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Short communication

Cheap and portable lab-free respiration assay

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ABSTRACT

A simple tool that can be set up just using agarose, cotton wool, a plastic test tube and a pH indicator are presented as a method to measure soil respiration and record its kinetics in time. The principle is based on the fact that CO_2 produced by respiration dissolves in the water of the gel and acts as an acid, lowering the pH in proportion to its intensity. Both basal and substrate-induced respiration can be measured and compared by wetting the soil with either water or glucose solution. The assay is done with 3 g of air dried soil and only requires a daily visual inspection of the progressively changing color of the agarose gel in the tube. It is also amenable to measures to be followed away from the lab (at home, in the field, during expeditions, exploratory campaigns etc.). The low cost and versatility of the principle allows to carry out environmental research monitoring in any country irrespective of laboratory facilities or complex supplies. The result is proportional to soil vitality, fertility and overall attitude to be responding to stimuli or to indicate pollution and overall impacts affecting its properties. Examples of respiration in organic farms soils vs. conventionally managed ones are shown.

The measurement of soil respiration, tightly linked to the assessment of soil microbial activity and biomass, has been pursued since the early seventies, starting from pioneering works that established the response to nutrient supplementation and the measurements of respiratory increase, as defined by the substrate induced respiration (Jenkinson and Powlson, 1976; Anderson and Domsch, 1973). As regards the quantification of microbial biomass the reference assay is based on the prior fumigation of soil (Anderson and Domsch, 1978). Subsequent work has introduced the concept of microbial metabolic quotient (Anderson and Domsch, 1993). The idea of linking the acidifying effect of the evolved CO_2 to an estimation of soil respiration, from which the present report is inspired, comes from the work of Doran and coworkers that devised the Solvita soil test (Doran et al., 1997), which yielded a currently a commercialized application (https://solvita.com/soil/). An analogous application fitted for microtiter plates is the Microresp™ (Campbell et al., 2003).

The new protocol hereby introduced, conceived as simple and economic alternative to lab-based and other more complex CO_2 analyses, uses pH variation as direct function of the respiration rate. Since soil is within a closed tube of known volume, CO_2 dissolves into the agarose-water gel and dissociates into carbonic acid. This liberation of protons (H^+) lowers pH (Fig. 1). In practice a hot solution of 5 ml of 0.7% agarose in water at pH 7, with 1% Carlo Erba universal pH indicator dye is poured in the 13 ml graduated falcon tube, then a small piece of cotton is put as spacer and holder in order to sustain the unsieved air-dried soil sample (3 g) and to prevent the contact between soil and agarose solution. After that the tube is accurately closed and incubated, observing daily the pH color variation with the reference scale and taking note of the volumes assuming the different pH over time.

Bacteria and fungi in soil degrade organic matter and produce CO_2 at a rate depending on their number, on resource availability and on soil health (absence of intoxicating compounds), the void volume contains enough atmosphere to ensure aerobic respiration for the 3 g of soil incubated. CO2 in the presence of water acts as a diprotic acid leading to the formation of H^+ and carbonate anion (CO_3^{2-}) with the following reactions:

$$H_2O + CO_{2(aq)} \Leftrightarrow H_2CO_3$$

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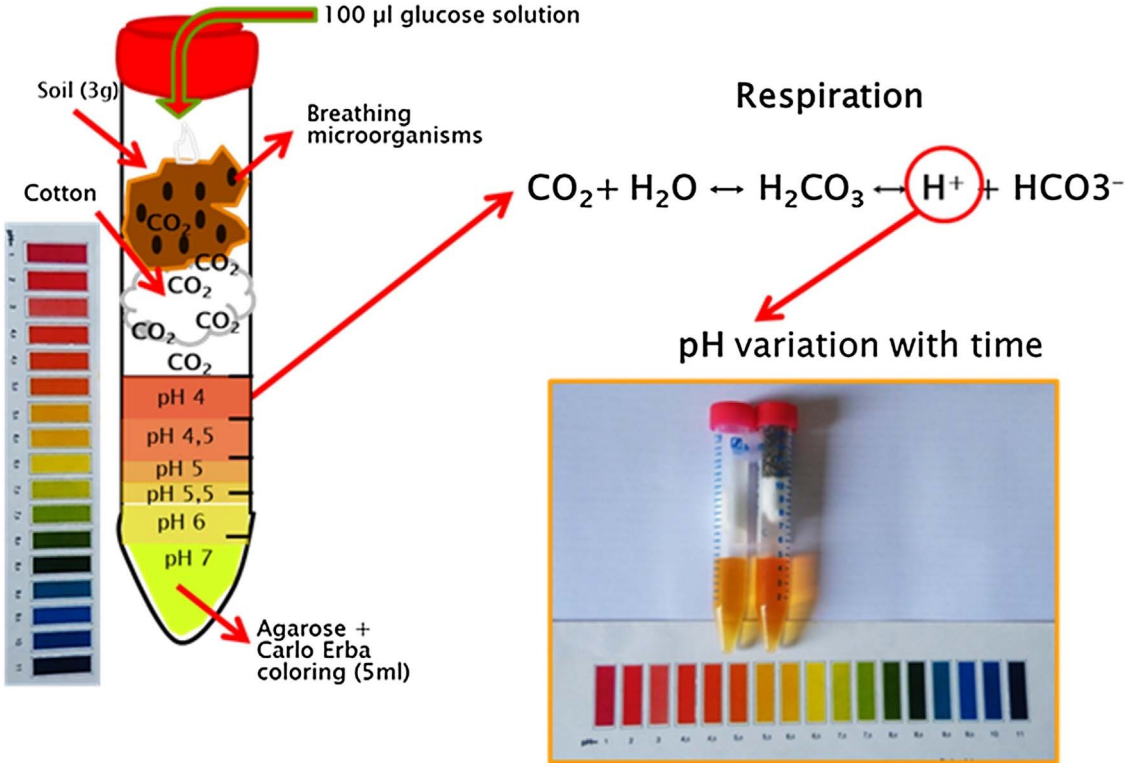

Fig. 1. Outline of the new method, based on pH variation with time.

$$H_2CO_{3(aq)} \Leftrightarrow H^+ + HCO_3^-$$

$$HCO_{3(aq)}^- \Leftrightarrow H^+ + CO_3^{2-}$$

The% presence of each of the formed ions is pH-dipendent (Fig. 2).

Thus the analysis relies on the calculation of the moles of protons [H^+] cumulated over time. This kinetics is the direct of the metabolic rate of the microorganisms present in those 3 g (dry weight) of soil.

Each pH value observed for each given volume (read on the graduated scale of the tube) is noted and converted into molar proton concentration with the following formulas of the chemical equilibrium:

$$pH = -log\,[H^+] \Rightarrow [H^+] = 10^{-pH}\left[moli/_l\right]$$

$$moli_{H^+} = [H^+]\frac{ml}{1000}$$

Once no further color changes are observed the graphs are plotted, reporting the number of proton moles cumulating over time. The initial number of protons present at the beginning (those pertaining to 5 ml of water at pH7) are subtracted and the Y axis of the plots reports the observed difference (dH^+) consisting in the net value of generated protons (Fig. 3).

The method allows simple application to soils of different types and also to deliberate experiments of addition of known amounts of water or glucose solution, allowing to perform also Substrate-Induced Respiration (S.I.R.) measurements, quantifying the respiration response to substrate supplementation efficiency of soil microorganisms being a quantitative estimation of CO_2 product by OM oxidation processes made by microbial community. S.I.R. is also a physiological method for estimation of the soil microbial biomass (Anderson and Joergensen, 1997; Stenstrom et al., 1998). The S.I.R. method principle is in fact is significantly correlated with Fumigation-Extraction (F.E.) method, which utilizes chloroform to kill the overall soil microflora in order to

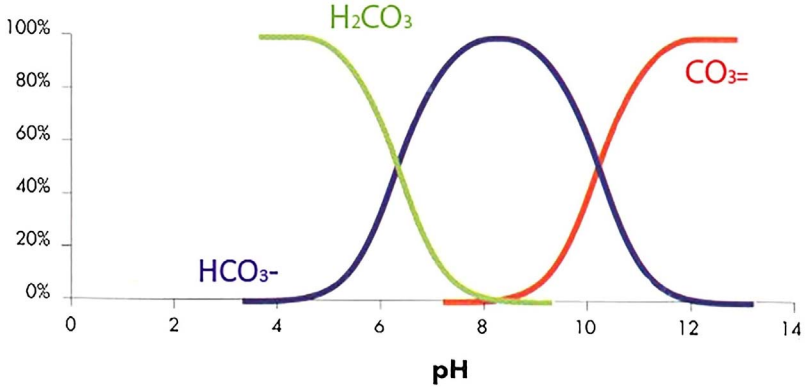

Fig. 2. Ions proportions upon pH change. The respective percentages of the three forms (carbonic acid, bicarbonate and carbonate ions) as a function of the solution pH are plotted.