
**Soil quality — Laboratory methods for
determination of microbial soil
respiration**

*Qualité du sol — Méthodes de laboratoire pour la détermination de
la respiration microbienne du sol*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 16072 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

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Introduction

This International Standard is derived from the German standard DIN 19737 (see [1]). It describes methods for the determination of microbial soil respiration in the laboratory.

Microbial soil respiration results from the mineralization of organic substances. In this process, organic substances are oxidized to the end products carbon dioxide and water, with concurrent uptake of O₂ for aerobic microorganisms. The soil respiration is measured by the determination of O₂ consumption and/or by CO₂ release. Respiration is a measure of the overall activity of soil microorganisms.

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Soil quality — Laboratory methods for determination of microbial soil respiration

1 Scope

This International Standard describes methods for the determination of soil microbial respiration of aerobic, unsaturated soils. The methods are suitable for the determination of O₂ uptake or CO₂ release, either after addition of a substrate (substrate-induced respiration), or without substrate addition (basal respiration).

This International Standard is applicable to the measurement of soil respiration in order to:

- determine the microbial activity in soil (see [3]);
- establish the effect of additives (nutrients, pollutants, soil improvers, etc.) on the metabolic performance of microorganisms;
- determine the microbial biomass (see [4]);
- determine the metabolic quotient $q\text{CO}_2$.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-6:1993, *Soil quality — Sampling — Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory*

ISO 11274:1998, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

basal respiration

microbial soil respiration without addition of nutrients

3.2

substrate-induced respiration

SIR

microbial soil respiration after addition of nutrients

NOTE Glucose is an example of an added nutrient.

**3.3
microbial activity**

metabolic performance of microorganisms

NOTE It can be measured, for example, as O₂ uptake or CO₂ release.

**3.4
metabolic quotient**

q_{CO_2}

specific metabolic activity of soil microorganisms, which can be calculated as the quotient basal respiration: microbial biomass

NOTE Metabolic quotient is usually expressed as milligrams of CO₂ carbon released per hour per gram of microbial biomass carbon.

**3.5
rate of CO₂ formation [O₂ consumption]**

R_{CO_2} [R_{O_2}]

amount of CO₂ released [O₂ consumed] per time unit from a mass unit of soil

NOTE 1 Soil respiration is usually measured as the rate of CO₂ formation or O₂ consumption.

NOTE 2 It is usually expressed as milligrams CO₂ [or O₂] per gram per hour (mg CO₂ [or O₂]·g⁻¹·h⁻¹).

**3.6
microbial biomass**

mass of intact microbial cells in a given soil

NOTE This is usually estimated from the measurement of carbon or nitrogen content of these cells.

4 Procedure

4.1 General conditions

4.1.1 Soil sampling and storage

Sample, store and pre-incubate soils in accordance with ISO 10381-6, independently of the choice of the procedure and the respiration parameter to be measured (basal respiration, SIR).

4.1.2 Measuring and incubation conditions

Soil respiration is strongly influenced by water content and temperature. Therefore these parameters should be recorded in the final report. At suction pressures > 0,03 MPa, the soil respiration will decrease considerably. The water content of the test soil is optimal when it corresponds with a pore water pressure of – 0,01 MPa to – 0,03 MPa (measured with an accuracy of 5 %, in accordance with ISO 11274) or 40 % to 60 % of the maximum water-holding capacity, respectively. A stable temperature should be used. Incubation temperatures between 20 °C and 30 °C are generally recommended, but other temperatures may be used if required. In the description of the methods, examples of incubation temperatures are given as well as the accuracy of temperature maintenance and measurement.

If a method is used for the determination of soil microbial biomass, a temperature of 22 °C is recommended because biomass calculations have been calibrated to this temperature.

When soil samples are compared with respect to soil respiration, they should have the same moisture status (pore water pressure or percentage of maximum water-holding capacity).

4.2 Choice of the measuring system

Each measurement method has its own advantages and disadvantages. Care is needed, because the results obtained by O_2 uptake and by CO_2 release are not strictly compatible. It is the responsibility of the investigator to decide which of these methods is to be used.

One of the systems described in Clause 5 should be used.

Systems for measuring CO_2 do not distinguish between CO_2 released from microbial activities and CO_2 resulting from abiotic processes. For alkaline soils and soils with a high organic matter content, which can release considerable amounts of abiotically released CO_2 , methods using O_2 uptake are recommended.

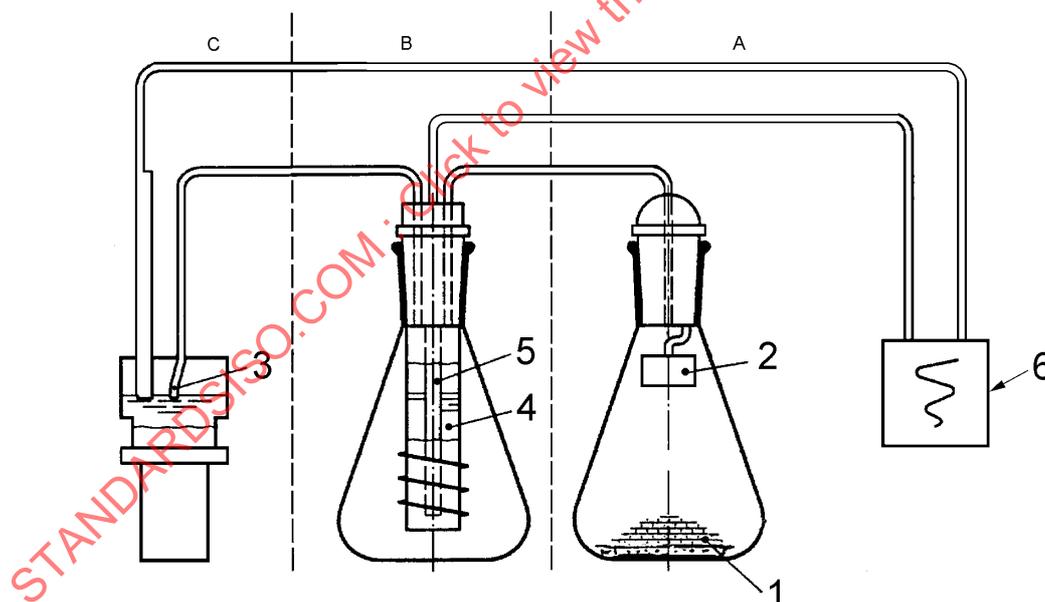
NOTE The advantages and disadvantages are described in the individual descriptions of the methods.

5 Measuring systems

5.1 Determination of O_2 consumption by static incubation in a pressure-compensation system

5.1.1 Principle

The determination is based on the measurement of O_2 consumption during incubation of a soil sample in a closed system. The O_2 in the system is replenished electrochemically. The CO_2 released is absorbed by calcium hydroxide [$Ca(OH)_2$].



Key

A	reaction vessel	1	soil sample	4	electrolyte
B	oxygen generator	2	CO_2 absorbent	5	electrodes
C	pressure indicator	3	pressure cell	6	recorder with display

Figure 1 — Determination of O_2 consumption (showing connection of a measuring unit)

5.1.2 Apparatus

A detailed description of the apparatus can be found in [4]; the essential features are as follows.

The measuring system (see Figure 1) consists of a water bath with temperature control containing measuring units each comprising a reaction vessel (A) in which a CO₂ absorption device (2) is suspended from the stopper, an O₂ generator (B) and a pressure indicator (C). The vessels (A, B, C) of the measuring unit form together a closed system, connected to each other by tubing. In this way fluctuations in atmospheric pressure will not influence the results. The CO₂ released is absorbed by the calcium hydroxide (2). The consumption of O₂ due to respiration results in a negative pressure which activates the pressure indicator (C). This drives the electrolytic O₂ formation as well as the display and graphical registration of measuring values on a recorder (6). The O₂ consumption is shown directly, in milligrams of oxygen (mg O₂) on a digital display.

The system can be obtained commercially¹⁾ and detailed instructions should be given in the supplier's manual.

5.1.3 Procedure

Use 50 g to 100 g of field-moist, sieved (2 mm) soil for the measurements. The O₂ consumption should not be measured during the first 2 h, the time needed to reach equilibrium in the system.

5.2 Determination of CO₂ release by titration in a static system

5.2.1 Principle

The soil is incubated in a closed vessel and the released CO₂ is absorbed in a solution of sodium hydroxide. After back-titration of the non-consumed sodium hydroxide, the amount of CO₂ released is calculated. The method is suitable for large numbers of samples, and up to 80 respiration measurements per working day are possible.

5.2.2 Reagents

5.2.2.1 CO₂-free water

Boil distilled water and after cooling store it in flasks closed with stoppers provided with absorption tubes containing calcium hydroxide.

5.2.2.2 Sodium hydroxide (NaOH) solution, $c = 0,05 \text{ mol}\cdot\text{l}^{-1}$.

5.2.2.3 Hydrochloric acid (HCl) solution, $c = 0,1 \text{ mol}\cdot\text{l}^{-1}$.

The concentrations of NaOH solution (5.2.2.2) and HCl (5.2.2.3) should be chosen so that less than 20 % of the NaOH is neutralized by CO₂. Higher percentages of neutralization will result in less reliable results (see [5]). If other concentrations are used, Equation (1) should be changed accordingly.

5.2.2.4 Barium chloride solution, $c = 0,5 \text{ mol}\cdot\text{l}^{-1}$.

Dissolve 10,4 g of BaCl₂ in 100 ml of CO₂-free distilled water (5.2.2.1).

5.2.2.5 Indicator.

Dissolve 0,1 g of phenolphthalein in 100 ml of aqueous ethanol (volume fraction ethanol 0,6).

5.2.3 Apparatus

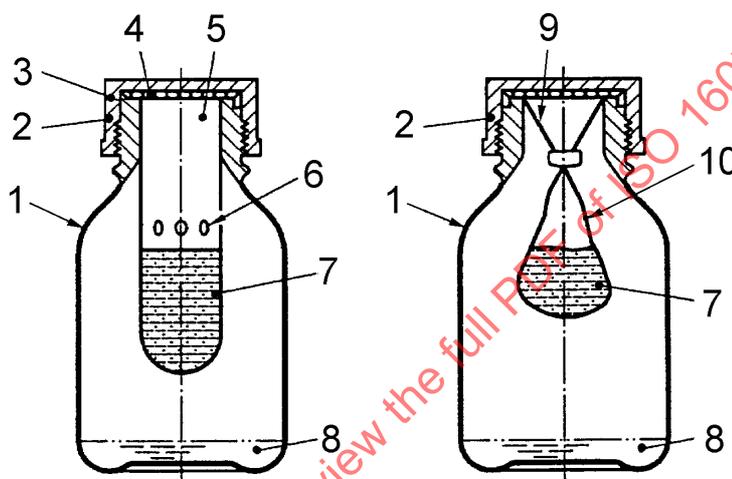
5.2.3.1 Wide-mouth flasks (250 ml content) with screw-caps and pour rim, or preserve flasks (1 l content) with rubber rings, covers and 2 universal clips.

1) Sapromat is the trade name of a product supplied by H+P Labortechnik AG. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

5.2.3.2 Centrifuge tubes or reaction tubes with a rim (e.g. polypropylene, external diameter 29 mm, length 120 mm). Small holes should be drilled in the tubes for gas exchange. Instead of tubes, fine-mesh nylon bags can also be suspended from the neck in the wide-mouth flasks.

5.2.4 Procedure

Weigh 20 g to 25 g of field-moist soil into the centrifuge tubes (5.2.3.2). Suspend the tubes in the wide-mouth flasks (5.2.3.1) (see Figure 2), in which 20 ml solution of sodium hydroxide (5.2.2.2) has been previously pipetted. Close the flasks tightly and incubate for 24 h in a temperature-controlled room at the temperature of choice, e.g. $22\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. Before closing the flasks, they should be flushed with clean air with low CO_2 content (e.g. from outdoors). Then remove the tubes. The CO_2 absorbed will precipitate as barium carbonate upon addition of 2 ml of barium chloride solution (5.2.2.4). Titrate the unused sodium hydroxide with hydrochloric acid (5.2.2.3) after addition of 3 or 4 drops of indicator solution (5.2.2.5).



Key

1	wide-mouth flask (250 ml)	6	openings for gas exchange
2	screw-cap	7	soil sample
3	pour rim	8	sodium hydroxide solution
4	closing pad	9	plastic thread
5	suspended centrifuge tubes	10	fine-mesh woven plastic bag

Figure 2 — Incubation flasks for the determination of soil respiration

The determination should be carried out at least in triplicate. Controls (triplicate flasks without soil) should be included.

If the soil respiration is measured in a long-term experiment (> 3 days), then the soil samples should be incubated in flasks in which the sodium hydroxide solution is renewed every 3 days. Also the water content of the soil has to be adjusted every 3 days.

Also suitable for incubation are preserve flasks (1 l content) with rubber rings, covers and 2 universal clips. Weigh the soil samples (up to 200 g) into crystallization disks, which are placed on the bottom of the preserve flasks. Place sodium hydroxide solution in a beaker. On the bottom of the flasks, place 4 ml of CO_2 -free water (5.2.2.1) to maintain air moisture.

NOTE When determining basal respiration in the laboratory, an increase in CO_2 release is often observed in the first hours. This can be caused by an increased availability of nutrients due to the moving and mixing of soil particles during sample preparation, but also by the short-term establishment of an equilibrium between gaseous and dissolved CO_2 . The incubation time necessary for reaching a steady basal respiration depends in the first instance on the soil's content of easily available carbon compounds. This applies to all methods measuring CO_2 release.

5.2.5 Calculation of results

Calculate the rate of CO₂ evolution using equation (1).

$$R_{\text{CO}_2} = \frac{2,2(\bar{V}_b - \bar{V}_p)}{24 \cdot m_{\text{sm}} \cdot w_{\text{sd}}} \quad (1)$$

where

- R_{CO_2} is the rate of CO₂ evolution a on soil dry mass basis (mg CO₂·g⁻¹·h⁻¹);
- \bar{V}_b is the average volume of HCl consumed in the control, in millilitres;
- \bar{V}_p is the average volume of HCl consumed in the test sample, in millilitres;
- m_{sm} is the mass of the moist soil sample, in grams;
- 2,2 is a factor (1 ml of 0,1 molar HCl corresponds to 2,2 mg of CO₂ per day) (mg·ml⁻¹·day⁻¹);
- 24 is a factor to convert daily evolution to hourly evolution (h·day⁻¹);
- w_{sd} is the dry mass fraction of the moist soil.

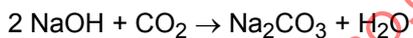
NOTE The dry mass fraction (w_{sd}) of the moist soil equals the percent dry mass divided by 100.

This calculation is only valid if the specified reagent concentrations (0,05 mol·l⁻¹ NaOH and 0,1 mol·l⁻¹ HCl) are used.

5.3 Coulometric determination of CO₂ release in a static system

5.3.1 Principle

The soil sample is incubated (e.g. in triplicate) in containers at constant temperature, and the CO₂ evolved is absorbed in sodium hydroxide solution. In the reaction:



hydroxyl ions are replaced by carbonate ions, which have a different electrical conductivity. The change in conductivity is registered electronically by the apparatus. A CO₂ content of 0,000 1 % (volume fraction) can be detected with certainty. With the apparatus, basal respiration can be measured as well as substrate-induced soil respiration after addition of glucose to determine biomass carbon. Depending on the type of apparatus, up to 90 samples can be measured simultaneously.

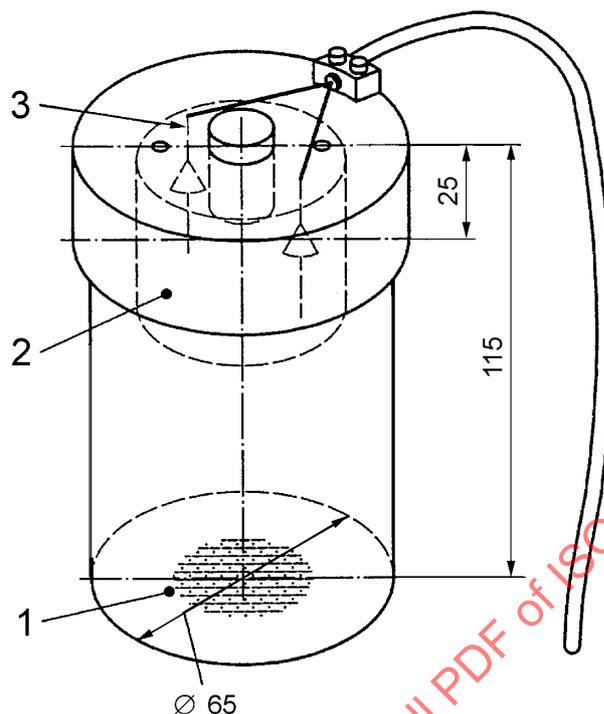
The mass of the soil samples used and the temperature setting should be established according to the manufacturer's instructions.

For more details see [6] and [8].

5.3.2 Test assembly

A simple type of apparatus is depicted schematically in Figure 3.

Dimensions in millimetres

**Key**

- 1 container with soil sample
- 2 conductivity cell (in the cover)
- 3 platinum electrodes

Figure 3 — Apparatus for coulometric measurement of soil respiration

5.4 Determination of CO₂ release using an infrared gas analyser in a flow-through system

5.4.1 Principle

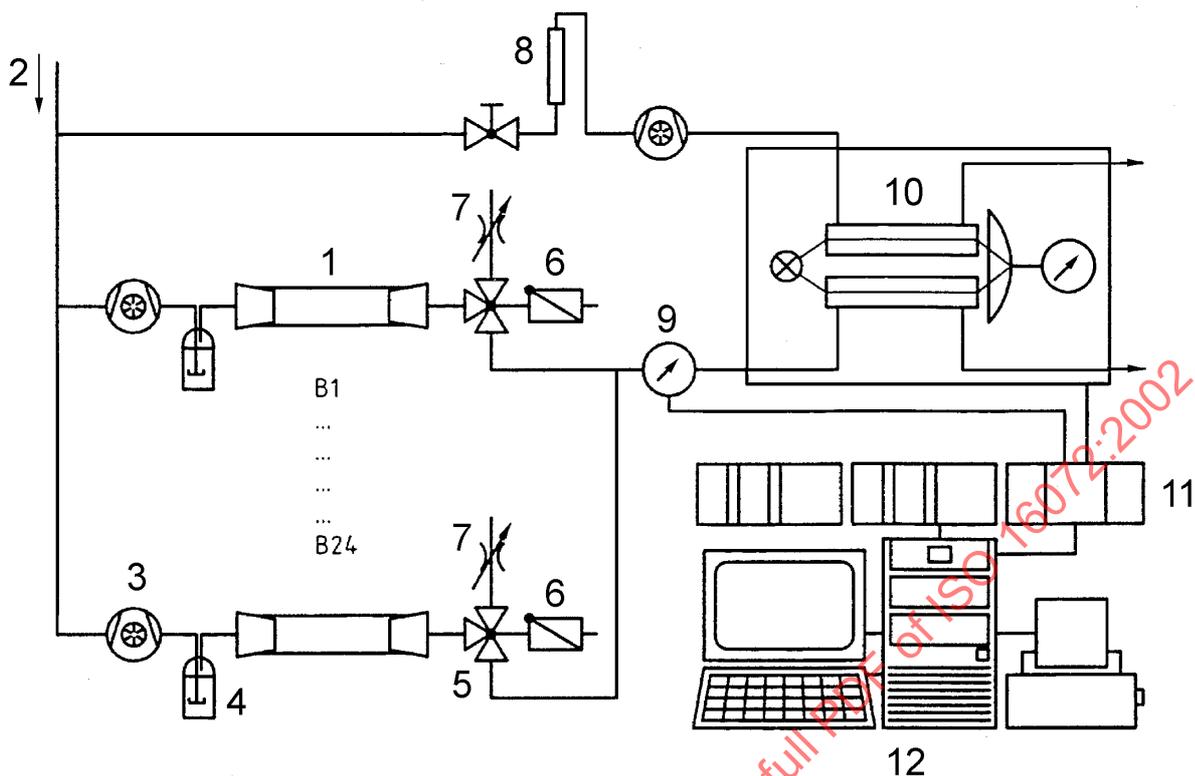
Soil samples are flushed with ambient air. The release of CO₂ from the soil samples during the incubation period is determined by an infrared gas analyser in combination with a flow meter. This method is frequently used for microbial biomass measurements, in which case a temperature of 22 °C is recommended. At a temperature other than 22 °C, routine evaluation of biomass measurements cannot be carried out, but must be recalculated using factors.

5.4.2 Reagent

5.4.2.1 CO₂ calibration gas (air with 350 µl CO₂·l⁻¹ and 400 µl CO₂·l⁻¹).

5.4.3 Apparatus

The usual laboratory equipment, as well as an infrared gas analyser (see Figure 4) placed in a temperature-controlled room at the temperature of choice, e.g. 22 °C ± 1 °C.



Key

- | | | | | | |
|---|-----------------------|---|----------------|----|-----------------------------------|
| 1 | soil sample container | 5 | valves (3-way) | 9 | flow adjustment and flow meter |
| 2 | air inlet | 6 | steering | 10 | infrared CO ₂ analyser |
| 3 | gas pump | 7 | closure valve | 11 | control unit |
| 4 | moistener | 8 | reference air | 12 | control and evaluation equipment |

Figure 4 — Example of configuration of an infrared gas analyser system for the determination of soil respiration

5.4.4 Procedure

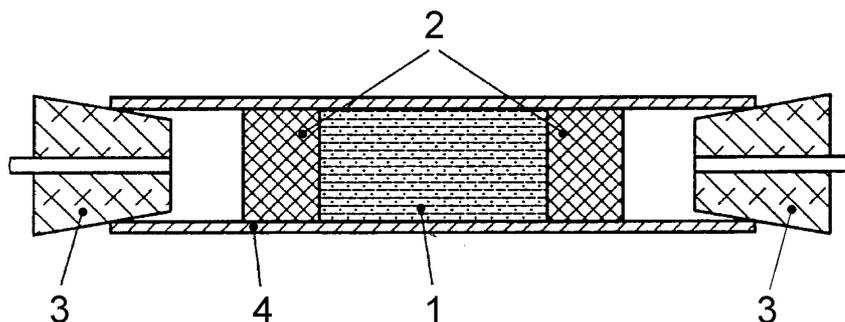
Sieve and weigh 10 g to 200 g, depending on the activity expected, of moist soil into the soil sample cylinders (e.g. in triplicate). To pack the soil sample so that it completely fills the diameter of the cylinder, the use of porous polyurethane foam stoppers is recommended as shown in Figure 5. To control the baseline of the apparatus, at least two sample cylinders should remain empty. After connecting the sample cylinders to the measuring equipment, start the gas pumps and adjust the gas flowrate at each measuring channel to the required value. Normally, the measured data are registered automatically by the evaluation software.

The infrared gas analyser usually requires recalibration every one to two weeks by means of the calibration gas.

The continuous flow of surrounding air in the soil sample reduces the disturbance of the soil carbonate equilibrium (especially in calcareous soils) and therefore reduces the risk of erroneous results due to abiotic release of CO₂.

A good linearity exists over a wide range between the mass of the soil samples (10 g to 100 g) and the CO₂ release for the measuring equipment described.

If this system is compared with flow-through systems which use CO₂-free air to measure CO₂ release, no inhibition of the citric acid cycle in microorganisms is observed.



Key

- 1 soil sample
- 2 polyurethane foam stopper
- 3 rubber stoppers
- 4 acrylic glass cylinder

Figure 5 — Sample container for determination of soil respiration using an infrared gas analyser

5.4.5 Calculation and results

The infrared gas analyser measures the CO₂ volume fraction in the differential mode, in microlitres per litre. The rate of formation of CO₂ (milligrams CO₂ per gram per hour) is calculated by the evaluation software taking into account the actually measured gas flowrate (millilitres air per minute) and the mass of the soil sample (grams dry mass).

$$R_{\text{CO}_2} = 0,00183 \frac{(\varphi_s - \varphi_e) \cdot q_g}{m_{\text{sm}} \cdot w_{\text{sd}}} \quad (2)$$

where

- R_{CO_2} is the rate of CO₂ formation on a soil dry mass basis (mg CO₂·g⁻¹ h⁻¹);
- φ_s is the read-out of CO₂ volume fraction in the soil sample cylinder (µl CO₂·l⁻¹);
- φ_e is the read-out of CO₂ volume fraction in the empty cylinder which has been analysed most recently before or afterwards the soil sample cylinder (µl CO₂·l⁻¹);
- q_g is the read-out of the gas flowrate, in litres per hour;
- 0,001 83 is the conversion factor for µl CO₂ to mg CO₂ (mg·µl⁻¹), if one molar volume of CO₂ is 24,057 l·mol⁻¹ at 22 °C and a pressure of 101,3 kPa;
- m_{sm} is the mass of the moist soil sample, in grams;
- w_{sd} is the dry mass fraction of the moist soil (see Note in 5.2.5).

5.5 Determination of CO₂ release using gas chromatography in a flow-through system and a static system

5.5.1 Principle

Soil samples are incubated in an incubator or incubation room at a constant temperature (e.g. 22 °C ± 1 °C) in the dark. Ambient air or synthetic CO₂-free air is drawn through the samples. The CO₂ released by the samples during the incubation period is quantified with the aid of a gas chromatograph with flame ionization detector (FID) or thermal conductivity detector (TCD) in combination with a gas flow meter. To prevent the condensation of water in on-line gas measurements, use of gas pipe which can be heated in the incubator is recommended.

5.5.2 Reagents

5.5.2.1 If ambient air is used: **CO₂ calibration gas** (300 µl·l⁻¹ to 500 µl·l⁻¹).

5.5.2.2 If synthetic air is used: **CO₂ calibration gas** (20 µl·l⁻¹ to 200 µl·l⁻¹).

5.5.3 Apparatus

5.5.3.1 **Temperature-controlled incubator or incubation room.**

5.5.3.2 **Gas flow meter.**

5.5.3.3 **Gas pressure meter.**

5.5.3.4 **Gas chromatograph with FID or TCD.**

NOTE The TCD is frequently used for inert gases because it detects many other gases besides CO₂. This is not the case for a hydrogenation column/FID. However, older gas chromatographs can be updated with the latter system if a TCD is no longer available. For the detection of CO₂, the FID has no disadvantages compared to the TCD.

5.5.3.5 **Computer for data evaluation and system control.**

5.5.4 Procedure

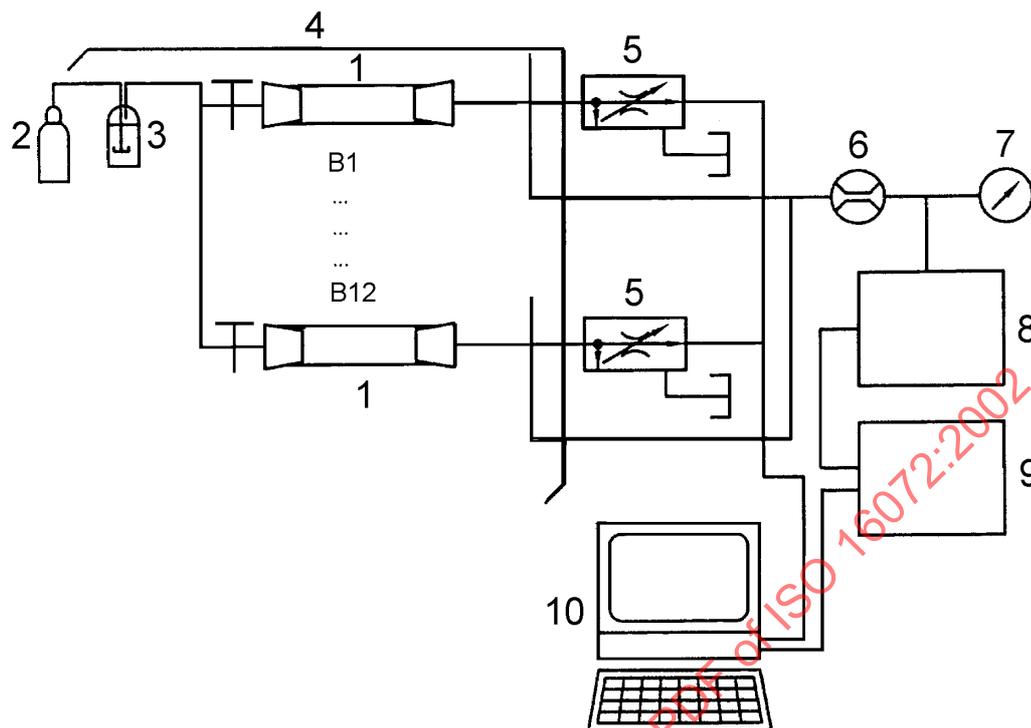
5.5.4.1 General

The incubation may be carried out continuously using a flow-through system (see Figure 6) or discontinuously using the gas sample injection method.

5.5.4.2 Flow-through method

For the method of Figure 6, weigh moist soil (10 g to 200 g, depending on the expected activity, e.g. in triplicate) into sample cylinders. To pack the soil sample so that it completely fills the diameter of the cylinder, the use of porous polyurethane foam stoppers is recommended (see Figure 5). To control the baseline of the apparatus, at least two sample cylinders should remain empty. After connecting the sample cylinders to the measuring equipment, start the gas pumps and adjust the gas flowrate at each measuring channel to the required value. The measured data are registered automatically by the evaluation software. The gas flowrate should be adjusted to between 80 ml·min⁻¹ and 300 ml·min⁻¹.

At regular time intervals (e.g. one or two weeks) the system should be recalibrated with calibration gas. Three concentrations should be used to check the linearity of the detectors in the required measuring range.

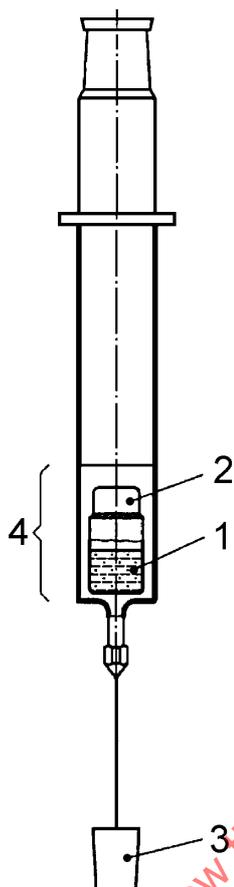
**Key**

1	soil sample cylinder	5	3-way valve	9	integrator
2	carrier gas inlet	6	flow meter	10	control and evaluation equipment
3	gas moistener	7	pressure gauge		
4	incubator	8	gas chromatograph		

Figure 6 — Configuration for measuring soil respiration using a gas chromatograph/flow-through system

5.5.4.3 Injection methods

For the gas sample injection method of Figure 7, weigh (e.g. in triplicate) 2 g to 4 g of moist soil [water content 40 % to 60 % (mass fraction) of the maximal water-holding capacity, or 0,01 MPa to 0,03 MPa suction pressure] into glass ampoules. Rinse a gas-tight glass syringe with clean air and place the ampoule on the bottom of the syringe (see Figure 7). With the plunger (lightly greased) press out part of the contents of the glass syringe and then inject 2 ml into the injection port of a gas chromatograph (this is the starting value of the measurement). Then adjust the volume of the syringe to 16 ml and press the needle into a rubber stopper, thus closing the syringe air-tight. Store the syringe in vertical position in a holder. Keep pressure and temperature constant during the incubation. After the required incubation time (about 20 h, temperature of incubation according to the experimental design) remove the stopper from the needle and inject again 2 ml (end value of the measurement). Remove, degrease and dry the ampoules containing soil to constant mass at 105 °C to determine the dry mass.



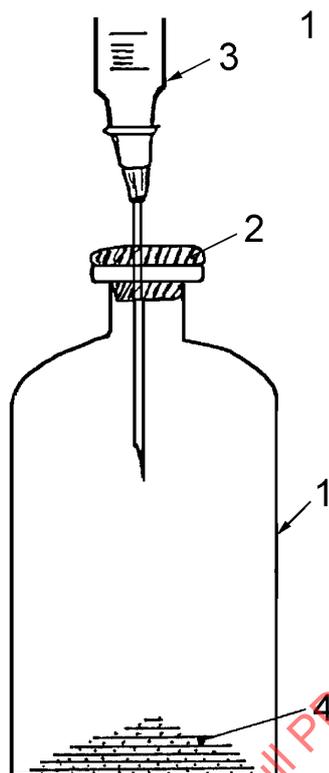
Key

- 1 soil
- 2 sample flask
- 3 rubber stopper
- 4 accumulation volume (16 ml)

Figure 7 — Glass syringe for the syringe method for incubation of soil samples

In an alternative method, serum bottles (volume about 116 ml, height about 95 ml, Ø 50 ml) are provided with 10 g of soil and closed with a septum (see Figure 8). Incubate the flasks as described in the previous paragraph. At the beginning of the incubation and after 20 h to 24 h, take gas samples from the gas phase with a disposable gas-tight syringe and inject into a gas chromatograph with TCD detector as described in 5.5.5.2.

The rate of CO₂ evolution can be calculated in a manner analogous to that described in 5.5.6.1 and 5.5.6.2.



Key

- 1 serum bottle
- 2 rubber septum
- 3 gas-tight syringe
- 4 soil sample

Figure 8 — Determination of soil respiration by gas chromatography

5.5.5 Detection

5.5.5.1 General

The detection of CO₂ can be carried out by a flame ionization detector (FID) or thermal conductivity detector (TCD) according to instructions of the manufacturer. If the latter is not available, the procedures in 5.5.5.1 or 5.5.5.2 can be followed.

5.5.5.2 Gas chromatograph with FID (flame ionization detector)

For detection of CO₂, a hydrogenation column is additionally needed to convert the CO₂ in the gas flow by catalytic hydrogenation into CH₄, which can then be detected by the FID. The detector temperature should be at least 350 °C. The H₂ feed into the hydrogenation column also functions as the combustion gas of the FID. Capillary columns or packed columns can be used for separation. Capillary columns with an internal diameter smaller than 0,53 mm cannot be used for this detection system because of the restricted flow. A temperature programme is not necessary, as the measurement is carried out isothermally at 80 °C. The manual measurement of single samples is also possible. For this purpose, gas-tight syringes are used to inject directly a gas sample by means of a septum into the carrier gas flow. For online gas measurements, an automatic sampling device is used. An 8-way gas sampling valve controlled by compressed air provided with a sample loop with defined volume is useful. The complete gas-sampling valve is situated in the column oven.

5.5.5.3 Gas chromatograph with TCD (thermal conductivity detector)

For this detection method, a gas chromatograph with a TCD is needed. The detector temperature should be 150 °C. Packed columns are used for separation. The measurement is carried out isothermally at 40 °C. Online measurements and single-probe measurements can be carried out. The sampling system is similar to that described in 5.5.5.1.

5.5.6 Calculation of results (method with syringe)

5.5.6.1 Determination of volume

The calculation of the volume of the glass ampoule (V_{amp}), the volume of the soil dry mass (V_{sd}), and the volume of the water (V_w) are carried out according to Equations (3), (4) and (5) respectively.

$$V_g = \frac{m_{amp}}{\rho_{quartz}} \tag{3}$$

$$V_{sd} = \frac{m_{sd}}{\rho_{sd}} \tag{4}$$

$$V_w = \frac{m_w}{\rho_w} \tag{5}$$

where

V_{amp} is the volume of the ampoule, in millilitres;

m_{amp} is the mass of the glass ampoule, in grams;

ρ_{quartz} is the specific density of quartz ($\text{g}\cdot\text{ml}^{-1}$) = 2,65 $\text{g}\cdot\text{ml}^{-1}$;

V_{sd} is the volume of the dry soil mass, in millilitres;

m_{sd} is the mass of the dry soil sample, in grams;

ρ_{sd} is the specific density of the dry soil mass ($\text{g}\cdot\text{ml}^{-1}$) = 2,65 $\text{g}\cdot\text{ml}^{-1}$ for mineral soils, 1,2 $\text{g}\cdot\text{ml}^{-1}$ for organic soils;

V_w is the volume of the water, in millilitres;

m_w is the mass of the water, in grams;

ρ_w is the specific density of water ($\text{g}\cdot\text{ml}^{-1}$) = 1 $\text{g}\cdot\text{ml}^{-1}$.

5.5.6.2 Calculation of the CO₂ formation

$$\varphi_2 - \varphi_1 = \varphi_{CO_2} \tag{6}$$

$$V_{syr} - (V_{amp} + V_{sd} + V_w) = V_{CO_2} \tag{7}$$

$$V_{CO_2} \cdot \varphi_{CO_2} \times 10^{-6} = V_{exp} \tag{8}$$

$$V_{exp} \cdot p_{exp} \cdot 273 / [1013,25 \cdot (273 + T_{exp})] = V_0 \tag{9}$$

$$R_{CO_2} = V_0 \cdot 44 / (22,4 \cdot t \cdot m_{sd}) \tag{10}$$