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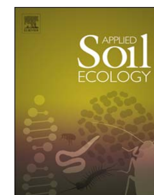

SituResp®: A time- and cost-effective method to assess basal soil respiration in the field

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ABSTRACT

The soil microbial activity is a key parameter in numerous studies aiming to assess soil quality in agricultural plots. Basal Soil Respiration (BSR) has been extensively used as an indicator of this soil microbial activity. However, available methods to measure BSR remain time- and labor- consuming and must be performed in the laboratory which may lead to result distortion due to the needed soil pre-treatments. The SituResp® method was developed to assess BSR in a time- and cost-effective way. This method was adapted from a laboratory methodology, the MicroResp™ method, in order to be implemented in the field on fresh soil samples. It is based on the color change of a pH-sensitive gel in reaction to the CO_2 concentration change in the headspace of a soil sample over the 24-h incubation. This study presents the calibration and validation of the SituResp® method in laboratory conditions, and a comparison in the field with the Solvita® tool, a comparable method used by agricultural scientists and advisors. The results of the calibration showed a high correlation between the air CO_2 concentration and the absorbance variation of the gel at 570 nm (R^2 = 0.95). The validation against the titration alkali-trap method, on 21 soil samples, showed a strong correlation between the two methods (R^2 = 0.90). In the field test on 9 agricultural-plots, the SituResp® method yielded similar results to the Solvita® tool. The SituResp® method is therefore a reliable method for performing a cheap, rapid but efficient assessment of soil microbial activity in the field which could be included in soil quality monitoring.

1. Introduction

Carbon dioxide (CO_2) emissions from the soil, also called soil respiration, are closely related to major soil ecosystem services such as primary production (Raich and Tufekciogul, 2000), climate regulation (Paustian et al., 2016), and nutrient cycling (Contosta et al., 2011). Soil respiration reflects a dynamic view of the ecosystem metabolism and provides information on the soil carbon transformation function (Gil-Sotres et al., 2005; Kibblewhite et al., 2008; Ryan and Law, 2005).

Soil respiration is a complex process that results from several sources of CO_2 in the soil (Kuzyakov, 2006). These sources can be grouped in two main ones: the autotrophic respiration linked to the activity of roots, and the heterotrophic respiration linked to the activity of soil microorganisms (Hanson et al., 2000; Epron, 2009). Basal soil respiration (BSR) is the main efflux of the heterotrophic respiration and is defined as the steady rate of CO_2 emissions linked to the microbial decomposition of soil organic matter in a root-free soil (Creamer et al., 2014; Kuzyakov, 2006).

For decades, BSR has been widely used as an indicator to monitor the impact of agricultural practices on soil biological activities (Elmholt, 1992). A recent survey on the most appropriate indicators for the monitoring of soil quality evolution showed that BSR is among the most used biological indicators of soil quality (Stone et al., 2016). BSR is often applied in combination with other indicators in multivariate

Abbreviations: BSR, Basal Soil Respiration; CV, Coefficient of Variation; DCR, Digital Score Reader; max, Maximum; min, Minimum; OD, Optical Density

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studies (Bastida et al., 2008; Obriot et al., 2016), such as the microbial biomass to indicate ecosystem disturbances (Insam and Haselwandter, 1989) or the labile pool of soil organic carbon to assess the potential of soil carbon sequestration (Hurisso et al., 2016).

Despite the importance of BSR in soil quality evaluations, there is still a lack of time- and cost-effective methods adapted to large-scale campaign of field monitoring. Most of the methods used to measure BSR are laboratory methods based on a four-step protocol: 1/the soil sampling and conservation, 2/the preparation of soil samples, 3/the incubation, and 4/the analysis of the CO_2 produced during the incubation. These laboratory-based methods have several advantages with respect to their high "technical factors" which make them appropriate to assess BSR (Stone et al., 2016). However, several methodological bottlenecks of these methods were stressed by Zornoza et al. (2007) and Swallow and Quideau (2015). Changes in soil temperature and water content during soil transportation to the laboratory and soil conservation may have considerable effects on the microbial activity. Soil sieving at 2 mm, as commonly done, may also remove large soil fractions thereby changing the soil microbial community structure and the size of the different carbon pools. These potential biases linked to the conservation and preparation of the soil samples may lead to some result distortion.

To our knowledge, Solvita-Field Test® (Haney et al., 2008) is the only method available for assessing BSR from fresh soil samples on-site. The US Department of Agriculture has included the Solvita-Field Test® method, further referred to as Solvita®, in its Soil Quality Test guide since 1999 (USDA, 1999). More recently, Solvita® was applied in scientific studies to characterize the impact of perturbations on the soil functions in various contexts (Muñoz-Rojas et al., 2016; Nkongolo et al., 2016; Ward et al., 2016, 2017). However, Solvita® is a commercial product with a selling price that might be a limiting factor to the large scale deployment of the method (Doran and Zeiss, 2000). In addition, the details of the Solvita® method, particularly the preparation of the gel tag and the calibration of the digital color reader (DCR) have not been published, so that the method cannot be reproduced without buying the license. So far, no method makes it possible to appraise the BSR through an easily and freely accessible proxy measured directly in the field, i.e. without the need of soil preparation, systematic spectrometer calibration and laboratory incubation.

Therefore, we developed a new method called SituResp® that provides an indication of the BSR for the purpose of soil quality monitoring. The ambition was to have a method that would be 1) cheap, 2) freely accessible, 3) rapid to prepare and perform; and 4) allowing for an on-site incubation and result reading. First, this paper presents the calibration and validation of the SituResp® method against a reference method in laboratory conditions. Second, it shows a comparison of the SituResp® method with the Solvita® method applied both in laboratory conditions and in agricultural plots. Third, the potential use of the SituResp® method within the framework of soil quality assessment is discussed.

2. Material and method

2.1. SituResp® principle

The SituResp® method aims to appraise the intensity of BSR but it is not a quantitative measurement of the actual soil CO_2 emissions. Indeed, the CO_2 emissions involve various complex variables (texture, moisture, temperature) that cannot be assessed easily (Ryan and Law, 2005). In the aim to develop a cheap on-site indicator of the soil microbial activity, an indication of potential BSR assessed promptly and repeatedly in the field is assumed to be sufficient.

The SituResp® method consists in a 24-h incubation in the field (at ambient field temperature) of a fresh soil sample coarsely sieved at only 5 mm and inserted in an airtight jar together with a pH-sensitive color gel filled in a 4.5 mL spectrophotometer macro-cuvette. The 24 -h incubation time coincide with the Solvita® test and allows for taking into account the same daily temperature variability. The detailed protocol is described in Appendix A in Supplementary materials. The color of the gel changes along the incubation process as the result of the reaction between bicarbonate in the gel and the CO_2 concentration in the headspace of the jar, which can be linked to CO_2 emitted from the soil (Rowell, 1995).

The change in the gel color is quantified by measuring the absorbance of the gel at 570 nm with a portable spectrophotometer (SpectroVis, Vernier Software and Technology, Beaverton, OR, USA) before (Abs_{T0}) and after (Abs_{T24}) the incubation process. The preparation of the gel strictly follows the MicroResp™ method (Campbell et al., 2003).

The output of the SituResp® measurement is the gel absorbance over the incubation time, which is an indicator of the potential BSR intensity. This absorbance difference is not converted into CO_2 emissions due to the high uncertainty in traducing this punctual absorbance into emissions without a more intensive and expensive measurement protocol (see Section 4).

2.2. Calibration

In order to establish the relative significance of the absorbance difference, a one-shot calibration was carried out in order to correlate the difference in the gel absorbance ($\Delta Abs = Abs_{T0}\text{-}Abs_{T24}$) with the CO_2 concentration in the jar headspace. First, thirty macro-cuvettes were filled with the gel (see 2.1) and their initial absorbances (Abs_{T0}) were determined at 570 nm with the SpectroVis spectrophotometer. The cuvettes were then inserted into thirty 310 mL glass bottles sealed with silicon septa. Second, CO_2 from a gas bottle (99.9% − Praxair Thailand Co. LTD) was injected at different volumes through the bottle septa using gastight syringes. In total, thirty values of carbon dioxide percentage injected in the bottle ranging from 0 to 3% were considered (Campbell et al., 2003). Third, the glass bottles were put into an incubator at a stabilized temperature of 30 °C for 24 -h (Haney et al., 2008). After the incubation, the absorbance was finally read again to obtain Abs_{T24}. The regression between ΔAbs and the CO_2 concentration of the bottles was then computed.

2.3. Laboratory comparison against the reference method, the alkali-trap chemical titration

In the laboratory, assessments of the amount of CO_2 emitted by soil samples with the SituResp® method were compared to measurements with the alkali-trap chemical titration method (Anderson, 1982; Zibilske, 1994), which is considered as one of the reference methods to measure the BSR in laboratory conditions (Pell et al., 2006).

40 g-air-dried and 2-mm-sieved soil samples were prepared in order to cover a wide range of soil respiration potentials. To do so, first, two soils with contrasted texture characteristics (Table 1) were mixed in six different proportions (0/100, 20/80, 40/60, 60/40, 80/20, and 100/0% of mass). Second, 40-g samples of each mix were humidified up to a given percentage of Water Holding Capacity (WHC; 0, 20, 50 or 80%). Unfortunately, 2 combinations of the mix, 20/80 and 80/20, could not be prepared at 20% WHC due to the limited amount of these two soil mix. Altogether, three replicates of a set of 22 different samples (6 soil mix x 4 soil water content level minus the 2 missing samples) were prepared. Third, two replicates of the 22 samples were used to assess the amount of CO_2 emitted by the soil with one of the two methods detailed hereafter:

The alkali-trap chemical titration method. The 22 soil samples were incubated in 580 mL air-tight jars (headspace of 533 mL) with 10 mL of 1 M NaOH at room temperature (30 °C). Back-titrations were performed manually 24 h after the beginning of the incubation. The CO_2 trapped in the alkali was precipitated with $BaCl_2$ and 0.1 M HCl were poured until the color of the phenolphthalein pH indicator changed (pH

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