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Dinitrogen emissions: an overlooked key component of the N balance of montane grasslands

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Abstract While emissions of nitric oxide (NO), ammonia (NH_3) and nitrous oxide (N_2O) from grassland soils have been increasingly well constrained, soil dinitrogen (N_2) emissions are poorly understood. However, N_2 losses might dominate total gaseous nitrogen (N) losses. Knowledge on N losses is key for the development of climate-adapted management that balances agronomic and environmental needs. Hence, we quantified all gaseous N losses from a montane grassland in Southern Germany both for ambient climatic conditions and for a climate change treatment (+ 2 °C MAT, − 300 mm MAP). Monthly measurements of soil N_2 emissions of intact soil cores revealed that those exceeded by far soil N_2O emissions and averaged at 350 ± 101 (ambient climate) and 738 ± 197 $\mu g\ N\ m^{-2}\ h^{-1}$ (climate change). Because these measurements did not allow to quantify emission peaks after fertilization, an additional laboratory experiment was deployed to quantify the response of NH_3, NO, N_2O, and N_2 emissions in sub daily temporal resolution to a typical slurry fertilization event (51 kg N ha^{-1}). Our results revealed that total N gas losses amounted to roughly half of applied slurry-N. Surprisingly, N_2 but not NH_3 dominated fertilizer N losses, with N_2 emissions accounting for 16–21 kg or 31–42% of the applied slurry-N, while NH_3 volatilization (3.5 kg), N_2O (0.2–0.5 kg) and NO losses (0–0.2 kg) were of minor importance. Though constraining annual N_2 loss remained uncertain due to high spatiotemporal variability of fluxes, we show that N_2 losses are a so far overlooked key component of the N balance in montane grasslands, which needs to be considered for developing improved grassland management strategies targeted at increasing N use efficiency.

Keywords Climate change · Denitrification · Dinitrogen · Nitrous oxide · Fertilizer emissions · temperate grassland

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Introduction

Grasslands represent about 30% of the total agricultural area of Germany, with the pre-alpine and montane grassland belt in S-Germany being one of the largest grassland regions in Germany (> 1 Mio ha) (BMLEV 2012). Soils of these montane grasslands, including even larger areas in the adjacent countries of Austria, Switzerland and Italy, store extraordinarily large amounts not only of soil organic carbon (SOC) but also of total nitrogen (TN), because cold temperatures and high precipitation slow down organic matter mineralization (Wiesmeier et al. 2013). At regional scale, SOC and TN stocks decrease with decreasing elevation, i.e. along gradients representing increasing temperature and decreasing precipitation. This suggests that, in response to climate change, existing SOC and TN stocks might decrease (Wiesmeier et al. 2013; Parton et al. 1995). Climate warming in montane and alpine regions has been twice as intense as the global average—a trend which is expected to continue in the 21st century (Böhm et al. 2001; Smiatek et al. 2009; Wagner et al. 2013).

While the role of grasslands and soils in general and their importance for the global carbon cycle in a changing climate has received considerable interest and thus is increasingly well known (Falkowski et al. 2000), knowledge on N cycling traditionally drags behind knowledge on C cycling (Schlesinger 2009). The N balance of managed montane grasslands is consisting of the components N input (e.g., via manure fertilization, atmospheric deposition and biological N fixation), internal N turnover (e.g., microbial mineralization-immobilization turnover) and N outputs (e.g., grass harvest, gaseous N losses due to NH_3 volatilization, or production of gaseous N compounds by microbial processes such as nitrification and denitrification, hydrological losses due to leaching or surface run off). Recent research showed that climate change in montane grasslands heavily increases N mineralization, which can promote plant N uptake and productivity and thus also N mowing exports (Wang et al. 2016). If fertilization is too low, this might lead to nutrient mining (negative nutrient balance, e.g. higher N export than import leading to a loss of N from SOM) of soil organic matter (Wang et al. 2016). However, total gaseous N losses from such grassland ecosystems have not yet been accurately quantified (Felber et al. 2012), mainly as a consequence of the severe difficulties in the quantification of denitrification and its terminal product N_2, which is the most severely understudied process of the N cycle (Groffman et al. 2006; Butterbach-Bahl et al. 2013). Denitrification is the stepwise reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to NO, N_2O and the terminal product N_2. By reducing N_2O to inert and harmless N_2, denitrification removes reactive N from the biosphere and closes the global N cycle. While many studies quantified the net exchange of the potent greenhouse gas and destructor of stratospheric ozone N_2O (Ravishankara et al. 2009) at the soil-atmosphere interface, studies on gross N_2O reduction to N_2 and associated N_2 emissions from soil are scarce (Wen et al. 2016; Butterbach-Bahl et al. 2013). In a review article, Butterbach-Bahl et al. (2013) concluded that N_2 losses have been accurately constrained only for five agricultural and eight natural/semi-natural terrestrial ecosystems. The lack of sound measurements of N_2 losses from soils is due to the extreme methodological difficulties in its quantification against the atmospheric background and the failure of the widely used acetylene inhibition method to accurately quantify N_2 emissions, (Bollmann and Conrad 1997; Groffman et al. 2006; Felber et al. 2012; Butterbach-Bahl et al. 2013; Yang and Silver 2012). Therefore, currently only the Helium soil core incubation technique (Scholefield et al. 1997; Butterbach-Bahl et al. 2002) and the ^{15}N tracing technique are available to directly quantify N_2 emissions from soils. Application of ^{15}N tracing or Helium atmosphere methodologies has resulted in estimates of N_2 emissions ranging from 0.51 to 198 kg N ha^{-1} $year^{-1}$ across terrestrial systems (Butterbach-Bahl et al. 2013). Due to limited N_2 data, also the regulators of denitrification and in particular N_2 loss are hardly known for most of the terrestrial systems. Existing laboratory studies with soils and/or pure cultures of denitrifying microorganisms and a few field studies which aimed to directly measure N_2 fluxes, suggest that main controls determining the extent of N_2O reduction to N_2 are the availability of NO_3^- and NO_2^- as electron acceptors, the available carbon to NO_3^-/NO_2^- ratio, temperature, oxygen and pH (Butterbach-Bahl et al. 2013; Saggar et al. 2013; Liu et al. 2014; Bakken et al. 2012; Zumft 1997).

Besides the gases produced by denitrification, NH_3 is another environmental relevant N-gas which is relevant for the N mass balance and fertilizer N use efficiency of agricultural systems and which is predominantly volatilized following synthetic or organic fertilizer applications (Sommer and Hutchings 2001; Bouwman et al. 1997; Amon et al. 2006). Ammonia volatilization is mainly regulated by the temperature and pH effects on the ratio of gaseous NH_3 over dissolved NH_4^+ (Freney et al. 1983). Besides its role for the N mass balance, and its atmospheric effects (Ciais et al. 2013), NH_3 plays a major role in respect to eutrophication of natural ecosystems as well as reduced air quality and the negative impact of N-aerosols on human health (Sutton et al. 2011; Sommer and Hutchings 2001). As a consequence, there is a widely recognized need to minimize NH_3 losses from agricultural soils.

Yet, the total N gas emissions from montane grassland soils and the contribution of different N gas species to gaseous output are unknown. However, a thorough characterization and quantification of gaseous N outputs from grassland soils is an indispensable prerequisite for the development of management measures targeted to increase fertilizer N use efficiency. Improved fertilizer N use efficiency is needed to minimize environmental N losses along hydrological and gaseous pathways, to avoid nutrient mining from soil organic matter and thus, to minimize environmental impacts of grassland management and to maintain key soil functions on the long-term.

Therefore, the main objective of our study was the quantification of gaseous N losses from montane grassland soils from a translocation study, allowing for studying processes and fluxes under varying climatic conditions.

Our study aimed at the (1) provisioning of first estimates of annual N_2 loss from montane grassland soils, (2) identification of the environmental regulators of N-gas emissions, (3) quantification of gaseous N loss after manure application, thereby disentangling the contribution of NH_3, N_2, N_2O and NO, and (4) identification of short-term climate change effects on N-gas loss.

We hypothesized that N_2 and N_2O gas background emissions would be lower than fertilizer emissions. Furthermore, we expected that after slurry application to soil, NH_3 emissions would be higher than N_2 emissions.

Materials and methods

Site description

The investigated extensively managed grassland site Graswang (11.03°E, 47.57°N) at an altitude of 870 m a.s.l. is a typical montane grassland in the calcareous Alps of Southern Germany and part of the TERENO alpine long-term ecosystem observatory (TERENO: http://teodoor.icg.kfa-juelich.de/overview-de). The investigated soil is a Haplic Cambisol-G from alluvial gravel under grassland with 9% organic carbon and 0.8% total nitrogen in the 0–20 cm topsoil and a pH of ca. 7 (Unteregelsbacher et al. 2013). The dominant vegetation are the perennial herbs *Plantago lanceolata* L., *Trifolium repens* L. and *Prunella vulgaris* L., and the perennial grass *Festuca rubra* L. Mean annual temperature and precipitation are 6.0 °C and 1438 mm (Unteregelsbacher et al. 2013) (Table 1).

To study climate change effects on gaseous N losses, we used a space-for-time climate change experiment with intact plant–soil mesocosms. Therefore, intact plant–soil mesocosms were taken at the high elevation (HE) site Graswang. Half of the mesocosms were translocated to a low elevation (LE) site (Fendt, 605 m a.s.l, 11.07°E, 47.83°N, in the following referred to as the climate change treatment LE) (Table 1), with the other half being treated equally but remaining as control at HE. Part of the mesocosms were used for monthly monitoring of N_2 and N_2O emissions under field-like conditions and for quantification of soil mineral N concentrations, soil temperature and moisture (Experiment I). The other mesocosms were used for a laboratory experiment targeted at quantifying manure-N losses through emission of N_2, N_2O, NO and NH_3 with sub daily temporal resolution (Experiment II).

Translocation from HE to LE increased annual air temperature by approximately 2 °C and decreased

Table 1 Mean air temperature (Ta), precipitation, soil temperature (Ts) and water filled pore space (WFPS) during the measurement period of experiment I (10.05.13–09.05.14)

	Ta (°C)	Precipitation (mm)	Ts/10 cm (°C)	WFPS
HE	7.0	1260	9.3	71.2
LE	9.1	995	10.4	63.3

HE high-elevation control site, *LE* low-elevation climate change site precipitation by almost 300 mm per year. More detailed information about the sites is provided by Unteregelsbacher et al. (2013) and (Wang et al. 2014).

Experimental design

Experiment I: background N_2 and N_2O emissions

Sampling of the mesocosms (12.5 cm diameter, 20 cm height) was conducted at four plots of 10 m^2 size at the HE site. At three spots of each plot, quadruplicated plant–soil cores were sampled in immediate vicinity to each other with two being transferred within HE and two being translocated to LE. Overall, 48 soil cores were translocated, 24 cores within HE and 24 from HE to LE (see Fig. 1), with twelve being used for N_2 and N_2O flux measurement and the other twelve for analysis of soil mineral N concentrations (Fig. 1). In addition 8 soil cores were equipped with soil moisture and temperature sensors and transferred within HE or to LE (i.e. four replicates per treatment), so that a total of 56 intact soil cores were used in the experiment. Details about coring and reburying procedures of plant–soil mesocosms are provided by Unteregelsbacher et al. (2013). Translocation of the soil cores was conducted April 17th 2013. Measurements started May 17th 2013 and lasted for 1 year with nine measurement dates in total.

The experimental setup was designed in order to minimize disturbance and thus to approach field-like conditions for N_2 and N_2O flux measurements, but also to account for the restricted capacity of the He incubation system to analyze N_2 and N_2O fluxes from four cores at a time. For measurement of N_2 and N_2O emissions, four soil cores were transferred from either HE or LE to the laboratory of KIT-IMK-IFU within 1 h and immediately incubated in the He soil core incubation system at in situ observed soil temperature in 10 cm depth. Subsequently to the flux measurement period of approximately 4 days, the soil cores were brought back to the in situ incubation site and four soil cores of the other site (HE or LE) were transferred to KIT-IMK-IFU for measurement (Fig. 1). The three sets of four cores each were undergoing an alternating measuring pattern so that the same set of soil cores was measured every 4th sampling date (Fig. 1). Due to restricted measurement capacities, delays of up to 6 days occurred between measurements for HE and LEs in the second half of the experimental year. Additional cores were analyzed simultaneously for soil mineral N concentrations (see below).

Experiment II: N-gas emissions due to manuring

For this experiment we used plant–soil mesocosms, which were translocated within HE and from HE to LE already in 2009 (Unteregelsbacher et al. 2013). In total, 24 mesocosms were retrieved in autumn 2015, 12 each from HE and LE site. The 12 cores of each site were further subdivided in two groups with six cores being used for N_2/N_2O measurements by the He soil core technique and the other six for $NO/N_2O/NH_3$ measurements in a custom-made laboratory incubation system. Prior to the measurements, all 24 soil cores were reburied in grassland soil at the facilities of the IMK-IFU in Garmisch-Partenkirchen (730 m

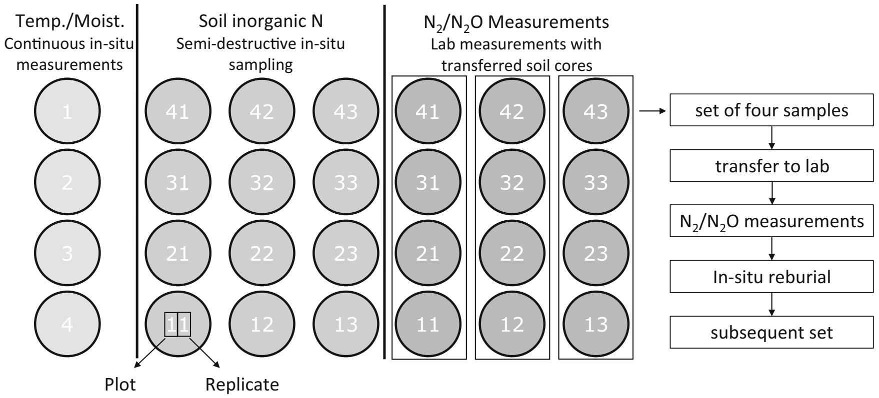

Fig. 1 Experimental design of experiment I, illustrating number and use of plant–soil mesocosms incubated either at the high elevation sampling site HE (control) or the low elevation climate change simulation site LE

a.s.l.) until start of measurements. Two weeks prior to the measurements, we adjusted the Water Filled Pore Space (WFPS) to 70% by adding a standard rain solution after Schierl (1991) in order to achieve comparable conditions for plant physiological-, microbial- and physicochemical processes. The soil cores were then placed in the incubation system, where the air temperature for N gas flux measurements was set to 18 °C, both for HE and LE. Following measurements of emissions from unfertilized soil cores over a period of 10 days, fertilizer in form of liquid cattle slurry was applied to the soil cores. Each soil core received 20 ml of slurry, which corresponded to an application of 51 kg N ha^{-1}, a typical amount used by farmers in the study region. The applied slurry was analyzed for total Nitrogen (TN), total organic nitrogen (TON) and ammonium (NH_4^+) according to DIN ISO 13878 and DIN 38406-5 by the independent Raiffeisen laboratory (Ormont, Germany). Subsequently to fertilization, the measurements were continued for another 14 days (N_2/N_2O by using the He-soil-core technique) and 20 days (NH_3/NO/N_2O/CO_2 fluxes by using the headspace gas flow system), i.e., until the fertilization-induced N gas emission peaks had diminished to pre-fertilization gas flux levels.

Simultaneous N_2 and N_2O flux measurements

The simultaneous N_2 and N_2O measurements were conducted using the He gas flux soil core incubation technique (Butterbach-Bahl et al. 2002; Cardenas et al. 2017). This method is based on the complete replacement of the soil and headspace atmosphere by an artificial gas mixture containing 20% O_2, 400 ppbv N_2O and 20 ppmv N_2 in He in a highly gastight incubation system. For this purpose, repeated evacuation and He/O_2 purging cycles are conducted for 48 h, followed by a final flushing of the headspace and sampling lines. Due to the complete replacement of the atmospheric N_2 background in the gastight system, N_2 evolution from soil can be measured by gas chromatography based on the increase of N_2 concentrations in the headspace of the system. The system is connected to a Gas chromatograph (GC 17 A, Shimadzu, Tokyo, Japan), equipped with an electron capture detector (ECD) for N_2O detection and a Pulse Discharge Helium Ionization Detector (PDHID, Valco Instruments Company, Inc., Houston, TX, USA) for analysis of sample air N_2 concentrations. Regular measurements with empty cuvettes showed a constant N_2 concentration increase in the system equivalent to 0.1 PPM h^{-1} or 10 μg N_2–N m^{-2} h^{-1}, which is representing the system-inherent leakage rate and detection limit for soil-born N_2 loss. The high gas tightness of the system was achieved by using double cuvette lid sealing, additionally purged with He, and by placing the entire incubation cuvettes and associated sampling lines under water, which also allowed to directly regulate the incubation temperature. Furthermore, all other potential sources of leakage of atmospheric N_2 into the system such as valves or tubing connections are placed in an additional gastight chamber purged with He. The detection limit for N_2O fluxes was 1 μg N_2O–N m^{-2} h^{-1}. Further details about the He soil core incubation system and the measurement setup as used in this study is provided by Butterbach-Bahl et al. (2002).

Simultaneous NO, N_2O and NH_3 measurements

Within experiment II, in addition to the He soil core technique for measuring N_2/N_2O fluxes an automated continuous headspace gas-flow system was used, which allowed the quantification of soil emissions of NO, N_2O and NH_3 (Zuazo 2016). Soil N gas emissions were calculated from the difference in concentrations between inlet and outlet of each headspace of the incubated soil cores (dynamic chamber approach). Nitrous oxide and NH_3 concentrations in inlet and outlet gas samples were detected by a cavity- ring-down spectrometer (CRDS G25008, PICARRO, Santa Clara, CA, USA), whereas a chemoluminescence detector (CLD88p, Eco Physics AG, Munich, Germany) was used for NO detection. Due to the tendency of NH_3 to stick to the surface of tubings, we were not able to fully exclude cross contamination between the cores. Therefore we excluded the comparison between treatments but solely show the average course of NH_3 emissions for all cores and the average cumulative NH_3 emissions. In order to assure equal and continuous conditions for all soil cores, soil cores were kept in incubation cabinets at constant air temperature (18 °C) during the measurement. The measurement interval to measure N gas fluxes from all 18 incubated soil cores was 3 h (6 min closure time per core and 4 min flushing of sample tubes), which led to a sub-daily resolution of eight measurements per core and day. With a total measurement time of 30 days, 240 measurements were conducted on each core. Flow rate of the dynamic measurements using the PICARRO spectrometer was 333 ml min^{-1}.

Soil mineral N concentrations

In experiment I, soil samples for mineral N determination were taken from the respective soil cores in the field with a small corer at the same time as N_2/N_2O flux measurements (i.e., in approx. monthly intervals). Mineral N contents of soil cores measured in experiment II were determined on soil samples taken 14 days before and 20 days after slurry application. Soil samples were homogenized by hand and roots removed. In both experiments, 30 g of soil were extracted with 60 ml of 0.5 M K_2SO_4. Soil extracts were immediately frozen until the determination of soil mineral N (NH_4^+ and NO_3^-) concentration by the commercial laboratory Dr. Janssen GmbH (Gillersheim, Germany). Results are displayed in Table 2.

Meteorological data

Soil environmental data was monitored in eight soil cores of Experiment I. Soil temperature (Thermistor SC32B, Campbell Scientific, Logan, Utah, USA) and moisture (ECH2O, EC-5, DECAGON, Pullman, Washington, USA) sensors were installed in 10-cm depth and recorded via a datalogger (21X, Campbell Scientific, Logan, Utah, USA) every 30 min during the measurements period. The soil cores in Experiment II were not monitored with respect to soil moisture and temperature, since all cores were brought to the same WFPS prior to the start of the incubation and were subsequently incubated under controlled conditions.

Statistical data analysis

Kolmogorov–Smirnov test for normal distribution was applied for data of any measured parameter. When required, data were log-transformed to meet the criteria of normal distribution and homogeneity of error variance. For experiment I, a 2-way ANOVA was applied to test the effect of (1) climate change induced by translocation, (2) and sampling time on the N_2, N_2O emissions. In experiment II, we compared treatment effects (control vs. climate change) and N loss via different gases (N_2 vs. NH_3 vs. N_2O vs. NO) also using ANOVA. Additionally, Pearson's correlation analysis was carried out for all N-gases and soil parameters and multiple linear stepwise regression served as a tool to identify the main drivers of N_2 emissions. For statistical analysis SPSS 24.0 (SPSS.Inc. Chicago, USA) was used and Origin 16 (Origin Lab Corporation, Northampton, USA) for graphical display. Results of statistical analyses are displayed in Table 3.

Results

Soil mineral N concentrations

The soil cores used in experiment I for estimating annual N_2/N_2O fluxes on basis of approx. monthly measurements did overall not show differences in mineral N concentrations between HE and LE

Table 2 Soil mineral N concentrations for experiments I (targeted for measurement of annual course of N gas emissions) and II (targeted for measurement of fertilizer induced N gas emissions) [μg N g^{-1} sdw]

	Experiment I		Experiment II			
	NO_3^-	NH_4^+	NO_3^-		NH_4^+	
			Pre-fertiliz.	Post-fertiliz.	Pre-fertiliz.	Post-fertiliz.
HE	4.1 ± 2.1	4.2 ± 2.0	30.0 ± 6.9	40.7 ± 6.0	8.5 ± 1.6	8.7 ± 1.5
LE	3.8 ± 1.6	3.2 ± 1.4	13.2 ± 3.6	19.6 ± 11.1	8.8 ± 1.91	16.9 ± 2.8

Uncertainty is given as standard error of the mean

Exp. I average concentration from eight samplings during the measurement period, *Exp. II* pre-fertiliz: data gained 2 weeks before slurry application, *post-fertiliz* data gained 20 days after slurry application, *HE* high elevation control site, *LE* low elevation climate change site

Table 3 Pearson's coefficients (R) obtained for correlation between emission rates of N_2O and N_2 and soil temperature (Tsoil) and –moisture (Msoil) in 10 cm depth for experiment I, ANOVA for comparison between climate treatments

	Pearson's correlation coefficient					ANOVA (climate change: HE vs. LE)		
	N_2O	Tsoil	Msoil	NH_4^+	NO_3^-	df	F	P
Annual emissions (exp. I)								
N_2	0.37	0.53*	0.41	0.01	0.06	65	1.0	0.32
N_2O		0.714**	0.29	0.00	0.06	67	0.4	0.51
Post fertilization (exp. II)								
N_2						11	0.61	0.45
N_2O						10	4.8	0.06
NO						10	30.0	0.00*

$^*P < 0.05$, $^{**}P < 0.01$

(Table 2). However, soil NO_3^- concentrations of cores used in experiment II tended to be lower at LE compared to HE both before fertilization and at the end of the laboratory incubation experiment (Table 2). In contrast, 20 days post-fertilization NH_4^+ concentrations tended to be increased at LE compared to HE, thereby still exceeding the values obtained before fertilization (Table 1).

Seasonality of N-gas emissions from unfertilized soil cores (experiment I)

N_2 emissions

Dinitrogen emissions were characterized by great variability in space and time with single fluxes ranging from 0 to 9601 µg N m^{-2} h^{-1}. Mean fluxes for different sampling dates ranged from 4.9 ± 1.6 to 1925 ± 1405.0 µg N m^{-2} h^{-1} for HE and 15.7 ± 23.9 to 4426.3 ± 1881.0 µg N m^{-2} h^{-1} for LE.

High N_2 emissions > 300 µg-N m^{-2} h^{-1} were only observed for four sampling dates between May and beginning of September, i.e., under conditions of high soil temperature and of moderate to wet soil moisture (Fig. 2a). Peak emissions at HE and LE sites were observed in August, though emission rates were highly variable between the four cores (HE range 332–3180 mean 1925 ± 702; LE range 692–9601, mean 4426 ± 1881 µg N m^{-2} h^{-1}) (Fig. 2a). Interestingly, these peak N_2 emissions occurred in a period of highest temperatures but decreasing WFPS (Fig. 2c), which contributed to our observation that

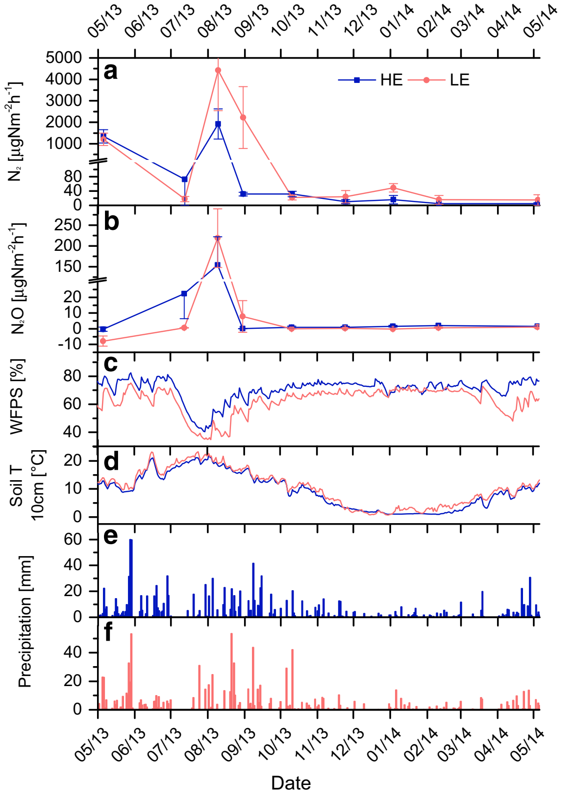

Fig. 2 N_2 emissions (**a**) and N_2O emissions (**b**) (± SE, n = 4) as obtained in experiment I from short-term transport of field cores to laboratory for measurements by use of the helium soil core incubation system, followed by subsequent post-measurement-reburial of cores at the field site. The panels **c**–**f** present meteorological data for the sampling period. *HE* high elevation incubation (control), *LE* low elevation incubation (climate change treatment), *WFPS* water filled pore space, *Soil T* soil temperature

temperature but not soil moisture had the highest predictive power for N_2 emissions (Table 3). For all other sampling dates, mean emissions were low ranging from ca. 0–50 μg N m^{-2} h^{-1} (Fig. 2a). Mineral N concentrations were not correlated with N_2 emissions (Table 3). The average annual N_2 flux was 350 ± 101 and 738 ± 197 μg m^{-2} h^{-1} for HE and LE, respectively. ANOVA did not reveal significant differences between HE and LE (Table 3).

N_2O fluxes

As described for N_2 fluxes also for N_2O fluxes high temporal and spatial variability was observed. Mean N_2O fluxes observed for HE varied between − 0.3 ± 4.7 and 153.8 ± 136.2 μg N m^{-2} h^{-1} while the range observed for LE soil cores was − 7.86 ± 6.5 to 218.8 ± 141.1 μg N m^{-2} h^{-1} (Fig. 2b).

N_2O fluxes for both treatments showed a pronounced seasonality with peak emissions in August (HE range 46–331, mean 154 ± 136; LE range 119–319, mean 219 ± 141 μg-N m^{-2} h^{-1}), i.e., at the same time as N_2 emissions peaked too. At all other sampling dates comparably low flux rates, ranging from − 7.9 to 7.8 μg N m^{-2} h^{-1}, were observed. Mean annual flux rate for HE was 16.4 ± 6.3 μg N m^{-2} h^{-1} and 10.9 ± 5.1 μg N m^{-2} h^{-1} for the LE site. N_2O fluxes did not show statistically significant differences between HE and LE.

Similar to N_2 emissions, Pearson's correlation analysis revealed that soil temperature was the main control of soil N_2O emissions ($R = 0.714$, $P < 0.01$) (Table 3).

N-gas emissions after fertilization (experiment II)

N_2 emissions

Before fertilization N_2 fluxes were with 585.6 ± 193.6 μg N m^{-2} h^{-1} at HE and 300.4 ± 106.3 μg N m^{-2} h^{-1} for LE (Fig. 3a) comparable to the fluxes at the same time for cores used in experiment I (Fig. 2a). Dinitrogen fluxes remained low until day 2 after slurry application. However, three days after fertilization, soil N_2 emissions from both treatments were found to be largely increased, with peak emissions being observed at day four (HE: 34739.7 ± 33376.0 μg N m^{-2} h^{-1}; LE: 33720.6 ± 29323.1 μg N m^{-2} h^{-1}). Seven days after the fertilization, N_2 emissions at both HE and LE soils had stabilized at constant emission levels, that were however larger than pre-slurry emissions (HE: 2057.2 ± 708.5 μg N m^{-2} h^{-1}; LE: 2942.2 ± 1376.9 μg N m^{-2} h^{-1}) (Fig. 3a). The response of N_2 emission dynamics to fertilization was overall quite similar for HE and LE with no statistically significant differences between the treatments. Cumulative post fertilization emissions amounted to 16.1 ± 8.3 and 21.4 ± 8.2 kg N ha^{-1} for HE and LE, respectively, or 31.4 and 41.7% of slurry-N application (Table 4).

N_2O emissions

Prior to fertilization, soil N_2O emissions were on average 35.9 ± 10.9 μg N m^{-2} h^{-1} for HE and

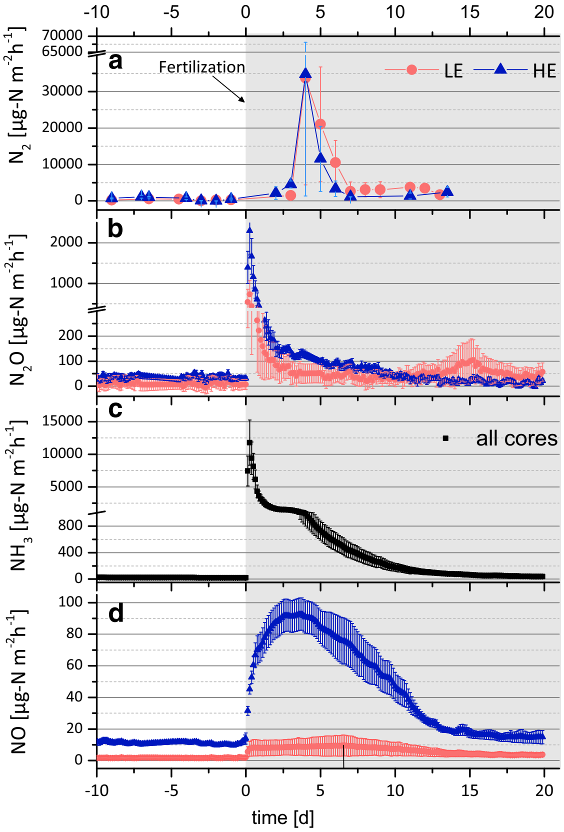

Fig. 3 Dynamics of slurry application induced N gas emissions (N_2, N_2O, NH_3 and NO) (experiment II) (± SE; N_2, N_2O, NO: $n = 6$; NH_3 $n = 12$). Surface addition of slurry is indicated by an arrow after the first 10 days of measurements. Blue symbols: HE, red symbols: LE, black symbols: all cores

Table 4 Post-fertilization N gas emissions over the incubation period of 2 weeks in experiment II (kg N ha^{-1}) with percentage of applied manure-N in brackets

	HE	LE	Difference LE-HE
Manure-N application	51.2	51.2	
N_2 emission	16.1 ± 8.3 (31.4%)	21.4 ± 8.2 (41.7%)	5.3
NH_3 emissions	3.6 ± 1.5 (7.1%)		
N_2O emissions	0.52 ± 0.04 (1.0%)	0.23 ± 0.11 (0.5%)	− 0.29
NO emissions	0.24 ± 0.01 (0.5%)	0.02 ± 0.02 (0.04%)	− 0.22
Total N gas emissions	20.6 (40.2%)	24.9 (48.7%)	4.3

Uncertainty is given as SE from then mean (n = 6)

9.3 ± 23.0 μg N m^{-2} h^{-1} for LE. Highest N_2O emissions were observed at the day of slurry application and emissions sharply declined thereafter (Fig. 3b). Maximum N_2O emissions were three times higher at HE as compared to LE ($P < 0.05$) (HE: 2297.65 ± 597.40 μg N m^{-2} h^{-1} LE: 735.09 ± 345.81 μg N m^{-2} h^{-1}). Nine days after fertilization, N_2O emissions reached a stable level of approx. 30 μg N m^{-2} h^{-1} both for HE and LE. However, during days 13–17 after fertilization, we observed a second emission peak for the LE soil only, with maximum N_2O emission of ca. 100 μg N m^{-2} h^{-1} (Fig. 3b). Towards the end of the measurement period, post fertilization fluxes for HE dropped below the starting conditions, whereas N_2O fluxes for LE soil cores still showed higher N_2O emissions as compared to fluxes observed before slurry application.

Cumulative N_2O emissions for HE (0.52 ± 0.04 kg N ha^{-1}) were marginally, but significantly ($P = 0.056$) higher than those observed for LE (0.23 ± 0.11 kg N ha^{-1}) (Table 4).

NH_3 emissions

Before fertilizer application, NH_3 losses were at a constant, very low level (22 ± 1 μg N m^{-2} h^{-1}) (Fig. 3c). Similar to N_2O, NH_3 emissions showed a sharp increase directly following the slurry application with peak emissions occurring 8 h after fertilization (11778 ± 3469 μg N m^{-2} h^{-1}). In the following days NH_3 fluxes sharply decreased, with fluxes being 37 ± 12 μg N m^{-2} h^{-1}, i.e. close to the pre-fertilization levels, at the end of the incubation period. Because of the methodological reasons described in Materials and methods, we abstained from a comparison between the treatments. Cumulative post-fertilization NH_3 losses amounted to 3.6 ± 1.5 kg N ha^{-1} representing 7.1% of applied fertilizer-N, i.e., significantly lower values than obtained for N_2 ($P < 0.01$).

NO emissions

Compared to other N gases, the NO emissions were low. Before slurry application NO emissions were higher for HE soil cores (11.12 ± 1.25 μg N m^{-2} h^{-1}) as compared to soil cores at LE (3.72 ± 1.45 μg-N m^{-2} h^{-1}). Following slurry application NO emissions peaked over several days, though distinct differences in magnitude were observed between control and climate change treatment. While the HE soil cores showed constantly growing NO emission rates, peaking after 3.75 days (92.83 ± 10.25 μg N m^{-2} h^{-1}), NO emissions for LE soil cores only increased by approx. a factor of two, thereby reaching the maximum emission of 9.59 ± 6.65 μg N m^{-2} h^{-1} after 6.5 days. For both treatments, NO fluxes were higher at the end of the measuring period as compared to the pre fertilization period. Statistical analysis showed significantly higher NO emissions for HE soil cores as compared to LE treatment.

For the N mass balance of both treatments, NO emissions were of marginal importance as cumulative NO emissions at HE were 0.24 ± 0.01 kg N ha^{-1} and 0.02 ± 0.01 kg N ha^{-1} at LE (Table 4).

Discussion

This study shows for the first time that soil N_2 emissions are the dominant pathway of gaseous N losses from a typical montane grassland, being even approx. five (HE) or nine times (LE) higher than NH_3 emissions. Moreover, we show that N gas emissions following slurry applications dominate the annual N gas emission budget, highlighting the need for high temporal resolution measurements in order to accurately quantify fertilizer emissions. While this still is a huge methodological challenge for N_2, such high frequency measurements are feasible for NO, N_2O and NH_3 fluxes, which, with the exception of NH_3, however are of smaller importance for the N mass balance.

Annual background emissions

The annual average N_2 emissions from unfertilized grasslands were 350 ± 101 and 738 ± 197 µg m^{-2} h^{-1} for intact soil cores taken from HE and LE sites, respectively. The average flux rates would add up to an annual N_2 emission estimate of 30.7 ± 8.8 at HE and 64.6 ± 17.3 kg N ha^{-1} at LE. However the huge spatiotemporal variability of N_2 emissions as well as potential methodological limitations make these estimates – despite the application of state-of-the-art-methodology—still uncertain. A potential methodological problem could be that the Helium soil core method relies on a virtually complete removal of N_2 from the soil core. If this is not achieved to sufficient extent, remaining N_2 in soil solution, sorbed on surfaces or trapped in micropores, could be released during the measurements, leading to an overestimation of soil born N_2 fluxes. In this study, a He purging/evacuation procedure over 48 h was used, which lowered headspace N_2 concentrations to hardly detectable N_2 concentrations of few PPM, suggesting sufficient N_2 removal. Furthermore, the very low N_2 flux rates measured (5–30 µg N m^{-2} h^{-1}) during several sampling dates between November 2013 and May 2014 suggest that physical degassing of N_2 indeed was not a significant problem.

The flux magnitude measured in this study is comparable to the few existing studies reporting on N_2 fluxes for unfertilized (semi-) natural upland ecosystems. Dinitrogen emissions from temperate forest ecosystems affected by atmospheric N deposition were reported to range between 11 and 2264 µg m^{-2} h^{-1} (Butterbach-Bahl et al. 2002, 2013; Rennenberg and Dannenmann 2015; Kulkarni et al. 2014; Uri et al. 2011; Soosaar et al. 2011). To our knowledge, only a few studies aimed at estimating annual N_2 emissions from temperate grasslands. E.g., the spring and summer emissions of 95–619 µg m^{-2} h^{-1} measured by Horgan et al. (2002) in a Kentucky bluegrass turf (*Poa pratensis* L.) and 34–2557 µg m^{-2} h^{-1} in a temperate fertilized lawn (Raciti et al. 2011) are comparable to our results. Other studies on grassland N_2 emissions were mostly based on indirect N_2 emission estimations based on the acetylene inhibition technique with its severe methodological shortcomings (e.g. Ammann et al. 2009).

Compared to grassland ecosystems, wetland ecosystems tend to show much higher soil N_2 emissions with up to 3401 µg m^{-2} h^{-1} for a fertilized forest swamp (Delaune et al. 1998), 2740 µg m^{-2} h^{-1} for a boreal peatland (Wray and Bayley 2007) or 342–2808 µg m^{-2} h^{-1} for a riparian wetland (Burgin and Groffman 2012). This can be explained by the combined occurrence of high nutrient availability and highly anaerobic conditions in wetlands, favoring complete denitrification with the terminal product N_2. In our study, soil temperature rather than soil moisture had the highest predictive power for N_2 emissions. This could be due to a faster consumption of oxygen at higher temperatures due to soil respiration (Butterbach-Bahl and Dannenmann 2011), which might add a significant volume of anaerobic pores due to the clayey texture of the studied soil. Furthermore, persistence of anaerobic soil pores might generally have been facilitated by the wet weather throughout the year, with WFPS values hardly dropping below 40% (Fig. 2).

Average Nitrous oxide fluxes (16.4 ± 6.3 and 10.9 ± 5.1 µg N m^{-2} h^{-1} for HE and LE, respectively) are higher compared to values reported earlier for the same experimental site by Unteregelsbacher et al. (2013). Due to higher temporal resolution, with weekly measurements over a period of 2 years, Unteregelsbacher et al. (2013) provided better constrained N_2O emissions from unfertilized grassland averaging at 3.5 µg N m^{-2} h^{-1} both for HE and LE. However the emissions, measured in this study are in the same magnitude as reported by several other studies in temperate grassland ecosystem ranging from 0.5 to 14.4 µg N m^{-2} h^{-1} (Horváth et al. 2010; Cantarel et al. 2012; Flechard et al. 2005). In contrast to IPCC projections (Ciais et al. 2013) and studies analyzing multiple soils from Europe (e.g., Schaufler et al. 2010), which predict a climate change induced N_2O emission increase, the N_2O emission measurements in this study did not increase in response of translocation of intact soil cores from a cooler and wetter (HE) to a warmer (+2 °C) and dryer (-300 mm rainfall) site (LE). This confirms previous findings of both Unteregelsbacher et al. (2013) and Cantarel et al. (2011) for temperate grasslands in Germany and France. We hypothesize that the potentially stimulating effect of increasing temperature might have been outbalanced by the concomitant average decrease in soil moisture (Butterbach-Bahl et al. 2014). Furthermore, the generally high potential for N_2O reduction to N_2 as observed for soils of this study might have prevented a significant increase in N_2O emissions. This assumption is further supported by a laboratory incubation study by (Chen et al. 2015), who observed for the same soils as used in our study, strong increases in N_2O emissions comparable to those of N_2 emissions only if the availability of NO_3^- (NO_3^- conc. > 100 mg N kg^{-1}ds) and labile C is at levels which were never observed in the field.

Fertilizer induced N-gas emissions

Contrary to the general assumption that NH_3 emissions largely dominate the gaseous N-loss induced by manure fertilization (Amon et al. 2006) and in contrast to our hypothesis, our results show that N_2 losses are the main gaseous N loss pathway. In our study, N_2 emissions are accounting for a loss of 32–43% of the applied fertilizer N of 51 kg ha^{-1}, N_2–N emission, thus being ca. 6–8 times higher than NH_3–N losses (3.6 kg or 7.1% of the applied N). In contrast to our results, EMEP/EEA emission factors suggest a loss of 55% of Total Ammoniacal Nitrogen (TAN) from spread dairy cattle slurry (EEA 2016). This would, in our case represent an emission of 13.7 kg N ha^{-1} or 27% of total applied N and thus giving NH_3 a significantly higher importance in the N balance than suggested by our results. However, Spirig et al. (2010) measured NH_3 emissions for a temperate grassland in Switzerland in the range of 4–19% of TAN after cattle slurry application, thereby suggesting a potential overestimation of EMEP/EEA emission factors for temperate grassland ecosystems. Estimating NH_3 losses in our study based on the 4–19% of TAN as found by Spirig et al. (2010) reveals a range 1–5 kg N ha^{-1} per fertilization event, thus being in the same magnitude as our measurements have shown. On the other hand, the NH_3 emissions measured in this study might be underestimated, because of the lack of solar radiation and relatively low air temperature in the incubation system, both being important drivers of NH_3 emissions in the field (Sommer and Hutchings 2001). Furthermore, the incubation system used in this study was not able to mimic the original in situ wind and precipitation conditions, with wind being another driver of NH_3 emissions and precipitation potentially reducing NH_3 emissions due to faster infiltration of slurry N into the soil. Nonetheless, our results provide convincing evidence that N_2 plays a bigger role in the N balance of temperate, montane grasslands compared to NH_3.

Assuming that the measured fertilizer N_2 emissions are representative for both of the seasonal cattle slurry applications of farmer's when practicing extensive management of grasslands, we calculated annual N_2 losses of 32.1 ± 16.6 and 42.8 ± 16.4 kg N ha^{-1} $year^{-1}$ (two fertilization events) for HE and LE, respectively. These numbers are—in contrast to our hypothesis—comparable to annual "background" emissions as obtained in experiment I. However, as for the background emissions of experiment I, also the estimates of fertilizer N_2 emissions of experiment II are affected by method-inherent uncertainties such as the constant temperature and moisture regimes without occurrence of precipitation during the incubation period. Hence, attempts to constrain total annual N_2 fluxes based on both experiment I and II (62.8 ± 25.4 and 107.4 ± 33.7 kg N ha^{-1} for HE and LE) remain highly uncertain. Fertilizer N_2 emissions as obtained in this study from experiment II are however in the same magnitude as found for an irrigated and fertilized cotton crop field in Uzbekistan (Scheer et al. 2009). In the latter study, seasonal N_2 emissions—measured with the He-gas-flow technique—were 92.2 ± 22.9 kg N ha^{-1}. Some studies using the ^{15}N gas flux method also revealed rather high N_2 emissions, ranging from 28.5 kg N ha^{-1} a^{-1} in a fertilized winter wheat field (Colbourn and Dowdell 1984) to 26–198 kg N ha^{-1} a^{-1} for a manured agricultural soil (Rolston et al. 1978). Nonetheless, most of the studies using the ^{15}N gas flux method have shown comparably low N_2 emissions between 0.7 and 24 kg N ha^{-1} $season^{-1}$, with all soils being fertilized at rather high addition rates of 200–300 kg N ha^{-1} (Rolston et al. 1978; Mosier et al. 1986) compared to our study (102 kg N ha^{-1} $year^{-1}$). Both the ^{15}N tracing technique and the He soil core technique have their specific advantages and shortcomings (Groffman et al. 2006; Butterbach-Bahl et al. 2013). In this study, a problem could have been the dark incubation of the intact plant-soil mesocosms in the He system, leading to only respiratory activity of plants. Hence, there likely was no significant plant competition for mineral N during the measurement period. Switching off the plant microbe competition for available N in soils, a major control of denitrification in the rhizosphere (Bohlen et al. 2001), has likely increased the N substrate availability for denitrification and possibly stimulated denitrification and associated N gas emissions. Consequently, the measured N_2 emissions in our study might rather represent upper limits. To obtain better N_2 emission measurements from intact plant-soil systems, a He soil core technique with a transparent chamber would be required. However, to our knowledge, such a system is currently not available due to the enormous technical challenges to ensure the indispensable gas tightness of such a system. Another limitation could be lack of N leaching losses due to absence of precipitation during the incubation period. However, the soils of this study are characterized by excellent filter capacity so that nitrate leaching rates are extraordinarily low with annual losses of only ca. 2 kg N ha^{-1} (Fu et al. 2017).

In contrast to N_2 and NH_3, N_2O and NO were of negligible importance regarding N losses from the studied montane grassland ecosystems. However, considering the large global warming potential of N_2O (Ciais et al. 2013; Carter 2007) and the huge areal importance of grasslands at European and global scales (Soussana et al. 2004), the observed magnitude of N_2O fluxes are nonetheless of significance as a source for atmospheric N_2O. In this study, fertilizer induced N_2O emissions ranged from 1% (HE) to comparably low ca. 0.5% at LE, thereby falling below the IPCC emission factor of 1%. The observed total N_2O emissions of 0.92–1.58 kg N ha^{-1} a^{-1} are in the same range as reported by Flechard et al. (2007) for various temperate grassland ecosystems.

Interestingly, the emission peaks for N_2O, NH_3 and NO fluxes immediately occurred after the application of the fertilizer, indicating microbial or abiotically driven gaseous losses originating directly from the slurry itself. In contrast, the N_2 emission peak occurred after a delay of 2–3 days post fertilization. This sequential initialization of emission peaks of N_2O and N_2 was also observed earlier by Wang et al. (2013), with a N_2 peak delay of up to 27 h, compared to N_2O. This could result from retarded triggering of full denitrification, because the nosZ harboring microorganisms might be more ubiquitous in the soil than in slurry also explaining the high variability between soil cores. Alternatively, the stimulated microbial activity due to slurry application could have resulted in a retarded full oxygen depletion and associated later onset of complete denitrification until the terminal product of denitrification N_2 is produced (Butterbach-Bahl and Dannenmann 2011). Molecular studies analyzing abundance and expression of functional N cycle genes could clarify how soil microorganisms are involved in the release of slurry N via denitrification.

Climate change effects and environmental controls

In the present study we could not detect statistically significant effects on soil N_2 and N_2O emissions by translocating intact soil cores from a cooler and wetter to a dryer and warmer site, though seasonal changes of temperature correlated best with observed changes in emissions. The notoriously high spatiotemporal variability of fluxes and the relatively low number of replicates might have prevented a detection of smaller climate change treatment effects. Furthermore, the lower soil moisture at LE could have outbalanced potential stimulating effects of temperature increases, since limited soil moisture can lead to reduced substrate availability for nitrification and denitrification. The lower substrate availability is caused by decreased microbial activity under dry conditions, hampering mineralization and the following N-turnover processes of nitrification and denitrification (Rennenberg et al. 2009). Also Cantarel et al. (2011) did not detect significant changes in N_2O emissions due to climate change for a temperate grassland in France. Notwithstanding this, also the latter study has identified temperature as the main driver for N_2O emissions.

In contrast to the emission measurements on unfertilized intact soil cores, which reflect mainly actual differences in environmental conditions between HE and LE, N gas emissions after slurry addition (exp. II) were observed for standardized soil temperature (18 °C) and moisture (70%WFPS) conditions for HE and LE. Consequently, differences between HE and LE might be related to long-term changes in the soil microbial community (Castro et al. 2010), which were facilitated due to the 6 year long pre-incubation time of the cores at HE or LE before the measurements started. Nonetheless, we did not find significant differences in fertilizer induced emissions of N_2 and NH_3. However, NO emissions were significantly reduced and N_2O emissions were marginally significantly reduced for soil cores taken from the LE compared to HE site. This could be a consequence of decreased abundance of denitrifying bacteria involved in NO and N_2O production like *cnor*B, thus hampering NO and N_2O production at LE site compared to HE (Chen et al. 2015).

Overall, the translocation (climate) effect on N gas fluxes was not significant, while fertilization massively increased N gas emissions from grassland soils. Consequently, improved grassland organic fertilizer management is key to reducing environmental N losses both under present and future climate.

Implications for the grasslands greenhouse gas and N mass balance

The efficient N_2O reduction to N_2 observed in the soils of this study generally point towards low greenhouse gas emission, caused by the high share of inert N_2 emissions and the relatively low emissions of the potent greenhouse gas N_2O. Consequently, complete denitrification until the terminal product is an important function of the soils of this study. However, with regard to the total N amount of measured gaseous losses, ca. 40–50% of the applied slurry N is lost in the first 2 weeks following slurry application and the high annual background emissions of N_2 are further adding up to this. Even considering the high uncertainties accompanying the annual N_2 background emissions, this represents a highly undesired, massive nutrient loss. The high gaseous N losses observed in this study lead to both a low slurry N use efficiency by plants and high environmental N losses which are of importance for the GHG footprint of grassland systems but also regional air quality, affecting human and environmental health (Sutton et al. 2011). In contrast to the gaseous N losses described in this study, Fu et al. (2017) have shown remarkably low NO_3^- leaching rates in the same soils, which again emphasizes the enormous importance of reducing N gas emissions. Since plants nonetheless show enormous productivity in the investigated grassland systems, with plant N uptake and thus N export, often exceeding total slurry N application rates (Unteregelsbacher et al. 2013), we assume for our study sites a high risk of soil N mining and possibly an associated C mining from the soil due to soil organic matter mineralization. This might on the long-term jeopardize economic and environmental functions of the investigated grassland ecosystems such as feed production, nutrient retention and C and N storage. This urgently requires modified climate-adapted management that leads to improved N fertilizer use efficiency, thereby reducing environmental losses and maintaining productivity and soil N resources. Generally, acidification of manure or manure injection techniques (Stevens et al. 1989; Hansen et al. 2003) could be suitable measures. However, still a more detailed process-based understanding of cycling and fate of fertilizer N in the grassland plant-soil system is needed as a basis for the development of such climate-smart management of montane grasslands.

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References

Ammann C, Spirig C, Leifeld J, Neftel A (2009) Assessment of the nitrogen and carbon budget of two managed temperate grassland fields. Agric Ecosyst Environ 133(3–4):150–162

Amon B, Kryvoruchko V, Amon T, Zechmeister-Boltenstern S (2006) Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. Agric Ecosyst Environ 112(2–3):153–162

Bakken LR, Bergaust L, Liu B, Frostegård Å (2012) Regulation of denitrification at the cellular level: a clue to the understanding of N2O emissions from soils. Philos Trans R Soc B 367(1593):1226–1234

BMLEV (2012) Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten der Bundesrepublik Deutschland 2012. Landwirtschaftsverlag GmbH Münster-Hiltrup 606

Bohlen PJ, Groffman PM, Driscoll CT, Fahey TJ, Siccama TG (2001) Plant–soil–microbial interactions in a northern hardwood forest. Ecology 82(4):965–978

Böhm R, Auer I, Brunetti M, Maugeri M, Nanni T, Schöner W (2001) Regional temperature variability in the European Alps: 1760–1998 from homogenized instrumental time series. Int J Climatol 21(14):1779–1801

Bollmann A, Conrad R (1997) Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. Soil Biol Biochem 29(7):1067–1077

Bouwman AF, Lee DS, Asman WA, Dentener FJ, van der Hoek KW, Olivier JG (1997) A global high-resolution emission inventory for ammonia. Glob Biogeochem Cycles 11(4):561–587

Burgin AJ, Groffman PM (2012) Soil O_2 controls denitrification rates and N_2O yield in a riparian wetland. J Geophys Res 117(G1):G01010

Butterbach-Bahl K, Dannenmann M (2011) Denitrification and associated soil N2O emissions due to agricultural activities in a changing climate. Curr Opin Environ Sustain 3(5):389–395

Butterbach-Bahl K, Willibald G, Papen H (2002) Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. Plant Soil 240(1):105–116

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos Trans R Soc B 368(1621):20130122

Butterbach-Bahl K, Diaz-Pines E, Dannenmann M (2014) Soil trace gas emissions and climate change. In: Rosenstock TS, Rufino MC, Butterbach-Bahl K, Wollenberg E, Richards M (eds) Global environmental change. Springer, New York, pp 325–334

Cantarel AAM, Bloor JMG, Deltroy N, Soussana J-F (2011) Effects of climate change drivers on nitrous oxide fluxes in an upland temperate grassland. Ecosystems 14(2):223–233

Cantarel AAM, Bloor JMG, Pommier T, Guillaumaud N, Moirot C, Soussana J, Poly F (2012) Four years of experimental climate change modifies the microbial drivers of N_2O fluxes in an upland grassland ecosystem. Glob Change Biol 18(8):2520–2531

Cardenas LM, Bol R, Lewicka-Szczebak D, Gregory AS, Matthews GP, Whalley WR, Misselbrook TH, Scholefield D, Well R (2017) Effect of soil saturation on denitrification in a grassland soil. Biogeosciences 14(20):4691–4710

Carter MS (2007) Contribution of nitrification and denitrification to N_2O emissions from urine patches. Soil Biol Biochem 39(8):2091–2102

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil microbial community responses to multiple experimental climate change drivers. Appl Environ Microbiol 76(4):999–1007

Chen Z, Wang C, Gschwendtner S, Willibald G, Unteregelsbacher S, Lu H, Kolar A, Schloter M, Butterbach-Bahl K, Dannenmann M (2015) Relationships between denitrification gene expression, dissimilatory nitrate reduction to ammonium and nitrous oxide and dinitrogen production in montane grassland soils. Soil Biol Biochem 87:67–77

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M (2013) Climate change 2013: the physical science basis. In: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change

Colbourn P, Dowdell RJ (1984) Denitrification in field soils. Plant Soil 76(1–3):213–226

Delaune RD, Lindau CW, Sulaeman E, Jugsujinda A (1998) Nitrification and denitrification estimates in a Louisiana swamp forest soil as assessed by 15 N isotope dilution and direct gaseous measurements. Water Air Soil Pollut 106(1–2):149–161

EEA (2016) EMEP/EEA emission inventory guidebook 2016. European Environment Agency, Office for Official Publications of the European Communities, Luxembourg. https://www.eea.europa.eu/publications/emep-eea-guidebook-2016/part-b-sectoral-guidance-chapters/4-agriculture/3-b-manure-management-2016/view. Accessed 27 Sept 2018

Falkowski P, Scholes RJ, Boyle EE, Canadell J, Canfield D, Elser J, Gruber N, Hibbard K, Högberg P, Linder S (2000) The global carbon cycle: a test of our knowledge of earth as a system. Science 290(5490):291–296

Felber R, Conen F, Flechard CR, Neftel A (2012) Theoretical and practical limitations of the acetylene inhibition technique to determine total denitrification losses. Biogeosciences 9(10):4125

Flechard CR, Neftel A, Jocher M, Ammann C, Fuhrer J (2005) Bi-directional soil/atmosphere N_2O exchange over two mown grassland systems with contrasting management practices. Glob Change Biol 11(12):2114–2127

Flechard CR, Ambus P, Skiba U, Rees RM, Hensen A, van Amstel A, Van Den Pol-Van Dasselaar A, Soussana J-F, Jones M, Clifton-Brown J (2007) Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. Agric Ecosyst Environ 121(1–2):135–152

Freney JR, Simpson JR, Denmead OT (1983) Volatilization of ammonia. In: Freney J, Simpson JR (eds) Gaseous loss of nitrogen from plant-soil systems. Springer, New York, pp 1–32

Fu J, Gasche R, Wang N, Lu H, Butterbach-Bahl K, Kiese R (2017) Impacts of climate and management on water balance and nitrogen leaching from montane grassland soils of S-Germany. Environ Pollut 229:119–131

Groffman PM, Altabet MA, Böhlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, Voytek MA (2006) Methods for measuring denitrification: diverse approaches to a difficult problem. Ecol Appl 16(6):2091–2122

Hansen MN, Sommer SG, Madsen NP (2003) Reduction of ammonia emission by shallow slurry injection. J Environ Qual 32(3):1099–1104

Horgan BP, Branham BE, Mulvaney RL (2002) Mass balance of 15 N applied to Kentucky bluegrass including direct measurement of denitrification. Crop Sci 42(5):1595–1601

Horváth L, Grosz B, Machon A, Tuba Z, Nagy Z, Czóbel SZ, Balogh J, Péli E, Fóti SZ, Weidinger T (2010) Estimation of nitrous oxide emission from Hungarian semi-arid sandy and loess grasslands; effect of soil parameters, grazing, irrigation and use of fertilizer. Agric Ecosyst Environ 139(1–2):255–263

Kulkarni MV, Burgin AJ, Groffman PM, Yavitt JB (2014) Direct flux and 15 N tracer methods for measuring denitrification in forest soils. Biogeochemistry 117(2–3):359–373

Liu B, Frostegård Å, Bakken LR (2014) Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. MBio 5(3):e01383

Mosier AR, Guenzi WD, Schweizer EE (1986) Soil losses of dinitrogen and nitrous oxide from irrigated crops in Northeastern Colorado 1. Soil Sci Soc Am J 50(2):344–348

Parton WJ, Scurlock JM, Ojima DS, Schimel DS, Hall DO, Scopegram Group Members (1995) Impact of climate change on grassland production and soil carbon worldwide. Glob Change Biol 1(1):13–22

Raciti SM, Burgin AJ, Groffman PM, Lewis DN, Fahey TJ (2011) Denitrification in suburban lawn soils. J Environ Qual 40(6):1932–1940

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326(5949):123–125

Rennenberg H, Dannenmann M (2015) Nitrogen nutrition of trees in temperate forests—the significance of nitrogen availability in the pedosphere and atmosphere. Forests 6(8):2820–2835

Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H (2009) Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biol 11:4–23

Rolston DE, Hoffman DL, Toy DW (1978) Field measurement of denitrification: I. flux of N_2 and N_2O 1. Soil Sci Soc Am J 42(6):863–869

Saggar S, Jha N, Deslippe J, Bolan NS, Luo J, Giltrap DL, Kim D-G, Zaman M, Tillman RW (2013) Denitrification and N_2O: N_2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. Sci Total Environ 465:173–195

Schaufler G, Kitzler B, Schindlbacher A, Skiba U, Sutton MA, Zechmeister-Boltenstern S (2010) Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature. Eur J Soil Sci 61(5):683–696

Scheer C, Wassmann R, Butterbach-Bahl K, Lamers JPA, Martius C (2009) The relationship between N_2O, NO, and N_2 fluxes from fertilized and irrigated dryland soils of the Aral Sea Basin, Uzbekistan. Plant Soil 314(1–2):273

Schierl R (1991) Saure Perkulation von Bodenproben aus dem Höglwald. Vergleich von Labor- und Freilandversuchen, Paul Parey

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. Proc Natl Acad Sci 106(1):203–208

Scholefield D, Hawkins JM, Jackson SM (1997) Development of a helium atmosphere soil incubation technique for direct measurement of nitrous oxide and dinitrogen fluxes during denitrification. Soil Biol Biochem 29(9–10):1345–1352

Smiatek G, Kunstmann H, Knoche R, Marx A (2009) Precipitation and temperature statistics in high-resolution regional climate models: evaluation for the European Alps. J Geophys Res 114(D19):78

Sommer SG, Hutchings NJ (2001) Ammonia emission from field applied manure and its reduction. Eur J Agron 15(1):1–15

Soosaar K, Mander Ü, Maddison M, Kanal A, Kull A, Lõhmus K, Truu J, Augustin J (2011) Dynamics of gaseous nitrogen and carbon fluxes in riparian alder forests. Ecol Eng 37(1):40–53

Soussana J, Loiseau P, Vuichard N, Ceschia E, Balesdent J, Chevallier T, Arrouays D (2004) Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use Manag 20(2):219–230

Spirig C, Flechard CR, Ammann C, Neftel A (2010) The annual ammonia budget of fertilised cut grassland—Part 1: micrometeorological flux measurements and emissions after slurry application. Biogeosciences 7(2):521–536

Stevens RJ, Laughlin RJ, Frost JP (1989) Effect of acidification with sulphuric acid on the volatilization of ammonia from cow and pig slurries. The Journal of Agricultural Science 113(3):389–395

Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B (2011) The European nitrogen assessment: sources, effects and policy perspectives. Cambridge University Press, Cambridge

Unteregelsbacher S, Gasche R, Lipp L, Sun W, Kreyling O, Geitlinger H, Kögel-Knabner I, Papen H, Kiese R, Schmid H (2013) Increased methane uptake but unchanged nitrous oxide flux in montane grasslands under simulated climate change conditions. Eur J Soil Sci 64(5):586–596

Uri V, Lõhmus K, Mander Ü, Ostonen I, Aosaar J, Maddison M, Helmisaari H-S, Augustin J (2011) Long-term effects on the nitrogen budget of a short-rotation grey alder (*Alnus incana* (L.) Moench) forest on abandoned agricultural land. Ecol Eng 37(6):920–930

Wagner S, Berg P, Schädler G, Kunstmann H (2013) High resolution regional climate model simulations for Germany: part II—projected climate changes. Clim Dyn 40(1–2):415–427

Wang R, Feng Q, Liao T, Zheng X, Butterbach-Bahl K, Zhang W, Jin C (2013) Effects of nitrate concentration on the denitrification potential of a calcic cambisol and its fractions of N_2, N_2O and NO. Plant Soil 363(1–2):175–189

Wang C, Dannenmann M, Meier R, Butterbach-Bahl K (2014) Inhibitory and side effects of acetylene (C_2H_2) and sodium chlorate (NaClO3) on gross nitrification, gross ammonification and soil-atmosphere exchange of N_2O and CH_4 in acidic to neutral montane grassland soil. Eur J Soil Biol 65:7–14

Wang C, Chen Z, Unteregelsbacher S, Lu H, Gschwendtner S, Gasche R, Kolar A, Schloter M, Kiese R, Butterbach-Bahl K (2016) Climate change amplifies gross nitrogen turnover in montane grasslands of Central Europe in both summer and winter seasons. Glob Change Biol 22(9):2963–2978

Wen Y, Chen Z, Dannenmann M, Carminati A, Willibald G, Kiese R, Wolf B, Veldkamp E, Butterbach-Bahl K, Corre MD (2016) Disentangling gross N_2O production and consumption in soil. Sci Rep 6:36517

Wiesmeier M, Hübner R, Barthold F, Spörlein P, Geuß U, Hangen E, Reischl A, Schilling B, von Lützow M, Kögel-Knabner I (2013) Amount, distribution and driving factors of soil organic carbon and nitrogen in cropland and grassland soils of southeast Germany (Bavaria). Agric Ecosyst Environ 176:39–52

Wray HE, Bayley SE (2007) Denitrification rates in marsh fringes and fens in two boreal peatlands in Alberta, Canada. Wetlands 27(4):1036–1045

Yang WH, Silver WL (2012) Application of the N2/Ar technique to measuring soil-atmosphere N2 fluxes. Rapid Commun Mass Spectrom 26(4):449–459

Zuazo P (2016) Development of a fully automated soil incubation and gas sampling system for quantifying trace gas emission pulses from soils at high temporal resolution, Albert-Ludwigs-Universität Freiburg im Breisgau

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61(4):533–616