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Selection of the most suitable sampling time for static chambers for the estimation of daily mean N_2O flux from soils

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ABSTRACT

Soil N_2O fluxes are frequently assessed by the use of static chambers with a single daily sampling. In this study, two experiments were conducted in two contrasting climatic locations, one in Edinburgh, UK, and the other at Seropédica, Rio de Janeiro State, Brazil. Soil N_2O fluxes were monitored every 6 h for 30 days during the summer in Edinburgh by the use of an automatic chamber system, and every 3 h for 5 days at Seropédica, using a manually-sampled static chamber. Air and soil temperatures were also measured at the same time as the N_2O fluxes. The principal driver of N_2O flux within any diurnal period was found to be soil temperature. Regression analysis showed that, for both places, the evenings (21:00–22:00 h) and mornings (09:00–10:00 h), were the times that the flux best represented the daily mean. The ability to work in daylight make the morning period the preferred one.

1. Introduction

Static chambers are widely used for measuring greenhouse gas fluxes from soils. The most common static chamber procedure involves manual sampling of chamber headspace gas using syringes (e.g. Ball et al., 1999; Du et al., 2006; Jantalia et al., 2008) or more advanced systems such as the use of vacuum pumps or automated flux monitoring systems (e.g. Akiyama et al., 2000; Dobbie and Smith, 2003).

The high spatial variability of N_2O fluxes, related to hotspots of production in soil, requires many chamber replicates to evaluate N_2O fluxes with reasonable precision. Moreover, it is considered a good practice to take four or five successive air samples (at 5 or 10 min intervals for example) after chamber deployment to examine possible deviations from linearity of N_2O flux measurements with time (Rochette and Eriksen-Hamel, 2008). It is also advisable that sampling regimes should be intensified after any event that raises mineral N levels in the soil or that might create an oxygen limitation in the pore space (Smith and Dobbie, 2001). However, compromises often have to be made in order to limit the number of samples to manageable quantities, so soil N_2O daily emission calculations are usually based on the extrapolation of a single daily measurement during a short period to represent the mean flux for a full 24 h period.

In most environments N_2O formation in soil is controlled mainly by available C and mineral N, soil O_2 concentration in the soil pore space and temperature (Granli and Bøckman, 1994). Available soil C and N are not expected to vary significantly during a period of one day, unless crop residues and fertilizers are added to the soil. However, soil O_2 concentration can decrease rapidly after rainfall events or irrigation, and soil temperature is likely to follow the diurnal fluctuation of air temperature. The N_2O flux generally increases exponentially with soil temperature, with high Q_{10} values sometimes observed (e.g. Brumme, 1995; Flessa et al., 2002; Dinsmore et al., 2009), which can be explained by a combination of an expansion in anaerobic zones triggered by the acceleration of soil respiration, and the increasing denitrification rate per unit of anaerobic volume (Smith et al., 2003). The saturation of soil pore space with water also leads to exponential changes in soil N_2O fluxes, but the effect seems not to be so rapid (Russow et al., 2000) as that demonstrated for changes in soil temperature.

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Several studies have found a close relationship between diurnal variations in air temperature and N_2O fluxes, with a general pattern of higher fluxes during the day and lower fluxes during the night, accompanying the trends of soil temperature (Ryden et al., 1978; Denmead et al., 1979; Akiyama et al., 2000; Livesley et al., 2008). However, other studies failed to find this relationship (Blackmer et al., 1982; Chao et al., 2000; Du et al., 2006) or have demonstrated a substantial lag between temperature and flux maxima (Thomson et al., 1997). Ryden et al. (1978) suggested that N_2O fluxes could be measured at any time of the day and night as long as the afternoon peak coinciding with maximal daily temperature was avoided.

The diurnal air temperature fluctuation generally follows a sinusoidal path and is described by well-established models (Parton and Logan, 1981; Ephrath et al., 1996). Hence the mean temperature for the day occurs sometime after sunrise and after sunset. If the air temperature is a powerful driver of the changes in N_2O fluxes observed during the 24 h of the day, it can be hypothesized that there are two times in the day when the chance of the observed N_2O flux is most representative of the mean N_2O flux for the day. We investigated this issue and tested the hypothesis at two sites in contrasting climates (Scotland, UK and Rio de Janeiro, Brazil).

2. Material and methods

2.1. Sites

The experiments were carried out under field conditions at two sites with contrasting climates. A first experiment was set up in Edinburgh, Scotland, UK, at 55° 56′58″N and 3°9′37″W, with a mean daylight of 17 h in the summer (June to August) which was the season when measurements were made. Another experiment was carried out at Seropédica, Rio de Janeiro State, Brazil, at 22°45′28″S 43°40′54″W, with a mean daytime of 12 h at the beginning of autumn (April). According to the World Meteorological Organization (www.worldweather.org) mean daily minimum and maximum temperatures for Edinburgh are about 9 °C and 18 °C, respectively, during June and July. Monthly mean rainfall is similar over the whole year, varying from 51 to 57 mm during the summer, with an average of 13 days with rain. In the case of Seropédica, Rio de Janeiro State, mean monthly minimum and maximum temperatures for April are 22 °C and 28 °C, respectively, and mean rainfall is approximately 138 mm with 10 days of rain.

2.2. Edinburgh experiment

The experiment in Edinburgh was set up in a small area of the science campus of the University of Edinburgh that had been used for cropping potatoes and vegetables. For the current experiment, the soil in an area of 3 m^2 was well mixed with a spade to the depth of 40 cm in order to get a homogenized profile. Gravel, visible roots and other plant parts were manually removed. To avoid excessive soil looseness, some compaction was applied to the soil at 20 cm depth and then at each 5 cm up to the soil surface. During this phase, a thermocouple was buried to a depth of 10 cm and a 30-cm-long CS615 time-domain reflectance (TDR) probe (Campbell Scientific, Edmonton, Alberta, Canada) was inserted diagonally from the soil surface in order to measure the water content of the top 10 cm of the soil. Both probes were connected to a CR10X datalogger (Campbell Scientific, Edmonton, Alberta, Canada). A volume of water equivalent to an irrigation of 20 mm was applied 3 days before starting measurements.

A sample of the soil layer of 0–20 cm presented a sandy-loam texture (58% sand; 38% silt; 4% clay), 4.84% total C, 0.34% total N and a soil pH of 5.02. The soil bulk density of the 0–10 cm layer was 1.02 Mg m^{-3}, after the compaction process.

The N_2O flux measurements were performed every 6 h using one automatic static closed chamber. Samples started to be taken at 03:00 GMT (04:00 British Summer Time) on the first day and thenceforth there were 4 samplings a day during 30 days (17 June – 16 July 2005).

The chamber design was exactly the same as that described by Dobbie and Smith (2003). Briefly, the automated chamber was of the base-lid type, made of galvanized steel. The base was a frame of 70 × 70 cm in area and 30 cm height, with the bottom edges of the walls inserted into the soil to a depth of 8 cm. The chamber lid was also made of galvanized steel with the same area dimensions as the base. When the lid was in the closed position, it compressed a rubber gasket cemented to its underside against a horizontal flange at the top of the base walls, thus providing a gas seal. A control unit contained an air flow pumping system, and a timer/programmer unit to control the opening and closure of the chamber. A second module accommodated a set of Tedlar bags and a switching valve that allowed evacuation of the bags and the pumping in of sufficient chamber head space air for the N_2O analysis. Immediately after lid closure, a sample of the headspace air was pumped to one of the empty Tedlar bags, and another sample into a second bag after 40 min, before the chamber lid was raised. The air sample in each Tedlar bag was later transferred to 20 mL pre-evacuated chromatography vials using gastight syringes and then analysed with a gas chromatograph (GC) fitted with an electron capture detector as described in Dobbie and Smith (2003).

Fluxes of N_2O were calculated on the basis of an analytical curve of N_2O standards in nitrogen used to transform the integrated area of each sample peak into N_2O concentration. Nitrous oxide fluxes were expressed in μg N–N_2O m^{-2} h^{-1} using the equation: N_2O flux $= (\delta C/\delta t)(M/Vm)V/A$, where $\delta C/\delta t$ is the change in N_2O concentration (in μL L^{-1}) in the chamber after the incubation time (in hours); M is the molecular weight and Vm is the molecular volume of N_2O at the sampling temperature, and V is the volume of the chamber in litres and A the area in m^2.

During the sampling period, air and soil temperatures at 10 cm depth below the chamber were monitored hourly along with the soil moisture. An extra thermocouple was fixed above the chamber, but protected from direct sunlight, to record the external air temperature.

2.3. Seropédica experiment

The experiment in Seropédica, Rio de Janeiro State, was performed on a soil covered with the grass *Paspalum notatum* [Flügge] cv. Batatais. No soil preparation was carried out in this area. A sample of the soil layer of 0–20 cm presented a sandy texture (72% sand; 8% silt; 20% clay), 0.94% total C, 0.01% total N and a soil pH of 5.41. The bulk density of the 0–10 cm soil layer was 1.34 Mg m^{-3}. The experiment was set up on 9 April 2008, with 5 days of gas sampling every 3 h starting from 01:00 h (Brazilian Standard Time). As the soil was of very low fertility, urea fertilizer was applied at a rate of 10 g N m^{-2} two days before starting the measurements, along with a 10 mm irrigation, to stimulate N_2O production.

Five manually-sampled closed static chambers were used for the soil N_2O flux measurement. Each was composed of a rectangular hollow metal frame, 38 cm wide × 58 cm long × 6 cm in height which was inserted 5 cm into the soil and left for the whole experimental period. A trough was made around the top of the frame, and filled with soft rubber to ensure the system could be sealed after coupling the top portion of the chamber. This was a polyethylene tray of the same width and length as the base, 9 cm high, and was only coupled to the base during the periods of gas sampling. The top of each chamber had a three-way tap with Luer fittings through which gas samples could be withdrawn. At sampling time, the chamber top was pressed against the soft rubber existing in the trough with strong rubber bands stretched over the top, with both ends clipped to the metal base.

Gas samples were taken every 3 h, and the standard time for sampling the chambers was set to 30 min, one sample immediately after closure and another one at the end of incubation time. The air accumulated in the head space of each chamber was transferred to 20 ml chromatographic vials using a vacuum pump adapted to be connected to the chamber and the vial simultaneously. Analysis of N_2O concentrations were performed using a gas chromatograph equipped with an electron capture detector and a back-flush system (Jantalia et al., 2008). Fluxes of N_2O were calculated the same way as described previously.

The temperatures of the external air of the chamber headspace and of the soil at 10 cm depth were registered manually at each gas sampling using digital thermometers. Rainfall data was collected from a meteorological station located about 500 m away.

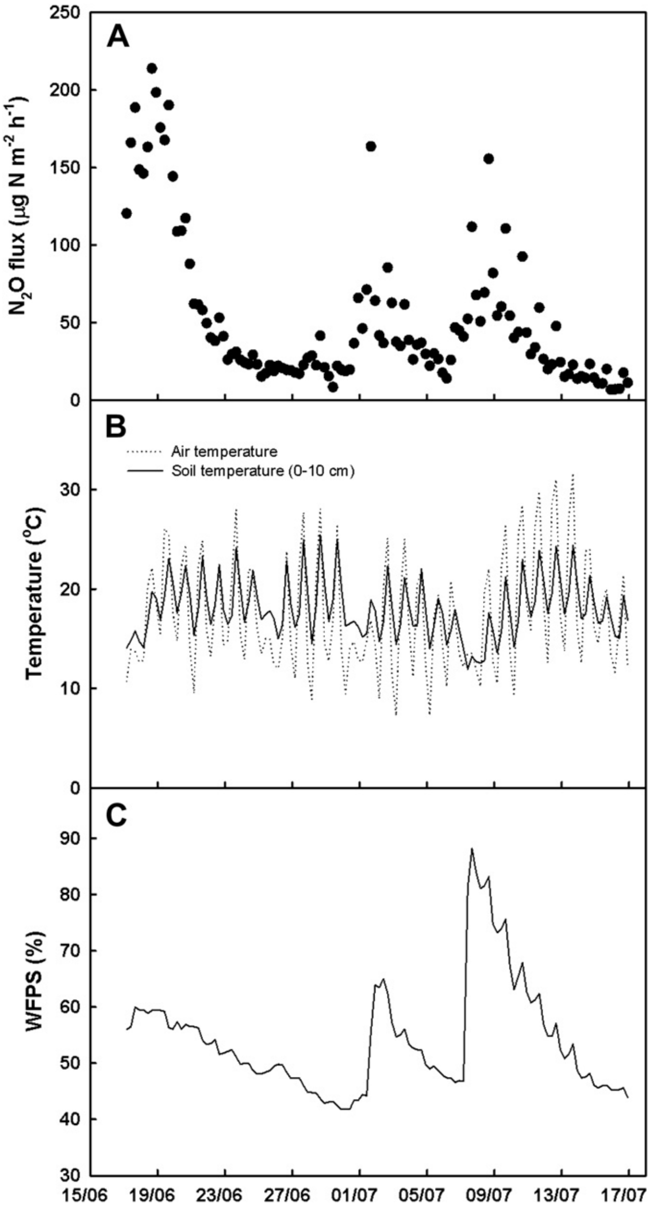

Fig. 1. Soil N_2O fluxes (A), soil and air temperature (B) and water filled pore space (C) measured every 6 h during the 30 days sampling period at Edinburgh, UK.

2.4. Statistical analysis

Descriptive statistic of data was performed. Pearson product-member correlations among N_2O fluxes and soil temperature and soil moisture were performed for the Edinburgh data, but only for air and soil temperature in the case of Seropédica data. Daily means of N_2O flux, soil temperature and soil moisture were calculated from the measured data in each day, the latter only for the Edinburgh experiment. Regressions of mean data against the data obtained for each sampling hour were made to find the most probable time for gas sampling that would represent the daily mean N_2O flux.

3. Results

At the Edinburgh site, fluxes of N_2O varied from 6.8 to 198.5 μg N m^{-2} h^{-1} during the 30 days of experimentation (Fig. 1A). Three periods of increased soil N_2O emissions were observed, the first at the beginning of the experiment, the second after a moderate rainfall and the last one after irrigation. Air temperature was recorded every 6 h and oscillated from about 7 to 31 °C approximating a daily sinusoidal pattern (Fig. 1B) which was accompanied by soil temperature at 0–10 cm, but with a smaller amplitude. Rainfall was not monitored, but events of mainly light rain (drizzle) were most frequent. On July 1st, a moderate rainfall provoked an increase in the soil water filled pore space (WFPS). A greater increase was observed 7 days later after an artificial irrigation of 10 mm (Fig. 1C).

The experiment at Seropédica presented N_2O fluxes varying from 17.1 to 249 μg N m^{-2} h^{-1}. Fluxes surpassed 100 μg N m^{-2} h^{-1} after the first 24 h and remained high for the rest of the monitoring period (Fig. 2A). Registered air temperatures were in the range of 22–37 °C, and also exhibited a sinusoidal pattern. Soil temperature showed the same trend but with an amplitude for the whole period of approximately 5 °C. The first day started with drizzle, but rains became heavier in the afternoon when in 3 h accumulated rainfall above 22 mm was registered. The rest of the 24 h period was rainy, but with an accumulated volume below 5 mm (Fig. 2B).

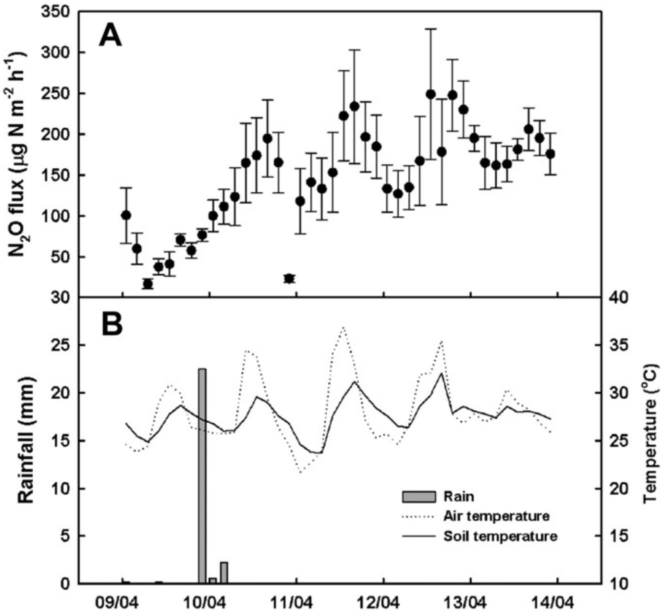

Fig. 2. Soil N_2O fluxes (A) and rainfall and soil and air temperatures (B) measured every 3 h during the 5 days sampling period at Seropédica, RJ, Brazil.

Table 1
Pearson product–member correlation among N_2O fluxes, soil and air temperatures and percentage of water filled pore space (WFPS), the latter included only for the Edinburgh data analysis.

Site variable	N_2O flux	Air temperature	Soil temperature
Edinburgh			
Air temperature	0.114	–	–
Soil temperature	−0.005	0.751***	–
WFPS	0.472***	0.071	−0.243**
Seropédica, RJ			
Air temperature	0.41**	–	–
Soil temperature	0.56***	0.74***	–

***, ** Represent statistical significance at $P < 0.001$ and 0.01 for the correlation coefficients, respectively.

Correlations between soil N_2O fluxes and soil and air temperatures were significant for the Seropédica site, but not for Edinburgh (Table 1). For the latter, a significant correlation was observed between soil N_2O fluxes and WFPS. Air temperature was also correlated with soil temperature and WFPS, the latter in an inverse relationship.

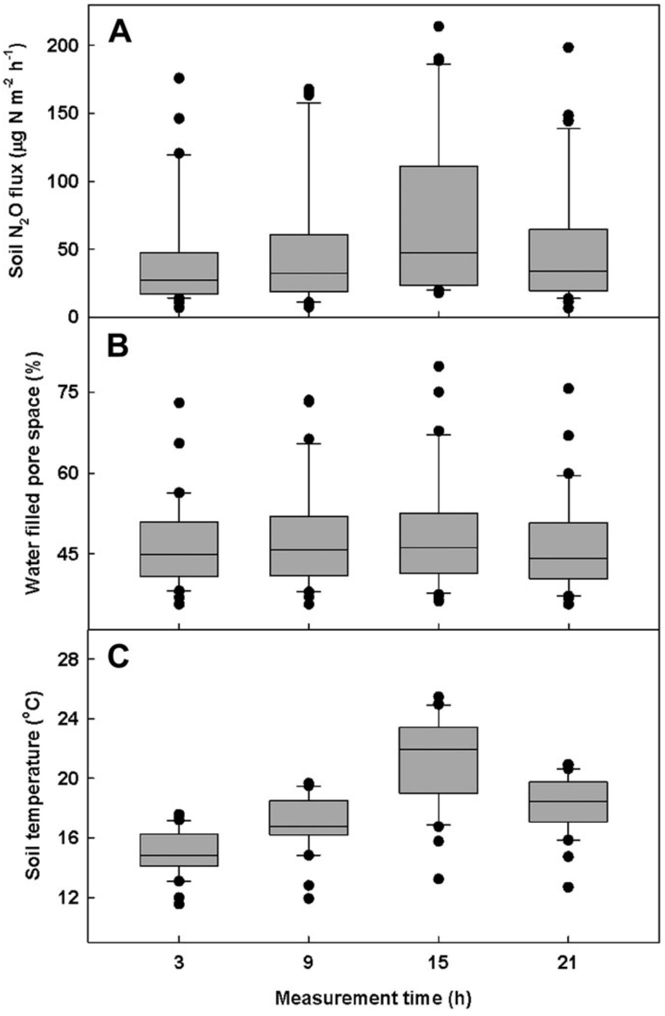

Fig. 3. Box plot showing the distribution of data for observed soil N_2O flux (A), water filled pore space (B) and soil temperature (C) during a 30-day period for each one of the four measurement hours of the day at Edinburgh, UK.

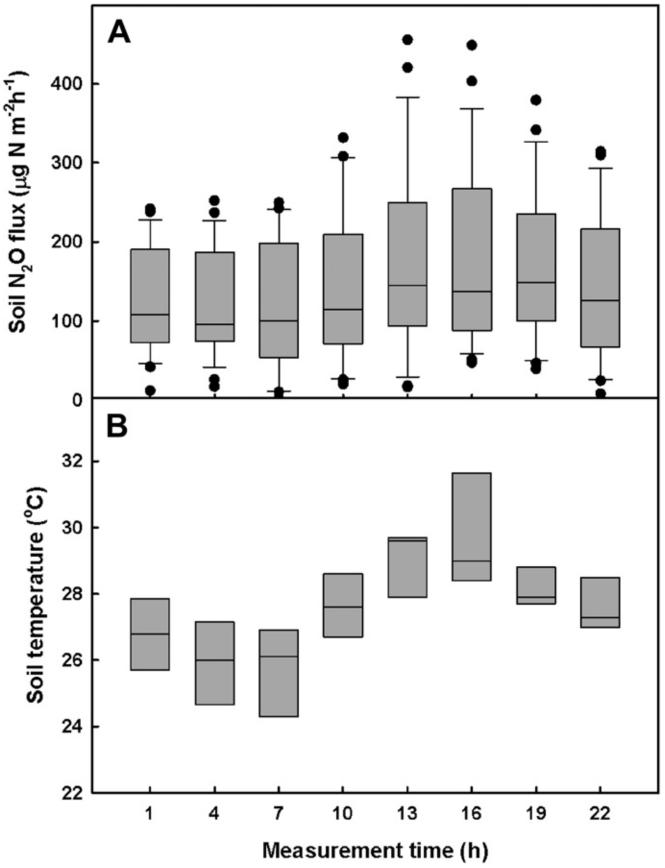

Fig. 4. Box plot showing the distribution of data for observed soil N_2O flux (A) and soil temperature (B) during a 5-day sampling period for each one of the eight measurement hours of the day at Seropédica, RJ, Brazil.

The data obtained in both places were grouped by sampling hour and exhibited as box plots in Figs. 3 and 4. At the Edinburgh site, soil N_2O fluxes were variable and presented a skewed distribution (Fig. 3A). The highest fluxes, exhibited above the upper whisker limits, tended to increase from night time to day time, the highest being at 15:00 h. The same trend was observed for the boxes and medians, especially for the upper quartile, the exceptions being the data for the lower whiskers and below them (Fig. 3A). The data for the WFPS of soil presented the same skewed distribution as for N_2O fluxes, but the trend of being defined by the sampling time was not evident (Fig. 3B). In contrast there was a stronger effect of sampling time on the magnitude of soil temperature (Fig. 3C). At 15:00 h the highest soil temperature would be expected, whilst the lowest would be found at 3:00 h. The latter presented the least variable soil temperature data with approximately 50% of the readings being within 2 °C. The largest variation was found for the measurements at 15:00 h, but most of the data indicated that highest fluxes coincided with highest soil temperature. Data asymmetry was only absent for the data obtained at 21:00 h and, when present, a common trend was not observed for the three groups per sampling hour.

At Seropédica, the distribution of N_2O fluxes within each sampling time was also skewed. Higher N_2O fluxes were more frequent during the afternoon (Fig. 4A), with the highest observed at 13:00 h and 16:00 h. Measurements made at 10:00 h, 19:00 h and 22:00 h were intermediate. The trend of increasing N_2O fluxes from night time to day time was more evident when the highest fluxes were taken into account, but it could also be observed from the data points within the boxes, containing 50% of the data. In the same way as observed for Edinburgh, the highest temperatures occurred in the afternoon, 12:00 h to 16:00 h, and the lowest late at night (Fig. 4B), which was also the observed trend for soil N_2O fluxes. This means there was a time during the day when the measured soil

N_2O flux would represent the daily mean N_2O flux, and this same reasoning applies to soil temperature.

Linear regression of mean soil temperatures and mean soil N_2O fluxes (Log transformed) for each sampling time at Edinburgh was strong with a probability of $p = 0.043$ (Fig. 5). A correlation was also observed for the same sort of data from Seropédica, but with a greater significance probability. From the linear function adjusted to Edinburgh data, a Q_{10} coefficient of 2.3 was calculated, whilst for Seropédica, the calculated Q_{10} was 3.5. This is within the range of estimates of Q_{10} of 2.2 and 7.1 reported by others (Flessa et al., 2002; Bagherzadeh et al., 2008; Mo et al., 2008; Abdalla et al., 2009; Dinsmore et al., 2009).

Regression analyses were performed between the mean daily N_2O flux (dependent variable) and the fluxes measured at each specific time of the day (independent variables). At Edinburgh, the slope of the regression of soil N_2O fluxes measured at 03:00 h was 1.08 or about 8% lower than the daily mean flux. When chambers were sampled at 09:00 h and 21:00 h the slopes of the regressions were the closest to the unity (Table 2). Gas sampling at 09:00 h returned soil N_2O fluxes 3% above the daily mean flux whilst at 21:00 h they were 2% higher. However, not surprisingly, large overestimates of the daily mean N_2O fluxes would result from chamber sampling at 15:00 h. The slope of the regression at this time indicated the estimates were over 30% of the daily mean fluxes. For this site in Edinburgh, all the regression coefficients were above 0.90 and intercepts were not significantly different from zero.

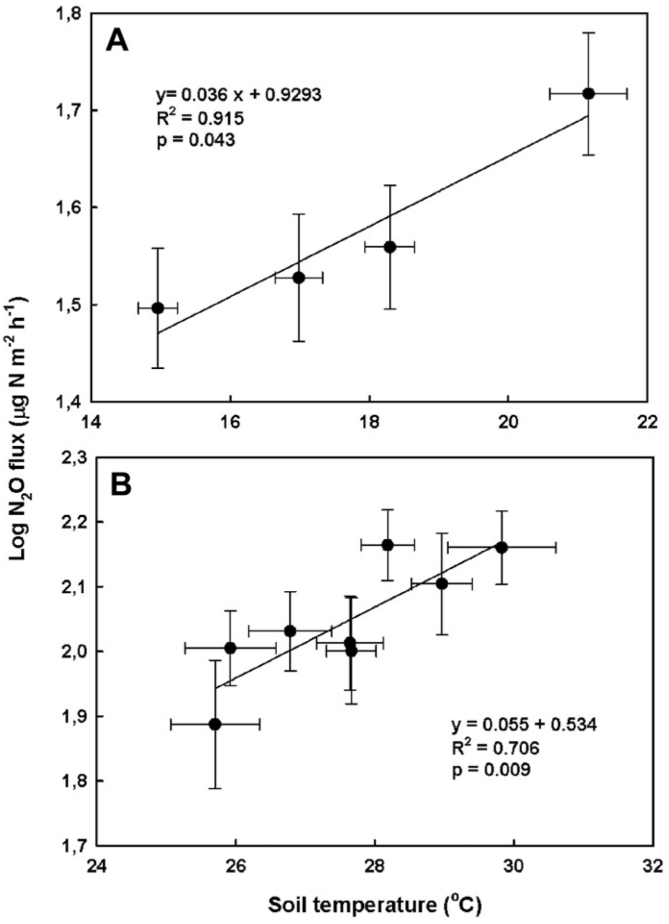

Fig. 5. Regression parameters between the means for each time of measurement of soil N_2O flux (log transformed) and soil temperature obtained from the 30 days of data at Edinburgh (A) and from the 5 days of data at Seropédica, RJ (B).

Table 2
Regression parameters and the regression coefficients (R^2) of the mean daily N_2O flux and the mean flux for the time given in Column 1.

Sampling time (hour of the day)	N_2O flux		
	a	*b*	R^2
Edinburgh			
03:00	1.08***	6.51	0.92***
09:00	0.97***	2.09	0.97***
15:00	0.75***	−0.55	0.93***
21:00	1.02***	3.15	0.94***
Seropédica, RJ			
01:00	1.19***	−30.76	0.54***
04:00	1.39***	−24.05	0.86***
07:00	1.15***	14.97	0.90***
10:00	0.93***	32.96	0.81***
13:00	0.71***	25.25*	0.90***
16:00	0.79***	7.20	0.86***
19:00	0.99***	28.27	0.87***
22:00	1.23***	26.95	0.56***

Data were adjusted to the linear model $F_{dm} = (aF_{time}) + b$, where F_{dm} is the mean daily N_2O flux and F_{time} is the mean flux for each time of the day evaluated.
***, * Represent statistical significance at $P < 0.001$ and <0.05 respectively, for the slope, intercept and regression coefficient.

At Seropédica, the slopes obtained from the regressions of soil N_2O fluxes measured in each one of the 8 sampling times varied from 0.71 to 1.39 (Table 2). The lowest was estimated for sampling at 13:00 h, which means the time when the measured N_2O flux was about 30% above the daily mean N_2O flux. The highest was estimated with data obtained at 04:00 h, when the measured fluxes underestimated the daily mean flux even more. Conversely, the soil N_2O fluxes measured at 19:00 h were the closest to the mean daily N_2O flux. At 10:00 h, the measured fluxes overestimated the daily mean flux by 8%. Soil N_2O fluxes measured at any other time ended up overestimating by 20% or underestimating the daily mean flux by 15%, at least. Regression coefficients (R^2) were all significant at $p < 0.001$ but varied from 0.54 to 0.90. Intercepts were not different from zero, with the exception of that obtained for the sampling at 13:00 h.

4. Discussion

For both experimental sites, soil N_2O fluxes presented an expected increase after changes in soil moisture provoked by irrigation and rainfall (Figs. 1 and 2). At Edinburgh, it was possible to observe the existence of a threshold close to 60% of WFPS (Fig. 1) as has already been reported (Dobbie et al., 1999; Flessa et al., 2002), beyond which N_2O fluxes rose abruptly (about 6–9 times). This relationship was confirmed by a significant correlation between both variables (Table 1) that was enhanced when an exponential function was used.

However, correlations between soil N_2O fluxes and soil (at 10 cm) and air temperatures were significant, but weak, for the data from Seropedica. All correlations were performed using the whole data set instead of daily means. Notwithstanding, when data from each site were grouped by sampling time, a clear trend of high temperatures and soil N_2O fluxes occurring in the afternoon and the opposite in the early morning was observed (Figs. 3 and 4). Soil temperature presents a more constant oscillation than WFPS and soil available N (especially when soil is disturbed or N fertilizer is applied), and there will be moments of similar soil temperatures but with differences in other key factors controlling N_2O emissions, which would explain the absence of correlations with temperature for Edinburgh data. Livesley et al. (2008) also found a diurnal reciprocity between soil temperature and soil N_2O fluxes but only when a short period of reasonably constant soil moisture was considered.

Table 3
Summary of results of studies which compare diurnal flux of N_2O and soil temperature and estimates of the sampling time which best represented the mean daily N_2O flux.

Local	Soil cover	Soil depth (cm)	Time resolution (h)	Date	µg $N-N_2O\ m^{-2}\ h^{-1}$		Period in day time which best represented average daily N_2O flux (h)	Time of lowest/highest temperature (h)	Ref[b]	Correlation with soil temperature
					N_2O range[a]	Mean daily flux				
Tsukuba, Japan	Carrots	5	4	25/Jun	10 to 40	28	08:00 to 12:00	06:00/16:00	1	Yes
			4	26/Jun	32 to 47	39	08:00 to 12:00	23:00/13:00		
			4	27/Jun	33 to 52	43	04:00 to 08:00	07:00/16:00		
			4	28/Jun	8 to 33	27	08:00 to 12:00	08:00/19:00		
			4	29/Jun	26 to 37	32	08:00 to 12:00	07:00/16:00		
			4	30/Jun	21 to 30	26	08:00 to 12:00	09:00/18:00		
Near Munich, German	Potatos	5	12	2 to 10/Aug	30 to 450	75 to 250	08:00 to 12:00	0:00/13:00 to 15:00	2	Yes
Cumbria, UK	Rye grass/White Clover	10	2,67	03/Apr	9 to 28	17	12:00 to 14:40	09:20/17:20	3	Yes
				04/Apr	6 to 26	15	12:00 to 14:40	04:00/17:20		
				05/Apr	8 to 28	16	12:00 to 14:40	09:20/17:20		
Inner Mongolia, China	Grasslands	15	2,67	06/Jun	1 to 10	3	10:00 to 15:00	03:00/12:00	4	No
			2,67	08/Jul	8 to 65	25	08:00 to 09:00	03:00/12:00		
			2,67	12/Aug	3 to 12	5	09:00 to 12:00	03:00/12:00		
			2,67	10/Sep	0 to 3	2	<09:00 and after 15:00	04:00/12:00		
Rio de Janeiro, Brazil	Grass sward	10	3	3 days/Nov	n.a.[c]	n.a.	07:00 to 10:00	n.a.	5	n.a.
Canberra, Australia	Grass sward	3	~1	08/Nov	72 to 180	126	09:00 to 12:00	07:00/17:00	6	Yes
			~1	09/Nov	65 to 144	97	09:00 to 12:00	06:00/15:00		

[a] Minimum and maximum daily flux.
[b] 1 – Akiyama et al. (2000), 2 – Flessa et al. (2002), 3 – Williams et al. (1999), 4 – Du et al. (2006), 5 – Jantalia et al. (2008), 6 – Denmead et al. (1979).
[c] Not available.

The high and significant determination coefficients between soil N_2O fluxes and soil temperature at both sites meant the variation in diurnal soil N_2O fluxes was largely explained by a soil temperature change when data were averaged per sampling hour (Fig. 5). The effect was more prominent for Seropédica, which presented a Q_{10} 1.5 times greater than that for the Edinburgh site, which is explained by a greater potential for conditions that lead to O_2 restriction (Dobbie and Smith, 2001), such as the high bulk density of Seropédica soil.

Diurnal variations in soil N_2O fluxes were not significantly correlated with WFPS, as the means for this changed on a much slower timescale. Ryden et al. (1978) reported a diurnal variation in soil N_2O fluxes with a peak in early afternoon, which led to the recommendation of choosing the mid-morning as the best period for a more reliable estimative of the daily N_2O flux.

Results from other publications on the relationship between temperature and soil N_2O fluxes and the most representative time for N_2O emission assay are summarized in Table 3. The data in these studies was all presented graphically and were extracted by measuring graphic points against the scales on the axes. Despite the fact that temperatures were measured at different depths (3–15 cm) some agreements could be observed. In most cases, a N_2O peak was detected in the afternoon with the lowest flux appearing early morning or late night. The mid-morning period (09.00–12.00 h) was frequently associated to the occurrence of the mean daily N_2O flux. Even though the peaks of lowest and highest temperatures varied among the sites or soil cover this did not greatly affect the period when daily mean N_2O fluxes occurred. The study of Du et al. (2006) on Mongolian grasslands showed on one occasion a close positive relationship between temperature and N_2O fluxes but when fluxes were very low no relationship of any type were observed.

In the present study soil temperature was measured at 10 cm soil depth where the amplitude of diurnal variation was attenuated compared to air temperature (Figs. 1 and 2) and it is possible that N_2O fluxes and temperature patterns will not be exactly phased in detriment to the correlations (Thomson et al., 1997; Akiyama et al., 2000).

As most of the studies to measure soil N_2O fluxes are carried out using static chambers with a daily gas sampling it is fundamental that the sampling time be appropriate to produce a N_2O flux that represents the daily mean N_2O flux. Taking the daily mean N_2O flux as the dependent variable of a regression analysis and the flux at each sampling time as the independent variable, the best time for gas sampling will be that with the slope closest to unity. In the case of Edinburgh, the best time would be at 21:00 h followed by 09:00 h, and for Seropédica, the most appropriate times were at 22:00 h and 10.00 h. The obvious advantages of sampling during the mornings, in daylight instead of darkness, and the associated greater convenience for research staff, point to the 09:00–10:00 h as most suitable.

The correlation of flux with soil temperature is an indication that the latter variable could be used as a reference for extrapolations for other locations, as in most of the cases it is a reflection of air temperature. Hourly air temperature is available for most places and the time the daily mean temperature occurs would be the most suitable for sampling static chambers to estimate daily N_2O fluxes. However, Blackmer et al. (1982) argued that there is no such suitable time even though they suggested that the lack of agreement could be related to similar N_2O concentration of soil atmosphere and air, as the effect of temperature on soil N_2O fluxes is partially related to the dissolution of this gas in water and not only to the sensitivity of biological activity to temperature.

Hence, it can be deduced that when the soil is at that level of pore saturation which stimulates N_2O production, the temperature is a valid parameter to help in evaluating the best time for sampling static chambers once a day. Moreover, the largest errors are associated with the highest N_2O fluxes caused by high temperature, irrespective of other factors involved, which means that air temperature is useful as a reference for the selection of a suitable sampling time in the day.

5. Conclusion

For most situations the use of static chambers with a single sampling per day is the best compromise for assessing N_2O fluxes by manual procedures. Practically all the published reports support the existence of diurnal variation in N_2O fluxes, which may be large, although the correlation with soil temperature suffers from the lack of phasing of the fluctuations of the two variables, which is a function of the depth at which soil temperature is measured. The results presented here that were obtained from two contrasting environments led to the same conclusion that morning (*c.* 09:00–10:00 h) and evening (*c.* 21:00–22.00 h) would be the most suitable times for sampling static chambers, and that other practical considerations made the morning the preferred option. In the cases of uncertainty, the correlation between the sampling time means of soil N_2O fluxes and soil temperature, and obviously the latter with air temperature, supports the use of the time that the mean air temperature occurs as the parameter to define the most suitable sampling time.

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References

Abdalla, M., Jones, M., Smith, P., Williams, M., 2009. Nitrous oxide fluxes and denitrification sensitivity to temperature in Irish pasture soils. Soil Use and Management 25, 376–388.

Akiyama, H., Tsuruta, H., Watanabe, T., 2000. N_2O and NO emissions from soils after the application of different chemical fertilizers. Chemosphere – Global Change Science 2, 313–320.

Bagherzadeh, A., Brumme, R., Beese, F., 2008. Temperature dependence of carbon mineralization and nitrous oxide emission in a temperate forest ecosystem. Journal of Forestry Research 19, 107–112.

Ball, B.C., Scott, A., Parker, J.P., 1999. Field N_2O, CO_2 and CH_4 fluxes in relation to tillage, compaction and soil quality in Scotland. Soil and Tillage Research 53, 29–39.

Blackmer, A.M., Robbins, S.G., Bremner, J.M., 1982. Diurnal variability in rate of emission of nitrous oxide from soils. Soil Science Society of America Journal 46, 937–942.

Brumme, R., 1995. Mechanisms of carbon and nutrient release and retention in beech forest gaps. III. Environmental regulation of soil respiration and nitrous oxide emissions along a microclimatic gradient. Plant and Soil 169, 593–600.

Chao, C.C., Young, C.C., Wang, Y.P., Chao, W.L., 2000. Daily and seasonal nitrous oxide fluxes in soils from hardwood forest and different agroecosystems of Taiwan. Chemosphere – Global Change Science 2, 77–84.

Denmead, O.T., Freney, J.R., Simpson, J.R., 1979. Studies of nitrous oxide emission from a grass sward. Soil Science Society of America Journal 43, 726–728.

Dinsmore, K.J., Skiba, U.M., Billett, M.F., Rees, R.M., Drewer, J., 2009. Spatial and temporal variability in CH4 and N2O fluxes from a Scottish ombrotrophic peatland: implications for modelling and up-scaling. Soil Biology & Biochemistry 41, 1315–1323.

Dobbie, K.E., McTaggart, I.P., Smith, K.A., 1999. Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors. Journal of Geophysical Research 104, 26891–26899.

Dobbie, K.E., Smith, K.A., 2001. The effects of temperature, water-filled pore space and land use on N2O emissions from an imperfectly drained gleysol. European Journal of Soil Science 52, 667–673.

Dobbie, K.E., Smith, K.A., 2003. Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. Global Change Biology 9, pp. 204–218.

Du, R., Lu, D., Wang, G., 2006. Diurnal, seasonal, and inter-annual variations of N_2O fluxes from native semi-arid grassland soils of inner Mongolia. Soil Biology & Biochemistry 38, 3474–3482.

Ephrath, J.E., Goudriaan, J., Marani, A., 1996. Modelling diurnal patterns of air temperature, radiation wind speed and relative humidity by equations from daily characteristics. Agricultural Systems 51, 377–393.

Flessa, H., Ruser, R., Schilling, R., Loftfield, N., Munch, J.C., Kaiser, E.A., Beese, F., 2002. N_2O and CH_4 fluxes in potato fields: automated measurement, management effects and temporal variation. Geoderma 105, 307–325.

Granli, T., Bøckman, O.C., 1994. Nitrous oxide from agriculture. Norwegian Journal of Agricultural Sciences Suppl. 12, pp. 1–128.

Jantalia, C.P., Santos, H.P., Urquiaga, S., Boddey, R.M., Alves, B.J.R., 2008. Fluxes of nitrous oxide from soil under different crop rotations and tillage systems in the South of Brazil. Nutrient Cycling in Agroecosystems 82, 161–173.

Livesley, S.J., Kiese, R., Graham, J., Weston, C.J., Butterbach-Bahl, K., Arndt, S.K., 2008. Trace gas flux and the influence of short-term soil water and temperature dynamics in Australian sheep grazed pastures of differing productivity. Plant and Soil 309, 89–103.

Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., Wang, H., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology 14, 403–412.

Parton, W.J., Logan, J.A., 1981. A model for diurnal variation in soil and air temperature. Agricultural Meteorology 23, 205–216.

Rochette, P., Eriksen-Hamel, N.S., 2008. Chamber measurements of soil nitrous oxide flux: are absolute values reliable? Soil Science Society of America Journal 72, 331–342.

Russow, R., Sich, I., Neue, H.U., 2000. The formation of the trace gases NO and N_2O in soils by the coupled processes of nitrification and denitrification: results of kinetic ^{15}N tracer investigations. Chemosphere – Global Change Science 2, 359–366.

Ryden, J.C., Lund, L.J., Focht, D.D., 1978. Direct infield measurement of nitrous oxide flux from soil. Soil Science Society of America Journal 42, 731–738.

Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A., 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. European Journal of Soil Science 54, 779–791.

Smith, K.A., Dobbie, K.E., 2001. The impact of sampling frequency and sampling times on chamber-based measurements of N_2O emissions from fertilized soils. Global Change Biology 7, 933–945.

Thomson, P.E., Parker, J.P., Arah, J.R.M., Clayton, H., Smith, K.A., 1997. An automated soil monolith/flux chamber system for the study of trace gas fluxes. Soil Science Society of America Journal 61, 1323–1330.

Williams, D.L., Ineson, P., Coward, P.A., 1999. Temporal variations in nitrous oxide fluxes from urine-affected grassland. Soil Biology and Biochemistry 31, 779–788.