Seasonality of soil microbial nitrogen turnover in continental steppe soils of Inner Mongolia

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Abstract. Annual estimates and seasonal patterns of gross nitrogen turnover in terrestrial soils are poorly understood due to the lack of experimental evidence. Based on year round sampling in wintergrazed and ungrazed steppe soils of Inner Mongolia, we show that measurements of net rates of ammonification (−9 to −6 kg N ha−1 year−1) or nitrification (19 to 31 kg N ha−1 year−1) do not at all reflect the pronounced dynamics of gross rates of ammonification (215–240 kg N ha−1 year−1) or nitrification (362–417 kg N ha−1 year−1).

Four different seasons with characteristic functional patterns of N turnover were identified: (1) Growing season dynamics as characterized by drying/rewetting cycles and negatively correlated temporal courses of net microbial growth and periods with intensive gross ammonification, contributing 40–52% and 29–32% to cumulative annual gross ammonification and nitrification, respectively. Net N mineralization was almost exclusively observed during the growing season. (2) Microbial N dynamics during the autumn freeze-thaw period was characterized by a sharp decline in microbial biomass in conjunction with a peak of gross nitrification contributing 19–36% to cumulative annual fluxes. (3) During winter at constantly frozen soil, a net build-up of microbial biomass was observed, whereas gross N turnover rates were low, contributing 7–10% and 6–11% to cumulative annual gross ammonification or gross nitrification, respectively. (4) The spring freeze-thaw period showed extremely dynamic changes in gross N turnover and soil nitrate concentrations. This period contributed 34–44% and 21–46% to cumulative annual gross ammonification and nitrification, respectively. This study highlights that freeze-thaw cycles are key periods for understanding patterns and magnitudes of gross N turnover in semi-arid continental steppe ecosystems. The results further imply that the observed patterns of microbial biomass and gross N turnover dynamics are likely the consequence of a seasonal succession of microbial communities and turnover of microbial biomass. Our findings emphasize the necessity for high resolution studies on gross N turnover as a prerequisite to infer functioning and annual budgets of ecosystem N cycling.

Key words: annual nitrogen budget; drying-rewetting; freeze-thaw; microbial biomass; nitrification; N mineralization; steppe.

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INTRODUCTION

Biogeochemical nitrogen (N) cycling in terrestrial ecosystems is complex, since it comprises many players ranging from microorganisms to higher plants performing and involving a wide range of processes with very different magnitudes of N turnover (Schimel and Bennett 2004, Booth et al. 2005, Kreutzer et al. 2009, Rennenberg et al. 2009, Butterbach-Bahl et al. 2011). The terrestrial N cycle is dominated by the soil microbial N turnover processes of ammonification (the conversion of organic N compounds to ammonium), nitrification (conversion of organic N or ammonium to nitrate), and the subsequent allocation of bioavailable N such as ammonium and nitrate to plants and microorganisms as well as to N loss pathways. Soil nitrogen cycling regulates plant productivity, and ecosystem N retention and loss. Hence, N cycling affects soil acidification, stream water quality, and eutrophication, as well as atmospheric chemistry and radiative forcing (Galloway et al. 2008, Butterbach-Bahl et al. 2011). Consequently, the ecological significance of N turnover processes has been well acknowledged. However, due to its complexity and methodological difficulties in the quantification of actual soil N turnover processes, our understanding of soil N cycling is still fragmentary. The current state of knowledge on N cycling may correspond to the state of knowledge on the carbon (C) cycle several decades ago (Schlesinger 2009).

In the past most studies focused on measurements of net rates of ammonification and nitrification across a wide variety of terrestrial ecosystems. However, since net rates comprise both production and consumption of inorganic N, such studies do not necessarily provide insight into actual gross rates of N turnover. Therefore, they are a poor approximation of the real N status of ecosystems (Davidson et al. 1991, 1992, Schimel and Bennett 2004). Since the determination of gross rates of ammonification and nitrification is based on time- and resource-intensive techniques mostly involving the use of stable 15N isotopes, actual gross rates of N turnover have been determined less often than net rates. Nevertheless, studies on gross N turnover are available for a range of terrestrial ecosystems (Booth et al. 2005), though the temporal resolution of these studies is strongly limited. The few available studies examining the temporal variability of gross N turnover in soils (e.g., Dannenmann et al. 2006, Rosenkranz et al. 2010) show that rates may markedly fluctuate across seasons. Hence, the available studies on gross N turnover in general neither allow for understanding seasonal dynamics or for calculating annual rates of gross N turnover. Actual gross rates of N turnover in soil subjected to winter conditions have only been determined a few times in laboratory studies (Müller et al. 2002, Ludwig et al. 2004, Freppaz et al. 2007), but not in situ. The latter studies determined significant rates of gross ammonification and/or nitrification in soil subjected to freeze-thaw events, though still being restricted to single point in time measurements. So far, no study has investigated gross N turnover in permanently frozen soil. However, based on the investigation of more indirect parameters of N turnover such as soil mineral N concentrations, several studies showed that significant biogeochemical C and N turnover can occur in frozen soils and during freeze/thaw periods (Vogt et al. 1986, Clein and Schimel 1995, Brooks et al. 1999). Soil microbial N turnover may occur in winter under snowpack in the soil, as indicated e.g., by measurements of significant net N turnover rates in continental steppe of China (Zhou et al. 2009, Zhao et al. 2010), boreal forests (Kielland et al. 2006), arctic tundra (Schimel et al. 2004), and temperate hardwood forests (Groffman et al. 2001b). Furthermore, microbial biomass and the activities of several soil enzymes were even found to peak in late winter in alpine soils (Lipson et al. 1999, 2002). Furthermore, significant plant litter decomposition was found in winter in seasonally snow-covered ecosystems (Taylor and Jones 1990, Hobbie and Chapin 1996, Schmidt and Lipson 2004).

Soil freeze-thaw cycles have been reported to kill a significant portion of the soil microbial biomass (Clein and Schimel 1995, Schimel and Clein 1996, DeLuca et al. 2002). The resulting increase of easily degradable N and C substrates in soil during spring snowmelt has been shown to prime fine root turnover, increase net N mineralization (Groffman et al. 2001a, Schmidt et al. 2007, Matzner and Borken 2008), nutrient leaching (Brooks et al. 1999), and N2O emissions.
The knowledge gap on gross N turnover in snow covered and or frozen soils still hampers a functional and quantitative understanding of soil N biogeochemistry at the annual scale. In particular, it remains uncertain whether our understanding of the contribution of the winter season to annual N flux is valid, as estimated from dynamics and magnitude of measurements of net rates of N turnover, enzyme activities and microbial community parameters.

The Inner Mongolia grassland, part of the Eurasian Steppe, is the largest contiguous grassland area in the world (Bai et al. 2004) covering approx. 8% of the Earth’s land surface. Rapid degradation and desertification have taken place in these grasslands, primarily caused by over-grazing that reduced vascular plant cover, accelerated soil loss, and decreased soil nutrient level (Graetz 1994, Kang et al. 2007). Few studies on gross N turnover are available for such ecosystems (Holst et al. 2007, Wu et al. 2011), and data that are available were based on a few measurements made during the growing seasons only and thus cannot provide insight into the temporal dynamics and environmental controls of N turnover at the annual scale. Hence, a functional understanding of the importance of the winter and freeze-thaw periods for annual soil N turnover has not yet been achieved for semi-arid continental steppe.

Sheep grazing could either decrease or increase gross ammonification, nitrification and nitrate concentrations in such soils (Holst et al. 2007, Wu et al. 2011). However, also these grazing studies were based on only one or few measurements during the growing season, and were additionally not supported by plot replication. In the light of the observation, that spring thaw is triggering $N_2O$ pulse emissions in steppe soils, which decrease with increasing grazing intensity (Wolf et al. 2010), it is essential to also understand the full annual course of N turnover and the dynamics of soil mineral N and microbial biomass concentrations as influenced by grazing.

Therefore, we measured gross and net N turnover as well as dynamics of soil inorganic N and microbial biomass concentrations in monthly or bi-weekly temporal resolution over an entire year at replicated plots of either winter grazed or ungrazed steppe. The aim of this study was to characterize and quantify the temporal dynamics, and season-specific patterns, as well as obtaining annual estimates of microbial N turnover over a full year. In particular, we tested the following hypotheses: (1) There is significant gross N turnover in frozen steppe soils, (2) both N turnover during the growing season as well as during freeze-thaw periods are of major importance for the annual budget of gross N fluxes; (3) grazing decreases microbial N turnover due to reduced plant cover and altered soil microclimate. Furthermore, we were interested in elucidating the significance of potential environmental controls (temperature, soil moisture, microbial biomass) on gross N turnover and to investigate whether net rates of N turnover provide insight into the magnitude and dynamics of gross N turnover over an annual cycle.

**Material and Methods**

**Study site description**

Our study was carried out in the Xilin River Basin, Inner Mongolia Autonomous Region, China (43°38’ N, 116°42’ E). The mean annual temperature is 0.3°C with mean monthly temperatures ranging from –21.6°C in January to 19.0°C in July. Mean annual precipitation is 346 mm (1984–2005) with 60–80% falling during the growing season from May to August. Only 10% of annual precipitation occurs during the winter months as snow. The soil is classified as alkaliescent Phaeozem with a loamy sand texture (Steffens et al. 2008). We investigated three ungrazed and three grazed plots (1 ha each) of *Leymus chinensis* grassland. The ungrazed plots (UG) were fenced in 1999 to prevent grazing by large animals. The winter grazed plots (WG) were fenced and grazed in the winter period from November to April by 3–4 sheep units per hectare, representing a heavy grazing intensity. Further site information is given by Wolf et al. (2010).

**Experimental and sampling design**

Soil sampling was conducted at the three ungrazed and grazed plots by use of soil cores of 5 cm diameter and 10 cm depth. At each of the six investigated plots, ten sampling spots were randomly selected over the entire plot every sampling date. For the determination of gross N turnover, enzyme activities and microbial community parameters.
rates of ammonification and nitrification, one soil core was taken per sampling spot, and subsequently the soil of the 10 cores of every plot was bulked for the $^{15}$N experiments, which immediately started after sampling. In order to avoid the necessity for soil storage, separate soil samples were used for determination of net rates of N turnover, soil mineral N concentrations and microbial biomass C and N. Samples for these analyses were taken at the same sampling spots after $^{15}$N experiments were finished, i.e., with a time shift of approximately five days. For this purpose, two paired soil cores were inserted at every sampling spot (i.e., 20 cores at each plot). One of the paired cores was immediately sampled and analyzed for soil mineral N concentrations as well as microbial biomass C and N. The second core was covered with plastic film that allowed gas exchange but prevented water penetration and was analyzed for soil mineral N concentrations after an in situ incubation period of two weeks in order to facilitate the determination of net rates of N turnover. Inorganic N concentrations, net rates of N turnover and soil microbial biomass were analyzed separately in the spatial replicates, i.e., samples were not bulked. All soil samples were immediately processed after sampling in order to avoid storage artifacts. Both paired intact soil cores for net rate assays and homogenized soil samples for the determination of gross N turnover were incubated in situ at the sampling spots. Soil inorganic N concentrations, net rates of N turnover, microbial biomass C and N and gross ammonification were measured 20–21 times at 8–38 day intervals from August 16, 2007 to October 13, 2008. On 15 occasions, gross nitrification was also determined simultaneously to gross ammonification.

Based on analysis of variance of the results of the first four sampling dates, the spatial replicates were reduced from ten to eight (net rates of N turnover, soil mineral N concentrations) and to five (microbial biomass C and N). Overall, net rates of N turnover and soil mineral N concentrations were determined for 952 samples and microbial biomass C and N were determined for 618 samples.

**Gross rates of microbial N turnover**

Gross rates of ammonification and nitrification were determined using an in situ $^{15}$N pool dilution technique described previously by Dannenmann et al. (2006) and Wolf et al. (2010). The experimental procedure was adapted to the logistical and climatic conditions at the remote experimental site. Samples bulked from the 10 cores were sieved (5 mm mesh width) and homogeneously sprayed with $(\text{NH}_4)_2\text{SO}_4$ or KNO$_3$ solution at 30 atom% $^{15}$N enrichment (Dannenmann et al. 2009) on the day of sampling. Label application increased soil water content by 3% and the total NH$_4^+$-N or NO$_3^-$-N content by approximately 1 mg N kg$^{-1}$ sdw, which is generally well below the measured mean pool sizes in unlabelled soil (compare Fig. 1F, G). Six subsamples of 30 g each for every labelling treatment and plot were placed in parafilm-sealed plastic bottles (250 ml volume) and buried in situ close to the sampling location after labelling. Eighteen hours (time 1) and 42 hours after labelling (time 2), half of the bottles, i.e., three bottles per labelling treatment and plot, were excavated and immediately extracted with 60 ml 1 M KCl as described by Dannenmann et al. (2006). Diffusion steps for trapping on acid filter traps and subsequent GC-IRMS analyses for $^{15}$N-enrichment were performed as described earlier (Dannenmann et al. 2009). Total NH$_4^+$ and NO$_3^-$ concentrations in the extracts were determined at Inner Mongolia Grassland Ecosystem Research Station (IMGERS) as described below. For frozen soil conditions, a different $^{15}$N labelling technique was applied (Wolf et al. 2010). Here, the labelling solution was amended with triple hot washed and autoclaved quartz sand at a ratio of 1:2.5, frozen, and subsequently crushed to a fine powder. This $^{15}$N-labelled quartz-sand/ice mixture was homogenously mixed with the still frozen soil for labelling. All experimental steps until the amendment of the KCl solution for soil extraction were performed outdoors at air temperatures below $-10^\circ$C in order to ensure that the investigated soil and the labelling solution/quartz sand mixture remained constantly frozen. Incubations took place in situ in the plots by burying the incubation bottles in winter and by maintaining a representative snow cover, if applicable.

Calculation of rates of gross ammonification and nitrification were based on the equations given by Kirkham and Bartholomew (1954).
did not calculate microbial NH$_4^+$ immobilization rates from $^{15}$N consumption rates (Davidson et al. 1991), since our data indicated the presence of heterotrophic nitrification (direct oxidation of organic N to NO$_3^-$ without dilution of $^{15}$NH$_4^+$). Similarly, NO$_3^-$ immobilization was not considered to equal $^{15}$NO$_3^-$ consumption, since the contribution of denitrification to NO$_3^-$ consumption remained unclear. In this context, the observations of dry soil and low N$_2$O emissions may be insufficient criteria to conclude that dinitrogen emissions and total denitrification are negligible for the NO$_3^-$ mass balance (Dannenmann et al. 2011).

Net rates of N turnover and mineral N concentrations

Net rates of N turnover were determined according to the paired soil core method described by Wang et al. (2006). One of the paired soil cores was immediately harvested, soil was removed from the core and organic debris and rocks were separated from the soil. Representative subsamples were analysed for inorganic N concentrations and microbial biomass C and N (see below). Soil extraction for mineral N concentrations was conducted with 30 g of soil by use of 1 M KCl (soil to solution ratio 1:2) as described in detail by Dannenmann et al. (2006). Concentrations of inorganic N (NH$_4^+$-N and NO$_3^-$-N) in the filtered extracts were conducted at IMGERS using a flow injection autoanalyzer (Zhou et al. 2009; FIAstar 5000 Analyzer, Foss Tecator, Denmark). Soil water content was determined gravimetrically at 105°C for 24 h. After two weeks, the remaining soil cores were harvested, extracted and analyzed for mineral N. The NH$_4^+$-N and NO$_3^-$-N concentrations from the first harvesting date are referred to in situ soil ammonium and nitrate concentrations (Fig. 1F, G). Net rates of ammonification and nitrification (Fig. 1H, I) were calculated from the difference between the two sampling times (Wang et al. 2006).

Under frozen soil conditions, the use of the polyvinyl chloride plastic (PVC) soil cores was not possible. Here, an in situ incubation buried bag technique (Dannenmann et al. 2006) was used for the determination of net N turnover instead of the soil core technique. For this purpose, paired intact portions of soil (50 cm$^2$ area, 10 cm depth) were obtained by use of a spate. One half was immediately extracted and analyzed as described above. The second half was packed into polyethylene bags with pinholes (Dannenmann et al. 2006) and reburied for the second extraction two weeks later. If applicable, the incubated soil was covered with snow of a representative height. In winter, soil samples were kept frozen during transport from the field sites to a nearby provisional laboratory until extraction.

Microbial biomass C and N

Microbial biomass C and N was estimated using the chloroform fumigation-extraction (FE) method (Brookes et al. 1985, Vance et al. 1987, Tate et al. 1988) as described in detail by Dannenmann et al. (2006) and Wu et al. (2011). After removal of coarse organic materials and stones, samples were divided into paired subsamples of 30 g each. One subsample was immediately extracted with 60 ml 0.5 M K$_2$SO$_4$ while the second subsample was fumigated under chloroform vapour for 24 h in a desiccator. Subsequently, ten vacuum/release purge cycles ensured the complete removal of chloroform, and fumigated subsamples were extracted as described above. Extracts were filtered using a 0.45 µm syringe filter (Schleicher and Schuell, Dassel, Germany) and immediately frozen until analysis for total organic carbon (TOC) and total chemically bound nitrogen (TNb) using a TOC analyzer with a coupled TNb module (Dimatec Analysentechnik GmbH, Essen, Germany). Total carbon (TC) and total inorganic carbon (TIC) were determined based on non-dispersive infrared photometrical detection of evolving CO$_2$ after thermic and chemical oxidation of the samples. TOC was calculated as TC – TIC. TNb was analyzed by use of a chemoluminescence detector. Correction factors (0.54 for microbial biomass N and 0.379 for microbial biomass C; Brookes et al. 1985, Vance et al. 1987) were applied to the difference in total extractable N and TOC between paired untreated and fumigated subsamples to estimate microbial biomass C and N.

Meteorological data

Soil temperature at 0.05 m depth was recorded continuously in the plots with PT100 thermometers (Th2-h, UMS GmbH, Muenchen, Germany)
at one minute intervals. Soil moisture was recorded with the same frequency using three FD probes (ECH2O-5, Decagon Devices, Pullman, WA, USA) per site. The FD sensors were installed in a way that they integrated soil moisture over a depth of 0–0.05 m. During wintertime when soil temperatures dropped below 0°C, soil samples from 0–0.05 m soil depth were taken by means of 100 ml core cutters at least twice a week. The samples were dried in the oven at a temperature of 105°C for 24 hours in order to determine volumetric water content. Precipitation data was provided by the IMGERS station in daily resolution.

Statistics and calculations
In general, the plot mean values were used as the statistical unit in this study (n = 3 for grazed and n = 3 for ungrazed). The Wilcoxon test was applied to test for significant differences between WG and UG at a given sampling date. For the calculation of cumulative annual N turnover on an area basis, gross and net N turnover was calculated on a daily basis by linear interpolation considering the bulk density of the uppermost 10 cm of soil. Thus, the given cumulative curves of N turnover are representative for the uppermost 10 cm of the soil.

Pearson’s correlation coefficients were calculated to illustrate dependency between N fluxes and potential controls. This was done (1) using data from specific seasons only (growing season, freeze-thaw periods, winter), and (2) using the whole annual dataset. When several potential environmental controls correlated significantly with N turnover rates at the annual scale, multiple linear regression models with a forward stepwise procedure were used to identify dominant environmental controls of N turnover processes. The threshold value for significant correlations or differences was set at $P < 0.05$. All statistical analyses were performed with SPSS 10.0 (SPSS, Chicago, USA).

RESULTS
Meteorological data
The start of our study in August 2007 was characterized by a sharp decrease in soil moisture from 25 vol% to values well below 10% (Fig. 1A). After a few weeks of such low soil moisture values, precipitation events during three consecutive days in mid September increased soil moisture again to almost 30 vol%, followed again by a rapid drying out of soil in the last week of September. Following these two drying/rewetting cycles, soil temperatures declined slowly towards 0°C. The first freeze-thaw event in topsoil took place in the first week of October (Fig. 1A). It needs to be noted, that the first night time freeze events in the uppermost cm of the topsoil occurred at temperatures well above 0°C as given in Fig. 1, since the presented temperature is daily mean temperature at 5 cm soil depth. From mid November 2007 to the beginning of March 2008 the soil down to at least 15 cm was permanently frozen.

The spring-thaw period with frequent freeze-thaw events lasted from the beginning of March until the beginning of May. The beginning of this period is clearly marked by a sharp increase in soil moisture, which was more pronounced in UG as compared to WG plots. Following these spring freeze-thaw cycles, the growing season of the year 2008 was characterized by pronounced soil drying-rewetting cycles as driven by episodic strong convective rainfall events (Fig. 1A).

Temporal dynamics of gross and N turnover and microbial biomass
Soil microbial biomass N as well as N turnover were characterized by pronounced temporal dynamics between and within seasons over the investigated time span of 14 months (Fig. 1).

During the growing season, microbial biomass oscillated along with drying-rewetting cycles (range approx. 20 to 100 mg N kg$^{-1}$ sdw; Fig. 1D). After the first topsoil freeze events in autumn, soil microbial biomass declined dramatically by more than 80% (Fig. 1D), while at the same time the microbial C:N ratio increased by approximately a factor of two (Fig. 1E). After an initial strong increase in winter, microbial biomass N declined in the beginning of January 2008 in conjunction with an intensification of soil frost, i.e., a drop in soil temperatures to approx. $-10^\circ$C at the ungrazed plots and to approx. $-15^\circ$C at the wintergrazed plots (Fig. 1A, D). Subsequently, the sudden increase in soil moisture due to spring thaw events coincided with increases in soil microbial biomass N.

Gross ammonification (Fig. 1B) during the
Fig. 1. Dynamics of gross rates of N turnover, net rates of N turnover, extractable soil inorganic N concentrations, soil microbial biomass N, microbial C:N ratio and meteorological data over the investigation period (August 2007 to October 2008).
growing season was characterized by huge variability following drying-rewetting cycles. However, it oscillated in opposite directions compared to soil moisture and microbial biomass, i.e., soil drying and microbial biomass decline were accompanied by the highest rates of gross ammonification (approximately 3 mg N kg⁻¹ sdw d⁻¹). In the autumn freeze-thaw period, a moderate increase of gross ammonification at low levels occurred (turnover rates of less than 0.5 mg N kg⁻¹ sdw day⁻¹; Fig. 1B). In winter, gross ammonification was either not significantly different from zero or slightly but significantly positive throughout the soil frost period. A pronounced temporal dynamic was not evident (Fig. 1B). In contrast, high rates of ammonification were observed in the spring freeze-thaw period (Fig. 1B).

Compared to gross ammonification, smaller temporal variation was observed for gross nitrification (rates between 0 and 1.5 mg N kg⁻¹ sdw d⁻¹) during the growing season (Fig. 1C). Due to the lower temporal resolution of measurements of gross nitrification, clear evidence for effects of summer drying-rewetting events on gross nitrification could not be established (Fig. 1B). However, gross nitrification dramatically increased to values of approximately 2.2–4 mg N kg⁻¹ sdw day⁻¹ after the first topsoil freeze events in autumn (Fig. 1C). Similar to gross ammonification, very small or non-significant rates of gross nitrification were observed in winter, followed by a strong increase of gross nitrification in the spring freeze-thaw period.

Net ammonification was close to zero over the whole year, but became significantly negative in summer 2008 (Fig. 1H). This did not affect extractable soil ammonium concentrations which showed little temporal variation over the whole year (Fig. 1F). However, peaks of soil nitrate concentrations were observed in the spring freeze-thaw period, whereas smaller soil nitrate levels were observed during the growing season (Fig. 1G).

Several peaks of net nitrification were observed during the growing season (Fig. 1I). However, despite pulses of gross nitrification and soil nitrate concentrations in the spring freeze-thaw period, a corresponding dynamic of net nitrification in that period was not observed (Fig. 1I).

**Cumulative annual N turnover and contribution of seasons**

To obtain cumulative rates of N turnover, linear interpolation between measuring points was used. These calculations illustrated the importance of both autumn and spring freeze-thaw periods for annual gross N turnover (Fig. 2). Neither magnitude nor dynamics of cumulative net N turnover were related to cumulative gross N turnover. For the period from October 1, 2007 to September 30, 2008, annual gross ammonification for the uppermost 10 cm of soil was 240 and 215 kg N ha⁻¹ year⁻¹, while annual gross nitrification was 417 and 362 kg N ha⁻¹ year⁻¹ for UG and WG, respectively (Table 1). In contrast, annual net ammonification was −9 and −6 kg N ha⁻¹ year⁻¹, while annual net nitrification was 31 and 19 kg N ha⁻¹ year⁻¹ at UG and WG, respectively (Table 1). The autumn freeze-thaw period (46 days) contributed 6% and 7% to annual sums of gross ammonification, but 19% and 36% to annual sums of gross nitrification at UG and WG, respectively (Table 1). Approximately one third (WG: 34%) to one half (UG: 44%) of annual gross ammonification was observed during the spring freeze-thaw period (79 days). Also with regard to gross nitrification the spring thaw period was of key importance: 46% and 21% of annual gross nitrification at UG and WG, respectively, was observed during this period of time. Both freeze-thaw periods contributed 50% and 41% to annual cumulative gross ammonification and 65% and 57% to annual cumulative gross nitrification. In contrast, the growing season contributed 40% and 52% to annual cumulative gross ammonification, 29% and 32% to annual cumulative gross nitrification, while the winter period was of minor importance for both gross rates of N turnover (Table 1). In contrast, different patterns were observed for seasonal contribution to annual net N turnover. Almost all net nitrification occurred during the growing season (Table 1). Net ammonification was negative at the annual scale and the calculated annual value was mainly influenced by net ammonium consumption during the growing season (Table 1).

**Season-specific environmental controls on gross N turnover**

The role of soil drying-rewetting cycles during
Fig. 2. Cumulative gross N turnover and cumulative net N turnover during the investigation period (August 2007 to October 2008).
Table 1. Contributions of seasons to annual gross and net N turnover (in kg N ha\(^{-1}\)y\(^{-1}\)) in winter-grazed (WG) and ungrazed (UG) sites.

<table>
<thead>
<tr>
<th>Season</th>
<th>Date</th>
<th>Gross ammonification Mean ± SE</th>
<th>Gross ammonification %</th>
<th>Gross nitrification Mean ± SE</th>
<th>Gross nitrification %</th>
<th>Net ammonification Mean ± SE</th>
<th>Net ammonification %</th>
<th>Net nitrification Mean ± SE</th>
<th>Net nitrification %</th>
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<tr>
<td>Autumn freeze-thaw</td>
<td>01.10.2007–</td>
<td>14 ± 4</td>
<td>7</td>
<td>130 ± 11</td>
<td>36</td>
<td>0.20 ± 0.34</td>
<td>3</td>
<td>1.53 ± 0.45</td>
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<td>Spring freeze-thaw</td>
<td>18.02.2007–</td>
<td>74 ± 7</td>
<td>34</td>
<td>77 ± 20</td>
<td>21</td>
<td>0.02 ± 0.59</td>
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<td>−6.92 ± 2.26</td>
<td>117</td>
<td>19.18 ± 5.18</td>
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<td>Growing season</td>
<td>08.05.2008–</td>
<td>112 ± 17</td>
<td>52</td>
<td>117 ± 18</td>
<td>32</td>
<td>−8.62 ± 2.26</td>
<td>111</td>
<td>19.18 ± 5.18</td>
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<td>Winter</td>
<td>16.11.2007–</td>
<td>16 ± 6</td>
<td>7</td>
<td>40 ± 109</td>
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<td>Whole year</td>
<td>01.10.2007–</td>
<td>215 ± 35</td>
<td>67</td>
<td>362 ± 158</td>
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<tr>
<td>Autumn freeze-thaw</td>
<td>01.10.2007–</td>
<td>15 ± 3</td>
<td>6</td>
<td>79 ± 36</td>
<td>19</td>
<td>−0.92 ± 0.19</td>
<td>10</td>
<td>0.67 ± 0.31</td>
<td>2</td>
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<tr>
<td></td>
<td>15.11.2007</td>
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<tr>
<td>Spring freeze-thaw</td>
<td>18.02.2007–</td>
<td>107 ± 5</td>
<td>44</td>
<td>191 ± 63</td>
<td>46</td>
<td>−0.56 ± 0.34</td>
<td>6</td>
<td>4.36 ± 0.54</td>
<td>14</td>
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<td></td>
<td>07.05.2008</td>
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<td>Growing season</td>
<td>08.05.2008–</td>
<td>95 ± 29</td>
<td>40</td>
<td>122 ± 31</td>
<td>29</td>
<td>−7.48 ± 3.2</td>
<td>84</td>
<td>27.72 ± 3.51</td>
<td>89</td>
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<td>30.09.2008</td>
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<td>Winter</td>
<td>16.11.2007–</td>
<td>23 ± 9</td>
<td>10</td>
<td>25 ± 47</td>
<td>6</td>
<td>0.10 ± 1.9</td>
<td>−1</td>
<td>−1.53 ± 3.11</td>
<td>−5</td>
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<td></td>
<td>17.02.2008</td>
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<td>Whole year</td>
<td>01.10.2007–</td>
<td>240 ± 45</td>
<td>11</td>
<td>417 ± 177</td>
<td>5</td>
<td>−8.86 ± 5.63</td>
<td>31.22</td>
<td>7.47</td>
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<tr>
<td></td>
<td>30.09.2008</td>
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**Notes:** Duration of seasons: autumn freeze-thaw period: 46 days; spring freeze-thaw period: 79 days; growing season: 146 days; winter: 94 days. Percentages are the contributions to annual flux.

The growing season as a driver of microbial biomass dynamics was confirmed by highly significant positive correlations between soil moisture and soil microbial biomass N (\(R = 0.93, P < 0.001\)). Remarkably the oscillations of soil microbial biomass N along drying-re-wetting cycles (range approx. 20 to 100 mg N kg\(^{-1}\)sdw; Fig. 1D) coincided with simultaneous counter rotating cycles of gross rates of ammonification (range from close to zero to >3 mg N kg\(^{-1}\)sdw d\(^{-1}\); Fig. 1B). That is, gross rates of ammonification during the vegetation period were highest following declines in microbial biomass. This inverse relationship was confirmed by a significant negative correlation between gross ammonification and soil microbial biomass N during the growing seasons (Fig. 3).

A similar relationship between microbial biomass dynamics and gross N turnover was evident during the first topsoil freeze events in fall, when microbial biomass declined dramatically, while at the same time the microbial C:N ratio increased by approx. a factor of two (Fig. 1E), followed by a minor increase in gross ammonification but a dramatic increase in gross nitrification (Fig. 1C). For the spring freeze-thaw period only, a positive relationship between soil moisture and gross ammonification, was observed (\(R = 0.778, P = 0.003\)).

**Environmental controls on soil N dynamics over the full annual course**

While within-season dynamics of gross ammonification was partly correlated with other parameters (see above), gross rates of ammonification rates were not correlated with any of the determined potential controls (i.e., soil temperature, soil moisture, microbial biomass C and N as well as microbial C:N ratio) when the full annual dataset was analyzed. In contrast, correlations at the annual scale were found for rates of gross nitrification and net N turnover. Multiple regression analysis indicated a positive relationship between microbial C:N ratio and gross nitrification, while other parameters, though in part positively correlated, were removed from the regression model (Table 2). Both for net nitrification and for net ammonification, soil moisture remained as a single parameter in the multiple regression models (Table 2). While the relationship between soil moisture and net nitrification was positive, it was negative for net ammonification.
cation, or, in other words, there was a positive relationship between net ammonium consumption and soil moisture (Table 2).

Grazing effects on N turnover
Wintergrazing significantly reduced microbial biomass N, soil nitrate concentrations and net nitrification at only a few single sampling dates during the growing season (Fig. 1D). A pronounced grazing-induced reduction in microbial biomass N was found in winter, when soil temperatures in the WG but not the UG plots dropped below −10°C (Fig. 1A, D). However, the most obvious effect was observed in the spring freeze-thaw period, with persistently reduced soil microbial biomass N in WG (Fig. 1D), accompanied by increased microbial C:N ratios (Fig. 1E). At the same time, gross rates of ammonification were significantly larger in WG than in UG plots (Fig. 1B). Gross nitrification was almost twice as large at UG than at WG in the spring freeze-thaw period (Fig. 1C), however, due to large variation between plots, this difference was not significant. As a consequence of different turnover rates in the freeze-thaw period, grazing reduced gross ammonification by 11% and gross nitrification by 13% on an annual basis (Fig. 3).

DISCUSSION
Temporal dynamics of N turnover
With a hitherto not realized temporal resolution of mostly sub-monthly measurements (8–38 day intervals) over a period of 14 months we followed the temporal dynamics of soil gross and net microbial N turnover in typical steppe soils of Inner Mongolia. Our measurements show an

Table 2. Results of multiple stepwise regression analysis using data from the whole investigation period in order to identify major environmental controls of gross and net N turnover. Gross ammonification was not correlated with any of the potential controls at the annual scale. The unit for gravimetric soil moisture was g H₂O g⁻¹ sdw.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Stepwise multiple regression model</th>
<th>R²</th>
<th>corrected R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross nitrification</td>
<td>0.106× microbial C:N ratio − 0.480</td>
<td>0.202</td>
<td>0.17</td>
<td>0.019</td>
</tr>
<tr>
<td>Net nitrification</td>
<td>1.652× gravimetric soil moisture − 0.100</td>
<td>0.351</td>
<td>0.333</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net ammonification</td>
<td>−0.453× gravimetric soil moisture + 0.003</td>
<td>0.356</td>
<td>0.338</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 3. Linear correlation between soil microbial biomass N and the gross rates of ammonification during the growing seasons. Points are means of treatments (wintergrazed, ungrazed) as calculated from three replicate plots. Error bars represent standard errors of the mean.
often assumed but rarely demonstrated high temporal variability of gross inorganic N turnover (Grenon et al. 2004, Dannenmann et al. 2006, Rennenberg et al. 2009) and microbial biomass, driven by periods of environmental stress or transition, such as drying-rewetting cycles or freeze-thaw events.

So far, most studies on gross N turnover in various ecosystems (see summary by Booth et al. 2005) were limited to growing seasons. For example, Holst et al. (2007) determined gross N turnover at adjacent unreplicated steppe sites during the growing seasons of the years 2004–2005 and determined mean gross ammonification rates of 0.6–4.1 mg N kg\(^{-1}\) sdw day\(^{-1}\) and gross nitrification rates of 0.5–3.1 mg N kg\(^{-1}\) sdw day\(^{-1}\), thus being of similar magnitude as determined in this study. Though measurements of Rosenkranz et al. (2010) also showed a high variability of gross inorganic N production between and within all sampling periods from February to November for an N-saturated temperate spruce forest in Southern-Germany, winter measurements of gross N turnover for frozen soil were not reported.

**Contribution of seasons to annual N turnover**

In our study four distinct seasons with characteristic patterns and magnitudes of N turnover could be identified: growing season, autumn freeze-thaw period, winter with permanently frozen soil, and spring freeze-thaw period. Here we demonstrate that not only the growing season, but also autumn and spring freeze-thaw periods were of major importance for gross N turnover. Though gross N turnover in permanently frozen soil was of little importance for the annual budget, significant and persistent activity and even microbial immobilization of inorganic N in frozen soil was found. Microbial immobilization was indicated by frequently significantly positive \(^{15}\)NO\(_3\)\(^{-}\) and \(^{15}\)NH\(_4\)\(^{+}\)-consumption rates (data not shown). Microbial activity in frozen soil is further confirmed by the net growth of microbial biomass over winter, and by net nitrate immobilization (Fig. 1I). To our knowledge, there is no other study investigating gross N turnover in frozen soil, but Zhang et al. (2011) reported significant net nitrification or net nitrate immobilization under frozen soil conditions for steppe sites of Duolun County, Inner Mongolia, in a
range as observed in this study.

Since the very minor net immobilization of inorganic N during winter (<0.03 mg N kg⁻¹ sdw d⁻¹) cannot explain the net increase in microbial biomass (up to 40 mg N kg⁻¹ sdw) (Fig. 1) and—if at all—only little gross inorganic N production was observed, we hypothesize that during conditions of frozen soils microbial growth was fuelled by the metabolism of monomeric organic N compounds. This view is supported by several studies detecting significant turnover of monomeric organic N compounds such as amino acids in seasonally frozen continental soils (Lipson and Monson 1998, Kielland et al. 2007, Näsholm et al. 2009).

A few studies have investigated gross N turnover rates in thawing soils, e.g., Müller et al. (2002) for grassland soil, Ludwig et al. (2004) for agricultural Luvisol, and Freppaz et al. (2007) for alpine forest soils. These studies—though restricted to single point in time measurements only—showed that significant gross N turnover may occur in freeze-thaw periods. Comparable results were also obtained in this study. With the onset of soil thawing a vigorous revival of gross N turnover was observed driving pulse emissions of N₂O, with N₂O emissions during the spring thaw period even dominating the annual N₂O budget (Wolf et al. 2010).

The observed variability of gross N turnover as well as the importance of the freeze-thaw periods for annual N turnover questions the low-temporal resolution approach of most previous studies on gross N turnover. It can be doubted if low-temporal resolution studies may provide a sufficient insight into functioning and magnitude of ecosystem N cycling. Though determination of gross N turnover remains time- and resource-intensive, we suggest that a concentration of scientific resources to determine gross N turnover with a reasonable temporal resolution of monthly values at a reduced number of sites may be more beneficial for a better understanding of magnitudes and controls of ecosystem soil N cycling than conducting abundant low temporal resolution studies.

**Environmental controls of gross N turnover**

It was very obvious that soil moisture exerted a dominant influence on soil microbial biomass N dynamics (Fig. 4). In close relationship with the seasonality of rainfall, which peaks in the vegetation period from May-August, we also observed high values for microbial biomass. Similarly, Liu et al. (2010) have shown for another semi-arid steppe in Duolun County, Inner Mongolia, that water availability and plant productivity regulate the interannual variability of soil microbial N and C pools. The few outliers observed for our relationship between soil moisture and microbial biomass at high soil moisture contents (see Fig. 4) were determined in soil samples taken during the first soil thaw event after the winter in the ungrazed plots. Hence, these outliers may arise from a retarded microbial growth at suddenly extremely high soil water content. Pronounced changes in environmental conditions such as freeze-thaw in autumn and spring, as well as drying events during the growing season triggered a rapid decline in microbial biomass, but stimulated gross N turnover. Hence, gross rates of N turnover were not related to soil moisture or temperature, but gross N turnover seems to depend on the availability of C and N substrates, most of which may originate from microbial residues. Significant changes in microbial C:N ratios in such periods of environmental stress or transition (Fig. 1E) indicate microbial succession of functional microbial groups well adapted to the new environmental conditions (Schmidt et al. 2007). For the autumn freeze-thaw period we calculated mean residence times of microbial N (estimated by microbial biomass N divided by the dominating process of gross N turnover) of 3–6 days only while it was 47–63 days on an annual scale. This confirms that such transition periods are characterized by a very active microbial community and that successions of microbial communities may be fuelled by feeding on the residues of the preceding microbial community. High microbial immobilization in periods of environmental transition was also indicated by the observation that the accompanying pulses of gross N turnover were usually not associated with increased net inorganic N production, also during absence of competing vegetation in freeze-thaw periods. The dynamic of gross N turnover and microbial biomass observed here supports a recent hypothesis about biogeochemical consequences of rapid microbial succession in soils, i.e., that turnover and succession of
microbial biomass are the major source of bioavailable N in soil (Schmidt et al. 2007). Similar to recent observations in a temperate beech forest in Austria (Kaiser et al. 2011), the observed net build-up of microbial biomass in winter steppe soils may represent a large pool of bio-available nitrogen for the consecutive growing season, thus improving ecosystem productivity.

Both the observed absence of effects of soil moisture on gross N turnover and the negative relationships between microbial biomass and gross N turnover over most periods of the year are contradictory to findings in other ecosystems. Gross nitrification in a temperate beech forest was found to be positively related to soil microbial biomass and to soil moisture content up to an optimum soil water content of approximately 60% of the water holding capacity (Dannenmann et al. 2006). Furthermore, a laboratory parameterization of gross ammonification in temperate hardwood forest soil revealed a significantly positive effect of soil moisture (Rennenberg et al. 2009). Positive relationships between soil moisture and gross ammonification as well as gross nitrification were also reported for the uppermost mineral soil of a nitrogen saturated temperate spruce forest of Southern Germany (Rosenkranz et al. 2010). These contrasting findings may imply that the temporally explicit mechanistic understanding of gross N turnover as developed in this study for semi-arid continental steppe with their extremely pronounced dynamics in environmental conditions is not directly transferable to temperate forests or other ecosystems.

**Gross nitrification exceeded gross ammonification during freeze-thaw periods and at the annual scale**

During freeze-thaw periods gross nitrification was considerably higher than gross ammonification (Fig. 1B, C). For several adjacent sites, Holst et al. (2007) also reported that monthly mean gross nitrification in August was several fold larger than the corresponding gross ammonification. However, since a depletion of the soil ammonium pool was not observed, nitrification must have largely been based on the direct oxidation of organic N (heterotrophic nitrification) and not on the oxidation of free soil ammonium (autotrophic nitrification). This interpretation explains why the $^{15}$N excess was only diluted in the nitrate pool but not in the ammonium pool (Huygens et al. 2008). Heterotrophic nitrification is likely to be performed by fungi characterized by higher microbial C:N ratios than bacteria (Landi et al. 1993, Paul and Clark 1996), which coincides with our finding of high microbial C:N ratios during key periods of gross nitrification (Table 2). Heterotrophic nitrification has been reported to dominate over autotrophic nitrification in several soils ranging from various forest ecosystems (Burton et al. 2007, Grenon et al. 2004, Pedersen et al. 1999, Koyama et al. 2010) to grassland (Cookson et al. 2006, Müller et al. 2002, 2009, Huygens et al. 2008, Rütting et al. 2008). Nonetheless, the actual contribution of heterotrophic nitrification to total gross nitrification remains unclear in our as well as in most other studies, unless $^{15}$NO$_3$ pool dilution studies combined with selective inhibitors of autotrophic or heterotrophic nitrification (Barraclough and Puri 1995) are performed.

**Grazing effects on N turnover**

Negative effects of winter grazing on N turnover and microbial biomass were only significant in the spring freeze-thaw period. Comparable results were also found by Wolf et al. (2010), who showed that at grazed sites spring-thaw N$_2$O pulse emissions were significantly lower or even missing. These authors showed that grazing-induced reductions in vegetation height, surface roughness length and snow capture resulted in a reduction of the insulation of the soil and thus lower winter soil temperatures (Fig. 1A, D). Furthermore, reduced snow cover reduces wintertime water retention, and thus strongly decreases soil moisture in the spring freeze-thaw period. The effects of grazing on both soil temperatures and soil moisture explain grazing-induced reduction of microbial biomass N, gross ammonification as observed in this study (Fig. 1B, D), and the strongly reduced N$_2$O emissions in the spring freeze-thaw period as described by Wolf et al. (2010). Here, we also found that gross nitrification was smaller at grazed plots in the spring freeze-thaw period by a factor of two, but these differences remained insignificant due to large variation between the three investigated plots (Fig. 1C). Since winter
grazing completely eliminated spring thaw N$_2$O pulse emissions (Wolf et al. 2010), but insignificantly reduced gross nitrification (Fig. 1C), this points to the fact that denitrification was the major source of freeze-thaw pulse emissions of N$_2$O in the investigated steppe soils.

Do net rates provide insight into ecosystem N dynamics and N status of semi-arid steppe?

The parallel full annual courses of gross and net N turnover represent a unique prerequisite to judge to what extent net rates of N turnover, which are much easier to determine, allow for insight into actual status and dynamics of N turnover. Growing season net N mineralization (calculated as the sum of net ammonification and net nitrification) of this study (year 2008) was 20.2 and 12.2 kg N ha$^{-1}$ year$^{-1}$ for UG and WG, respectively. These values are in the range of the values reported by Zhou et al. (2009) for comparable ungrazed steppe sites. These authors reported net N mineralization in the uppermost 10 cm of 13.2 kg N ha$^{-1}$ year$^{-1}$ for the growing season of 2007 and 30.7 kg N ha$^{-1}$ year$^{-1}$ for the growing season of 2006. In our study, net N mineralization was approximately one order of magnitude smaller than gross N turnover (Table 2). More surprisingly, the dynamics of net N turnover were not related to gross N turnover (Figs. 1–3, Table 2) either. In particular, pulses of gross N turnover during the freeze-thaw periods were not accompanied by a concomitant increase in net N turnover (Fig. 1). Thus, N$_2$O peak emissions during spring freeze-thaw (Wolf et al. 2010) were not indicated by net rates of N turnover. This is contradictory to the suggestion that net rates of N turnover may be predictors of N loss (e.g., Holmes and Zak 1999, Venterea et al. 2003, Matejek et al. 2009) as well as to successful competition for bioavailable N of plants over microorganisms in the rhizosphere (Schimel and Bennett 2004, Chapman et al. 2006).

In sum, our study shows that the determination of net N turnover is of little use in steppe ecosystem studies, since they provided only a very poor approximation to the magnitude, dynamics and status of net rates of N turnover may be of limited use in steppe ecosystem studies, since they provided only a very poor approximation to the magnitude, dynamics and status of

CONCLUSIONS

Four different seasons with characteristic patterns of gross N dynamics could be distinguished in the investigated semi-arid continental Asian grassland. Both freeze-thaw cycles and the growing season were key periods for understanding patterns and magnitudes of gross N turnover. Various patterns of biogeochemical N turnover between seasons appeared to be closely related to microbial succession in periods of environmental stress or transition. In this context, turnover and succession of soil microbial biomass appeared to be the major driver of gross N fluxes, and hence, are of outstanding importance for nutrient retention and availability and may mitigate nutrient shortage in drought periods of the growing season. Net rates of N turnover may be of limited use in steppe ecosystem studies, since they provided only a very poor approximation to the magnitude, dynamics and status of...
actual N turnover in soil. The observed high temporal variation of N turnover and between seasons emphasizes the necessity for high resolution sampling of gross N turnover as a prerequisite to infer functioning and annual budgets of N turnover. Furthermore such high temporal resolution data on gross N turnover process dynamics are an indispensable prerequisite to test and improve process-oriented biogeochemical ecosystem models.

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