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Spatial variability and hotspots of soil N₂O fluxes from intensively grazed grassland

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Abstract

One hundred N₂O flux measurements were made from an area of intensively managed grazed grassland in central Scotland using a high resolution dynamic chamber method. The field contained a variety of features from which N₂O fluxes were measured including a manure heap, patches of decaying grass silage, and areas of increased sheep activity. Individual fluxes varied significantly across the field varying from 2 to 79 000 µg N₂O-N m⁻² h⁻¹. Soil samples were collected at 55 locations to investigate relationships between soil properties and N₂O flux. Fluxes of N₂O correlated strongly with soil NO₃⁻ concentrations. Distribution of NO₃⁻ and the high spatial variability of N₂O flux across the field are shown to be linked to the distribution of waste from grazing animals and the resultant reactive nitrogen compounds in the soil which are made available for microbiological processes. Features within the field such as shaded areas and manure heaps contained significantly higher available nitrogen than the rest of the field. Although these features only represented 1.1 % of the area of the field, they contributed to over 55 % of the total estimated daily N₂O flux.

1 Introduction

Nitrous oxide (N₂O) is the single largest contributor to global stratospheric ozone depletion (Ravishankara et al., 2009) and a potent greenhouse gas (GHG). N₂O is formed naturally in soils and aquatic environments, primarily as a by-product of the microbial processes of nitrification and denitrification (e.g. Davidson et al., 2000; Wrage et al., 2001). Agricultural activities such as the use of nitrogen fertilisers and livestock farming have dramatically altered the natural nitrogen cycle in agricultural environments resulting in significantly increased global emissions of N₂O since pre-industrial times (IPCC, 2007). Agriculture is believed to be the largest source of global anthropogenic N₂O emissions with estimates as high as 80 % of all anthropogenic emissions due directly or indirectly to agricultural activities (Isermann, 1994; IPCC, 2007).

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Large scale N₂O flux estimates for terrestrial sources are often subject to large and poorly defined uncertainties which can limit the effectiveness of mitigation efforts in the agricultural sector (e.g. Bouwman et al., 1995; Oenema et al., 2005). Even estimates of N₂O fluxes from agricultural sources at much finer scales (i.e. the plot and farm scale) can be highly uncertain. This is predominately caused by the large temporal and spatial variability of N₂O fluxes due to the high heterogeneity of soil properties and microbiological processes (Parkin, 1987; Zhu et al., 2013; Chadwick et al., 2014). Soil properties which are believed to increase N₂O emissions by influencing the nitrification and denitrification processes include available nitrogen (in the form of ammonium (NO₄⁺) and nitrate (NO₃⁻)), available organic carbon, oxygen supply and pH (Bateman and Baggs, 2005; Davidson and Verchot, 2000). Although it is known that these properties can alter N₂O production in soils, it is still difficult to accurately simulate the net effect on N₂O fluxes from areas (that are often considered to be homogeneous land cover) such as agricultural fields used for arable crops and grazing of livestock due to the heterogeneous nature of microbial populations and nitrogen availability in soils (Conen et al., 2000; Jarecki et al., 2008; Oenema et al., 1997).

The two main flux measurement methods applied to the field scale for N₂O in agricultural areas are the flux chamber method and the eddy covariance method (e.g. Jones et al., 2011; Skiba et al., 2012). Chamber fluxes are measured over a number of enclosed areas (typically < 1 m²) on a field, and a mean or median flux estimate is extrapolated to the farm, field or regional scale: the combination of upscaling with the large spatial variability of N₂O sources often results in very significant uncertainty when estimating N₂O fluxes (Velthof et al., 1996). The advantage of using the eddy covariance method is that it can measure and integrate flux data directly over areas greater than 100 m² continuously without disturbing the soil or air environment. For large homogeneous areas, which are well represented by an integrated value of flux, the eddy covariance approach is ideal, but it does not address the issue of spatial variability on reported fluxes within the measurement area. Eddy covariance also requires fast, sen-

sitive equipment that often demands high power supply and so it can be an expensive option (Hensen et al., 2013).

In this experiment a high precision dynamic chamber method (Cowan et al., 2014) was used to make 100 flux measurements of N₂O from an intensively managed grassland field which contained several features associated with elevated N₂O fluxes. Soil NH₄⁺, NO₃⁻, total carbon, total nitrogen, water filled pore space (WFPS %), bulk density and pH were recorded from 55 out of 100 flux measurement locations. The aims of the experiment were: (i) to measure the spatial variability of N₂O fluxes at a field scale, (ii) to try to identify the main drivers of this variability, and (iii) to provide better understanding of how N₂O flux estimates from agricultural soils can be improved.

2 Materials and methods

2.1 Field site

Flux measurements were carried out at an intensively managed grassland field owned by the University of Edinburgh (55°52'1.2144" N, 3°12'39.564" W) (Fig. 1). This 6.78 ha field contained approximately 140 sheep (a mixture of ewes and lambs) during the 3 day measurement period between the 8 to the 10 July 2013. This field had been used to graze predominately sheep for at least the last decade with regular nitrogen fertiliser application. The field contained several interesting features that provided the opportunity to measure N₂O fluxes from soils with a wide range of properties. The vast majority of the field (98.62 % of the study area) could be classed as typical grazed grassland in which sheep were free to roam during the measurement period. The sheep had been present on the field for several months giving us the opportunity to measure from suspected hotspots of N₂O flux where sheep droppings had collected on the grass. A drinking trough was situated in a shaded area under several large mature trees with wide leaf coverage at the north end of the field. The sheep had spent a lot of time in this shaded area due to the warm weather during the past 2 to 3 weeks before

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measurements were made. This behaviour was observed during recent measurements carried out in adjacent fields unrelated to this study. Several flux measurements were made in the shaded area to investigate the effect that the recent increase in sheep density in this area had on N₂O flux.

Patches of decayed grass silage were visible in two small areas of the field. These patches remained after silage bales had been placed in the fields to feed sheep over the winter months. The patches had scarred the grassland leaving small areas of bare soil with decayed grass matter still present. Fluxes from both of these patches were measured during the experiment. A small running stream crosses the north side of the field which helped with drainage. Several flux measurements were made from the stream using the dynamic chamber to investigate if it was a significant source of N₂O.

One particular area of interest was a large manure heap which was situated in the Northeast corner of the field. This heap was a semi-permanent feature which had been used to fertilise a nearby barley field on several occasions. The heap reached a height of up to 3 metres and covered approximately 100 m² of the field, with a wider perimeter of contaminated soil. The area of influence of the manure heap contamination was uncertain due to consistent build up and removal of the heap over several years. A scarred area around the heap was visible with no grass present for several metres. The scarred grassland was used as an indicator of the area of contamination of the manure heap. Measurements were made on the heap, from soils near the base of the heap and on the contaminated soils surrounding the heap at varying distances to investigate the spatial variability of this particular feature of the field.

2.2 Dynamic chamber method

N₂O flux measurements were made using a non-steady-state flow-through (or closed dynamic) chamber system which circulated air between a flux chamber and a quantum cascade laser (QCL) gas analyser via an air pump (SH-110, Varian Inc, CA, USA) (for a full description of the system see Cowan et al., 2014). A compact continuous wave QCL (CW-QC-TILDAS-76-CS, Aerodyne Research Inc., Billerica, MA, USA) was

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used to measure gas mixing ratios within the dynamic chamber system (with a detection limit of approximately 30 nmol mol⁻¹ s⁻¹ for N₂O). The instrument was secured inside a 4 wheel drive vehicle to allow mobile measurements. A diesel generator was kept on a tow trailer which provided electricity to the system. The chamber was placed onto circular aluminium collars which were inserted several cm into the soil (on average 5 cm) and almost flush to the soil, prior to each measurement. Neoprene sponge formed an airtight seal between the chamber and the collar. When used to measure from the stream in the field the chamber was held steady in place by hand with the bottom slightly under the surface of the water. Two 30 m lengths of 3/8 inch ID Tygon[®] tubing were attached to both the inlet of the analyser and the outlet of the pump. This provided a 30 m radius from the vehicle in which the chamber could be placed. A flow rate of approximately 6 to 7 L min⁻¹ was used between the analyser and the chamber.

Fluxes of N₂O were calculated using linear and non-linear asymptotic regression methods using the HMR package for the statistical software R (Levy et al., 2011; Pedersen et al., 2010). Using a mixture of goodness-of-fit statistics and visual inspection the regression method that provided the best fit for the time series of concentration was chosen for each individual measurement. The rate of change in concentration of a particular gas can then be used to calculate the soil flux for each measurement according to Eq. (1).

$$F = \frac{dC}{dt_0} \cdot \frac{\rho V}{A} \quad (1)$$

Where F is gas flux from the soil ($\mu\text{mol m}^{-2} \text{s}^{-1}$), dC/dt_0 is the initial rate of change in concentration with time in $\mu\text{mol mol}^{-1} \text{s}^{-1}$, ρ is the density of air in mol m^{-3} , V is the volume of the chamber in m^3 and A is the ground area enclosed by the chamber in m^2 .

2.3 Soil sampling and analysis

Fifty five of the one hundred locations from which dynamic chamber measurements were made were selected for soil analysis. From these locations 5 cm deep soil samples were taken from inside the chamber collar using a 2 cm wide corer immediately after the flux measurement was completed. These soils were used to calculate soil pH and available nitrogen in the form of ammonium (NO_4^+) and nitrate (NO_3^-) via KCl extraction (see below). Soil cores were taken immediately after the flux measurement using a sharp metal cutting cylinder (7.4 cm diameter, 5 cm deep) which was carefully hammered into undisturbed soil. Samples were used to calculate total carbon and nitrogen content of the soil, soil moisture content (via oven drying at 100°C) and WFPS % as well as bulk density. WFPS% was calculated from the bulk density soil samples using Eq. (2) (Rowell, 1994).

$$\text{WFPS}\% = \frac{V_{\text{cont}} \times 100}{1 - \left(\frac{r_b}{r_d}\right)} \quad (2)$$

Where WFPS% is the percentage of porous volume in the soil filled by water, V_{cont} is the volumetric water content of the soil, r_b is the bulk density of the soil in g cm^{-3} and r_d is the particle density of the soil (assumed as 2.65 g cm^{-3}) (Rowell, 1994).

KCl extractions were carried out on 15 g un-dried soil samples (kept frozen until extraction) using 1 mol KCl solution. Concentrations of NH_4^+ and NO_3^- were measured using a Bran and Luebbe AutoAnalyser (SPX Flow Technology, Norderstedt, Germany). The mass of available nitrogen in the soil was calculated using Eq. (3).

$$N = \frac{C \times V}{m} \quad (3)$$

Where N is the mass of nitrogen in the form of NH_4^+ or NO_3^- in g (per kg of soil), C is the concentration of NH_4^+ or NO_3^- measured in the analysis of KCl extract in mg L^{-1} ,

V is the volume of solution in which the soil sample was mixed with KCl in L, and m is the mass of dry soil mixed with the KCl solution in g.

3 Results

3.1 Variation in N_2O fluxes at the field scale

5 The 3-day measurement period (8 to 10 July 2013) was very dry with no rainfall and relatively low soil moisture contents (ranging from 9 to 50 % WFPS). Daily temperatures were similar, with mean daytime soils temperatures recorded as 15.7, 16.6 and 15.9 °C on the 8, 9 and 10 July respectively. Flux measurement locations were chosen using a mixture of a grid approximately 30 m × 30 m across the field and a selection of fea-
10 ture areas in which multiple measurements were made in close proximity (See Fig. 1). 50 measurements were made on what was considered “normal” grassland across the field. This provided an estimate of the spatial variability of N_2O flux across the field without interference from the hotspot features. Chamber placement on the grassland area included some locations where sheep droppings were present. These locations
15 were noted during measurements when visible. Fluxes from the grassland followed a geometric (log-normal) distribution ranging between 2 to 227 $\mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$, with an arithmetic and geometric mean value of 25 and 13 $\mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$ respectively (Fig. 2). No negative fluxes of N_2O were measured during this experiment at any of the locations. Droppings were present at locations where the two largest fluxes were
20 measured from the grassland (227 and 132 $\mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$), although fluxes measured at other locations which contained droppings were not always larger than those observed on clear (dropping-free) grassland (Fig. 2).

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3.2 Silage and Shaded Patch Fluxes

Two features which were measured in more detail were patches of the field which contained the remains of decayed grass silage and a large area shaded by trees in which the sheep had spent much of their time due to the warm weather. A total of 7 flux measurements were made over 2 patches of decayed grass silage (Fig. 3a). Only small residues of the grass silage were visible, mixed in with the soil in these areas as the sheep had consumed the majority of it months before the measurement period. The patches were easily visible due to the lack of grass on the bare soil where the silage bales had been left. N₂O fluxes measured from these plots were higher than those measured from the grassland area. Fluxes varied from 1160 to 13 393 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ (Fig. 3a). The arithmetic and geometric mean values of these fluxes were 3745 and 2664 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ respectively.

5 flux measurements were made in the shaded area in which the sheep had access to a water trough. These fluxes varied between 200 and 9600 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ (Fig. 3b). The arithmetic and geometric mean values of these fluxes were 2983 and 1217 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ respectively. The precise area which had been influenced by increased sheep activity was difficult to measure for certain, although an increased number of animal droppings, clumps of wool and damp urine patches were visible in this area of the field. The two measurements made in the centre of the shaded area appeared to contain more animal droppings and emit higher fluxes, whereas the outer perimeter appeared more similar to the surrounding grassland area and fluxes were lower. It was likely that the additional presence of sheep had influenced N₂O production in this area, although the effect of the shade (on soil moisture content) and a difference in organic material composition (due to leaf litter) provided by the tree may have also contributed.

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3.3 Drainage stream fluxes

Flux measurements were made using the chamber from a stream: nine sampling points were chosen where the stream was wide enough to fit the chamber onto the surface of the water with flux values shown in Fig. 4. The stream was approximately 5 m away from the North edge of the study area. These measurements of flux were not as reliable as the measurements made on the soil, due to the unavoidable disturbance on water pressure and flow caused by the chamber. These flux estimates can still be used as a rough approximation of the N_2O which is emitted from the stream as it passes through this field. Fluxes from the stream varied from 1 to $22 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ with arithmetic and geometric mean values of 9.5 and $7.1 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ respectively. These fluxes were similar in magnitude to some of those measured from the grassland area, although hotspots were not observed in the stream, even in areas with higher turbulence in which de-gassing of N_2O would be expected to increase (Reay et al., 2003). Uncertainty in flux measurements from the stream was generally larger than for equivalent fluxes measured from the grassland soils due to higher uncertainty in the regression analysis. The concentration change within the chamber did not follow the linear and non-linear models as well as fluxes measured from soils. The surface area of the stream crossing the field was approximated at 183 m^2 using a combination of GPS coordinates and water body width measurements. It is not possible to determine whether N_2O fluxes from the stream were a result of nitrogen input from the grazed field in the experiment, or from sources further up the stream. It is also not possible to determine the magnitude of N_2O fluxes which may have occurred further downstream as a result of inputs from the field. The measurements were made only as an indicator of the fluxes from the stream within the field area.

3.4 Manure heap fluxes

10 N_2O flux measurements were made directly on top of the manure heap located on the field at differing heights (0.5 to 3 m). Care was made not to physically dis-

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turb the chamber during measurements to prevent additional gases escaping from the porous manure surface. Fluxes varied in magnitude significantly across the heap with measured values ranging between approximately 660 to 79 000 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ (Fig. 5). Two of the measurements recorded very high N₂O fluxes exceeding 35 000 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$. No relationship between height of the heap and N₂O flux was observed from these measurements. 7 sampling points were taken near the foot of the heap: fluxes recorded from these locations showed a similar mixture of very large and comparatively small fluxes of N₂O, varying by up to three orders of magnitude, between 85 and 31 250 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$. Again, no clear spatial pattern was observed in the fluxes around the heap. A further 6 flux measurements were made at distances of 5 to 10 metres and 5 more were made at 10 to 20 metres from the heap. The arithmetic and geometric mean fluxes recorded from the 5 to 10 metre range were 6759 and 1986 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ respectively. The arithmetic and geometric mean fluxes recorded from the 10 to 20 metre were 466 and 91 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ respectively. These results suggest that the influence of the manure heap on N₂O fluxes decreases dramatically after a distance of approximately 10 metres (See Fig. 5).

3.5 Variation in soil properties at the field scale

Soil measurements were made from 55 of the 100 flux measurement locations (Table 1). The majority of these samples ($n = 38$) were taken from the grassland area to assess the natural heterogeneity of the soil throughout the field. The remaining soil samples were taken from the visible hotspot features of the field to investigate the causes of elevated N₂O emissions ($n = 17$).

The most variable of the soil properties across the grassland area were the concentrations of the available reactive nitrogen in the form of NH_4^+ and NO_3^- (see Table 1). Locations with elevated NH_4^+ also generally recorded higher NO_3^- concentrations, although this relationship was not consistent at all locations ($R^2 = 0.56$). Soil samples taken from patches of decayed grass silage and the shaded area indicated that these

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small areas had significantly greater concentrations of NH₄⁺ and NO₃⁻ ($p < 0.001$) compared to the grassland area. Reactive nitrogen concentrations in soils from the perimeter of the manure heap also showed wide variations, with some extremely large (2.2 g N kg⁻¹) and small (0.1 g N kg⁻¹) values being measured (Table 1).

Total carbon and nitrogen content of the soil from the grassland area showed less variation than the reactive nitrogen content, with a small number of elevated outlier values. The ratio of carbon to nitrogen content of the soils (12 : 1) was consistent across the measurement locations ($R^2 = 0.94$). Total soil carbon and nitrogen concentrations from the shaded area and silage remains were similar in magnitude to the grassland area measurements. The manure heap perimeter was the exception to this, presenting some very high concentrations of carbon and nitrogen. Total carbon and nitrogen content of the soils around the manure heap varied from small concentrations similar to the grassland soil (8 g N kg⁻¹ and 107 g C kg⁻¹) to concentrations as large as 34 g N kg⁻¹ and 355 g C kg⁻¹ (Table 1).

Soil pH varied little between most of the measurement locations in the grassland area with the majority of the grazed field confidently estimated at pH levels of 5.6 ± 0.34 ($n = 38$), in agreement with measurements made in similar managed grazed fields in this area. Soil pH from the silage remains and tree shaded area was generally more alkaline (pH 6.9 ± 1.5) than from the grassland area. The soils from the manure heap perimeter were highly alkaline (pH 8.3 ± 0.85) (Table 1).

WFPS% values were relatively consistent across the field with the majority of measurements ranging between 20 to 25 %. The bulk density of the soil in the field was also fairly consistent ranging between 0.65 to 0.80 g cm⁻³. Due to the heterogeneous nature of soils there were several outliers for each of the soil properties measured across the field (Table 1).

3.6 Correlation between soil properties and N₂O flux

Multiple linear regression was used to investigate the relationships between the soil properties presented in Table 1 (also soil porosity) and N₂O flux. Due to the wide rang-

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ing and uneven distribution of values measured for both N₂O flux and soil properties, the common logarithm (hereafter referred to as log₁₀) of several of these measurements (N₂O flux, NH₄⁺, NO₃⁻, total carbon and total nitrogen content) was used for the multiple linear regression. Correlations of soil properties were carried out using multiple linear regression in the statistical software R. The soil properties from all of the features in the field were processed together as one group ($n = 55$).

Linear regression was first of all carried out using all of the measured soil properties for each of the fits. After the initial fit, the properties which were not statistically significant ($p > 0.1$) were removed and the fit was run again using only the significant values (See Table 2). Concentrations of NH₄⁺ in soils were found to correlate well with pH and total carbon and nitrogen ($R^2 = 0.64$) (Fig. 6a). High total carbon and nitrogen contents were indicative of an increased presence of total organic carbon (TOC) in the soils.

Concentrations of NO₃⁻ correlated strongest with TOC and NH₄⁺ present in the soil ($R^2 = 0.77$) (Fig. 6b). NO₃⁻ concentrations were presumed to be indicative of microbial nitrification activity in the soil as it is the primary product of this process. Fluxes of N₂O (log₁₀(N₂O)) correlated strongly with NO₃⁻, pH and WFPS% ($R^2 = 0.86$) (Fig. 6c). The soil property with the most significant correlation with N₂O flux was NO₃⁻ (See Table 2).

3.7 Interpolation of N₂O fluxes at a field scale

The simplest way to estimate the total daily N₂O flux from the field during the measurement period is to combine the relevant area and mean flux recorded for each of the features of the field. Due to the uneven distribution of flux magnitude and the many large hotspots of flux measured using the chamber method in this experiment, geometric mean values are most suitable to determine fluxes across the field scale (Table 3). Using the geometric mean values an estimate of 47.7 g N₂O–N d⁻¹ was emitted from the field site during the measurement period. (See Table 3) (122.5 g N₂O–N d⁻¹ estimated using the arithmetic mean). The grassland area of the field which accounts for 98.62 % of the study area contributed 45 % (21.3 g N₂O–N) of the estimated daily

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N₂O flux from the field. The silage remains and shaded area contributed 5 and 13 % to the total emissions, respectively. The manure heap and soils contaminated by the heap contributed a very large 38 % (18 g N₂O–N) of the total flux estimate which comes from a relatively small area of the field (0.8 %) (Table 3).

4 Discussion

4.1 Variation in N₂O fluxes at the field scale

N₂O fluxes measured from the grazed grassland area of the field (excluding the hotspot areas) were highly variable (between 2 to 227 μg N₂O–N m⁻² h⁻¹). This is a common phenomenon which is verified in many N₂O flux measurement experiments (e.g. Oenema et al., 1997; Skiba et al., 2012). Flux magnitude was unpredictable across the grassland and in some cases varied by 2 orders of magnitude across relatively short distances (< 10 m). 80 % of the fluxes measured from the grassland area were below 30 μg N₂O–N m⁻² h⁻¹. Fluxes of N₂O comparable to this magnitude are often measured from grazed fields in different climates in between fertilisation events (Clayton et al., 1997; Luo et al., 2013; Oenema et al., 1997). The advantage of using the closed loop dynamic chamber (Cowan et al., 2014) in this experiment was that the extremely high precision (1 μg N₂O–N m⁻² h⁻¹) allowed us to confidently report very low individual N₂O fluxes across the field and compare these measurements with the relevant soil properties collected from within the measurement plot at each individual location.

The largest fluxes in the field were measured from the hotspot features present (up to 79 000 μg N₂O–N m⁻² h⁻¹). Fluxes from the shaded area and the silage heap remains were consistently higher than those measured on the grassland area. The shaded area presented an increased number of sheep, with the resultant increase in animal waste freshly deposited there (NH₄⁺). Fluxes measured from the silage heap remains were surprisingly high. Decaying plant matter is known to emit N₂O (Hellebrand, 1998), but it is unclear whether the emissions from these patches are due to the additional

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organic materials present in the soil or to the increased sheep activity and resultant urine and faeces deposits. The larger pH values from the shaded areas, as well as the manure heap and perimeter suggest that animal waste was the most likely source of N₂O. The combination of large concentrations of mineral N and organic C in a high pH environment are ideal conditions for denitrification (Hofstra and Bouwman, 2005; Sagar et al., 2013), which is most probably the main source of the N₂O here.

Fluxes of N₂O from the stream were relatively small (1 to 22 μg N₂O–N m⁻² h⁻¹) compared with those measured from the rest of the field. Significantly higher fluxes have been measured from drainage streams at the Bush Estate in previous experiments (100 to 1000 μg N₂O–N m⁻² h⁻¹) using different methodology (Reay et al., 2003). Dry conditions in the run up to the measurement period had decreased any leachate from the soils entering the stream. Past experiments have reported N₂O flux measurements from agricultural streams similar in magnitude to those made in the surrounding soils (Baulch et al., 2011); however, it is likely that the N₂O fluxes measured in this experiment are lower than they would have been had the measurements taken place on a wetter date when drainage waters containing N₂O and other nitrogen compounds from surrounding fields would also have been entering the stream.

Flux measurements made on and around the manure heap were on average 420 times higher than the fluxes measured for the grassland area of the field. The large spatial variability of N₂O flux observed from the heap was similar to that of a previous experiment carried out on the farm estate using static chamber measurements, although reported fluxes are an order of magnitude smaller in this study (Skiba et al., 2006). Solid manure heaps are a known large source of N₂O emissions and several studies have estimated emission factors for such heaps (Amon et al., 2001; Chadwick et al., 1999; Skiba et al., 2006). Emission factors for manure heaps are often calculated by volume of stored manure. This implies a large degree of variability, following from different components of animal waste as well as the age of the waste and how it is stored (Amon et al., 2001). Application of the manure as fertiliser is often considered in the emission factor of animal waste as well as storage (Chadwick et al., 1999,

2011; Velthof et al., 2003). Measurements made in this experiment did not account for manure volume or calculate an emission factor for the heap; however, this study highlights that an additional factor may also need to be taken into account for a more accurate estimate of the emission factor of solid manure storage (i.e. the legacy emissions of a manure heap). Very high N₂O fluxes (up to 10 825 µg N₂O–N m⁻² h⁻¹) were measured from the area around the manure heap which had become contaminated with the animal waste. Our data have shown that these areas that are highly enriched with available nitrogen compounds and organic matter remain after the manure heap has been removed, and can continue to emit N₂O for months, as was observed for the patches of silage heap remains (manure was spread in Autumn, nine months prior to measurements). The high emissions and lasting effect of these areas may contribute significantly to the overall emission factor of solid manure heaps and agriculture as a whole when the large volumes of animal waste and storage from livestock farms are considered.

4.2 Correlation between soil properties and N₂O flux

High concentrations of NH₄⁺ and NO₃⁻ are known to increase N₂O fluxes from soils as they are the primary nutrients required for the microbial processes of nitrification and denitrification in which N₂O is produced and then released to the atmosphere (Davidson et al., 2000). Animal urine and droppings are a known source of urea (CO(NH₂)₂) and ammonia (NH₃) which are both alkaline and convert to NH₄⁺ in the presence of water (Freney et al., 1983). A strong positive correlation between NH₄⁺ concentrations and soil pH was observed across the field (See Table 2). As ruminant (sheep and cattle) urine is normally slightly alkaline the increased pH in the small hotspot areas suggested that increased alkaline animal waste deposition was the reason for the increase in pH and resultant available NH₄⁺ in the soil. This relationship has also been observed in other studies (e.g. Haynes and Williams, 1992). Organic matter in the soils (Total C and N) also correlated with NH₄⁺ concentrations in the soils (See Table 2). Mineralisation of animal waste, and plant materials such as silage, continues to pro-

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vide NH_4^+ to soils over extended periods (Martins and Dewes, 1992; Van Kessel and Reeves, 2002). All of the N_2O flux hotspot features of the field contained elevated concentrations of NH_4^+ in the soil (See Table 1); however the concentration of NH_4^+ was not found to correlate significantly with N_2O fluxes (See Table 2).

NO_3^- concentrations in the soil correlated well with available NH_4^+ and organic matter (See Fig. 6b). The physical properties of the soil were also influential as NO_3^- correlated strongly with WFPS%, and weakly with bulk density and soil porosity. Elevated NO_3^- concentrations in the soil can be associated with high rates of nitrification, as NO_3^- is the primary product of the nitrification process. The strong correlation between NO_3^- with the available NH_4^+ and organic material present in the hotspot features of the field provides strong evidence that elevated concentrations of NO_3^- in these areas is due to nitrification occurring at an increased rate. The soils measured in this study were relatively dry (9–50 % WFPS), therefore more conducive for nitrification than denitrification (Bateman and Baggs, 2005; Davidson et al., 2000). However the presence of organic matter would have created the necessary anaerobic conditions required for denitrification in localised microsites, through increased O_2 consumption required for organic matter decomposition (Sexstone et al., 1985). No significant correlation between organic carbon and N_2O flux was observed in this data set. Organic carbon is known to be a limiting factor of denitrification rates in some soils (McCarty and Bremner, 1992); however it is possible that the lack of correlation between carbon and N_2O flux measured in this experiment is due to the abundance of carbon available in the soils.

Correlation between N_2O flux and the measured soil properties showed that NO_3^- concentrations were the most significant factor (Table 2). The strength of the correlation with NO_3^- and lack of correlation with NH_4^+ does not explain if fluxes are predominantly caused by either microbial nitrification or denitrification. The presence of NO_3^- indicates that nitrification is definitely happening at these sites; however, the lack of correlation between NH_4^+ and N_2O flux suggests that denitrification may be the primary source of emissions. The correlations indicate that areas in which the concentrations of available nitrogen compounds are higher emit more N_2O , and therefore, available nitrogen input

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is likely the primary driver of the spatial variability observed in N₂O flux measurements in this study. This relationship between soil NO₃⁻ and NH₄⁺ concentrations and N₂O flux is also observed in similar studies (e.g. Turner et al., 2008). Our conclusion from the correlation analysis is that the high spatial variability of N₂O flux across the grazed field is primarily due to the uneven distribution of nitrogen deposition in the form of animal waste.

There remains a high degree of uncertainty in the relationship between the soil properties and N₂O flux. This study suggests NH₄⁺, NO₃⁻ and organic matter can be used as proxies to predict where fluxes will be higher in the field; however, exact fluxes are more difficult to estimate due to the large number of variables which affect the rates of microbial processes. Similar studies carried out in different environments predicted very different significance values for each of the measured soil properties depending on environmental factors (Šimek et al., 2006; Turner et al., 2008). In order to better understand these processes more detailed experiments would be required in a variety of geographical and environmental conditions to better predict the behaviour of microbial processes in soils with high available nitrogen concentrations. Alternatively, a more controlled analysis of individual soil properties and microbial processes can be examined under laboratory conditions using similar high precision chamber methodology. Ideally the use of this equipment could be paired with 15N labelled nitrogen compounds (such as urea) and denitrification inhibitors to investigate the biological mechanisms in N₂O production and determine relationships between these processes and soil properties.

4.3 Interpolation of N₂O fluxes at a field scale

Using mean values to interpolate N₂O flux at the field scale results in very high uncertainty values due to the high spatial variability of the N₂O fluxes (Table 3). From this experiment the total daily flux is estimated to be between 12.8 and 215.1 g N₂O–N d⁻¹. These high uncertainties highlight the weakness of the chamber methodologies inability to account for spatial variability of N₂O flux over large areas and the importance of

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spatial variability when N₂O flux estimates are made using simple interpolation methods on a large scale. These results also highlight the need for a better understanding of how agricultural flux measurements are made using current methodology. Flux chamber placement is vital in understanding the variability of N₂O flux across a field. Without a good understanding of N₂O hotspots and the appropriate positioning of chambers to include (or exclude) these areas, chamber methods will not be able to provide effective comparable results between experiments.

Other methods of interpolation exist when using chamber measurements; however, these also struggle to account for the spatial variability of N₂O at larger scales. Fluxes measured from the field in this experiment showed some predictability in spatial patterns as fluxes were higher in certain hotspot locations, although knowledge of these locations is required to observe this predictability as there was little relationship observed between N₂O flux and distance between measurements. Hotspot locations which are not visible by eye are much more difficult to investigate. Variance diagrams highlight this lack of predictability across the field, showing a random distribution with no clear spatial pattern visible in the flux or the corresponding soil properties across the field scale (Fig. 7). The nature of the unpredictable spatial variability of N₂O fluxes is a huge barrier which limits the use of many methods of spatial interpolation of the flux across a large scale such as a field. Taking many chamber measurements across a small area is one way to improve this method (Turner et al., 2008); however this becomes impractical at larger scales and a compromise needs to be made between field coverage and the number of chamber measurements taken.

Another method of measuring N₂O fluxes at a field scale which has advanced in recent years due to the increasing precision of rapid gas analysers would be eddy covariance (Eugster et al., 2007; Kort et al., 2011). Eddy covariance does not suffer from the same interpolation issues as the chamber method and can provide a relatively confident estimate of mean N₂O flux across a large area (> 100 m²). The weakness of the eddy covariance method is that it would not be able to distinguish between sources and provide information on hotspot fluxes. Areas in which animals spend a lot of time

to shelter from the elements such as the shaded area in this field scale study present problems for eddy covariance measurements as any physical objects which alter turbulence in the air (such as trees or foliage in our case) can prevent measurements from taking place. From the results in this experiment we would suggest that both methods should be deployed in tandem to investigate N₂O flux on a field scale as both methods have significant weaknesses that the other can compliment.

5 Conclusions

Spatial variability remains one of the largest sources of uncertainty when measuring N₂O flux from agricultural soils. Results from this study suggest that additional nitrogen applied to fields in the form of animal waste is the primary source of anthropogenic N₂O emissions from grazed agricultural soils (with the exception of fertiliser events). The wide and often random distribution of this nitrogen in the soils is one of the major causes of the spatial variability observed in N₂O emissions. This inherent variability of soil properties limits the ability to reduce uncertainty in N₂O emission estimates that can be achieved by taking a practical number of flux measurements using a chamber method. In order to reduce uncertainties in large scale emission budgets it is effective to identify hotspots of N₂O fluxes and determine the causes of these increased emissions. Identifying areas in which N₂O fluxes are significantly higher than the majority of the experimental area can reduce overall uncertainty in results by defining different emission factors.

This study highlights the requirement of a better understanding of spatial variability of N₂O fluxes from intensively grazed grasslands. Without a basic understanding of how hotspots of N₂O are formed and the lifetime of these hotspots it is difficult to determine the true effect of these areas, which may be significant over wider areas such as the farm scale. Field, farm, national and global scale emission budgets of agricultural contributions to N₂O emissions are often dominated by emission factors which account for the soil conditions of the majority of the area of a field. These budgets

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may be significantly underestimating N₂O fluxes in some cases, especially for livestock farms with high stocking densities.

Author contributions. N. Cowan, P. Norman and U. Skiba designed the measurement strategy for the experiment. The field measurements and soil analysis were carried out by N. Cowan and P. Norman. N. Cowan carried out the statistical analysis of the data with help from D. Famulari and P. Levy. D. Famulari and P. Levy also provided help with instrumentation and methodology. N. Cowan and U. Skiba prepared the manuscript with contributions from all of the co-authors. The study was carried out under the supervision of U. Skiba and D. Reay.

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Table 1. Summary of relevant soil properties of all 55 soil measurements made during flux measurements. Soil samples were taken from inside the chamber area immediately after flux measurements were completed. The mean values and range (in brackets) of measurements from each variable within the field are included in the table.

Feature	Soil Samples	Area (m ²)	NH ₄ ⁺ (g N kg ⁻¹)	NO ₃ ⁻ (g N kg ⁻¹)	Total Carbon (g C kg ⁻¹)	Total Nitrogen (g N kg ⁻¹)	pH	WFPS (%)	Bulk Density (g cm ⁻³)
Grass	38	66861	0.060 (0.008–0.745)	0.017 (0.001–0.198)	60.269 (43.458–103.707)	4.708 (3.368–9.494)	5.63 (4.74–6.62)	24.7 (8.9–36.7)	0.754 (0.566–0.968)
Silage re- mains	5	36	0.247 (0.037–0.934)	0.161 (0.046–0.243)	77.010 (44.252–118.652)	5.872 (3.779–8.501)	6.42 (5.21–8.28)	42.8 (38.0–50.0)	0.848 (0.667–1.061)
Shaded area	3	210	0.287 (0.037–0.489)	0.087 (0.009–0.239)	51.841 (9.678–105.96)	4.277 (0.835–9.178)	7.38 (6.1–3.18)	33.6 (24.2–44.3)	0.953 (0.833–1.079)
Stream	0	183	NA	NA	NA	NA	NA	NA	NA
Manure heap	0	102	NA	NA	NA	NA	NA	NA	NA
Manure heap perimeter	7	^a	0.987 (0.089–2.175)	0.103 (0.002–0.587)	216.996 (107.652–354.828)	18.750 (8.045–34.099)	8.33 (6.97–9.41)	22.8 (14.1–31.9)	0.423 (0.172–0.846)
Manure perimeter (5–10 m)	1	^b	0.036	0.398	52.346	5.440	6.00	33.6	0.955
Manure perimeter (10–15 m)	1	406	0.008	0.002	111.563	9.641	7.21	10.7	0.792

NA No samples recorded,

^a As Manure heap

^b Total manure perimeter area of influence estimated as 406 m

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Table 2. Multiple linear regression correlation of soil properties and N₂O flux as plotted in Fig. 6

	Estimate	SD	<i>p</i> value
(a) $Y = \log_{10}(\text{NH}_4^+)$			
(Intercept)	-2.56	0.76	<0.01
pH	0.37	0.05	<0.001
$\log_{10}(\text{Carbon g kg}^{-1})$	-1.14	0.62	<0.1
$\log_{10}(\text{Nitrogen g kg}^{-1})$	1.53	0.79	<0.1
(b) $Y = \log_{10}(\text{NO}_3^-)$			
(Intercept)	-402.47	205.04	<0.1
$\log_{10}(\text{NH}_4\text{-N g kg}^{-1})$	0.48	0.130	<0.001
$\log_{10}(\text{Carbon g kg}^{-1})$	-6.7	0.87	<0.001
$\log_{10}(\text{Nitrogen g kg}^{-1})$	8.58	1.13	<0.001
WFPS %	0.04	0.01	<0.001
Soil porosity	403.81	205.12	<0.1
Bulk density g cm^{-1}	154.86	77.39	<0.1
(c) $Y = \log_{10}(\text{N}_2\text{O})$			
(Intercept)	-4.33	1.29	<0.01
$\log_{10}(\text{NH}_4\text{-N g kg}^{-1})$	-0.25	0.20	<0.1
$\log_{10}(\text{NO}_3\text{-N g kg}^{-1})$	0.76	0.10	<0.001
pH	0.60	0.10	<0.001
WFPS%	0.04	0.01	<0.001
Soil porosity	3.85	1.34	<0.01

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Table 3. Geometric mean flux values and estimated cumulative flux from each of the measured features across the field scale. 95 % confidence intervals (CI's) are included.

Field Feature	Area (m ²)	Geometric Mean Flux (µg N ₂ O–N m ⁻² h ⁻¹)	95 % CI (µg N ₂ O–N m ⁻² h ⁻¹)	Cumulative Flux (g N ₂ O–N d ⁻¹)	95 % CI (g N ₂ O–N d ⁻¹)
Grazed Grassland	66861	13.3	(4.7–37.2)	21.3	(7.6–59.8)
Silage Remains	36	2663.6	(1220–5815)	2.3	(1.1–5.0)
Shaded Area	210	1217.1	(252–5881)	6.1	(1.3–29.6)
Stream	183	7.1	(2.9–17.5)	0	(0.0–0.1)
Manure heap	102	3195.2	(656–15 562)	7.8	(1.6–38.1)
Manure perimeter	50	4469.7	(573–34 875)	5.4	(0.7–41.9)
Manure outer perimeter	366	550.9	(66–4628)	4.8	0.6
Total	67808			47.7	(12.8–215.1)

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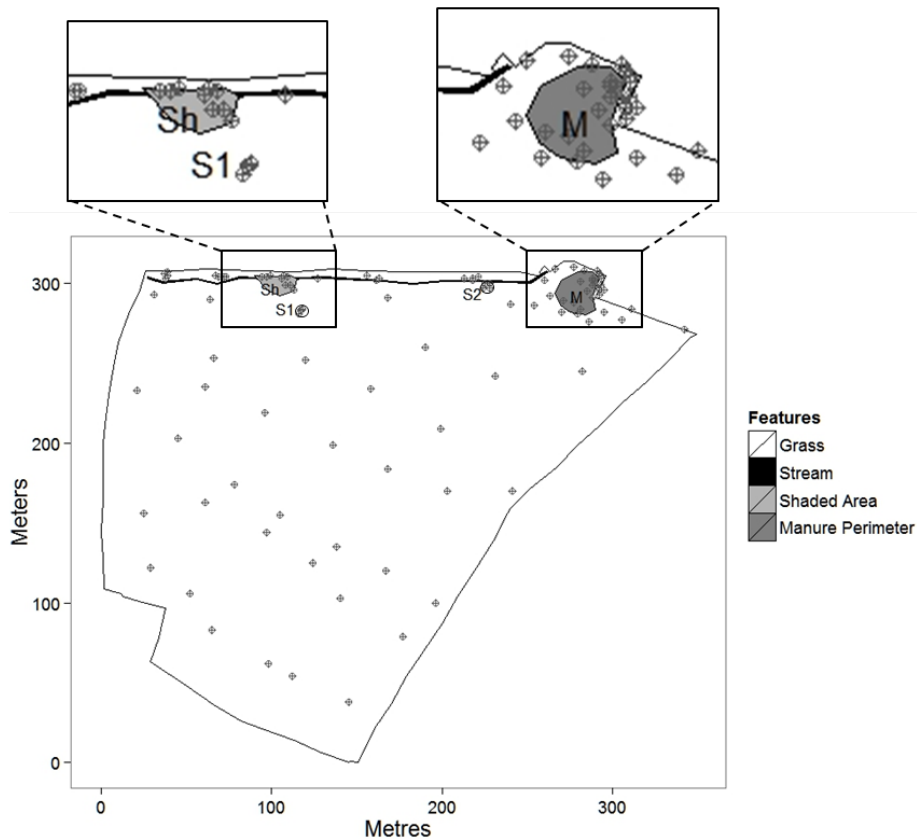


Figure 1. The locations of 100 flux measurements (markers) made over a 6.78 ha grazed grassland field using the closed loop dynamic chamber method (bottom). Details of the high density measurement areas in the north of the field are expanded (top). Features present in the field are outlined, including the tree shaded area (Sh), the two small patches of silage remains (S1 and S2) and the manure heap (M). The stream runs across the North of the field through the shaded area.

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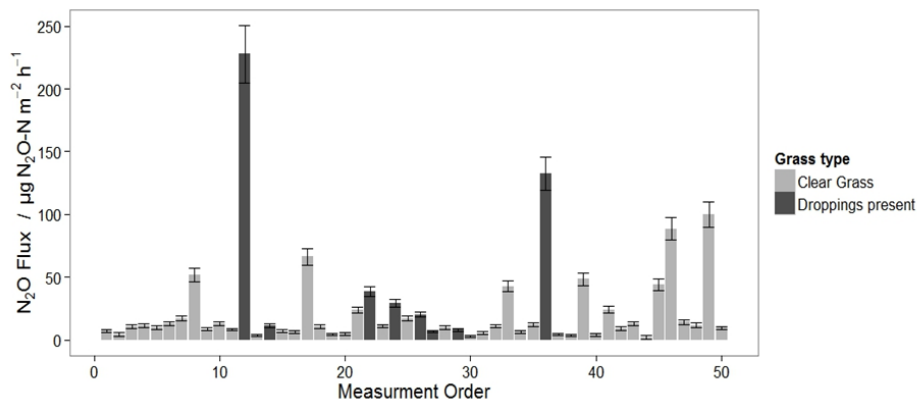


Figure 2. 50 flux measurements of N_2O were made on grazed grassland: the sampled locations which contained visible sheep droppings are represented by the darker bars. Error bars represent the uncertainty in each flux measurement which was calculated using a propagation of regression, volume, temperature and pressure uncertainties (See Cowan et al., 2014).

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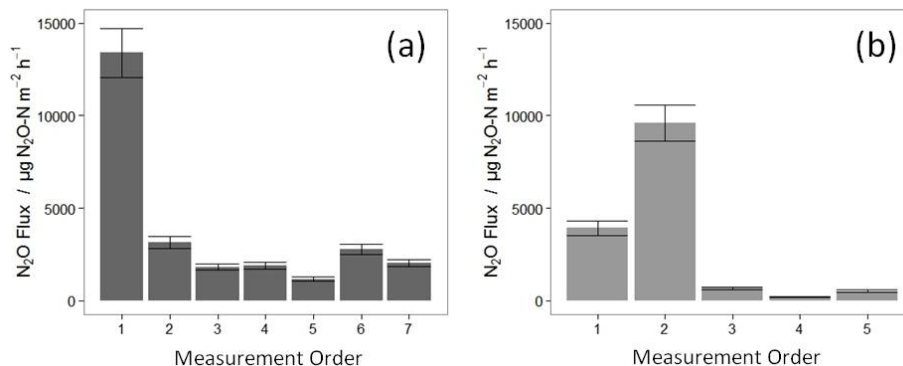


Figure 3. (a) Flux measurements made on patches of decayed grass silage. Measurements 1–3 were taken from the first patch (referred as S1 in Fig. 1) and the remaining 4 were measured from the second (referred as S2 in Fig. 1). (b) Flux measurements made from a shaded area with increased sheep density. The first two of these measurements were made near the centre of the shaded area. Fluxes from both features were made during the same 3 d measurement period between the 8 to the 10 July 2013. Error bars represent the uncertainty in flux measurement calculated using a propagation of errors from regression, volume, temperature and pressure.

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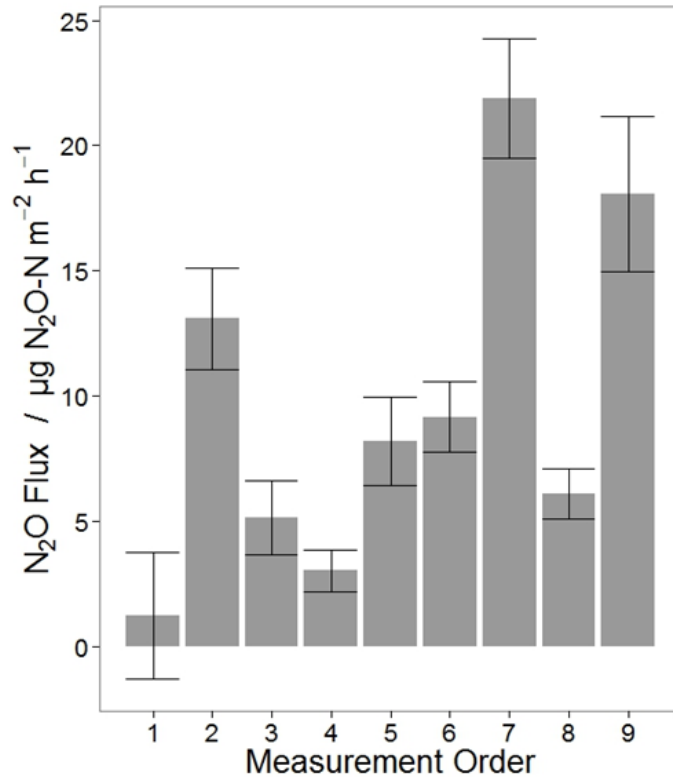


Figure 4. N₂O fluxes measured from different locations in a drainage stream in the grazed grassland field. Hotspots of N₂O flux were not observed in the stream measurements. Uncertainty was calculated for each measurement, as was done for the fluxes measured from soils in the field.

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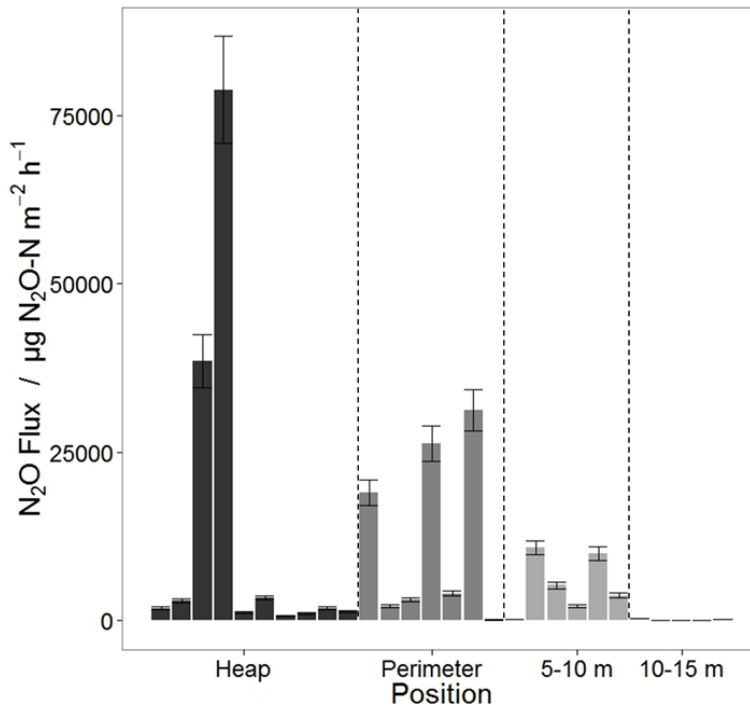


Figure 5. N₂O flux measurements from a semi-permanent manure heap located on the grass-land field. Vertical dashed lines split the measurements into groups separated by distance from the heap with the left side of the figure being the nearest and right side the furthest from heap. The darkest bars in the figure represent measurements made on top of the actual manure heap. Next are the measurements made from the base of the heap, then those made 5 to 10 m and 10 to 15 m from the heap.

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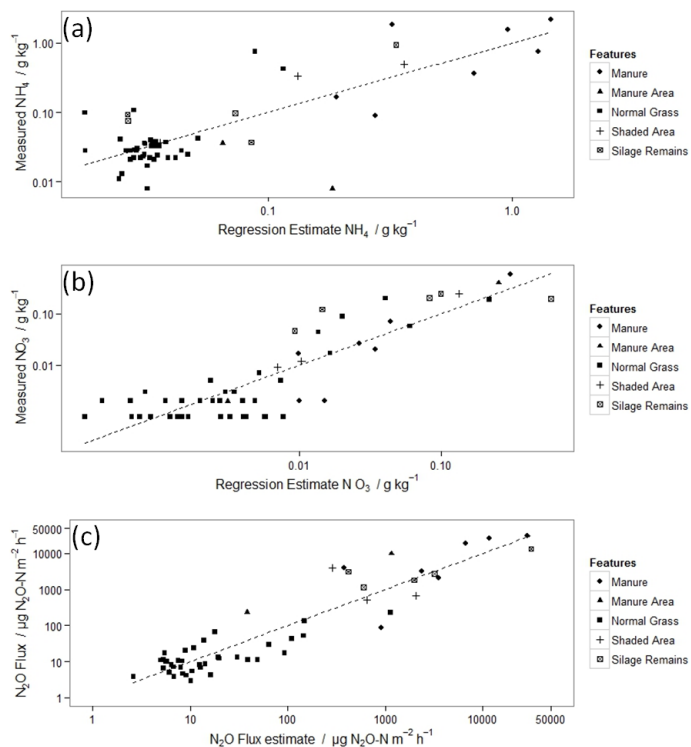


Figure 6. Multiple linear regression used to identify relationships between NH_4^+ (a), NO_3^- (b) and N_2O flux (c) with soil properties measured during flux measurements from grazed grassland (See Table 2 for fitting parameters). All 55 soil samples collected from multiple features present in the field were included in the regression analysis.

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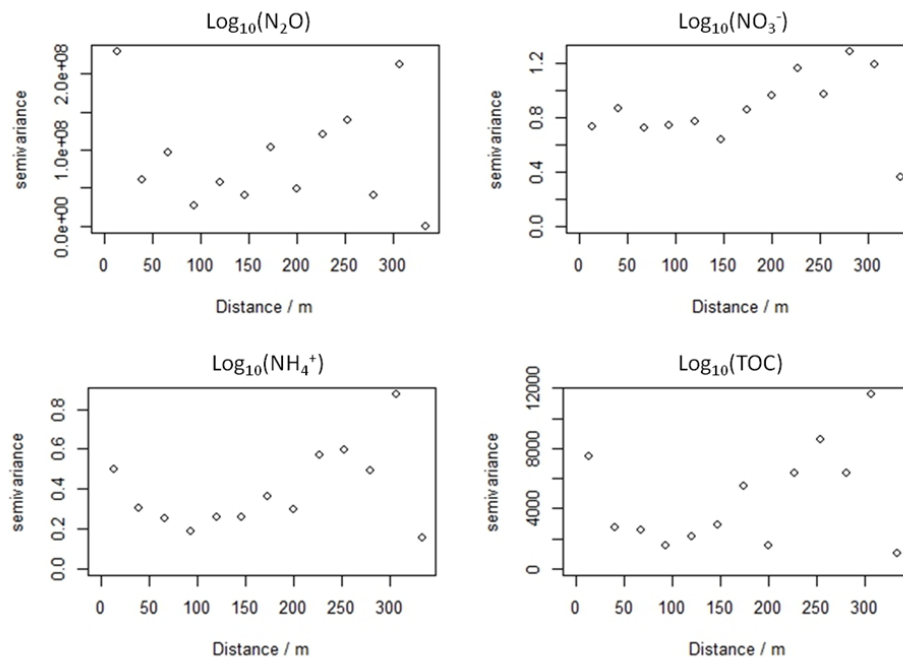


Figure 7. Variograms for N_2O flux, NO_3^- , NH_4^+ and total carbon measured across the field scale. Log-normal distributions were used as in Fig. 6 and Table 2. The x-axis is the distance between measurement locations in m and the y-axis is the semivariance in all of the respective measurements made for the entire field.

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