

TECHNICAL ADVANCE

Evaluation of photoacoustic infrared spectroscopy for simultaneous measurement of N₂O and CO₂ gas concentrations and fluxes at the soil surface

JAVED IQBAL*, MICHAEL J. CASTELLANO* and TIMOTHY B. PARKIN†

Department of Agronomy, Iowa State University, Ames, IA 50011, USA, †National Laboratory for Agriculture and the Environment, US Department of Agriculture-ARS, Ames, IA 50011, USA*Abstract**

Simultaneous measurement of N₂O and CO₂ flux at the soil surface with photoacoustic infrared spectroscopy (PAS) is gaining popularity due to portability, low maintenance, and ease-of-operation. However, the ability of PAS to measure N₂O with accuracy and precision similar to gas chromatography (GC) is uncertain due to overlap in N₂O, CO₂, and H₂O absorbance spectra combined with the large range in analyte concentrations. We tested the ability of six PAS units to simultaneously measure N₂O and CO₂ gas concentrations and fluxes with accuracy and precision similar to two GC units. We also evaluated H₂O vapor and CO₂ interferences with N₂O measurement. The accuracy and precision of standard gas concentration measurements with PAS and GC were similar. High water vapor (~26 600 ppm) and CO₂ concentrations (~4500 ppm) did not interfere with N₂O measurement across the concentration range typically observed in static flux chambers at the soil surface (~0.5–3.0 ppm N₂O). On average, N₂O fluxes measured with the six PAS were 4.7% higher than one GC and 9.9% lower than the second GC.

Keywords: carbon dioxide, gas chromatography, humidity, nitrous oxide, photacoustic infrared gas analyzer

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Introduction

Reports of N₂O fluxes at the soil surface are growing rapidly due to increasing interest in global change and the potential economic value of reducing greenhouse gas emissions. Accurate estimates of N₂O fluxes at the soil surface require frequent and widespread sampling due to high spatial and temporal variation (Yanai *et al.*, 2003; Parkin, 2008; Groffman *et al.*, 2009). However, the conventional method of N₂O measurement, gas chromatography (GC), requires significant time and cost. As a result, a number of recent studies have used photoacoustic infrared spectroscopy (PAS) to measure N₂O fluxes because it permits relatively rapid, simultaneous measurement of N₂O and CO₂ fluxes *in situ* (Flechard *et al.*, 2005; Adviento-borbe *et al.*, 2007, 2010; Castellano *et al.*, 2010; Iqbal *et al.*, 2012).

Although PAS may be an attractive alternative to GC, there are trade-offs between the two methods. External GC calibration allows easy and frequent calibration checks by measurement of samples with known gas concentrations. However, GC requires sample transport from field to laboratory, pressurized gas standards, and relatively high equipment maintenance. The number of

samples collected for GC analysis is also limited by sampling time and cost: point estimates of N₂O flux with GC are typically derived from three to four individual gas concentration measurements from a static flux chamber deployed over the soil surface for 40–60 min (Parkin & Venterea, 2010; Parkin *et al.*, 2012). Few gas concentration measurements over long periods of time increase the likelihood of nonlinear relationships between gas concentration and time (flux rates) due to analytical error, changes in microclimate, or changes in gas diffusion. Regardless of cause, nonlinear changes in gas concentration over time complicate selection of an appropriate flux calculation model, potentially increasing experimental error (Venterea *et al.*, 2009; Venterea, 2010).

In contrast with GC, PAS is field portable and capable of measuring multiple gas concentrations in a closed loop system every 2 min without need for subsampling, pressurized carrier gases, and chromatographic separation. Rapid analysis permits multiple gas concentration measurements during brief chamber closure (6–14 min) thereby increasing statistical power in flux calculation model fitting and decreasing chamber effects on microclimate and gas diffusion. Despite these benefits, internal calibration of PAS (typically set by the vendor; Ambus & Robertson, 1998; Flechard *et al.*, 2005; Adviento-borbe *et al.*, 2007) does not permit calibration

Correspondence: Javed Iqbal, tel. + 001 515 294 3963, fax + 001 515 294 9985, e-mail: jiqbal.soil@yahoo.com

checks during gas measurement in the field. Moreover, the proximity of absorption wavelengths among H₂O vapor (6.25, 10, 20 μm), CO₂ (15 μm), and N₂O (7.78, 17, 4.5 μm), combined with the large range of analyte concentrations that spans six orders of magnitude (e.g., 26 000 ppm H₂O vs. 0.320 ppm N₂O), interferes with accurate N₂O concentration measurement (Wang *et al.*, 1976). Although some reports suggest numerical correction for H₂O vapor and CO₂ interferences with N₂O measurement can produce similar accuracy among GC and PAS methods (Ambus & Robertson, 1998; Yamulki & Jarvis, 1999; Flechard *et al.*, 2005), other reports suggest PAS cannot accurately measure N₂O concentrations despite such correction factors (Akdeniz *et al.*, 2009).

Here, we build on previous evaluations of PAS by comparing six PAS and two GC units in four experiments that explicitly evaluate CO₂ and H₂O vapor interferences with N₂O measurement. The experiments evaluated as follows: (i) The accuracy and precision of PAS and GC measurement of National Institute of Standards and Technology-certified N₂O and CO₂ concentrations in 'compressed air'; (ii) The effects of high H₂O vapor and CO₂ concentrations on the accuracy of N₂O concentration measurement across the range typically observed in static chambers deployed over the soil surface; (iii) The effects of H₂O vapor and CO₂ removal on measurement of N₂O fluxes at the soil surface with PAS; and (iv) The consistency among simultaneous PAS and GC measurements of a broad range of N₂O and CO₂ fluxes without CO₂ or H₂O vapor removal.

Materials and methods

Measurement of gas concentrations

In this study, six PAS gas analyzers and two gas chromatographs were used to measure gas concentrations. The PAS analyzers (1412 Photoacoustic multi-gas monitors; INNOVA Air Tech Instruments, Ballerup, Denmark) were equipped with optical filters for measurement of N₂O, CO₂, and H₂O vapor concentrations. The PAS units analyze a 0.754 cm³ gas sample in a sealed cell that is irradiated by modulated ('chopped') infrared (IR) light at a preselected wavelength using a specific optical filter for each gas. As the gas absorbs energy, it expands, increasing cell pressure. Because the IR light beam is chopped, the pressure alternately increases and decreases, creating an acoustic signal. The acoustic signal is detected by microphone and converted into a voltage differential that is proportional to the concentration of the gas in the cell. The particular instrument used in our experiments has a rotating optical filter carousel that can hold six gas filters, and is thus capable of automatically measuring six gases every 2 min. Water vapor must be measured to correct for absorbance spectra overlap (interference) with other gases such as

N₂O and CO₂. Similarly, CO₂ must be measured to correct for interference with N₂O. Optical filters should be arranged in order of increasing water vapor interference with gas detection. Gas concentrations are reported at user-selected temperature and pressure.

The PAS analyzers were calibrated by the vendor (California Analytical Instruments, Inc., Orange, CA, USA) with National Institute of Science and Technology-certified (NIST)-traceable standard gases ($\pm 5\%$) according to expected concentrations in soil chambers (optical filter ranges 0.030–12.4 ppm N₂O and 5–5010 ppm CO₂). Optical filters were ordered: N₂O, CO₂, and H₂O. Calibrations were conducted at 20 °C and 1 atm. Tube flushing time was set to 8 s and chamber flushing to 11 s. Details of the calibration procedure are described by Moody *et al.* (2008). The calibration accuracy is checked by measuring combinations of N₂O and CO₂ standards that are expected to occur in static flux chambers at the soil surface and maximize potential interferences of CO₂ and H₂O vapor on N₂O measurement. Similar to most research, we did not recalibrate the instruments upon receipt from the vendor (Adviento-borbe, 2005; Castellano *et al.*, 2010).

Although PAS gas concentration detection limits of 0.030 ppm N₂O and 5 ppm CO₂ are a function of optical filters and instrument capabilities, gas flux detection limits are variable and a function of: (i) analytical precision at ambient gas concentration, (ii) the number of gas concentration measurements used to calculate flux, (iii) the amount of time the chamber was closed, and (iv) the selected flux calculation model (e.g., linear, quadratic, etc.). Using the methods of Parkin *et al.* (2012), we calculated minimum detectable N₂O and CO₂ fluxes for PAS based on: (i) 4.1% ambient N₂O measurement precision and 1.3% ambient CO₂ precision, (ii) five individual gas concentration measurements including time zero, (iii) 8 min chamber closure, and (iv) linear model flux calculation. Resultant flux detection limits were 0.0033 ppm N₂O min⁻¹ and 1.32 ppm CO₂ min⁻¹.

Two gas chromatographs in separate laboratories were compared with PAS analyzers. One gas chromatograph (Agilent 7890, Santa Clara, CA, USA) was maintained by the Department of Agronomy at Iowa State University (hereafter GC-1). Nitrous oxide and CO₂ were analyzed on GC-1, which was operated with an electron capture detector (ECD) at 350 °C for N₂O detection and a thermal conductivity detector at 200 °C for CO₂ detection. Gas species separation was accomplished with stainless steel columns packed with Porapak Q, 80/100 mesh (Restek Corporation, Lancaster, PA, USA) and maintained at 85 °C. Carrier gas was 10% CH₄ and 90% Ar. The second gas chromatograph (SRI Instruments, Model 8610C, Torrance, CA, USA) was maintained by the US Department of Agriculture National Laboratory for Agriculture and the Environment (hereafter GC-2). Only N₂O concentrations were analyzed on GC-2, which was operated with an ECD at 325 °C for N₂O detection. Gas species separation was accomplished with stainless steel columns packed with Haysep D and maintained at 50 °C. Carrier gas was N₂.

The GCs were calibrated with independent sets of NIST-traceable standard gases with accuracies certified at $\pm 5\%$ (Scott Specialty Gas, Plumsteadville, PA, USA). Calibration

concentrations were as follows: $0.101 \pm 5\%$, $1.02 \pm 5\%$, and $10.0 \pm 5\%$ ppm N_2O for both GC; and $510 \pm 5\%$, $1000 \pm 5\%$, and $2000 \pm 2\%$ ppm CO_2 for GC-1. Calibrations always produced linear relationships between voltage output and gas concentration with $r^2 > 0.99$. Standard gas calibration check samples were inserted between every 10 unknown samples and were measured within 5% of known concentration.

Gases were sampled from SamplePro[®] PVDF (Eighty Four, PA, USA) gas bags and PVC static flux chambers with stainless steel fittings. The cylindrical PVC static flux chamber used in this study was built according to specifications of Parkin & Venterea (2010). It consisted of a chamber base (anchor) and lid. The base had an inner diameter (ID) of 25.2 cm and height of 28.5 cm. The base was inserted 5 cm into 2492.5 cm³ of soil so that the chamber base extended 23.5 cm above the soil surface. The soil inside the base was sampled from the Iowa State University Research Farm (Boone County, IA, USA). The vented PVC chamber lid had an ID of 25.2 cm and a height of 11.2 cm. To simultaneously connect the six PAS machines to the flux chamber, we added one inlet and one outlet (3 cm diameter) to the chamber design. Six 1 m long Teflon[®] tubes (McMASTER-CARR, Elmhurst, IL, USA) (3 mm ID) were sealed in 3 cm diameter rubber stoppers with silicone gel and fitted to the inlet and outlet ports, providing a gas-tight fit. A rubber septum in the lid facilitated manual gas sampling for GC sampling. At the time of gas flux measurements, six PAS gas analyzers were connected in a closed loop air circulation system with the chamber. The chamber lid was sealed to the base with a tire inner tube (Parkin & Venterea, 2010). The entire loop volume (including chamber) was 19.21 L.

Experiment 1: accuracy and precision

A 'compressed air' tank (synonyms: 'breathing gas', 'synthetic air', 'medical air') was purchased from Airgas (Part Number AI B300GE) and shipped to the National Institute of Standards and Technology (Gaithersburg, MD, USA, www.NIST.gov) where N_2O and CO_2 concentrations in the tank were measured and certified at 0.7148 ± 0.0017 ppm N_2O and 473.45 ± 0.44 ppm CO_2 . The tank was then returned to our laboratories and air from this tank was used to evaluate the accuracy and precision of six PAS and two GC units. Two gas bags (3 L SamplePro[®] PVDF) with dual stainless steel fittings were flushed with helium and evacuated before filling with the compressed air. Each gas bag was paired with three PAS units. Each PAS unit was individually connected to a gas bag in a closed loop circulation system (except for the first measurement with each PAS when the outlet port was not connected to the bag to flush the analysis cell and tubing that connected the PASs and gas bags). After PAS connection, N_2O and CO_2 were measured every 2 min for 22 min. The first sampling point was discarded to ensure the system was completely flushed. The subsequent 10 sampling points, representing 20 min of measurement, were analyzed. For GC analysis, samples were drawn from the NIST-certified compressed air tank in a 10 mL polypropylene syringe and injected into 20 evacuated glass serum vials. Ten vials were analyzed on each GC unit. The mean of each of the 10

replicate samples from each PAS or GC unit was used to determine accuracy; the coefficient of variation was used to determine precision.

Experiment 2: effects of H_2O vapor and CO_2 on measurement of N_2O concentrations

To evaluate potential CO_2 and H_2O vapor interferences with N_2O measurement, we compared PAS and GC measurements of three approximate N_2O concentrations (0.5, 1.0, and 3.0 ppm) at high CO_2 (4500 ± 300 ppm) and a wide range of H_2O vapor concentrations. The three concentrations of N_2O at high CO_2 were measured at one constant low H_2O vapor concentration (4100 ± 100 ppm) and across a range of variable high H_2O vapor concentrations that were reduced from 26 600 to 15 500 ppm during continuous N_2O measurement. The combinations of N_2O , CO_2 , and H_2O vapor were prepared in 3 L SamplePro[®] PVDF gas bags. A total of six gas bags were prepared (3 levels $\text{N}_2\text{O} \times 1$ level of $\text{CO}_2 \times 2$ levels of H_2O vapor). The approximate N_2O and CO_2 concentrations were achieved by filling gas bags with laboratory air (2.86 L) and 0.1, 0.3, or 0.9 mL of $10\,000 \pm 5\%$ ppm N_2O standard gas in addition to 135 mL of $100\,000 \pm 2\%$ ppm CO_2 standard gas. Calibrated syringes were used to prepare the bags. The same high CO_2 concentration was used at all N_2O concentrations because a positive CO_2 interference on N_2O concentration measurement has been reported at high CO_2 (Zimmerman & Rasmussen, 1975).

After each of the six gas bags was prepared, each of five PAS units was connected to the gas bag for 22 min during which gas concentrations were measured every 2 min (110 total minutes of PAS measurement per gas bag). The sixth PAS unit was not available for this experiment due to multiple user demands. The first of the eleven 2 min gas concentration measurements with the PAS was discarded to ensure system flushing (see above) and the last 10 measurements were analyzed (2–22 min, see Experiment 1 methods). The order in which individual PAS units were connected to each gas bag was randomized. To compare PAS measurements with GC, three replicate gas samples were drawn from each of the gas bags immediately before the first PAS was connected (initial samples) and immediately after the last PAS was connected (final samples). The gas samples were analyzed on GC-1 and the average of the three initial and final samples ($N = 6$) was used for comparison with PAS. There was no statistical difference between the 'initial' and 'final' gas concentrations. Only GC-1 was used in this experiment because GC-2 calibration checks indicated that the calibration drifted $>5\%$ from standard gas check samples.

We attempted to hold all combinations of N_2O , CO_2 , and low H_2O vapor constant throughout the 110 min of measurement per bag. However, in the three gas bags with high H_2O vapor concentrations, we intentionally produced a 34–39% linear decline in H_2O vapor concentrations during the 110 min measurement period by changing the gas temperature and condensing the H_2O vapor. This allowed us to determine if rapid change in H_2O vapor concentration affects N_2O measurement.

In the three bags with low H₂O vapor, no additional water was added beyond that contained in the laboratory air (4100 ± 100 ppm H₂O). However, in the three bags with high H₂O vapor concentrations, we added 3 mL of deionized water and evaporated it by moving the bags to a 40 °C incubator for 3 h. Subsequently, the gas bags were returned to the ~22 °C laboratory for immediate PAS gas concentration measurements. We did not measure H₂O vapor in the gas bags independent of PAS because our objective was to evaluate the effects of high CO₂ and H₂O vapor concentrations on N₂O measurements. The percent difference between mean PAS and GC gas concentration measurements was compared.

Experiment 3: effects of CO₂ and H₂O vapor removal on N₂O flux measurement at the soil surface

Using PVC flux chambers and soil as described above, we evaluated the ability of PAS calibration to eliminate CO₂ and H₂O vapor interferences with N₂O flux measurements. We compared measurements among four methods: (i) GC, (ii) PAS with calibration corrections for H₂O vapor and CO₂ interference, (iii) PAS with H₂O vapor removal, and (iv) PAS with CO₂ removal. To produce N₂O and CO₂ fluxes that are observed in the field, we simulated a 10 mm rainfall event including 150 kg KNO₃ N ha⁻¹ and 12 mg glucose C kg per soil dissolved in solution. The solution was added to the chamber soil 8 h before the start of gas measurements. During gas flux measurements, H₂O vapor concentrations in the chamber were at the high end of the range normally encountered in the field (approximately 27 000 ppm H₂O vapor).

At the start of gas flux measurements, six PAS gas analyzers were connected to the chamber. Upon closure of the chamber, all the PAS gas analyzers were started simultaneously and measured gas concentrations every 2 min for 30 min (15 measurements). During PAS measurements, chamber gas was sampled at 7.5 min intervals (five sampling points including time zero) with two 10 mL syringes and stored in separate 10 mL glass serum vials for analysis on GC-1 and GC-2 within 48 h.

During each chamber closure, one of the six PAS gas analyzers was connected to an in-line soda lime (Ca(OH)₂, KOH, NaOH, ethyl violet) trap for CO₂ removal or an in-line silica gel trap for H₂O vapor removal so that N₂O measurements from one PAS with CO₂ or H₂O vapor removal could be compared to N₂O measurements from five PAS without CO₂ or H₂O vapor removal. Accordingly, there were 12 discrete chamber closures: During each of the six closures, five PAS had no traps and one PAS had a CO₂ trap; during each of the other six closures, five PAS had no trap and one PAS had a H₂O trap.

Soda lime traps contained sufficient material to trap 13 times the mass of CO₂ that was passed through the traps. Silica gel traps had a water removal capacity of 10 times the H₂O that was passed through the trap during the six, 30 min gas measurement periods. The five PAS units without CO₂ or H₂O vapor traps were maintained in closed loop with the flux chamber. However, the PAS unit with either a CO₂ or H₂O vapor trap was not maintained in the closed loop and vented to the laboratory atmosphere so that it would not dilute CO₂

or H₂O vapor concentrations in the chamber. Accordingly, gas flux measurements in this experiment represent production from soil minus dilution from laboratory air that entered the pressure equilibration vent on the flux chamber lid (~0.2 L min⁻¹). Gas fluxes (ppm min⁻¹) were calculated from the slope of linear increase in gas concentrations over time. All data were best fit by a linear model ($r^2 = 0.98\text{--}0.99$). The mean N₂O flux from five PAS units without traps was compared with the N₂O flux from one PAS with a CO₂ or H₂O trap, and the N₂O flux from each GC. In addition, the mean flux for all six chamber closures with each method was compared. For each CO₂ and H₂O trapping trial that consisted of six discrete chamber closures, the means of 30 PAS flux measurements without traps were compared with six PAS flux measurements with traps, and six GC flux measurements with each GC unit.

Experiment 4: comparison of N₂O and CO₂ fluxes measured with PAS and GC

In addition to Experiment 3, gas flux measurements were compared among PAS and GC in PVC soil chambers with all six PAS connected to the chamber in a closed air circulation loop without CO₂ or H₂O vapor traps. Gas fluxes in the chamber were promoted by adding a simulated 10 mm rainfall with dissolved KNO₃ (150 kg N ha⁻¹) and glucose (12 mg C kg per soil). Gas measurements with PAS were made at 2 min intervals during discrete chamber closures that spanned 14, 30, 46, and 60 min (7, 23, and 30 PAS gas concentration measurements, respectively). For GC comparison, samples were manually drawn from the chamber headspace every 4.4 min during 14 min chamber closures (four sampling points including time zero) and every 7.5 min during 30 min (five sampling points including time zero), 46 min (seven sampling points including time zero), and 60 min chamber closures (nine sampling points including time zero). Samples were stored in 10 mL glass vials for GC measurement within 48 h. In total, there occurred 26 discrete chamber closures with 26 N₂O (PAS vs. GC-1 and GC-2) and 14 CO₂ (PAS vs. GC-1) gas flux measurement comparisons. Relationships between gas concentrations and time were best fit by a linear model. We compared PAS-measured against GC-measured fluxes with linear regression.

Results and Discussion

Experiment 1: PAS accuracy and precision

All GC and PAS units measured N₂O within 0.5–8.8% of the NIST-certified concentration. Repeated measurements of the NIST-certified N₂O concentration on individual PAS units produced coefficients of variation (CV) that ranged from 1.20% to 2.52% and were not correlated with accuracy (Table 1). This variability in N₂O measurement from recirculated gas is lower than that reported for older PAS models from the same manufacturer (CV = 6%; Ambus & Robertson, 1998), and comparable to variability in repeated, automated, N₂O

Table 1 Accuracy and precision of six photoacoustic infrared gas analyzers (PAS) and two gas chromatographs (GC-1 and GC-2) indicated by measurement of compressed air that contained National Institute of Science and Technology-certified (NIST) concentrations of N₂O (0.7148 ± 0.0017 ppm) and CO₂ (473.45 ± 0.44 ppm). Measured values represent averages of 10 consecutive measurements ± standard deviation for each PAS and GC unit

Unit	N ₂ O			CO ₂		
	Measured (ppm)	Coefficient of variation (%)	% Deviation from NIST standard	Measured (ppm)	Coefficient of variation (%)	% Deviation from NIST standard
PAS 1	0.719 ± 0.016	2.22	0.6	514.70 ± 2.76	0.54	8.0
PAS 2	0.679 ± 0.013	1.91	-4.9	493.53 ± 3.34	0.68	4.0
PAS 3	0.668 ± 0.008	1.20	-6.5	493.33 ± 2.54	0.51	4.0
PAS 4	0.675 ± 0.017	2.52	-5.5	488.84 ± 4.77	0.98	3.1
PAS 5	0.711 ± 0.014	1.97	-0.5	517.05 ± 2.99	0.58	8.4
PAS 6	0.651 ± 0.016	2.45	-8.8	518.26 ± 2.38	0.46	8.6
PAS 1-6 Average	0.683 ± 0.028	4.09	-4.5	504.28 ± 16.03	3.18	6.1
GC-1	0.762 ± 0.015	1.97	6.73	464.03 ± 16.8	3.62	-1.98
GC-2	0.734 ± 0.038	5.18	2.68	N.D.	N.D.	N.D.

N.D., no data.

analysis with GC observed by Parkin (1985) CV = 1.9–4.9% and Yamulki & Jarvis (1999) CV = 1.5%. All GC and PAS units measured CO₂ within 2.0–8.6% of the NIST-certified concentration (Table 1). The GC measured the NIST-certified CO₂ concentrations with greater accuracy than any of the PAS units (Table 1). However, the CV of repeated NIST-certified CO₂ concentration measurements with individual PAS units (range: 0.46–0.98%) was lower than the CV of repeated NIST-certified CO₂ concentration measurements with the GC.

Four of the six PAS analyzers produced a mean N₂O concentration within the ±5% of calibration standards. Additional error in PAS-3 and PAS-6 (Table 1) could have resulted from: (i) CO₂ and H₂O interference with N₂O measurement; (ii) Contamination of NIST-certified N₂O and CO₂ concentrations, and (iii) PAS calibration error. Experiments presented below demonstrate that CO₂ and H₂O vapor interference with N₂O measurements was not significant. Neither the accuracy nor precision of N₂O measurements was correlated with the accuracy or precision of CO₂ measurements. Contamination of the NIST-certified N₂O and CO₂ concentrations with laboratory atmosphere could have occurred, but was unlikely; the contamination required to dilute N₂O by 4.5% and concentrate the CO₂ by 6.1% (Table 1) was 5.06 and 2.35 L. In contrast, errors in instrument calibration and standard gas concentrations (±5%) were likely the largest source of error in N₂O and CO₂ concentration measurement. All PAS units were calibrated with the same NIST-traceable standards, potentially explaining the consistent positive bias among all units (Table 1).

Experiment 2: water vapor and high CO₂ effects on N₂O concentration measurements

Water vapor and CO₂ can interfere with accurate N₂O measurement by PAS (De Klein *et al.*, 1994). However, correction factors developed during PAS calibration appear to have eliminated H₂O vapor and CO₂ interferences with N₂O measurement across the range of N₂O concentrations in our experiments. Measurements of N₂O concentrations typically observed in static flux chambers at the soil surface (~0.5–2.8 ppm) in combination with high CO₂ (~4500 ppm) and a broad range of H₂O vapor (~4100 ppm or ~26 600–15 500 ppm) were similar among PAS and GC (Fig. 1).

As a proportion of the GC measurement, absolute differences between N₂O concentration measurements with PAS and GC appeared to be larger at low N₂O concentrations, but independent of humidity (Fig. 1). Nevertheless, the observed range of differences between N₂O measurements with PAS and GC (0.0–7.4%) is consistent with PAS accuracy reported in Experiment 1 (Table 1 vs. Fig. 1). These data further suggest that differences between N₂O measurements with the two methods were largely a result of calibration errors rather than CO₂ or H₂O vapor interferences with PAS.

However, we further tested potential H₂O vapor interference with N₂O concentration measurements by evaluating the consistency of N₂O measurements among five PAS units as a function of decreasing H₂O vapor concentrations that encompassed the highest concentrations typically observed in static flux chambers. Despite the 34–39% decrease in H₂O vapor

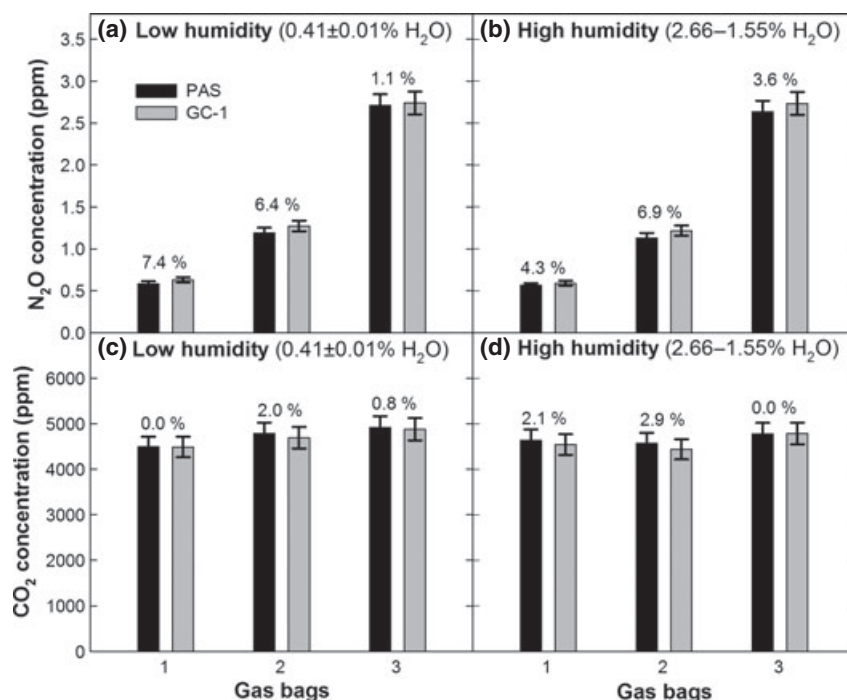


Fig. 1 Consistency among photoacoustic infrared spectroscopy (PAS) and gas chromatography (GC) for measurement of a wide range of N₂O concentrations at high CO₂ concentrations and high or low humidity. During gas measurements, H₂O vapor at low humidity (a and c) was constant whereas H₂O vapor at high humidity (b and d) was intentionally decreased from 2.66% to 1.55%. PAS measurements represent the average of five units. Only one gas chromatograph was used in this experiment (GC-1). To facilitate comparison among methods, 'error bars' indicate $\pm 5\%$ of each of each column. The percent difference between paired PAS and GC-1 measurements is displayed above columns and is the difference between PAS and GC measurements as a proportion of the GC measurement [(PAS–GC)/GC]. See methods section for details.

concentrations, repeated N₂O and CO₂ measurements remained stable within and across PAS units throughout the tested range (Fig. 2).

Experiment 3: effects of CO₂ and H₂O vapor removal on N₂O flux measurements at the soil surface

Removal of H₂O vapor and CO₂ during gas transport from static flux chambers to PAS units did not affect N₂O flux measurements (Figs. 3 and 4). During six independent chamber closure events, simultaneous N₂O concentration measurements from PAS units with and without silica gel H₂O traps produced flux rates within 5% (Fig. 3). Moreover, PAS N₂O flux rate estimations during these chamber closure events were also within 5% of N₂O flux rate estimations from hand-drawn gas samples that were analyzed by GC-1. However, N₂O flux rate estimations based on gas samples measured by GC-2 were 15.6–19.3% higher than PAS and GC-1 flux rate estimations (Fig. 3). The reason for this variability remains unclear, although it could result from positive bias on N₂O concentration measurements due to the N₂ carrier gas method (Zheng *et al.*, 2008).

However, this large difference among PAS, GC-1, and GC-2 was only apparent during this part of our experiment, suggesting another cause. From the time of collection to analysis, hand-drawn gas samples could have been contaminated during evacuation, sampling or storage; GC-1 and GC-2 required different sample vials.

Similar to H₂O vapor removal, CO₂ removal with soda lime traps during gas transport from chamber to PAS did not affect N₂O flux measurements during six independent chamber closure events (Fig. 4). With the exception of two gas flux estimations from samples analyzed by GC-1, hand-drawn gas samples that were collected during the same chamber closure events and analyzed by GC-1 and GC-2 produced N₂O flux estimations that were within 5% of PAS (Fig. 4). Coupled with results from Experiment 2, these results suggest that correction factors for H₂O vapor and CO₂ interference provided by the vendor are accurate and produce N₂O flux measurements that are similar to GC. Nevertheless, our results highlight the potential importance of calibration errors among individual GC and PAS units.

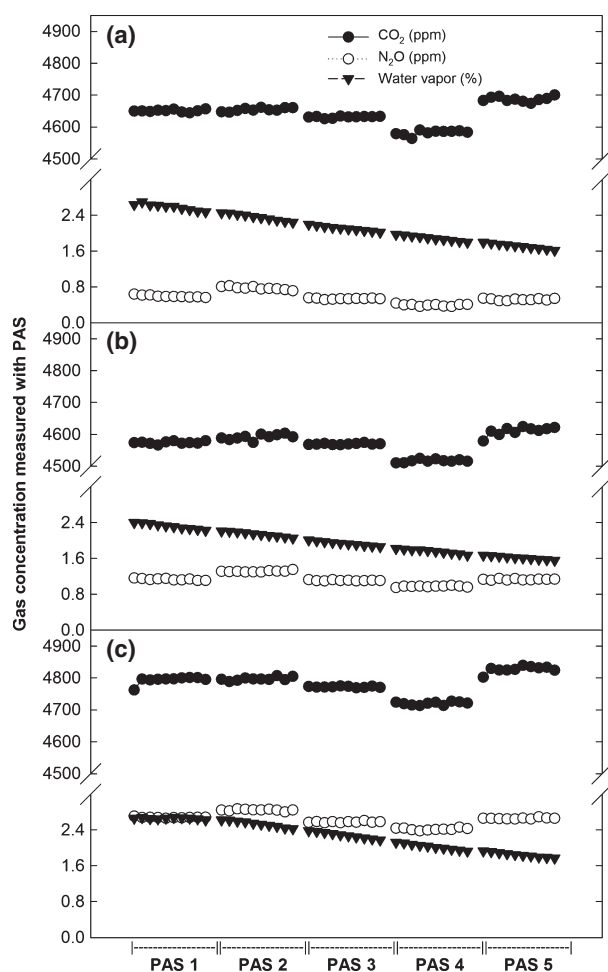


Fig. 2 Consistency of N_2O measurements at low (a), medium (b), and high (c) concentrations with photoacoustic infrared spectroscopy (PAS) at high CO_2 concentration, across a wide range of H_2O vapor concentration. At each N_2O concentration (displayed in panels a–c), five PAS units were sequentially connected to a gas bag for 20 min while 10, 2 min measurements were made. The decline in H_2O vapor concentration was controlled by decreasing the temperature throughout the 100 min experiments. Gas samples at time zero and 100 min were analyzed with gas chromatography (GC) and compared to the mean PAS concentrations (Fig. 1b and d) displayed above.

Experiment 4: consistency of N_2O and CO_2 fluxes measured with PAS and GC

Flux estimations of N_2O and CO_2 developed from simultaneous gas concentration measurements with PAS and GC methods were similar. Simultaneous gas flux estimations with the two methods were significantly correlated ($r^2 > 0.99$; Figs. 5 and 6) across an N_2O flux range of approximately $0.07\text{--}0.5 \text{ ppm min}^{-1}$. These results complement results reported by Ambus & Robertson (1998) who demonstrated similar N_2O flux measurements among PAS and GC at low fluxes from

0.0005 to $0.0202 \text{ ppm min}^{-1}$. Our data extend these results by demonstrating high CO_2 and humidity do not affect N_2O flux measurements.

Although we expected differences in N_2O concentration measurements between PAS and GC units would be attenuated during flux calculations, differences in flux measurements were similar to differences in concentration measurements: Mean N_2O fluxes measured with PAS were 4.7% higher than GC-1 and 9.9% lower than GC-2 across a range $0.07\text{--}0.47 \text{ ppm min}^{-1}$ ($N = 26$ independent flux measurements with each method). On the other hand, mean N_2O concentration measurements with PAS were 5.6% and 2.3% higher than GC-1 and GC-2, respectively, across a range $0.44\text{--}17.3 \text{ ppm N}_2\text{O}$. This difference between PAS and GC measurements, although small, could easily be accounted by the sum of calibration errors among the two methods and eight individual instruments (six PAS and two GC).

Estimation of CO_2 flux with PAS and GC were also similar ($r^2 = 0.97$; Fig. 6). Fluxes of CO_2 measured with PAS were 6.44% lower than GC-1 across a range typically observed in the field ($9.7\text{--}58.8 \text{ ppm min}^{-1}$). However, CO_2 concentrations measured with PAS were 11.4% lower than GC measurements.

Summary comparison of PAS and GC

Given the potential error associated with each independent set of gas standards used to calibrate GC-1, GC-2, and the six PAS units, this study demonstrated similarity among PAS and GC methods for measuring the broad range of N_2O and CO_2 fluxes that are typically observed in static flux chambers at the soil surface. Nitrous oxide and CO_2 flux measurements with PAS and GC were comparable. Other than the comparison of NIST-certified standard gas measurements (Table 1), it is impossible to determine which method is more accurate. Although both methods demonstrated similar variation in N_2O and CO_2 measurement, our individual comparisons of PAS and GC were limited to one GC calibration. Because GC units are calibrated at least daily, the direction and magnitude of calibration error will change daily, particularly with use of new calibration standards. Accordingly, sample-to-sample variation in N_2O and CO_2 measurement may be greater with GC than PAS.

Water vapor and CO_2 are well known to interfere with PAS analysis of N_2O (De Klein *et al.*, 1994) and previous research has concluded that H_2O vapor and CO_2 interference are the largest source of error in N_2O measurement with PAS (Akdeniz *et al.*, 2009). However, we observed no effect of H_2O vapor or CO_2 removal on N_2O concentration measurement (Figs. 3 and 4). Moreover, measurements of a broad

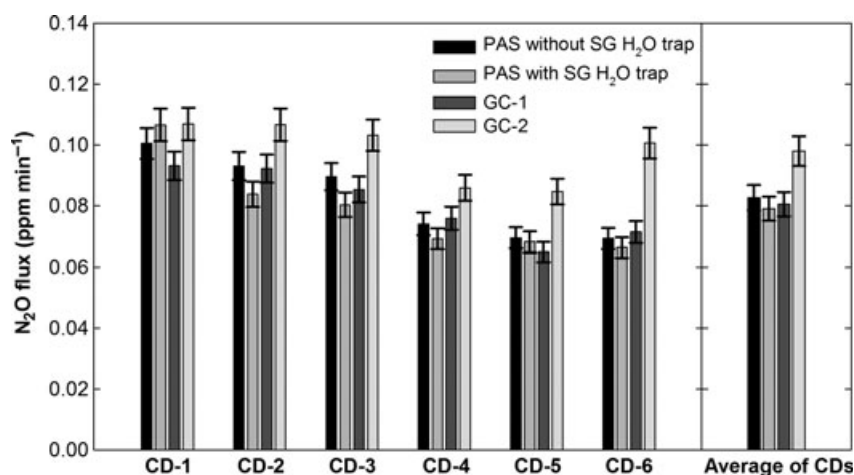


Fig. 3 Comparison of N₂O fluxes measured during six discrete static flux chamber deployment events (CD-1–CD-6) with three methods: (i) photoacoustic infrared spectroscopy (PAS) without silica gel (SG) H₂O trap (average of five PAS units per CD is displayed); (ii) PAS with SG H₂O trap (one PAS unit per CD); and (iii) Gas chromatography with electron capture detector (two GC units; GC-1, GC-2). Static chambers were closed for 30 min during each deployment. Fluxes were linearly calculated from gas concentration measurements every 2 min with PAS and every 7.5 min with GC. Average fluxes of all chamber closures (Average of CDs) are also displayed on the far right panel. To facilitate comparison among methods, ‘error bars’ indicate $\pm 5\%$ of each of each column. A unique PAS unit was connected to the H₂O trap for each CD event. See methods for details.

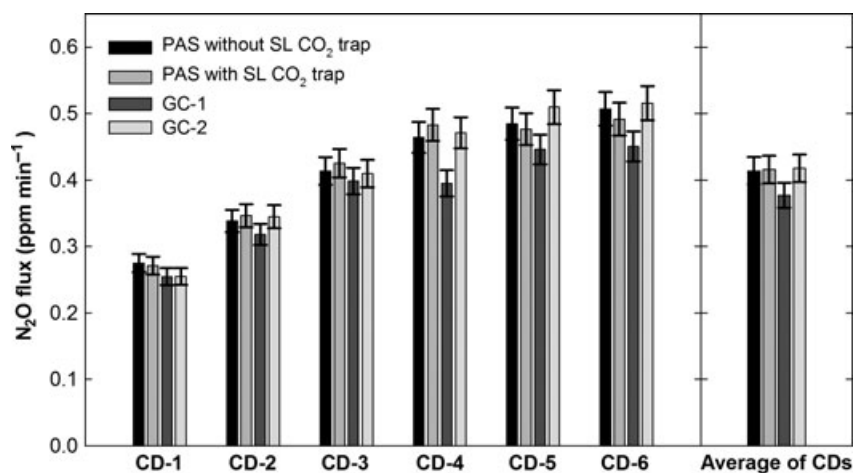


Fig. 4 Comparison of N₂O fluxes measured during six discrete static flux chamber deployment events (CD-1–CD-6) with three methods: (i) photoacoustic infrared spectroscopy (PAS) without soda lime (SL) CO₂ trap (average of five PAS units per CD is displayed); (ii) PAS with SL CO₂ trap (one PAS unit per CD); and (iii) Gas chromatography with electron capture detector (2 GC units; GC-1, GC-2). Static flux chambers were closed for 30 min during each deployment. Fluxes were linearly calculated from gas concentration measurements every 2 min with PAS and every 7.5 min with GC. Average fluxes of all chamber closures (Average of CDs) are also displayed on the far right panel. To facilitate comparison among methods, ‘error bars’ indicate $\pm 5\%$ of each of each column. A unique PAS unit was connected to the CO₂ trap for each chamber deployment event. See methods for details.

range of N₂O concentrations were similar among PAS and GC despite high CO₂ and H₂O vapor. Thus, we conclude that CO₂ and H₂O vapor correction factors introduced during PAS calibration were effective.

Nevertheless, there are a number of trade-offs associated with the decision to measure N₂O and CO₂ fluxes with PAS or GC. The major weakness of PAS is that calibration stability over time is unknown. Few PAS

users calibrate the units themselves and rely on vendor calibration due to significant complexity (e.g., Moody *et al.*, 2008). Although vendor calibration of PAS units tested herein appears to be accurate, it must be checked upon receipt and with regularity. Our personal experience and vendor recommendations suggest that calibration is stable for approximately 1 year of continuous monitoring, but we are unaware of reports that

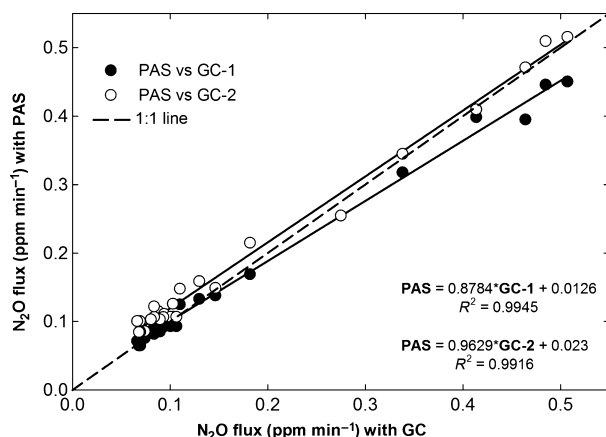


Fig. 5 Consistency of N_2O fluxes measured with photoacoustic infrared spectroscopy (PAS) and gas chromatography (GC) across various soil conditions ($N = 26$ discrete gas flux measurements calculated from simultaneous PAS and GC measurements of N_2O concentrations). Data are from six PAS units and two GC units (GC-1 and GC-2). Fluxes were linearly calculated from increase in N_2O concentration over time. Solid lines are regression lines while dashed line represents 1 : 1 line.

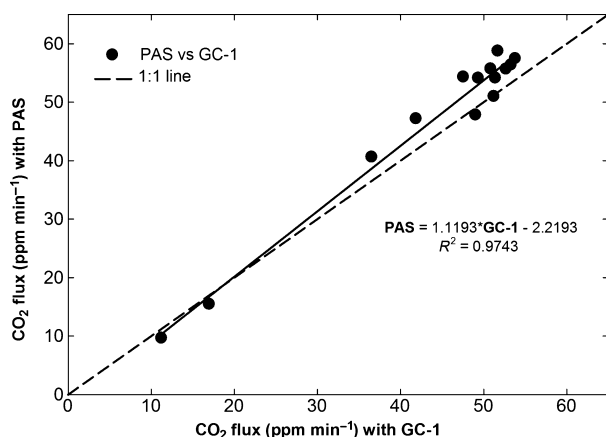


Fig. 6 Consistency of CO_2 fluxes measured with photoacoustic infrared spectroscopy (PAS) and gas chromatography (GC) across various soil conditions ($N = 14$ discrete gas flux measurements calculated from simultaneous PAS and GC measurements of N_2O concentrations). Data are from six PAS units and one GC unit (GC-1). Fluxes were linearly calculated from increase in CO_2 concentration over time. Solid line is regression line while dashed line represents 1 : 1 line.

examine the duration of calibration stability. The accuracy of N_2O and CO_2 calibrations can be checked with a two-step process: (i) measurement of 100% N_2 gas and (ii) measurement of the minimum N_2O and CO_2 concentrations that are expected to be observed (in a gas mixture similar to that encountered in a soil chamber). The calibration should be accurate across the full measurement range if the PAS measures within 5% of the target gas concentrations and the zero N_2 measurement is

not off by >5% of the minimum target gas concentration. For example, if 0.3 ppm N_2O is the minimum concentration, the PAS should read zero N_2O within 0 ± 0.015 ppm. The major strengths of PAS include portability, low maintenance, and rapid gas analysis.

Minimum PAS N_2O flux detection for an 8 min chamber closure consisting of five gas concentration measurements including time zero is $0.0033 \text{ ppm min}^{-1}$ (see methods). Minimum GC N_2O flux detection limit for a typical 45 min chamber closure with three gas concentration measurements is $0.0007 \text{ ppm min}^{-1}$ (Parkin *et al.*, 2012). A number of options are available to manage gas flux detection limits, regardless of analytical technique. Flux detection limits can be reduced by increasing the number of gas concentration measurements or chamber closure time. Alternatively, flux rate can be increased by increasing the ratio of soil surface area to chamber volume.

A significant trade-off between PAS and GC gas analysis methods is the number of measurements that can be made in a working day (3 h measurement period). Human labor and analytical processing rate limit GC analysis. A typical GC with autosampler can run approximately $150 \text{ samples day}^{-1}$ excluding calibration and check standards. Thus, if each flux calculation is based on 3–4 gas concentration measurements, ~35–50 flux calculations could be produced from one GC each day. Gas sampling for 50 flux measurements in a 3 h measurement period requires several workers. In contrast, one PAS operator can produce ~12–25 flux measurements each day depending on length of measurement (which should vary with expected flux rates, but is typically 6–14 min). No subsequent laboratory analysis is required.

The PAS units used in this study are functionally capable of measuring six gases at once, potentially including those of interest to environmental biologists such as CH_4 , NH_3 , and SF_6 . Moreover, water trapping did not produce accurate CH_4 measurements (M. Neti, unpublished data) and low molecular weight hydrocarbons can interfere with detection of low concentrations of CH_4 (M. Neti, personal communication). Thus, we recommend careful calibration checks and experimentation prior to measurement of additional gases

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