

Microbial N Turnover and N-Oxide (N₂O/NO/NO₂) Fluxes in Semi-arid Grassland of Inner Mongolia

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ABSTRACT

Gross rates of N mineralization and nitrification, and soil–atmosphere fluxes of N₂O, NO and NO₂ were measured at differently grazed and ungrazed steppe grassland sites in the Xilin river catchment, Inner Mongolia, P. R. China, during the 2004 and 2005 growing season. The experimental sites were a plot ungrazed since 1979 (UG79), a plot ungrazed since 1999 (UG99), a plot moderately grazed in winter (WG), and an overgrazed plot (OG), all in close vicinity to each other. Gross rates of N mineralization and nitrification determined at in situ soil moisture and soil temperature conditions were in a range of 0.5–4.1 mg N kg⁻¹ soil dry weight day⁻¹. In 2005, gross N turnover rates were significantly higher at the UG79 plot than at the UG99 plot, which in turn had significantly higher gross N turnover rates than the WG and OG plots. The WG and the OG plot were not significantly different in gross ammonification and in gross nitrification rates. Site differences in SOC content, bulk density

and texture could explain only less than 15% of the observed site differences in gross N turnover rates. N₂O and NO_x flux rates were very low during both growing seasons. No significant differences in N trace gas fluxes were found between plots. Mean values of N₂O fluxes varied between 0.39 and 1.60 µg N₂O-N m⁻² h⁻¹, equivalent to 0.03–0.14 kg N₂O-N ha⁻¹ y⁻¹, and were considerably lower than previously reported for the same region. NO_x flux rates ranged between 0.16 and 0.48 µg NO_x-N m⁻² h⁻¹, equivalent to 0.01–0.04 kg NO_x-N ha⁻¹ y⁻¹, respectively. N₂O fluxes were significantly correlated with soil temperature and soil moisture. The correlations, however, explained only less than 20% of the flux variance.

Key words: mineralization; nitric oxide; nitrification; nitrogen dioxide; nitrous oxide; overgrazing; steppe

INTRODUCTION

China is covered by approximately 400 million hectare of grassland (42% of the total area), of which 87 million hectare belong to Inner Mongo-

lia. The Inner Mongolian steppe is a part of approximately 12.5 million km² of temperate grasslands, which is more than 8% of the Earth's land surface. The temperate grasslands comprise the Eurasian steppe belt that ranges from Eastern Europe to Eastern Asia at mid-latitudes, with a remarkable branch extending into Inner Mongolia and further to the Southwest of China (Tibet). They

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play a substantial role in balancing the global greenhouse gas budget due to their ability to sequester carbon in the soil (Batjes 1998), storing some 300 Gt C – that is, approximately one-eighth of the global terrestrial carbon stock –, of which 97% is localized below ground (IPCC 2001). However, the role of grasslands in the global N cycle, including the nitrous oxide budget, is less clear.

In steppe areas of Asia, a nomadic land-use system prevailed for the past centuries but is only in practice today in some areas. The greatest recent changes in this practice were the sedentarization of the nomads, the collectivization of their livestock and, after re-privatization, the increase of livestock and the establishment of arable land which resulted in over-cropping and over-grazing of some areas. This has led to a severe decrease in soil organic matter, depletion of soil nutrients, facilitation of erosive processes and finally desertification (Chuluun 2001). It is estimated by the UNEP (United Nations Environment Programme) that 60–70% of the grasslands in China, Mongolia and the Asian parts of the former USSR are affected by deterioration and desertification mainly due to over-grazing, but in some areas also due to over-cropping (Graetz 1994).

Grazing can lead very quickly to changes in nutrient pools and fluxes (for example, Ross and others 1999; Augustine and Frank 2001), in vegetation cover (Paruelo and others 2001), plant community composition in grasslands (Li 2001; Oba and others 2001; Zhang and Skarpe 1996), and in the worst case even to desertification (Li and others 2000). Recent investigations of key microbial N turnover processes show that gross rates of N-mineralization and nitrification can be substantial in steppe ecosystems (Chen and Stark 2000; Corre and others 2002; Verchot and others 2002), comparable in the range to temperate forest ecosystems (for example, Davidson and others 1991; Stark and Hart 1997). Intensive studies in steppe ecosystems in North America have revealed that the N cycle and thus the involved consumptive and productive N cycling processes, such as mineralization, nitrification and immobilization, are in most cases tightly coupled in semi-arid temperate grasslands (Davidson and others 1990), and, thus, the N₂O formation does not appear to be a significant pathway in the N cycle (Billings and others 2002) of such ecosystems. Reported mean emission rates are mostly below 6.5 µg N₂O-N m⁻² h⁻¹ or smaller than 0.6 kg N ha⁻¹ y⁻¹ (for example, Mosier and others 1991, 1996, 2002; Epstein and others 1998; Mummey and others 1997, 2000). But due to the

high spatial variability of N₂O fluxes (for example, Billings and others 2002; Corre and others 1996) and a potential importance of wintertime fluxes in steppe ecosystems for total annual N₂O losses, which are mostly associated with snow melting events (Mosier and others 1996), estimates are still uncertain.

In contrast to N₂O fluxes, emission rates of NO-N from steppe ecosystems (0–200 µg N m⁻² h⁻¹, Epstein and others 1998; Smart and others 1999; Mosier and others 2002) have been reported to be significantly higher, in a range of 0.8–1.8 kg N ha⁻¹ y⁻¹ for a short-grass steppe in Colorado, USA (Martin and others 1998). NO fluxes have been shown to be strongly correlated with temperature (Martin and others 1998), but rainfall events can initiate short-term pulses of up to 400-fold increased NO fluxes (Martin and others 1998; Smart and others 1999).

Our knowledge about rates of microbial N cycling and biosphere–atmosphere exchange of N trace gases in East Asian steppe ecosystems, which are significantly different from steppe ecosystems in North America with respect to floristic composition and rangeland management, is still rudimentary. To contribute to constraining the uncertainties associated with N cycling and N trace gas exchange in East Asian steppe ecosystems, a study was conducted in a typical steppe area in Inner Mongolia, P. R. China, by means of chamber measurements of soil–atmosphere N₂O, NO and NO₂ exchange with high time resolution in the field, accompanied by laboratory incubation studies for the quantification of gross N turnover rates.

MATERIALS AND METHODS

Site Characteristics

The study was performed at four differently managed *Leymus chinensis* (Trin.) Tzvel. steppe sites in a typical steppe ecosystem of the Xilin river catchment, Inner Mongolia, China, approximately 450 km north of Beijing. Three of the sites, that is, a site fenced and ungrazed since 1979 (“UG79”, 25 hectare), a site fenced and ungrazed since 1999 (“UG99”, 35 hectare), and a site fenced and occasionally grazed in the winter period from November to April by 3–4 sheep units per hectare (“WG”, 40 hectare), are permanent. These three sites belong to the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 116°42′ E, 43°38′ N), a station within the Chinese Ecological Research Network (CERN) managed by the Institute of Botany, Chinese Academy of Sciences. To our knowledge, these sites are the only sites in this region

Table 1. Main Characteristics of the Different Experimental Sites

	UG99	WG	UG79	OG
Geographic coordinates	43°33.0'N, 116°40.1'E	43°33.0'N, 116°40.0'E	43°33.1'N, 116°40.5'E	43°34.7'N, 116°40.6'E
Height above sea level (m)	1,268	1,267	1,252	1,218
Maximum vegetation height 2005 (cm)	n.d.	19.6 ± 3.0	24.2 ± 2.8	6.9 ± 1.9
Slope (°)	2.2–2.5	2.5–2.7	1.9–2.9	Flat
pH, 0–4 cm, ± s.d.	6.8 ± 0.3	6.7 ± 0.3	6.6 ± 0.2	6.6 ± 0.3
Bulk density, 0–4 cm, ± s.d. (g cm ⁻³) ¹	1.09 ± 0.12	1.09 ± 0.08	0.94 ± 0.10	1.28 ± 0.08
C to N ratio, 0–4 cm, ± s.d.	9.7 ± 0.7	9.5 ± 0.4	9.8 ± 0.3	9.7 ± 0.4
Organic C content, 0–4 cm, ± s.d. (%)	2.55 ± 0.63	2.59 ± 0.45	3.10 ± 0.55	1.70 ± 0.42
Soil texture, 0–10 cm: sand (%)	48.3	54.9	64.2	66.2
Silt (%)	25.8	18.2	13.5	15.8
Clay (%)	25.9	27.0	22.3	18.0
Grazing intensity (sheep units ha ⁻¹ y ⁻¹)	0	1.5–2.0	0	~4

n.d. = not determined.

¹For further details on soil data see Steffens and others (2007).

with a well-documented management history. Floristic composition and seasonal variation of aboveground biomass at the UG79 site were described previously (Chen and others 2003; Bai and others 2004). It is noteworthy to mention that the ungrazed sites were moderately grazed before fencing.

The fourth site, which is approximately 2 km away from the other sites, is an unfenced and overgrazed site, located in the vicinity of a hamlet and used by a local farmer for grazing ('OG', approximately 5–6 sheep units per hectare during the vegetation period). At this site grazing has already led to changes in vegetation composition. For example, the abundances of *Potentilla acaulis* L. and *Artemisia frigida* Willd. were found to be significantly increased, and also the abundances of C₄ grasses [for example, *Cleistogenes squarrosa* (Trin.) Keng] increased at the cost of C₃ grasses [for example, *L. chinensis* (Trin.) Tzvel., *Stipa grandis* P. Smirn.]. These vegetation changes are typical indicators of overgrazing (Wang and Ripley 1997; Wang 2002; Tong and others 2004). High evaporation and transpiration rates during summer reduce plant available water, which is, besides temperature, the most limiting factor for plant growth in this semi-arid grassland ecosystem. A short growing season of approximately 150 days, a frost-free period of less than 100 days, and low mean monthly temperatures October–March further limit plant growth. Precipitation, mainly of convective nature, is restricted mostly to the summer months. For the period 1982–2003, the mean annual temperature of this area was 0.7°C, and mean annual precipitation was 343 mm. The soil

type at all sites is a calcic chernozem soil on a loess parent material, with a soil organic carbon (SOC) content of 1.8–2.2% in the uppermost 10 cm, and of 1.7–3.1% in the uppermost 4 cm. The C to N ratio of all sites was approximately 10 and, hence, rather narrow. The soil texture was a sandy loam, with pH values of 6.6–6.8. For further site details see Table 1.

Experimental Design

Gross ammonification and nitrification rates, and soil inorganic N concentrations were determined in the 2004 and 2005 growing season at the UG99 and WG sites, and additionally at the UG79 and WG sites in the 2005 growing season in at least monthly intervals.

N₂O fluxes were measured during the 2004 and 2005 growing season at the adjacent WG and UG99 sites with a fully automated measuring system for every 3 h, using three chambers for each site. At the UG79 and OG sites manual N₂O measurements were performed every 3–4 days during the 2005 growing season with four chambers for each site. At the latter two sites no automated measurements were possible because only one automated measuring system was available, and the UG79 and OG sites were too far away from the location of the automated system. Therefore, automated and manual N₂O flux measurements were considered as two separate sets of two treatments each.

NO_x flux measurements were exclusively conducted at the UG99 and WG sites because, like for the N₂O measurements, only one automated measuring system was available, and the other two

sites were too far away with respect to maximum tube length. As manual NO_x measurements with static chambers do not yield results comparable with dynamic chamber measurements, they were not performed at the UG79 and OG sites. Technical problems with the chemoluminescence detector restricted our measurements of NO_x fluxes and ambient air concentrations of NO and NO_2 to a few weeks in August and September 2004.

Determination of Gross N Turnover Rates

Soil samples of 0.5 kg from the top 0.05 m at three randomly chosen positions were taken from each treatment and immediately processed at IMGERS. Soil samples were homogenized by sieving carefully with a mesh width of 3.15 mm. The moisture content of the soil samples was determined gravimetrically by drying an aliquot of each unlabeled and labeled soil sample at 105°C for 24 h. Gross N mineralization and gross nitrification rates were determined according to Rosenkranz and others (2006a) with the ^{15}N pool dilution technique at in situ soil temperature and moisture conditions. $^{15}\text{N}/^{14}\text{N}$ isotope ratios were determined at the mass spectrometer laboratory at IMK-IFU, Garmisch-Partenkirchen, Germany, using an elemental analyzer (Flash EA 1112, Thermo Electron, Milan, Italy) coupled to a Delta^{Plus}XP mass spectrometer (Thermo Electron, Bremen, Germany). Ammonium and nitrate contents of the soil extracts were directly analyzed at the IMGERS using a Foss FIA Star 5000 Analyzer (Foss Inc., Hillerød, Denmark). Gross N mineralization and gross nitrification rates were calculated from the isotope ratios and NH_4^+ soil contents as described by Kirkham and Bartholomew (1954).

Gas Flux Measurements

The automated system for N_2O measurements was installed in a bus, which was placed approximately 10 m from the border between the WG and UG99 sites. All chambers were installed at a distance of 20–40 m from the bus. The chambers (side length 0.7×0.7 m, height 0.3 m at the UG99 site; side length 0.5×0.5 m, height 0.15 m at the WG site) were fixed on stainless steel frames, which were driven approximately 0.1 m deep into the soil. The static chambers were pneumatically closed gas-tight for 90 min, followed by a period of 90 min with open lids to allow for the aeration of the chambers. Thus, one measurement cycle lasted 180 min resulting in eight flux measurements per day. Alternately, five air samples were taken by the automated sampling system from each closed

chamber during the closure period (flow rate 100 ml min^{-1} , sampling time 3 min), followed by five reference gas injections (400 ppbv N_2O in synthetic air, Air Liquide, Munich, Germany). Samples were automatically injected into a gas chromatograph (SRI 8610C, Texas Instruments, Torrance, CA, USA), equipped with an electron capture detector (ECD) for N_2O detection. A pre-column filled with Ascarite (Sigma Aldrich, Munich, Germany), which was renewed in weekly intervals, was installed upstream of the ECD to remove CO_2 . The carrier gas for N_2O analysis was N_2 (99.999% purity). The ECD was heated to 350°C , while the column temperature was kept at 60°C . The detection limit of the automated system was approximately 2 ppbv N_2O concentration difference to ambient air when using five sample injections for one flux calculation. This is equivalent to an N_2O flux of $0.4 \mu\text{g N m}^{-2} \text{ h}^{-1}$. For further details of the automated measuring system and the calculation of fluxes, see Butterbach-Bahl and others (1998 and 2004), Papen and Butterbach-Bahl (1999), Breuer and others (2000), and Rosenkranz and others (2006b).

For the manual measurements, we installed four stainless steel frames (size 0.4×0.4 m) at each of the two sites, driven approximately 0.15 m into the soil. In intervals of 3–4 days, we mounted temperature-isolated chambers (chamber height 0.4 m) gas-tight onto the frames. Over a period of 80 min five gas samples were taken in 20 min intervals from the chambers using plastic syringes with a stopcock. Directly after sampling, the gas samples were brought to a laboratory at the IMGERS, where they were immediately analyzed for N_2O . Analytical conditions were the same as described above. The system used for manual N_2O analyses was described in detail by Wang and Wang (2003) and Wang and others (2005). However, to avoid interference with CO_2 during N_2O analysis we installed a pre-column filled with Ascarite upstream of the analytical column. The detection limit for manual N_2O flux measurements using five data points for flux calculation was significantly higher than that for the automated system due to uncertainties associated with manual sampling and injection into the gas chromatograph. Using multiple injections of calibration standard, we calculated the precision of sampling and gas-chromatography analysis to be 5 ppbv concentration change of N_2O , equal to a flux of approximately $1 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. A comparison between measurements with the automated system and manual measurements at the same spot indicated no differences in N_2O fluxes determined

independently with the two methods (data not shown).

For the determination of NO_x fluxes six dynamic measuring chambers and one reference chamber (side lengths 0.5×0.5 m; height 0.3 m) with a gas-tight perspex bottom were used. Three chambers were placed at the UG99 site, whereas the other three were located at the adjacent WG site. Each of the measuring chambers and the reference chamber were sampled alternately for 6 min, thus, within 72 min all six chambers had been sampled once. Besides the chambers, the measuring system consisted of a sampling device, an air pump and flow controller to achieve a controlled sample air flow of approximately 50 l min^{-1} , a chemoluminescence detector for the determination of NO (CLD 770 AL ppt, Ecophysics AG, Dürnten, Switzerland), a photolysis converter for the determination of NO_2 (PLC 760, Ecophysics AG, Dürnten, Switzerland) and an ozone analyzer (TE 49 C, Thermo Environmental Instruments Inc., Franklin, MA, USA). The chemoluminescence detector was calibrated weekly using 10 ppbv NO in synthetic air made by dilution of a reference gas (0.998 ppmv NO in N_2 , Air Liquide, Munich, Germany) with synthetic air (79% N_2 , 21% O_2), using a computerized multi-gas calibrator (EnviroNics 100, EnviroNics Inc., West-Wellington, USA). The efficiency of photolytic conversion of NO_2 into NO was determined weekly as described by Butterbach-Bahl and others (1997). NO_x fluxes were calculated as the difference between measured NO and NO_2 mixing ratios of sample and reference chamber air, multiplied by the flow rate, and were corrected for the reaction of NO with O_3 to NO_2 in the chambers and the sample lines. Details of flux calculation procedures are described by Butterbach-Bahl and others (1997), and Rosenkranz and others (2006b). The detection limit of the NO_x measuring equipment was equivalent to fluxes of 0.2 and $0.4 \mu\text{g N m}^{-2} \text{ h}^{-1}$ for NO and NO_2 , respectively (Gasche and Papen 1999).

Auxiliary Measurements

Daily rainfall was measured at the climate station at the IMGERS, approximately 10 km from the experimental sites. During the growing season, air and soil temperatures were automatically recorded every 1 min at UG99 and WG sites using PT100 thermocouples. In addition, soil moisture was measured daily at all experimental sites using a portable TDR probe (ThetaKit, Delta-T Devices, Cambridge, UK). At each site the TDR probe was inserted vertically at least five times. From the

individual readings a mean value was calculated representing the soil moisture in 0–6 cm soil depth.

Statistical Analysis

Statistical analysis was performed as a blocked analysis with the different plots serving as the blocks, using SPSS 8.0 (SPSS Inc., Chicago, IL, USA) and Origin 7.0 (OriginLab Corp., USA) software packages. Tests of significant differences ($P < 0.05$) between the experimental sites were either performed with the multiple range test (LSD) of the ANOVA, with a parametric t -test or a non-parametric U -test (Mann–Whitney). Because no treatment replications were available, a separate analysis of the effect of grazing on soil N turnover and N trace gas exchange was not possible.

RESULTS

Meteorology

The year 2004 was a “normal” year with a total sum of rainfall of 265 mm within the period June 1 to September 30 (average sum for the years 1982–2003: 262.3 mm). In contrast, rainfall in 2005 during the same period was only 119 mm, and, thus, far below average. The mean air temperature during the period June to September was $17.7 \pm 0.7^\circ\text{C}$ in 2004 and $19.3 \pm 0.8^\circ\text{C}$ in 2005, but the difference was not significant. However, it should be noted that daily air temperatures varied widely within a range from 2.5°C up to 35°C .

Inorganic Soil N Concentrations and Microbial N Turnover Rates

Ammonium concentrations in the uppermost 5 cm of the soil ranged from 0.1 to 5.0 mg N kg^{-1} soil dry weight (SDW) during the 2005 growing season, with mean values (\pm s.e.) of $0.51 \pm 0.05 \text{ mg N kg}^{-1}$ SDW for the UG99 site, $0.67 \pm 0.09 \text{ mg N kg}^{-1}$ SDW for the WG site, $0.73 \pm 0.16 \text{ mg N kg}^{-1}$ SDW for the OG site, and $1.71 \pm 1.06 \text{ mg N kg}^{-1}$ SDW for the UG79 site. At all sites soil nitrate concentrations were significantly higher than soil ammonium concentrations and ranged from 0.4 to $14.5 \text{ mg N kg}^{-1}$ SDW. The highest mean nitrate concentration ($6.96 \pm 2.64 \text{ mg N kg}^{-1}$ SDW) was found at the UG79 site, and was significantly higher than at all other three sites. The lowest soil nitrate concentrations were found at the WG site ($1.59 \pm 0.15 \text{ mg N kg}^{-1}$ SDW). Mean nitrate concentrations at the UG99 and OG sites were $2.77 \pm 0.17 \text{ mg N kg}^{-1}$ SDW and $1.75 \pm 0.29 \text{ mg N kg}^{-1}$ SDW, respectively.

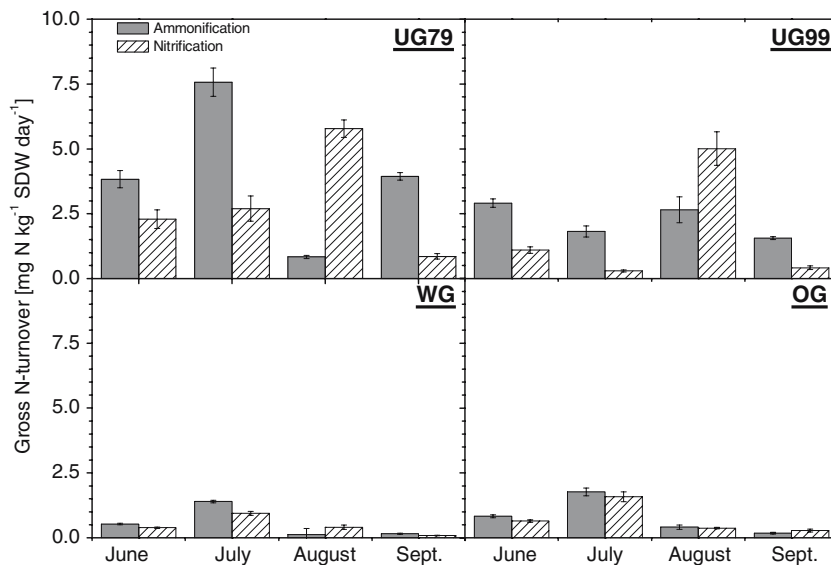


Figure 1. Rates of gross ammonification and gross nitrification (\pm s.e.) at the differently managed *Leymus chinensis* steppe sites during the 2005 growing season. Values represent mean values of at least three independent and replicated measurements per month.

Table 2. Mean Values (\pm s.e.) of Gross Microbial N Turnover Rates ($\text{mg N kg}^{-1} \text{SDW day}^{-1}$) at the Four Different Experimental Field Sites

	UG99		WG		UG79		OG	
	N	Mean \pm s.e.	N	Mean \pm s.e.	N	Mean \pm s.e.	N	Mean \pm s.e.
2004								
Ammonification	21	1.4 \pm 0.4 ^a	21	1.3 \pm 0.2 ^a	–	–	–	–
Nitrification	21	1.0 \pm 0.2 ^a	21	0.8 \pm 0.2 ^a	–	–	–	–
NH ₄ ⁺ immobilization	18	1.4 \pm 0.5 ^a	20	1.2 \pm 0.2 ^a	–	–	–	–
NO ₃ ⁻ immobilization	19	0.5 \pm 0.1 ^a	17	0.5 \pm 0.1 ^a	–	–	–	–
2005								
Ammonification	12	2.2 \pm 0.4 ^b	11	0.6 \pm 0.6 ^a	11	4.1 \pm 0.9 ^c	12	0.8 \pm 0.8 ^a
Nitrification	12	1.7 \pm 0.7 ^b	10	0.5 \pm 0.1 ^a	11	3.1 \pm 0.7 ^c	12	0.7 \pm 0.2 ^a
NH ₄ ⁺ immobilization	10	2.2 \pm 0.3 ^b	12	0.6 \pm 0.1 ^a	9	4.9 \pm 0.3 ^c	11	1.3 \pm 0.2 ^a
NO ₃ ⁻ immobilization	8	0.9 \pm 0.2 ^{ab}	10	0.5 \pm 0.1 ^a	12	1.2 \pm 0.3 ^b	11	0.6 \pm 0.2 ^a

Different superscripts indicate significant differences between the individual microbial N turnover rates for the different sites and measuring years. N = number of measurements; – = not determined.

Monthly mean ammonification rates at the different steppe sites varied within a range of 0.1–7.5 $\text{mg N kg}^{-1} \text{SDW day}^{-1}$ (Figure 1). Mean ammonification rates were significantly higher at the ungrazed sites than at the grazed sites (Table 2). The highest growing season mean ammonification rate was found at the UG79 site (Table 2). A comparable difference between ungrazed and grazed sites and between the sites UG79 and UG99 was also found for gross nitrification (Table 2, Figure 1).

N₂O Fluxes

The N₂O fluxes observed at the UG99 and WG sites were close to zero in both years, showing both net

emission and net uptake (Figures 2, 3). Net emission of N₂O was mainly observed in periods with volumetric soil water contents higher than 15%, whereas uptake of atmospheric N₂O was especially observed when volumetric soil water content was low (Figures 2, 3). N₂O fluxes were significantly correlated with soil moisture and soil temperature. However, the correlations explained only less than 20% of the variance of the measured fluxes. The linear regression of all data from the automated measurements in 2004 and 2005 at WG and UG99 plotted against volumetric soil water content resulted in an r^2 value of 0.166 ($f(x) = 0.098 + 0.05x$, $P < 0.01$), and the linear regression with soil temperature yielded an r^2 value of 0.19 ($f(x) = 2.24 - 0.094x$, $P < 0.01$). The mean N₂O

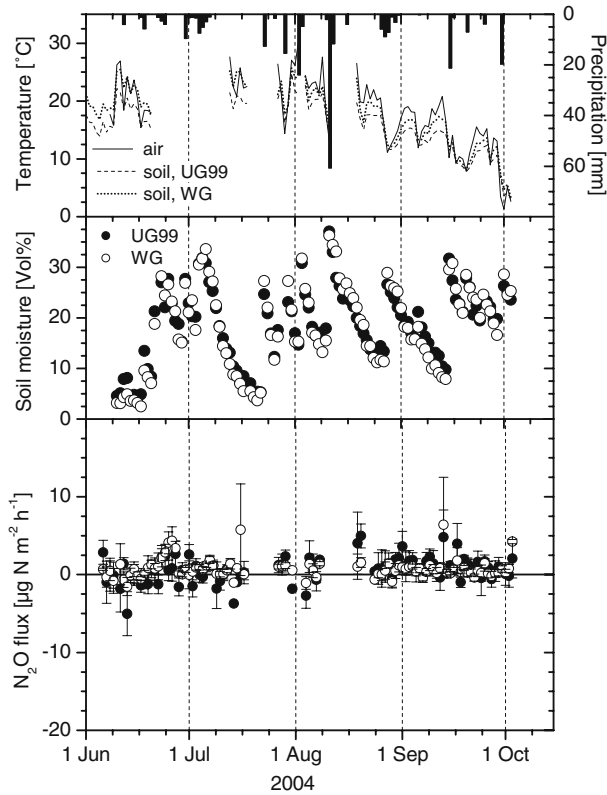


Figure 2. Temporal course of soil (5 cm soil depth) and air temperatures, soil moisture (0–6 cm) and daily mean N_2O fluxes during the 2004 growing season at the sites UG99 (filled circle) and WG (open circle). N_2O fluxes are daily mean values (\pm s.e.), representing measurements with three chambers and eight flux measurements chamber⁻¹ day⁻¹.

flux at the UG99 site was slightly, but not significantly lower than at the WG site in 2004 (Table 3). N_2O fluxes during the much drier 2005 growing season tended to be lower than that in the 2004 growing season at both sites (Figure 3, Table 3). However, these differences were not statistically significant. The manual N_2O flux measurements performed in 2005 at the UG79 and OG sites revealed somewhat higher mean N_2O fluxes (Figure 4, Table 3). Nevertheless, N_2O fluxes at both sites were not significantly different from N_2O fluxes at the UG99 and WG sites, neither in 2004 nor in 2005.

NO_x Fluxes

Ambient air concentrations of NO and NO_2 were mostly well below 1 ppbv during the measurement period in August and September 2004. Only during some days when wind was blowing predominantly from the direction of a nearby country road (distance approximately 1 km) did we observe slightly

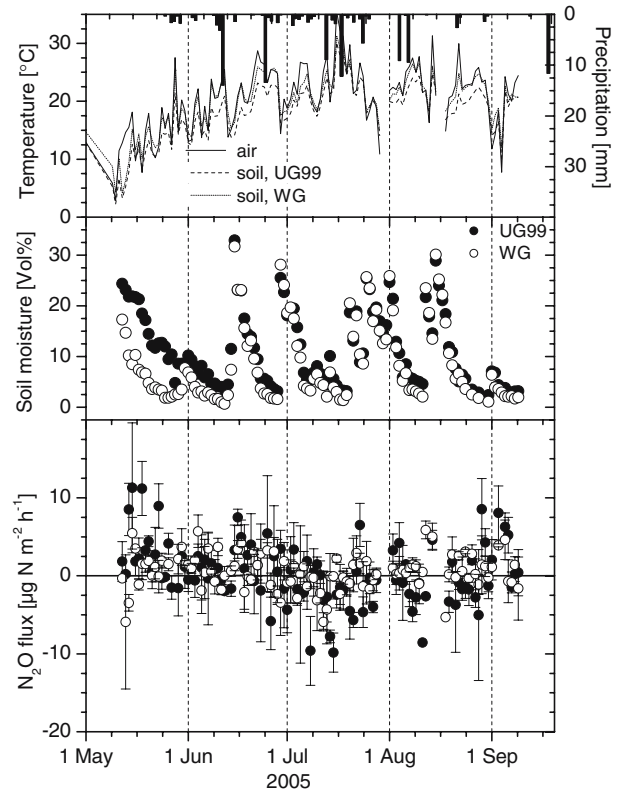


Figure 3. Temporal course of mean daily N_2O fluxes, soil moisture (0–6 cm) and soil and air temperature (5 cm soil depth) during the 2005 growing season at the sites UG99 (filled circle) and WG (open circle). N_2O fluxes are daily mean values (\pm s.e.), representing measurements with three chambers and eight flux measurements chamber⁻¹ day⁻¹.

higher mean daily NO_2 concentrations of up to 2.1 ppbv (Figure 5). Ambient air O_3 concentrations varied in a range of 15–50 ppbv. Soil–atmosphere NO_x fluxes at the UG99 site and the WG site were always close to the detection limit of the system (Figure 5). The magnitude of mean NO_x fluxes was as low as observed for N_2O fluxes (Table 3). However, it should be noted that NO_x fluxes, like N_2O fluxes, tended to be higher at the WG site than at the UG99 site.

DISCUSSION

Meteorology

Although the 2005 growing season was much drier and warmer than in 2004 – such a year-to-year variability of rainfall and temperature is not unusual in Inner Mongolia and the Xilin river basin (Liang and others 2003) – no significant effect on gross ammonification or on gross nitrification rates could be found. Also the growing season means of

Table 3. Mean Values (\pm s.e.) of N_2O and NO_x (sum of NO and NO_2) Fluxes ($\mu\text{g N m}^{-2} \text{h}^{-1}$) at the Differently Managed *Leymus chinensis* Steppe Sites in Inner Mongolia

	UG99		WG		OG		UG79	
	<i>N</i>	Mean \pm s.e.	<i>N</i>	Mean \pm s.e.	<i>N</i>	Mean \pm s.e.	<i>N</i>	Mean \pm s.e.
2004								
N_2O	93	0.65 ± 0.20	93	0.79 ± 0.10	–	–	–	–
NO_x	36	0.16 ± 0.16	36	0.48 ± 0.27	–	–	–	–
2005								
N_2O	107	0.39 ± 0.40	107	0.56 ± 0.22	33	0.95 ± 0.56	33	1.60 ± 0.47

N = number of measuring days; – = not determined.

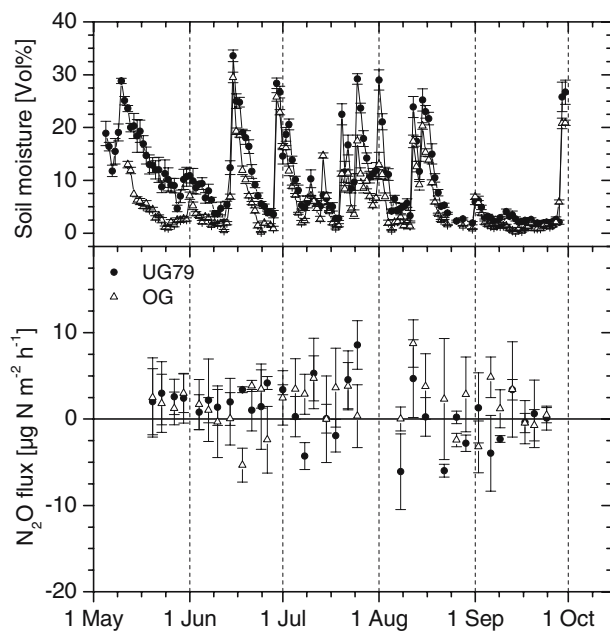


Figure 4. Temporal course of N_2O fluxes and soil moisture (0–6 cm) during the 2005 growing season at the sites UG79 (filled circle) and OG (triangle). N_2O fluxes represent mean values (\pm s.e.) for three chambers. Fluxes were obtained by manual measurements.

N_2O fluxes were not significantly different in the two years. However, given the very low level of N_2O emission rates, there was obviously not a sufficiently high potential of N gas formation to be stimulated.

Inorganic Soil N Concentrations and Microbial N Turnover Rates

In the present study, total inorganic N (nitrate + ammonium) concentrations tended to be higher at the ungrazed sites as compared to the grazed sites. At all sites nitrate concentrations in the top soil were on average significantly higher than ammonium concentrations with concentra-

tions of up to $14.5 \text{ mg N kg}^{-1}$ soil at the ungrazed sites. Our results are in agreement with a previous study carried out at the ungrazed and grazed sites (Wang and others 2006), but are in contrast to findings by Frank and others (2000) who found no significant effect of fencing on soil inorganic N concentrations in Yellowstone Park grasslands. Nitrate concentrations exceeding ammonium concentrations as found in this study were also reported for autumn measurements in *Bromus*-dominated shrub-steppe ecosystems in northern Utah (Booth and others 2003). The authors hypothesized that increased inorganic N concentrations in the topsoil of *Bromus*-dominated steppe sites as compared to *Artemisia* and *Elymus* steppe sites are most likely associated with the dense thatch at *Bromus* sites resulting in a more homogenous litter input as compared to litter inputs under perennials. Also the ungrazed sites of the present study had a well-developed thatch, whereas the thatch of the grazed sites was sparse and inhomogeneously distributed.

Changes in soil inorganic N concentrations are a result of differences in production, consumption and N loss processes, that is, ammonification, nitrification, denitrification, plant and microbial N uptake, gaseous N losses, and nitrate and DON leaching. Gross mineralization and gross nitrification rates, and, thus, N availability were significantly higher in the topsoil of the ungrazed plots of this study than that in the grazed plots. This was especially pronounced at the site, which had been protected from grazing for 26 years (UG79), but was still significant at the UG99 site (protected from grazing for 6 years) in the year 2005, that is, the year with strongly reduced rainfall during the growing season. In contrast, there was no significant difference between moderately (winter-)grazed and heavily (over-) grazed plots.

The significant differences in gross N turnover rates between the grazed and ungrazed plots of our

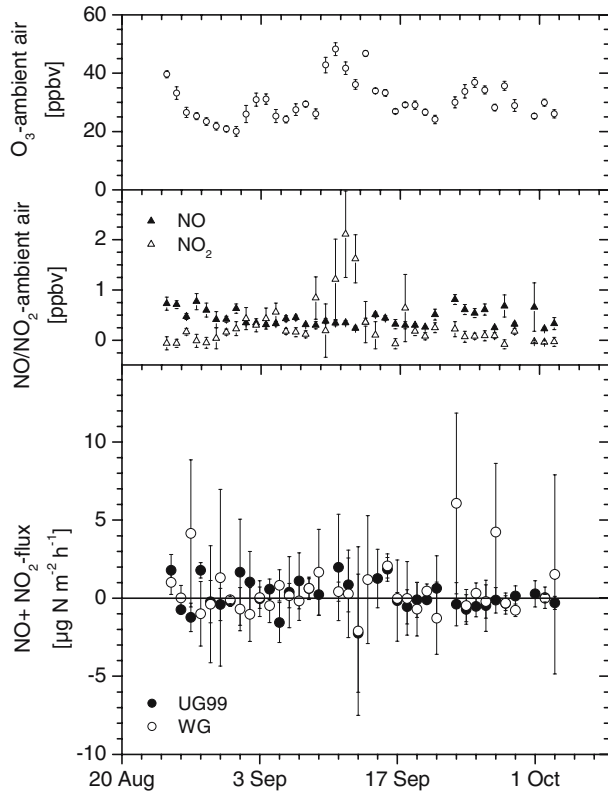


Figure 5. Temporal course of mean daily NO_x fluxes at the sites UG99 (filled circle) and WG (open circle), and ambient air concentrations of NO, NO_2 and O_3 in late August and September 2004. NO_x fluxes are daily mean values (\pm s.e.), representing measurements with three chambers and 20 flux measurements chamber $^{-1}$ day $^{-1}$.

study could have potentially been a result of differences in soil physical and chemical site properties, concealed by sufficient rainfall during the 2004 growing season, but unveiled by the very dry conditions in 2005. The sites exhibited small but measurable differences in soil texture, soil organic C (SOC) content and bulk density (Table 1). Although it is likely the differences in soil properties had been caused by grazing and sheep trampling (for example, Golodets and Boeken 2006), this could not be proved in the present work due to the lack of replication.

Our findings are in agreement with a livestock exclusion experiment in the Columbia Basin, USA, in which 32-fold and 149-fold higher potential net mineralization and net-nitrification rates, respectively, were found for ungrazed riparian meadow sites as compared to grazed sites (Kauffman and others 2004). In contrast, in a study including the determination of net mineralization and net nitrification rates under grazing disturbance at the sites of the present study in Inner Mongolia, Wang and

others (2006) could not observe differences between grazed and ungrazed plots, supporting our finding that microbial inorganic N immobilization rates were in the same range as gross ammonification and gross nitrification rates and therefore masking differences in gross N turnover rates during the determination of net N turnover rates. Yet our results are in contradiction to most measurement and modeling studies in grasslands of North America, where grazing was mostly found to stimulate mineralization, nitrification and denitrification (Groffman and others 1993; Frank and Groffman 1998; Frank and others 2000; Le Roux and others 2003) which may be due to the stimulation of rhizosphere microbial mineralization activity as a result of increased root exudation after defoliation (Holland and others 1996; Hamilton and Frank 2001). However, a statistically sound testing of an effect of grazing on soil N turnover and N trace gas exchange in steppe ecosystems of Inner Mongolia was not possible in this study due to the lack of experimental plot replication.

N_2O and NO Fluxes

Emission rates of N_2O and NO_x were in the range of 0.03–0.14 kg $\text{N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$ and 0.01–0.04 kg $\text{NO-N ha}^{-1} \text{ y}^{-1}$, respectively, and, thus, at the lower detection limit of the equipment used in the field experiments. N_2O fluxes showed a highly significant correlation with soil moisture and soil temperature. The correlations, however, explained only less than 20% of the variance of the fluxes. However, other factors contributing to N_2O flux variance could not be identified in this work.

The average growing season N_2O emission rate found in this study was approximately 30–50% lower than was previously reported by Wang and others (2005), who calculated a range of 0.06–0.21 kg $\text{N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$ as the average annual N_2O emission for the same region. Wang and others (2005) found differences in N_2O emission rates between grazed and ungrazed plots in the same region at certain times during the growing season, especially during flowering of the grass. Whenever N_2O fluxes were different, they were higher at the ungrazed plot. Xu and others (2003) found an annual average value for N_2O emissions of 0.76 g $\text{N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ for the typical steppe of Inner Mongolia, equivalent to 3.2 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ and to 0.28 kg $\text{N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$, respectively, and Du and others (2006) even reported a mean total annual N_2O flux of 0.73 ± 0.52 kg $\text{N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$. The higher values in these studies could be a result of not using sufficient means to

exclude cross-sensitivity of electron capture detectors for CO₂ if the gas-chromatographic separation of N₂O and CO₂ is insufficient. This can especially become a problem in studies with static soil chambers, leading to high CO₂ accumulation during the measurements and, hence, to a higher bias in the N₂O measurements. In our study ascarite, that is, sodium hydroxide on a silica carrier, was used as a CO₂ absorbent. In the studies cited above the use of a CO₂ absorbent was not mentioned in the respective "Materials and methods", and by discussions with the involved colleagues we could confirm that a complete removal of CO₂ could not be assured. Tests using both methods, that is, no or an ascarite pre-column for CO₂ absorption revealed that at low N₂O fluxes (<10 µg N m⁻² h⁻¹) not using an ascarite pre-column and N₂ as carrier and purge gas will result in an overestimation of N₂O fluxes up to a factor of 10 due to the cross-sensitivity with CO₂. Therefore, we hypothesize that those previous methods used to measure N₂O fluxes in Inner Mongolia grasslands tended to overestimate N₂O fluxes. This is especially supported by the high number of single chamber flux rates determined in this study (approximately 10,000). N₂O emission rates previously reported for temperate semi-arid grassland of North America were also low, albeit not as low as the ones found in this study, with emission rates below 6.5 µg N m⁻² h⁻¹ or 0.6 kg N ha⁻¹ y⁻¹ (for example, Mosier and others 1991, 1996, 2002; Epstein and others 1998; Mummey and others 1997). Mummey and others (2000) estimated an annual mean N₂O emission of 0.24 kg N₂O-N ha⁻¹ y⁻¹ from the Great Plain States of the US, that is, almost twice as high as the upper limit of the range of emission rates in this study. The higher rates found in North America could be a result of differences in vegetation composition between the two different steppe types and, hence, differences in soil nitrogen cycling. This is also supported by the very low average NO emission rates found in the present study, which are approximately two orders of magnitude lower than those found in a shortgrass steppe in northeastern Colorado (Epstein and others 1998), with the highest rates found for C₄-dominated plots, whereas the Inner Mongolian typical steppe and likewise the experimental plots of the present study were dominated by C₃ plants.

CONCLUSION

N₂O and NO_x fluxes were at a very low level at all four experimental sites in a typical steppe ecosystem of Inner Mongolia. No significant differences in

N trace gas fluxes between the experimental plots, both grazed and ungrazed, were found. In contrast, at the grazed study sites microbial gross N turnover was significantly lower than at the ungrazed plots. Although the N₂O fluxes were at least 30% lower than previously reported for the same region, the contribution of Eurasian steppe ecosystems to regional and global emission budgets could nonetheless be significant due to the vast extension of this ecosystem. For a statistical confirmation of our preliminary results on potential grazing effects on soil N cycling and N trace gas exchange further work on replicated sites and in other regions of Central and East Asian steppe is required.

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