A new CO_2 analyzer for measuring respiration rates in organic material

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In this article, we present a new CO_2 analyzer which can be used for monitoring respiration rates in organic material. To demonstrate the potentiality of the analyzer, CO_2 evolution of soil samples collected from three different edaphic environments were measured. The results obtained with this compact CO_2 analyzer were compared with those carried out simultaneously using the chemical method, and the correlation between them has been established. © *1996 American Institute of Physics.* [S0034-6748(96)02709-8]

I. INTRODUCTION

Many ecological studies involve estimates of the biological activity in mineral soil and forest ground.[1–3] These studies are usually carried out by the measurement of the O_2 uptake or CO_2 evolution rates from the soil. These measurements provide valuable information regarding the kinetics of the organic matter mineralitation with respect to the several temperature and moisture regimes,[4] as well as the evaluation of the response of the microorganisms living in the soil to the addition of organic compounds.[5]

The usefulness and advantages of the wide variety of experimental methods for measuring the respiration activity of biological materials has been documented in several publications.[6] The most commonly used methods[6,7] include the NaOH absorption of CO_2 with acid tritation (chemical method), the Warburg respirometer, infrared gas analysis, and gas chromatography. The adequacy and usefulness of these methods for each particular application is essentially dictated by its sensibility, costs and simple handling capability.

In this article, we present a new, compact, CO_2 analyzer, which can be successfully used for assessing the respiration of organic materials, as indicated by the CO_2 evolution. The potentialities of this analyzer are demonstrated by monitoring the CO_2 time evolution of soil samples collected from three different edaphic environments. These three edaphic environments belonging to a semiarid mexican region corresponds to a cultivated land, and the areas just below, and outside, the crown area of native trees. The interest in these particular environments relies on the wide spread concept of regarding the native trees of semiarid regions as ''fertility islands.''[8–11] In order to compare the results obtained with this compact CO_2 analyzer, parallel monitoring of the carbon dioxide evolution by means of the chemical method was performed, and the correlation between them has been established.

II. EXPERIMENT

A. CO_2 analyzer

In Fig. 1, we present the schematic view of the CO_2 analyzer. It consists basically of three screwable units, namely, the infrared source housing (**a**), the closed gas chamber unit (**c** and **d**), and the detection unit (**e** and **f**). The outside dimensions of this CO_2 analyzer are 155.0 mm long and 35.0 mm diam.

The infrared source consists of a cylindrical heating element (**g**) axially inserted into a Cr-coated conical brass reflector (**h**). The heating element used is a 2.8 mm diam, 13.0 mm long, ceramic coated Pt resistance (100 ohms). The electric power supplied to the heating element (28 V, 0.11 A) is such that it works at a temperature of 407 Celsius. At this temperature, the black-body irradiance peaks at 4.26 microns, which corresponds to the characteristic absorption band for the CO_2 molecule. The emerging infrared radiation can be modulated by means of a mechanical chopper (**b**) positioned just in front of the gas cell unit. This gas cell unit consist of two cylindrical chambers (25.0 mm long, 10.0 mm diam) (**c** and **d**) which are sealed to the outside environment by means of 12.0 mm diam sapphire windows. Both, the active and the reference chambers have feedthroughs on their bodies to allow for gas exchange with the sample compartment and purge as well. Behind each gas chamber exit window, it is positioned a home-made PVDF pyroelectric detector with a 4.26 micron interference optical filter cemented on its entrance window. These infrared detectors sense the radiation outputs from the active and reference gas chamber at the CO_2 peak absorption band. The operational principle of this gas analyzer is then clear. Any increase in the amount of CO_2 along the optical path of the infrared radiation in the active chamber will decrease the intensity of the output ra-

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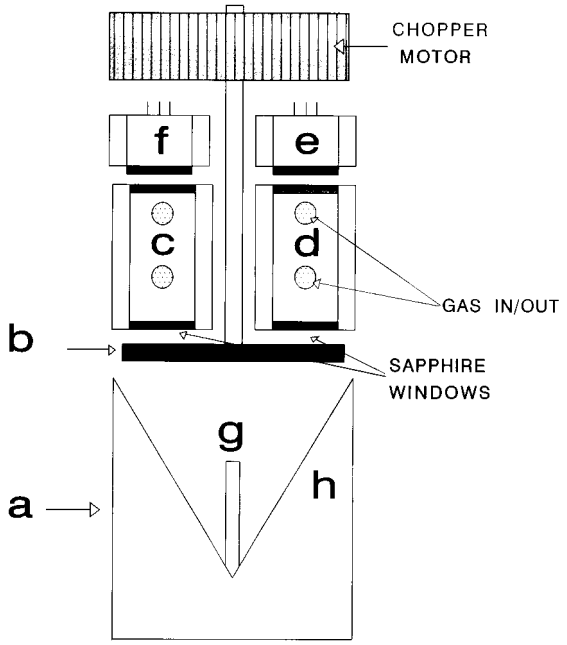

FIG. 1. Schematic design of the CO_2 analyzer. The main components of the detection unit are: The infrared source housing (**a**), the closed gas chamber unit (**c** and **d**), and the detection unit (**e** and **f**). The cylindrical heating element and the conical brass reflector of the infrared source are denoted by **g** and **h**, respectively. The shaded regions delimiting the chambers represent sapphire windows and the shaded circles denote feedthroughs for allowing gas exchange with the compartment and purging.

diation leaving this chamber. In other words, denoting by P_0 the infrared power entering the active cell, at the CO_2 peak absorption wavelength, the corresponding output power leaving the exiting windows P, sensed by the pyroelectric sensor, can be written as

$$P = P_0 \exp(-C\sigma l), \tag{1}$$

where C is the CO_2 concentration in the cell, σ is the CO_2 extinction coefficient at 4.26 microns, and l is the cell length.

The block diagram of our experimental arrangement is shown in Fig. 2. The active chamber is connected to the sample container used to collect the CO_2 evolved from the soil material. The reference chamber, which is not exposed to the evolved CO_2, has a double function. One, is the monitoring of the source intensity fluctuations, if any. The other role played by the reference compartment is to provide a reference signal to the lock-in amplifier used in the detections of the active chamber signal.

B. Soil material

The soil used as testing medium was collected in August 1995 at a ranch located in a semiarid region, which has a forest tree vegetation type dominated by mesquite (*Prospis laevigata*). This region is characterized by a dry-temperate climate with an annual mean temperature of about 17 °C and average rainfall of 400–500 mm per year. The wet season goes from June until August. The dominant soil in this region is of the pheozem type up to 150–400 mm depth, having the following average composition: 60% sand, 20% loam, and 20% clay.

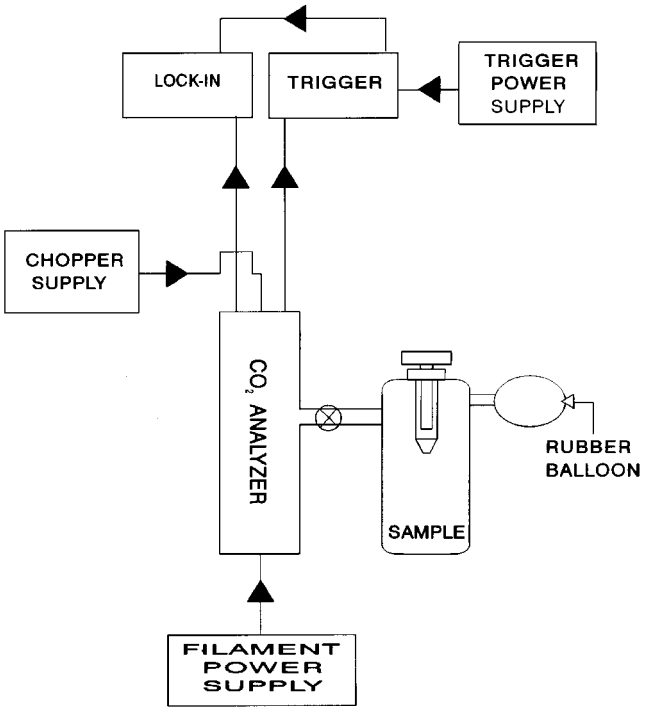

FIG. 2. Block diagram of the experimental setup.

As previously mentioned, the samples were collected from three different edaphic environments. One, taken within the vertical projection of the mesquite crown, hereafter denoted by I (inside) sample, another, taken outside the mesquite crown area, roughly 1 m away, denoted by O sample, and another one from a cultivated land, designated, from now on, as C sample. This last sample was taken from a piece of land which was open to agricultural production 14 years ago. The soils collected from the top 100.0 mm of each edaphic environment were sieved through a 2.0 mm mesh screen. The *p*H and soil holding capacity for the I, O, and C samples were (7.0, 5.73 ml/10 g), (7.3, 5.06 ml/10 g), and (7.5, 4.5 ml/10 g), respectively.

From each soil, samples of 20.0 g were placed into identical 330 ml transparent plastic bottles, to which 6 ml of 3% glucose aqueous solution was added. In this way, each sample of soil had a 1% glucose content. A similar set of reference samples, without any glucose addition was also prepared. For these reference samples we have added just 6 ml of distilled water so that the level of additional moisture was maintained the same in both type of samples. Each sample container was then sealed by a cap having a 20 ml syringe, as indicated in Fig. 2. The syringe was used to homogenize the gas before the active chamber of the CO_2 analyzer is filled with it. To keep at constant atmospheric pressure, the bottles were provided with a small rubber balloon and were maintained at constant room temperature of 25 Celsius. To monitor the CO_2 evolution during ten days, we have prepared ten sets of six soil samples (three with glucose added, and three reference samples).

The CO_2 evolution monitoring by means of the chemical method was carried out using 10 g of soil sample from each edaphic environment into a 100×10 mm petri dish. Similarly to the case of the CO_2 analyzer experiment, the samples in this case consisted also of 1% glucose content and bare ref-

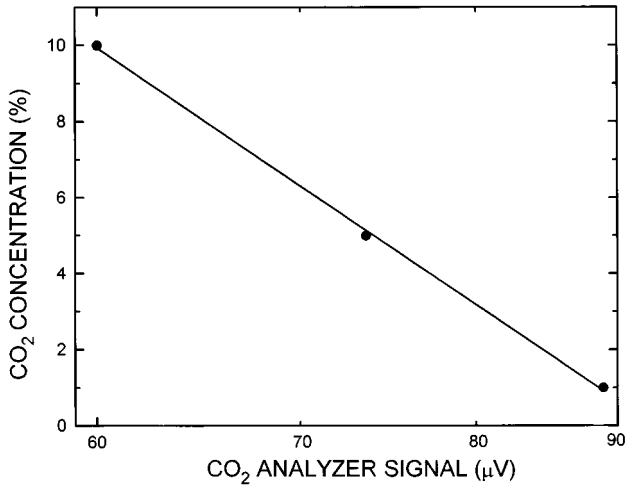

FIG. 3. Calibration curve in the (1%–10%) CO_2 concentration regime.

erence samples, prepared as described above. A small glass flask containing 10 ml of NaOH 0.2 N solution was placed inside each petri dish, which were then sealed with paraffin paper and stored in the laboratory at constant temperature. The CO_2 monitoring in this case of the chemical method was carried out during six days. Accordingly, six sets of soils samples (three with and three without glucose) were prepared. The surface NaOH solution exposed within each chamber was 36% of the area of the chamber cross section. The amount of CO_2 was determined by adding three drops of phenylphthalein and 1.0 ml of 50% BaCl solution to the flasks with NaOH, followed by tritation with 0.2 N HCl, as described in Ref. 12.

III. RESULTS AND DISCUSSION

Before carrying out the CO_2 monitoring of our soil samples, we have performed the calibration of our analyzer. This was done by filling the active chamber of the analyzer with mixtures of CO_2 and N_2 of known concentrations, and recording the lock-in output signal. The experiments were conducted at modulation frequency of 19 Hz, and the CO_2 concentration range in the gas mixtures used were restricted to the expected range of CO_2 production in our soil samples, namely, 1%–10%. In Fig. 3, we present the correlation between the measured signal and the CO_2 concentration. The solid line in this figure corresponds to the data line regression given by the equation

$$C(A) = 103.48 - 22.85 \ln(A). \tag{2}$$

Here $C(A)$ represents the CO_2 concentration (%) and A is the recorded signal amplitude (μV). Equation (2) means that minimum detectable changes in the CO_2 concentration is related to the signal resolution by

$$|\Delta C| = 22.85 \frac{|\Delta A|}{A}. \tag{3}$$

For the CO_2-free cell, A is roughly 110 μV and the correspond signal resolution is of the order of 0.1 μV. Under these conditions the minimum detectable changes in the CO_2 concentration is of the order of 0.021% (210 ppm).

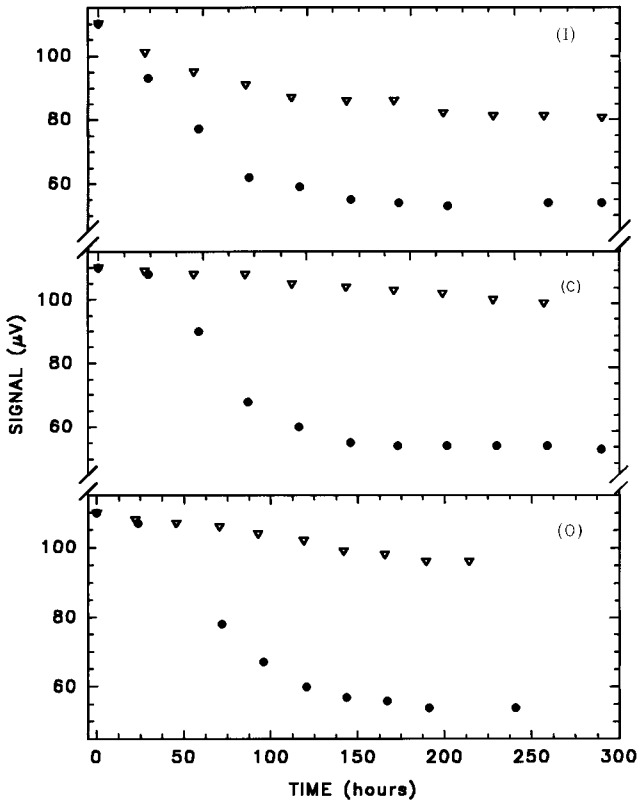

FIG. 4. Signal amplitudes as function of time generated by the inside (I), outside (O) and cultivated (C) soils samples, with (closed symbols) and without (open symbols) glucose addition.

Having calibrated our detection unit, we have next carried out the monitoring of the CO_2 evolution of our samples. In Fig. 4, we show a typical response of the active detector signal amplitude, as a function of time, for our soil samples. The closed symbols in this figure correspond to the glucose added samples, whereas the open symbols refer to the corresponding reference samples. Three aspects call our attention in these plots. First, that the signal amplitude changes are more prominent for the glucose-added samples than for the corresponding reference samples. Second, during the first 24 h, sample I exhibits a larger change than those of samples O and C. Finally, we note that the relative changes between the glucose added and reference O and C samples begin to be significant after the first 24 h. The faster decrease of the analyzer signal amplitude for soil sample I, as compared to the samples from the other two edaphic environments, is, indeed, associated with the idea of ''fertility islands'' for the role played by the semiarid native trees.

Using the calibration curve, given by Eq. (2) above, together with the signal amplitude data, we can then find the CO_2 concentration as a function of time, for the samples investigated. This is shown in Fig. 5, in which the closed symbols refer to the glucose-added soil samples. The open symbol curve in this figure corresponds to the reference sample of the I soil type, and it was included just to show the influence of the glucose addition on the soil respiration measurements. The overall behavior of the soil respiration shown in Fig. 5 follows an S-shape curve. That is, starting from lower initial value, it eventually reaches a higher saturation

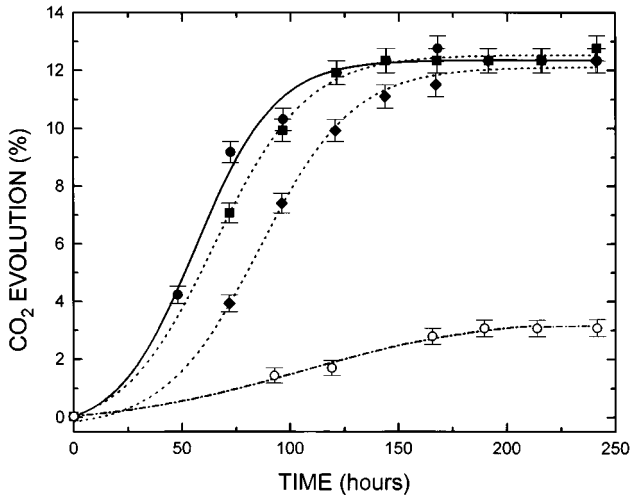

FIG. 5. CO_2 evolution curves for I (●), O (♦), and C (■) soils. Closed (open) symbols indicate (non) glucose addition to the soils.

level, after passing through a transition region where it changes its curvature. The data in Fig. 5 were best fitted to one type of S-shape curve, namely, a logistic function, which may be written as

$$C(t)=\Delta C\ \exp\left(\frac{(t-t_0)}{\Delta t}\right)\Bigg/\left[1+\exp\left(\frac{(t-t_0)}{\Delta t}\right)\right]. \qquad (4)$$

Here ΔC represents the CO_2 concentration excursion to reach the saturation level, which takes place during a time interval Δt, and t_0 is the time at which the fractional change $(C-\Delta C)/\Delta C$ equals to 0.5, corresponding to halfway the saturation excursion. In Table I we summarize the values of the fitting parameters we got from the corresponding data fitting procedure. Apart from showing the improved CO_2 production in the glucose-added soils, as already noted in Fig. 4, these results clearly indicate that the CO_2 saturation is more rapidly reached in soil I than in the other two types of soils investigated.

TABLE I. Logistic parameters of the CO_2 temporal evolution of I, O, and C glucose amended soils.

Soil	ΔC (%)	t_0 (h)	Δt (h)	$\Delta C/\Delta t$ (%/h)
I	12.88	56.51	18.04	0.71
C	13.05	65.12	21.00	0.62
O	12.44	86.40	22.70	0.55
I[a]	3.74	105.51	44.11	0.08

[a]Inside soil.

Finally, we have made a comparison between the above results, using the gas analyzer, with those obtained with the more traditional NaOH absorption (chemical) method. The results obtained, following the procedure outlained in the previous section, are summarized in Table II. In Fig. 6, we present the correlation between the CO_2 evolution from C soil, as determined by the CO_2 analyzer and by the chemical method. All other two types of soils exhibited essentially the same behavior as the one shown in this figure. The solid line in Fig. 6 represents the data linear regression expressed by

$$Q=0.597\Delta A \qquad (5)$$

with a correlation factor of 0.99. Here Q is the averaged CO_2 content (given in mg CO_2/10 g of soil) as determined by the chemical method and ΔA is the decrease in the amplitude of the CO_2 analyzer signal relative to the air signal (expressed in μV). The reason the correlation between these two measurements turn out to be linear is that for small concentration levels, as in our case, the CO_2 gas analyzer signal changes, with respect to the normalizing air signal, is essentially proportional to the CO_2 concentration. In other words, for small concentrations, the difference P_0-P in Eq. (1) is proportional to C.

IV. DISCUSSION

In this article we have presented a new, compact, CO_2 analyzer for measuring the respiration rate in organic material. Among the great variety of application possibilities, we

TABLE II. Carbon dioxide temporal evolution data obtained through NaOH absorption of CO_2 analysis.

Soil	Glucose	24 h	48 h	72 h	96 h	120 h	144 h
		6.61	23.30	33.10	33.30	31.09	31.70
I	yes	6.17	27.70	33.22	33.70	32.56	34.40
		6.50	25.00	34.98	33.40	31.24	34.40
		0.89	4.84	9.24	9.24	11.00	10.20
I	no	0.84	1.79	6.16	11.00	11.88	15.92
		0.45	1.32	6.60	6.16	9.24	3.16
		1.27	7.48	15.18	23.18	28.16	28.24
O	yes	3.00	9.68	16.94	25.38	29.04	32.20
		5.00	7.04	14.30	24.04	23.08	28.68
		1.30	3.08	3.76	6.90	11.00	8.00
O	no	0.45	3.56	4.18	4.70	5.28	5.80
		0.09	7.00	2.42	4.18	6.16	11.52
		3.97	13.20	22.66	31.98	29.04	34.40
C	yes	4.41	11.44	29.26	31.54	31.68	34.40
		5.29	15.40	23.98	29.78	29.48	34.40
		0.01	1.32	2.00	0.74	0.74	5.80
C	no	0.89	0.88	0.84	0.74	0.74	7.50
		0.01	0.88	2.00	0.74	0.73	7.10

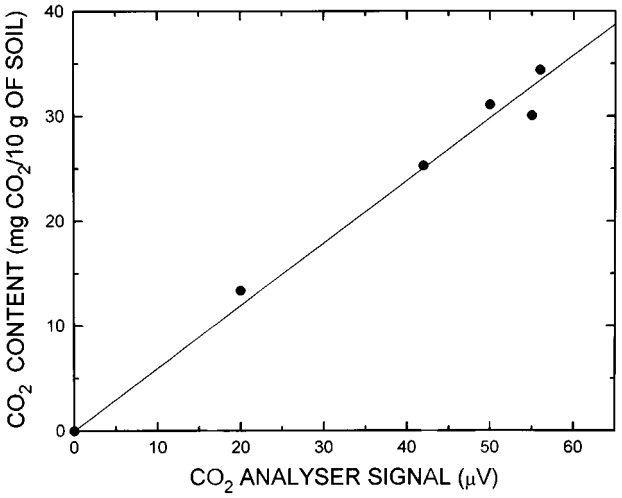

FIG. 6. Correlation between CO_2 evolved from C soil as determined by the chemical method and through measurement with the CO_2 analyzer. The solid line in this figure represents the data linear expression given in Eq. (3).

have chosen to demonstrate its potentialities by monitoring the CO_2 evolution of soil samples from a mexican semiarid region. The correlation between the results of the CO_2 evolution measurements using this gas analyzer and the more conventional chemical method has been found to be in good agreement. Even though the results shown in this article had the main purpose of illustrating the use and the kind of information one can get with this analyzer, it may be stressed that this kind of investigation may be important for gaining insight regarding the understanding of the concept of semiarid native trees as ''fertility islands.''

One common and important aspect of both type of CO_2 evolution measurements reported here, is that the main differences among the edaphic environments are manifested at the early stages of the CO_2 evolution from the soil samples. In other words, it is the rate of change at the early stage of the CO_2 evolution, and not its final saturation value, that distinguish between the different type of soils, as can be seen by close look at Tables I and II. This sort of studies may eventually lead to the development of a more ecological agriculture in semiarid regions.

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