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Comparison of field measurement methods of nitrous oxide soil emissions: from the chamber to the vial

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ABSTRACT: Nitrous oxide (N₂O) is a greenhouse gas that contributes substantially to global climate change. The N₂O soil emissions have a large uncertainty because of its low atmospheric concentration levels and enormous spatial and temporal variability, which hinders its correct field measurement. For this reason, there are many papers focused on improving the N₂O measurements in the field, which focus on different parts of the measurement process. However, no studies have focused on determining the appropriate method, in terms of simplicity and precision, for the sample extraction from inside of the chambers and its transfer to the storage vials, although this step is key in the sampling process. This study aimed to assess and compare the accuracies of three simple and economical methods in transfer soil emitted N₂O from inside of the chambers to the vials. For this, a highly accepted method (vacuum by manual pump) and two simpler alternative methods (gas exchange by displacement and vacuum by syringe) were compared. Thirty static chambers were assessed with the quantified N₂O emission values varied from 0 to 450 μg m⁻² h⁻¹ of N-N₂O. Out of the three assessed methods, the vacuum method through the use of a manual vacuum pump was the best to quantifying N₂O soil emissions (capturing 57 % of the highest emission values), followed by the gas exchange method by displacement (30 %), and finally by the vacuum method by syringe extraction (13%).

Keywords: vacuum by manual pump, gas exchange by displacement, vacuum by syringe.

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INTRODUCTION

Nitrous oxide (N_2O) is the main greenhouse gas emitted from the soil by the agricultural sector (IPCC, 2014) and participates in the depletion of stratospheric ozone (Del Grosso et al., 2008). The global warming potential (GWP) of N_2O is 265 times higher than CO_2 , and it possesses a half-life time in the atmosphere of 121 years (IPCC, 2014), resulting in a N_2O contribution of approximately 6 % to the global warming effect (Rapson and Draques, 2014). Added to this, the emissions of N_2O from the soil have a considerable uncertainty compared to other greenhouse gases because of its low atmospheric concentration levels and enormous spatial and temporal variability (Hensen et al., 2013). In this context, it is imperative to correctly assess whether soils are a source or a sink for this gas (Oertel et al., 2016) and to establish the magnitude of N_2O flow under different environments.

Most of the papers about field N_2O fluxes determinations reported in the literature have been focused on reviewing aspects such as chamber design, the sampling method, and the sample storage appropriate for the correct determination of N_2O (Rochette and Eriksen-Hamel, 2008). Despite this step is key, studies about the method of transferring the sampled gas from inside of the chamber to the storage vials were not found in the literature (Rochette and Eriksen-Hamel, 2008; Hensen et al., 2013; Klein and Harvey, 2015).

The sampling method with pre-evacuated vials (with an automatic pump) and the vacuum with manual vacuum pumps method are among the most used to take gas samples from inside of the chamber and to transfer them to the vials (Alves et al., 2012; Cosentino et al., 2013; Coverdale et al., 2016; Della Chiesa et al., 2018; Sá et al., 2019). However, the pre-evacuated vials are costly and not accessible in many cases; therefore, it is necessary to assess whether alternative methods are equally appropriate. As accessible methods to take gas samples from inside of the chamber and to transfer them to the vials, we can mention the manual vacuum pump, the vacuum by syringe, and the gas exchange by the displacement method proposed by the US Department of Agriculture (Collier et al., 2014, 2016). Of these three methodologies, the manual pump is the most laborious but the most widely accepted (Alves et al., 2012; Morais et al., 2013; Paredes et al., 2014), while the vacuum by syringe and the gas exchange by the displacement methods arises as cheaper alternatives. The aim of this study was to assess and compare the accuracies of three relatively simple and economical methods in transfer soil emitted N_2O from inside of the static chambers to the vials.

MATERIALS AND METHODS

Study site

The experiment included thirty static chambers located in different environments with contrasting soil textural classes and soil uses (forest and pasture). The N_2O determinations were conducted in different seasons to generate thermal differences during measurements. The textural classes included: sandy, silt-loam, silty-clay-loam, and sandy-loam textures (INTA, 2019). Sampled soils under forests correspond to the province of Entre Ríos ($32^\circ 58' 16''$ S and $58^\circ 14' 34''$ W), and soils under pastures correspond to Buenos Aires province ($34^\circ 36' 10''$ S and $58^\circ 40' 11''$ W), Argentina. The variability in soil and climate conditions allowed measuring a wide range of N_2O soil emissions.

N_2O measurements

Nitrous oxide soil emission was determined by using static chambers according to Rochette and Eriksen-Hamel (2008). Before the experiment, measurement times were calibrated to avoid gas saturation inside the chamber. Chosen measurement times (0, 15, and 30 min) were adjusted by a linear function.

Nitrous oxide flow from the soil to the atmosphere was calculated with the following equation 1:

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{V_m} \quad \text{Eq. 1}$$

in which $\Delta C/\Delta t$ is the variation in the N_2O concentration inside the chamber during incubation time (Δt), V is the chamber volume (0.0167 m^3), A is the soil area covered by the chamber (0.13 m^2), m is the molecular weight of N_2O , and V_m is the molar volume of N_2O . Gas flow was calculated as the increase in gas concentration during the incubation time.

Evaluated methods

For the correct comparison between the three methods evaluated, at each measurement time (0, 15, and 30 min), samples were taken directly from the chamber with 60 mL syringes. The syringes have a needle and a valve to prevent the loss of the gas sample during the transfer from the chamber to the vial. The gas samples were immediately transferred from the syringes to vials of 10 mL according to the procedure corresponding to each of the method described below:

1 - Gas Exchange by Displacement (GED): This method was proposed by the United States Department of Agriculture (USDA; Collier, 2014) and it consists in displacing the original gas inside the vial through a small needle inserted in the septum of the vial, by injecting over-pressurized gas with a needle syringe (gas sampling inside the chamber). For this, the volume of the syringe (used to purge and to displace the gas that is originally inside the vial) has to be at least three times larger than the volume of the storage vial. In our case, 10 mL vials and 60 mL syringes were used. This method consists of three steps: 1) a small needle has to be inserted into the septum of the vial. Then, with the syringe needle inserted into the septum of the vial, open the syringe's valve and inject approximately 2/3 of the volume of the gas sample (the content of the vial is expelled through the small needle and replaced by the gas samples inside the chamber); 2) remove the small needle and continue injecting the remaining gas sample (approximately 1/3) until reaching the over-pressurized of the vial, ensuring the integrity of the sample and to allow the analysis of multiple samples if necessary; and 3) finally remove the syringe needle from the septum (Collier, 2014).

2 - Vacuum by Manual Pump (VP): this method consists in extracting the gas inside the vial (by negative pressure) and replace it with the syringe gas (gas samples inside the chamber) using a manual vacuum pump. The procedure for gas exchange consists of three steps: 1) vacuum is generated inside the vial until the pump pressure gauge indicates a fixed value (-80 Kpa); 2) by opening and closing different valves, the air is passed by negative pressure from the syringe into the vial; 3) put the valves back to their initial position. Each time these three steps are conducted, approximately 80 % of the air volume from the vial is replaced by the same air volume that comes from the inside of the chamber (syringe). To assure the replacement of approximately 100 % of the original air with the air from inside the chamber, this procedure must be repeated three times (Cosentino, 2015).

3 - Vacuum by syringe (VS): This method consists in extracting the gas inside the vial with a syringe (by negative pressure) and replaces it with the gas inside the chamber using another syringe. The procedure for gas exchange inside the vial consists of four steps: 1) introduce a syringe needle into the septum of the vial and pull the plunger outwards generating vacuum inside the vial; 2) remove the syringe used to perform the vacuum and insert into the vial the syringe containing the gas sample from the chamber; 3) the gas contained in the syringe (gas inside the chamber) pass by negative pressure into the vial; 4) remove the syringe.

Data treatment criteria and method selection

Maximum emission: For each sampling situation (in each measured chamber), three N₂O emission values were obtained, one for each method evaluated. Of the three emission values obtained, the maximum value was considered as the real value. This was performed under two assumptions: 1) lower N₂O emission value is observed when N₂O concentration inside the vial is lower; 2) N₂O concentration lower than the maximum are because the gas replacement inside the vial (the original gas by the gas coming from within the chamber) is not complete.

Gas Exchange Method Comparison: For the 30 sampling chambers, the N₂O emission values obtained from each method were compared to the maximum N₂O emission value registered, this was done through the method of the Standardized Major Axis (SMA; Warton et al., 2006). The SMA method has been pointed out as more appropriate than the linear regression method when the aim is to describe the relationship between two variables measured with an error or to test whether if two measurement methods match (Warton et al., 2006).

Estimation of the relationship between the maximum emissions and the emissions measured in each method: The maximum emission and the emission determined from each method were related through linear models, and measures of goodness of fit and error in the prediction were estimated (i.e., R², MSE, and RSME). Since the slope of the best-fitting linear regression was used not only for estimating the calibration parameter but also to evaluate the rest of the methods, a crossed validation strategy was performed to avoid the double use of the data (LooCV, cf. James et al., 2013). The statistical analyses were performed using the statistical software R (R Development Core Team, 2018).

RESULTS

When comparing the complexity and time that each of the methods evaluated demand, we can mention that of the three described methods, VP was the most laborious since it has more complex steps, mainly when opening and closing the different valves. This method consumed, on average, 120 seconds. The GED and VS methods were similar in complexity and time spent to exchange the gas from the syringe (gas inside the chamber) to the vial, with an average time of 40 seconds for the whole procedure.

The quantified N₂O emission values ranged from 0 to 450 μg m⁻² h⁻¹ of N-N₂O. The VS and GED methods never detected emission rates over 250 and 365 μg m⁻² h⁻¹ of N-N₂O, respectively (Table 1). The VP method showed the highest average emission of 71.24 μg m⁻² h⁻¹ of N-N₂O, followed by the method of GED with average N₂O emissions of 59.31 μg m⁻² h⁻¹ of N-N₂O and finally the VS method with average N₂O emission of 39.50 μg m⁻² h⁻¹ of N-N₂O. Added to this, the VP method quantified 57 % of maximum emission values, while GED and VS quantified 30 and 13 %, respectively (Table 1). The average linear regression adjustments (R²) was high in the three methods, with values higher than 0.80 for VP and GED methods. The R²>0.80 is usually used as a criterion for quality check for accumulation of gas inside the chamber is adequate (Klein and Harvey, 2015). However, despite the fact that the N₂O emission rates were linear, they differed in the emission values, ordering from highest to lowest according to 0.89, 0.84, and 0.78 for VP, GED, and VS methods respectively.

However, the GED and VS methods underestimated the maximum measurements. The VS method underestimated N-N₂O emissions above ~100 μg m⁻² h⁻¹ of N-N₂O, while the GED method did so above higher emission values (i.e., ~250 μg m⁻² h⁻¹ of N-N₂O). Added to this, the adjusted functions for the different methods were different from each other (p<0.05; Figure 1). The VP method showed a high correspondence with

Table 1. N-N₂O emission values and linear regression adjustments (R²) for the Vacuum by Manual Pump (VP), Vacuum by syringe (VS), and Gas Exchange by Displacement (GED) methods for each of the 30 studied chambers. The maximum emission values are shown in bold font

Chambers	VS		VP		GED		maximum value
	R ²	N ₂ O emissions	R ²	N ₂ O emissions	R ²	N ₂ O emissions	N ₂ O emissions
1	0.41	0.02	0.63	0.14	0.02	0.12	0.14
2	0.09	0.08	0.02	0.13	0.19	0.09	0.13
3	0.49	0.29	0.03	0.24	0.09	0.36	0.36
4	0.50	0.83	0.12	0.19	0.07	0.25	0.83
5	0.12	0.27	0.88	5.39	0.84	4.23	5.39
6	0.83	1.50	0.77	3.08	0.37	1.30	3.08
7	0.90	4.71	0.79	1.10	0.94	1.20	4.71
8	0.72	4.46	0.67	5.18	0.71	5.96	5.96
9	0.79	2.50	0.65	2.30	1.00	3.71	3.71
10	0.01	0.20	0.77	5.28	0.79	8.82	8.82
11	0.99	5.18	0.99	10.02	0.80	2.40	10.02
12	0.78	6.04	1.00	6.80	0.96	9.28	9.28
13	0.67	18.60	0.92	18.10	0.74	18.21	18.60
14	0.38	1.41	0.92	10.78	0.88	26.34	26.34
15	0.90	16.22	0.92	18.94	0.87	22.47	22.47
16	1.00	21.14	0.94	27.26	1.00	22.18	27.26
17	0.91	13.01	0.94	45.55	0.80	16.60	45.55
18	0.90	29.33	0.99	68.37	0.94	65.94	68.37
19	0.76	54.31	1.00	59.75	0.96	54.55	59.75
20	0.80	59.36	0.98	114.38	0.98	85.71	114.38
21	0.92	64.22	1.00	104.91	0.79	91.34	104.91
22	0.73	112.17	0.74	132.74	0.71	116.11	132.74
23	0.95	169.70	0.92	252.87	0.98	229.94	252.87
24	0.95	153.10	0.93	325.95	0.53	225.20	325.95
25	0.91	181.43	1.00	444.21	1.00	362.74	444.21
26	1.00	21.57	0.87	9.01	0.65	7.08	21.57
27	0.99	4.35	0.97	70.57	0.98	78.30	78.30
28	0.94	15.25	0.97	30.43	0.85	26.00	30.43
29	0.97	24.90	0.75	25.90	0.92	26.16	26.16
30	0.96	42.15	0.89	53.25	0.79	30.26	53.25
Best method fit		4 times		17 times		9 times	
Average emisión		39.50		71.24		59.31	73.23
Emission %		13.0		57.0		30.0	100.0
Average R ²	0.78		0.89		0.84		

the maximum emissions determined without significant differences with the 1:1 line. The GED method underestimated N₂O emissions by an average of ~19 %, while the VS method underestimated nitrous emissions by an average of ~52 % (Table 2). The underestimation of the emission values by the VS method becomes more evident at higher emission values (Figure 1).

The linear model adjusted between the maximum values and the VS method had a wide confidence interval, the highest MSE and RMSE values, and the lowest R² (Table 2). The linear model adjusted between the maximum values and the VP method had a slope

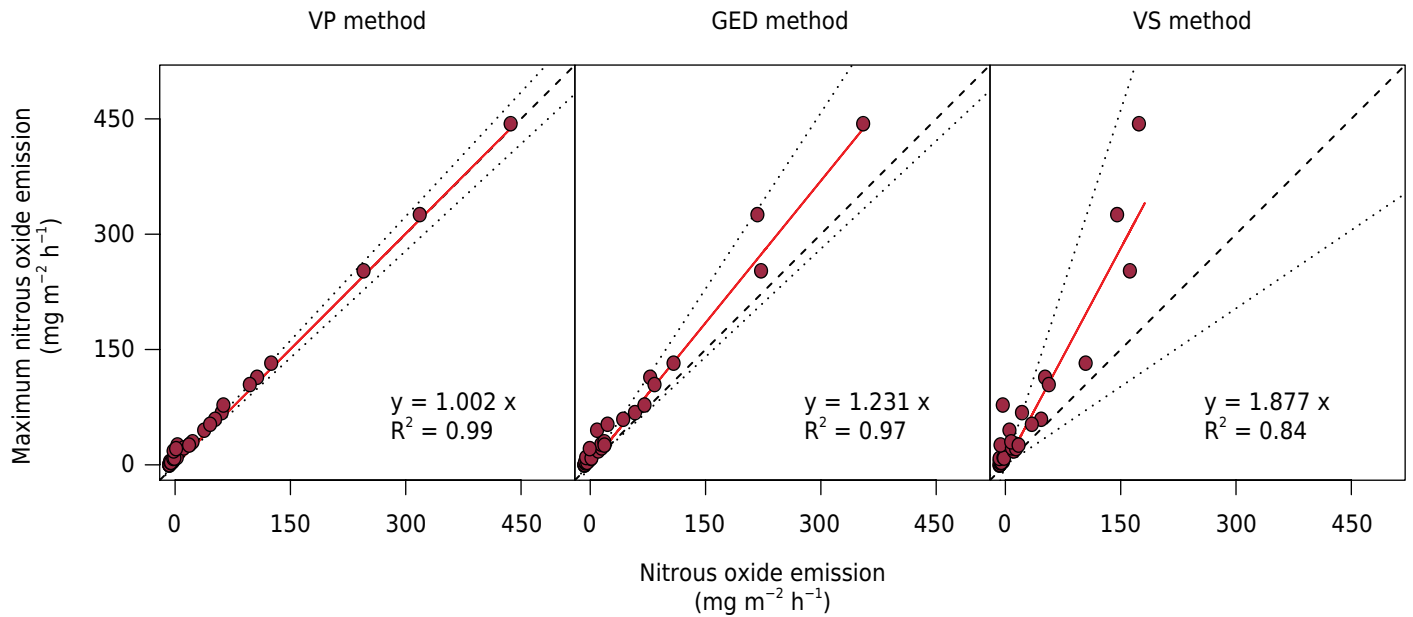


Figure 1. Relationship between maximum nitrous oxide emission and N-N₂O emission by the Vacuum by Manual Pump (VP) method (left), Gas Exchange by Displacement (GED) method (middle) and the Vacuum by syringe (VS) method (right). The discontinuous line (---) represents the 1:1 relationship, the dashed lines (···) represent the relative RMSE and the red continuous line represents the adjustment of the assessed variables.

Table 2. Linear models adjusted for the maximum emission values (Max.) and the Vacuum by Manual Pump (VP), Vacuum by syringe (VS) and Gas Exchange by Displacement (GED) methods emissions; and associated statistical parameters

	Slope	CI 95%	R ²	MSE	RMSE	Comparison with the 1: 1 line (p-value)
Max. vs VP	1.00	(0.99; 1.02)	0.99	22.46	4.74	0.67
Max. vs GED	1.23	(1.18; 1.29)	0.97	131.15	15.22	< 0.05
Max. vs VS	1.88	(1.96; 2.17)	0.84	1646.58	40.58	< 0.05

value of 1.00, a small confidence interval, lower MSE and RMSE values and a high R². Finally, the linear model adjusted between the maximum emission values and the GED method presented a slope of 1.23, a narrow confidence interval and relatively low MSE, and RMSE values with a high R² (Table 2).

DISCUSSION

The VS method never detected emission rates over 250 μg m⁻² h⁻¹ of N-N₂O, probably due to the vacuum generated by the syringe inside the vial was not complete, resulting in a partial gas replacement; so, the sample inserted in the vial was diluted with the gas that was not extracted. Similarly, the GED method never detected emission rates over 365 μg of N-N₂O m⁻² h⁻¹, probably because the volume used to purge did not reach the full replacement of the original gas inside the vial by the gas coming from inside the chamber (syringe).




The linear model fitted between the maximum values and the VS method had a wide confidence interval, the highest MSE and RMSE values, and the lowest R². This suggests that the use of the VS method would not be appropriate for determining soil N₂O emission values compared with the other methods tested. The linear model fitted between the maximum values and the VP method had a slope value of 1.00, a small confidence interval, lower MSE and RMSE values, and a high R². This suggests that the VP method is the one that best represents the maximum emission values. Finally, the linear model




adjusted between the maximum emission values and the GED method presented a slope of 1.23, a narrow confidence interval and relatively low MSE, and RMSE values with a high R^2 . The GED method adjusted to the maximum emission values could be an alternative to the VP method for obtaining reliable values when the emission values are lower than $250 \mu\text{g N-N}_2\text{O m}^{-2} \text{h}^{-1}$. This method shortens the extraction time and allows the sampling of a larger number of chambers simultaneously.

CONCLUSIONS





This study gives a first approach to the selection of an accurate soil N_2O extraction method from static chambers at the field. Discrimination of the soil N_2O detection of the three proposed methods was performed on different soil types, emission rates, and environments. The VP method presented the highest accuracy in N_2O detection. The values obtained with this method were statistically equal to the maximum emission values because this method showed the largest number of maximum rates. The VS method underestimated the maximum emission values in $\sim 52\%$ and did not adjust correctly to the maximum N_2O emission values. Therefore, this method seems to be inadequate to measure N_2O from the soil, especially if the emissions are higher than $100 \mu\text{g m}^{-2} \text{h}^{-1}$ of $\text{N-N}_2\text{O}$. Nitrous oxide emission values determined directly by the GED method underestimated in $\sim 19\%$ the maximum emission values but presented a correct adjustment to the maximum N_2O emission values. This suggests that using the GED could be a recommendable method if the data is adjusted to obtain the maximum values, providing an efficient and economical alternative to measure the N_2O fluxes when the emission values are lower than $250 \mu\text{g N-N}_2\text{O m}^{-2} \text{h}^{-1}$.







AUTHOR CONTRIBUTIONS






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