Food and Agriculture Organization of the United Nations

Standard operating procedure for soil respiration rate

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Standard operating procedure for soil respiration rate

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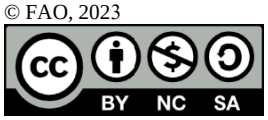

SOIL RESPIRATION RATE

VERSION HISTORY

N°	Date	Description of the modification	Type of modification
01	12 January 2023	All comments by RESOLANs and reviewers to the draft SOP were addressed	Finalization of the SOP
02			
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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 (no nutrient or organic material addition), there is an ecological balance between soil micro- and mesofauna and their activities. The respiration is then called “basal respiration” which is defined as the respiration without the addition of carbon(C)-containing substrate. On the other hand, substrate-induced respiration (SIR) is the 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, e.g. 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, an acceleration, an exponential, a delay, a stationary and a decreasing phase (Freytag, 1977). Substrate addition can also lead to a priming effect, i.e. an accelerated decomposition of native soil organic matter. Estimating soil respiration periodically/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 (pollution, fertilizers, and chemical fungicides/pesticides/herbicides). Air-drying reduces soil respiration significantly. Remoistened soils, however, typically results in very high initial soil respiration rates, as a result of an 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, 1978; Wilson and Griffin, 1975; Kowalenko *et al.,* 1978; Krockel and Stolp, 1986; Kieft *et al.,* 1987), being recommend a period longer than ten days of pre-incubation at 25 °C and 40-50 percent water holding capacity when air-dried soil samples are used (Franzluebbers *et al.,* 1996).

2. Scope and field of application

The off-site measurement indicates the microbial activity, which is an important indicator of soil health and 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 (in 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

- drying oven
- jar or preserve flasks 1 L content with a rubber ring and peg or wide-mouth flasks (250 mL content) with screw caps and pout rim.
- incubator adjustable at 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
- magnetic stirrer

5. Materials

5.1 Deionized water/distilled water: it 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) and ASTM D1193-06 (2018), respectively, if this is the quality of water produced in the laboratory);

5.2 NaOH 0.05M: dissolve 2 g of NaOH in a final volume of 1 L with deionized water; NaOH 1 M: dissolve 19.95 g of NaOH in a final volume of 500 mL with deionized water; potassium hydroxide (KOH) 0.5 M: dissolve 28.05 g of KOH in a final volume of 1 L with deionized water.

5.3 $BaCl_2$ 0.5M: dissolve 104.2 g of $BaCl_2$ in a final volume of 1 L with deionized water.

5.4 Phenolphthalein ($C_{20}H_{14}O_4$). Dissolve 0.1 g of phenolphthalein in 80 mL of ethanol (C_2H_6O, 95%) and add sufficient water to a final volume of 100 mL with deionized water.

5.5 HCl 0.05 M: dilute 0.83 mL 6 M HCl (or 4.3 mL conc. HCl, 37% or 12 M) to a final volume of 100 mL with deionized water/distilled water.

6. Health and safety

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

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

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

7. Sample preparation

7.1 Take moist samples;

7.2 The soil is sieved through a 2 mm mesh;

7.3 After the moist sample has passed through the sieve, it must be mixed again thoroughly;

7.4 The moist soil samples can be stored for 10 days at 20-25 (Anderson and Domsch, 1978);

7.5 For longer periods of storage, the soil samples should be air-dried.

8. Procedure

8.1 For air dried samples, the soils need to be remoistened and pre-incubated for a period of ≥ 10 days at 25 °C and 40-50 percent water holding capacity, and the determination should be carried out in duplicate or triplicate;

8.2 Weigh 20 g soil into a beaker and place it in the bottom of a jar;

8.3 Measure into another beaker either 20-25 mL NaOH (0.05 M), or 10 mL NaOH (1 M) or 9 mL KOH (0.5 M) and place it the bottom of jar;

8.4 Immediately close the jar airtight using a rubber ring and tow crossing pegs;

8.5 Determine the moisture content of the soil;

8.6 For the blanks, use three to five jars with the same chemical base but without soil;

8.7 Incubate all jars during three days at 25 °C (longer incubation periods could result in anaerobic conditions);

8.8 Open the jars, take out 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;

8.9 Add 5 mL barium chloride (0.5 M) and 3-4 drops of the phenolphthalein indicator;

8.10 Add HCl (0.05 M, dropwise) under continuous stirring until the colour changes from red to colourless.

9. Calculation

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

$$\text{Soil Respiration SR (3 Days)} = \frac{(B - S) \times E}{w}$$

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)
E = CO_2 Equivalent (In this case 1:1 conversion factor due HCl 0.05 M)

10. 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.

10.1. Precision test

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

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

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

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

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

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10.2. Internal quality control

One internal reference sample per batch of 11 samples must be analyzed to assure the QC by means of 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).

10.3. Proficiency tests

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

11. Reference documents

Alef, K., & Kleiner, D. 1986. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. *Soil Biology and Biochemistry*, 18(2): 233–235.

Anderson, J.P.E. & Domsch, K.H. 1978. Mineralization of bacteria and fungi in chloroform-fumigated soils. *Soil Biology & Biochemistry*, 10: 207–213.

Anderson, J.P.E. & Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry*, 10: 215–221.

Anderson, J.P.E. 1982. Soil respiration. In: A.L. Page, R.H. Miller, D.R. Keeney, eds. *Methods of soil analysis, part 2. Chemical and* microbiological properties, 2nd edition, pp. 831–871. Madison, Wisconsin, American Society of Agronomy.

ASTM International. 2018. Standard Specification for Reagent Water (D1193-06). West Conshohocken, USA.

Clark, F.E. & Kemper, W.D. 1967. Microbial activity in relation to soil water and soil aeration. *Agronomy*, 11: 472–480.

FAO & ITPS. 2020. *Protocol for the assessment of Sustainable Soil Management*. Rome, FAO. https://www.fao.org/fileadmin/user_upload/GSP/SSM/SSM_Protocol_EN_006.pdf

Franzluebbers, A.J., Haney, R.L., Hons, F.M., & Zuberer, D.A. 1996. Active fractions of organic matter in soils with different texture. *Soil Biology and Biochemistry*, 28(10-11): 1367–1372.

Freytag, H.E. 1977. Analyse von Bodenatmungskurven. *Zentralbl Bakt Abt II*, 132: 452–460.

ISO. 1987. Water for analytical laboratory use. Specification and test methods (3696). Geneva, Switzerland.

Kieft, T.L. & Rosacker, L.L. 1991. Application of respiration- and adenylate-based soil microbiological assays to deep subsurface terrestrial sediments. *Soil Biology & Biochemistry,* 23: 563–568.

Kieft, T.L., Soroker, E. & Firestone, M.K. 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology & Biochemistry,* 19: 119–126.

Kowalenko, C.G., Ivarson, K.C. & Cameron, D.R. 1978. Effect of moisture content, temperature, and nitrogen fertilization on carbon dioxide evolution from field soils. *Soil Biology & Biochemistry*, 10: 417–423.

Wilson, J.M. & Griffin, D.M. 1975. Water potential and respiration of microorganisms in the soil. *Soil Biology & Biochemistry*, 7: 199–204.

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Appendix I – Acknowledgment

GLOSOLAN would like to thank Ms Rosalina Gonzalez from Colombia for leading the harmonization of this SOP and the members of the working group that served as leaders for their regions and contributed to the writing of this SOP (appendix II). GLOSOLAN would also like to thank Mr João Coutinho and the other experts who were part of the Review Panel and who ensured the finalization of the SOP, all the laboratories that provided inputs for the harmonization of this method, all the experts from the GLOSOLAN Technical Committee and the International Network on Soil Biodiversity (NETSOB) who technically reviewed this SOP.

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, University of Natural Resources and Life Sciences - Institute for Soil Research (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 - Universidade de Trás-os-Montes e Alto Douro, **Portugal**

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From the GLOSOLAN Technical Committee (in alphabetical order):

- Wu Huijun, Institute of Agricultural Resources and Regional Planning (IARRP) of Chinese Academy of Agricultural Sciences (CAAS), **China**
- Md. 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**

Appendix III – Contributing laboratories

GLOSOLAN thanks 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**
- 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**

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- 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**

The Global Soil Partnership (GSP) is a globally recognized mechanism established in 2012. Our mission is to position soils in the Global Agenda through collective action. Our key objectives are to promote Sustainable Soil Management (SSM) and improve soil governance to guarantee healthy and productive soils, and support the provision of essential ecosystem services towards food security and improved nutrition, climate change adaptation and mitigation, and sustainable development.

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GLOSOLAN is a Global Soil Laboratory Network which aims to harmonize soil analysis methods and data so that soil information is comparable and interpretable across laboratories, countries and regions. Established in 2017, it facilitates networking and capacity development through cooperation and information sharing between soil laboratories with different levels of experience. Joining GLOSOLAN is a unique opportunity to invest in quality soil laboratory data for a sustainable and food secure world.

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