Talanta 81 (2010) 849–855

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A simple automated system for measuring soil respiration by gas chromatography

Claudio Mondini[a,*], Tania Sinicco[a], Maria Luz Cayuela[b], Miguel Angel Sanchez-Monedero[b]

[a] CRA - RPS Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo, Gruppo di Ricerca di Gorizia, Via Trieste 23, 34170 Gorizia, Italy
[b] CEBAS - CSIC Campus Universitario de Espinardo, Apartado de Correos 164, 30100, Espinardo, Murcia, Spain

ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form 9 January 2010
Accepted 17 January 2010
Available online 25 January 2010

Keywords:
Soil respiration
Greenhouse gases
Gas chromatography
Dynamic closed chamber
Soil microbial biomass

ABSTRACT

An automated dynamic closed chamber system for CO_2 sampling and analysis was developed for the measurement of soil respiration under laboratory conditions. The system is composed by a gas chromatograph linked to a fully computerised sampling system composed by 16 sample jars and 2 multiposition valves. Besides CO_2, the system can automatically and simultaneously measure CH_4, N_2O and other gases of environmental interest.

The detection limits of the system for CO_2, N_2O and CH_4 were 2, 1 and 4 ppmv, respectively. The accuracy of the system, expressed as percent bias, was −0.88, −0.94 and −3.17% for CO_2, N_2O and CH_4, respectively, with relative standard deviation of 0.42, 0.68 and 0.61%. Measurement of CO_2 evolved following acidification of a known amount of reagent grade $CaCO_3$ showed a standard recovery of 96.8 ± 2.5% reached within 30 s after acidification.

A linear response of CO_2 respiration was obtained for a wide range of operative conditions (5–60 min accumulation time, 10–80 g soil sample size, 10–60 mL min^{-1} air flow rate, 15–25 °C temperature of incubation) demonstrating the flexibility of the system, which allows for the measurement of soil samples characterised by different rates of gas evolution. Moreover, the results obtained with soil samples showed that within the above conditions the proposed system is not affected by potential limitations of static closed chamber systems such as CO_2 dissolution in the soil solution, reduced rate of CO_2 diffusion from soil to headspace and CO_2 inhibition of microbial activity. The system was also capable to detect significant changes in N_2O emissions from soil amended with different amounts of glutamic acid.

The automatic and frequent measurements provided by the system make possible an accurate description of the dynamics of gas evolution from soil samples under laboratory conditions.

1. Introduction

Soil respiration is among the most utilised parameters in soil ecology and biology to determine the level of the microbial activity. Since all heterotrophs need to degrade organic matter to satisfy their energetic requirements, the measurement of CO_2 evolution is considered a good indicator of overall biological activity of the soil. Due to the fundamental functions exerted by microorganisms in the soil ecosystem, microbial activity measurement is important to assess the level of soil quality (i.e. the capacity of a soil to regulate element and water cycles, support plant growth and degrade pollutants) and health (i.e. lack of degradation and contamination and overall strength for responding to environmental stresses). Soil respiration has also been used for measuring the size of soil microbial biomass as in the fumigation-incubation [1] and substrate induced respiration (SIR) [2] methods and to estimate maintenance requirements of microorganisms [3]. Other applications of soil respiration involve the evaluation of the impact of xenobiotics on soil functions and the effect of environmental conditions and soil management on the rate of soil organic matter mineralisation.

Measurement of soil respiration has gained further importance for the implications of atmospheric CO_2 on climate change. Soil respiration is the major pathway of C transfer from soil to atmosphere and, due to the size of C reservoir in the soil, a small variation in soil respiration rate may have profound impact on the atmospheric CO_2 budget. Atmospheric concentration of CO_2 has progressively increased due to anthropogenic activities [4] and in particular because of anthropogenic depletion of soil organic carbon (SOC) [5]. On the other hand, soil can offset climate change by incorporation of atmospheric C in SOM for a long period (C sequestration). Therefore, given the current concerns over global warming it is important to understand when soils serve either as a source or sink for atmospheric CO_2 [6]. Measurement of CO_2 evolution could

* Corresponding author. Tel.: +39 0481 522041; fax: +39 0481 520208.
E-mail address: claudio.mondini@entecra.it (C. Mondini).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.01.026

be useful to evaluate the effects of different soil management on CO_2 emission and to estimate C sequestration.

For many years the most widely utilised methodology to measure soil respiration in laboratory consisted in trapping the evolved CO_2 in a NaOH solution with subsequent titration [7,8], but this method is time consuming, not very accurate and does not allow for frequent measurement.

Continuous measurement of soil respiration can provide a better evaluation of variables affecting microbial activity and it is required for some methods for measuring the size of soil microbial biomass such as the SIR method. Several soil respiration systems have been developed that allow for automated and frequent analysis of sample.

The first set of systems with such characteristics was based on the variation in the conductivity of a hydroxide solution capturing the CO_2 evolved from soil [9–11]. However, conductimetric detectors are temperature sensitive and the solutions used require accurate thermostating [11]. Furthermore, conductimetric detectors tend to be non-linear over a wide concentration range [12], therefore limiting the versatility of these systems.

To date, the most widely utilised methods for automatic and frequent measurement of soil respiration are based on infrared gas analysis (IRGA) and gas chromatography due to the sensitivity and speed of the detectors that allow for a fast and accurate measure of soil CO_2 effluxes [7,13–17].

Both IRGA analyser and gas chromatograph (GC) can be linked to fully computerised sampling systems to frequently measure the respiration of soils incubated under laboratory conditions [14,17–21].

IRGA and GC methods are based on three main chamber techniques: open chamber, static closed chamber and dynamic closed chamber.

In open chamber systems, ambient air is continuously passed through the soil [14,17] or the chamber headspace [19] and soil CO_2 efflux is calculated using the difference in CO_2 concentration between air entering and leaving the chamber. Such systems had demonstrated to be effective, but they present the limitation that the accuracy of the measure strongly depends on atmospheric pressure, air humidity and the rate of air flux and consequently are sensible to changes in the laboratory environmental conditions. Therefore, these systems need a strict flow control and a continuous correction for changes in temperature and atmospheric pressure [22]. In addition, open chamber systems are usually expensive and complicate to operate, requiring specific skills and well trained staff.

Static closed chamber systems are usually less expensive and more simple to operate [18,21,23–25], but present a limitation related to CO_2 dissolution in the soil solution. In non-continuous flushing systems (as for example the system proposed by Brooks and Paul [18]) it has been demonstrated that CO_2 can accumulate in the soil aqueous phase if the sample is not aerated, especially in neutral and alkaline soil [10]. The respiration of the microbes increases the partial pressure of the CO_2 in the soil and, according to the aqueous carbonate equilibrium, increases the amount of CO_2 that dissolves in the soil solution. This accumulated CO_2 can be released during measurement and detected in addition with CO_2 produced by microorganisms giving an overestimation of CO_2 evolution.

Such limitation could be overcome by utilising dynamic closed chamber systems in which air is circulated in a closed loop between the chamber and the gas detector during measurement. Soil CO_2 efflux is then calculated using the difference in CO_2 concentration between the beginning and end of the measurement period. Dynamic closed chamber systems are commonly utilised for measuring soil respiration in the field and advantages and drawbacks of such system are well known [26–31], but, to our knowledge, they have not been utilised for the measurement of soil respiration in laboratory.

Apart from CO_2, the emissions of CH_4 and N_2O from soils and organic waste management have been demonstrated to significantly contribute to the overall anthropogenic emissions of greenhouse gases (GHG) [32]. Soil emissions of CH_4 and N_2O are relevant in terms of contribution to climate change because of their average global warming potentials 23 and 296 times larger than CO_2 [4]. Consequently, systems allowing for the simultaneous and automated measurement of different gases of environmental interest enhance their range of applicability and are increasingly required in researches dealing with GHG emissions.

Therefore, the aim of the present work was the evaluation of the reliability and functioning requirements of a dynamic closed chamber gas chromatographic system for the simultaneous, automated and frequent measurement of CO_2, N_2O and CH_4 emitted from soil under laboratory conditions.

2. Materials and methods

2.1. Materials

All reagents were of analytical reagent grade. Two mixtures of standard gases were provided by Praxair Rivoira (Milan, Italy). Mixture 1 contained 5000, 50 and 1000 ppmv of CO_2, N_2O and CH_4, respectively. Mixture 2 contained 500, 2 and 30 ppmv of CO_2, N_2O and CH_4, respectively. $CaCO_3$ was oven dried (105 °C, 24 h) before utilisation.

Three different bulk soil samples were selected to be used in the optimisation and validation experiments. The soils were sampled at 5–25 cm depth, sieved at 2 mm and stored at 4 °C until utilisation. Main properties of the soils are reported in Table 1.

2.2. Apparatus

The automated system for continuous gas sampling and analysis is basically composed by 16 sample jars connected to a micro-gas chromatograph through 2 multiposition valves. The set-up of the systems is outlined in Fig. 1.

Soil samples are enclosed in 16 polypropylene jars with screw cap (112 mm height, 40 mm outer diameter, 120 mL volume, Sarstedt, Nümbrecht, Germany) positioned in a thermostat operating in the 5–50 °C temperature range (M250-TBR, MBM Instruments, Bernareggio, Italy). Every jar is provided with an inlet and an outlet positioned at the bottom and top of the flask to allow for soil

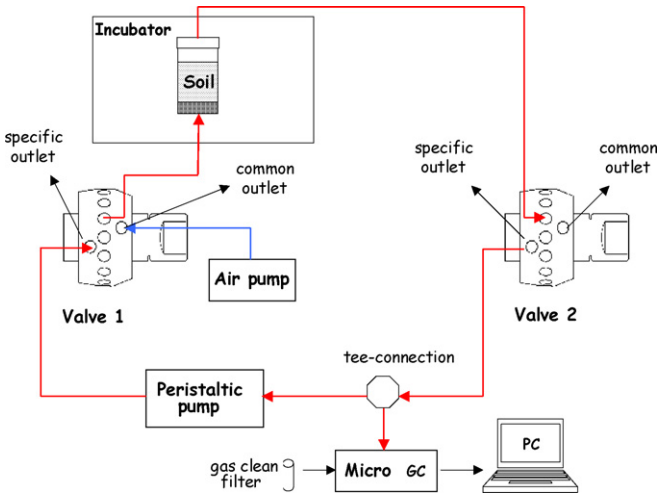

Fig. 1. Diagram of the automatic gas sampling and measurement system set-up.

Table 1
Main characteristics of the soils used for system optimisation and validation.

Soil	Management	Sand (%)	Silt (%)	Clay (%)	pH (H_2O)	$CaCO_3$ ($g\,kg^{-1}$)	N_{TOT} ($g\,kg^{-1}$)	C_{org} ($g\,kg^{-1}$)	B_C[a] ($\mu g\,g^{-1}$)
Gorizia	Grassland	37	48	15	8.0	46	2.4	25.4	795
Jumilla	Arable	52	21	17	7.8	415	1.0	10.4	119
Reana	Arable	55	28	17	5.0	–	1.2	8.7	118

[a] Microbial biomass C.

aeration and air sampling. A circular disk of foam rubber is placed on the bottom of each jar to prevent clogging of the inlet. The disk is moistened before the start of the analysis to avoid soil drying. A cellulose acetate syringe filter is positioned on the outlet to avoid contamination of the system with soil airborne particulates.

The outlet and inlet of sample jars are connected to the ports of two multiposition valves (EMTCSC16MWE, Vici Valco Instruments, Houston, TX, USA) by polypropylene fittings and PTFE tubing (4 m length, 1.0584 mm/1.5875 mm ID/OD) (Fig. 1). The valves are automated with electric actuators and managed by a specific software (Valve Control 32, Varian, Palo Alto, CA, USA). Each valve holds 16 ports connected with the sample jars, a specific outlet and a common outlet (Fig. 2). The valve is configured so that the selected stream flows through the specific outlet, while the non-selected streams flow to the common outlet (Fig. 2).

The common outlet of valve 1 is connected by PTFE tubing (1.0584 mm/1.5875 mm ID/OD) with a membrane air pump (Optimal 250, Schego, Offenbach am Main, Germany), while the common outlet of valve 2 is set free.

The specific outlet of the two valves are connected by PTFE tubing (1.8 m length, 1.0584 mm/1.5875 mm ID/OD) passing through the pump head of a peristaltic pump (Fig. 1). The peristaltic pump was in-house constructed by assembling a pump head (Watson Marlow, Wilmington, MA, USA) and a brushless motor (Croeuzet, Valence, France).

A micro-GC (CP-2003P, Varian, Palo Alto, CA, USA), especially designed for continuous gas analysis, is used to measure gas concentrations. The micro-GC is equipped with two capillary columns, PoraPlot Q (fused silica, 10 m length, 0.25 mm ID, 8 μm Df) and Molsieve (fused silica, 10 m length, 0.32 mm ID, 30 μm Df), in which head pressure and temperature can be electronically programmed. The micro-GC can simultaneously measure CO_2, CH_4 and N_2O by means of a TCD detector. The concentration operating range is from 1 ppmv to 100%, with a linear dynamic range of 10^6. The precision and reproducibility of the instrument satisfy the ISO 6976 Standard [33]. The micro-GC is managed by a specific software (Star 5.5 for Windows, Varian, Palo Alto, CA, USA) operating in synchronicity with the multiposition valves software.

2.3. Procedure

During system operation, both valves are set to select the same stream. In this way, the corresponding sample jar is made a "dynamic closed chamber" in which the air is re-circulated for a specific accumulation time (usually in the range 10–60 min) by means of the peristaltic pump. At the beginning and at the end of the accumulation period, the gas contained in the sample jar is automatically sampled through a diversion on the interconnecting tubing (Fig. 1) and measured by means of the micro-GC. The difference between the final and initial content provides the rate of gas production for the selected time interval. When the analysis of a sample is completed, the system automatically switches to the following sample jar and this cycle is run continuously. The frequency of sample analyses mostly depends on the number of samples and the accumulation period. With 16 samples and an accumulation time of 10 min, adequate for most of the applications, the sampling frequency is every 4 h.

The non-selected sample jars are continuously aerated with ambient air by means of the membrane pump connected with the common outlet of valve 1 (Fig. 1). The incoming air in valve 1 is split into the 15 non-selected streams, flows through the soil samples and vents to the outside through the common outlet of valve 2. Both peristaltic and membrane pumps are adjusted, utilising a soap bubble flow meter, to have similar fluxes through the sample in both flow configuration (accumulation and stand by period). The usual setting for standard analysis is 20 mL min^{-1}.

Usual operative conditions of micro-GC are: 30 s sampling time, 30 ms injection time, 120 s running time, 40 and 60 °C column temperature for PoraPlot Q and Molsieve, respectively, 30 °C injector temperature.

The chromatograph is calibrated by injecting a mixture of pure standard gases of CO_2, N_2O and CH_4 at a concentration of 5000, 50 and 1000 ppmv, respectively. A new recalibration is performed weekly. Observed drift during 1 week was usually lower than 0.5%.

Gas fluxes are calculated from the rate of change in gas concentration during the measurement period, the molecular weight of

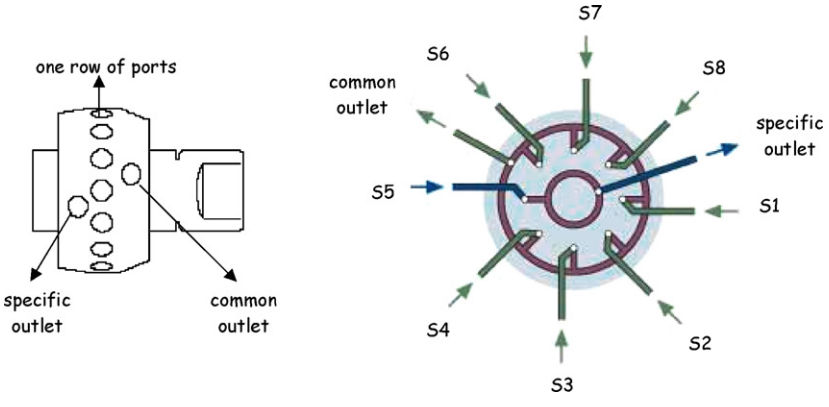

Fig. 2. Configuration of the multiposition valve (Vici Valco Instruments). Each valve holds several ports connected with the sample jars (8 in this example denoted by the letter S), a specific outlet and a common outlet. The valve is configured so that the selected stream flows through the specific outlet, while the non-selected streams flow to the common outlet.

the gas, the dynamic chamber volume and the weight of the soil sample as follows:

$$F(\mu g\, g^{-1} \text{soil}\, min^{-1}) = \frac{\Delta C \times M \times P \times V}{R \times T \times W}$$

where ΔC is the change in gas concentration (ppmv) in time interval Δt (min), M is the molecular weight of the gas, P is the atmospheric pressure (atm), V is the volume of the enclosed chamber (L), R is the ideal gas constant (0.082054 atm L mol^{-1} $°K^{-1}$), T is the ambient temperature (°K) and W is the weight of the soil sample (g).

The volume of the chamber (including all the interconnecting tubing and valves dead end) without soil sample is 135.4 mL. The actual volume is calculated by subtracting from the volume of the chamber the volume occupied by the soil. The latter is calculated taking into account soil weight, density and water content.

Cumulative gas production ($\mu g\, g^{-1}$ soil) is calculated from single measurements by integrating the area under the curve of gas efflux versus time.

2.4. System performance

In this work a set of experiments were carried out to evaluate the detection and quantification limits, accuracy, precision and reproducibility of the system with both the utilisation of standard gas mixture and reagent grade $CaCO_3$.

Since the measurement of the gas concentration in the proposed system is based on the difference between the final and the initial gas concentration during the accumulation time, the system detection limit can be defined as the minimum increase in gas concentration in the sample jar that can be measured with a defined confidence limit. Accordingly to this definition, the system detection and quantification limits for CO_2, N_2O and CH_4 were calculated by injecting a known volume of gaseous standard mixture (500, 2 and 30 ppmv of CO_2, N_2O and CH_4, respectively) in 4 samples jars and performing 10 consecutive measurement cycles. The standard gas concentrations utilised were close to the estimated system detection limit, with the exception of CO_2, which concentration was similar to that in ambient air.

The difference of the gas concentration between the end and the beginning of the accumulation time (10 min) of each measurement cycle was considered as a blank value. The limits of detection and quantification of the system for the 3 gases were calculated by multiplying the standard deviation of the blank mean (σ) for 3.29 and 10 times, respectively [34]. In the case of a normal distribution, 99.95% of the measurements lie within $\pm 3.29\sigma$ away from the mean. Therefore, for a mean blank of 0, the probability that the analyte is present if the measured value is larger than 3.29σ is 99.95%. The value of 10σ for the quantification limit represents a IUPAC default value chosen to be approximately 3 times the detection limit.

The accuracy and precision of the system was tested by injecting in an empty sample flask a known volume of gas standard mixture containing CO_2, N_2O and CH_4 at a concentration of 5000, 50 and 1000 ppmv, respectively, through a three way valve positioned in the inlet of the sample jar. The sample jar was flushed by the peristaltic pump and ten consecutive analysis of the sample jar content were performed. The inter-run reproducibility was checked by performing an identical trial a few days later.

A further experiment was performed to test the ability of the system to quantitatively measure the CO_2 evolved by $CaCO_3$ following acidification. An exactly weighted amount of $CaCO_3$ was transferred on the bottom of a sample jar along with a vial containing 2 mL of 5 M HCl. After connecting the sample jar to the system, the HCl was mixed with $CaCO_3$ by tilting and rotating the jar. The evolved CO_2 was determined at different times after the acidification up to stabilisation of the measured value. The measurement was performed in quadruplicate for each sampling time.

2.5. System optimisation and validation

A second set of experiments was designed to validate and optimise the system for the measurement of CO_2 and N_2O fluxes at normal operative conditions with soil samples and to show some example of the system application.

2.5.1. Accumulation time

A sample of Gorizia moist soil (50 g on oven dried basis) was pre-incubated for 7 days at 25 °C and 40% water holding capacity (WHC) and subsequently analysed for CO_2 evolution at 25 °C using 5, 10, 20, 30 and 60 min of accumulation time. For any accumulation period, the measurement cycle was repeated 15 times on three sample replicates.

2.5.2. Soil sample weight

Reana moist soil was pre-incubated for 1 week at 25 °C and 40% WHC and then analysed for CO_2 evolution for 70 h (20 times) at 25 °C utilising the following amounts of soil (oven dried basis) in triplicate: 10, 25, 50, 65 and 80 g.

2.5.3. Air flow rate

Samples of Gorizia, Reana and Jumilla moist soils (40% WHC, 50 g on oven dried basis) were pre-incubated at 25 °C for 7 days and afterwards analysed for CO_2 evolution for 65 h at 25 °C utilising a flow rate of 10, 20, 40 and 60 mL min^{-1}. A soap bubble flow meter was used to set up the air flow rate. Four subsequent cycles of analyses were necessary as is not possible to simultaneously set different flow rates for different sample streams. For each flow rate, five replicates of each soil were analysed 18 times for CO_2 emission.

2.5.4. Temperature of incubation

Triplicate samples of Reana moist soil (40% WHC – 50 g on oven dried basis) were separately pre-incubated for 1 week at 15, 20 and 25 °C and then analysed for CO_2 evolution for 2 weeks at the temperature of conditioning.

2.5.5. Addition of glutamic acid

Reana moist soil (40% WHC – 80 g on oven dried basis) was pre-incubated for 1 week at 25 °C and then amended in triplicate with glutamic acid at the following rates: 1.05, 2.1, 4.2 and 8.4 mg g^{-1} soil. The rates were calculated to reproduce the soil addition of a N-rich organic waste at the rate of 4, 8, 16 and 24 ton ha^{-1}, respectively. The soil samples were then incubated at 25 °C and analysed for gas emissions for 48 h utilising an accumulation period of 20 min.

2.6. Statistics

The relationships between variables were analysed using the Pearson's correlation coefficient calculated with SPSS version 9.0 statistical package.

3. Results and discussion

3.1. System performance

The detection limit of the system for CO_2, N_2O and CH_4, with a confidence level of 99.95%, were 2, 1 and 4 ppmv, respectively, with corresponding quantification limits of 5, 2 and 11 ppmv. This indicates that the system is suitable to measure the basal respiration of soils with low microbial activity such as the Jumilla and Reana

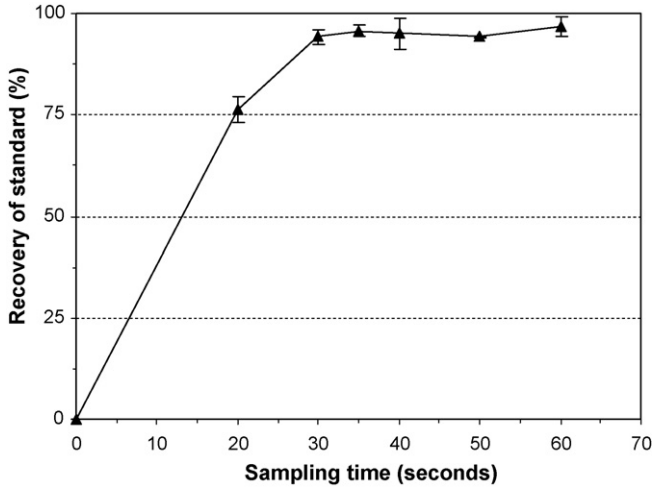

Fig. 3. Percentage of recovery of CO_2 evolved from reagent grade $CaCO_3$ following acidification with 5 M HCl. Bars represent standard deviation ($n = 4$).

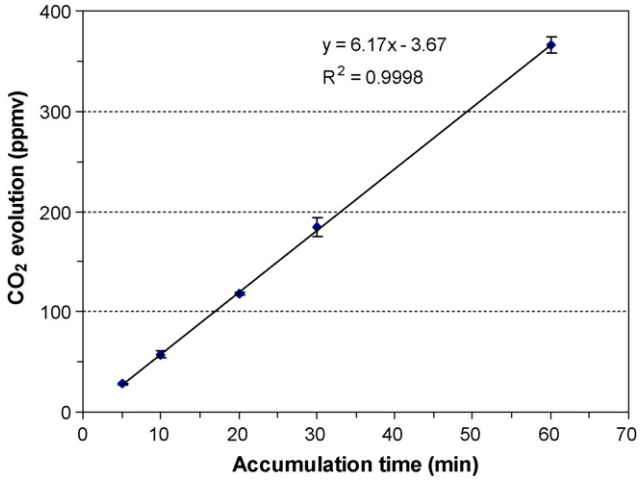

Fig. 4. CO_2 evolution (ppmv) from Gorizia soil utilising different accumulation times. Bars represent standard deviation ($n = 3$).

soil utilised in this work that present a low soil microbial biomass content at around 100 μg microbial biomass C per g of soil.

Results of the test performed with a mixture of calibration standard gases demonstrated the ability of the system to correctly measure the gas content in the sampling jar. The accuracy, determined from the reference values and expressed as percent bias was −0.88, −0.94, −3.17% for CO_2, N_2O and CH_4, respectively. The precision, expressed as percent relative standard deviation (%RSD), was 0.42, 0.68 and 0.61% for CO_2, N_2O and CH_4, respectively.

A similar test performed few days later showed percent bias of −0.61, 0.05 and 2.62% for CO_2, N_2O and CH_4, respectively, with a corresponding %RSD of 0.05, 0.32 and 2.17%.

Reproducibility, expressed as inter-run percent relative standard deviations, was 0.19, 0.71 and 4.11% for CO_2, N_2O and CH_4, respectively.

Results of test performed with standard grade $CaCO_3$ showed that a stable and almost complete recovery of the standard was achieved 30 s after the acidification of $CaCO_3$ (Fig. 3). Measurements performed 1 min after the acidification showed a percent bias of 3.20% and a %RSD of 2.55% with a flow rate of 20 mL min^{-1}. These results showed the capacity of the system to quantitatively recover the CO_2 generated in the sample jar following acidification of the solid reagent. In fact, the standard recovery of the proposed system (96.8 ± 2.5%) was significantly higher than 88.3% recorded by the system described by Martens [10]. In addition, the results of the test with $CaCO_3$ showed the good and fast (less than 30 s) homogenisation of the CO_2 concentration in the whole sampling system, which includes the sampling jar, the valves dead end and the connecting tubes volume.

3.2. System optimisation and validation

Results obtained utilising different accumulation times in the range 5–60 min showed a direct relationship with CO_2–C evolution ($r = 0.999$; $P < 0.001$) (Fig. 4). Therefore, already 5 min of accumulation time is sufficient to give reliable results of basal respiration (CO_2–C evolution: 24.2 ± 1.0 ppmv) analysing 50 g of a soil with a good level of biological activity.

A linear relationship was observed between CO_2 evolution and amount of soil sample. As shown in Fig. 5, CO_2–C evolution was significantly correlated to the amount of soil sample ($r = 0.997$; $P < 0.001$) in the range 10–80 g. Results showed that 10 g (oven dry basis) of a soil characterised by a low microbial biomass content (118 μg microbial biomass C g^{-1} soil) are sufficient to give reliable results with an accumulation period of 10 min.

Results of analysis performed with different accumulation times and sample weight showed that results of CO_2 evolution are not affected by these two parameters, at least for the range of values utilised in the present work. The possibility to utilise different accumulation times increases the flexibility of the system: higher accumulation time allows to accommodate soil samples with low biological activity or low size. On the other hand, lower accumulation time increases the sample frequency resulting in a more detailed description of the respiration dynamics. The independence of CO_2 evolution from the sample size, is another factor that increases the flexibility of the system.

Results of analysis performed with different air flow rates in 3 soils characterised by very distinct values of basal respiration showed a linear relationship between CO_2 evolution and air flow rate (Fig. 6). The relationships were highly significant, even if the slope of the correlation line was different for the 3 soils.

The increase in CO_2 production with increasing air flow rate could be explained as the response of microbial pool to the changed environmental conditions. In fact, it is well known that aeration status affects microbial activity and can either increase or decrease the rate of decomposition of organic matter in soil [35,36]. These

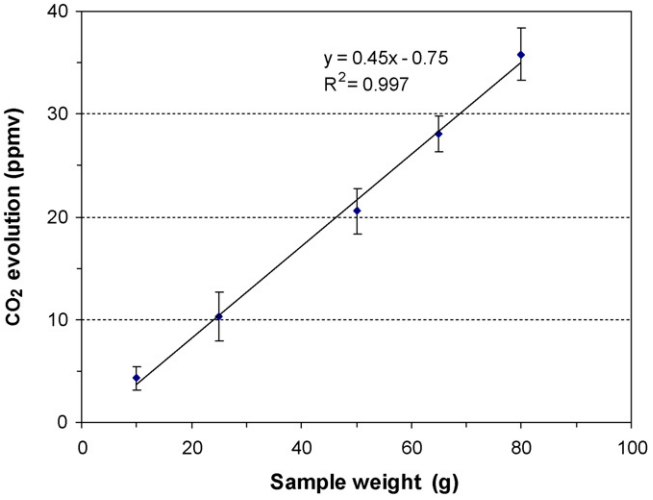

Fig. 5. CO_2 evolution (ppmv) from Reana soil utilising different sample weights. Bars represent standard deviation ($n = 3$).

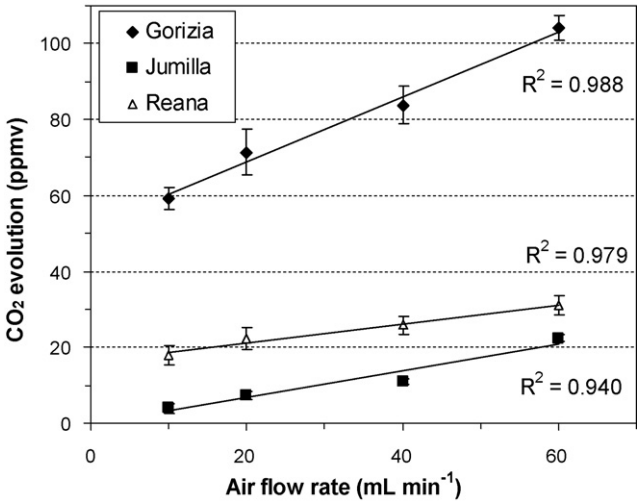

Fig. 6. CO_2 evolution (ppmv) from 3 different soils utilising different air flow rates. Bars represent standard deviation ($n = 5$).

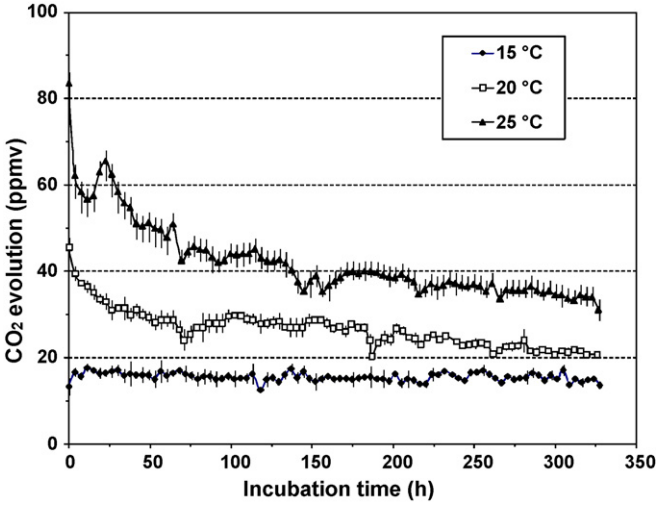

Fig. 7. CO_2 evolution (ppmv) from Reana soil utilising different incubation temperatures. Bars represent standard deviation ($n = 3$).

results indicate the need to utilise the same air flow rate (usually 20 mL min^{-1}) for comparison of microbial respiration among different experiments. In particular, a low flow rate, still ensuring reliable results, would be recommended since it minimises environmental disturbance and drying of the soil sample.

The measurements of soil CO_2 fluxes by closed chamber systems (as the proposed system) may be limited by CO_2 dissolution in the soil solution [10]. This problem is overcome in our system by the continuous flushing of the soil samples, both during stand by and accumulation time, that subtracts the CO_2 from dissolution in the aqueous phase. In the experiment with Gorizia soil, characterised by an alkaline pH (8.0), varying the accumulation time caused an increase in CO_2 concentration in the chamber that could have favoured the dissolution of CO_2 in the soil solution. Nevertheless, the relationship of the respired CO_2 with accumulation time was linear over a range of 350 ppmv and this would have not occurred if significant amount of CO_2 had been dissolved in the soil solution. In addition, during the stand by period the samples are continuously aerated with ambient air instead of CO_2 free air, as in the Wosthoff apparatus [7], decreasing the time needed to reach equilibrium between CO_2 in soil solution and in the soil atmosphere.

The linear relationship between sample size and CO_2 emission indicates that the increase of CO_2 concentration in the headspace did not affect CO_2 diffusion from soil to the headspace [27]. This is in agreement with several works demonstrating that the rate of CO_2 diffusion is affected only at high concentrations of CO_2 in the headspace of the chamber. Keith and Wong [31] showed that increasing the CO_2 concentration in the headspace of a chamber to 1000 ppmv has a relatively small effect on the concentration gradient, and hence on the transient rate of CO_2 efflux. Similarly, Bekku et al. [37] found that the critical CO_2 concentrations affecting the rate of CO_2 diffusion ranged from 1000 to 1500 ppmv in the chamber. Therefore, the flow rate commonly used in the proposed system (20 mL min^{-1}) is adequate to prevent problems related to CO_2 diffusion. Such value of air flow rate is in agreement with those utilised in other soil respiration systems. In the apparatus proposed by Martens [10], a flush rate of 15 mL min^{-1} was found to be sufficient for the quantitative measurement of CO_2 with a sample size of 100 g. Similarly, van Afferden et al. [22] found that an air flow between 10 and 30 mL min^{-1} was adequate to quantitatively determine CO_2 evolution of biologically active soil samples in the range 50–100 g.

A further problem in closed chamber systems is that the increase in CO_2 concentration may alter the rate of CO_2 emission by inhibition of the respiratory activity of microorganisms [38]. Such problem is overcome in the alkali method by entrapment of evolved CO_2 in the alkali solution, that avoids the increase in CO_2 concentration in the chamber. On the contrary, in the proposed system, the CO_2 concentration in the sample jar is allowed to increase during the accumulation period (usually 10 min). Nonetheless, increasing the air flow rate caused a linear increase in microbial respiration (Fig. 6) [35,36], suggesting that the increase in CO_2 concentration did not significantly affect the rate of microbial activity. Conversely, a deviation of experimental data points from the straight line would have been observed. As a matter of fact, the limiting effect of increased CO_2 content on the activity of microorganisms has been found at high concentrations of CO_2 (around 2% and above) [39].

In addition, the above underlined potential drawbacks of closed chambers systems could be made negligible in the proposed system by optimising the operative conditions (i.e. amount of soil sample, accumulation time and air flow rate) in order to minimise the CO_2 accumulated in the jar headspace.

As a whole we consider that the linear response between CO_2 respiration and the operative parameters we have examined demonstrates that, within the range of operative conditions utilised in the present work, the proposed system is not affected by the main problems biasing CO_2 measurement in closed chambers.

Fig. 7 reports the dynamics of basal respiration of Reana soil incubated at three distinct temperatures. As expected, the increase in temperature caused a significant enhancement in the basal respiration. Cumulative respiration at the end of the experiment was 17.7 (±0.2), 29.2 (±0.2) and 44.8 (±0.4) μg CO_2–C g^{-1} soil for soil incubated at 15, 20 and 25 °C, respectively. Cumulative respiration and temperature were significantly correlated ($r = 0.997$; $P < 0.01$). Results of basal respiration measured at different temperatures showed that the system is capable to detect significant changes in the dynamics and amount of CO_2 emission induced by the different incubation conditions.

Dynamics of N_2O emission in soil samples amended with glutamic acid (Fig. 8) was characterised by a sharp peak of gas emission occurring within 10–30 h after glutamic addition, in correspondence with the substrate induced microbial growth, as indicated by dynamics of CO_2 emissions (data not shown). The moment in time and the shape of the peaks of maximum emission significantly varied with the different rates of glutamic acid. Cumulative N_2O emissions were 0.38 ± 0.06, 3.47 ± 0.25, 4.26 ± 0.14 and

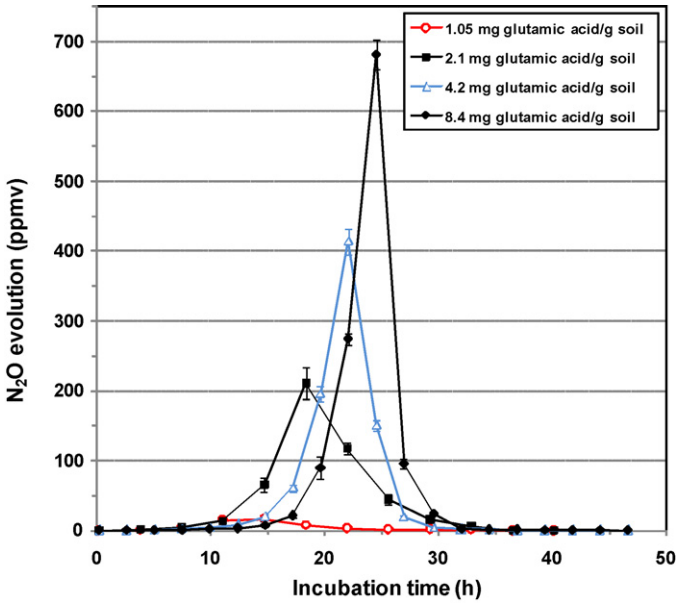

Fig. 8. N_2O evolution (ppmv) from Reana soil amended with different rates of glutamic acid. Bars represent standard deviation ($n = 3$).

$5.82 \pm 0.17\ \mu g\ N_2O\text{-}N\ g^{-1}$ for soil amended with 1.05, 2.1, 4.2 and 4.8 mg of glutamic acid for g of soil, respectively. The automated and frequent N_2O measurement allowed to detect significant changes in the dynamics and cumulative emission of the gas, suggesting the reliability of the system for N_2O measurements for specific researches, like for instance the study of N_2O emissions following soil amendment with compost and N-rich organic wastes. Furthermore, the performance of the system can be enhanced by increasing the content of N_2O in the headspace by increasing the size of the sample and/or the accumulation time.

4. Conclusions

The results presented in the present work demonstrate the reliability of the proposed system to measure the respiratory response and GHG emission of soil samples under laboratory conditions. The system is automated and can perform frequent analysis of the sample allowing for an accurate description of the respiration dynamics. It can be utilised in a range of sample size, air flow rate and accumulation time. The flexibility of the system makes possible to create the appropriate condition for the analyses of samples with different requirements. Furthermore, it presents the possibility to measure gas evolution in a wide range of temperature.

Compared with non-continuous flushing systems it avoids problems related to the dissolution of CO_2 in soil solution and variation in the rate of gas diffusion from soil to headspace. As the gas evolution is determined by the difference between the initial and final gas concentration during the accumulation time, the system is less sensible of systems based on open chamber technique to variations in air flow rate, temperature and atmospheric pressure.

Finally, it allows for the simultaneous measurement of other gases of environmental interest.

Acknowledgments

We are grateful to Dr. Alessandro Peressotti (University of Udine) for theoretical background and Mr. Riul Ennio and Riul Luca for technical assistance in the operation of the automated system.

This research was partially financed by a grant from the Spanish Ministry of Science and Innovation (Ref. CTM2005-05324).

M.L. Cayuela thanks Fundación Séneca (Agencia Regional de Ciencia y Tecnología de la región de Murcia) for financing her post-doctoral grant (Ref. 02534/PD/05) at CRA-RPS (Italy).

References

[1] D.S. Jenkinson, D.S. Powlson, Soil Biol. Biochem. 8 (1976) 209–213.
[2] J.P.E. Anderson, K.H. Domsch, Soil Biol. Biochem. 10 (1978) 215–221.
[3] T.H. Anderson, K.H. Domsch, Soil Biol. Biochem. 17 (1985) 197–203.
[4] IPCC, in: J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell, C.A. Johnson (Eds.), Climate Change 2001: The Scientific Basis, Cambridge University Press, Cambridge, 2001, pp. 1–83.
[5] R. Lal, Prog. Environ. Sci. 1 (1999) 307–326.
[6] W.N. Smith, R.L. Desjardins, E. Pattey, Glob. Change Biol. 6 (2000) 557–568.
[7] K. Alef, in: K. Alef, P. Nannipieri (Eds.), Methods in Soil Microbiology and Biochemistry, Academic Press, London, 2005, pp. 214–219.
[8] W. Cheng, D.C. Coleman, Soil Biol. Biochem. 21 (1989) 385–388.
[9] J.M. Anderson, P. Ineson, Soil Biol. Biochem. 141 (1982) 415–416.
[10] R. Martens, Soil Biol. Biochem. 19 (1987) 77–81.
[11] A. Nordgren, Soil Biol. Biochem. 20 (1988) 955–957.
[12] S. Satienperakul, T.J. Cardwell, R.W. Cattrall, I.D. McKelvie, D.M. Taylor, S.D. Kolev, Talanta 62 (2004) 631–636.
[13] E. de Jong, H.J.V. Schappert, Soil Sci. 153 (1972) 328–333.
[14] K. van Cleve, P.I. Coyne, E. Goodwin, C. Johnson, M. Kelley, Soil Biol. Biochem. 11 (1979) 237–246.
[15] K. Ljungholm, B. Norén, G. Odham, Oikos 34 (1980) 98–102.
[16] G.P. Sparling, B.G. Ord, D. Vaughan, Soil Biol. Biochem. 13 (1981) 455–460.
[17] O. Heinemeyer, H. Insam, E.A. Kaiser, G. Walenzik, Plant Soil 116 (1989) 191–195.
[18] P.D. Brooks, E.A. Paul, Plant Soil 101 (1987) 183–187.
[19] R.E. Hantschel, H. Flessa, F. Beese, Soil Sci. Soc. Am. J. 58 (1994) 401–404.
[20] N. Loftfield, H. Flessa, J. Augustin, F. Beese, J. Environ. Qual. 26 (1997) 560–564.
[21] R.L. Haney, W.F. Brinton, E. Evans, Renew. Agric. Food Syst. 23 (2008) 171–176.
[22] M. van Afferden, A.M. Hansen, C. Kaiser, N. Chapelain, Int. J. Environ. Pollut. 26 (2006) 220–233.
[23] F. Gardini, L. Vittori Antisari, M.E. Guerzoni, P. Sequi, Biol. Fertil. Soils 12 (1991) 1–4.
[24] B. Heilmann, F. Beese, Soil Sci. Soc. Am. J. 56 (1992) 596–598.
[25] F.C. Hoyle, D.V. Murphy, P.C. Brookes, Biol. Fertil. Soils 44 (2008) 571–579.
[26] S.M. Nay, K.G. Mattson, B.T. Bormann, Ecology 75 (1994) 2460–2463.
[27] Y. Bekku, H. Koizumi, T. Oikawa, H. Iwaki, Appl. Soil Ecol. 5 (1997) 247–254.
[28] N.T. Edwards, J.S. Riggs, Soil Sci. Soc. Am. J. 67 (2003) 1271–1366.
[29] J. Pumpanen, P. Kolari, H. Ilvesniemi, K. Minkkinen, T. Vesala, S. Niinistö, A. Lohila, T. Larmola, M. Morero, M. Pihlatie, I. Janssens, J. Curiel Yuste, J.M. Grünzweig, S. Reth, J.-A. Subke, K. Savage, W. Kutsch, G. Østreng, W. Ziegler, P. Anthoni, A. Lindroth, P. Hari, Agric. Forest Meteorol. 123 (2004) 159–176.
[30] J.R. Butnor, K.H. Johnsen, C.A. Maier, Biogeochemistry 73 (2005) 283–301.
[31] H. Keith, S.C. Wong, Soil Biol. Biochem. 38 (2006) 1121–1131.
[32] A. Mosier, D. Schimel, D. Valentine, K. Bronson, W. Parton, Nature 350 (1991) 330–332.
[33] Y.I. Yashin, A.Y. Yashin, J. Anal. Chem. 56 (2001) 794–805.
[34] IUPAC, Pure Appl. Chem. 67 (1995) 1699–1723.
[35] T. Picek, M. Simek, H. Santruckova, Biol. Fertil. Soils 31 (2000) 315–322.
[36] H. Santruckova, T. Picek, R. Tykva, M. Simek, B. Pavlu, Biol. Fertil. Soils 40 (2004) 386–392.
[37] Y. Bekku, H. Koizumi, T. Nakadai, H. Iwaki, Biol. Res. 10 (1995) 369–373.
[38] H. Koizumi, T. Nakadai, Y. Usami, M. Satoh, M. Shiyomi, T. Oikawa, Ecol. Res. 6 (1991) 227–232.
[39] G.P. Sparling, Soil Biol. Biochem. 19 (1981) 93–98.