LETTERS

Grazing-induced reduction of natural nitrous oxide release from continental steppe

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Atmospheric concentrations of the greenhouse gas nitrous oxide (N_2O) have increased significantly since pre-industrial times owing to anthropogenic perturbation of the global nitrogen cycle[1,2], with animal production being one of the main contributors[3]. Grasslands cover about 20 per cent of the temperate land surface of the Earth and are widely used as pasture. It has been suggested that high animal stocking rates and the resulting elevated nitrogen input increase N_2O emissions[4–7]. Internationally agreed methods to upscale the effect of increased livestock numbers on N_2O emissions are based directly on per capita nitrogen inputs[8]. However, measurements of grassland N_2O fluxes are often performed over short time periods[9], with low time resolution and mostly during the growing season. In consequence, our understanding of the daily and seasonal dynamics of grassland N_2O fluxes remains limited. Here we report year-round N_2O flux measurements with high and low temporal resolution at ten steppe grassland sites in Inner Mongolia, China. We show that short-lived pulses of N_2O emission during spring thaw dominate the annual N_2O budget at our study sites. The N_2O emission pulses are highest in ungrazed steppe and decrease with increasing stocking rate, suggesting that grazing decreases rather than increases N_2O emissions. Our results show that the stimulatory effect of higher stocking rates on nitrogen cycling[4,7] and, hence, on N_2O emission is more than offset by the effects of a parallel reduction in microbial biomass, inorganic nitrogen production and wintertime water retention. By neglecting these freeze–thaw interactions, existing approaches may have systematically overestimated N_2O emissions over the last century for semi-arid, cool temperate grasslands by up to 72 per cent.

In temperate ecosystems with long frost periods, distinct freeze–thaw periods can occur. These periods can contribute significantly to annual N_2O budgets[10–12] and need to be better understood in relation to alterations in land use and agricultural practice. To address these interactions, we deployed several N_2O flux measurement systems in the steppe grassland of Inner Mongolia. Our measurement strategy was designed to address both sub-daily and seasonal dynamics, and to consider the relationships between N_2O fluxes, grassland management and the underlying plant and soil processes.

The data set we report represents year-round (August 2007–August 2008) N_2O flux measurements at high and low temporal resolution at ten sites differing in stocking rate (Table 1). All sites are typical steppe grasslands belonging to the Inner Mongolia Grassland Ecosystem Research Station, Chinese Ecosystem Research Network (43° 33′ N, 116° 42.3′ E). At two of the sites, fluxes were measured every three hours using an automatic system combining triplicate automatic chambers with online analysis. These two sites compared grassland that had been ungrazed since 1999 (UG99) with grassland that was grazed only during winter (WG). At the remaining eight sites, manual replicated chambers were deployed, allowing samples to be taken weekly. Supporting measurements included soil temperature and water-filled pore space (WFPS), gross rates of nitrogen mineralization and soil microbial-biomass nitrogen.

Our automated N_2O flux measurements show that N_2O fluxes at both the ungrazed and the winter-grazed sites were low and close to the detection limit during both the growing season and most of the winter. However, at the end of the winter, during the spring thaw, a large pulse of N_2O emissions, lasting for approximately eight weeks, was observed at the ungrazed site. Contrary to expectations, there was no matching pulse of N_2O emissions at the winter-grazed site (Fig. 1a, b). Our sub-daily measurements also showed pronounced diurnal variations

Table 1 | Annual and seasonal cumulative fluxes of N_2O

Site	Number of chambers	Grazing intensity*; stocking rate (sheep per hectare)	Duration of grazing period (d)	AE ± s.e. (kg N ha^{-1})	SE ± s.e. (kg N ha^{-1})	Group mean of SE ± s.e. (kg N ha^{-1})	GE ± s.e. (kg N ha^{-1})	Ratio of SE to AE (%)
UG99	3	Ungrazed; 0	—	0.22 ± 0.07	0.15 ± 0.04	0.17 ± 0.01†	0.06 ± 0.03	68
UG1	3	Ungrazed; 0	—	0.28 ± 0.05	0.23 ± 0.06		0.02 ± 0.01	81
UG2	3	Ungrazed; 0	—	0.17 ± 0.03	0.11 ± 0.03		0.04 ± 0.01	66
L1	4	Light; 0.92	93	0.20 ± 0.03	0.15 ± 0.03	0.10 ± 0.01†‡	0.01 ± 0.01	77
L2	4	Light; 0.51	93	0.10 ± 0.02	0.03 ± 0.1		0.04 ± 0.02	35
M1	4	Moderate; 1.45	93	0.15 ± 0.05	0.09 ± 0.03	0.06 ± 0.01‡§	0.04 ± 0.05	60
M2	4	Moderate; 0.99	93	0.11 ± 0.04	0.04 ± 0.02		0.05 ± 0.01	34
WG	3	Heavy; 2.05	166	0.01 ± 0.03	−0.01 ± 0.003	0.01 ± 0.01§	0.03 ± 0.01	0
H1	4	Heavy; 2.24	93	0.12 ± 0.01	0.02 ± 0.01		0.06 ± 0.02	16
H2	4	Heavy; 1.94	93	0.17 ± 0.03	0.01 ± 0.001		0.11 ± 0.03	9

$N = 3$ for UG99, UG1, UG2, WG; $N = 4$ for other sites. AE, annual emission; GE, growing-season emission; SE, spring-thaw emission.
* Grazing intensity classes derived from herbage allowance. Herbage allowance (HA; kilograms of dry biomass per kilogram of life weight) is a measure of the amount of biomass available per mass of grazing animal. Classes are as follows: heavy grazing (HA < 2.5); moderate grazing (2.5 < HA < 7.5); light grazing (HA > 7.5).
†‡§ Quantities marked with different symbols differ significantly ($P < 0.05$) with respect to grazing intensity.

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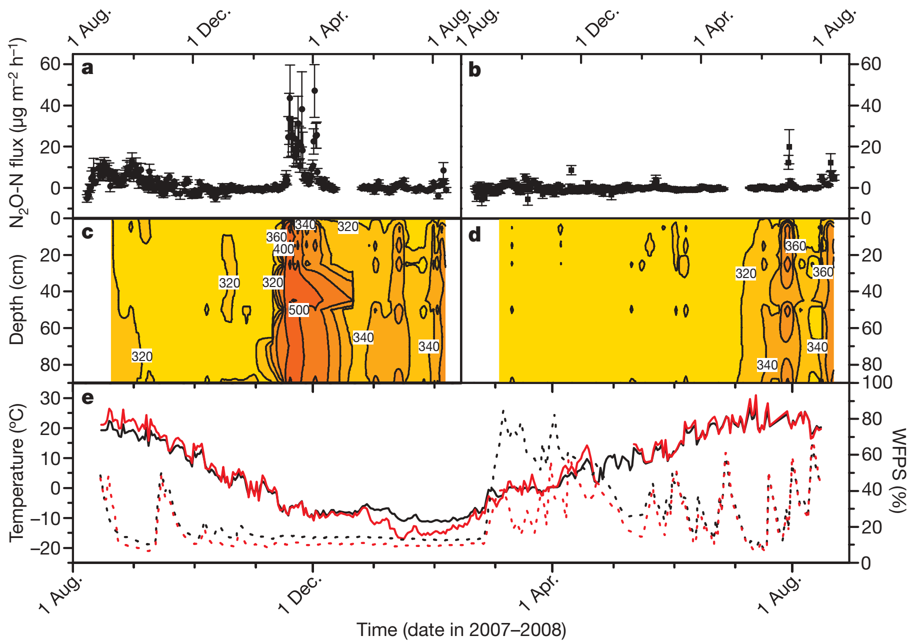

Figure 1 | Dynamics of N_2O fluxes, soil air concentrations and environmental parameters. **a**–**d**, Steppe grassland ungrazed since 1999 (UG99; **a**, **c**) is contrasted with a winter-grazed site (WG; **b**, **d**). Measured fluxes of nitrogen from N_2O (N_2O-N) are shown in **a** and **b** (error bars, s.e.; $N = 3$), and soil N_2O concentrations are shown in parts per 10^9 (contours) as a function of depth in **c** and **d**. **e**, Soil environmental conditions, contrasting temperature (solid lines) and WFPS (dashed lines) for UG99 (black) and WG (red).

of N_2O fluxes at the ungrazed site during this period (Supplementary Fig. 2).

Similar results highlighting the influence of grazing were also obtained at the sites where manual N_2O flux measurements were made. Pulse emissions of N_2O were most pronounced at sites that were either ungrazed or grazed at low intensity, but were hardly detectable or not detectable at higher stocking rates (Fig. 2). Correlation analysis revealed that N_2O fluxes during spring thaw were significantly negatively correlated with stocking rate ($r^2 = 0.57$ (coefficient of determination), $P < 0.05$; Supplementary Information). The spring-thaw N_2O pulses dominated the total annual N_2O emission and on average accounted for 72% of the annual emission for ungrazed sites and 8% of the annual emission for the heavily grazed sites (Table 1). Other studies show that increasing stocking rates stimulate soil N_2O emissions during the growing season[4–7]. These reports are not in contradiction to our findings, as in our study we also found a significant positive correlation ($P < 0.05$) between the stocking rate and the contribution of the growing-season emissions to the annual N_2O budget. However, because the effect of the stocking rate in suppressing spring-thaw emissions was much more pronounced, our results demonstrate that grazing decreased rather than increased annual N_2O fluxes.

Weekly measurements of N_2O in the gas profile showed that soil N_2O concentrations increased only at the start of soil thawing. Therefore, the observed peak N_2O emission during spring thaw was not due to an accumulation of N_2O in the profile during the preceding winter. Measurements of N_2O production rates in stratified soil samples further support the notion that spring-thaw N_2O fluxes were the result of instantaneous microbial production (Fig. 1c, d), with the main production of N_2O occurring in the topmost layer (Supplementary Figs 3 and 4). Therefore, grazing must have changed the soil and environmental conditions that determine emissions of N_2O during the spring thaw. With increasing stocking rate, the aboveground biomass and vegetation height decreased at our sites[13]. Vegetation height is the determining factor in snow-holding capacity, such that snow is more quickly eroded at grazed sites with sparse or low vegetation than at sites with denser and taller vegetation[14], as also observed in our study (Supplementary Table 1). Moreover, we found that the grazed sites with lower vegetation/snow cover have significantly higher freezing rates (Wilcoxon rank-sum test, $P < 0.05$; Supplementary Fig. 6) and lower winter soil temperatures but increased soil temperature fluctuations on daily and annual scales. The decreased snowpack (analysis of variance, $P < 0.05$) at the grazed sites was associated with reduced soil moisture during snow melt (Fig. 1e). At site UG99, WFPS values were on average 61% in spring (March to April) 2008, whereas at site WG, WFPS was on average 37%. Accompanying measurements of soil microbial-biomass nitrogen showed that at UG99 and WG, microbial populations decreased by 80% with the onset of winter. However, the recovery of the microbial biomass during the winter was faster at UG99, most probably because soil temperatures were not as low as at WG. In addition, gross nitrogen mineralization at the ungrazed site was significantly higher than at the winter-grazed site (Supplementary Fig. 1).

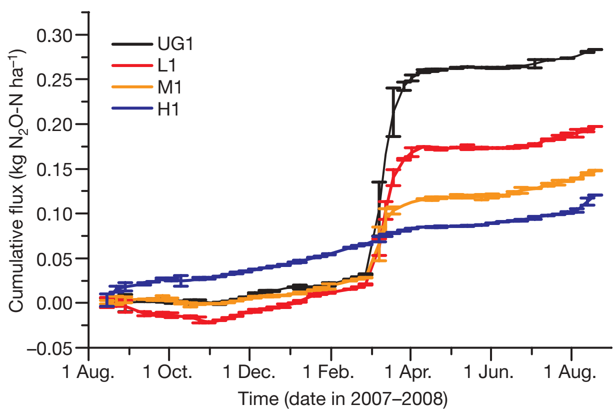

Figure 2 | Effect of stocking rate on cumulative N_2O fluxes, as recorded using the manual-chamber approach. Error bars show uncertainty over the time span between two consecutive measurements. Uncertainty calculations were based on the spatial variability of observed fluxes (s.e.; $N = 4$ for L1, M1 and H1; $N = 3$ for UG1). Further site information is provided in Table 1.

The dominating role of soil moisture in controlling soil nitrogen cycling and N_2O losses is indicated by strong positive correlations between soil water content and both microbial-biomass nitrogen ($r^2 = 0.90$, $P < 0.001$ at WG; $r^2 = 0.81$, $P < 0.001$ at UG99) and N_2O emissions. The correlations between N_2O fluxes and soil moisture were weaker but were still significant ($r^2 = 0.11$, $P < 0.001$ at WG; $r^2 = 0.32$, $P < 0.001$ at UG99).

On the basis of our results, a general mechanism can be identified that drives the interaction between grazing and freeze–thaw N_2O emissions. It is well established that tall vegetation accumulates snow[14] and that, with increasing snow cover, the soil is effectively insulated from low winter temperatures[15]. The temperature effect is of importance for supporting microbial growth during winter, because a critical threshold seems to exist at approximately −10 °C (ref. 16). Above this temperature, a vital microbial community can survive, and is further activated by the onset of thawing in spring[17]. Our data, as well as earlier studies[18,19], demonstrate that freeze–thaw N_2O emissions increase with increasing soil moisture values and are highest at a WFPS value of around 60%. Our explanation that snowpack temperature insulation and soil moisture effects are driving freeze–thaw N_2O pulses is also supported by a study at a prairie site in Colorado, USA[20]. Relative to a grazed site, the site with re-established grassland vegetation showed larger winter N_2O emissions, quantitatively consistent with our own findings for Asian steppe (Supplementary Information)[20].

To investigate whether our finding of grazing-induced reductions in N_2O emission could be meaningful on a global scale, we identified grazed grasslands[21] that are climatically similar (data set CRU CL 2.0; ref. 22) to our study sites and the prairie site in Colorado[20]. For this, we defined semi-arid, cool temperate grasslands as follows: at least 90% of the days during three consecutive winter months have frost (for sensitivity analysis we also used values of 92.5% and 95%); winter precipitation is >5 mm; annual precipitation is <600 mm. We further excluded North American tallgrass prairie as it may capture snow even if grazed (Supplementary Figs 7 and 8).

For the selected grasslands (29–35% of grasslands in temperate zones), we calculated historic and recent livestock-related N_2O emissions using internationally agreed methodology[8] (Supplementary Information) and tested two possible situations: in the first (S1), we considered the effects of livestock numbers and management practices, without application of synthetic nitrogen fertilizer, this being representative of many developing countries; in the second (S2), we considered the additional effect of fertilizer application rate. In S2 we assumed that application of synthetic fertilizer to pastures in temperate grasslands started in 1951 and increased linearly to a value corresponding to 23 kg N ha^{-1} yr^{-1} (ref. 23). We did not consider run-off of nitrate and downstream production of N_2O. We have not observed this process at our sites, but cannot exclude its occurrence elsewhere.

The main driving factor in our calculations was the numbers of grazers. We calculated livestock-related N_2O emissions for 1890–2000 in two ways per situation (Fig. 3): on the basis of the number of grazing animals (cattle, buffalo, goats and sheep)[24–27] according to the approach of the IPCC[8]; and considering the decreasing effect of increasing intensification of livestock farming (expressed by animal number) on background spring-time N_2O emissions, as demonstrated in our measurements (Table 1; see Supplementary Information for the calculation methodology). These estimates suggest that, according to the IPCC methodology[4], the N_2O emission from semi-arid, cool temperate pasture systems could be overestimated by 72% (S1) or, if fertilization is considered, 36% (S2).

The measurements presented here reveal a mechanism that may be relevant to grasslands in semi-arid, cool temperate regions in general. Our results are therefore likely to be pertinent in the context of the current debate on the global magnitude of N_2O emissions from agriculture[3,28]. The anthropogenic modification of so-called 'background fluxes', which we report here, demonstrates the complexity and high degree of uncertainty in constraining the global N_2O budget, highlighting the need to develop more sophisticated upscaling approaches. As our measurements in the steppe of Inner Mongolia show, human intervention—here grazing of livestock—has the potential to reduce natural background N_2O fluxes significantly. An understanding of these controlling mechanisms also provides pointers to identifying improved N_2O mitigation strategies. In addition to the effects of grazing, it is likely that cutting and haymaking at the end of the growing season have the potential to decrease spring-thaw N_2O emissions owing to the above-mentioned effect of vegetation height on snow accumulation. Although the possible trade-offs with water balance, snow drift and grass productivity would need to be quantified for different landscape types, such simple management practices could have a significant role in reducing N_2O emissions from temperate grasslands on a global scale.

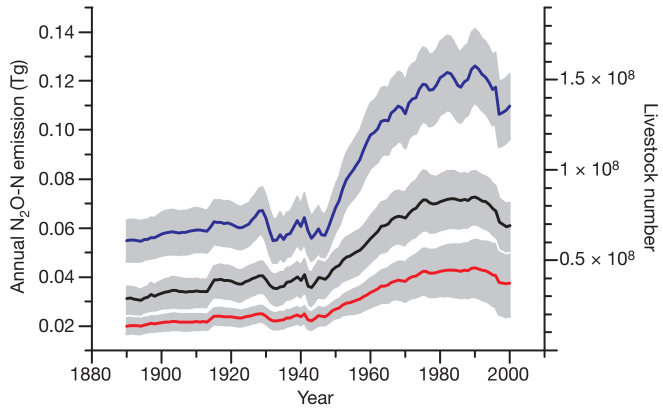

Figure 3 | Annual N_2O emissions and livestock numbers between 1890 and 2000 for situation S1. N_2O emissions due to increases in animal numbers determined using the default methodology of the Intergovernmental Panel on Climate Change[8] (IPCC; black); livestock numbers (cattle, buffalo, sheep and goats; blue); and N_2O emissions corrected for the decrease in spring-thaw fluxes as calculated here (red). The traces and the grey shaded areas represent mean ± s.d. of calculations for different frost-day climate criteria (90, 92.5 and 95% frost days in three consecutive winter months). The shaded areas thus indicate differences in the results due to changes in the extent of the projection area. The decrease in animal numbers since 1980 is due to more efficient livestock farming in developed countries.

METHODS SUMMARY

In the automatic-chamber system, N_2O analysis was conducted using an online gas chromatograph with electron capture detection (SRI 8610C, Texas Instruments), calibrated against N_2O standards with concentrations of 400 parts per 10^9 by volume (Air Liquide). In the manual-chamber system, air samples were collected in 100-ml syringes and analysed the same day by gas chromatography (6820D, Agilent Technologies). We calculated fluxes from the increase in N_2O concentration in the chamber and corrected them for atmospheric pressure and chamber air temperature. Each site was equipped with at least three chambers of at least 40 cm × 40 cm base area. The flux detection limit was <2 μg N_2O-N m^{-2} h^{-1}. We measured soil N_2O concentrations using gas-permeable tubes at different depths.

The ^{15}N pool dilution technique[29] was applied to measure gross rates of nitrogen mineralization (ammonification) at three replicated plots for each of the sites UG99 and WG at fortnightly to monthly intervals. Microbial-biomass nitrogen was determined by use of the chloroform-fumigation extraction method[29]. We determined the microbial biomass and related parameters within the top 10 cm of the soil. Soil temperature was determined at depths of 1, 5, 10 and 20 cm, and soil moisture was determined in the top 5 cm.

For statistical analysis of N_2O fluxes from the ten sites during spring thaw, we aggregated UG99 and WG data to the same measurement frequency as the sites with low temporal measurement resolution ($n = 7$). In the case of non-normality (Shapiro–Wilk test) and different variances (Bartlett test) of the N_2O flux data, we used multiple pairwise Wilcoxon tests to test for significant differences in spring-thaw fluxes between grazing intensities. We corrected P values for multiple testing. The Wilcoxon test was further applied to test for significant differences in microbial-biomass and gross nitrogen mineralization between UG99 and WG.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions K.B.-B., N.B., X.Z. and M.D. designed the experiment. B.W., W.C. and Z.Y. carried out the flux measurements. H.W. conducted the microbiological measurements. B.W., W.C., H.W. and M.D. performed data analysis. B.W. carried out the upscaling for temperate grasslands. M.A.S., K.B.-B., N.B., M.D. and B.W. drafted the manuscript.

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