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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

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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*.

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 O_2 for aerobic microorganisms. The soil respiration is measured by the determination of O_2 consumption and/or by CO_2 release. Respiration is a measure of the overall activity of soil microorganisms.

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 O_2 uptake or CO_2 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 qCO_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 O_2 uptake or CO_2 release.

3.4
metabolic quotient
qCO_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 CO_2 carbon released per hour per gram of microbial biomass carbon.

3.5
rate of CO_2 formation [O_2 consumption]
R_{CO_2} [R_{O_2}]
amount of CO_2 released [O_2 consumed] per time unit from a mass unit of soil

NOTE 1 Soil respiration is usually measured as the rate of CO_2 formation or O_2 consumption.

NOTE 2 It is usually expressed as milligrams CO_2 [or O_2] per gram per hour (mg CO_2 [or O_2]$\cdot g^{-1} \cdot h^{-1}$).

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 CO_2 absorption device (2) is suspended from the stopper, an O_2 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 CO_2 released is absorbed by the calcium hydroxide (2). The consumption of O_2 due to respiration results in a negative pressure which activates the pressure indicator (C). This drives the electrolytic O_2 formation as well as the display and graphical registration of measuring values on a recorder (6). The O_2 consumption is shown directly, in milligrams of oxygen (mg O_2) on a digital display.

The system can be obtained commercially[1)] 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 O_2 consumption should not be measured during the first 2 h, the time needed to reach equilibrium in the system.

5.2 Determination of CO_2 release by titration in a static system

5.2.1 Principle

The soil is incubated in a closed vessel and the released CO_2 is absorbed in a solution of sodium hydroxide. After back-titration of the non-consumed sodium hydroxide, the amount of CO_2 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 CO_2-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 mol·l^{-1}.

5.2.2.3 Hydrochloric acid (HCl) solution, c = 0,1 mol·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 CO_2. 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 mol·l^{-1}.

Dissolve 10,4 g of $BaCl_2$ in 100 ml of CO_2-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.

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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 °C ± 1 °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 preserver 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 CO_2 evolution using equation (1).

$$R_{CO_2} = \frac{2{,}2\left(\bar{V}_b - \bar{V}_p\right)}{24 \cdot m_{sm} \cdot w_{sd}} \quad (1)$$

where

R_{CO_2} is the rate of CO_2 evolution a on soil dry mass basis (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$);

$\bar{V}_b$ 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 CO_2 per day) ($mg \cdot ml^{-1} \cdot day^{-1}$);

24 is a factor to convert daily evolution to hourly evolution ($h \cdot day^{-1}$);

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 $mol \cdot l^{-1}$ NaOH and 0,1 $mol \cdot l^{-1}$ HCl) are used.

5.3 Coulometric determination of CO_2 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 CO_2 evolved is absorbed in sodium hydroxide solution. In the reaction:

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

hydroxyl ions are replaced by carbonate ions, which have a different electrical conductivity. The change in conductivity is registered electronically by the apparatus. A CO_2 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 CO_2 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 CO_2 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 CO_2 calibration gas (air with 350 µl $CO_2 \cdot l^{-1}$ and 400 µl $CO_2 \cdot l^{-1}$).

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 CO_2.

A good linearity exists over a wide range between the mass of the soil samples (10 g to 100 g) and the CO_2 release for the measuring equipment described.

If this system is compared with flow-through systems which use CO_2-free air to measure CO_2 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 CO_2 volume fraction in the differential mode, in microlitres per litre. The rate of formation of CO_2 (milligrams CO_2 per gram per hour) is calculated by the evaluation software taking into account the actually measured gas flowrate (millitres air per minute) and the mass of the soil sample (grams dry mass).

$$R_{CO_2} = 0{,}001\,83 \frac{(\varphi_s - \varphi_e) \cdot q_g}{m_{sm} \cdot w_{sd}} \quad (2)$$

where

R_{CO_2} is the rate of CO_2 formation on a soil dry mass basis (mg $CO_2 \cdot g^{-1}\ h^{-1}$);

φ_s is the read-out of CO_2 volume fraction in the soil sample cylinder (µl $CO_2 \cdot l^{-1}$);

φ_e is the read-out of CO_2 volume fraction in the empty cylinder which has been analysed most recently before or afterwards the soil sample cylinder (µl $CO_2 \cdot l^{-1}$);

q_g is the read-out of the gas flowrate, in litres per hour;

0,001 83 is the conversion factor for µl CO_2 to mg CO_2 ($mg \cdot \mu l^{-1}$), if one molar volume of CO_2 is 24,057 $l \cdot mo^{-1}$ 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 CO_2 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 CO_2-free air is drawn through the samples. The CO_2 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: **CO_2 calibration gas** (300 µl·l^{-1} to 500 µl·l^{-1}).

5.5.2.2 If synthetic air is used: **CO_2 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 CO_2. 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 CO_2, 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 CO_2 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 CO_2 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 CO_2, a hydrogenation column is additionally needed to convert the CO_2 in the gas flow by catalytic hydrogenation into CH_4, which can then be detected by the FID. The detector temperature should be at least 350 °C. The H_2 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 ($g \cdot ml^{-1}$) = 2,65 $g \cdot ml^{-1}$;

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 ($g \cdot ml^{-1}$) = 2,65 $g \cdot ml^{-1}$ for mineral soils, 1,2 $g \cdot ml^{-1}$ 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 ($g \cdot ml^{-1}$) = 1 $g \cdot ml^{-1}$.

5.5.6.2 Calculation of the CO_2 formation

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

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

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

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

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

where

R_{CO_2} is the rate of CO_2 formation on a soil dry mass basis (mg $CO_2 \cdot g^{-1} \cdot h^{-1}$);

φ_{CO_2} is the enrichment volume fraction with CO_2 ($\mu l \cdot l^{-1}$);

φ_1 is the starting volume fraction of CO_2 ($\mu l \cdot l^{-1}$);

φ_2 is the end volume fraction of CO_2 ($\mu l \cdot l^{-1}$);

V_{CO_2} is the enrichment volume of CO_2 , in millilitres;

V_0 is the enrichment volume of CO_2 at standard conditions of 1 013,25 hPa and 273 K, in millilitres;

V_{exp} is the enrichment volume of CO_2 at the experimental conditions, in millilitres;

p_{exp} atmospheric pressure at the experimental conditions, in hectopascals;

T_{exp} is the temperature at the experimental conditions (°C);

V_{syr} is the volume of the syringe, in millilitres;

44 is the molar mass of CO_2 ($g \cdot mol^{-1}$);

22,4 is the molar volume of CO_2 under standard conditions ($l \cdot mol^{-1}$);

t is the incubation time, in hours;

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

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

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

V_w is the volume of the water, in millilitres.

5.6 Determination of soil respiration by pressure measurement in a static system

5.6.1 Principle

The process of soil respiration results in O_2 consumption and simultaneous formation of CO_2. When this process takes place in a closed vessel containing, in addition to the solid substance (e.g. soil), a sufficiently large air-filled gas phase as well as an absorbent for CO_2, the O_2 consumption will lead to a reduction in the gas pressure. In a closed system, the pressure reduction takes place independently of the atmospheric pressure. The change in pressure is proportional to the mass of O_2 consumed.

5.6.2 Apparatus (see Figures 9 and 10)

5.6.2.1 Reaction vessel (e.g. wide-mouth flask in accordance with ISO 4796, or a preserve flask of capacity between 0,5 l and 1 l).

5.6.2.2 Flask closure with connection for manometer (of accuracy at least ± 0,5 %).

5.6.2.3 Container for absorbent material.

5.6.2.4 Pressure-measuring apparatus (manometer).

5.6.2.5 Thermostrated incubator.

5.6.2.6 Laboratory balance.

5.6.2.7 Drying oven.

Key

1 manometer
2 container with absorption material
3 adapter
4 reaction vessel with gas phase
5 soil sample

Figure 9 — Test configuration for measuring soil respiration by means of pressure reduction (autoclavable vessel)

Key

1 manometer
2 cover
3 suspension
4 NaOH, KOH or $Ca(OH)_2$ in beaker
5 reaction vessel
6 soil sample

Figure 10 — Test configuration for measuring soil respiration by means of pressure reduction (preserve flask)

5.6.3 Reagents

5.6.3.1 Absorbing material for CO_2: calcium hydroxide, potassium hydroxide solution or sodium hydroxide solution, depending on the required measurement. Calcium hydroxide is not recommended for measurement intervals less than 1 day (see Figure 9). For preserve flasks, potassium hydroxide solution or sodium hydroxide solution is recommended.

5.6.3.2 Deionized water.

5.6.4 Procedure

5.6.4.1 Preparation of soil and measuring apparatus

5.6.4.1.1 Determine the dry mass fraction of the soil in accordance with ISO 11465.

5.6.4.1.2 Adjust the water content of the soil to a required value [e.g. 50 % (mass fraction) of the maximum water-holding capacity]. This can be done by adding deionized water (5.6.3.2) or by air-drying at 19 °C to 22 °C.

5.6.4.1.3 Determine the volume of the sample of the soil with adjusted moisture content.

Place 100 ml of deionized water in a graduated cylinder (200 ml), and then add and suspend 100 g of soil (with adjusted moisture content) in the water by gentle stirring. The replaced water volume is equal to the soil volume (V_{Sm}).

If not known, the following quantities should be measured:

— total volume of the measuring vessel with closure;

— volume of the absorption vessel (V_{av});

— volume of the absorption material (V_{am}).

5.6.4.2 Measurement

Loosely pack the soil under investigation (e.g. 50 g to 300 g) into the reaction vessel. Introduce sufficient quantities of absorption material into the absorption vessel. Close the reaction vessel air-tight by connecting the manometer and incubate for the specified time period in the dark at the required constant temperature. The pressure changes in the first 3 h may be caused by temperature equilibration and are usually not taken into consideration, unless the equipment has been assembled under thermostatted conditions.

5.6.5 Evaluation

5.6.5.1 Calculation of the free gas volume

Calculate the free gas volume according to equation (11).

$$V_{\mathrm{fg}} = V_{\mathrm{tot}} - V_{\mathrm{av}} - V_{\mathrm{am}} - V_{\mathrm{sm}} \qquad (11)$$

where

V_{fg} is the free gas volume, in litres;

V_{tot} is the total volume of the measuring vessel without soil, adsorption vessel and absorption material, in litres;

V_{av} is the volume of the absorption vessel, in litres;

V_{am} is the volume of the absorption material, in litres;

V_{sm} is the volume of the moist soil, in litres.

5.6.5.2 Calculation of the mass of dry soil

Calculate the mass of dry soil according to equation (12).

$$m_{sd} = m_{sm} \cdot w_{sd} \quad (12)$$

where

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

m_{sm} is the mass of moist soil, in kilograms;

w_{sd} is the dry mass fraction of the moist soil sample (see Note in 5.2.5).

5.6.5.3 Calculation of the rate of O_2 consumption

Calculate the rate of O_2 consumption according to equation (13).

$$R_{O_2} = 32\,000 \; V_{fg} \cdot \Delta p / (t \cdot R \cdot T \cdot m_{sd}) \quad (13)$$

where

R_{O_2} is the rate of O_2 consumption on a soil dry mass basis (mg $O_2 \cdot g^{-1} \cdot h^{-1}$);

32 000 is the molar mass of oxygen (mg/mol);

V_{fg} is the free gas volume, in litres, from equation (11);

R is the gas constant (83,14 hPa $l \cdot mol^{-1} \cdot K^{-1}$);

T is the measuring temperature, in kelvins);

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

Δp is the measured pressure reduction, in hectopascals;

t is the elapsed time, in hours.

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