)$$

The Arrhenius equation is more accurate than the *van´t Hoff* law. In the Arrhenius equation, not only the two ´edge´ temperatures T_1 and T_2 are integrated but the whole temperature range (e.g., in the following experiments 5 different temperature steps were implemented) is depicted in the Arrhenius plot.

It is also possible to use the activation energy E_a as a parameter similar to the Q_{10} value in order to describe temperature dependencies. Craine et al. (2010) used the Arrhenius equation

(2) and calculated the activation energy E_a (in kJ mol^{-1}) by multiplying the slope m of the regression line with the universal gas constant R (equation 4):

$$E_a = - m * R \quad (4)$$

Besides the *van´t Hoff* law and the Arrhenius equation, several other functions exist to calculate the relationship between temperature and the reaction rate. Temperature responses decline at warmer temperatures, i.e. different Q_{10} values are required for 5-15°C, 15-25°C and 25-30°C (Ellert and Bettany, 1992). Therefore, Ellert and Bettany (1992) incorporated temperature functions (the Arrhenius function) into kinetic models and implemented a quadratic function to determine net N or S release from organic matter (equation 5):

$$\mathrm{d}\ln(k) / \mathrm{d}T = B + 2C / T \quad \text{with} \quad k = \mathrm{e}^{(A + BT + CT^2)} \quad (5)$$

Herein, k is the rate coefficient, T is the temperature in °C, and A, B, C are empirical constants. In this model, not only temperature but also time and length of the incubation interval are accounted for.

Dessureault-Rompé et al. (2010) developed a logistic function which has characteristics of both, the Arrhenius function and the Q_{10} function. Their logistic function is applicable over a wide temperature range and incorporates an exponential increase in the temperature response at low temperatures as well as a plateau in the temperature response at higher temperatures. Further mathematical functions that relate soil respiration to temperature are presented in Tuomi et al. (2008) and Rodrigo et al. (1997). Although several temperature functions (Arrhenius, Q_{10}, logistic) exist, the choice of the temperature response function has only a minor effect on the prediction of soil N mineralization (Dessureault-Rompé et al., 2010).

1.3 Temperature sensitivity of SOM mineralization as affected by substrate quality and substrate availability

The soil organic matter (SOM) pool represents one of the largest reservoirs of C on the global scale, consisting of a broad spectrum of diverse materials with different molecular structures (Thiessen et al., 2013). The temperature sensitivity of its decomposition is of prior interest as it significantly impacts global warming (Wagai et al., 2013). But up to now, there is still no consensus of how temperature controls all the enzyme-driven reactions that are involved in SOM decomposition and global simulation models differ largely in predicting the response of the soil C pool to future warming (Conant et al., 2011; Kirschbaum, 2006; Friedlingstein et

al., 2006). One leading assumption is based on the Arrhenius enzyme kinetic theory that predicts a higher temperature sensitivity with increasing activation energy of the substrate. Thus, the decomposition of a low-quality substrate (i.e. with a more complex, recalcitrant molecular structure) responds stronger to temperature compared to a simple (labile) substrate, as a higher activation energy is required to fully mineralize the former (Bosatta and Ågren, 1999). `C quality` can thus be defined by the molecular structure of the substrate and this `quality-temperature-hypothesis` (QTH) has been supported by several studies (Fierer et al., 2005; Conant et al., 2008; Xu et al., 2010; Hartley and Ineson, 2008; Craine et al., 2010). Wagai et al. (2013) reported a significant positive correlation between the decomposition Q_{10} value and the abundance of aromatic plus alkyl-C relative to O-alkyl-C groups in the light fraction of a temperate agricultural soil. However, when taking microbial respiration as an indirect C quality parameter in the same experiment, no correlation was found and the QTH was not affirmed. A considerable number of studies reported a decreasing temperature sensitivity when soil organic matter was more recalcitrant (Liski et al., 1999; Luo et al., 2001; Rey and Jarvis, 2006). Other studies found no difference in the temperature responses between labile and more stabilized organic matter (Fang et al., 2005; Conen et al., 2006; Reichstein et al., 2005). Karhu et al. (2010) reported Q_{10} values to vary with turnover times of SOC and found highest Q_{10} values (4.2-6.9) for the decadally cycling SOC fraction whereas lower Q_{10} were reported for the annually (Q_{10} <2) and centennially (Q_{10} of 2.4-2.8) cycling fraction.

Consensus is made that not only substrate quality but also substrate availability influences temperature sensitivity (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009; Conant et al., 2011). When substrate availability is low, e.g. SOM is protected from mineralization by physico-chemical stabilization mechanisms or microorganisms are constrained by pH value, water or oxygen supply, principles of Michaelis-Menten kinetics largely influence mineralization rates (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009; Auyeung et al., 2013). Under these conditions, the ´apparent´ temperature response may be low. In an old-field ecosystem, Auyeung et al. (2013) observed a strong decrease in the apparent Q_{10} of net N mineralization and nitrification after a combined warming and drought treatment over 2 years. The authors attributed this to diffusion limitations during moisture stress. Thus, substrate quality on the one hand and substrate availability on the other hand influence the temperature sensitivity of SOM mineralization. Gaining knowledge about their relevance is highly decisive for predicting effects of future warmer winters on C and N cycling in temperate forest soils.

1.4 Temperature sensitivity of gross ammonification and C mineralization

Commonly, gross ammonification is directly linked to C mineralization in soil organic matter decomposition. Though, the ratio of these processes may be altered at low temperatures. In high latitude soils, microbial biomass was reported to remain constant or even to increase during winter under a thick snow cover (Schadt et al., 2003; Brooks et al., 1998). Catabolic and anabolic processes were shown to continue between -4 and +9°C (Harrysson-Drotz et al., 2010). In temperate soils, microbial activity may decrease by several orders of magnitude when temperature drops below 5°C (Pietikäinen et al., 2005; Ranneklev and Bååth, 2001), while microbial maintenance respiration still continues (Campbell et al., 2005; Schindlbacher et al., 2007). However, N acquisition especially for the synthesis of amino acids for growth during winter may be less important. If the need of N for microbial growth is reduced, the temperature sensitivity of gross ammonification could be different from that of C mineralization at low temperatures.

1.5 Frost effects on C mineralization and soil microbial biomass

Besides an increase in the average annual temperature, the IPCC (2013) predicts an increasing frequency in the occurrence of extreme meteorological events in mountainous regions. Thus, a spring or summer drought may likely follow a winterly soil frost in temperate forests. Through this, ecosystems C and N fluxes may be altered on an annual scale, as freezing and drought are two common ecosystem stressors that impact microbial physiology and community composition (Schimel et al., 2007). The ability of microorganisms to survive soil frost was addressed in various freeze-thaw studies in a broad range of ecosystems. Short-lived pulses of N_2O and CO_2 are usually observed within a few days after thawing and commonly ascribed to the decomposition of microbial necromass (Herrmann and Witter, 2002; Dörsch et al., 2004; Koponen and Martikainen, 2004). After these pulses, CO_2 production of frozen soils usually equilibrates within several days to unfrozen control samples in temperate forest and grassland soils (Goldberg et al., 2008; Feng et al., 2007). In temperate forest soils, freeze-thaw cycles are usually not relevant for the CO_2 emissions on an annual scale (Matzner and Borken, 2008). However, effects of soil frost on microbial biomass may differ from those on soil respiration. Pesaro et al. (2003) reported that freezing an agricultural soil for four days at -20°C decreased soil DNA contents and direct cell counts by 24% and 22% compared to unfrozen controls (values remained low over 40 days), whereas the degradation of a crop protection product was not affected in the same study. Schmitt et al. (2008) reported the PLFA concentrations to decrease with increasing soil frost intensity in a Norway spruce

mineral soil. Haei et al. (2011) induced multiple freeze-thaw cycles at temperatures of -6 and -12°C to a boreal riparian soil and found an increase in the fungal-to-bacterial growth ratio after freeze-thaw cycles compared to a 0°C sample, indicating that freeze-thaw events affected specific microbial groups differently. In the long term, soil frost may induce a change in the microbial community composition. In a forest field study, Muhr et al. (2009) studied the response of soil respiration to the combined stressors of frost and drought over >1 year and found significant differences between plots that were subjected to soil frost and controls. In that experiment, winter soil frost was followed by a severe summer drought. In total, soil frost lowered C mineralization by 1100 kg C ha^{-1} a^{-1}, at which 14% could be ascribed to a reduction in soil respiration during the frost phase, whereas 63% could be ascribed to the dry summer 2006. Heterotrophic soil respiration was considerably reduced in the plots that received soil frost whereas autotrophic root respiration was not affected. The authors suggested that low soil water contents from June to October 2006 may have inhibited the recovery of the fungal biomass from frost and caused an enhanced sensitivity of heterotrophic respiration towards summer drought.

2. Objectives of this study

This study aimed firstly at quantifying overwinter rates of C and gross and net N mineralization and secondly, at quantifying the temperature sensitivity of these processes at low soil temperatures. The influence of substrate quality and substrate availability on temperature sensitivity was also determined. Therefore, one field and two laboratory studies were conducted. The effects of soil desiccation following soil frost on soil respiration and microbial biomass were determined in a third laboratory experiment. The following hypotheses were tested:

1. Considerable net N mineralization takes place during the dormant season in temperate forest soils.

2. The temperature sensitivity of C mineralization, gross and net N mineralization is higher in soil horizons of low substrate quality than in soil horizons of high quality substrates.

3. The temperature sensitivity of gross ammonification and nitrification is influenced by substrate availability. Removal of substrate shortage will increase the temperature sensitivity.

4. The temperature sensitivity of C mineralization is higher than of gross ammonification.

5. Soil frost reduces C mineralization during a subsequent desiccation period.

3. Materials and methods

3.1 Site description

The *in situ* incubation as well as soil sampling for the laboratory incubations were conducted in a European beech site (Steinkreuz) and a Norway spruce site (Coulissenhieb II). Both sites have been intensively investigated with respect to biogeochemical cycles by the ecosystem research at the University of Bayreuth (Matzner et al., 2004).

The Steinkreuz site is a 130-year-old hardwood stand composed of European beech (*Fagus silvatica*, 75% of area) and sessile oak (*Quercus petraea*, 25% of area), located in the Steigerwald Nature Park, Germany (49°52´N, 10°27´E) at 430 m a.s.l. Mean annual precipitation is 750 mm and mean annual air temperature is 7.5°C (Gerstberger et al., 2004). Sandy to loamy Dystric Cambisols prevail as soil types, and hydromorphic soils cover about 10% of the catchment area (classification according to FAO (IUSS, 2007)). The forest floor has an average thickness of 3 cm and is moder type, comprising Oi and Oe horizons and a patchy Oa horizon, of which the thickness is highly variable. The understory vegetation is sparse and comprises mainly acidophytic species like *Deschampsia flexuosa*, *Luzula albida, Oxalis acetosella* and *Calamagrostis arundinaceae* (Gerstberger et al., 2004). The pH ($CaCl_2$) is 3.2-4.0 in the mineral soil. The C stock of the Oi+Oe horizons amounts to 14.8 Mg C ha^{-1} and to 34.9 Mg C ha^{-1} in the A horizon whereas the N stock amounts to 0.8 Mg N ha^{-1} in the Oi+Oe horizon and to 2.2 Mg N ha^{-1} in the A horizon. TOC/TON ratios amount to 21.8 for the Oi layer and to 15.6 for the A layer (Gerstberger et al., 2004).

Soil sampling and the *in situ* incubation in the spruce site were conducted near to the Coulissenhieb II site, a 140-year-old Norway spruce stand located in the Lehstenbach catchment in the Fichtelgebirge, Germany (58°08´N, 11°52´E) at 770 m a.s.l.. The mean

annual precipitation is about 1160 mm and the mean annual temperature is 5.3°C (Gerstberger et al., 2004). Haplic Podzols with a sandy to loamy texture are the prevailing soil types (classification according to FAO (IUSS, 2007)) with a well-stratified mor-like forest floor. The forest floor has an average thickness of 6-10 cm and comprises Oi, Oe and Oa layers with a pH ($CaCl_2$) of 2.6 in the Oa horizon. pH values ($CaCl_2$) of the mineral soil are between 2.9 and 4.3. No ground vegetation occurred on the spot where samples were taken, but in the Coulissenhieb II site the understory vegetation is abundant and comprises mainly acidophytic species like *Deschampsia flexuosa*, *Vaccinium myrtillus*, *Calamagrostis villosa* and *Dryopteris dilatata* (Gerstberger et al., 2004). Soil C stocks of the Oi+Oe and Oa horizons are similar, amounting to 21 and 24 Mg C ha^{-1}. Soil N stocks amount to 0.8 Mg N ha^{-1} in the Oi+Oe horizon and to 1.24 Mg N ha^{-1} in the Oa horizon (Schulze et al., 2009). The C and N contents of the Oi horizon are 46% and 1.7%, of the Oe horizon 42% and 1.8%, of the Oa horizon 21% and 1.1% and of the EA horizon 8.3% and 0.4% (Schulze et al., 2009).

3.2 *In situ* measurement for net N mineralization

In situ N net mineralization was determined by the sequential coring method (Raison et al., 1987) from November 2011 to April 2012 in the beech and the spruce site. In total, 4 incubation periods were established, each lasting for about 6 weeks. PVC or stainless steel cores were driven down to 20 cm into the soil and ten replicates were taken immediately (t_0 sampling) and 10 were left in the field for 6 weeks (t_1 sampling). The cores were stratified in to Oi/Oe, Oa and A (or EA) horizons. The gravimetric water contents were determined at 60°C for organic layers and 105°C for the mineral horizons. Afterwards, soil samples were extracted with 1 M KCl solution. Net N mineralization rates were calculated by difference between t_0 and t_1. For further details on the method, see Study 2, section 2.2.

3.3 Laboratory incubations for gross and net N mineralization and C mineralization

Soil samples for the laboratory experiments were taken from the spruce site in April 2011 and from the beech site in March 2012 to assure that the microbial population is adjusted to winter temperatures. Mixed samples (2-4 kg fresh weight) were taken from an area (10 m^2) without ground vegetation from the Oi/Oe, Oa and A horizons. Before samples were adjusted to field capacity, as this reflects soil moisture conditions at ours sites during winter, soil samples were homogenized carefully by hand and leaves were cut into pieces of <1 cm^2. The incubation

experiments were conducted in freezers with constant temperatures of -4, -1, +2, +5 and +8°C (±0.3°C).

3.3.1 ^{15}N pool dilution technique for gross ammonification and gross nitrification

Gross ammonification and nitrification were determined by the ^{15}N pool dilution technique (Kirkham and Bartholomew, 1954) labeling the soil NH_4^+ and soil NO_3^- pool respectively. In the last decade, this technique was widely used to study gross N fluxes (Murphy et al., 2003). By the application of a ^{15}N solution (e.g., in form of $^{15}(NH_4)_2SO_4$ to measure gross ammonification or in form of $K^{15}NO_3$ for gross nitrification), the natural abundance of $^{15}NH_4^+$ or $^{15}NO_3^-$ of the soil (that is assumed at 0.36 atom%) is enriched, ideally to 20-40 atom%. By mineralizing the soil organic matter, microorganisms release NH_4^+ in natural abundance. The dilution of the ^{15}N enrichment of the NH_4^+ pool and the change in the size of the NH_4^+ pool is then traced through time (Murphy et al., 2003). Likewise, when measuring gross nitrification, nitrification of NH_4^+ occurs at natural abundance and leads to a dilution of the ^{15}N-enrichment of the NO_3- pool (Murphy et al., 2003). The equation of Kirkham and Bartholomew (1954) is used to calculate gross rates (equation 6). Herein, *m* is the gross ammonification rate in mg N kg^{-1} d^{-1}, *M* is the NH_4^+ total mass of tracing plus non-tracing NH_4^+-N in mg N kg^{-1}, *H* stands for the NH_4^+ tracer mass of tracing NH_4^+-N in mg N kg^{-1} and *t* is the time in d that refers to the time interval between the initial (M_0, H_0) and post-incubation analysis (M_1, H_1) (Murphy et al., 2003). For gross nitrification, the denotation is equivalent.

$$m = \frac{M_0 - M_1}{t} * \frac{\log\left(\frac{H_0 * M_1}{H_1 * M_0}\right)}{\log\left(\frac{M_0}{M_1}\right)} \qquad (6)$$

In total, 3 (spruce) to 4 (beech) replicates of homogenized soil were used for determination of gross ammonification and nitrification. The ^{15}N label ($(^{15}NH_4)_2SO_4$ for gross ammonification, $K^{15}NO_3$ for gross nitrification) was sprayed on the soil with a pump spray bottle. Manually mixing of soil improved the distribution of the ^{15}N tracer. After a t_0 time of 24h at 2°C, the t_0 samples were extracted with 1M KCl. The t_1 samples were sealed with flexible film and extracted after another 72 h. The ^{15}N abundance and the concentrations of NO_3^- and NH_4^+ in KCl-extracts were measured by the SPINMAS technique (Stange et al., 2007). Equation (6)

was used to calculate gross rates. For further information on the method see Study 1, section 2.3.

3.3.2 Addition of glycine and ammonium (substrate availability experiment)

In order to enhance the substrate availability and thus to test hypothesis 3, varying amount of glycine for gross ammonification and ammonium sulfate for gross nitrification were added to the samples together with the ^{15}N solution (they were stirred into the ^{15}N solution). For gross ammonification, 10 mg glycine-N per kg DM (treatment called ´glycine 1´) and 100 mg glycine-N per kg DM (treatment called ´glycine 2´) were added to a subset of soil samples. One subset of samples without glycine addition (´glycine 0´) served as control samples. Equally, for gross nitrification, 10 mg NH_4^+-N per kg DM (in form of $(NH_4)_2SO_4$ solution) and 100 mg NH_4^+-N per kg DM were added to the soil samples (treatments called ´ammonium 1´ and ´ammonium 2´) and samples without NH_4^+ addition served as control (´ammonium 0´). This experiment was conducted with soil from the spruce site only (Oi+Oe and Oa horizons).

3.3.3 Net N mineralization

Net N mineralization in the laboratory incubation was determined using homogenized soil as described in section 3.3. An initial extraction with 1M KCl was conducted to yield a t_0 concentration of NH_4^+ and NO_3^-. Five samples per horizon were then incubated in 930 ml airtight glass jars for 28 days (t_1, beech) and 29 days (t_1, spruce), at -4°C, -1°C, +2°C, +5°C and +8°C. For further information on the method see Study 1, section 2.3.

3.3.4 C mineralization

For the measurement of C mineralization, five replicates per horizon were incubated in 930 ml glass jars at the above listed temperatures. CO_2 measurements were conducted twice a week over an incubation period of 28 (beech) to 35 (spruce) days and C mineralization was calculated from the linear increase in CO_2 in the glass jars over the measurement interval. CO_2 concentrations were measured with a gaschromatograph (SRI 8610C, SRI Instruments Europe GmbH, Germany) and a linear calibration curve was generated by using standards of 380, 600, 1000, 3000 and 10000 ppm. For further details see *Study 1, section 2.2*.

3.3.5 Calculation of Q_{10} values

The calculation of Q_{10} values for gross and net N mineralization and C mineralization in the temperature range from -4°C to +6°C was conducted according to the Arrhenius equation (equation (2) and (3), section 1.2) as 5 measurement points existed. In the temperature range of +2°C to +8°C, only three measurement points existed and a linear function was fitted to calculate Q_{10} values. For calculation of the Q_{10} values in the *substrate availability experiment* (section 3.3.2), the *van´t Hoff* law (equation (1), section 1.2) was used with R_1 representing the reaction rate at T_1 (-4°C) and R_2 representing the reaction rate at T_2 (+8°C).

3.4 Experimental design of the frost and desiccation experiment

Soil samples of the Oi/Oe, Oa and (E)A horizons were taken from the beech and spruce site in January 2012, carefully homogenized and filled into glass jars for the laboratory incubation. Half of the samples was exposed to intensive frost of -8°C from day 0-20, the other half of the samples (control samples) were incubated at +2°C at field capacity. Afterwards, the previously frozen and control samples were warmed up stepwise to 20°C from day 21-90. From day 139-161, previously frozen and control samples were dried out to a pF of 4-4.7. C mineralization was monitored twice a week, and the microbial biomass was determined by the SIR method after the warming phase at day 90 and at the end of the experiment at day 161. For further details see *Study 3, section 2.2.*

3.5 Statistics

For the *in situ* incubation (section 3.2) one-way analysis of variance was used for testing statistical significance between soil horizons. For the soil frost and desiccation experiment (section 3.4) one-way and two-way analysis of variances were used for testing statistical significance between treatments. The Shapiro-Wilk test was used to test data distribution. All analyses were done with R 2.13.1 (R Development Core Team 2011).

4. Results and discussion

4.1 Gross and net N mineralization and C mineralization at low temperatures

4.1.1 *In situ* net N mineralization during the dormant season

Results from our field study confirm that considerable net N mineralization takes place during the dormant season in both temperate forest soils. In total, 44.3 kg N ha^{-1} 6 $months^{-1}$ were mineralized in the beech soil, and 10.9 kg N ha^{-1} 6 $months^{-1}$ were mineralized in the spruce

soil. In the beech soil net nitrification contributed by 1.5 kg N ha^{-1} 6 $months^{-1}$ to the total net N mineralization (3% of total net N mineralization, *Study 2, Fig. 2a,b*) whereas in the spruce soil, net nitrification contributed by 54% to total net N mineralization (5.9 kg N ha^{-1} 6 $months^{-1}$, *Study 2, Fig. 2c,d*). Rates under beech exceeded those under spruce by about 4 times. Rates measured under beech corresponded very well with rates measured in other deciduous temperate forests in the dormant season (Groffman et al., 2001). In order to emphasize the importance of the dormant season, net N mineralization in summer months were compared. Chang and Matzner (2000) reported in the beech soil a *in situ* N net mineralization of 44 kg N ha^{-1} 3 $months^{-1}$ during summer 1997, which is equal to the rates in the dormant season. However, net nitrification was higher by one order of magnitude in their study, giving evidence that nitrifiers at this site were constrained at low temperatures which was also supported by findings from Park et al. (2002). In the spruce soil, Hentschel et al. (2009) determined net N mineralization by *in situ* incubations of 21 kg N ha^{-1} 60 d^{-1} from mid of May to mid of July 2006 at soil temperatures between 7 and 11°C. Assuming this rate for the total growing season and including the dormant season, the annual N net mineralization in the spruce site would be around 74 kg N ha^{-1}. Thus, N net mineralization during the dormant season in the spruce soil contributes only about 15% to the annual mineralization.

In line with the present results, Kanerva and Smolander (2007), Booth et al. (2005) and Mueller et al. (2012) also reported higher net N mineralization under deciduous than under coniferous trees. This is generally attributed to a higher inherent litter quality in deciduous forests (Reich et al., 2005). Furthermore, in deciduous forests the leaf fall in autumn prior to the dormant season, providing easily decomposable substrates, seems responsible for the huge rates of net N mineralization in the dormant season. This conclusion is confirmed by the depth gradients of mineralization. Differences between the two tree species in the *in situ* study resulted mainly from the mineralization in the Oi/Oe horizons comprising different amounts of fresh litter. Furthermore, the beech Oi/Oe is less acidic compared to spruce Oi/Oe which may enhance N mineralization under beech (Högberg et al. 2007) and it also was on average 2°C warmer during the dormant season which partly explains the differences between beech and spruce.

Net nitrification rates are higher in the spruce than in the beech soil and enhance the risk of nitrate leaching in the former site. This was not only observed during the *in situ* study, but also during the laboratory incubation for gross and net nitrification (see below). Higher nitrification in the spruce soil can be attributed to two reasons. Firstly, evidence exists for heterotrophic nitrification in the spruce soil as a shift from autotrophic nitrification towards

heterotrophic nitrification occurs when substrate quality is decreasing (Trap et al., 2009). Heterotrophic nitrifiers are faster growing than autotrophic bacteria and less susceptible to frost damage (De Boer and Kowalchuk, 2001; Neilsen et al., 2001). In contrast, slow growth and small activity of autotrophic bacteria in the freshly fallen beech litter prevented rapid nitrification in this site. Secondly, microbial immobilization of NO_3^- is positively correlated to availability and quality of C compounds (Tahovská et al. 2013) and - although these parameters were not directly measured - immobilization of NO_3^- may have been smaller in the spruce than in the beech soil.

4.1.2 Temperature responses of gross and net N turnover and C mineralization at low temperatures

Results from our laboratory study indicate that considerable gross ammonification, gross nitrification and C mineralization take place at low temperatures (*Study 1, Fig. 1*). Gross ammonification, nitrification and C mineralization almost ceased at -4°C, but already increased at -1°C in Oi/Oe horizons (*Study 1, Fig. 1a-d, g-h*). Gross ammonification and C mineralization in the beech soil were much larger than in the spruce soil, whereas gross nitrification was in the same order of magnitude. Net ammonification in both Oi/Oe horizons was low at -4 and -1°C and increased strongly between +2 and +8°C and was about 2.4-fold higher in beech Oi/Oe compared to spruce Oi/Oe (*Study 1, Fig. 1e,f*). Net nitrification was low in both soils, but increased in the spruce soil at temperatures >2°C whereas no temperature response occurred in the beech soil.

Gross ammonification and nitrification from our study were comparable to rates measured at higher temperatures in the forest floor of the same spruce site (Chen et al., 2011). Gross ammonification was similar at 8°C (our study) and 15°C (Chen et al., 2011), however, gross nitrification was reduced by more than 50% at 8°C. Grenon et al. (2004) found gross ammonification and nitrification rates of 60 and 20 mg N kg^{-1} DW d^{-1} during July in forest floor of a subalpine fir stand, which are similar to rates in our study. Soil from calcareous A horizon of a beech forest had a gross ammonification rate of 50 mg N kg^{-1} DW d^{-1} during July at 14°C (Dannenmann et al., 2007) which were about 5 times higher than our rates. Differences among the studies may be attributed not only to temperature gradients but also to seasonal pattern of microbial population and varying soil properties.

In line with results from the *in situ* incubation, slightly higher gross and net nitrification rates were observed in the spruce compared to the beech soil in the laboratory incubation. As

described above, this may be due to a shift towards heterotrophic nitrification when the substrate quality decreases (Trap et al., 2009). Also Staelens et al. (2011) measured lower gross nitrification rates in broad-leaf than in coniferous forest soil and attributed this to a significant contribution of heterotrophic nitrification in the coniferous forest. Furthermore, in the beech forest soil temperatures rarely drop below 0°C and possibly hampered the microbial adaptation whereas the nitrifiers in the spruce forest were better adapted to the low temperature range from -4°C to +8°C.

4.2 Effects of soil substrates on Q_{10} values

Apparent Q_{10} values for C mineralization in the Oi/Oe horizon were around 11 (in the range of -4 to +6°C) in both sites, but were smaller in the Oa (4.1) and A horizons (3.5) (*Study 1, Erratum Table 2*). For gross ammonification, Q_{10} in Oi/Oe horizons were 11.1 for beech and 5.0 for spruce whereas in Oa and A horizons Q_{10} were again lower (2.9 in spruce Oa, 2.4 in beech A).

Q_{10} were lowest in soil horizons of low substrate quality and thus, the hypothesis that Q_{10}s of C mineralization, gross and net N mineralization are higher in soil horizons of low substrate quality could not be confirmed. Likewise, higher Q_{10}s for gross ammonification and nitrification in beech Oi/Oe than in the spruce Oi/Oe horizon did not confirm the hypothesis either. The Arrhenius kinetic theory, that predicts a higher temperature sensitivity with increasing activation energy of the substrate was not supported in our soils, in line with findings from Liski et al. (1999), Luo et al. (2001) and Rey and Jarvis (2006) about the temperature sensitivity of old soil organic matter. As described in the introduction (section 1.3), von Lützow and Kögel-Knabner (2009) and Davidson et al. (2012) suggest that substrate availability rather than quality is the main influencing factor. At low substrate availability, e.g. when soil enzymes are physically or chemically separated from the substrate, "apparent" Q_{10} values are low (von Lützow and Kögel-Knabner, 2009) due to substrate limitation. Under these conditions, principles of Michaelis-Menten kinetics largely influence mineralization rates (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009) and thus temperature responses. It cannot be excluded that substrate availability affected temperature sensitivity in the beech and spruce soil. However, neither water availability nor binding to mineral surfaces should have limited the substrate availability in the organic or A horizons. In a theoretical framework on temperature sensitivity of SOM decomposition, Sierra (2011) underlines that a differentiation between *relative* and *absolute* terms of temperature sensitivity

is highly needed. Low quality substrates are - according to the Arrhenius theory - more temperature sensitive in *relative* terms. However, their decomposition is slow and thus also their temperature response is low when regarded in *absolute* terms (e.g. on an annual scale) (Sierra, 2011). Biotic effects like the priming effect and microbial growth may also largely influence C turnover in soils (Kuzyakov 2010; Thiessen et al., 2013) and have received increasing attention recently. In a long-term incubation over 199 days, where an agricultural soil was amended with crop litter, Thiessen et al. (2013) reported the temperature sensitivity of soil respiration to increase slowly but significantly over time, accompanied by an increase in the PLFA amounts. The authors request to explicitly consider microbial processes like growth and priming effects, rather than sticking to the simple view of a physico-chemically derived substrate-temperature sensitivity relationship of decomposition. Furthermore, Ågren and Wetterstedt (2007) suggested in a modeling exercise that specific uptake kinetics of different organism groups, substrate diffusion rates and the rates at which substrates are made available in the environment might further affect temperature responses and explain the deviance of our findings from theory.

Generally, Q_{10} values of C mineralization at low temperatures were very high, in line with findings from Mikan et al. (2002), Tilston et al. (2010) and Öquist et al. (2009). For C mineralization in a boreal spruce soil, Tilston et al. (2010) reported a Q_{10} of 5.8 between -2°C and +10°C, which is well in line with a Q_{10} of 4.1 measured in our spruce Oa between -4°C and +6°C. Q_{10} values decreased when soil frost was excluded from the calculation (*Study 1, Erratum Table 2*). A similar decrease has been observed in boreal forest and tundra soils (Tilston et al., 2010; Mikan et al., 2002) and was attributed to changes in the availability of liquid water (Öquist et al., 2009). Water availability constraints the microbial activity in several ways, for example by related substrate limitation caused by extracellular diffusion barriers (Mikan et al., 2002). Öquist et al. (2009) found Q_{10} values below freezing point that were well comparable to those at higher temperatures after the influence of water reduction upon temperature responses was factored out. According to Tilston et al. (2010), unfrozen water content is an important driver of soil respiration below 0°C and makes a contribution similar to that of temperature in boreal organic forest soils.

4.3 Effects of substrate availability on Q_{10} values

The addition of glycine increased gross ammonification considerably in amended spruce Oi/Oe and Oa horizons. At +8°C, gross ammonification amounted to 231 mg N kg^{-1} DM d^{-1} in

the ´glycine 2´ treatment whereas 63 and 41 mg N kg^{-1} DM d^{-1} were mineralized in the ´glycine 1´ and ´glycine 0´ samples of the Oi/Oe horizon (Fig. 1a). Also in the Oa horizon, gross ammonification was about 6-fold higher in the ´glycine 2´ treatment compared to the control samples (Fig. 1b).

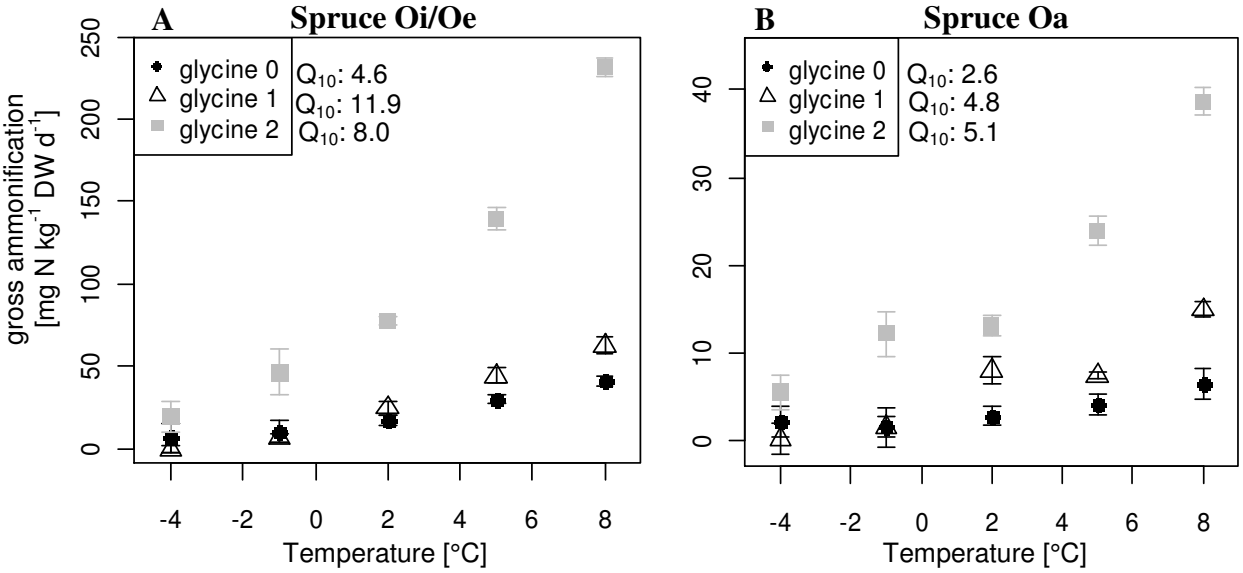

Fig. 1 Gross ammonification (in mg N kg^{-1} DW d^{-1}) in spruce Oi/Oe (A) and Oa (B) after the amendment with 10 and 100 mg glycine-N kg^{-1} DW.

Q_{10} values, calculated according to equation (1), increased after addition of glycine by 1.7 to 2.6-fold in the Oi/Oe horizon and by around 2-fold in the Oa horizon. Thus, the hypothesis, that increasing substrate availability enhances temperature responses could be affirmed for gross ammonification in the spruce soil. After sucrose amendments to a boreal spruce and pine organic soil, Tilston et al. (2010) observed a 2- to 3-fold increase in soil CO_2 efflux in a temperature range of -2 to +10°C. Likewise, the activation energy (E_a) and Q_{10} values were enhanced after sucrose amendment in their experiment. An abundant supply with labile C compounds changes the dominance of other environmental constraints acting on microbial respiration by reducing diffusion limitations of respiratory substrates and thus leads to an increase in the temperature dependence of soil respiration (Tilston et al., 2010).

However, although the hypothesis was affirmed, that an enhanced substrate availability increases temperature sensitivity, a gross ammonification rate of 231 mg N kg^{-1} DM d^{-1} is rather high, and indicates that the added amount of 100 mg glycine-N per kg DM would be turned over more than two times per day. Most likely, the addition of glycine induced

microbial growth and fuelled priming effects. Under these circumstances, besides substrate availability and temperature, also microbial parameters would have affected the temperature responses. After amending an arable soil with fresh organic matter, the temperature sensitivity of soil respiration increased slightly but significantly, concomitantly with increasing PLFA amounts (Thiessen et al., 2013, see above) and thus microbial growth parameters may have considerable effects on the decomposition temperature responses. By inducing microbial growth, one assumption of the ^{15}N pool dilution technique is injured. The ^{15}N pool dilution technique assumes a steady state between the applied and the indigenous N pools (Murphy et al., 2003) and that all soil N transformations like immobilization, nitrification, gaseous loss pathways, diffusion would equally affect the applied and indigenous pools. Thus, by adding glycine with a C:N ratio of 2 to needle litter with a C:N ratio of around 20-25, microbes would preferentially ammonify the added glycine.

In contrast to glycine, the addition of ammonium sulfate did not increase gross nitrification in the spruce horizons, compared to unamended control samples (Fig. 2). Gross nitrification was in the same order of magnitude between Oi/Oe and Oa horizon and ranged between 0.6 and 6.6 mg N kg^{-1} DM d^{-1} for the unamended samples (Fig. 2). At +8°C, gross nitrification of ´ammonium 1´ and ´ammonium 2´ samples reached only 67-74% of the ´ammonium 0´ samples in the Oi/Oe horizon (Fig. 2a), this was also true for the Oa horizon (Fig. 2b).

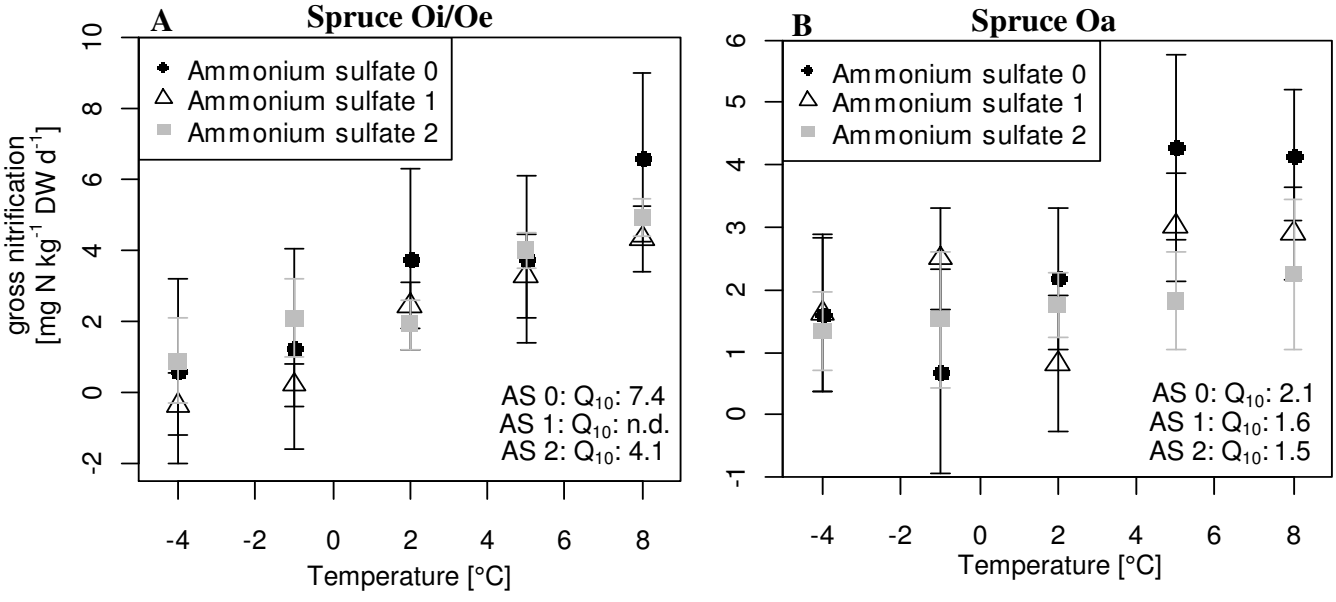

Fig. 2 Gross nitrification (in mg N kg^{-1} DW d^{-1}) in spruce Oi/Oe (A) and Oa (B) after the amendment with 10 and 100 mg NH_4^+-N kg^{-1} DW.

Likewise, Q_{10} values of the ammonium-amended samples were lower compared to the control samples. This may be due to two reasons. Firstly, the membrane-bound enzyme ammonia-mono-oxygenase (AMO) which is the key enzyme for autotrophic nitrifiers requires NH_3 rather than NH_4^+ as a substrate (Suzuki et al., 1974). After a conversion of NH_3 to hydroxylamine, the latter is further oxidized to nitrite by hydroxylamine oxidoreductase (Hooper et al., 1997). Consequently, in dystric soils, autotrophic nitrification - that may contribute between 8 and 100% to total nitrification in forest soils (De Boer et al., 1992; Venterea et al., 2003; Grenon et al., 2004) - occurs in pH neutral or alkaline micro niches (Hankinson and Schmidt, 1984). Heterotrophic nitrifiers possess enzymes that have strong similarities with the autotrophic AMO enzyme (Moir et al., 1996). Additional protons were added to our soils with the $(NH_4)_2SO_4$ solution, thus a pH reduction in alkaline micro niches is most likely (though only minor pH changes by <0.1 units were measured in the bulk soil after addition of the $(NH_4)_2SO_4$ solution (data not shown)). For NH_3 production, the cytoplasmatic pH value needs to be high (De Boer et al., 1992).

The contribution of both heterotrophic and autotrophic organisms to nitrification in our spruce soil remains unclear. In recent years, also archaea were shown to oxidize ammonium (Wessén et al., 2011; Treusch et al., 2005). In microcosms of an acid forest soil, ammonia oxidation was dominated by thaumarchaea (Levičnik-Höfferle et al., 2012) and was not enhanced by the addition of inorganic N, but it was significantly stimulated by the addition of organic N. In this experiment, an acetylene treatment inhibited ammonia oxidation completely, indicating AMO-dependent activity (Levičnik-Höfferle et al., 2012). Consequently, if thaumarchaeal ammonium oxidation was present in our soil samples, the addition of $(NH_4)_2SO_4$ solution would not have affected it - instead the decrease in pH of microniches after the addition would have hampered NH_3 formation for the thaumarchaeal AMO-like enzyme system.

Secondly, high salt concentrations are as well harmful to autotrophic nitrifiers (Lang et al., 1993; Martikainen, 1985). Lang et al. (1993) reported a complete inhibition of nitrification when 2 mM of a $(NH_4)_2SO_4$ or $(NH_4)_2HPO_4$+Na_2SO_4 solution were added to a dystric Cambisol under beech. However, in the same experiment, the nitrification was 3-fold enhanced when a 2mM $(NH_4)_2HPO_4$ solution was added to the samples. Consequently, the form of the salt plays also a role as it affects the pH value and ionic concentration of the liquid soil phase and through this influences autotrophic nitrification (Martikainen, 1985). It was observed that autotrophic nitrification either remained unaffected or was reduced after the addition of sulfate or chloride in form of $(NH_4)_2SO_4$ or NH_4Cl, whereas phosphate addition led to enhanced autotrophic nitrification (Martikainen, 1985; Lang et al., 1993).

Consequently, this experiment was repeated at temperatures of -4°C and +8°C with soil samples from the same spruce site and applied a 1.2 mM ammonium phosphate solution ($(NH_4)_3PO_4$) together with the $K^{15}NO_3^-$ label. Generally, gross nitrification was not enhanced by the phosphate addition neither in the spruce Oi/Oe horizon nor in the Oa horizon (Fig. 3). In the Oi/Oe horizon, gross nitrification was much higher during this experiment (~22 mg N kg^{-1} DM d^{-1} compared to~6 mg N kg^{-1} DM d^{-1} in the $(NH_4)_2SO_4$ experiment), and this could be ascribed to the sampling date, as sampling was conducted in July 2011 at a soil temperature of around 12°C.

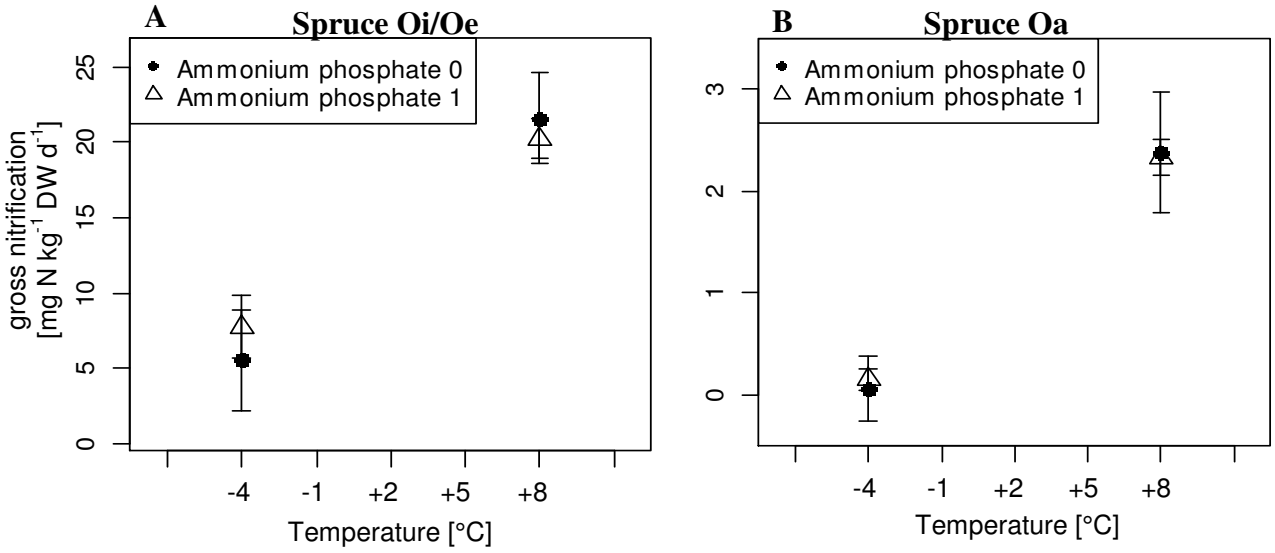

Fig. 3 Gross nitrification (in mg N kg^{-1} DW d^{-1}) in spruce Oi/Oe (A) and Oa (B) after the amendment with 1.2 mM $(NH_4)_3PO_4$.

Gross nitrification in acid coniferous soils seems to be not limited by the substrate (ammonium) and rates of the control samples can be considered as maximum, at least at low temperatures from -4°C to +8°C. Nugroho et al. (2009) suggests that besides unfavorable abiotic conditions (low pH, low temperature), unknown biotic factors hamper nitrification activity in acid forest soils.

4.4 Q_{10} values of gross ammonification compared to C mineralization

Q_{10} values of gross ammonification in our study ranged from 2.4 to 11.1 and were in most horizons lower than Q_{10}s for C mineralization, confirming our hypothesis that C mineralization is stronger temperature dependent at low temperatures than gross

ammonification. Koch et al. (2007) reported higher Q_{10}s for enzymes involved in the C cycle compared to enzymes involved in the N cycle with decreasing temperature in organic alpine soils. In their experiment, the relative temperature sensitivity of β-glucosidase, β-xylosidase and N-acetyl-β-glucosaminidase (chitinase) activity increased with decreasing temperatures whereas no such increase was reported for tyrosine and leucine amino peptidase. The authors suggested that the use of different substrates is largely regulated by temperature. Hopkins et al. (2006) reported higher Q_{10} values of glucose-induced respiration compared to basal respiration in a temperature range of -0.5 to +20°C in Arctic dry soils and suggest the turnover of easily degradable compounds (e.g., glucose) to be more temperature sensitive than the turnover of complex compounds.

4.5 Comparison of Q_{10} values of net N mineralization determined in laboratory vs. *in situ* incubations

Since net N mineralization was measured in undisturbed samples during the *in situ* incubation and in homogenized samples during the laboratory incubation, the temperature sensitivity (Q_{10}s) of net N mineralization in both methods could be compared. In *Study 2, Fig. 3* the actual *in situ* net N mineralization is depicted (open triangles) as well as the interpolated net N mineralization from the laboratory incubation at the present field temperatures (filled circles) (for interpolation, see *Study 2, section 2.3*). Apparent Q_{10} values for net N mineralization of laboratory samples were rather high, ranging between 7 and 25. However, as the calculation of Q_{10} values with a linear function is very sensitive towards a low initial value at +2°C (as it was in case of spruce Oi/Oe) Q_{10} values may be rather high with a high degree of uncertainty. Generally, the temperature response of net N mineralization was more pronounced in the laboratory samples than in the *in situ* samples in all soil horizons (*Study 2, Fig. 3*). Homogenization of the laboratory samples of beech A, as well as cutting leaves from beech Oi/Oe, might have increased substrate availability. Substrate availability increases temperature sensitivity of mineralization (von Lützow and Kögel-Knabner, 2009; Hamdi et al., 2013; Davidson et al., 2012). As discussed also in section 4.2, these findings strengthen the assumption, that in our soils, the temperature sensitivity of mineralization may strongly be influenced by the substrate availability. However, the less pronounced temperature dependency of the *in situ* rates could also be attributed to temperature fluctuations that occur in the field. As *in situ* rates were calculated to average monthly soil temperatures, the influence of temperature variations at time scales of days to weeks was thereby ignored.

4.6 Effects of rising winter temperatures on net N mineralization

From results of the laboratory incubations, overwinter net N mineralization in both forest sites was calculated with a linear function for net N mineralization based on the laboratory measurements at +2, +5 and +8°C from November to April. In the beech soil (Oi/Oe+A), net N mineralization would sum up to 47.4 kg NH_4-N ha^{-1} 6 $months^{-1}$ at an actual average winter temperature of 3.7°C (net nitrification was negligible at low temperatures). This is in the same order of magnitude as it was determined during the *in situ* incubation (here, a total amount of 44 kg N ha^{-1} 6 $months^{-1}$ was mineralized). A temperature increase during the winter period of 3°C would result in a net N mineralization of 93.8 kg NH_4-N ha^{-1} 6 $months^{-1}$ (*Study 1*, *Erratum Table*). The additional amount of 46.4 kg NH_4-N ha^{-1} 6 $months^{-1}$ in the beech soil could substantially change overwinter N fluxes, and it remains an open question, if net nitrification stays negligible when winter temperatures increase.

In the spruce soil with an actual average winter temperature of 1.9°C, net N mineralization would amount to 6.0 kg N ha^{-1} 6 $months^{-1}$ (net nitrification would contribute less than 1 kg NO_3-N ha^{-1} 6 $months^{-1}$ and the spruce Oa horizon revealed no net N mineralization in the laboratory study at low temperatures). This is slightly lower than net N mineralization determined during the *in situ* study, here 10.9 kg N ha^{-1} 6 $months^{-1}$ were mineralized. An increase of the winter temperature by 3°C would be also important for the spruce soil and would enhance net N mineralization by +20.3 kg N ha^{-1} 6 $months^{-1}$ resulting in a total net N mineralization of 26.3 kg N ha^{-1} 6 $months^{-1}$ at an average temperature of 4.9°C (*Study 1*, *Erratum Table*). Rising winter temperatures might have substantial effects on net N mineralization, but effects are tree species specific and decrease with soil depth, likely due to decreasing substrate quality of organic matter.

4.7 Effects of soil frost and subsequent desiccation on C mineralization and microbial biomass

Results from the frost experiment revealed that microbial biomass and C mineralization recover quickly from freezing in temperate soils. C mineralization of previously frozen samples increased within 1-7 days after thawing and continued at rates comparable to those of unfrozen soils (*Study 3, Fig. 2a-b, 3a-c*). Already 5 (spruce EA) to 13 days (spruce Oa, beech A) after thawing, cumulative C mineralization was equal between +2°C and -8°C samples of the respective horizons (*Study 3, Fig. 2b, 3b-c*). This thawing pulse - usually occurring within a time scale of 5-10 days - has been frequently reported and is ascribed to the decomposition

of necromass (Schimel and Clein, 1996; Goldberg et al., 2008; Koponen and Martikainen, 2004). After 90 days, cumulative C mineralization of the -8°C samples of the spruce EA horizon exceeded that of the +2°C by 18 mg C kg^{-1} DM 85 d^{-1} (*Study 3, Fig. 3c*). This overcompensation (or rapid compensation in beech A and spruce Oa) might be due to the additional release of previously occluded substrates due to physical disruption (Kurganova et al., 2007; Matzner and Borken, 2008) as temperatures of -8°C represent an exceptional soil frost for the Oa and (E)A horizons in our sites.

On the contrary, the compensation of low soil respiration during the frost phase in Oi/Oe horizons took longer (84 days in beech Oi/Oe, *Study 3, Fig. 2a*) or did not occur (spruce Oi/Oe, *Study 3, Fig. 3a*). An explanation may be that these responses might be due to the horizon-specific microbial biomass and activity (Schimel and Clein, 1996). Studying the stratification and dynamics of bacteria and fungi in organic layers of a Scots Pine forest, Berg et al. (1998) reported the hyphal length to decrease significantly with soil depth. Likewise, the fungal-to-bacterial-biomass ratio declined with increasing decomposition stage (lower fungal biomass in humus compared to fresh litter). In line with these results, the fungal mycelia was likely more negatively affected than bacteria by soil frost in the Oi/Oe horizons (Feng et al., 2007; Schimel et al., 2007; Muhr et al., 2009). Thus, bacteria that likely prevailed in deeper soil horizons recovered faster from the soil frost and could faster compensate C losses during freezing.

After the warming phase at day 90, microbial biomass was not significantly different between previously frozen and control samples in any horizon (*Study 3, Fig. 4*). Microbial biomass was highest in beech Oi/Oe (around 63 mg C_{mic} g^{-1} DM), and smallest in spruce EA (around 0.9 mg C_{mic} g^{-1} DM) (*Study 3, Fig. 4*).

From day 139-161, C mineralization was significantly lower in previously frozen samples of the spruce soil and the beech A horizon compared to the unfrozen samples and - despite C mineralization and microbial biomass had apparently recovered from soil frost until day 90 - indicated a potential `lag` effect of soil frost, when samples were kept permanently moist (*Study 3, Fig. 2d, 3d-f*). A fraction of the active microbial biomass was likely in the long-term impaired by the soil frost that was not visible until day 90. As no measurements of CO_2 emissions between day 90 and 139 were conducted, the exact day where C mineralization rates started to differ between -8°C and +2°C samples could not be defined. Furthermore, as no qualitative assessment of the microbial community (e.g., in PLFA analyses) was carried out, it remains unclear if any/which microbial group is in the long-term impaired. At least in beech A, spruce Oa and EA a lower microbial biomass in the previously frozen samples may

explain the lower cumulative C mineralization on day 161 (*Study 3, Fig. 4*). Reasons for this late change in C mineralization under optimal moisture conditions remain speculative. Mackelprang et al. (2011) reported a rapid shift in the abundance of many phylogenetic, microbial and functional genes that were involved in C and N cycling when a permafrost soil was at the transition from a frozen to a thawed state. Several authors reported late changes in the microbial biomass or activity in long-term incubation experiments, which are generally ascribed to a decreasing availability of easily decomposable substrates (Hart et al., 1994; Steinweg et al., 2008). Exposing a boreal riparian surface soil to multiple freeze-thaw cycles at -6 and -12°C, Haei et al. (2011) reported the duration (2-6 months) of a multiple freeze-thaw experiment to be negatively correlated with fungal and bacterial PLFAs. In the same experiment, the freeze-thaw events increased the fungal-to-bacterial growth ratio compared to a constant 0°C sample, giving evidence that soil frost affects specific microbial groups differently. Subjecting a Norway spruce soil (Oa, EA and B horizon) to three freeze-thaw cycles over 220 days significantly decreased the content of plant and microbial sugars compared to control samples that were kept at +5°C (Schmitt et al., 2008). Schmitt et al. (2008) suggested an alteration of sugar molecules leading to SOM stabilization in the frozen samples, as no concomitant increase in CO_2 emission was reported in this experiment (Goldberg et al., 2008). In the later stage of our experiment, a reduced abundance of sugars could have also provoked a reduced respiration activity in the previously frozen samples but this remains very speculative.

Desiccation to a pF of 4-4.7 reduced C mineralization in all horizons except beech Oi/Oe by 19-47% (*Study 3, Fig. 2f, 3g-i*), but no effect of previous freezing was observed. Thus, the hypothesis, that soil frost reduces C mineralization in the subsequent desiccation phase, could not be affirmed. Desiccation likely affected solely the active microbial biomass and superimposed the belated frost stress (if it was also present in these samples). Thus, the active microbial groups in previously frozen and unfrozen samples were weakened in an equal measure by desiccation.

The present results contrast the findings by Muhr et al. (2009) who hypothesized that soil frost leads to an increased sensitivity of heterotrophic soil respiration towards summer drought. Deviances of the results from the findings of Muhr et al. (2009) likely result from the conditions implemented during the laboratory experiment. In the field experiment, a pF of ~2.6 was measured in 20 cm soil depth and the Oa horizons dried out to a pF of ~5 (Muhr et al., 2009; Muhr and Borken, 2009). Desiccation in the laboratory study was likely not intensive and not long enough. Due to the lack of substrate inputs by plant roots and other

(a)biotic effects that prevail *in situ* the microbial community in the laboratory incubation likely differed largely from the persisting microbial community in the field study and thus both studies are hardly comparable. Furthermore, extensive summer droughts frequently occur in our sites and microbial processes are known to continue even at extreme low water potentials (Muhr et al., 2010; Chen et al., 2011). Barnard et al. (2013) proved a high degree of fungal resistance to decreasing water potentials down to -22 MPa in Mediterranean grassland soils. This might be an explanation for the minor response of C mineralization to desiccation, especially in the Oi/Oe horizon of the beech soil.

5. Conclusions

In summary, the present results indicate that in temperate forests, C and gross and net N mineralization take place at considerable rates during the dormant season, with overwinter net N mineralization contributing 15% (spruce) to 30% (beech) to the annual net N mineralization. Microbial processes proceed at -1°C and the soil microbial community as well as soil respiration recover quickly from soil frost in temperate forest soils. The temperature response of gross ammonification, gross nitrification, net N mineralization and C mineralization was generally high at low temperatures. When soil frost was excluded from the calculation, Q_{10}s decreased and gave evidence for additional constraints acting on microbial processes at subzero temperatures. Our findings suggest that the apparent temperature dependency of C and N mineralization of low quality substrates is smaller than of high quality substrates. C and N mineralization in the forest floor would respond stronger to rising winter temperatures than in the A horizon. Not only substrate quality but also substrate availability affects temperature responses in our soils. Low nitrification rates in both forest soils represent a moderate risk of increasing NO_3^- leaching during winter, with a slightly higher risk of leaching in spruce stands.

Generally, this work underlines the great importance of overwinter C and N mineralization as well as their large temperature response at low temperatures. Increasing winter temperatures are expected to have a huge effect on the C and N cycle in temperate forest soils with highest relevance for microbial processes in the Oi/Oe horizons. High Q_{10} values of net N mineralization at low temperatures suggest a huge effect of increasing winter temperature on the N cycle, especially in deciduous forests where the leaf fall in autumn provides a huge amount of easily decomposable substrates directly before the dormant season. Future studies

should focus more on the identification of the effects of different microbial groups and their activity on the temperature sensitivity of decomposition.

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Study 1

Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures

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Published in Soil Biology and Biochemistry (2014) 69, 320-327.

ERRATUM

In the Publication "Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures" (SBB Vol. 69, pp 320-327), the calculation of Q_{10} values for gross ammonification, gross nitrification, net N mineralization and C mineralization was done erroneously and is now corrected. We submit a new *Table 2*. In *Table 2* we fitted the exponential Arrhenius function to calculate Q_{10} values in the temperature range of -4°C to +6°C where 5 measurement points existed. In the temperature range of +2°C to +8°C, only three measurement points existed and we fitted a linear function to calculate Q_{10} values.
Furthermore, the estimation of winterly net N mineralization under average temperature and a +3°C enhanced winter temperature (chapter 4.4) was changed accordingly. We submit a new *Table* to correct the values in chapter 4.4. The corrected values were calculated with a linear function for net N mineralization based on the measurements at +2°C, +5°C and +8°C.

Table 2 Q_{10} values, activation energy E_a (kJ mol^{-1}) and r² of Arrhenius plots for gross and net N mineralization and C mineralization in beech and spruce soil. Regressions are significant at $p<0.05$ when indicated by * (n=5).

	Beech								Spruce							
	Oi/Oe horizon				A horizon				Oi/Oe horizon				Oa horizon			
Temp. range -4-6°C	Q_{10}	E_a	r²	A	Q_{10}	E_a	r²	A	Q_{10}	E_a	r²	A	Q_{10}	E_a	r²	A
Gross ammonific.	11.1	151	0.84*	71.5	2.4	55	0.98*	26.9	5.0	101	0.99*	47.9	2.9	68	0.82*	31.6
Gross nitrification	11.1	151	0.93*	66.9	3.8	84	0.63	37.0	7.2	123	0.93*	55.8	3.5	78	0.60	36.0
Net N mineralization	4.6	95	0.65*	46.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C mineralization	11.1	150	0.91*	74.5	3.5	78	0.98*	39.0	11.0	150	0.97*	72.6	4.1	88	0.97*	44.0
Temp. range 2-8°C																
Gross ammonific.	2.1	n.d.	0.96	n.d.	2.5	n.d.	0.99	n.d.	3.3	n.d.	0.99	n.d.	3.3	n.d.	0.98	n.d.
Gross nitrification	9.1	n.d.	0.99	n.d.	4.6	n.d.	0.94	n.d.	2.5	n.d.	0.75	n.d.	2.3	n.d.	0.70	n.d.
Net N mineralization	10.8	n.d.	0.92	n.d.	7.1	n.d.	0.92	n.d.	25.5	n.d.	0.92	n.d.	n.d.	n.d.	n.d.	n.d.
C mineralization	4.0	n.d.	0.91	n.d.	2.4	n.d.	0.96	n.d.	3.4	n.d.	0.99	n.d.	3.5	n.d.	0.97	n.d.

n.d. = not determined

Table Net N mineralization in the beech and spruce soil under average winter temperature and a +3°C-enhanced winter temperature.

	Beech			Spruce	
	incorrect value	corrected value		incorrect value	corrected value
average winter temperature of 3.7°C	34.2 kg N ha^{-1} 6 months^{-1}	47.4 kg N ha^{-1} 6 months^{-1}	average winter temperature of 1.9°C	4.2 kg N ha^{-1} 6 months^{-1}	6.0 kg N ha^{-1} 6 months^{-1}
average winter temperature of 6.7°C	58 kg N ha^{-1} 6 months^{-1}	93.8 kg N ha^{-1} 6 months^{-1}	average winter temperature of 4.9°C	5.6 kg N ha^{-1} 6 months^{-1}	26.3 kg N ha^{-1} 6 months^{-1}
additional release of N	+23.8 kg N ha^{-1} 6 months^{-1}	+46.4 kg N ha^{-1} 6 months^{-1}	additional release of N	+1.4 kg N ha^{-1} 6 months^{-1}	+20.3 kg N ha^{-1} 6 months^{-1}

Abstract

Climate models predict warmer winter in temperate regions, but little is known about the temperature sensitivity of soil carbon (C) and nitrogen (N) mineralization at low temperatures. Here, we assess the temperature sensitivities of gross ammonification, gross nitrification, C and net N mineralization of top soil horizons, under a European beech and a Norway spruce temperate forest. We tested the hypotheses that (1) substrate quality affects the temperature sensitivity of C and N mineralization and (2) that temperature sensitivity of C mineralization is higher than of gross ammonification. Soil incubations were conducted at constant temperatures of -4, -1, +2, +5 and +8 °C. Gross ammonification and nitrification were measured by the ^{15}N pool dilution technique. Temperature sensitivities of C, gross and net N mineralization were calculated using the Arrhenius equation and C mineralization was taken as proxy for substrate quality.

Gross ammonification and C mineralization was much larger in the beech than in the spruce soil, while gross nitrification was in the same order of magnitude. Gross ammonification, nitrification and C mineralization almost ceased at -4 °C, but already increased at -1 °C. Net ammonification in Oi/Oe horizons was low at -4 and -1 °C and increased strongly between +2 and +8 °C. Net nitrification was low in both soils, but increased in the spruce soil at temperatures >2 °C whereas no temperature response occurred in the beech soil.

Apparent Q_{10} values of gross ammonification and C mineralization in the temperature range of -4 to +8 °C were in the range of 3-18. Q_{10} were lowest in soil horizons of low substrate quality. The ratio of C mineralization to gross ammonification varied between 0.5 and 2.9, suggesting preferential mineralization of N rich organic substrates or rapid turnover of the N pool in microbial biomass. Rising winter temperatures might have substantial effects on net N mineralization, but effects decrease with soil depth, likely due to decreasing substrate quality of soil organic matter.

1. Introduction

Cold-season processes substantially contribute to annual soil carbon (C) and nitrogen (N) mineralization in high-latitude and high-altitude ecosystems (Clein and Schimel, 1995; Schimel et al., 2004; Miller et al., 2007). Soil microbial activity throughout the winter can account for a large proportion of annual C (21 - 50%) and net N mineralization (30 - >50%) (Monson et al., 2006; Zimov et al., 1996; Kielland et al., 2006; Vestgarden et al., 2003) as microbes remain physiologically active in unfrozen water films (Coxson and Parkinson, 1987; Mikan et al., 2002). Unfrozen water films in soils exist even below -17°C (Sparrman et al., 2004). Only a few studies have addressed C and net N mineralization (hereafter, the sum of net ammonification and net nitrification) during soil frost in temperate forests (Schindlbacher et al., 2007; Hentschel et al., 2008; Goldberg et al., 2008). In temperate forests, topsoils rarely freeze and are often subjected to temperatures >0 °C because of the insulating snow cover. In a laboratory incubation with soil columns of the forest floor (Oi+Oe+Oa horizon) from a temperate Norway spruce stand, a total amount of 8.6 g N m^{-2} was leached in 170 d at a temperature of 5 °C (Hentschel et al., 2008), emphasizing the magnitude of net N mineralization at relatively low soil temperatures. Similarly, CO_2 production during the dormant season ranged from 12 -15% in cold-temperate deciduous forests (Mariko et al., 2000; Schindlbacher et al., 2007). In response to global warming the frequency and intensity of soil frost and snow covers likely change in temperate forests (Campbell et al., 2005; IPCC, 2007). A rise of soil temperatures in the lower range is of special importance as the temperature sensitivity of microbial processes is enhanced at low temperatures (Dalias et al., 2002; Davidson and Janssens, 2006; Wetterstedt et al., 2010). Kirschbaum (1995) indicated a Q_{10} value of almost 8 at 0 °C for soil respiration in a relationship fitted to literature data. Substrate quality and availability are known to influence the temperature sensitivity of soil organic matter (SOM) mineralization. The Arrhenius equation implicates a higher temperature sensitivity of stable SOM than of labile SOM (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009). Hence, temperature sensitivity of SOM mineralization should differ among soil horizons and litter types; for example coniferous litter contains more recalcitrant organic compounds than deciduous litter (Reich et al., 2005). This, often referred to as the "*quality-temperature hypothesis*", has been supported by studies on C mineralization (e.g. Fierer et al., 2005; Conant et al., 2008; Xu et al., 2010), but has also been questioned by other studies (Conen et al., 2006; Gershenson et al., 2009; Zhu and Cheng, 2011).

Net N mineralization in acid beech-oak litter had a Q_{10} value of 1.4 in the range of 0 to 30 °C (Emmer and Tietema,1990). Grenon et al. (2004) determined a Q_{10} value of 3.2 for

gross ammonification in a subalpine forest soil in a range of +10 °C – +21 °C. Gross nitrification did not reveal a clear temperature response in their study. According to Dalias et al. (2002), responses of nitrifying microbial communities to temperature vary with climate due to microbial acclimation to the prevailing climatic conditions.

At soil temperatures below 10 °C, ammonification takes place more readily than nitrification (Emmer and Tietema, 1990). Autotrophic nitrifying bacteria are sensitive to cold temperatures and take longer to acclimatize to cold conditions than ammonifying microorganisms (Cookson et al., 2002). Autotrophic nitrifiers are slow growing bacteria (De Boer and Kowalchuk, 2001), and their contribution to total nitrification in forest soils is highly variable (8 to 100%) (De Boer et al., 1992; Venterea et al., 2003; Grenon et al., 2004). In recent years, also archaea were shown to oxidize ammonium (Wessén et al., 2011; Treusch et al., 2005). In the bulk soil, a large variety of functional microbial groups (e.g. fungi, archaea, autotrophic bacteria) interacts in the N cycle and their specific contribution can hardly be differentiated. However, the interaction between different organisms is crucial for determining overall temperature responses in N and C cycling (Wetterstedt et al., 2010). So, the temperature sensitivity of nitrification and ammonification might differ in soils or soil horizons if distinct microbial communities contribute to these processes.

Gross ammonification is directly linked to C mineralization though the ratio of these processes is possibly altered at low temperatures. In high latitude soils, microbial biomass was reported to remain constant or even increase during winter under a thick snow cover (Schadt et al., 2003; Brooks et al., 1998). Both catabolic and anabolic processes were shown to continue between -4 and +9 °C (Harrysson Drotz et al., 2010) and the microbial N demand may thus be high, especially in boreal soil with wide C/N ratios. In temperate soils, microbial activity may decrease by several orders of magnitude when temperature drops below 5 °C (Pietikäinen et al., 2005; Ranneklev and Bååth, 2001) and N acquisition during winter seems less important. If the need of N for microbial growth is reduced, the temperature sensitivity of gross ammonification could be different from that of C mineralization at low temperatures. Soil frost affects the temperature sensitivity of both processes through microbial death or synthesis of frost protection agents (Stres et al., 2010; Schimel et al., 2007). Another constraint on microbial activity under frozen conditions emanates from the reduced liquid water availability (Öquist et al., 2009).

In our study, we investigated the response of soil processes to changes in winterly temperatures and tested the following hypotheses: (1) the temperature sensitivity of C mineralization, gross and net N mineralization is higher in soil horizons of low substrate

quality than in soil horizons of high quality substrates, and (2) the temperature sensitivity of C mineralization is higher than of gross ammonification. Cumulative C mineralization was used as proxy for substrate quality of soil organic matter.

2. Materials and methods

2.1 Study sites and sample preparation

Soil samples were taken from the site "Steinkreuz" in the Steigerwald Nature Park, Germany (49°52´N, 10°27´E), dominated by European beech (*Fagus sylvatica* L.), hereinafter referred to as beech site. Furthermore, samples were taken from the site "Coulissenhieb II" in the Fichtelgebirge, Germany (58°08´N, 11°52´E), dominated by Norway spruce (*Picea abies* L.) and in the following referred to as spruce site. Mean annual precipitation and air temperature is 750 mm and 7.5 °C at the beech and 1160 mm and 5.3 °C at the spruce site (Gerstberger et al., 2004). The soil of the beech site is classified as Dystric Cambisol, with a shallow moder type forest floor. Haplic Podzols with a well stratified, moor-like forest floor of 6-10 cm thickness prevail at the spruce site (classification according to FAO (IUSS, 2006)). The mean throughfall deposition rates of inorganic N are 22.2 kg ha^{-1} a^{-1} in the spruce site and 15.4 kg ha^{-1} a^{-1} in the beech site (Matzner et al., 2004). Further soil characteristics are available in Table 1. A detailed description of the sites is given in Gerstberger et al. (2004).

Soil samples were taken from the spruce site in March 2012 and from the beech site in April 2011 to assure that the microbial population is adjusted to winter temperatures. An area (10 m^2) without ground vegetation was selected for soil sampling at both sites. Mixed samples (2-4 kg fresh weight) were taken each from the Oi/Oe, Oa and A horizons. Soil samples were homogenized by hand and roots, twigs, branches and stones were removed. Leaves were cut by hand into pieces of <1 cm^2. Samples were adjusted to field capacity as this reflects soil moisture conditions at our sites during winter. Alternating frost and warm periods with rainfall and snow melt mostly prevent drying of forest soils.

Samples were stored at 2 °C for up to 3 days before incubation. Initial gravimetric water contents were determined by drying at 60 °C for organic layers and 105 °C for the mineral horizon. Gravimetric moisture contents at field capacity were 261% in spruce Oi/Oe and 338% in beech Oi/Oe horizon, 82% in beech A horizon and 209% in spruce Oa horizon.

Table 1 Soil characteristics of forest floor and A horizons at beech and spruce site (Data from Gerstberger et al., 2004).

		depth (cm)	pH* ($CaCl_2$)	C* (%)	N* (%)	C/N*	CEC_{eff}	BS (%)	NH_4^+ §,* mg kg^{-1}	NO_3^- §,* mg kg^{-1}
Beech	Oi/Oe	3-0.5	4.4	40.3	1.5	26.3	567.9	86.7	19.2	2.0
	Oa	0.5-0	n.d	n.d.	n.d.	n.d.	172.3	65.7	n.d.	n.d.
	A	0-5	3.1	9.5	0.5	20.7	87.8	34.5	0.97	0.62
Spruce	Oi/Oe	8.5-3	2.7	45.9	2.1	22.3	245.8	22.7	118	51.4
	Oa	3-0	2.6	28.5	1.1	26.9	274.2	56.8	11.4	40.8

CEC_{eff} = effective cation exchange capacity; BS = base saturation; n.d. = not determined; § = 1M KCl extract (1:10 resp. 1:5 v/w for organic and mineral horizons); * = own data

2.2 C mineralization

Five replicates per horizon (each 5-15 g dry weight (DW)) were incubated in 930 ml glass jars at constant temperatures of -4, -1, +2, +5 and +8 °C with a precision of +/- 0.3 °C. Measurements were conducted twice a week over an incubation period of 28 days (beech Oi/Oe and A horizons), 32 days (spruce Oi/Oe) and 35 days (spruce Oa). The jars were kept closed during the incubation and were flushed with synthetic air (80% N_2, 20% O_2) only when headspace CO_2 concentration exceeded 3000 ppm. The respiration rates were calculated from the linear increase in CO_2 in the glass jars over the measurement interval. CO_2 concentration was measured as CH_4 with a gaschromatograph (SRI 8610C, SRI Instruments Europe GmbH, Germany) equipped with a flame ionization detector. Gas samples of 30 µl were withdrawn from the headspace of the jars through a septum by a 50 µl syringe and injected into the GC. CO_2 standards of 380, 600, 1000, 3000 and 10000 ppm were used to generate a linear calibration function.

2.3 Gross and net rates of ammonification and nitrification

Gross ammonification and nitrification were determined by the ^{15}N pool dilution technique (Kirkham and Bartholomew, 1954) labeling soil NH_4^+ and soil NO_3^- respectively, and using 3 (spruce) or 4 (beech) replicates of homogenized soil for each incubation temperature (see below). For addition of ^{15}N label, homogenized samples were divided into subsamples. To about 150 g fresh weight of soil, 1.8 ml solution was sprayed onto the soil, comprising 0.15-0.6 mg $(^{15}NH_4)_2SO_4$ (98.0 atom%) or 0.015-0.15 mg $K^{15}NO_3^-$ (99.2 atom%) depending on soil NH_4^+or NO_3^- concentration. Manually mixing of soil improved the distribution of the added ^{15}N tracer. Depending on the initial soil NH_4^+ or NO_3^- concentration ^{15}N label of soil NH_4^+ and NO_3^- were enriched to 20-40 atom% above the natural abundance level of ^{15}N (0.36 atom%) after tracer application. Samples were incubated in 100 ml polyethylene bottles at constant temperatures of -4, -1, +2, +5 and +8 °C with a precision of +/- 0.3 °C. Following a t_0 incubation time of 24 h at 2 °C, subsamples (3 replicates for spruce, 4 replicates for beech) of 5 g DW for organic horizons and 10 g DW for mineral soil were extracted with 1.0 M KCl solution at a soil/solution ratio of 1:10 for organic horizons and 1:5 for mineral soil. KCl-treated samples were shaken on a horizontal shaker at 225 min^{-1} and the supernatant filtered (cellulose folded filters $595^1/_2$, 4-7 µm, Whatman, Germany). Filtered KCl-extracts were stored frozen at -20 °C. Pre-incubated samples were sealed by flexible film (Parafilm M, Alcan Packaging, USA) and incubated at temperatures given above for a t_1 incubation time of

72 h (hereafter referred to as t_1-samples) and then extracted in the same way as for t_0. All extractions were conducted at 5 °C.

By incubating all soil samples for 24h (t_0) at 2 °C, we assured similar conditions for pre-mineralization/pre-decomposition and infiltration of the ^{15}N label into the soil matrices. The latter would have not been possible if samples were frozen. We consider the adjustment of incubation temperatures (-4 to +8 °C) after 24h (t_0) for another 72h (t_1) adequate as microbial mineralization responds very quickly to temperature changes (Andrews et al., 2000; Dalias et al., 2001). We assume that abiotic processes, if present, occur within few minutes or hours after application of ^{15}N tracer. As t_0 is taken 24 h after ^{15}N application, abiotic processes are unlikely between t_0 and t_1.

The ^{15}N abundance and the concentrations of NO_3^- and NH_4^+ in KCl-extracts were measured by the SPINMAS technique (Sample Preparation unit for Inorganic Nitrogen and Mass Spectrometer) (Stange et al., 2007). Gross N ammonification and nitrification were calculated using the equation from Kirkham and Bartholomew (1954). As we used mixed samples (pseudo-replicates), we chose the approach of Luxhøi and Brockhoff (2004) to calculate gross rates and to account for error propagation in the standard errors. Any negative values (only observed for gross nitrification) were set to zero.

Net N mineralization was determined using homogenized soil as described in the previous section. Prior to incubation, samples were stored at 2 °C for up to 3 days. Extractions were done as described above to yield an initial t_0 concentration of NH_4^+ and NO_3^-. Five samples per horizon (each 5-15 g DW) were incubated in 930 ml airtight glass jars for 28 days (t_1, beech) and 29 days (t_1, spruce), respectively, at temperatures given above and then extracted as described earlier. Filtered KCl-extracts were analyzed for concentrations of NH_4^+ (FIAmodular MLE, Dresden, Germany) and of NO_3^- (FIAcompact, MLE, Dresden, Germany) by colorimetric methods. Net N mineralization rates were then calculated by difference between t_0 and t_1.

2.4 Q_{10} calculation

The Arrhenius equation (equation 1) was applied to calculate the temperature sensitivity of C and N mineralization in a first-order linear regression in the temperature interval between -4 °C and +8 °C and between +2 °C and +8 °C:

$$k = A\, e^{-\frac{E_a}{R*T}} \qquad (1)$$

where A is the fitted Arrhenius constant (no dimension), E_a (kJ mol^{-1}) is the fitted activation energy, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the absolute temperature in K and k is the reaction rate (gross ammonification, gross nitrification, net N mineralization or C mineralization rate). The natural logarithm of C or N mineralization rate was plotted versus the reciprocal of soil temperatures (1/T in K) in Arrhenius plots. E_a was obtained from the slope of the regression line and Q_{10} values were calculated according to equation 2:

$$\ln Q_{10} = \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T+10}\right) \qquad (2)$$

Log-transformations and calculation of Q_{10} values were only possible for positive mineralization rates, thus Q_{10} were not calculated for net N mineralization in beech A horizon and in the spruce site from -4 to +8 °C. At -4 and -1 °C, gross nitrification in beech Oi/Oe was negligible and not included in the Q_{10} calculation.

3. Results

In both soils, C mineralization was much larger in the Oi/Oe than in the Oa and A horizons (Fig. 1g,h). Furthermore, C mineralization in beech Oi/Oe exceeded that of the spruce soil, while rates of the Oa and A horizons were similar in both soils. At -4 °C, C mineralization was close to zero for all soil horizons, but rates increased at -1 °C in case of the Oi/Oe horizons. The increase continued to +8 °C, reaching maximum rates of 22.3 (beech) and 4.4 (spruce) g C kg^{-1} DW 30 d^{-1}. C mineralization in the Oa and A horizons increased also with temperature, but at a much smaller level.

Temperature patterns of gross ammonification largely coincided with those of C mineralization (Fig. 1a,b) and beech exceeded spruce by almost one order of magnitude. Rates at -4 °C were close to zero for all soils. For beech Oi/Oe, a rate of 79.5 mg N kg^{-1} DW d^{-1} was observed at -1°C.

Rates of gross nitrification in the beech horizons and spruce Oi/Oe reached 1 to 22% of gross ammonification. In spruce Oa, gross nitrification rates amounted to 44-100% of gross ammonification (Fig. 1c,d). In all horizons, a distinct increase with temperature was observed. In the beech Oi/Oe gross nitrification was zero at negative temperatures, while low rates were observed at temperatures <0 °C in the spruce soil.

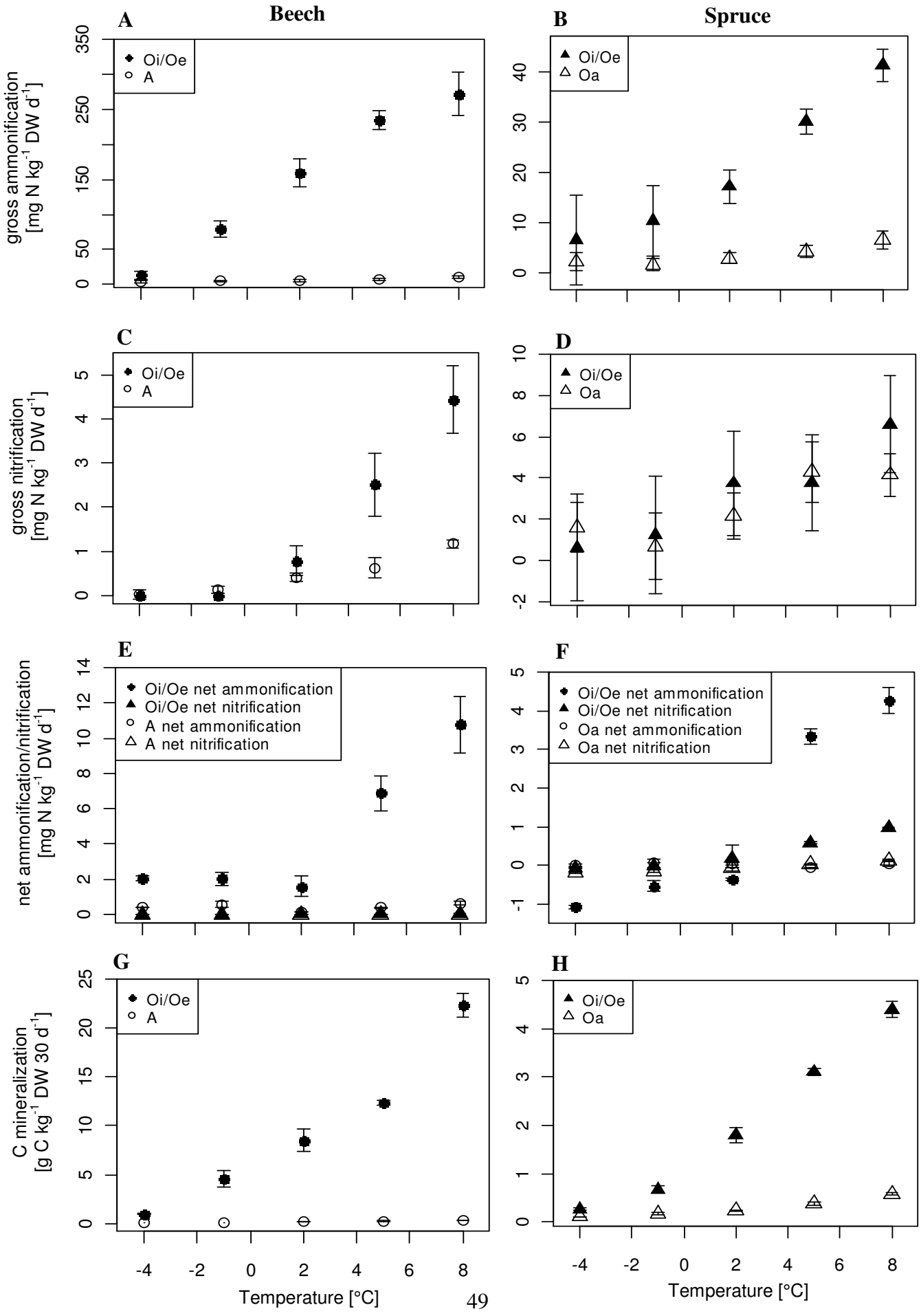
A
Beech
B
Spruce
gross ammonification [mg N kg-1 DW d-1]
Oi/Oe
A
Oa
C
D
gross nitrification [mg N kg-1 DW d-1]
E
F
net ammonification/nitrification [mg N kg-1 DW d-1]
Oi/Oe net ammonification
Oi/Oe net nitrification
A net ammonification
A net nitrification
Oa net ammonification
Oa net nitrification
G
H
C mineralization [g C kg-1 DW 30 d-1]
Temperature [°C]

Fig. 1 Gross ammonification rates, gross nitrification rates, net N mineralization rates (in mg N kg^{-1} DW d^{-1}) and C mineralization rates (in g C kg^{-1} DW 30 d^{-1}) of beech Oi/Oe (●) and spruce Oi/Oe (▲) and beech A (○) and spruce Oa (Δ). Error bars represent SE.

The response of net ammonification to temperature differed from that of gross ammonification (Fig. 1e,f). Rates were small at temperatures between -4 and +2 °C and strongly increased between +2 and +8 °C in Oi/Oe horizons. Net nitrification was negative or small at subzero temperatures in both soils. The beech soil did not respond to higher temperatures, whereas there was a strong increase in net nitrification from +2 to +8 °C in the spruce soil.

Apparent Q_{10} values for C mineralization in the Oi/Oe horizon were around 17 (-4 - +8 °C) in both sites, but were smaller in the Oa (5.3) and A horizons (4.4) (Table 2). A close positive relationship was found between mineralization rates and soil temperature but not all regressions were significant because of only 3 temperature steps (n=3 when Q_{10} was calculated between +2 - +8 °C). For gross ammonification, Q_{10} in Oi/Oe horizons were 18 for beech and 6.8 for spruce whereas in Oa and A horizons Q_{10} were again lower (3.6 in spruce Oa, 2.9 in beech A).

Q_{10} of gross nitrification revealed a large variation among study sites and soil depth. In Oa and A horizons, coefficients of correlation (r^2) of the Arrhenius graphs were rather weak (0.63 for beech A and of 0.60 for spruce Oa (Table 2)) which coincided with a high heterogeneity of the sample replicates. Q_{10} values calculated from +2 to +8 °C were lower than Q_{10} values calculated from -4 to +8 °C for all processes.

Table 2 Q_{10} values, activation energy E_a (kJ mol^{-1}) and r^2 of Arrhenius plots for gross and net N mineralization and C mineralization in beech and spruce soil. Regressions are significant at $p<0.05$ when indicated by *.

	Beech								Spruce							
	Oi/Oe horizon				A horizon				Oi/Oe horizon				Oa horizon			
Temperature range -4-8°C	Q_{10}	E_a	r^2	A	Q_{10}	E_a	r^2	A	Q_{10}	E_a	r^2	A	Q_{10}	E_a	r^2	A
Gross ammonification	17.7	151	0.84*	71.5	2.9	55	0.98*	26.9	6.8	101	0.99*	47.9	3.6	68	0.82*	31.6
Gross nitrification	17.7	151	0.93*	66.9	5.0	84	0.63	37.0	10.5	123	0.93*	55.8	4.4	78	0.60	36.0
Net N mineralization	6.10	95	0.65*	46.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C mineralization	17.6	150	0.91*	74.5	4.4	78	0.98*	39.0	17.3	150	0.97*	72.6	5.3	88	0.97*	44.0
Temperature range 2-8°C																
Gross ammonification	1.7	58	0.94	31.4	1.9	68	0.99*	32.5	2.3	90	0.97	44.4	2.2	86	0.99*	40.5
Gross nitrification	5.7	187	0.96	82.5	2.9	113	0.99	49.4	1.8	61	0.75	28.8	1.9	69	0.72	32.3
Net N mineralization	7.0	209	0.87*	95.4	4.7	165	0.93*	73.5	2.6	104	0.77*	450	n.d.	n.d.	n.d.	n.d.
C mineralization	2.6	103	0.94*	54.0	1.9	67	0.92*	34.5	2.5	97	0.96*	49.8	2.4	96	0.98*	47.5

n.d. = not determined

The ratio of C mineralization (in mg C kg^{-1} DW d^{-1}) to gross ammonification (in mg N kg^{-1} DW d^{-1}) was from 0.5 to 2.9 (Fig. 2) with an increasing trend with rising temperatures. The C/N ratio of mineralization in the different soils was in the same order as the C/N ratio of SOM.

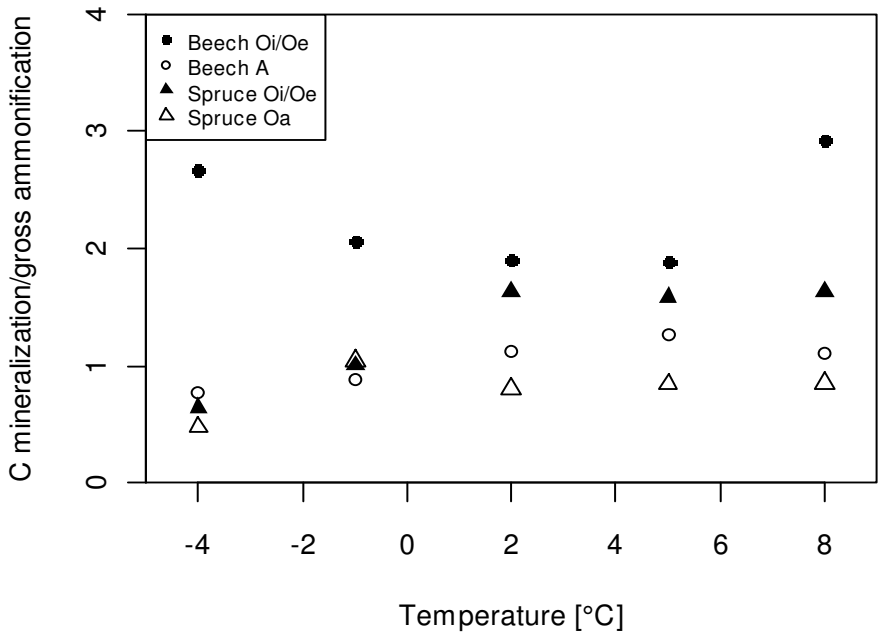

Fig. 2 Ratios of C mineralization to gross ammonification (C/N ratio of mineralization in mg kg^{-1} DW d^{-1}) in beech and spruce Oi/Oe (●,▲) and A and Oa (○,Δ) horizons.

4. Discussion

4.1 Comparing gross N turnover and C mineralization at low temperatures

Our results confirm that considerable gross ammonification, gross nitrification and C mineralization take place at low temperatures. Gross ammonification and nitrification from our study were comparable to rates found at higher temperatures in the forest floor of the same spruce site (Chen et al., 2011). While gross ammonification was similar at 8 °C (our study) and 15 °C (Chen et al. 2011), gross nitrification was reduced by more than 50% at 8 °C. In forest floor of a subalpine fir stand, Grenon et al. (2004) found gross ammonification and nitrification rates of 60 and 20 mg N kg^{-1} $soil_{DW}$ d^{-1} during July, which are similar to rates in our study. Soil from calcareous A horizon of a beech forest had a gross ammonification rate of 50 mg N kg^{-1} $soil_{DW}$ d^{-1} during July at 14 °C, exceeding our rates by a factor of 5 (Dannenmann et al., 2007). We attribute the differences among the studies not only to temperature gradients but also to seasonal pattern of microbial population and varying soil

properties. The ratio of C mineralization to gross ammonification (in mg kg^{-1} DM d^{-1}) was around 0.5-2.9 at low temperatures, giving evidence for rapid microbial N turnover. Throughout the literature, ratios of C mineralization to gross ammonification were only reported for higher temperatures, and displayed greater values of 5 - 10 (Hart et al., 1994; Chen et al., 2012). The small ratios might be typical for low temperatures. The synthesis of frost protection agents (e.g. glycine, betaine) as well as maintaining the fluidity of cell wall membranes requires a continuing microbial uptake of N at temperatures below 0 °C (Rilfors and Lindblom, 2002; Schimel et al., 2007). If frost protection agents contain N (e.g. amino acids), the C to N ratio of the microbial biomass would decrease at low temperatures (Mihoub et al., 2003; Bae et al., 2004). As the ^{15}N pool dilution technique mainly quantifies the N turnover of the microbial biomass, a high microbial biomass N will tend to increase gross ammonification and contribute to the low ratios of C to N mineralization.

4.2 Q_{10} values at low temperatures

Q_{10} values of C mineralization at low temperatures were very high, corroborating other findings (Mikan et al., 2002; Tilston et al., 2010; Öquist et al., 2009). In a review, Hamdi et al. (2013) reported Q_{10}s of 1.9-7 for C mineralization in forest soils between 5 and 10 °C, which corresponds roughly to our findings (Q_{10}s of 1.9 - 2.6 at +2 °C to +8 °C). For C mineralization in a boreal spruce soil, Tilston et al. (2010) reported a Q_{10} of 5.8 between -2 °C and +10 °C, which fits well to a Q_{10} of 5.3 measured in our spruce Oa between -4 °C and +8 °C. Our Q_{10} values for C mineralization were possibly superimposed by decreasing substrate quantity during the 30-day incubation. The depletion of easily available substrates was likely greater at higher temperatures due to stronger microbial activity. As a consequence, the Q_{10} determined for the 30 day period might be lower than for shorter incubation periods.

Q_{10} values of gross ammonification in our study ranged from 2.9 to 17.7 and were in most horizons lower than Q_{10}s for C mineralization, confirming our hypothesis that C mineralization is stronger temperature dependent at low temperatures than gross ammonification. This corresponds to findings by Koch et al. (2007), who reported higher Q_{10}s for enzymes involved in the C cycle compared to enzymes involved in the N cycle. As indicated also by the C/N ratio, rising winter temperature would have a stronger effect on C mineralization than on gross ammonification.

We found an increase in Q_{10} when soil frost was included in the calculation (e.g., for C mineralization in beech Oi/Oe, Q_{10} was 17.6 at -4 to +8 °C and 2.6 at +2 to +8 °C, Table 2). A similar increase has been observed in boreal forest and tundra soils (Tilston et al., 2010;

Mikan et al., 2002). It has been attributed to changes in the availability of liquid water (Öquist et al., 2009) that constraints the microbial activity in several ways, for example by related substrate limitation caused by extracellular diffusion barriers (Mikan et al., 2002). Excluding the influence of reduced water availability in frozen soils, Öquist et al. (2009) calculated Q_{10} values (1.4-2.6) similar to Q_{10} values of unfrozen soils. However, in our experiment, we observed only small changes in rates between -4 and -1 °C. As our samples were moistened to field capacity, representing the winter conditions at our sites, we assume that liquid water availability at -4 °C was similar to -1 °C. Hence, the constraints of liquid water availability might have only marginally affected our Q_{10} values.

4.2.1 Effects of soil substrates on Q_{10} values

Our hypothesis that Q_{10} of C mineralization, gross and net N mineralization is highest in soil horizons with low substrate quality was not confirmed. In contrast, Q_{10}s (-4 to +8 °C) of the Oa and A horizons were markedly lower than those of the respective Oi/Oe horizons. Higher Q_{10}s for gross ammonification and nitrification in beech compared to spruce soil horizons do not confirm our hypothesis. With increasing soil depth, average substrate quality generally decreases by enrichment of humified, recalcitrant organic substrates and declining production of easily decomposable compounds. As described earlier, theory (Arrhenius, 1889) predicts a stronger temperature dependency of mineralization of low quality substrates due to their high inherent activation energies. Using the horizon-specific C mineralization rate as a proxy for substrate quality (high C mineralization means high substrate quality), this theory is not affirmed. Von Lützow and Kögel-Knabner (2009) and Davidson et al. (2012) suggest that substrate availability rather than quality is the main influencing factor. At low substrate availability, e.g. when soil enzymes are physically or chemically separated from the substrate, "apparent" Q_{10} values are low (von Lützow and Kögel-Knabner, 2009) due to substrate limitation. Under these conditions, principles of Michaelis-Menten kinetics largely influence mineralization rates (Davidson and Janssens, 2006; von Lützow and Kögel-Knabner, 2009). Both together, quality and availability of substrates influence the temperature sensitivity of SOM decomposition in the field as conjunct parameters and contribute to the large variability in Q_{10} (Davidson and Janssens, 2006; Hamdi et al., 2013). We cannot exclude that substrate availability affected temperature sensitivity in the beech and spruce soil. However, substrate availability was not or barely limited by water availability or binding to mineral surfaces in the organic horizons or A horizons.

Besides substrate quality and quantity, other factors modify temperature responses and also may explain the deviance of our findings from theory. In a modeling exercise Ågren and Wetterstedt (2007) suggested that specific uptake kinetics for different organism groups, substrate diffusion rates and the rates at which substrates are made available in the environment might affect temperature responses.

4.3 Nitrification at low temperatures

Gross nitrification was generally low and similar in different soil horizons. Rates under spruce slightly exceeded rates under beech. In spruce Oa, gross ammonification and nitrification were in the same order of magnitude. Here, nitrifiers were possibly limited by NH_4^+ supply from ammonification. In line with our results, Staelens et al. (2011) measured lower gross nitrification rates in broad-leaf than in coniferous forest soil and attributed this to a significant contribution of heterotrophic nitrification in the coniferous forest, forming NO_3^- directly from N_{org} compounds. Trap et al. (2009) suggested a shift towards heterotrophic nitrification when substrate quality is decreasing and fungi were shown to have heterotrophic nitrification ability under acid soil conditions (De Boer and Kowalchuk, 2001). In the beech soil, no data exists about the contribution of heterotrophic nitrifiers to gross nitrification. Furthermore, soil temperatures rarely drop below 0 °C in our beech forest and possibly hampered the microbial adaptation whereas the nitrifiers in our spruce forest were better adapted to low temperatures. A general conclusion about the temperature sensitivity of gross nitrification can hardly be drawn, since the temperature sensitivity of nitrification depends on the temperature sensitivity of ammonification and immobilization of NH_4 by other organisms.

4.4 Effects of rising winter temperatures on net N mineralization

An estimate of winter rates by applying the exponential equations with Q_{10}s from +2 to +8 °C (Table 2) from November to April, net ammonification in the beech soil (Oi/Oe+A) with an actual average winter temperature of 3.7 °C would sum up to 34.2 kg NH_4-N ha^{-1} 6 $months^{-1}$. In this soil, net nitrification was negligible from -4 to 8 °C (Fig. 1e). A mean temperature increase during the winter period of 3 °C would amount to a net ammonification of 58 kg NH_4-N ha^{-1} 6 $months^{-1}$. The additional amount of 24 kg NH_4-N ha^{-1} 6 $months^{-1}$ in the beech soil could substantially change overwinter N fluxes, and it remains an open question, if net nitrification stays negligible when winter temperature increases.

In the Oi/Oe horizon of the spruce forest with average winter temperature of 1.9 °C, net N mineralization would amount to 4.2 kg N ha^{-1} 6 $months^{-1}$ where net nitrification contributes

less than 1 kg NO_3-N ha^{-1} 6 $months^{-1}$. An increase of winter temperature by 3 °C would be of minor importance and increase net N mineralization by +1.4 kg N ha^{-1} 6 $months^{-1}$. Because the Oa horizon revealed no net N mineralization rising winter temperature (+3°C) displays a moderate risk of increasing NO_3^- leaching from the organic horizons in the spruce forest.

5. Conclusion

Gross ammonification and C mineralization in organic horizons continued at considerable rates around freezing point. Q_{10}s were generally high at low temperatures, ranging between 3 and 18. When soil frost was excluded from the calculation, Q_{10}s decreased and gave evidence for additional constraints acting on microbial processes at subzero temperatures.

Our findings suggest that the apparent temperature dependency (Q_{10}) of C and N mineralization of low quality substrates is smaller than of high quality substrates, despite large differences in the absolute mineralization rates. C and N mineralization in the forest floor would respond stronger to rising winter temperatures than in the A horizon. Ratios of C mineralization to gross ammonification around 1.0 gave evidence for a rapid microbial N turnover at temperatures around 0 °C. Rising winter temperatures might have substantial effects on net N mineralization, but effects decrease with soil depth, likely due to decreasing substrate quality of organic matter.

Acknowledgements

This research was financially supported by the *Deutsche Forschungsgemeinschaft* (DFG). We thank Andrea Schott, Helga Hertel-Kolb and Uwe Hell for their help during field and laboratory works and Bernd Apelt (Department of Soil Physics, Helmholtz Center for Environmental Research – UFZ) for the ^{15}N analyses. We are grateful to the Central Analytic Department of Bayreuth Center of Ecology and Environmental Research (BayCEER) for the chemical analysis of samples.

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Study 2

Substantial net N mineralization during the dormant season in temperate forest soils

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In press in Journal of Plant Nutrition and Soil Science.

Abstract

In temperate forest soils, nitrogen (N) net mineralization has been extensively investigated during the growing season, whereas N cycling during winter was barely addressed. Here, we quantified net ammonification and nitrification during the dormant season by *in situ* and laboratory incubations in soils of a temperate European beech and a Norway spruce forest. Further, we compared temperature dependency of N net mineralization in *in situ* field incubations with those from laboratory incubations at controlled temperatures.

From November to April, *in situ* N net mineralization of the organic and upper mineral horizons amounted to 10.9 kg N ha^{-1} 6 $months^{-1}$ in the spruce soil and to 44.3 kg N ha^{-1} 6 $months^{-1}$ in the beech soil, representing 65% (beech) and 26% (spruce) of the annual above ground litterfall. N net mineralization was largest in the Oi/Oe horizon and lowest in the A and EA horizons. Net nitrification in the beech soil (1.5 kg N ha^{-1} 6 $months^{-1}$) was less than in the spruce soil (5.9 kg N ha^{-1} 6 $months^{-1}$).

In the range of soil temperatures observed in the field (0 – +8°C), the temperature dependency of N net mineralization was generally high for both soils and more pronounced in the laboratory incubations than in the *in situ* incubations. We suggest that homogenization of laboratory samples increased substrate availability and thus enhanced the temperature response of N net mineralization.

In temperate forest soils N net mineralization during the dormant season contributes substantially to the annual N cycling, especially in deciduous sites with large amounts of litterfall immediately before the dormant season. High Q_{10} values of N net mineralization at low temperatures suggest a huge effect of future increasing winter temperature on the N cycle in temperate forests.

1 Introduction

Biological processes in soils are known to continue at low temperatures in winter and even below freezing point (*Clein* and *Schimel*, 1995; *Schimel* et al., 2004; *Miller* et al., 2007; *Ueda* et al., 2013) as microorganisms remain active in unfrozen water films (*Coxson* and *Parkinson*, 1987; *Mikan* et al., 2002). In high-altitude and high-latitude ecosystems, overwinter mineralization can account for a large proportion of annual C (21 - 50%) and net N mineralization (30 - >50%) (*Monson* et al., 2006; *Zimov* et al., 1996; *Kielland* et al., 2006; *Vestgarden* et al., 2003). Snow cover often prevents soils from freezing and maintains relatively high microbial turnover during winter. In deciduous temperate forest soils, the N net mineralization in the dormant season (fall and winter) was substantial amounting to 34 to 49 kg N ha^{-1} 200 d^{-1} (*Groffman* et al. 2001). Large mineral N pools in forest floors in spring have been reported for temperate forests (*Ueda* et al., 2013) giving evidence for N net mineralization rates in the dormant season. *Hentschel* et al. (2008) reported 86 kg N ha^{-1} 170 d^{-1} that was leached from Norway spruce forest floor columns in a laboratory study at constantly 5°C, emphasizing the potentially large N net mineralization at rather low temperatures. In some climatic regions, winter may be an especially important period for ecological processes, with microbial processes accounting for a considerable percentage of annual rates (*Campbell* et al., 2005).

Norway spruce and European beech represent common species in European forests. Coniferous and deciduous litter differ in their quality and higher amounts of recalcitrant compounds as well as less calcium contents are reported for coniferous litter (*Reich* et al., 2005). Beside litter quality, the temporal patterns of litterfall differ substantially in beech and spruce stands. The leaf fall in autumn in deciduous sites provides almost the total annual litterfall immediately prior to the dormant season, whereas leaf fall in coniferous forests occurs throughout the year. The litterfall in beech sites provides huge amounts of easily decomposable substrate for the following cold period, suggesting differences in the relevance of N net mineralization in the dormant season between spruce and beech.

Field and laboratory incubations are commonly used to quantify N net mineralization (*Raison* et al., 1987). Disturbance of the soil structure and microbial community is minimized using intact soil cores during *in situ* field incubations. In laboratory incubations with homogenized samples temperature and moisture conditions can easily be controlled. Homogenization breaks up soil aggregates which may increase substrate availability which in turn will influence N turnover (*Kaur* et al., 2010) and temperature dependency of N mineralization, as increased substrate availability likely increases the temperature dependency (*Davidson* and

Janssens, 2006; *von Lützow* and *Kögel-Knabner*, 2009; *Hamdi* et al., 2013). Higher net mineralization and nitrification was found in disturbed samples at temperatures between +2°C to +21°C and in a broad range of ecosystems such as arable, forest and steppe soil (*Stenger* et al., 1995; *Kaur* et al., 2010; *Ross* and *Hales*, 2003; *Booth* et al., 2006). However, homogenization did not increase net rates in all studies. Removal of roots and adherent mycorrhizal fungal hyphae likely lowered N net mineralization in sieved soil as compared to intact soil cores (*Persson* et al., 2000).

Here we measured net ammonification and nitrification at low soil temperatures by *in situ* and laboratory incubations to estimate the contribution of overwinter N net mineralization to annual N net mineralization and to quantify the temperature dependency of both processes at low temperatures. We expected rates under beech being larger than under spruce. Further, we expected that the apparent temperature dependency of N net mineralization at low temperatures is larger in homogenized soil samples than in *in situ* incubations.

2 Material and methods

2.1 Site description

Soil N net mineralization was determined in a 130-year-old European beech (*Fagus sylvatica* L.) and a 140-year-old Norway spruce forest (*Picea abies* L.). The beech site "Steinkreuz" is located in the Steigerwald Nature Park, Germany (49°52´N, 10°27´E) and the spruce site "Coulissenhieb II" is located in the Fichtelgebirge, Germany (50°08´N, 11°52´E). Mean annual precipitation and air temperature is 750 mm and 7.5°C for the beech site and 1160 mm and 5.3°C for the spruce site (*Gerstberger* et al., 2004). In the beech site, Dystric Cambisols prevail as soil types, with a shallow moder type forest floor. Haplic Podzols with a well stratified, mor-like forest floor of 6-10 cm thickness prevail in the spruce site (classification according to FAO (*IUSS*, 2007)). The mean throughfall fluxes of inorganic N are 22.2 kg ha^{-1} a^{-1} in the spruce and 15.4 kg ha^{-1} a^{-1} in the beech site (*Matzner* et al., 2004). The soil was more acidic under spruce (pH($CaCl_2$) from 3.3 to 3.6) than under beech (pH($CaCl_2$) from 3.9 to 4.7) and the C stocks of the upper soil horizons were larger under spruce than under beech. Stocks of N were however similar (Table 1). A detailed description of the sites is given in *Gerstberger* et al. (2004). Soil temperatures at the sites were recorded automatically in intervals of 30 min (beech) or 1h (spruce) in 2, 5 and 10 cm soil depth.

Table 1 Soil characteristics of forest floor and A horizons at beech and spruce site (Data from *Gerstberger* et al., 2004; *Berg* and *Gerstberger*, 2004; *Schulze* et al., 2009).

		depth (cm)	pH ($CaCl_2$)	C stock t ha^{-1}	N stock t ha^{-1}	C %	N %	C/N	CEC_{eff}	N return† kg N ha^{-1} a^{-1}
Beech	Oi/Oe	3-0.5	4.7*	14.8	0.8	40.3*	1.5*	26.9*	568	68.3
	Oa	0.5-0	4.1	12.1*	0.7*	16.4*	0.9*	18.2*	172	
	A	0-5	3.9	34.9	2.2	9.5*	0.5*	19.0*	88	
Spruce	Oi/Oe	8.5-3	3.6*	20.6	0.8	44.0	1.8	24.4	246	41.2
	Oa	3-0	3.3	24.0	1.2	21.2	1.1	19.3	274	
	EA	0-10	3.4	23.9	1.2	8.3	0.4	21.0	98	

CEC_{eff} = effective cation exchange capacity
†: total N return from litter (foliar+woody parts+cones+nuts)
*: own data

2.2 N net mineralization

In situ N net mineralization was determined by the sequential coring method (*Raison* et al., 1987) from November 2011 to April 2012. On each sampling date, twenty cores (made from PVC or stainless steel, 6.5 cm diameter) were driven down to 20 cm into the soil, ten were immediately taken to determine initial soil NH_4^+ and NO_3^- contents (t_0) and ten were left in the field (covered by lid) for approx. 6 weeks (t_1). In total, 4 incubation periods were established. The cores were kept cool during transport to the laboratory and then stratified into Oi/Oe (combined sample), Oa and A (or EA) horizons (upper 5 cm). Gravimetric water contents were determined by drying at 60°C for organic layers and 105°C for the mineral horizons. 5-10 g dry weight (DW) were extracted with 1 M KCl at a soil/solution ratio of 1:10 for organic horizons and 1:5 for mineral soil and the supernatant was filtered (cellulose folded filters $595^1/_2$, 4-7 µm, Whatman, Germany). Extractions were conducted at 5°C. KCl-extracts were stored at -20°C and analyzed for NH_4^+ (FIAmodular MLE, Dresden, Germany) and NO_3^- (FIAcompact, MLE, Dresden, Germany) by colorimetric methods. N net mineralization rates were calculated by difference between t_0 and t_1.

N net mineralization in laboratory incubations was measured over 4 weeks at constantly -4, -1, +2, +5 and +8°C (with a precision of +/- 0.3°C). Mixed samples (2-4 kg fresh weight) were taken from the Oi/Oe and A horizons in the beech site in March 2012 and from Oi/Oe and Oa horizon in the spruce site in April 2011. After removal of roots, twigs and stones soil samples were mixed by hand whereas beech leaves of the Oi/Oe horizon were cut by hand into pieces of <1 cm^2. As soil moisture is near field capacity in our sites during winter, we adjusted samples to field capacity. Extractions for t_0 (immediately after sampling) and t_1 (after 4 weeks) were conducted as described above.

2.3 Data analysis, calculations and statistics

In order to extrapolate N net mineralization rates measured in the laboratory incubation to the actual average monthly soil temperatures in the field, a linear function was fitted to the laboratory rates measured at +2, +5 and +8°C. Rates from -4 and -1°C were not included as negative rates (immobilization) occurred at these temperatures. We then calculated N net mineralization (sum of ammonification and nitrification) for the average soil temperatures in the field and compared those with the *in situ* N net mineralization rates. Rates were calculated in kg N ha^{-1} by multiplying rates in g N kg^{-1} DW with C stocks and C% (Table 1). Q_{10} values for N net mineralization in the laboratory incubation were estimated by a linear function in the temperature interval of +2 to +12°C. One-way analysis of variance was used for testing

statistical significance between soil horizons (Fig. 2). The analyses were done with R 2.13.1 (*R Core Team* 2011).

3 Results

3.1 *In situ* incubation

Gravimetric water contents ranged from 130 to 300% in the Oi/Oe horizons. In Oa horizons, water contents varied between 77 and 150%, in beech A between 26 and 34% and in spruce EA between 37 and 58% (data not shown). Soil temperatures dropped from +9 to <0°C in the beech site and from +6 to <0°C in the spruce site. After soil frost in February 2012, approximately 3.5 weeks in beech, and 5.5 weeks in spruce, temperatures raised in March to +4 - +8°C in the beech soil and to +1 - +6°C in the spruce soil (Fig. 1).

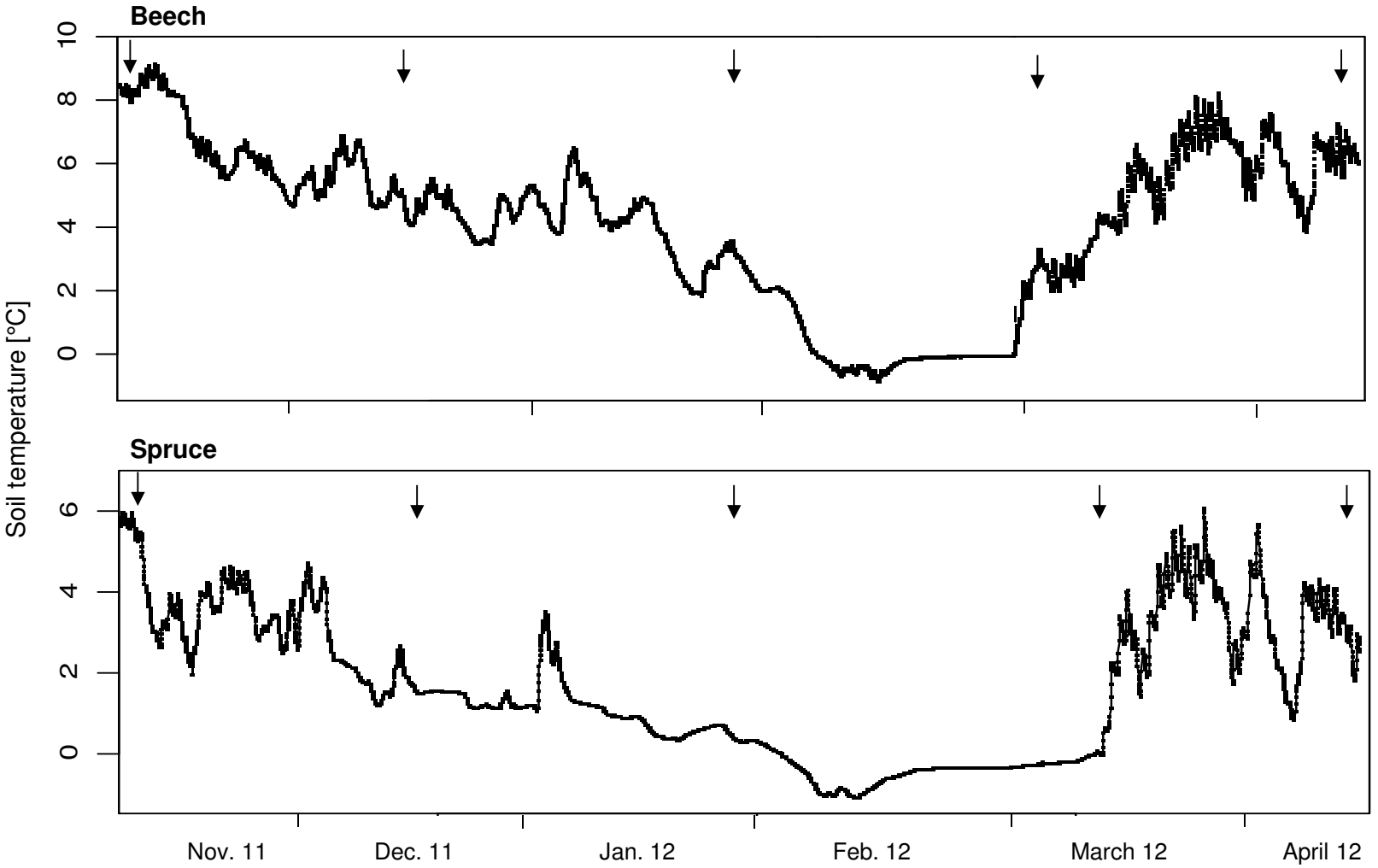

Figure 1 Soil temperatures at the beech and spruce site at 5 cm depth. Arrows indicate the sampling dates.

Net ammonification in Oi/Oe horizons was highest in late autumn and lowest after soil frost in March (Fig. 2a,c). Net ammonification was between 7.6 and 10.6 kg N ha^{-1} 6 $weeks^{-1}$ in beech Oi/Oe, and between 2.4 and -1.6 kg N ha^{-1} 6 $weeks^{-1}$ in spruce Oi/Oe (Fig. 2a,c). Net ammonification was similar in beech Oa and A, and decreased steadily from approx. 2.0 in November to 0.4 (Oa) and -0.6 (A) kg N ha^{-1} 6 $weeks^{-1}$ in April. Net nitrification in the beech soil was very low and similar among horizons, a maximum amount of 0.5 kg N ha^{-1} 6 $weeks^{-1}$ was measured in April for Oi/Oe (Fig. 2b). Net nitrification in the spruce soil differed between horizons, with Oi/Oe exceeding Oa and EA horizons in December and January (Fig. 2d), and ranged between -0.3 and 1 kg N ha^{-1} 6 $weeks^{-1}$.

In total, 44.3 kg N ha^{-1} 6 $months^{-1}$ were mineralized in the beech soil and 10.9 kg N ha^{-1} 6 $months^{-1}$ in the spruce soil. The Oi/Oe, Oa and A in the beech soil contributed 37, 4.1 and 3.2 kg N ha^{-1} 6 $months^{-1}$ and 4.1, 4.3 and 2.5 kg N ha^{-1} 161 d^{-1} in the spruce soil, respectively. Net ammonification was always significantly higher in beech Oi/Oe compared to spruce Oi/Oe, except during the last incubation period in April 2012. In the spruce soil, net nitrification amounted to 54% of total N net mineralization (5.9 kg N ha^{-1} 6 $months^{-1}$) whereas in the beech soil, net nitrification amounted to 3% (1.5 kg N ha^{-1} 6 $months^{-1}$) of total N net mineralization (Fig. 2b,d).

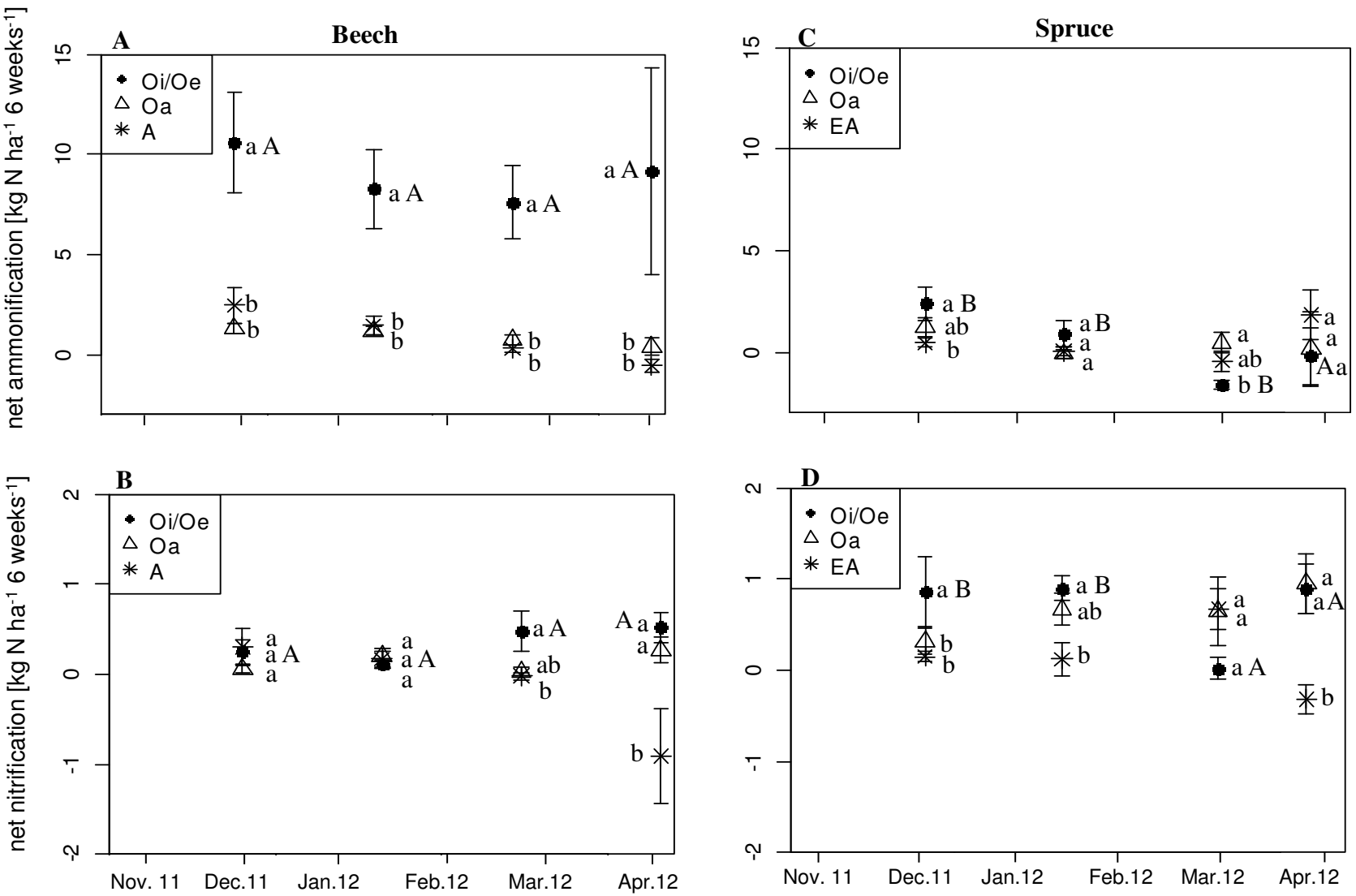
A
Beech
C
Spruce
B
D
Oi/Oe
Oa
A
EA
net ammonification [kg N ha-1 6 weeks-1]
net nitrification [kg N ha-1 6 weeks-1]
Nov. 11
Dec.11
Jan.12
Feb.12
Mar.12
Apr.12

Figure 2 *In situ* net ammonification and net nitrification rates in the dormant season. Error bars represent standard error of the mean. Different letters indicate significant differences between soil horizons at α=0.05. Different capital letters indicate differences between beech and spruce.

3.2 Comparison of laboratory and *in situ* incubation

In the beech soil, N net mineralization generally increased with temperature, though the increase was more pronounced for the laboratory incubations (Fig. 3a,b). In beech Oi/Oe, *in situ* rates exceeded laboratory rates by 8.8 kg N ha^{-1} 6 $weeks^{-1}$ at +0.3°C. Around 6°C, laboratory rates exceeded *in situ* rates on average by 2.4 kg N ha^{-1} 6 $weeks^{-1}$. Only at +3.9°C both rates were similar (Fig. 3a). In beech A, laboratory rates exceeded *in situ* rates on average by 4.2 kg N ha^{-1} 6 $weeks^{-1}$ over the whole temperature range from +0.6 to +6.4°C (Fig. 3b).

In spruce Oi/Oe, the increase of N net mineralization with temperature was clearly observed only for *in situ* rates in the range of 0 to +3°C (Fig. 3c). Rates from laboratory incubations were lower than *in situ* rates by 1.8 kg N ha^{-1} 6 $weeks^{-1}$ at +1°C and higher by 3.7 kg N ha^{-1} 6 $weeks^{-1}$ at +3.7°C and generally showed a more pronounced increase with temperature than the *in situ* rates (Fig. 3c). No N net mineralization was found in the laboratory incubation for spruce Oa, and *in situ* rates were also very low (around 1 kg N ha^{-1} 6 $weeks^{-1}$) (Fig. 3d).

Apparent Q_{10} values of N net mineralization calculated from the laboratory incubations were 10.8 for beech Oi/Oe, 7.0 for beech A and 25.4 for spruce Oi/Oe.

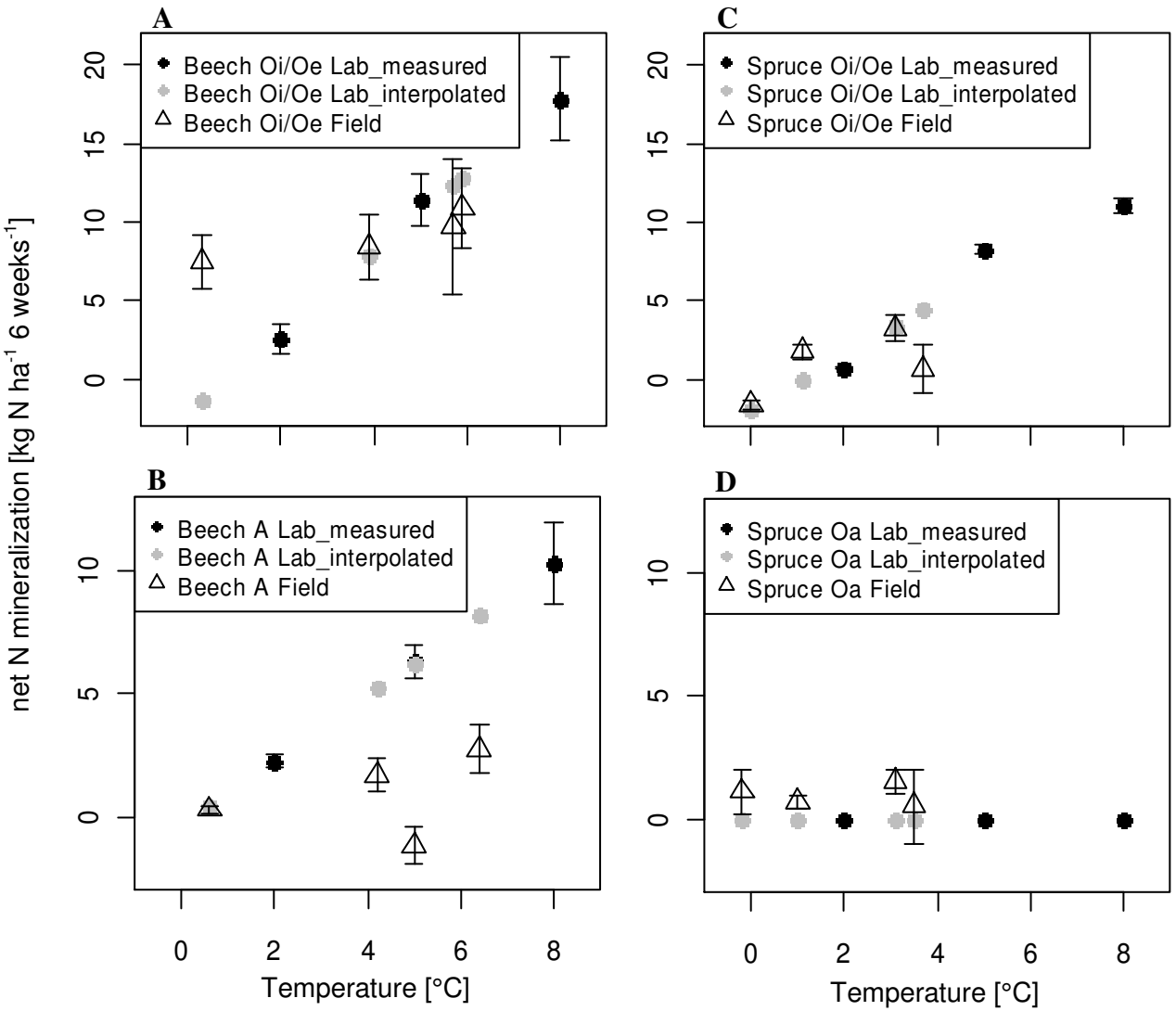

Figure 3 Net N mineralization derived from *in situ* and laboratory incubations as dependent on soil temperature. Error bars represent standard errors. For the field data: average soil temperatures for the sampling periods.

4 Discussion

4.1 *N net mineralization in the dormant season*

Our results confirm that considerable N net mineralization takes place during the dormant season in both temperate forest soils. This is especially true in case of broadleaf sites. The rates of N net mineralization in the dormant season under beech exceeded those under spruce by about 4 times. The rates measured under beech (44.3 kg N ha^{-1} 166 d^{-1}, of which 37 kg N ha^{-1} 166 d^{-1} were mineralized in the Oi/Oe horizon) correspond well to rates measured in other deciduous temperate forests in the dormant season (*Groffman* et al., 2001). The leaf fall in autumn in our beech site amounts to about 68 kg N ha^{-1} (*Berg* and *Gerstberger*, 2004). Hence, the N mineralization in the beech soil during the dormant season accounts for 65% of the annual litterfall, while this relation is only 26% for the spruce soil. To emphasize the importance of the dormant season we can compare N net mineralization rates in summer months. *Chang* and *Matzner* (2000) reported in our beech soil a *in situ* N net mineralization of 44 kg N ha^{-1} 3 $months^{-1}$ during summer 1997, which is equal to the rates in the dormant season. However, in their study net nitrification was higher by one order of magnitude, giving evidence that nitrifiers at this site were constrained at low temperatures which was also supported by findings from *Park* et al. (2002). In our spruce soil, *Hentschel* et al. (2009) determined N net mineralization by *in situ* incubations of 21 kg N ha^{-1} 60 d^{-1} from mid of May to mid of July 2006 at soil temperatures between 7 and 11°C. Assuming this rate for the total growing season and including the dormant season, the annual N net mineralization in the spruce site would be around 74 kg N ha^{-1}. Thus, N net mineralization during the dormant season in the spruce soil contributes only about 15% to the annual mineralization.

Higher N net mineralization rates under deciduous than under coniferous trees are in line with results from other studies (*Kanerva* and *Smolander*, 2007; *Booth* et al., 2005; *Mueller* et al., 2012) and are generally attributed to a higher inherent litter quality in deciduous forests (*Reich* et al., 2005). Furthermore, in deciduous forests the leaf fall in autumn prior to the dormant season, providing easily decomposable substrates, seems responsible for the huge rates of net mineralization in the dormant season. This conclusion is confirmed by the depth gradients of mineralization. Differences between the two trees species in our study resulted mainly from the mineralization in the Oi/Oe horizons. The beech Oi/Oe is less acidic compared to spruce Oi/Oe which may enhance N mineralization under beech *Högberg* et al. 2007). Lastly, the beech soil was on average 2°C warmer during the dormant season which also partly explains the differences between beech and spruce.

We attribute the higher net nitrification in the spruce soil to two reasons. Firstly, evidence exists for heterotrophic nitrification by forming NO_3^- directly from N_{org} compounds in coniferous soils. A shift from autotrophic nitrification towards heterotrophic nitrification occurs when substrate quality is decreasing (*Trap* et al., 2009). Heterotrophic nitrifiers are faster growing than autotrophic bacteria and less susceptible to frost damage (*De Boer* and *Kowalchuk*, 2001; *Neilsen* et al., 2001). By contrast, slow growth and small activity of autotrophic nitrifiers in fresh fallen beech litter, where 84% of total N mineralization took place, prevented rapid nitrification. Secondly, microbial immobilization of NO_3^- is positively correlated to availability and quality of C compounds (*Tahovská* et al. 2013). Although we have no direct measurements of C availability, we assume that immobilization of NO_3^- was less in the spruce than in the beech soil.

4.2 Temperature dependency of N net mineralization

The temperature sensitivity of N net mineralization determined in the laboratory incubation was generally high with apparent Q_{10} values between 7 and 25 in both soils. However, as these Q_{10} values were calculated by a linear function between +2°C to +12°C, they are very sensitive towards the initial value at +2°C. The low initial value in the case of spruce Oi/Oe creates a large Q_{10} with a high degree of uncertainty. Generally, the temperature dependency of N net mineralization was larger in the laboratory samples than in the *in situ* incubation for all soil horizons. Homogenization of the laboratory samples of beech A, as well as cutting leaves from beech Oi/Oe, might have increased substrate availability. Substrate availability increases temperature sensitivity of mineralization (*von Lützow* and *Kögel-Knabner*, 2009; *Hamdi* et al., 2013; *Davidson* et al., 2012). Other reasons for the less pronounced temperature dependency of the *in situ* rates could be attributed to temperature fluctuations that occur in the field. We related the *in situ* rates to average monthly soil temperatures and thereby ignoring the influence of temperature variations at time scales of days to weeks.

In summary, our results indicate that in temperate forest soils N is mineralized at considerable rates during the dormant season, which is of outmost relevance for deciduous forests. In our study, rates under beech exceeded those under spruce 4-fold. The temperature dependency of N net mineralization was more pronounced in laboratory incubations at constant temperature and with homogenized samples than in the *in situ* incubations. Q_{10} values of N net mineralization at low temperatures were very high, suggesting a huge effect of increasing winter temperatures in the future on the N cycle in temperate forest soils.

Acknowledgements

This research was financially supported by the *Deutsche Forschungsgemeinschaft* (DFG). We thank Helga Hertel-Kolb, Petra Eckert, Alexander Guhr and Uwe Hell for their help during field and laboratory works. We are grateful to the Central Analytic Department of Bayreuth Center of Ecology and Environmental Research (BayCEER) for the chemical analysis of samples and to Prof. T. Foken (Department of Micrometeorology, University of Bayreuth) for providing soil temperature data of the Coulissenhieb II site.

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Study 3

Effects of soil frost and subsequent drying on C mineralization in temperate forest soils

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In preparation.

Abstract

In temperate forests, winterly soil frost is often followed by a spring or summer drought. Soil freezing may severely impact the microbial community. Here, we tested the hypothesis, that severe soil frost reduces C mineralization during a subsequent desiccation period.

In laboratory incubations, homogenized soil samples of organic and upper mineral horizons from a temperate European beech and a Norway spruce stand were exposed to intensive frost of -8°C from day 0-20. Control samples were incubated at +2°C at field capacity. The previously frozen and control samples were warmed up stepwise to 20°C from day 21-90. Samples were dried out to pF 4-4.7 from day 139-161. C mineralization was monitored twice a week. Microbial biomass was determined after the warming phase at day 90 and at the end of the experiment at day 161.

C mineralization in previously frozen soils increased within 1-7 days after thawing and continued at rates comparable to those of the unfrozen samples. Microbial biomass and C mineralization was equal between frozen and control samples after 90 days. On day 161, cumulative C mineralization was significantly lower in previously frozen, moist samples of the spruce Oi/Oe, Oa and EA and beech A horizon, and microbial biomass was likewise lower (except in spruce Oi/Oe) by 17-42%. During desiccation, C mineralization decreased in all horizons by 7-47% but no effect of previous freezing was observed.

Our results suggest that microbial biomass and C mineralization recover quickly from freezing in temperate soils. Freeze-thaw cycles likely have no effects on C mineralization during a subsequent moderate desiccation phase, but frost effects may occur belated (after 90 days), leading to a reduction of CO_2 emissions in previously frozen soils when soils are kept at optimal moisture conditions.

1. Introduction

In addition to an increase in the mean annual temperature, the IPCC (2013) predicts a changing frequency in the occurrence of meteorological extreme events. Consequently, the likelihood of a severe spring or summer drought following winterly soil frost is enhanced in temperate forests. Soil freezing and drought are two common ecosystem stressors that impact soil microbial physiology and community composition, and through this may alter ecosystems C and N fluxes (Schimel et al., 2007).

Numerous freeze-thaw studies in a broad range of ecosystems addressed the ability of microorganisms to survive soil frost. Short-lived pulses in gaseous losses of N_2O and CO_2, were often reported during thawing and more in arable than in alpine and tundra soils (Herrmann and Witter, 2002; Feng et al., 2007; Matzner and Borken, 2008). In many studies, the CO_2 flush after thawing was reported to be due to decomposition of microbial necromass (Herrmann and Witter, 2002; Dörsch et al., 2004; Koponen and Martikainen, 2004). For temperate and Northern hardwood forest soils it was often reported that, after a short thawing pulse, the CO_2 production of frozen soils equilibrated within several days (usually 2-14 days) and was then in the same order as unfrozen control soil (Goldberg et al., 2008; Feng et al., 2007; Pesaro et al., 2003). However, effects of soil frost on microbial biomass may differ from those on soil respiration. Pesaro et al. (2003) reported that freezing an agricultural soil for four days at -20°C decreased soil DNA contents and direct cell counts by 24% and 22% compared to unfrozen controls (values remained low over 40 days), whereas the degradation of a crop protection product was not affected in the same study. Schmitt et al. (2008) reported PLFA concentrations to decrease with increasing soil frost intensity (-3, -8, -13°C) in a Norway spruce mineral soil.

Decreasing water potentials also impose physiological stress on microbes. In order to prevent cell dehydration and dying, microbes need to accumulate osmolytes, mostly in form of simple organic acids, amino acids (bacteria) or polyols (fungi) (Mihoub et al., 2003; Tibett et al., 2002). Drought stress decreases C mineralization and may also induce changes in the microbial community composition (Fierer and Schimel, 2002; Schimel et al., 2007). E.g., in a drought-sensitive oak forest soil that was exposed to multiple drying-rewetting cycles, Fierer and Schimel (2002) reported substantially lower respiration rates in the frequently stressed soil compared to the control soil six weeks after the treatment. This was attributed to a shift in the bacterial community composition (Fierer et al., 2003).

Muhr et al. (2009) studied the response of soil respiration to the combined stress of frost and drought in a forest field study. The severe soil frost in this experiment was followed by a

pronounced summer drought. Soil frost significantly lowered C mineralization by 1100 kg C ha^{-1} a^{-1}. 14% of this difference was ascribed to a reduction in soil respiration during the frost phase, whereas 63% was ascribed to the dry summer 2006. The authors reported a considerable reduction in the heterotrophic soil respiration in the soil frost plots whereas autotrophic respiration was not affected. They suggested that low soil water contents from June to October 2006 may have inhibited the recovery of the fungal biomass from frost, causing an enhanced sensitivity of heterotrophic respiration to summer drought.

Here, we conducted an incubation experiment with soils from two temperate forests over 161 days where a severe soil frost was followed by a desiccation phase. We tested the hypothesis that soil frost reduces C mineralization during a subsequent desiccation period.

2. Materials and methods

2.1 Study sites

Soil samples were taken from the site "Steinkreuz" in the Steigerwald Nature Park, Germany (49°52´N, 10°27´E), dominated by European beech (*Fagus sylvatica* L.), hereinafter referred to as beech site. Furthermore, samples were taken from the site "Coulissenhieb II" in the Fichtelgebirge, Germany (58°08´N, 11°52´E), dominated by Norway spruce (*Picea abies* L.) and in the following referred to as spruce site. Mean annual precipitation and air temperature is 750 mm and 7.5°C at the beech and 1160 mm and 5.3°C at the spruce site (Gerstberger et al., 2004). In the beech site, Dystric Cambisol prevail, with a shallow moder type forest floor. In the spruce site, the dominating soils are Haplic Podzols with a well stratified, mor-like forest floor of 6-10 cm thickness (classification according to FAO (IUSS, 2007)). C stocks of the upper soil horizons were larger under spruce than under beech, however, stocks of N were similar between both sites (Table 1[1]). The soil was less acidic under beech than under spruce. Further soil characteristics are available in Table 1. A detailed description of the sites is given in Gerstberger et al. (2004).

2.2 Soil sampling and experimental design

Soil samples were taken in January 2012. At both sites an area (10 m^2) without ground vegetation was selected for sampling and mixed samples (4-6 kg fresh weight) were taken from the Oi/Oe, Oa and (E)A horizons. Before homogenizing the samples by hand, roots, twigs, branches and stones were removed and leaves were cut by hand into pieces of <1 cm^2.

[1] see study 1 Table 1

Samples were moistened to field capacity and stored at 5°C for 3 weeks prior to the incubation. Gravimetric water contents were determined by drying at 60°C for organic layers and 105°C for the mineral horizon. Gravimetric moisture contents were 387% in beech Oi/Oe and 339% in spruce Oi/Oe horizon, 78% in beech A horizon, 132% in spruce Oa and 48% in spruce EA horizon.

For the incubation, 20 replicates per horizon (each 10-35 g dry weight (DW), in total 100 samples) were weighted into 830 ml glass jars and slightly compacted. A schematic overview of the experiment is given in Fig. 1. For the soil frost phase 10 replicates per horizon were incubated at -8°C for 20 days (hereinafter referred to as -8°C samples) and 10 replicates per horizon were kept at 2°C (hereinafter referred to as +2°C samples). After the frost period, the samples were thawed and warmed stepwise: At day 21, the -8°C samples were placed in the +2°C freezer. At day 33, all samples were placed in the +5°C freezer, at day 46 all samples in the +10°C freezer, at day 62 all samples in the +15°C freezer and at day 74 all samples in the +20°C climate chamber, where they remained until the end of the experiment. Temperatures in the different freezers were adjusted with a precision of ±0.3°C.

From day 139 on, 5 replicates per horizon of the -8°C and +2°C samples were stepwise desiccated. From day 139 to 143 the samples were kept moist, at day 144 samples were desiccated to pF 3 and at day 153 to pF 4.0-4.7. The remaining 5 replicates per horizon and per treatment remained constantly moist. In total, four different treatments per horizon were established: a) +2°C wet, b) -8°C wet, c) +2°C dry, d) -8°C dry.

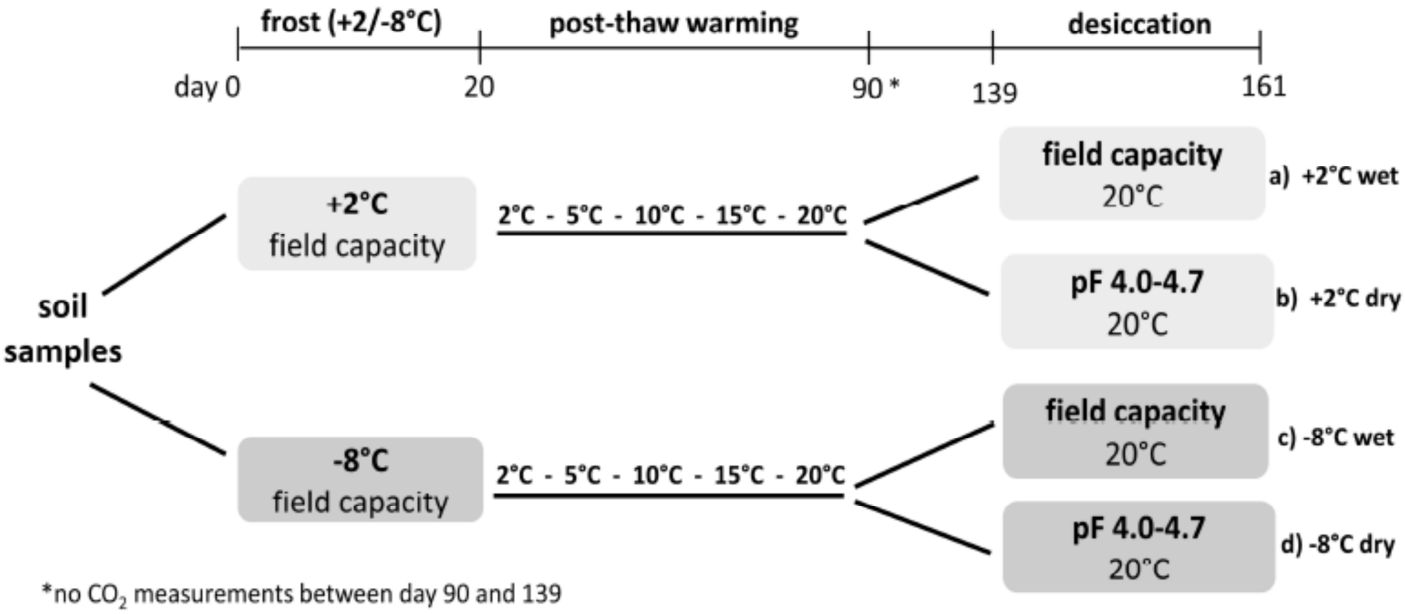

Fig. 1 Schematic overview of the experimental design.

In order to achieve a homogeneous desiccation, the glass jars were placed open into a ventilated drying cabinet at ambient air temperature (around 20°C). pF values during the desiccation procedure were monitored regularly with a potentiometer (WP4-T, UMS, Munich, Germany). pF values of the samples that were kept permanently moist were measured with a micro tensiometer (INFIELD7, UMS, Munich, Germany). pF values of the four treatments at the end of the incubation period are given in Table 2.

Table 2 pF values at day 161 for wet and dry samples used for measuring C mineralization (mean of 5 replicates ±SD).

Treatment	+2°C, wet	-8°C, wet	+2°C, dry	-8°C, dry
Beech Oi/Oe	1.41 (±0.03)	1.41 (±0.02)	4.35 (±0.07)	4.20 (±0.15)
Beech A	1.75 (±0.03)	1.63 (±0.06)	4.08 (±0.09)	3.90 (±0.06)
Spruce Oi/Oe	1.50 (±0.02)	1.53 (±0.02)	4.63 (±0.24)	4.03 (±0.21)
Spruce Oa	1.73 (±0.02)	1.60 (±0.06)	4.64 (±0.16)	4.69 (±0.10)
Spruce EA	1.67 (±0.12)	1.61 (±0.05)	4.73 (±0.20)	4.64 (±0.16)

2.3 C mineralization

Measurements of CO_2 production were conducted two to three times per week over the whole incubation period, except from day 90 to 139. Gas samples of 30 µl were withdrawn from the headspace of the jars through a septum by a 50 µl syringe and injected into a gas chromatograph (SRI 8610C, SRI Instruments Europe GmbH, Germany). CO_2 concentration was measured as CH_4 with a GC equipped with a flame ionization detector. The respiration rates were calculated from the linear increase in CO_2 in the glass jars over the measurement interval. CO_2 standards of 380, 600, 1000, 3000 and 10000 ppm were used to generate a linear calibration function. The jars were kept closed during the incubation and only flushed with synthetic air (80% N_2, 20% O_2) when headspace CO_2 concentration exceeded 3000 ppm. When no CO_2 production was measured from day 90 to 139 the jars were closed by flexible film (Parafilm M, Alcan Packaging, USA) to allow a gas exchange but prevent desiccation. Cumulative C mineralization was calculated on a g C kg^{-1} DM basis for the frost and warming phase (day 0-90) and for the desiccation phase (day 139-161).

2.4 Microbial biomass

Microbial biomass was measured by the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978) at day 90 after the thawing and warming phase and at the end of the experiment at day 161 with 3 replicates per treatment and horizon. Glass jars for the SIR measurements were treated in the same way as those for C mineralization.

2.5 Statistics

We used one-way and two-way analysis of variances for testing statistical significance between treatments. The Shapiro-Wilk test was used to test data distribution. All analyses were done with R 2.13.1 (R Development Core Team 2011).

3. Results

3.1 C mineralization and microbial biomass in post-thaw warming phase (day 21-90)

C mineralization during the warming phase from day 21 to day 90 (to day 85 in spruce EA) increased with time and temperature in both previously frozen and control samples (Fig. 2a-b, 3a-c). C mineralization was higher in beech Oi/Oe compared to spruce Oi/Oe and decreased in both sites with soil depth**.** C mineralization of previously frozen samples increased within 1-7 days after thawing and continued at rates comparable to those of unfrozen soils (Fig. 2a-b, 3a-c). In both Oi/Oe horizons, the space between both curves (Fig. 2a, 3a) emanated from the frost period and persisted in spruce Oi/Oe until day 90, where cumulative C mineralization amounted to 14.7 and 12.9 g C kg^{-1} DM 90 d^{-1} in +2°C and -8°C samples (Fig. 3a). In beech Oi/Oe, lower C mineralization of -8°C during the frost phase was compensated at day 84 (parallel curves until day 84) and on day 90, cumulative C mineralization of both -8 and -2°C samples of beech Oi/Oe was almost similar (ca. 40 g C kg^{-1} DM 90 d^{-1}). A faster compensation of less C mineralization during the soil frost occurred in Oa and (E)A horizons. Already 5 (spruce EA) to 13 days (spruce Oa, beech A) after thawing, cumulative C mineralization was equal between +2°C and -8°C samples (Fig. 2b, 3b-c). In spruce EA, reduced C mineralization during the frost phase was even overcompensated, in total, 131 mg C kg^{-1} DM 85 d^{-1} were emitted from the -8°C samples and 113 mg C kg^{-1} DM 85 d^{-1} were emitted from +2°C samples (Fig. 3c).

After 90 days, microbial biomass was highest in beech Oi/Oe (around 63 mg C_{mic} g^{-1} DM), lower in spruce Oi/Oe (15 mg C_{mic} g^{-1} DM) and smallest in spruce EA (around 0.9 mg C_{mic} g^{-1} DM) (Fig. 4) and was not significantly different between previously frozen and control samples in any horizon.

3.2 C mineralization and microbial biomass during the desiccation phase (day 139-161)

3.2.1 Permanently moist samples

Between day 139-161, C mineralization was significantly lower in the -8°C samples compared to +2°C samples in all horizons except beech Oi/Oe (Fig. 2c-d, 3d-f). On day 161, cumulative C mineralization of -8°C samples reached only 78, 83, 70 and 80% of the CO_2 that was emitted from the +2°C samples in beech A, spruce Oi/Oe, Oa and EA (Fig. 2d, 3d-f).

Microbial biomass of the Oi/Oe horizons decreased significantly between day 90 and day 161 to around 10 and 5 mg C_{mic} g^{-1} DM in beech Oi/Oe and spruce Oi/Oe whereas in Oa and mineral horizons, microbial biomass remained fairly constant over the experiment (Fig. 4). Microbial biomass was not significantly different between previously frozen and unfrozen samples on day 161 in any horizon, however microbial biomass was lower in -8°C compared to +2°C samples in spruce Oa, EA and beech A by 40, 17 and 42% (Fig. 4).

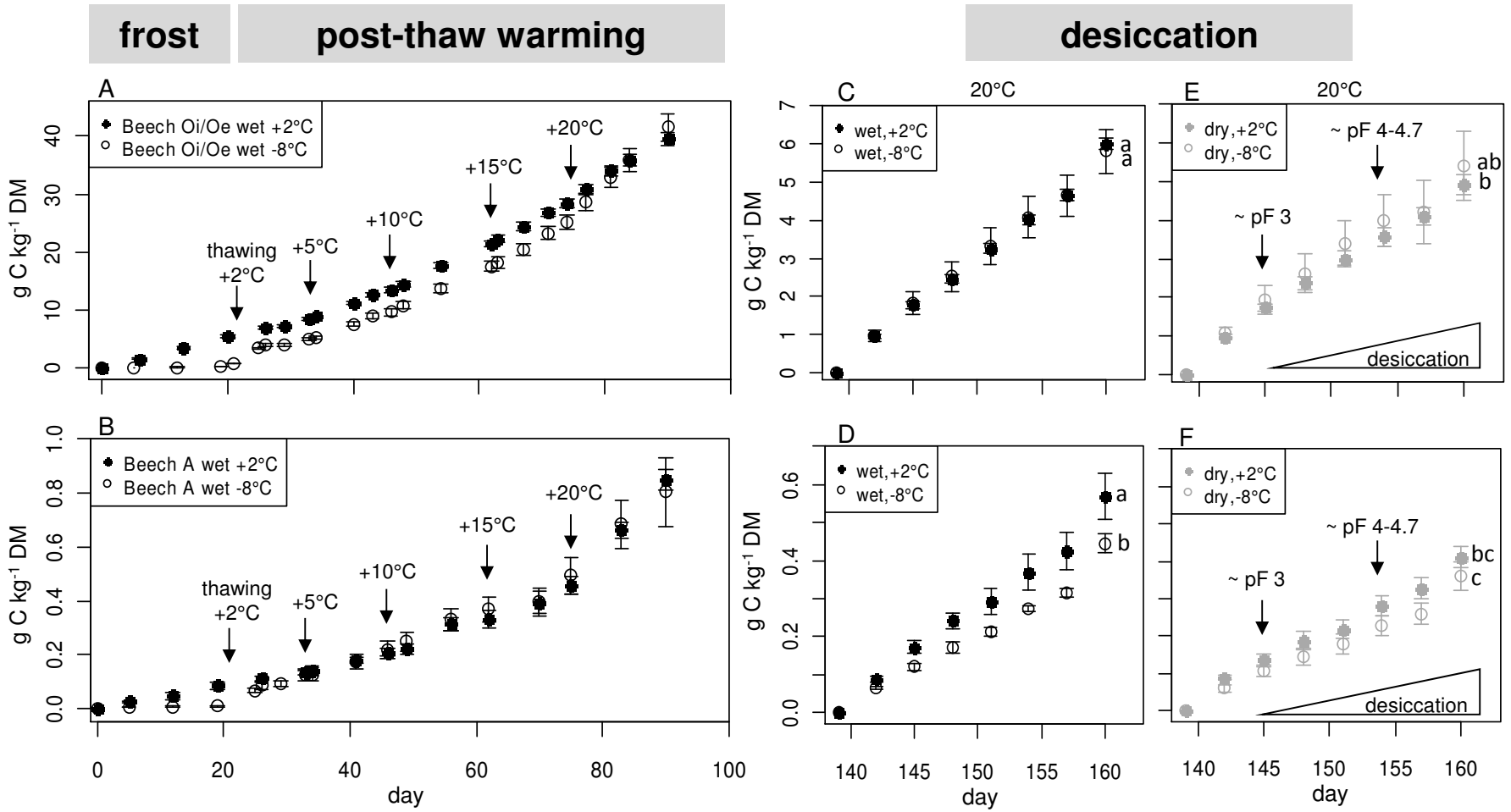

Fig. 2 Cumulative C mineralization (g C kg^{-1} DM) in the beech soil during the frost and post-thaw warming phase (a,b), and desiccation phase (c-f). Error bars represent SD and different letters indicate significant differences at α=0.05.

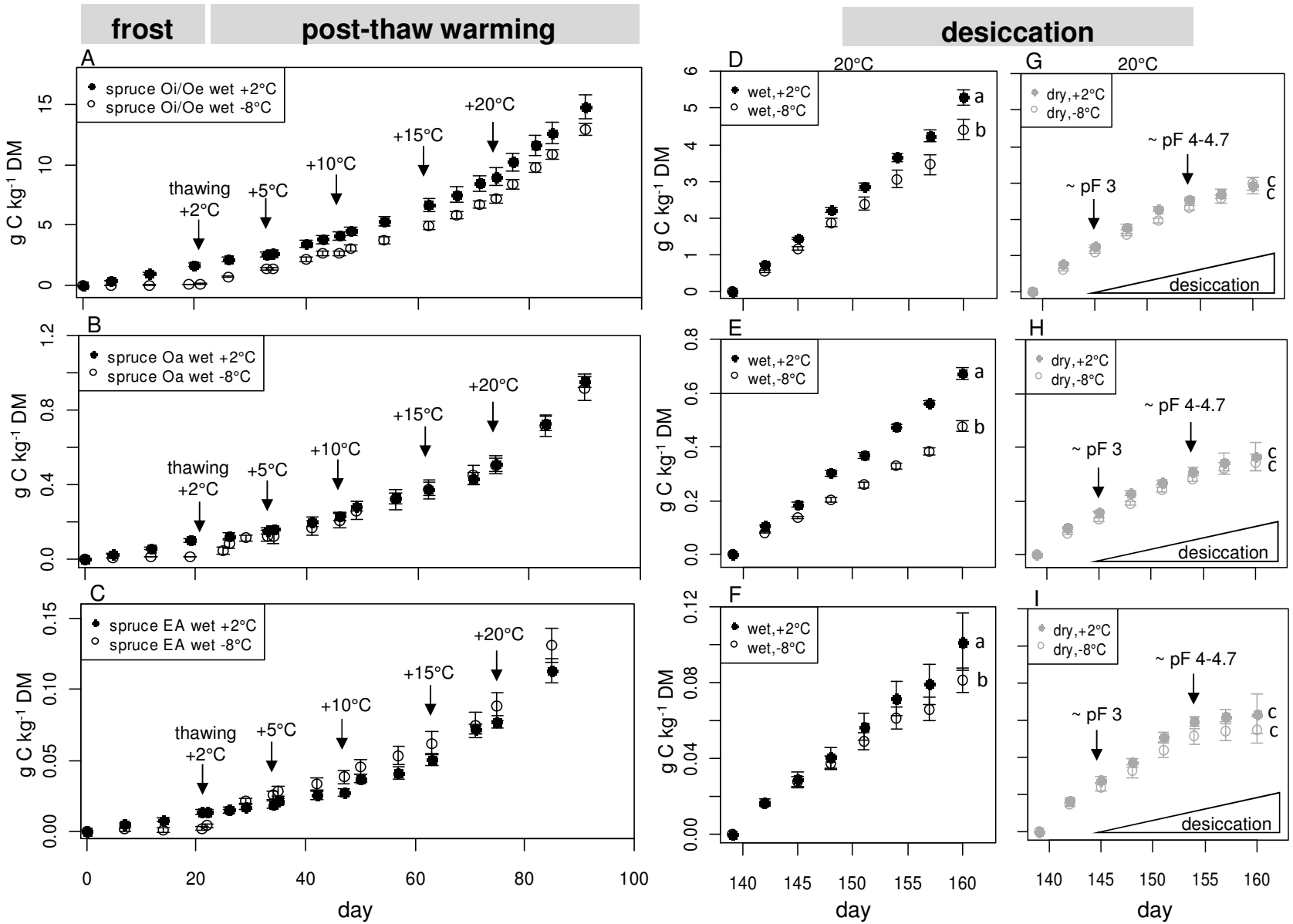
frost
post-thaw warming
desiccation
A
B
C
D
E
F
G
H
I
spruce Oi/Oe wet +2°C
spruce Oi/Oe wet -8°C
spruce Oa wet +2°C
spruce Oa wet -8°C
spruce EA wet +2°C
spruce EA wet -8°C
wet,+2°C
wet,-8°C
dry,+2°C
dry,-8°C
thawing +2°C
+5°C
+10°C
+15°C
+20°C
20°C
~ pF 3
~ pF 4-4.7
desiccation
a
b
c
g C kg-1 DM
day

Fig. 3 Cumulative C mineralization (g C kg^{-1} DM) in the spruce soil during the frost- and post-thaw warming phase (a-c), and desiccation phase (d-i). Error bars represent SD and different letters indicate significant differences at α=0.05.

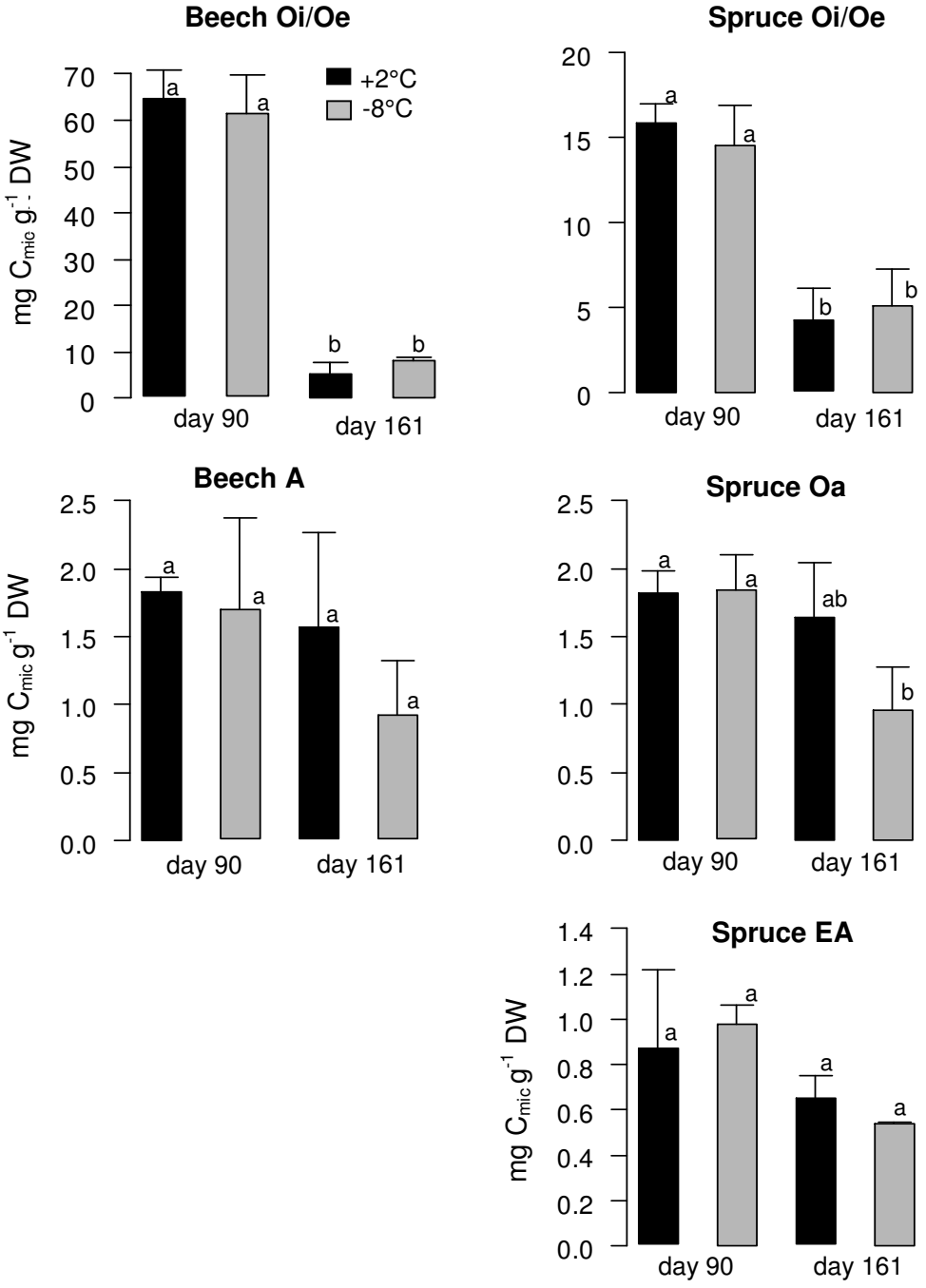

Fig. 4 Soil microbial biomass (mg C_{mic} g^{-1} DW) in permanently moist beech and spruce samples. Error bars represent SD and different letters indicate significant differences at α=0.05.

3.2.2 Desiccated samples

Desiccation decreased C mineralization in +2°C and -8°C samples of the spruce soil and beech A by 19-47% (Fig. 2f, 3g-i) whereas C mineralization in beech Oi/Oe was barely affected by drying (Fig. 2e). On day 161, differences in cumulative C mineralization between desiccated -8°C and +2°C samples were not significant in any horizon.

4. Discussion

4.1 C mineralization and microbial biomass in the post-thaw warming phase (day 21-90)

C mineralization during the frost phase was very low in frozen samples and reached only 5% of the cumulative C mineralization of the unfrozen samples after 20 days in the Oi/Oe horizons. This is in line with findings from Coxson and Parkinson (1987), Brooks et al. (1997) and Clein and Schimel (1995) who reported the lower temperature limit for CO_2 production at a temperature of -5 to -6 °C in cool-temperate forest and alpine tundra soils.

CO_2 production increased quickly after thawing and equilibrated to the level of unfrozen controls. This thawing pulse - usually occurring within a time scale of 5-10 days - has been frequently reported and is ascribed to the decomposition of necromass (Schimel and Clein, 1996; Goldberg et al., 2008; Koponen and Martikainen, 2004). In a mesocosm experiment with organic horizons (Oi+Oe+Oa) from the same spruce site, Goldberg et al. (2008) reported increasing CO_2 emissions during thawing that equalized to emissions of unfrozen controls approx. 12 days after thawing, which is well in line with our findings. Also in a Swiss agricultural soil that was frozen for four days at -20°C, Pesaro et al. (2003) reported the substrate-induced respiration to increase shortly after thawing and returning back to control levels within 10 days. Similar microbial biomass, determined as SIR, on day 90 in our experiment indicates that the microbial population and likewise their respiratory activity completely recovered from the soil frost. However, the SIR method detects only the active, glucose- and O_2-consuming fraction of the microbial community (Pesaro et al., 2003). Direct cell counts or DNA contents (being not restrictive to the active fraction) were significantly reduced by freezing an agricultural soil for four days at -20°C and stayed low for over 42 days (Pesaro et al., 2003).

Generally, the microbial biomass in our study was comparable to microbial biomass determined in boreal aspen forest and boreal Norway spruce stands by the chloroform-fumigation extraction or SIR method (Mariani et al., 2006; Smolander et al., 1994). According to Mazur (1980), a temperature of less than -15°C is necessary to significantly disrupt the microbial biomass pool. In line with findings from Goldberg et al. (2008),

Stenberg et al. (1998), Koponen et al. (2006) and Lipson et al. (2000) who conducted freeze-thaw studies in temperate, boreal and tundra soils, we suggest that in temperate forests, soil frost has only minor impacts on the microbial community as it is - especially in the upper horizons - well adapted to freezing stress.

Temperatures of -8°C represent an exceptional soil frost for the Oa and (E)A horizons in our sites. Thus, the observed rapid compensation of low soil respiration during frost (and even overcompensation in spruce EA) during the warming phase might be due to the additional release of previously occluded substrates due to physical disruption (Kurganova et al., 2007; Matzner and Borken, 2008). On the contrary, the compensation of lower soil respiration during the frost phase in Oi/Oe horizons took longer (84 days in beech Oi/Oe) or did not occur (spruce Oi/Oe). We suggest that these responses might be due to the horizon-specific microbial biomass and activity (Schimel and Clein, 1996). The fungal-to-bacterial-biomass ratio is higher in less decomposed organic matter (thus Oi/Oe horizons) compared to more decomposed organic matter and the hyphal length is known to decrease significantly with soil depth (Berg et al., 1998). The fungal mycelia is highly sensitive to physical disturbance during frost and evidence exists that it is more negatively affected than bacteria by soil frost (Feng et al., 2007; Schimel et al., 2007; Muhr et al., 2009). Thus, bacteria that likely prevailed in lower soil horizons recovered faster from the soil frost and could faster compensate C losses during freezing.

4.2 C mineralization and microbial biomass during the desiccation phase (day 139-161)

4.2.1 Permanently moist samples

Despite C mineralization and microbial biomass had apparently recovered from soil frost until day 90, lower C mineralization rates in previously frozen samples from day 139-161 indicated a potential `lag` effect of soil frost, when samples were kept permanently moist. This is surprising and we suggest that a fraction of the active microbial biomass was in the long-term impaired by the soil frost that was not visible until day 90. Unfortunately, CO_2 measurements were not conducted between day 90 and 139, so we missed the exact day where C mineralization rates started to differ between -8°C and +2°C samples and furthermore, we did no qualitative assessment of the microbial community and cannot tell *if any/which* microbial group is in the long-term impaired. At least in beech A, spruce Oa and EA the lower cumulative C mineralization on day 161 can be explained by a generally lower microbial biomass (Fig. 4). Reasons for this late change in C mineralization remain speculative. In a metagenomic assay, Mackelprang et al. (2011) revealed rapid shifts in many phylogenetic,

microbial and functional gene abundances when a permafrost soil was at the transition from a frozen to a thawed state. Many of these rapidly shifting genes were involved in the cycling of soil organic C and N.

Late changes in the microbial biomass or activity in long-term incubation experiments have been reported by several authors and are generally ascribed to a decreasing availability of easily decomposable substrates (Hart et al., 1994; Steinweg et al., 2008). During an incubation experiment over 456 d at 25°C with an old-growth coniferous forest, Hart et al. (1994) reported the microbial growth efficiency and biomass C and N pools to decline exponentially over time. Exposing a boreal riparian surface soil to multiple freeze-thaw cycles at -6 and -12°C, Haei et al. (2011) reported the duration (2-6 months) of a multiple freeze-thaw experiment to be negatively correlated with fungal and bacterial PLFAs. In the same experiment, freeze-thaw events increased the fungal-to-bacterial growth ratio compared to a constant 0°C control sample, giving evidence that soil frost affects microbial groups differently. Furthermore, in a freeze-thaw experiment over 220 days the content of plant and microbial sugars decreased significantly in a Norway spruce soil (Oa, EA and B horizon) that was subjected to frost compared to control samples at +5°C (Schmitt et al., 2008). As no concomitant increase in CO_2 emission was reported in this experiment (Goldberg et al., 2008), Schmitt et al. (2008) suggested an alteration of sugar molecules leading to SOM stabilization in the frozen samples. It remains very speculative, but in the later stage of our experiment a reduced abundance of sugars could have also provoked a reduced respiration in the previously frozen samples.

4.2.2 Desiccated samples

Desiccation to a pF of 4-4.7 reduced C mineralization in all horizons except beech Oi/Oe, which is in line with findings from e.g. Beare et al. (2009), Guo et al. (2012) and Muhr et al. (2010). However, desiccation did not lead to a stronger reduction of C mineralization in -8°C samples and we could not affirm our hypothesis, that soil frost reduces C mineralization in the subsequent desiccation phase. We assume that desiccation affected solely the active microbial biomass and superimposed the belated frost stress (if it was also present in these samples). Thus, the microbial groups with high respiration activity in previously frozen and unfrozen samples were weakened in an equal measure by desiccation.

Deviances of our results from the findings of Muhr et al. (2009) could be due to the conditions implemented in our lab experiment. In the field experiment, a matric potential of –40 kPa was measured in 20 cm soil depth (pF ~ 2.6) and the Oa horizons dried out to a pF of

~5 (Muhr et al., 2009; Muhr and Borken, 2009) during the subsequent summer. We assume that desiccation in the laboratory study was not intensive and not long enough. Due to the lack of substrate inputs by plant roots and other (a)biotic effects that persist *in situ* we assume that the microbial community in our laboratory incubation differed largely from the persisting microbial community in the field study and thus both studies were hardly comparable.

Generally, a cumulative CO_2 production of 3.3 g C kg^{-1} DM 22 d^{-1} measured in the spruce Oi/Oe+Oa horizons in our experiment at pF 4.6 was well in line with a cumulative CO_2 production determined after a drying period of 80 days (pF 3.8) at 15°C in a mesocosm experiment with organic horizons from the same spruce site (Muhr et al., 2010). In the same experiment, Muhr et al. (2010) reported a cumulative C mineralization of 5.4 g C kg^{-1} SOC after a 80-day desiccation period in samples that were dried to a pF of 6.6, giving evidence that C mineralization continues even at extreme low water contents. This was supported by findings from Chen et al. (2011) for gross ammonification. Extensive summer droughts frequently occur in our sites and especially in the beech Oi/Oe horizon, this might be an explanation for the minor response of C mineralization to desiccation.

Our results suggest that microbial biomass and C mineralization quickly recover from soil frost in temperate forests and all frost-related effects disappear until day 90. Freeze-thaw cycles have no effects on C mineralization during a subsequent moderate desiccation phase, but - under optimal soil moisture conditions - frost-related effects may occur belated (after day 90), impacting certain microbial groups and leading to a reduction of CO_2 emissions in previously frozen soils.

Acknowledgements

This research was financially supported by the *Deutsche Forschungsgemeinschaft* (DFG). We thank Helga Hertel-Kolb and Uwe Hell for their help during field and laboratory works.

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Appendix

Own contributions of the candidate

Study 1: **Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures.**

Marianne Schütt, Werner Borken, Oliver Spott, Claus Florian Stange, Egbert Matzner. Published in Soil Biology and Biochemistry (2014) 69, 320-327.

M. Schütt:	65%	laboratory and field works, data analysis and calculation, manuscript preparation
W. Borken	10%	discussion of results, manuscript preparation
O. Spott	5%	discussion of results
C. F. Stange	5%	discussion of results, calculations
E. Matzner	15%	interpretation and discussion of results, manuscript preparation

Study 2: **Substantial net N mineralization during the dormant season in temperate forest soils.**

Marianne Schütt, Werner Borken, Claus Florian Stange, Egbert Matzner. In press in Journal of Plant Nutrition and Soil Science (2014).

M. Schütt:	70%	laboratory and field works, data analysis and calculation, manuscript preparation
W. Borken	5%	discussion of results, manuscript preparation
C. F. Stange	5%	discussion of results, calculations
E. Matzner	20%	interpretation and discussion of results, manuscript preparation

Study 3: **Effects of soil frost and subsequent drying on C mineralization in temperate forest soils.**

Marianne Schütt, Carolin Lotz, Werner Borken, Egbert Matzner. In preparation (2014).

M. Schütt:	55%	concepts, laboratory works, data analysis and calculation, manuscript preparation
C. Lotz	30%	laboratory works
W. Borken	5%	concepts, interpretation and discussion of results
E. Matzner	10%	interpretation and discussion of results, manuscript preparation

Publications

Marianne Schütt, Werner Borken, Oliver Spott, Claus Florian Stange, Egbert Matzner (2014): Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures. Soil Biology and Biochemistry 69, 320-327.

Marianne Schütt, Werner Borken, Claus Florian Stange, Egbert Matzner (2014): Substantial net N mineralization during the dormant season in temperate forest soils. Journal of Plant Nutrition and Soil Science, in press.

Marianne Schütt, Carolin Lotz, Werner Borken, Egbert Matzner (201X): Effects of soil frost and subsequent drying on C mineralization in temperate forest soils. In preparation.

Danksagung

Viele liebe Menschen haben mich während meiner Doktorarbeit hier in Bayreuth unterstützt und ich möchte mich bei allen sehr bedanken. Mein besonderer Dank gilt:

- Prof. Dr. Egbert Matzner für die sehr gute Betreuung meiner Arbeit, die Unterstützung bei Labor- und Feldexperimenten, für eine jederzeit offene Tür, die schnelle und viele Hilfe beim Paper schreiben und vor allem die unermüdliche Geduld, alles immer wieder zu lesen und zu verbessern.

- PD Dr. Werner Borken für die ebenfalls sehr gute Betreuung und für die vielen Ratschläge zu Labor- und Feldexperimenten. Vielen Dank für die Unterstützung beim Paper schreiben, beim Diskutieren der Ergebnisse und besonders für die Hilfe bei der Themenfindung zur Masterarbeit.

- Ingeborg Vogler für die ganze Hilfe bei allem Organisatorischen, besonders bei den Dienstreisen, für das Korrekturlesen, Verbessern und die stets gute Laune!

- Uwe Hell, Helga Hertel-Kolb, Karin Söllner, Andrea Schott und Petra Eckert vom Lehrstuhl für Bodenökologie für die Hilfe bei Feld- und Laborarbeiten und die netten Fahrten zu den Versuchsflächen. Danken möchte ich auch allen weiteren Mitarbeiter/innen und Student/innen am Lehrstuhl für Bodenökologie, die während meiner Zeit da waren, für jederzeit Hilfsbereitschaft und das sehr nette Miteinander! Im besonderen gilt mein Dank Carolin Lotz für ihren unermüdlichen Einsatz während der Masterarbeit und Andreas und Alexander für die Lösung sämtlicher statistischer Probleme in unserer `Selbsthilfegruppe R`.

- der Zentrale Analytik der Universität Bayreuth und Bernd Apelt und Oliver Spott vom UFZ Halle/Leipzig für die Messung meiner Proben. Florian Stange vom BGR Hannover möchte ich danken für die Diskussionen zu meinen Ergebnissen und für die Hilfe bei den Berechnungen.

- ganz besonders danken möchte ich meiner Familie und meinem Freund Philipp für die ganze Unterstützung und Ermutigung, alles anzupacken was ich mir vorgenommen habe, Aufmunterung, Optimismus und immer eine Schulter zum Anlehnen

(Eidesstattliche) Versicherungen und Erklärungen

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

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