Food and Agriculture Organization of the United Nations

Standard operating procedure for soil respiration rate

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GLOBAL SOIL LABORATORY NETWORK

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Standard operating procedure for soil respiration rate

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SOIL RESPIRATION RATE

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1. Introduction

Soil respiration is one of the longest established and most frequently used parameter for quantifying microbial activity in soils (Kieft and Rosacker, 1991). It is defined as oxygen (O_2) uptake or carbon dioxide (CO_2) evolution by soil microorganisms and includes the gas exchange of aerobic and anaerobic metabolism (Anderson, 1982). Soil respiration results from the mineralization of organic matter by soil micro- and mesofauna in which the organic compounds are oxidized to carbon dioxide and water, with simultaneous uptake of oxygen for aerobic microorganisms. In natural, undisturbed soils (with no nutrient or organic material addition), there is an ecological balance between soil micro- and mesofauna and their activities. Respiration is then called "basal respiration" which is defined as respiration without the addition of carbon (C)-containing substrate. On the other hand, substrate-induced respiration (SIR) is soil respiration measured following the addition of a C-containing substrate such as sugars, organic acids or amino acids and it used as measure of soil microbial biomass.

By changing the natural soil system, such as through disturbance or the addition of organic matter, it is possible to observe a change in soil respiration due to higher C mineralization by soil microorganisms and, at longer timescales, a change in microbial growth rates. This increase in soil respiration is characterized by several phases: an initial phase, an acceleration phase, an exponential phase, a delay phase, a stationary phase and a decreasing phase (Freytag, 1977). Substrate addition can also lead to a priming effect (an accelerated decomposition of native soil organic matter). Estimating soil respiration periodically or seasonally in terms of CO_2 evolution from soil is a powerful tool in such studies since it can be used as a measure of total soil biological activity per unit time (day, month, and season).

From an analytical point of view, soil respiration is influenced by soil moisture, temperature, the availability of nutrients and soil structure (including human-made soil amendments [such as pollution, fertilizers, and chemical fungicides, pesticides and herbicides]). Air-drying reduces soil respiration significantly. Remoistened soils, however, typically result in very high initial soil respiration rates, resulting from the increased release and mineralization of easily degradable organic compounds such as amino and organic acids caused by chemical and physical processes during the remoistening of dry soils (Clark and Kemper, 1967; Anderson and Domsch, 1978a; Anderson and Domsch, 1978b; Wilson and Griffin, 1975; Kowalenko, Ivarson and Cameron, 1978; Alef and Kleiner, 1986; Kroeckel and Stolp, 1986; Kieft, Soroker and Firestone, 1987). A period longer than ten days of pre-incubation at 25 °C and 40–50 percent water holding capacity is recommended when air-dried soil samples are used (Franzluebbers *et al.,* 1996).

2. Scope and field of application

The off-site measurement indicates microbial activity, which is an important indicator of soil health and the sustainability of soil management (FAO-ITPS, 2020). Soil respiration rate allows for the estimation of nutrient cycling in the soil and the ability of the soil to sustain plant growth and biological activity. Moreover, this parameter can be used as an indication of the turnover of nutrients contained in organic matter to forms available for plant uptake.

3. Principle

The method is based on the respiratory activity of soil microbial populations in absence of an excess of a carbon and energy source. It is an analytical method that integrates the biological response of the soil with the physicochemical environment. The soil is incubated in a closed vessel while the CO_2 is measured. The conventional method to determine CO_2 is an acid-base titration, where the CO_2 released during respiration in soils is trapped in an alkaline solution (sodium hydroxide [NaOH]) and measured as an index of soil respiration:

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

The amount of CO_2 adsorbed is equivalent to the amount of NaOH consumed. To determine this, the carbonate CO_3^{2-} is precipitated with barium chloride ($BaCl_2$) and the remaining NaOH is titrated with standardized hydrochloric acid (HCl).

The amount of NaOH initially present minus the amount remaining at the end of the incubation period is used to calculate the amount of CO_2 released from the soil.

4. Apparatus

The apparatus needed is as follows:

- drying oven;
- jars or preserve flasks with 1 L content with a rubber ring and peg, or wide-mouth flasks (250 mL content) with screw caps and pout rim;
- incubator adjustable to 25 °C;
- equipment for titration;
- CO_2 absorption tubes/50 mL beaker;
- plastic beakers;
- glass burette with a precision of 0.01 mL or digital burette; and
- magnetic stirrer.

5. Materials

The materials needed are as follows:

- Deionized water or distilled water. The water should have a specific conductivity not higher than 0.2 mS/m at 25 °C and a pH greater than 5.6 (or grade 2 water or type II water according to ISO 3696:1987 [ISO, 1987] and ASTM D1193-06 [ASTM International, 2018], respectively, if this is the quality of water produced in the laboratory).
- For NaOH (0.05 M): dissolve 2 g of NaOH with deionized water in a final volume of 1 L.
- For NaOH (1 M): dissolve 19.95 g of NaOH with deionized water to a final volume of 500 mL.
- Potassium hydroxide (KOH) (0.5 M). Dissolve 28.05 g of KOH with deionized water to a final volume of 1 L.
- $BaCl_2$ (0.5M). Dissolve 104.2 g of $BaCl_2$ with deionized water to a final volume of 1 L.
- Phenolphthalein ($C_{20}H_{14}O_4$). Dissolve 0.1 g of phenolphthalein in 80 mL of ethanol (C_2H_6O, 95%) with deionized water and add sufficient water to a final volume of 100 mL.
- HCl (0.05 M). Dilute 0.83 mL 6 M HCl (or 4.3 mL conc. HCl, 37 percent or 12 M) with deionized water or distilled water to a final volume of 100 mL.

6. Health and safety

Personal safety aids should be used, such as laboratory coats, protective gloves, safety glasses, face shields and proper footwear.

Waste should never be disposed of in the laboratory sink. Collect in a metal or PVC container for proper disposal at the specified places and in the manner described in national legislation for waste disposal.

Acids and other chemicals must be labelled properly (especially solutions containing $BaCl_2$), indicating their hazardous nature. In the preparation of HCl solutions, remember to add the acid to the water and not the opposite.

7. Sample collection and storage

At each location, five sampling points should be created: one at the georeferenced centre of the location, and one at each of the four cardinal points (north, east, south and west), 15 m from the centre. Make sure all sampling points have the same soil type and management history. Within one square metre of each sampling point, collect five soil cores from the topsoil, at 0–10 cm depth. Combine all five cores in a plastic container or resealable bag and mix well, removing roots and large

stones. Label the container or bag and prepare it for transport. For each location, you should have five labelled samples, each consisting of five mixed soil cores.

It is highly recommended that several composite samples are taken at the same time to be analysed separately in the laboratory (e.g. triplicates). It is important to specify the sampling depth in the analysis report.

Once taken from the field, the samples should be kept at the field temperature. If needed, a cool box could be used. It is also important to leave samples in the sampling container so that they do not dry out excessively. Avoid exposing the samples to direct sunlight, as this could change the balance of the microbial community. To avoid compromising the results, do not store samples for more than two to three days before analysis.

8. Sample preparation

The sample preparation is as follows:

1. Sieve soil through a 2 mm mesh.
2. After the moist sample has passed through the sieve, it must be mixed again thoroughly.

9. Procedure

The procedure is as follows:

1. Weigh 20 g soil into a beaker and place it in the bottom of a jar.
2. Measure into another beaker either 20–25 mL NaOH (0.05 M), 10 mL NaOH (1 M), or 9 mL KOH (0.5 M) and place it the bottom of the jar.
3. Immediately close the jar, ensuring it is airtight by using a rubber ring and two crossing pegs.
4. Determine the moisture content of the soil.
5. For the blanks, use three to five jars with the same chemical base but without soil.
6. Incubate all jars for three days at 25 °C (longer incubation periods could result in anaerobic conditions).
7. Open the jars, remove the beaker with the trap solution (NaOH or KOH) and wash the internal surfaces of the beaker with CO_2-free water to bring all trap solution to the bottom of the beaker.

8. Add 5 mL barium chloride (0.5 M) and three or four drops of the phenolphthalein indicator.
9. Add HCl (0.05 M, dropwise) under continuous stirring until the colour changes from red to colourless.

10. Calculation

Back-calculate the CO_2 release from soil by the results from the titration as follows:

$$\text{Soil respiration SR (three days)} = \frac{(B - S) \times E}{w}$$

where:

SR = mg CO_2/g dry soil;
B = average HCl volume used in the titration of the blanks (mL);
S = HCl volume used in the titration of the sample (mL);
w = weight of dry soil (g); and
E = CO_2 equivalent (In this case 1:1 conversion factor due to HCl 0.05 M).

11. Quality assurance/quality control

When a special reagent is used (for example, a reference material for equipment control), consult the material safety data sheet (MSDS) and conduct a risk assessment.

A minimum of three blank replicates should be included in each setting. The acceptance requirements for precision testing must be defined by the equipment used, environmental conditions, and other testing factors and by the specifications or requirements for the information use and agronomic criteria.

If the precision test fails, the cause of the failure must be identified, and corrective or preventive actions must be developed.

11.1. Precision test

Samples should be analysed in duplicate or triplicate. Calculate the percentage of relative standard deviation (%RSD) to determine precision:

$$\%RSD = \frac{s}{\bar{x}} \times 100$$

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where:
s = standard deviation
$\bar{x}$ = mean

If the values of %RSD for each sample is larger than 10 percent, then the sample must be retested.

The control chart is plotted from the analytical value of control sample.

11.2. Internal quality control

One internal reference sample per batch of 11 samples must be analysed to assure the quality control by means of a Z-score chart. If the difference between the daily values and the accumulated mean of the internal reference sample is larger than 3 × standard deviation, reject the daily values. If the problems recur on successive days or batches, carry out an evaluation to identify the core cause of the problem, and develop corrective and preventive actions (see GLOSOLAN basic guidelines on how to prepare a sample for internal quality control).

11.3. Proficiency tests

For external quality control, the laboratory must participate in a proficiency test at least once a year (also called “interlaboratory comparisons” or “ring tests”). The PT z-score should be less than two. If not, identify the root cause, and develop corrective and preventive actions.

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Appendix I: acknowledgments

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Appendix II: list of authors

Leading author:

- Rosalina Gonzalez, Laboratorio de Suelos Unisalle, **Colombia**

Main authors (in alphabetical order):

- Daniel Carreira, Laboratorio del Instituto de Suelos CIRN-CNIA-INTA, **Argentina**
- Miriam Ostinelli, Laboratorio del Instituto de Suelos CIRN-CNIA-INTA, **Argentina**
- Erich Inselsbacher, Institute for Soil Research, University of Natural Resources and Life Sciences (BOKU-IBF), **Austria**
- Gina Nilo, Bureau of Soils and Water Management Laboratory Services Division (BSWM-LSD), **Philippines**
- Jamie Ann Tumolva, Bureau of Soils and Water Management Laboratory Services Division (BSWM-LSD), **Philippines**
- Aurora Manalang, Philippines, Bureau of Soils and Water Management Laboratory Services Division (BSWM-LSD), **Philippines**
- Thembinkosi Mbedzi, Department of Soil Science and Environment, University of Zimbabwe (SSE-UZ), **Zimbabwe**

Members of the review panel:

From the SOP working group:

- João Coutinho, Laboratório de Solos e Plantas – University of Trás-os-Montes and Alto Douro, **Portugal**

From the GLOSOLAN Technical Committee (in alphabetical order):

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- Wu Huijun, Institute of Agricultural Resources and Regional Planning (IARRP) of Chinese Academy of Agricultural Sciences (CAAS), **China**
- Abdul Kader, School of Agriculture and Food Technology, the University of the South Pacific, **Samoa**

From the International Network on Soil Biodiversity (NETSOB):

- Monique Carnol, Plant and Microbial Ecology, University of Liège, **Belgium**
- Nishita Ivy, Nalanda University, **India**
- Isabel Campos, University of Aveiro, **Portugal**
- Knowledge Mushonga, University of Pretoria, **South Africa**
- Marisa Phiromtan De Bels, Loei Rajabhat University, **Thailand**
- Kusum Naithani, University of Arkansas-Fayetteville, **United States of America**

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- George Gardner Brown, Empresa Brasileira de Pesquisa Agropecuária (Embrapa), **Brazil**
- Ieda de Carvalho Mendes, Empresa Brasileira de Pesquisa Agropecuária (Embrapa), **Brazil**

Appendix III: contributing laboratories

The Global Soil Laboratory Network would like to thank the following laboratories for completing the GLOSOLAN form on the method and providing information on their standard operating procedure for soil respiration rate. This information was used as a baseline for the global harmonization.

From the African region:

- National Agricultural Soil Laboratories– KALRO Soil Labs, **Kenya**
- Federal College of Land Resources Technology, **Nigeria**

From the Asian region:

- Soil Laboratory, Royal University of Agriculture, **Cambodia**
- Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences (CAAS), **China**
- Laboratorium Pengujian, Balittanah (Soil Test Laboratory, Indonesian Soil Research Institute), **Indonesia**
- Bureau of Soils and Water Management Laboratory Services Division (BSWM-LSD), **Philippines**

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- Division of Soil Biotechnology, Land Development Department, **Thailand**

From the European region:

- Central Institute for Supervising and Testing in Agriculture, **Czechia**
- Research Group Soil Quality Assessment, Universidade de Santiago de Compostela, **Spain**
- Ankara University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, **Türkiye**
- Ministry of Agriculture and Forestry - Soil, Fertilizer And Water Resources Central Research Institute, **Türkiye**

From the Latin American region:

- Laboratorio del Instituto de Suelos CIRN-CNIA-INTA, **Argentina**
- Instituto Geográfico Agustín Codazzi – IGAC, **Colombia**
- Unidad Científico Técnica de Base (UCTB) de Suelos Camaguey, **Cuba**
- Laboratorio de Análisis de Suelos y Agua - Centro de Investigaciones en Geografía Ambiental UNAM, **Mexico**
- Laboratorio de suelos y agua comandante Fidel Castro Ruz/INTA, **Nicaragua**

From the Near East and North African region:

- KIMIA AB Environmental and Agricultural Consulting Laboratory, **Islamic Republic of Iran**
- Soil Characterization Lab - American University of Beirut, **Lebanon**

From the Pacific region:

- Environmental Chemistry Laboratory, Manaaki Whenua - Landcare Research, **New Zealand**
- School of Agriculture and Food Technology - The University of the South Pacific, **Samoa**

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