TOOL WORKSHEET NO.5

Oxitop® respirometric

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DESCRIPTION OF THE INDICATOR

Name of the indicator: Determination of soil respiration using the Oxitop® automated system.

Ecological role of the tested indicator: Respiration is a universal process that enables living cells, whether they be animal, plant or microbial, to meet their energy needs. There are several types of respiration mechanisms, but the most usual and best publicly-known is the aerobic respiration of chemoorganotrophic organisms. It consists in oxidising organic matter, which serves as a source of electrons and energy, and to channel electrons in a respiratory chain until a final acceptor, in this case oxygen. At the end of this process, mineralisation products (oxidation) of organic matter (CO_2) and of oxygen reduction (H_2O) are rejected by the organism. Respiratory activity can therefore be measured by quantifying the CO_2 produced or the O_2 consumed.

The first soil respiration measurements were conducted 60 years ago, which makes it one of the oldest techniques implemented to quantify biological activities in soils. This activity can stem from the energetic metabolism in roots, but it is generally accepted that respiration of microorganisms prevails. Soil respiration is commonly used as an indicator of the impact of agricultural practices or chemical inputs (e.g. pesticides, metals, HAP) on the biological functioning of soils.

Type of indicator: Bioindicator of effect: soil respiration is measured on a periodic basis in a sealed jar using an Oxitop® autonomous pressure device. Contrary to other respirometric techniques, the Oxitop indicator gives a kinetic vision of respiration through consumption in oxygen by soil microorganisms. During incubation, CO_2 is trapped by soda and can be quantified at the end of experimentation by chemical titration.

DESCRIPTION OF THE METHODE

Reference standards and/or protocols

The Oxitop® control system is a trade mark (WTW, Weilheim, Germany) that allows the continuous measurement of microbial respiration of soils. The systems and its measurement protocols are available from the provider and distributors. They have been subjected to standardisation procedures; ISO 16072:2002 and DIN 19 737 Standards.

Sampling plan and method: Depending on the goal of the study, sampling strategies and techniques can greatly vary. For full details of these various techniques, see “Manual of Soil Analysis. Monitoring and Assessing Soil Bioremediation” R. Margesin, F. Schinner (Eds.) Springer-Verlag, Berlin Heidelberg 2005 (366 pp). During the Ademe Bioindicateurs Program, soils were sampled at a 15 cm depth, and then sieved to 2 mm at the laboratory before analysis. As for forest soils, O horizons were ruled out prior to sampling. A common sampling strategy was applied to all the plots in the program. For each modality, an average composite sample was prepared by randomly collecting 9 elementary sub-samples and by mixing them together. Four replicates of composite samples were prepared for each modality (plot). After collection, samples were temporarily stored at 4°C (ice compartment) then sent as briefly as possible to the laboratory.

Sampling storage and pre-treatment: After they are received at the laboratory, samples are stored at 4°C and analysed as quickly as possible and for a period of time no longer than 3 months. Other procedures are available, should one prefer to store samples for longer before the analyses: freezing at -20°C (max. 1 year); freezing at -80°C or liquid nitrogen (max. 10 years). For more details, see NF ISO 10381-6 Standard "Soil quality - Sampling - Part 6: Guidelines for the collection, handling and storage, under aerobic conditions, of soils for the laboratory evaluation of microbial processes, biomass and diversity".

Simplified description of the measurement method: The measurement of soil respiratory activity is conducted using the OxiTop® control system. This system differs from traditional end-point incubation as it allows an automatic and continuous monitoring of the oxygen consumption of a sample. Equipment is made of a jar (B6M model) with a sealing system hermetic to ambient air, a test head to record pressure variations, and an Oxitop® OC 110 controller for the recording and the retrieval of data by infrareds (Figure 1). Incubation is realised in a thermoregulating cabinet with a resolution no higher than 0.1°C.

Two types of respiration can be measured on a soil sample: basal respiration and induced respiration. Basal respiration refers to soil respiration without the addition of organic matter. Substrate-induced respiration (SIR) refers to soil respiration after the addition of a source of C easy to metabolise for microorganisms, most often glucose.

Respiration is measured by following the curb of O_2 pressure in a sealed jar containing soil; CO_2 is trapped by soda. The pressure curb makes it possible to determine the amount of O_2 consumed during respiration. In practice, 20 g of fresh soil or soil with water content from 50 to 70% of its field capacity are inserted in the jar. It is tightly sealed with a joint, silicone grease and brackets. Pressure variations are continuously measured during 3 days at a constant temperature of 20°C. After 3 days, data is downloaded and a linear model is calculated in order to establish the exact gradient of loss of pressure in the jar. Results are expressed in mg of consumed O_2 / day per gram of dry soil using the formula:

:

$$SR = \frac{M(O2).Vfr\,.\,dP}{R\,.T\,.\,M(DS).Xd}$$

where SR: soil respiration; M(O_2): molecular mass of O_2; Vfr: free volume; dP: pressure difference; R: perfect gas constant; T: Kelvin temperature; M(DS): dry soil mass; Xd: number of days.

At the end of experimentation, the amount of CO_2 liberated can also be quantified by titration if the CO_2 trap contained a 0.2 N NaOH solution.

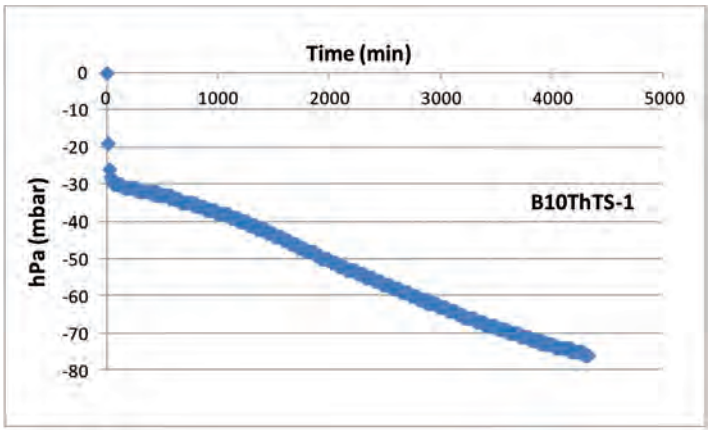

Figure 1. Left: Oxitop system with, from top to bottom, the recording head of pressure, the joint + brackets, the glass jar, the CO_2 trap, the soil sample. *Right:* example of depression kinetic obtained by soil microorganisms' O_2 consumption; the initial drop corresponds to a systematic artefact resulting from the time needed for temperature stabilisation in the jar.

EXAMPLE OF APPLICATION - INTERPRETATION OF RESULTS

Need for a global reference system using a database

Despite being practical and easy-to-use, Oxitop technology is still relatively little-used in fundamental studies of soil science communities. It is however more widely used in research firms and in applied research on degradability of xenobiotics. The Bioindicateurs program unveiled a first frame of reference for data variability of soil respiration under varied conditions (Figure 2). The yellow box plots refer to agricultural practices, the green ones, forest soils, and the red ones, soils polluted by metals or HAP.

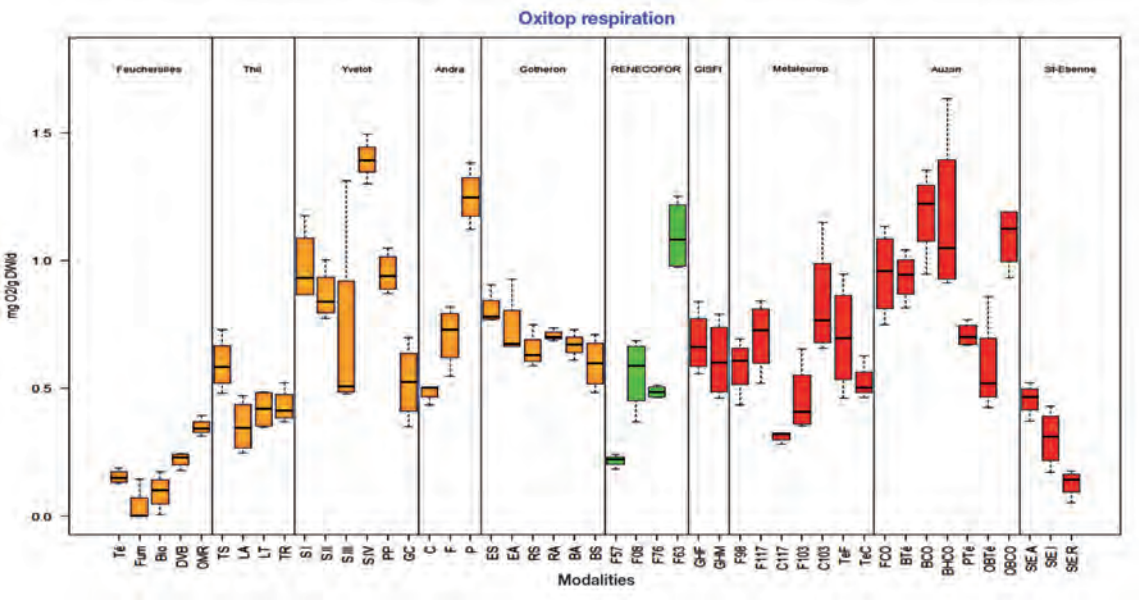

Figure 2: Multi-site comparison of the OxiTop® respiration indicator

Database availability/access

The Oxitop® respiratory activity values are indexed in the "Bio 2 BD" database, hosted by the Ecobiosol site of the University of Rennes 1: http://ecobiosol.univ-rennes1.fr. The physic-chemical characteristics of soils as well as the levels of pollution or the agricultural practices they are exposed to are also available. Access to the database is subject to prior authorisation.

Necessary supplementary information (ex: climate, use, type of soil...)

If soil respiration is sensitive to management methods and chemical inputs, it also depends on environmental natural factors. It is therefore essential to have data relative to physic-chemical properties of soils and climatic conditions. In fact, specific variables, such as content in organic matter, can significantly influence respiration and be confounding factors of the effect of a disruption. To compensate for this ambiguity, it is necessary to be able to distinguish between the disruption-caused variance and the variance due to intrinsic properties of soils. (Floch et al., 2009).

Floch C., Capowiez Y, Criquet S. (2009) Enzyme activities in apple orchard agroecosystems: How are they affected by management strategy and soil properties. Soil Biology and Biochemistry 41, 61-68.

INTERESTS AND LIMITS OF THE INDICATOR

+ Having a kinetic vision of soil respiration and not an end point.
+ Very easy use and accessible to everyone.
+ Possibility to titrate CO2 at end point.
+ Possibility to use jars with lateral tubular openings to allow the addition of ecotoxics and monitor their effects on respiration.
+ Very low costs of functioning.

– Acquisition cost.
– It is essential that soils be not saturated with water to avoid anaerobiosis. Adequate water content should be determined beforehand.
– Need for more data to calibrate it in regard to other respirometric techniques.

PARTICIPANTS

The following people were involved in this study: Virgile Calvert, Stéven Criquet, Vincent Poujol

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