Measurement of greenhouse gas fluxes in agricultural soils with a flexible, open-design automated system

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Abstract

Over the last decades and due to the current climate change situation, the study of the impacts of human activities on climate has reached great importance, being agriculture one of the main sources of soil greenhouse gas. There are different techniques to quantify the soil gas fluxes, such as micrometeorological techniques or chamber techniques, being the last one capable to assess different treatment at the same site. Manual chambers are the most common one. However, due to the low sampling frequency, this approach cannot resolve short-term emission events, like fertilization or rewetting. For this reason, automated chamber systems are an opportunity to improve soil gas flux determination, but their distribution is still scarce due to the cost and challenging technical implementation. The objective of this study was to develop an automated chamber system for agricultural systems under Mediterranean conditions and compare measured GHG flux rates to those derived using manual chambers. A comparison between manual and automated chamber systems was conducted to evaluate the soil gas fluxes obtained by the automated system. Moreover, over a period of one month the soil gas fluxes were determined by both systems to compare their capabilities to capture the temporal variability of soil gas emissions. The automated system reported higher soil GHG fluxes compared to the manual chamber system. Additionally, the higher sampling frequency of the automated chamber system allowed for the capture of daily flux variations, resulting in a more accurate estimation of cumulative soil gas emissions. The study emphasises the importance of chamber dimension and shape, as well as sampling frequency, in the development of chamber systems, especially when using the manual chamber system.

1. Introduction

Agriculture and land-use changes are significant contributors to climate change, accounting for a quarter of total global emissions of greenhouse gases (GHG) (IPCC, 2014). Moreover, agricultural emissions are expected to increase along with food demand (Wiebe et al., 2019). Microbial activity is the main responsible of the production and emission of the different soil GHG. Microbial processes are influenced by several abiotic factors such as soil water content, soil temperature or nutrient availability. The different farming practices – i.e. crop rotation, fertilization, irrigation – have a significant impact on these factors, and, therefore, they can have a great influence on soil GHG emissions (Oertel et al., 2016). By accurately measuring soil GHG emissions, it is possible to identify the major sources and understand the impact associated with various farming practices. This valuable information can be provided to policymakers and regulators to develop science-based policies and regulations that incentivize farmers to adopt more sustainable practices. Thus, measuring soil GHG emissions in agriculture is crucial to promote sustainable farming practices, that can mitigate climate change.

The use of manual chambers is one of the most widespread methods for studying soil GHG emissions on a small spatial and temporal scale (Collier et al., 2014). These chambers are designed to establish an enclosed environment, facilitating the periodic collection of gases emitted from or consumed in the soil using syringes. Subsequently, the gathered gas samples are subjected to laboratory analysis through gas chromatography (Harvey et al., 2020). These analyses determine the concentration of GHG within the chamber headspace and allow the calculation of emission rates based on the change in gas concentration over a given time span. This method is characterized by its simplicity and versatility as they are relatively simple to use and can be employed across diverse ecosystems and soil types (de Klein et al., 2020). Manual chambers are relatively simple to construct and can be tailored to fit specific research requirements. Besides, compared to alternative methods, they entail relatively low cost. However, they have as well some limitations.

For instance, their measurement frequency is restricted due to the time-intensive nature of manual sampling and subsequent analysis, making high-frequency sampling impractical. Usually, sampling frequency is not higher than one sampling per day, but it's well stablished that sampling frequency affects annual GHG estimations (Barton et al., 2015). For this reason, efforts are often concentrated on intense sampling frequencies during short periods (hours to days) when significant emissions peaks are expected, but later, during the rest of the campaign, samplings are carried out every 1 to 4 weeks (or even sometimes not considered). Another aspect to consider involves the notable soil disruption caused when samples need to be collected, such as after an irrigation event.

In contrast to manual chambers, the utilization of automated chambers coupled with an in-situ gas analyzer allows sampling at a higher temporal frequency. Consequently, these automated systems more comprehensively capture temporal variations, enhancing insight into the dynamics of soil GHG emissions on a daily and seasonal basis (Grace et al., 2020). Automation also ensures capturing fluxes linked to unexpected events (such as rainstorms), obtaining data in areas of difficult access, and reducing the impact of soil disturbance on measurements. However, this method requires costly equipment and skilled operators, and implies different infrastructure constraints. Over recent decades, several groups have crafted automated systems (Lognoul et al., 2017, Lawrence and Hall, 2020).

To date, the number of experiences using automated chambers coupled with in situ gas analyzer under Mediterranean conditions is scarce (Forte et al., 2017, Ferrara et al., 2021, Isla et al., 2022) and, as far as we have been able to find out, none of these previous studies used chamber systems consisting in a total of 12 individual chambers. The objective of this paper is to present an innovative non-commercial soil GHG measurement system based on automated chambers linked to an in situ photoacoustic multigas analyzer and describe its operational details. Besides, a comparison between this automated system and the manual static chamber methodology is presented.

2. Materials and Methods

2.1. Automated system description

In this section, we present an automated chamber system tailored for monitoring soil gas emissions. By integrating openness, cost-effectiveness, and versatility, this system facilitates precise and dynamic measurements of soil GHG fluxes. Our design principles focused on building an adaptable configuration and real-time functionality, alluding to its potential importance in agricultural and environmental research. The system consists of three main parts: the chambers, the set of solenoid valves controlled by a computer (central control unit) and the multigas analyzer (Figure 1).

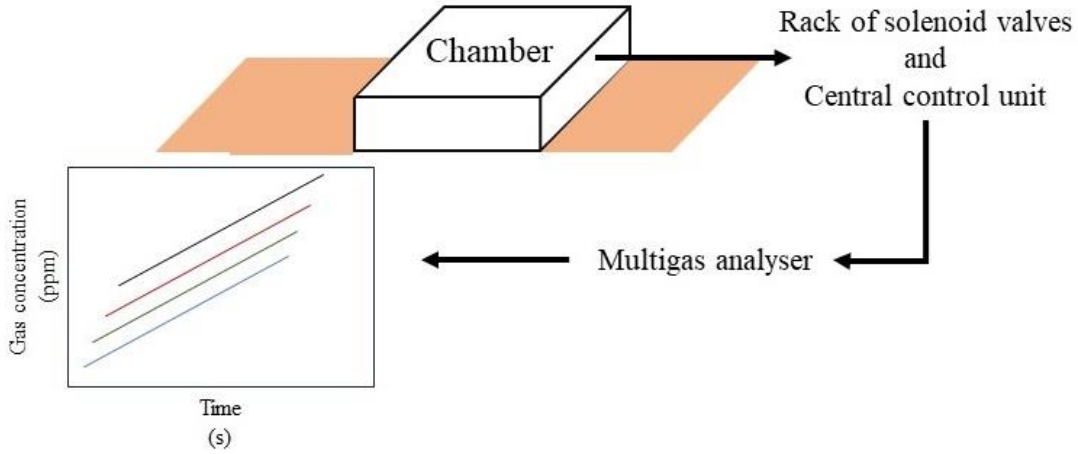

Figure 1. General scheme of the automated soil GHG measuring system.

2.2. Soil chamber design

Soil chambers, 'Queensland' design, have been built following a model provided by the Terrestrial Bio-Geo-Chemistry Division (Institute of Meteorology and Climate Research, Atmos. Environ.al Research (IMK-IFU), Karlsruhe Institute of Technology (KIT)). Chambers consisted of an aluminum structure of 0.50 x 0.50 m length and width and 0.15 m height closed with methacrylate panels and two lids 0.50 x 0.25 m width and length that are controlled by four pneumatic actuators, two per lid (Figure 2a). Besides, lids open at a 90º angle allowing rainfall or

irrigation water supply to reach the soil surface of the area covered by the chambers. All methacrylate panels were coated with an aluminum bubble foil to keep the internal chamber temperature homogeneous during the enclosure time. Moreover, a rubber seal was fixed to the lids and the bottom part of each chamber to ensure a hermetic close and avoid gas leakage during the sampling process.

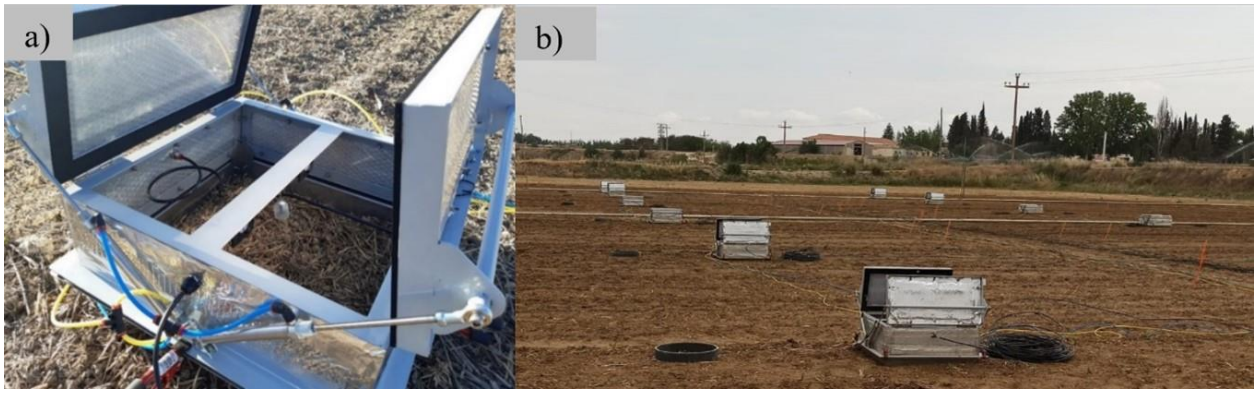

Figure. 2. (a) Open automated chamber deployed in the field trial ('Queensland' design). (b) Set of chambers deployed in the field trial. Dark rings next to chambers are the bases for manual chambers.

The gas sample line (polyethene coated aluminum tube, Eaton Sinflex. 6/4mmm external internal diameter, respectively) entered each chamber via one of the side panels, positioned approximately halfway up. In the central area of the chamber, the tube was bent facing downwards and the tip was protected by a small PVC funnel to prevent water condensation at the tube inlet. A vent (matching the material and diameter of the gas sampling line) was positioned on the opposite side panel to equalize pressure between the chamber's interior and exterior during flux measurements. Moreover, each chamber has two small fans (60x60x25 mm 12V) to promote air mixing inside the chamber.

Three chambers were equipped with a threaded cable gland on a lateral methacrylate panel for mounting a thermistor (107, Campbell Scientific Ltd., UK) to monitor internal chamber temperature. Chambers were attached by clamps to stainless steel bases (0.5 x 0,5 x 0.1) with sharp

edges at the bottom that were inserted 0.10 m into the soil . Plants (crop and weeds) growing inside the chambers were cut since the crop during this experiment was maize (*Zea mays* L.) (Figure 2b).

2.3. Automated chamber operation

The chambers opened and closed by means of pneumatic actuators. This setup comprised an air compressor delivering pressure to the pneumatic actuators. Inside a shed located next to the field trial, three solenoid valves installed in a panel, received air from the compressor (6 bar) and directed compressed air to the chambers. Routing of compressed air was facilitated by an external relay controller (8 relay board, 24V 6.5A, YWBL-WH) directly linked to the computer. In the configuration of this study, three sets of four chambers each opened and closed simultaneously. Similarly, each sampling line from each chamber was connected to a two-way solenoid valve that regulated the entry of the gas sample from each of the chambers to the photoacoustic multi-gas analyzer (Gasera One, Gasera Ltd, Finland). The two-way solenoid valves were connected to a relay board (16 relay board, 24V 6.5A, YWBL-WH) that controlled which valve was activated (Figure 3).

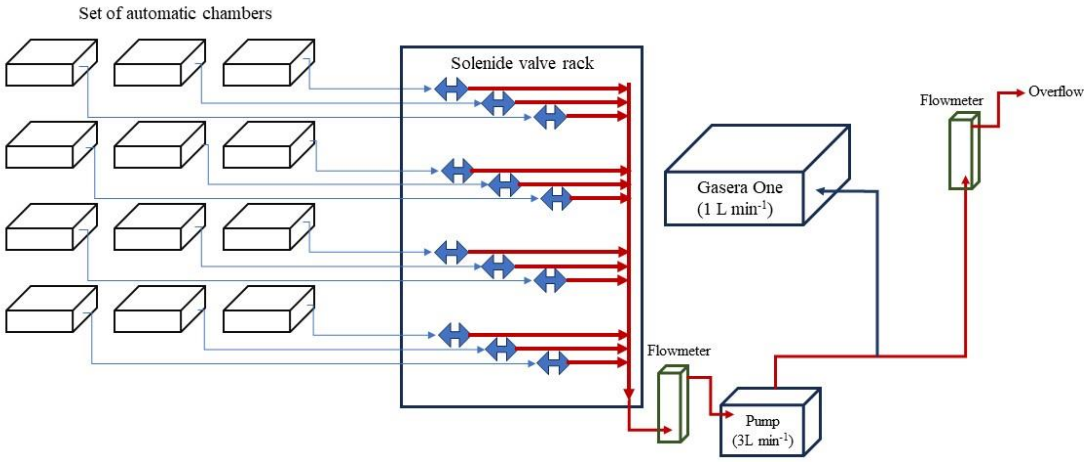

Figure. 3. Description of the automated chamber system.

To bring the gas from the chamber to the gas analyzer, an external diaphragm pump (KNF NMP830KNDC 12V, KNF Neuberger, Inc, Freiburg im Breisgau, Germany) was coupled to the two-way solenoid valve bank. This pump continuously drew air from the activated sampling line, maintaining a flow rate of 3L min^{-1}. The gas analyzer (Analysis cell volume 30 mL) drew sample gas from this primary line at a rate of 1 L min^{-1} for a duration of six seconds every one and a half minutes (Figure 3c). Two flowmeters were attached to the main line. The initial one, positioned after the pump and preceding the gas analyzer, regulated the gas flow delivered to the analyzer. The second flowmeter ensured a continuous overflow greater than 1 L min^{-1}, guaranteeing sufficient gas flow from the active sampling line to the gas analyzer (Figure 3). The solenoid valve banks, pneumatic system, chamber sampling lines, and gas analyzer were all managed through a custom script created using R statistical software version 4.2.2 (R Core Team, 2022).

The current setup consists of 3 blocks of four chambers each block. This configuration responds to the needs of the current experimental design, however, since it is an open system, the configuration is variable and can be individualised for each of the chambers.

2.4. Evaluation of the automated measurement system

Over the last decade, the current research team members have successfully conducted several GHG flux studies using a manual closed chamber system (Álvaro-Fuentes et al., 2016, Franco-Luesma et al., 2019,2020a, 2020b, 2022). Based on that, an evaluation experiment was carried out to compare the soil gas fluxes obtained via the newly developed automated chamber system against the conventional manual chamber system used regularly by the research group. This evaluation experiment was aimed to evaluate the impact of i) the chamber design and ii) the sampling frequency and time on the differences in soil GHG fluxes between a manual and an automated chamber measurement system.

Manual chambers consisted of a Polyvinyl Chloride (PVC) cylinder of 0.315 m diameter and 0.2 m height coated by white thermal paint to avoid internal air temperature increasing during the deployed time. A rubber septum was affixed atop the chamber to enable gas sampling via a plastic syringe equipped with a needle. Gas samples from each chamber were transferred to a 12 mL pre-evacuated glass vial (Exetainer Labco®). The concentrations of CO_2, CH_4 and N_2O in the gas samples were determined by gas chromatography Agilent 7890B (Agilent, Santa Clara, CA, United States) equipped with an autosampler (PAL3 autosampler, Zwingen, Switzerland). Soil gas fluxes were determined based on the increase of the gas concentration during the deployment period. Further details of the gas chromatography method and manual chamber design could be found in Franco-Luesma et al. (2022).

The evaluation experiment took place in a maize (*Zea mays* L.) field trial sown on 10/05/2023 under irrigation conditions. The soil is a *Typic Xerofluvent* (Soil Survey Staff, 2015) with a silty loam texture, characterized by a basic pH of 8, a calcium carbonate content ($CaCO_3$) of 48%, a total organic carbon content of 0.6% and a bulk density of 1.33 g cm^{-3} in the first 0.25 m soil depth. The area is characterized by a Mediterranean semiarid climate with a mean annual air temperature of 14.1 °C, mean annual precipitation of 298 mm and mean annual reference evapotranspiration (ETo) of 1,243 mm.

The evaluation experiment had two different steps. The first step consisted of simultaneous gas sampling with both manual and automated chamber systems on four different dates (i.e. 19/06/2023, 20/06/2023, 21/06/2023 and 28/06/2023). On June 19th and 20th, chambers were sampled once during 06:00 to 07:30 GMT. On June 21st and June 28th, chambers were sampled four times between 06:00 to 12:00 GMT. Conseqeuntly, a total of ten samplings were performed, covering four different days and different hours of the day to capture the possible diurnal variation of soil gas emission.

In this short time experiment, two chambers of each block were selected to compared with the manual chambers. The sampling sequence for the automated system was programmed to sample each chamber every five minutes, with a total enclosure time of 28 minutes. However, due to the sequence configuration, the computable time for determining the soil gas fluxes was 20 minutes as described in the sequence diagram (Figure S1). The two manual chambers of each block were closed at the same time as the automated chamber and gas sampling was done at time 0 (first automated chamber sampling), at time 10 minutes and at time 20 minutes (coinciding with the last automated chamber sampling).

Finally, in order to complement the results obtained in the first step of the evaluation experiment, a second step consists of compared the soil gas fluxes obtained by each chamber system for one month were carried out. For that propose, from 22 of May 2023 to 29 of June 2023, soil CO_2, CH_4 and N_2O fluxes were measured by the manual and automated chamber systems, to assess the impact of the sampling time (i.e. hour of the day) and sampling frequency on the estimation of the soil gas fluxes.

During this period, the sampling frequency and configuration of the automated chamber system was the same as it was used during the step one of the evaluation experiment. The twelve chambers were grouped in three set of four chambers each, being sampling every five minutes for 28 minutes, resulting in a total of 5 sampling points per chamber (Figure S1). However, the procedure followed in the manual chamber system was different and it consisted of the collection of three gas samples at time 0, 20 and 40 minutes after closing the chamber. The sampling frequency followed a daily frequency over the first five days and, afterwards, weekly measurements till the end of the experiment. For both chamber systems, the measuring instrument (i.e. photoacoustic multi-gas analyzer and gas chromatography for automated and manual chamber systems, respectively) were calibrated by using 4 different ultra-high purity gas standards (Carburos Metálicos, Barcelona, Spain, standard 1, 400 ppm CO_2, 1.5 ppm CH_4, 0.3 ppmN_2O,

standard 2, 800 ppm CO_2, 2 ppm CH_4, 1 ppmN_2O, standard 3, 1500 ppm CO_2, 4 ppm CH_4, 3 ppmN_2O, standard 4, 3000 ppm CO_2, 6 ppm CH_4, 6 ppmN_2O) in order to standardize the concentration values obtained.

2.5. Data analysis

Soil gas flux (mg of gas m^2 day^1) of CO_2, CH_4 and N_2O, i.e., f_{CO2}, f_{CH4} and f_{N2O} was calculated using the following equation (Eq. 1)

$$f_{gas} = \frac{Fit * MW * p * h}{R * T} * fT * fU \quad (Eq. 1)$$

where *Fit* represents the increase of gas concentration in the chamber over the enclosure time, *MW* is the molar weight of the atom in the gas molecule (i.e. 12 g mol^{-1} for CO_2 and CH_4 and 28 g mol^{-1} for N_2O), *p* is the atmospheric pressure in Pa, *h* is the chamber height in m, *R* is the ideal gas constant in J K^{-1} mol^{-1}, *T* is the chamber air temperature in K, *fT* is the correction factor of time units, 1440 minutes day^{-1} and *fU* is the unit correction factor, 10^3. Cumulative soil CO_2, CH_4 and N_2O emissions were calculated using the trapezoid rule (Levy et al., 2017). Comparison between systems was done by linear fitting considering only soil gas fluxes that presented a R^2 higher than 0.8. All analyses were done using the R statistical software.

3. Results and Discussion

3.1. Automated system comparison

The comparison between the automated and manual measurement systems showed a linear response for the three gases compared. In the case of soil CO_2, the automated system presented an average flux 58% greater compared to the manual system with a minimal flux difference of 425 mg CO_2-C m^2 day^1 (Figure 4a). Data exhibited moderate dispersion (R^2=0.60) revealing increased accuracy when manual fluxes were greater than 500 mg CO_2-C m^2 day^1 (Figure 4a). Regarding CH_4 fluxes, the automated chamber system showed values greater than the fluxes obtained in the manual chamber system, showing a better fitting when fluxes were positive (Figure 4b). However, the lowest data dispersion between both measurement systems was obtained for soil N_2O fluxes (R^2> 0.87) but as observed for the other two gases, the automated chamber system reported fluxes values 40% greater than the manual chamber system (Figure 4c).

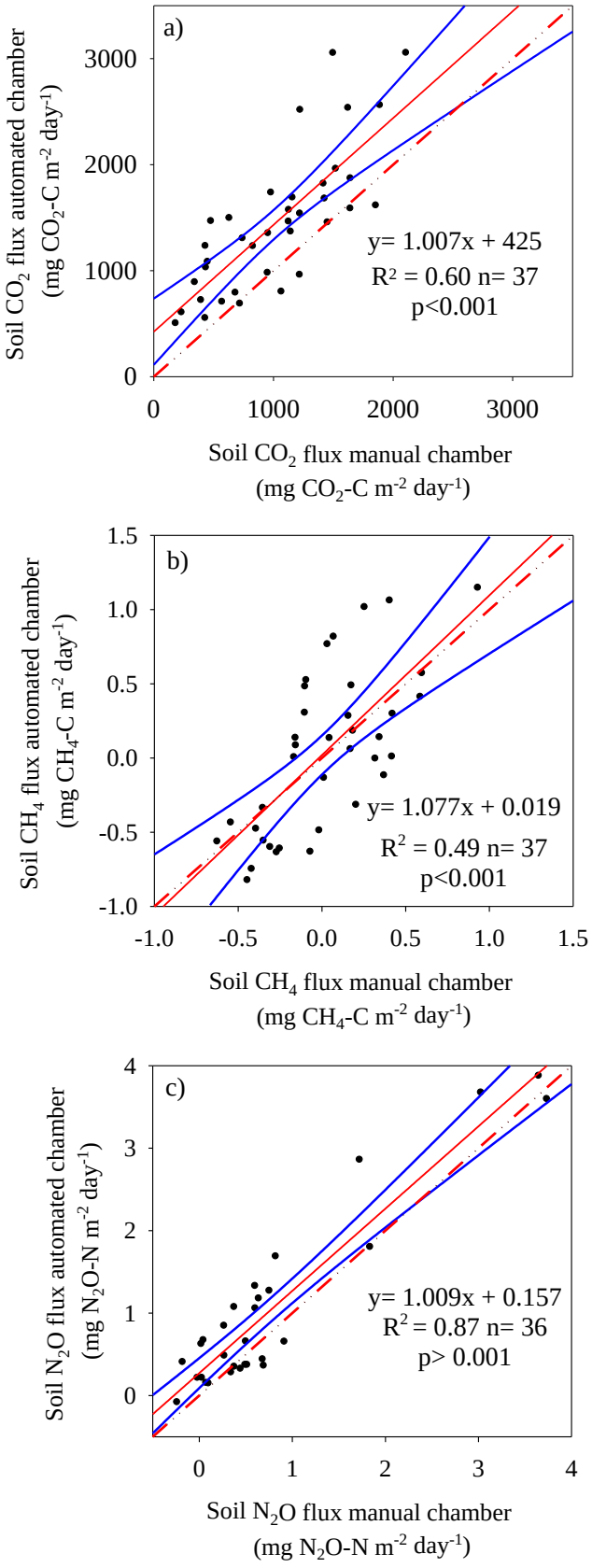

Figure 4. Comparison of soil gas flux between automated and manual chamber systems for carbon dioxide (CO_2) fluxes (a), methane (CH_4) fluxes (b) and nitrous oxide (N_2O) fluxes (c). Blue solid lines represent 95% confidence intervals. Red dotted lines represent 1:1 line.

These differences between both measurement systems in flux magnitude and for the three studied gases may probably be a consequence of the different chamber shapes and dimensions that presented both systems. Hoffmann et al. (2018) found that the shape and dimension of the chamber have a significant effect on CO_2 fluxes, observing that small and cylindrical chambers tend to result in higher underestimation of CO_2 fluxes compared with large and squared chambers. In line with the previous authors, Pihlatie et al. (2013) also found a significant effect of the chamber shape and dimension on soil CH_4 flux determination. Similarly, Rochette and Eriksen-Hamel (2008) also concluded that chamber shape and dimensions are critical factors in the estimation of GHG fluxes.

All previous studies agreed that the area/perimeter ratio is a key factor in soil gas flux estimation and, hence, they recommended a ratio greater than 0.10 m (Clough et al., 2020). In our work, the two types of chambers compared presented different area/perimeter ratios with values of 0.125 and 0.089 m for the automated and the manual chamber systems, respectively. This difference in the area/perimeter ratio could explain the greater CO_2, CH_4 and N_2O fluxes measured by the automated chamber system compared with the manual system. Moreover, the use of fans to mix the internal air of the automated chambers might have also explained the higher fluxes measured in this system compared with the manual system. Air-mixing by fans is highly recommended to homogenize the internal air of the chamber, ensuring that the air sample aliquot is representative of the chamber headspace air (Clough et al., 2020).

3.2. Sampling time and frequency comparison

The effect of sampling time and frequency on cumulative soil gas emissions was compared between the automated and the manual measuring systems. This analysis was performed during one month in which the automated chamber system ran continuously over the entire month, while in the manual chamber system sampling was only performed on nine different dates.

As expected, the automated chamber system was able to capture daily flux fluctuations, a fact that was not possible for the manual chamber system, because only one gas sampling was done for each of the selected dates (Figure 5). However, when fluxes temporal dynamics for each gas were evaluated, it had been observed differences for each gas.

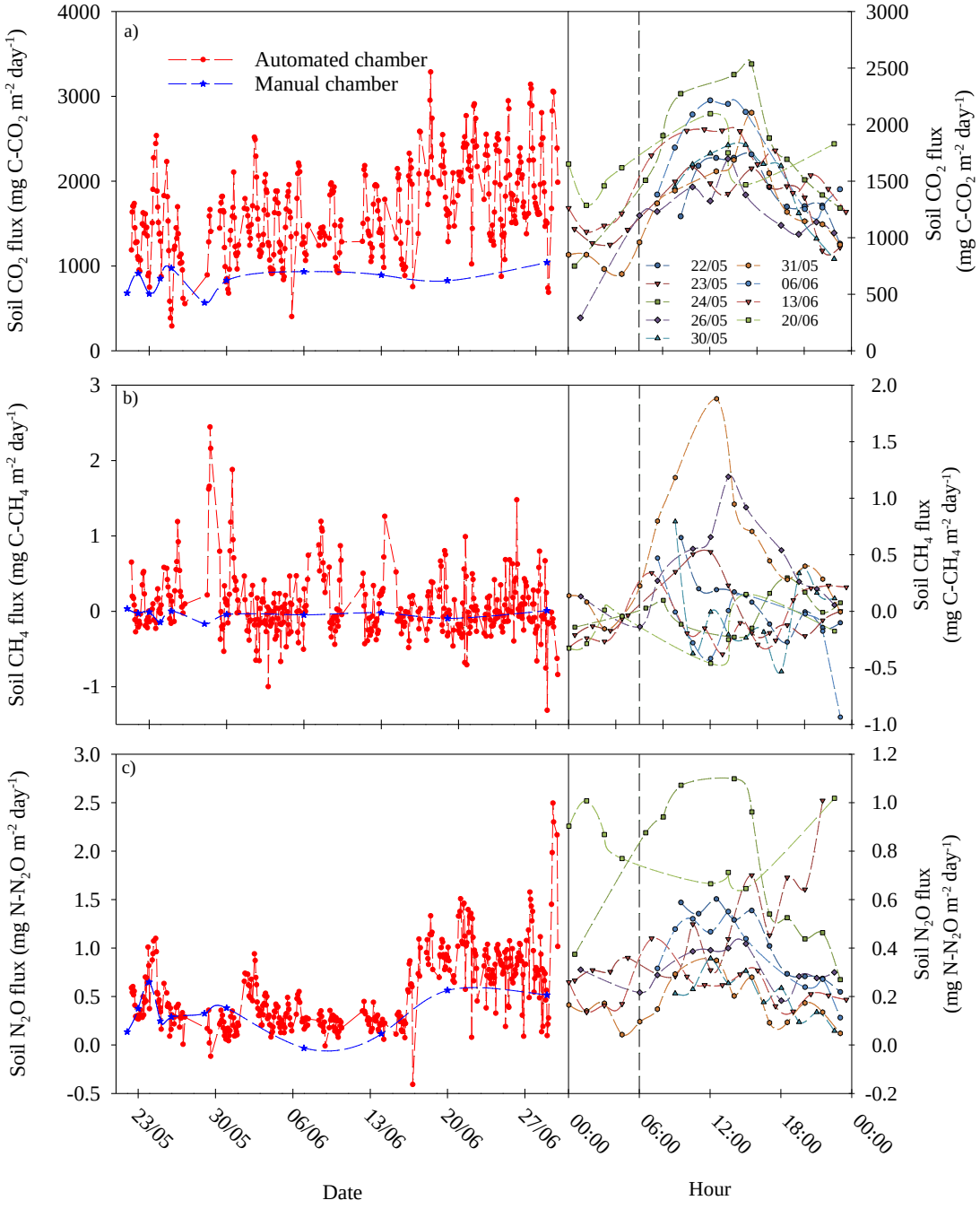

Figure 5. Comparison of soil gas flux and cumulative soil gas emissions between the automated (blue line and bar) and the manual (red line and bar) chamber system for carbon dioxide (CO_2) fluxes (a), methane (CH_4) fluxes (b) and nitrous oxide(N_2O) fluxes (c) (left panel).and daily soil flux of the automated chamber systems on nine different dates Vertical dotted line indicates manual sampling hour (i.e., 6 am) (right panel).

Soil CO_2 and CH_4 fluxes determined by the manual chamber system showed similar behaviour, presenting a low variation in the fluxes magnitude over the evaluated period, being more pronounced for soil CH4 fluxes (Figure 5a, 5b). For example, this was clearly observed in the CH_4 in which the automated system captured flux peaks greater than 2 mg CH_4-C m^{-2} day^{-1} while the manual fluxes kept close to 0 mg CH_4-C m^{-2} day^{-1} over the entire measuring period (Figure 5b). Interestingly, the manual system was able to capture the temporal emission trend shown by the automated system for soil N_2O fluxes, the gas that showed the greatest temporal variability over the period studied (Figure 5c).

Moreover, when the daily emission pattern of the automated chamber was evaluated for the manual sampling dates, it was observed that soil CO_2 fluxes presented the maximum fluxes rate between 12:00 and 16:00 GMT, a daily pattern similar to the results reported by Pumpanen et al. (2003) and Yu et al. (2013). The maximum soil CO_2 fluxes of one day were a factor of three higher than the minimum fluxes measured (Figure 5a). Differences between the maximum and the minimum CH_4 fluxes were lower since soil CH_4 fluxes only ranged between -0.5 to 0.5 mg CH_4-C m^{-2} day^{-1} for most of the nine selected dates, expected for May 26th and 31st when soil CH4 fluxes above 1 mg CH_4-C m^{-2} day^{-1} were observed at midday (Figure 5b).

Soil N_2O fluxes also presented a daily emission pattern characterized by reaching the maximum soil N_2O from 08:00 to 16:00 GMT and the minimum during nighttime, but not being as clear as emission pattern observed for soil CO_2 fluxes (Figure 5c). This daily emission pattern was also observed by Wu et al. (2021) in a metanalysis which evaluated global daily N_2O emission patterns. A possible explanation to the daily pattern observed in all three gases would be the temperature dependence of the biological process that governs the production and emission of soil GHG (Lloyd and Taylor, 1994, Smith and Dobbie, 2001, Davidson and Janssens, 2006,). This dependence would explain the higher emissions observed during daytime compared to nighttime (Fig. 5c).

The cumulative soil gas emissions of the three gases tended to be greater for the automated than the manual measuring system (Fig. 6). For example, cumulative soil CO_2 emissions presented significant differences between both sampling systems. The automated chamber system showed average values 16% more than the manual chamber system (Fig 6a). Indeed, this difference was even greater in CH_4 (more than 3-fold greater cumulative emissions in the automated than in the manual measuring system, Fig. 6b). Cumulative CH_4 emissions showed positive values for the automated chamber system while the average value for the manual chamber system was negative. However, the variability observed for the manual chamber system was 10 times greater rather than for the automated chamber system, a fact that resulted in the absence of significant differences between both sampling systems. Cumulative soil N_2O emissions did not show significant differences between sampling systems despite that the average cumulative N_2O emissions were 20% greater for the automated chamber system (Fig. 6c). As occurred with cumulative CH_4 emissions, the manual chamber system showed a greater variability than the automatic chamber system, reason that could explain the absence of significant differences between sampling systems.

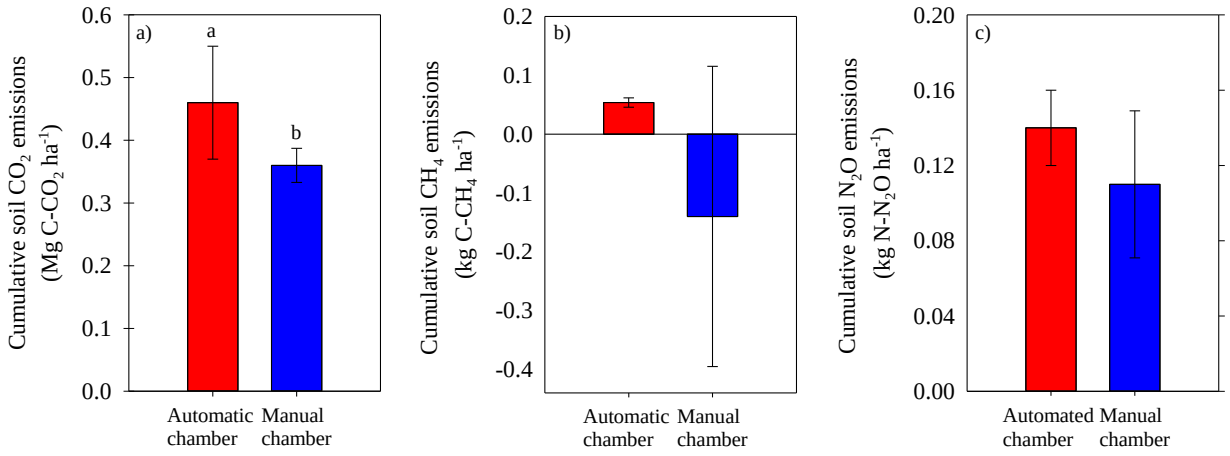

Figure 6. Comparison of soil cumulative soil gas emissions between the automated (blue bar) and the manual (red bar) chamber system for carbon dioxide (CO_2) (a), methane (CH_4) (b) and nitrous oxide(N_2O) (c). Error bars represent standard error. Different letters indicate significant differences at $p< 0.05$.

Differences in the different cumulative emissions found between measuring systems might have been explained by the next three points: (i) construction differences, (ii) the sampling time in the manual system, and (iii) the height/enclosure time ratio (Clough et al., 2020). The automated chamber presented higher area/perimeter ratios and air-mixing by fans which could contribute to the greater fluxes found in this system compared with the manual system. Regarding the sampling time, this was especially critical for CO_2 and CH_4. For both gases, manual sampling was performed at 06:00 GMT, resulting in an underestimation of the average daily emission (Pumpanen et al., 2003, Yu et al.,2013). In contrast, for N_2O, underestimation was lower since 06:00 GMT is considered a sampling time close to the optimal time for this gas (Wu et al., 2021). Finally, height/enclosure time ratio is also an important factor that affect the sensibility of the flux determination. As a recommendation, height/enclosure time ratio greater than 0.40 m $hour^{-1}$ is suggested to increase the minimum detectable flux and to reduce the impacts on air humidity, temperature and the gas diffusion process, variables that govern the soil gas fluxes between soil and atmosphere (Clough et al., 2020). In our study, the automated system resulted in height/enclosure ratios of 0.60 m $hour^{-1}$, while in the manual system the ratios dropped to 0.30 m $hour^{-1}$, explaining the lower cumulative emissions reported by the manual system.

4. Conclusion

The presented system features an open design, cost-effective components, and adaptable configuration, offering benefits in flexibility, compatibility, and affordability, which in the end resulted in a more precise monitoring of the time flux variability. Moreover, it has been highlighted that the shape, dimension, and configuration of the chamber system are critical factors that must be considered in the design of the chambers, being critical in setting area/perimeter and height/enclosure time ratios greater than 0.10m and 0.40m h^{-1}, respectively. Likewise, in case there is not option to implement an automated system, the sampling time of the manual measuring system is critical resulting in significant over or underestimation. Our results showed that 06:00GMT was an optimal sampling time for soil N_2O emissions but resulted in an underestimation of soil CO_2 and CH_4 emissions. Therefore, based on the results presented in this work, automated chamber systems are a powerful tool for quantifying GHG fluxes from the soil, allowing to capture the large temporal variability that characterizes them. Moreover, open configuration systems, such as the one presented in this study, are more suitable for use in agricultural systems, allowing the number of chambers to be easily modified to cover as much variability as possible.

Data availability

All raw data can be provided by the corresponding authors upon request.

Author contributions

SF-L: Conceptualization, formal analysis, software, data acquisition, writing—original draft, data curation, methodology, formal analysis, investigation. MA-A: Conceptualization, methodology, writing—review; editing. BW: Writing—review. BL: Conceptualization ,methodology, software, writing—review, funding acquisition. JA-F: Conceptualization ,methodology, writing—review; editing, supervision, project administration, resources, funding acquisition

Competing interests

The authors declare that they have no conflict of interest.

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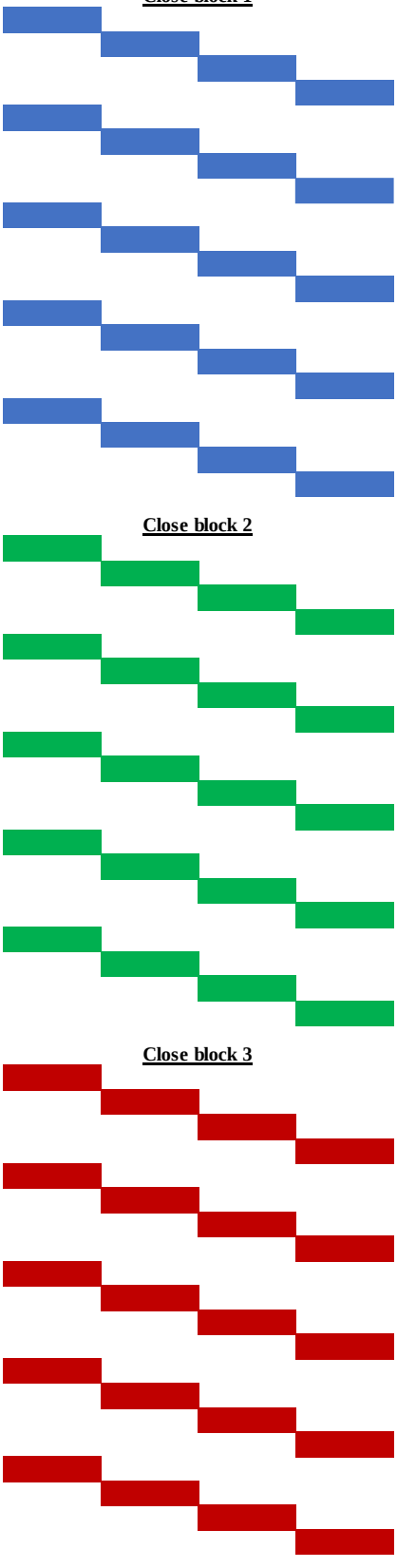
511 Supplementary Materials

Time valve on (s)	Time valve off (s)	Chamber		Gasera (s)
0	0		**Close block 1**	0
10	28	1		0
28	111	2		83
111	194	3		166
194	277	4		249
277	360	1		332
360	443	2		415
443	526	3		498
526	609	4		581
609	692	1		664
692	775	2		747
775	858	3		830
858	941	4		913
941	1024	1		996
1024	1107	2		1079
1107	1190	3		1162
1190	1273	4		1245
1273	1356	1		1328
1356	1439	2		1411
1439	1522	3		1494
1522	1605	4		1577
1800	1800		**Close block 2**	1800
1810	1828	5		1800
1828	1911	6		1883
1911	1994	7		1966
1994	2077	8		2049
2077	2160	5		2132
2160	2243	6		2215
2243	2326	7		2298
2326	2409	8		2381
2409	2492	5		2464
2492	2575	6		2547
2575	2658	7		2630
2658	2741	8		2713
2741	2824	5		2796
2824	2907	6		2879
2907	2990	7		2962
2990	3073	8		3045
3073	3156	5		3128
3156	3239	6		3211
3239	3322	7		3294
3322	3405	8		3377
3600	3600		**Close block 3**	3600
3610	3628	9		3600
3628	3711	10		3683
3711	3794	11		3766
3794	3877	12		3849
3877	3960	9		3932
3960	4043	10		4015
4043	4126	11		4098
4126	4209	12		4181
4209	4292	9		4264
4292	4375	10		4347
4375	4458	11		4430
4458	4541	12		4513
4541	4624	9		4596
4624	4707	10		4679
4707	4790	11		4762
4790	4873	12		4845
4873	4956	9		4928
4956	5039	10		5011
5039	5122	11		5094
5122	5205	12		5177



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513 Figure S1. Scheme of the sampling sequence