



## Short communication

## Gas pooling: A sampling technique to overcome spatial heterogeneity of soil carbon dioxide and nitrous oxide fluxes



Cristina Arias-Navarro<sup>a,1</sup>, Eugenio Díaz-Pinés<sup>b,\*,1</sup>, Ralf Kiese<sup>b</sup>, Todd S. Rosenstock<sup>c</sup>, Mariana C. Rufino<sup>d</sup>, David Stern<sup>e</sup>, Henry Neufeldt<sup>c</sup>, Louis V. Verchot<sup>f</sup>, Klaus Butterbach-Bahl<sup>b,d</sup>

<sup>a</sup> Center for International Forestry Research (CIFOR), Nairobi, Kenya

<sup>b</sup> Institute of Meteorology and Climate Research, Atmospheric Environmental Research, Karlsruhe Institute of Technology, Kreuzeckbahnstr. 19, 82467 Garmisch-Partenkirchen, Germany

<sup>c</sup> World Agroforestry Centre (ICRAF), Nairobi, Kenya

<sup>d</sup> International Livestock Research Institute (ILRI), Nairobi, Kenya

<sup>e</sup> Maseno University, Maseno, Kenya

<sup>f</sup> Center for International Forestry Research (CIFOR), Bogor, Indonesia

## ARTICLE INFO

## Article history:

Received 22 February 2013

Received in revised form

14 August 2013

Accepted 16 August 2013

Available online 28 August 2013

## Keywords:

Composite sample

Pooling technique

Soil CO<sub>2</sub> and N<sub>2</sub>O fluxes

Spatial variability

Static chamber

## ABSTRACT

Small-scale spatial variability in soil carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) fluxes poses serious challenges to the experimental design, and number of gas samples needed to provide a reliable estimate of flux usually exceeds analytical capacities. We pooled gas samples – analogously to soil pooling – to overcome this challenge. Our sample pooling technique collects a composite gas sample from several chambers instead of the conventional practise of analyzing samples from chambers individually, thus reducing numbers of gas samples. The method was verified to be reasonably accurate in forest, grassland and agricultural fields over a four week measurement campaign. Pooling technique results differed by 2–8% for CO<sub>2</sub> and by 3–4% for N<sub>2</sub>O when compared to individual chamber means. That shows pooling of gas samples across individual static chambers is an acceptable approach to integrate spatial heterogeneity.

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Practical methods are needed to quantify soil CO<sub>2</sub> and N<sub>2</sub>O fluxes in order to better understand magnitudes, spatial and temporal variability of soil-atmosphere CO<sub>2</sub> and N<sub>2</sub>O exchange. This information is needed to develop improved management practices aiming towards lower CO<sub>2</sub> and N<sub>2</sub>O emissions. Static chambers are the most commonly used approach for measuring soil greenhouse gas (GHG) fluxes (Grahammer et al., 1991; Livingston and Hutchinson, 1995; Smith et al., 1995) because relatively low cost, simple operation, and portability (Butterbach-Bahl et al., 2011; Denmead, 2008). Furthermore, the simple technique and deployment protocol can be adapted to a wide range of ecosystems and experimental designs (Rochette, 2011).

The basic principle of static chambers measurements is that a number of gas samples (three to six) are taken over a period of time

from the headspace of a gas-tight chamber enclosing the soil surface. GHG fluxes are calculated from the rate of change in the headspace gas concentration over time.

Soil fluxes of CO<sub>2</sub> and N<sub>2</sub>O vary significantly over space and time driven by microbiological processes, environmental conditions, heterogeneity of soil properties and spatial variation in available nutrients and root distribution (Butterbach-Bahl et al., 2011; Davidson et al., 2000; Verchot et al., 1999). Specifically, small-scale spatial variability – within a few meters – commonly exceeds 100% (Parkin and Venterea, 2010). Thus, replicated chamber measurements on sites investigated is required to achieve robust representative emission rates. Furthermore, when investigating CO<sub>2</sub> and N<sub>2</sub>O fluxes in landscapes with a mosaic of land uses and land covers, the total number of samples needed to provide a reliable flux estimate quickly exceeds analytical capacities.

Pooling is accepted and widely used for soil sampling, but it was so far not tested as a method to overcome the limitations imposed by time-consuming (and hence cost intensive) analytics

\* Corresponding author. Tel.: +49 (0) 8821 183 309; fax: +49 (0) 8821 183 294.

E-mail address: [eugenio.diaz-pines@kit.edu](mailto:eugenio.diaz-pines@kit.edu) (E. Díaz-Pinés).

<sup>1</sup> These authors contributed equally to the work.

and procedures in CO<sub>2</sub> and N<sub>2</sub>O measurements. Here we propose gas sample pooling to reduce the number of gas samples required while maintaining the reliability of the estimated CO<sub>2</sub> and N<sub>2</sub>O flux.

We selected three different experimental sites for measuring N<sub>2</sub>O and CO<sub>2</sub> soil-atmosphere fluxes with five chambers each over a four-week period: a forest (a 30-years old *Eucalyptus* plantation) a non-grazed grassland and a kale (*Brassica oleracea* L.) cropland. Each site had at least an area of 0.25 ha. We compared the emission rates obtained with the gas sample pooling technique (Fig. 1) with the mean rates of each observation date calculated from sampling the five individual chambers following the traditional method. The number of chambers was chosen in order to allow one operator to conduct the gas sampling within chamber closure period (40 min).

The study sites were located on the Maseno University Campus (Kenya) (0°, 34° 36'E). The experiment was conducted from 29 October to 29 November in 2012. Site characteristics are presented in Table 1. At each site, five 35 by 25 cm<sup>2</sup> PVC frames (collars) were inserted prior to the first measurement and remained in place throughout the experimental period. For CO<sub>2</sub> and N<sub>2</sub>O measurements, a PVC chamber (12 cm high), equipped with a fan, a non-forced vent and a sampling port was affixed to the frame by metal clamps and a rubber sealing between frame and chamber to assure air-tight seal.

For individual chamber measurements 50 ml gas sample was taken from the chamber headspace with a gas tight syringe through a stopcock valve at 10 min intervals (0, 10, 20, 30, and 40 min after chamber closure). If the use of a fan is not possible, an extra syringe should be used to carefully pump several times before taking the gas sample to obtain homogenous mixing of the headspace air.

For the gas pooling technique, a 10 ml sample was collected from each of the five chambers with the same syringe at each time interval equaling 50 ml in total (Fig. 1a). The 50 ml gas samples were then immediately transferred into 10 ml sealed glass vials (Fig. 1b) and analyzed by gas chromatography (<sup>63</sup>Ni-Electron capture detector for N<sub>2</sub>O and Flame ionization detector equipped with a methanizer for CO<sub>2</sub>). Detailed information about the analytical procedure can be found in Gauder et al. (2012) Flux rates of N<sub>2</sub>O and CO<sub>2</sub> were calculated from the linear change in gas concentrations in the chamber headspace with time.

It was our aim to test the applicability of the gas pooling technique across a range of N<sub>2</sub>O and CO<sub>2</sub> emission rates. Therefore, on November 5th, cropland and grassland experimental sites were fertilized with granular urea dissolved in water at a rate of 100 kg N ha<sup>-1</sup> simulating a 10 mm rainfall event.

Results from individual chamber measurements and gas sample pooling technique highly agreed for CO<sub>2</sub> measurements, capturing temporal variations of fluxes over the observation period (Fig. 2).

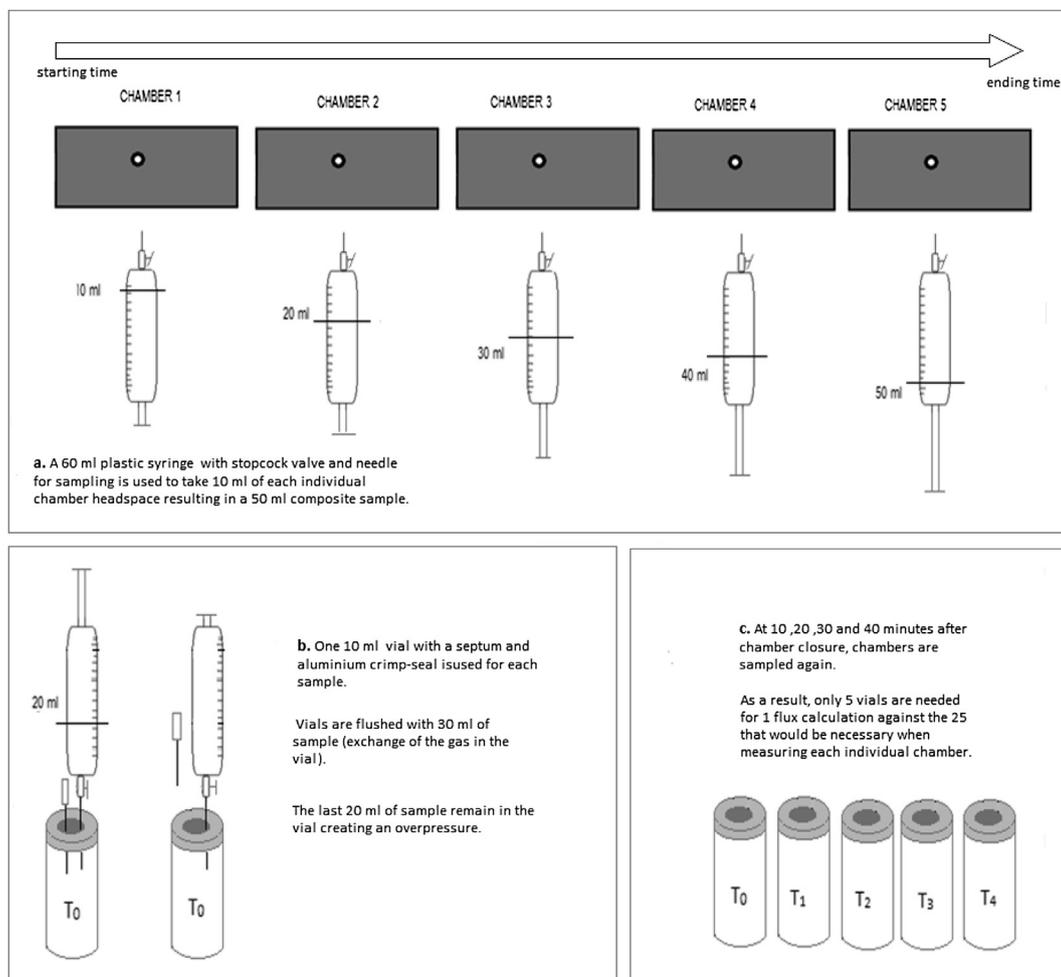
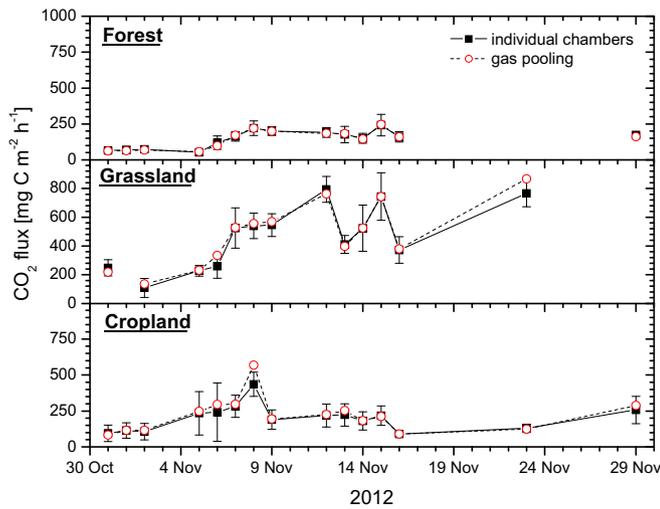


Fig. 1. Concept of gas sample pooling.

**Table 1**  
Soil properties of the sites.

Site	Depth (cm)	C (g kg <sup>-1</sup> )	N (g kg <sup>-1</sup> )	pH	Sand (%)	Silt (%)	Clay (%)
Forest	0–5	28.6 ± 6.8	2.14 ± 0.39	5.0 ± 0.6	18.7 ± 1.7	29.2 ± 1.6	52.1 ± 3.1
	5–20	9.0 ± 0.5	0.93 ± 0.05	4.8 ± 0.1	10.2 ± 2.6	21.8 ± 2.0	68.0 ± 3.5
Grassland	0–5	26.1 ± 4.0	2.47 ± 0.38	6.1 ± 0.1	17.5 ± 1.6	28.3 ± 1.9	54.2 ± 3.0
	5–20	20.4 ± 1.9	1.96 ± 0.17	6.0 ± 0.1	16.5 ± 0.6	23.8 ± 1.6	59.7 ± 2.2
Cropland	0–5	15.6 ± 1.1	1.44 ± 0.12	5.2 ± 0.2	20.6 ± 5.3	20.1 ± 2.0	59.3 ± 6.1
	5–20	13.9 ± 1.9	1.28 ± 0.14	4.8 ± 0.1	16.5 ± 5.2	17.4 ± 1.6	66.1 ± 4.8

**Fig. 2.** Soil CO<sub>2</sub> Fluxes in the three experimental sites over the observation period. Vertical bars denote one standard error of the mean of the individual chambers.

CO<sub>2</sub> fluxes ranged between 50 and greater than 700 mg CO<sub>2</sub>–C m<sup>-2</sup> h<sup>-1</sup> (Table 2) and absolute differences in single chamber means and gas pooling values for individual measuring days and CO<sub>2</sub> fluxes were in average 21, 22 and 6 mg CO<sub>2</sub>–C m<sup>-2</sup> h<sup>-1</sup> for cropland, grassland and forest, respectively. The mean values across the entire observation period calculated for single chambers and the gas sample pooling method differed by 8.3, 2.5 and 1.6% (Table 2). Linear regression between conventional sampling and gas pooling revealed no systematic bias ( $f(x) = 1.043 \pm 0.024 x$ ;  $R^2 = 0.98$ ).

Comparable results were obtained for N<sub>2</sub>O fluxes (Fig. 3) ranging between 0 and 400 µg N<sub>2</sub>O–N m<sup>-2</sup> h<sup>-1</sup> (Table 2): Mean fluxes for individual observation days differed by 14, 7, 5 µg N<sub>2</sub>O–N m<sup>-2</sup> h<sup>-1</sup>, representing a difference between single chambers and gas sample pooling between 3 and 4% for the whole observation period (Table 2). Linear regression results for N<sub>2</sub>O fluxes comparing conventional sampling and gas sample pooling were:  $f(x) = 0.996 \pm 0.018 x$ ;  $R^2 = 0.99$ .

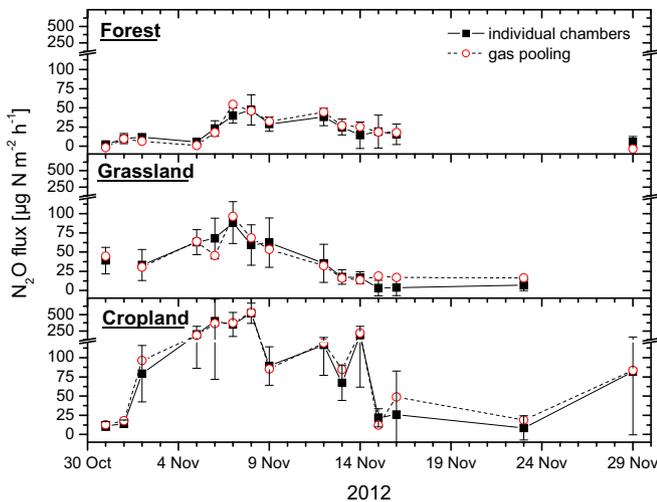
**Table 2**  
Summary of soil CO<sub>2</sub> and N<sub>2</sub>O fluxes, soil temperature and soil moisture during the measurement campaign.

Site	CO <sub>2</sub> flux pooling	CO <sub>2</sub> flux individual chambers	CV CO <sub>2</sub> flux individual chambers	N <sub>2</sub> O flux pooling	N <sub>2</sub> O flux individual chambers	CV N <sub>2</sub> O flux individual chambers	Soil temperature °C	Soil moisture m <sup>3</sup> m <sup>-3</sup>
	mg CO <sub>2</sub> –C m <sup>-2</sup> h <sup>-1</sup>		%	µg N <sub>2</sub> O–N m <sup>-2</sup> h <sup>-1</sup>		%		
Forest	144.2 (57.4–247.1)	146.7 (51.7–241.7)	25 (8–40)	21.2 (–3.5–54.4)	20.5 (2.4–47.3)	63 (24–123)	20.7 (19.9–21.7)	0.40 (0.24–0.49)
Grassland	480.3 (135.2–867.2)	466.6 (108.8–793.7)	52 (12–97)	39.8 (13.7–96.3)	38.2 (3.1–88.5)	90 (26–326)	24.3 (22.0–27.7)	0.43 (0.37–0.48)
Cropland	219.2 (83.5–568.4)	201.1 (89.5–435.6)	25 (14–84)	150.9 (12.2–530.6)	145.5 (8.5–520.5)	72 (28–220)	23.3 (20.2–26.9)	0.36 (0.24–0.44)

Mean (minimum–maximum) daily values for the three sites under investigation along the measurement campaign. CV denotes coefficient of variation.

Based on these results we conclude that the gas sample pooling method provides an opportunity for an optimized targeted sampling design to overcome heterogeneity of soil CO<sub>2</sub> and N<sub>2</sub>O exchange. The method has advantages over ultra large chambers (e.g. Galle et al., 1994), which are more expensive, difficult to handle and can cover the spatial variability up to only a few square meters. Though our results show good agreement between the conventional and the gas pooling method, a careful application is required since reliable CO<sub>2</sub> and N<sub>2</sub>O flux estimates still highly depend on a scientific sound experimental design. The gas sample pooling method can be used in, mainly, two ways: 1) gas samples from several chambers can be pooled from one plot, obtaining a single spatial representative flux; or 2) several sets of chambers can be placed within a plot, and each of the sets can be pooled separately. The former will dramatically decrease the analytical capacities needed, while losing the information of the spatial variability of the fluxes at the plot. With the same analytical capacity as when analyzing individual chambers, the latter will increase both the area effectively sampled and the human resources needed on the field. Thus, the design of the gas pooling technique needs to be specifically adjusted, based on the characteristics of the environment under investigation and the research questions of each specific experiment. To spatially locate the chambers in representative spots, we recommend to measure the soil CO<sub>2</sub> and N<sub>2</sub>O fluxes from individual chambers prior to the start of the experiment in order to assess the spatial variability of the site and eventually replace chambers, since subsequent removal of outliers upon results (Crill et al., 2000) is not feasible. Furthermore, the spatial variability should be characterized at regular intervals by applying the standard method of single chamber based measurements, since the spatial variability may seasonally vary.

The approach of sample pooling has been widely applied for ecosystem studies e.g. plant and soil material and water (Patty, 2013; Pennock et al., 2006; Wilde, 2006). Our results demonstrate that pooling is a sensible, low cost and easy to deploy technique for monitoring soil CO<sub>2</sub> and N<sub>2</sub>O fluxes with potential to radically increase the amount of information on the biosphere–atmosphere exchange of CO<sub>2</sub> and N<sub>2</sub>O.



**Fig. 3.** Soil  $N_2O$  Fluxes in the three experimental sites over the observation period. Vertical bars denote one standard error of the mean of the individual chambers.

### Acknowledgments

This work was funded by the SAMPLES project under the CCAFS program of the Consultative Group on International Agricultural Research, the German Science Foundation under the contract BU 1173/12-1 and the EU funded IA project InGOS. CAN acknowledges financial support by CLIFFS, CCAFS. Funding for RK and KBB has been partly provided by DFG in the frame of the research group ICON (FOR 1701).

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