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Investigating uptake of N_2O in agricultural soils using a high-precision dynamic chamber method

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Abstract. Uptake (or negative flux) of nitrous oxide (N_2O) in agricultural soils is a controversial issue which has proved difficult to investigate in the past due to constraints such as instrumental precision and methodological uncertainties. Using a recently developed high-precision quantum cascade laser gas analyser combined with a closed dynamic chamber, a well-defined detection limit of 4 $\mu g\, N_2O\text{-}N\, m^{-2}\, h^{-1}$ could be achieved for individual soil flux measurements. 1220 measurements of N_2O flux were made from a variety of UK soils using this method, of which 115 indicated uptake by the soil (i.e. a negative flux in the micrometeorological sign convention). Only four of these apparently negative fluxes were greater than the detection limit of the method, which suggests that the vast majority of reported negative fluxes from such measurements are actually due to instrument noise. As such, we suggest that the bulk of negative N_2O fluxes reported for agricultural fields are most likely due to limits in detection of a particular flux measurement methodology and not a result of microbiological activity consuming atmospheric N_2O.

1 Introduction

N_2O is a naturally occurring greenhouse gas (GHG) which is formed predominantly in soils and aquatic environments as a by-product of the microbial processes of nitrification and denitrification (e.g. Davidson et al., 2000). Atmospheric N_2O has increased from pre-industrial concentrations of 280 to over 320 $nmol\, mol^{-1}$ (IPCC, 2013). This increase is believed to be primarily due to agricultural activities such as the production and subsequent application of reactive nitrogen fertilisers to agricultural soils, which increases microbial activity and the production of N_2O on a global scale (IPCC, 2007, 2013). It is estimated that agriculture contributes either directly or indirectly to over 80 % of all anthropogenic N_2O emissions; however, a large uncertainty is associated with this figure (IPCC, 2007). Emission estimates of N_2O from various soils often have large uncertainties due to the large spatial and temporal variability of N_2O flux measurements (Velthof et al., 1996; Zhu et al., 2013; Chadwick et al., 2014). Accurate measurement of N_2O flux from various agricultural soils can also be difficult to perform due to the relatively low concentrations of N_2O in the atmosphere ($nmol\, mol^{-1}$). With the exception of nitrogen fertiliser application events, fluxes of N_2O from agricultural soils are often small, verging on the detection limits of gas analysers ($< 20\ \mu g\, N_2O\text{-}N\, m^{-2}\, h^{-1}$) (Smith et al., 1994; Jones et al., 2007).

Observations of negative fluxes (or uptake) of N_2O from the atmosphere into various soil types are relatively common in the literature and have been reported in several studies using different methodologies (sometimes exceeding values as high as 50 $\mu g\, N_2O\text{-}N\, m^{-2}\, h^{-1}$) (Ryden, 1981; Papen et al., 2001; Butterbach-Bahl et al., 2002; Flechard et al., 2005). In these studies the authors attribute the uptake of N_2O to microbial denitrification, which is biologically plausible (Okereke, 1993; Davidson et al., 2000). However, there has been much debate over whether the observed negative fluxes of N_2O are genuinely a result of microbial uptake or merely experimental or instrumental artefacts (Chapuis-Lardy et al., 2007).

The static chamber approach is generally deployed to monitor N_2O fluxes from agricultural soils (Jones et al., 2007; Hensen et al., 2013). Fluxes derived from static cham-

ber methods are often prone to high instrumental noise from gas chromatograph (GC) instruments, the regression method used and temperature and pressure changes within the chamber (Venterea et al., 2009; Levy et al., 2011). N_2O fluxes also show very high spatial variability, which makes it more difficult to judge whether any individual measurement is an erroneous outlier or valid (Cowan et al., 2014b, d).

Recent advances in infra-red laser technology have resulted in the commercial availability of high-precision trace gas analysers such as quantum cascade lasers (QCLs) capable of measuring N_2O concentrations with very high precision and accuracy. Here, we used a QCL gas analyser with a closed dynamic chamber, resulting in a measurement system with a significantly lower detection limit than GC-based static chamber methods. We used this system to measure a total of 1220 fluxes at five field sites across the UK at different times of the year. This study aimed to investigate the occurrence and validity of negative fluxes of N_2O within this data set, and their relationship with commonly measured soil properties.

2 Materials and methods

2.1 Dynamic chamber method

All of the N_2O flux measurements reported in this paper were made using a non-steady-state flow-through (or closed dynamic) chamber system which circulated air between a flux chamber and a QCL gas analyser (as described in Cowan et al., 2014a, based on a similar design to that described in Hensen et al., 2006). A compact continuous wave QCL (CW-QC-TILDAS-76-CS, Aerodyne Research Inc., Billerica, MA, USA) was used to measure gas mixing ratios within the dynamic chamber system. The instrumentation was either placed in a stationary cabin or secured inside a four-wheel-drive vehicle to allow for mobile measurements. The system could be powered from a main power supply when available; when it was used in mobile conditions, a diesel generator was required which was kept on a tow trailer to provide a constant supply of electricity to the system.

The chamber consisted of a cylindrical polyvinyl chloride (PVC) plastic pipe of 48 cm inner diameter (ID) and 22 cm height with closed cell neoprene sponge attached to the underside. It was placed onto circular stainless steel collars which were inserted (approximately 5 cm) into the soil; the neoprene sponge formed an airtight seal between the chamber and the collar. Clips were added to the chamber to increase the strength of this seal. Two 30 m lengths of 3/8 inch ID Tygon® tubing were attached to both the inlet of the QCL and the outlet of the pump. This provided a 30 m radius from the analyser in which the chamber could be placed. A flow rate of approximately 6 to 7 L min^{-1} was used between the QCL and the chamber.

The dynamic chamber method records gas mixing ratios at a rate of 1 Hz during flux measurements, which allows detailed investigation of an individual flux measurement. During the 180 s enclosure time of each chamber measurement the first 60 s of measurements are discarded to give the system time to mix air between the chamber and the analyser. A total of approximately 120 mixing ratio measurements are then used to calculate fluxes of N_2O from each chamber location

Fluxes of N_2O were calculated using linear and non-linear asymptotic regression methods using the HMR package for the statistical software R (Pedersen et al., 2010; Levy et al., 2011). Using a mixture of goodness-of-fit statistics and visual inspection, the regression method that provided the best fit for the time series of mixing ratio was chosen for each individual measurement. The rate of change in mixing ratio of a particular gas can then be used to calculate soil flux for each measurement:

$$F = \frac{\mathrm{d}C}{\mathrm{d}t}\,\frac{\rho V}{A}, \tag{1}$$

where F is gas flux from the soil (μmol m^{-2} s^{-1}), $\mathrm{d}C/\mathrm{d}t$ is the rate of change in mixing ratio with time in μmol mol^{-1} 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.

2.2 Field sites

The dynamic chamber method was developed to improve the precision of N_2O flux measurement from soils and verify other chamber methodologies in a national project (InveN_2Ory; http://www.ghgplatform.org.uk/) to improve the agricultural GHG emissions inventory in the UK (Skiba et al., 2012). The dynamic chamber has been used at a variety of field sites run by different research groups across the UK between 2011 and 2014 where N_2O flux experiments were taking place using more common static chamber methodologies (see Table 1; Chadwick et al., 2014). The majority of measurements made during the project were from areas within Easter Bush Farm Estate (Penicuik, Midlothian), which is run jointly by the Scottish Rural University College (SRUC) and the University of Edinburgh (UoE).

Soil samples were collected for individual flux measurements during the farm and grazed grassland field experiments at Easter Bush in order to investigate which soil properties were driving N_2O fluxes. 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 determine soil pH, soil moisture content (via oven drying at 100 °C) and available nitrogen in the form of ammonium (NH_4^+) and nitrate (NO_3^-) via KCl extraction (as outlined in Rowell, 1994, p. 226). Bulk density soil samples also were taken immediately after the flux measurement using a sharp metal cutting

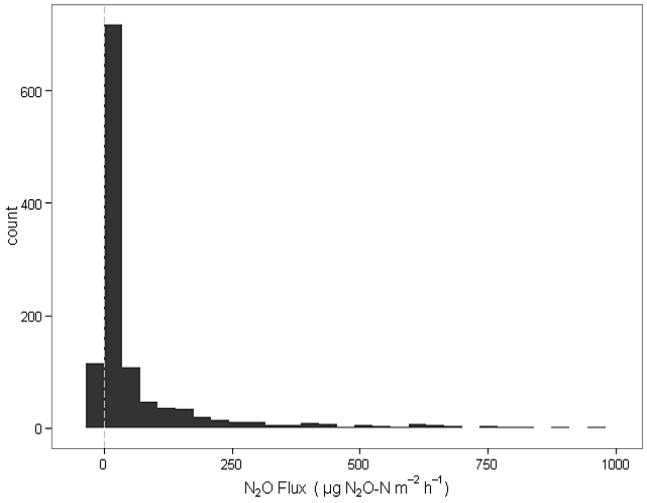

Figure 1. Frequency of all N_2O fluxes measured from all locations excluding those above 1000 µg N_2O-N m^{-2} h^{-1}.

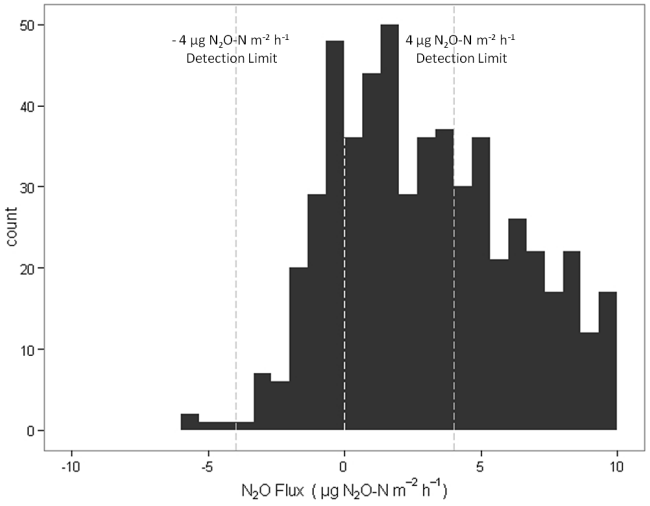

Figure 2. Frequency of all N_2O fluxes measured from all locations excluding those above 10 µg N_2O-N m^{-2} h^{-1}. The estimated zero flux detection limit of ±4 µg N_2O-N m^{-2} h^{-1} is included.

cylinder (7.4 cm diameter, 5 cm deep), which was carefully hammered into undisturbed soil. Bulk density samples were used to calculate total carbon and nitrogen content of the soil, soil moisture content and water-filled pore space (WFPS) percentage (Rowell, 1994). All soils were frozen after collection from the field sites for preservation before lab analysis was carried out. This provided 455 soil samples with individual flux measurements associated with each of them, 61 of which were from locations that reported negative N_2O flux. It was not possible to take destructive soil samples directly from the nitrogen fertiliser manipulation chambers, as this would have interfered with the very frequent (at least weekly) flux measurement programme.

3 Results

3.1 Measured fluxes of N_2O

Flux magnitude measured from the different field sites across the UK varied from –5.5 to 27 475 µg N_2O-N m^{-2} h^{-1}. A large variety of soil types, fertiliser treatments and agricultural fields that contained different crops and grazing animals were all measured during the experiments, which provided many areas of high and low N_2O fluxes. The vast majority of the N_2O fluxes measured were below 50 µg N_2O-N m^{-2} h^{-1}. Of the 1220 measurements, 887 (73 %) fell into this category (Fig. 1). Of these, 115 showed negative fluxes of N_2O, accounting for 9.4 % of all of the measurements made.

The detection limit of the dynamic chamber system (as defined by double the typical standard deviation (SD) of a zero flux measurement reported in Cowan et al., 2014a) is approximately 2 to 4 µg N_2O-N m^{-2} h^{-1}. Uncertainty in flux in each chamber measurement is calculated by propagating the uncertainty associated with each of the terms in Eq. (1) to estimate the total uncertainty in the flux. Uncertainty in dC/dt was obtained from the 95 % confidence interval in the regression slope parameter. As 1 Hz mixing ratios provide approximately 120 measurements over the 2 min enclosure period and both linear and non-linear regression methods are applied for each individual measurement to see which fits best, the uncertainty in dC/dt caused by the choice of regression method is far less significant in than previous studies which used three to five mixing ratio measurements over a period of an hour (Parkin et al., 2012).

Uncertainty in the chamber volume could be estimated by taking several measurements of height in each chamber, and taking the 95 % confidence interval in the calculated chamber volume. Including estimates of the volume of vegetation, this gave values of approximately 10 % of the total volume. Uncertainty in the air density term (ρ) arises from uncertainties in the temperature and pressure measurements. The 95 % confidence interval for the mean temperature and pressure was calculated from the 1 Hz data, and added to the instrumental precision of the temperature probe (0.4 °C) and pressure sensor (50 Pa). Of the apparent negative fluxes recorded during all of the experiments, only four exceed the negative limit of detection (0.3 %) (Fig. 2). Moreover, these fluxes (three of which are shown in Fig. 3a, b and c) only slightly exceeded the detection limit of the system, varying from −4.1 to −5.5 µg N_2O-N m^{-2} h^{-1}.

The 1 Hz mixing ratio measurements show that in some cases there is a definite and consistent negative flux occurring in the chamber during the measurement period (see Fig. 3a, b and c); however, these changes are often very small (less than 1 nmol mol^{-1} over 120 s) and several events can distort these measurements, such as a small leak within the chamber or a gas analyser issue. In certain conditions the sensitivity of the

Table 1. A summary of all InveN_2Ory field sites from which N_2O fluxes were made using the dynamic chamber method.

Location	Dates of measurements	Measurement details	Soil texture	Total annual rainfall (mm)	Annual average air temperature (°C)	No. of m'mnts	No. of negative fluxes
Nitrogen fertiliser manipulation plots[a]							
Dumfries (SRUC)[b]	Oct–Nov 2012	Grazed grassland, SW Scotland, Mineral N, or manure applications	Sandy loam	1211	10.2	282	12
Rosemaund (ADAS)[c]	Mar 2013	Barley, SW England Slurry, manure applications	Silty clay loam	418	10.4	49	0
Easter Bush (SRUC)	Apr–May 2013	Barley, central Scotland Slurry, manure applications	Clay loam	937	10.2	105	3
On-farm measurements (UoE and SRUC)							
2 grazed grasslands[d]	Apr–Jul 2012	Adjacent tilled and untilled sheep grazed grasslands	Clay loam	937	10.2	329	39
Autumn-Farm[e]	Sep 2012	Mixture of grazed and arable fields across Easter Bush Farm Estate	Clay loam	937	10.2	80	34
Winter-Farm	Feb 2013	As above	Clay loam	937	10.2	55	23
Spring-Farm	May 2013	As above	Clay loam	937	10.2	127	4
Summer-Farm	Jul 2013	As above	Clay loam	937	10.2	120	0
Grazed grassland[f]	Jul 2013	Grassland with high stocking density of sheep on Easter Bush Farm Estate	Clay loam	937	10.2	73	0
				Total		1220	115

[a] Overall experimental design is described in Chadwick et al. (2014). [b] Bell et al. (2015). [c] J. Williams et al., ADAS, personal communication, 2013. [d] Cowan et al. (2014b). [e] Cowan et al. (2014c). [f] Cowan et al. (2014d).

QCL can change due to a rapid temperature change or, for example, electronic noise from a generator or power supply. In these situations, at near zero flux conditions, it is difficult to determine whether a negative flux of N_2O is real or an artefact of instrumental noise (Fig. 3d). Of the 115 apparently negative fluxes measured, a mixture of the two is likely to have taken place.

3.2 Soil analysis of low-flux locations

Soil samples were available for 190 chamber measurements which measured N_2O flux below $10\,\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}$. Multiple linear regression analysis was used to investigate the relationship between flux and soil properties for fluxes reported in the range of -10 to $10\,\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}$ (see Table 2). The results of the regression analysis suggest that a weak relationship does exist between the measured soil properties and fluxes measured ($R^2 = 0.38$) (Fig. 4a). The properties which correlate strongest with measured flux are WFPS %, available NO_3^-, pH and bulk density. Individual comparison between flux and each of these soil properties reveals no clear indication of which soil conditions would provide ideal conditions for negative flux observations (Fig. 4b, c and d). From the soil analysis results it could be suggested that in general, negative fluxes of N_2O tend to contain very low concentrations of NO_3^- (below $0.01\,mg\,kg^{-1}$), and are

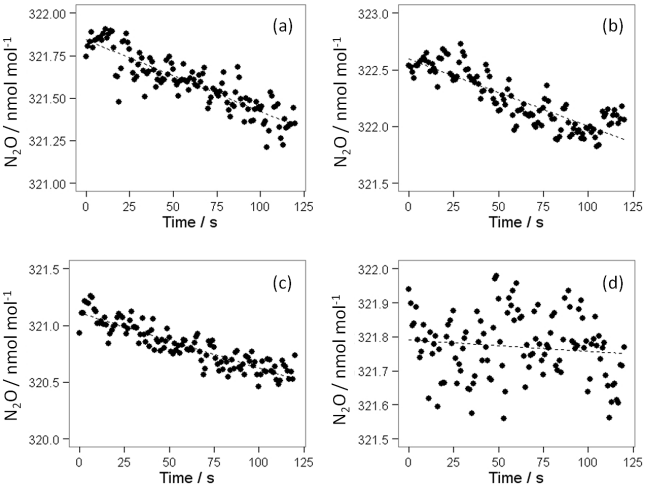

Figure 3. Examples of 1 Hz N_2O mixing ratio data recorded during four separate negative flux measurements made using the dynamic chamber method. Each flux measurement uses over 120 individual measurements to calculate the rate of change of N_2O mixing ratio within the chamber over a 3 min measurement period. **(a, b, c)** are examples of clear and consistent negative fluxes; **(d)** is an example of negative flux likely attributable to instrumental noise.

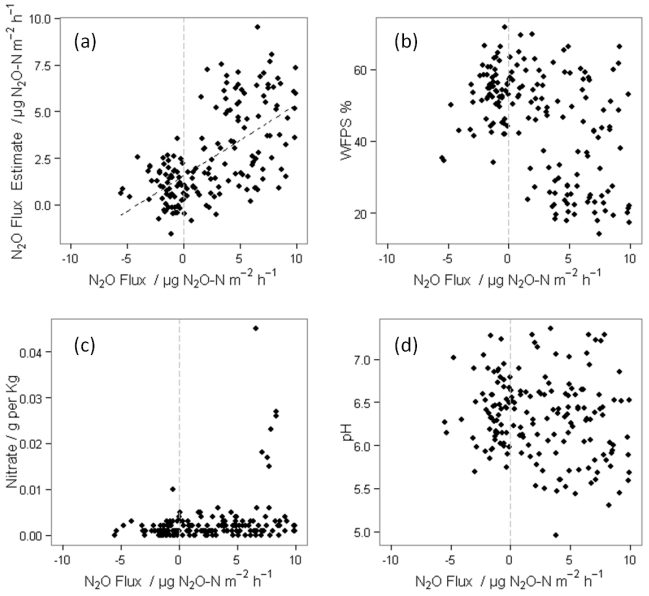

Figure 4. Multiple linear regression was carried out to correlate all N_2O fluxes measured below 10 µg N_2O-N m^{-2} and the soil properties measured from these locations **(a)** (see Table 2). Individual comparisons with the three strongest correlating properties are shown **(b, c, d)**.

Table 2. Multiple linear regression correlation of soil properties and N_2O flux below 10 µg N_2O-N m^{-2} h^{-1} as plotted in Fig. 4a.

	Estimate	SD	*p* value	Statistical significance
(Intercept)	−1512.96	779.79	0.054	.
NH_4-N g Kg^{-1}	14.87	16.23	0.361	
NO_3-N g Kg^{-1}	163.84	54.91	0.003	**
pH	−1.33	0.60	0.027	*
WFPS %	−0.17	0.02	7.85×10^{-11}	***
Bulk density g cm^{-1}	593.01	299.99	0.050	*
Soil porosity	1526.82	779.63	0.052	.
Carbon g Kg^{-1}	−0.02	0.02	0.358	
Nitrogen g Kg^{-1}	−0.10	0.07	0.179	

Significance of *p* value: 0, "***", 0.001 "**", 0.01 "*", 0.05 "." 0.1, " " 1.

more likely to occur in damper soils (WFPS > 40 %) with a pH of approximately 6.5; however, the lack of observable difference in the soil properties measured between slightly positive and slightly negative fluxes may indicate that measurement uncertainty in both flux and soil property measurements is too large to investigate these relationships in detail.

4 Discussion

The results in this paper show that even with a high-precision flux measurement methodology a relatively high proportion of apparently negative fluxes are recorded; however, these measurements rarely exceed the detection limit of the measurement method (Fig. 2). The frequency of near zero fluxes

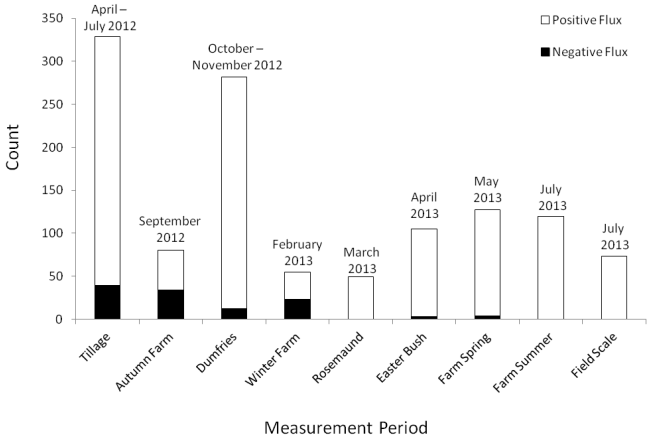

Figure 5. The number of negative and positive fluxes measured from all InveN_2Ory field sites using the dynamic chamber method in chronological order (see Table 1 for details).

below the detection limit is very high (28 % of measurements reported fluxes below 4 µg N_2O-N m^{-2}), and many of the negative flux measurements in this experiment are likely to be caused by noise in the gas analyser (as shown in Fig. 3d). A look at the change in ratio of negative fluxes with time also supports this theory. In the development stages of the dynamic chamber method (2012 measurements), the signal to noise ratio of the system was slightly lower due to unstable temperature conditions for the analyser and lack of a stable source of power supply. As the system logistics were optimised, the flux detection limit improved slightly and the number of negative fluxes recorded fell rapidly (see Fig. 5).

It would be simplistic to assume that instrumental noise is the cause of all of the negative fluxes of N_2O measured in these experiments, as can be seen in Fig. 3a, b and c. In these examples it is clear that concentrations of N_2O decreased below the ambient concentrations of N_2O in the atmosphere. It is highly unlikely that an increase in N_2O concentration followed by a leak could cause this effect over the short 120 s measurement period in our dynamic chamber method. However, this explanation would be plausible over the much longer, 30–60 min incubation periods required by static chamber methods (Cowan et al., 2014a). Although very rare, consistent decline in N_2O concentrations is observed in some of the measurements (as in Fig. 3) and the reasons for these observations are yet to be found.

One hypothesis which was tested in laboratory conditions using this methodology is that N_2O may dissolve in moisture in the tubing of the dynamic chamber system in wetter and colder conditions. This theory was tested early in the development of the dynamic chamber system and no effect on N_2O concentration measurements was observed when water was added to the system; however, the effect that humidity may have on the system in different temperature conditions may be very small and difficult to detect in lab conditions.

Some interference in N_2O measurements caused by moisture and high humidity remains one explanation of N_2O uptake in the system using this methodology. Very slight laser drift and spectra fitting caused by rough environmental conditions and transportation of the delicate instrumentation are other possible reasons for this uptake effect, although we see no evidence from any of the recorded data that this is the case; moreover, there is no reason to believe that the decreased signal to noise ratio due to these disturbances would not produce an equal distortion in the positive range of the fluxes.

It is believed that uptake of N_2O in agricultural soils may be the result of denitrification occurring at the surface layer of soils, which converts atmospheric N_2O into N_2 (Yu et al., 2000; Wrage et al., 2004). Past experiments have linked negative fluxes to soil properties such as moisture content, temperature, pH, oxygen and available nitrogen (Heincke and Kaupenjohann, 1999; Khalil et al., 2002). N_2O uptake has also been recorded from forest soils, with similar links observed between N_2O flux and available nitrogen (Rosenkranz et al., 2006; Goldberg and Gebauer, 2009); however, the influence of these factors seems to vary between experiments and no clear set of conditions that would favour negative fluxes from different soil types can be established. It remains plausible that various microbial processes in soils are able to remove N_2O from the atmosphere; however, the mechanisms and triggers for N_2O uptake need to be studied further to understand these processes (Chapuis-Lardy et al., 2007).

The analysis of soil samples taken from locations where negative and low fluxes were measured in this study showed no clear relationship between flux and soil properties. This lack of correlation may be due to the heterogeneous nature of soils at small scales, which may mask the relationship between soil properties if samples are not exactly co-located. The 5 cm sample depth may have been too deep if the causal relationship between N_2O uptake and soil properties is determined by the first few mm of soil, as suggested by Neftel et al. (2000). A more significant concern in this study is that the high number of negative fluxes measured during the wetter period in autumn and winter may bias any relationships with soil properties. It is unclear whether the higher ratio of negative fluxes measured during autumn and winter (2012/2013, as shown in Fig. 4) is caused by higher instrumental uncertainty which was improved in subsequent measurements or if it is a genuine effect of the wetter soil properties at the time. A moisture effect on the methodology could also have increased the possibility of negative flux measurements during these wetter periods.

What is clear from this study is that true negative fluxes of N_2O from the agricultural soils examined are rare and very small. The issues that still exist in identifying when negative fluxes of N_2O are real or caused by instrumental noise using a high-precision QCL instrument suggests that more commonly used N_2O flux measurement methodologies, such as the static chamber method, would have been unable to measure negative fluxes of N_2O with the precision required to identify if they are real or not. The results of this study suggest that large negative fluxes reported in the literature may in fact indicate a larger detection limit of an individual methodology than previously thought, which may explain many reports of negative flux measurements in the literature (Jordan et al., 1998; Flechard et al., 2005; Jones et al., 2011). Certainly, the majority of negative fluxes reported in this study were most likely caused by instrumental noise (as shown by Figs. 2 and 3d). The high frequency of near zero fluxes of N_2O from soils highlights the need for higher precision measurements (able to detect in the region of 0 to 10 $\mu g\, N_2O\text{-}N\, m^{-2}\, h^{-1}$) when one wants to characterise the N_2O exchange processes between soil and air in background or unperturbed conditions.

When negative fluxes of N_2O are measured during field experiments it can be detrimental to the study as it complicates the calculation of cumulative fluxes and emission factors from certain soils and agricultural practices. This issue has been addressed several ways in the past. Negative fluxes are sometimes treated as real and left in all calculations or declared false measurements and removed or set to zero flux values. In theory, when flux chambers are used a larger number of measurements should help reduce the uncertainty in an average flux measurement, thus reducing the likelihood of measuring a negative flux; however, this is not always the case, especially when detection limits are large. It is our recommendation that propagation of error be investigated thoroughly where negative fluxes are concerned. When calculating cumulative flux estimates over long periods of time it is important to propagate the large uncertainty in measurements with time as well as the average fluxes measured. This may lead to very large uncertainties in these types of experiments; however, if this is the case then it may indicate that a particular cumulative flux methodology is not suitable for purpose.

5 Conclusions

Four small negative fluxes of N_2O out of 1220 have been recorded in this study greater than the defined detection limit of the measurement methodology. The reason for these four negative fluxes is still not fully understood and these observations do not provide strong evidence for the occurrence of microbial net uptake of N_2O. This study suggests that it is likely that many recorded negative fluxes of N_2O are significantly smaller and rarer than reported in previous literature. We also highlight the need to fully understand whether negative flux measurements are real or simply readings below the detection limit of the measurement methodology. For these reasons we wish to highlight the importance of specifying the "real" flux detection limit associated to each data set, as opposed to a theoretical detection limit associated exclusively with the factory-declared precision of the gas analysers: this would allow a more robust estimate of the net contribution of each agricultural environment investigated.

The drivers of true negative N_2O flux in agricultural soils cannot be identified in this study. We suggest that, from the evidence presented here, it can be assumed that negative fluxes measured from agricultural soils are a good indicator of the true detection limit of a flux measurement methodology. The results of this study provide strong evidence against the theory that negative fluxes of N_2O in agricultural soils can be a significant sink of atmospheric N_2O, as most of the negative N_2O fluxes reported are likely to be an artefact of measurement methodology.

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