Design and application of a novel calorespirometer for measuring soil microbial activity

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Abstract:

Calorespirometry combines calorimetric and respirometric measurements, allowing the simultaneous measurement of the total metabolic activity (heat production rate, R_q) of microorganisms in soils and the metabolic processes during which CO₂ is respired (CO₂ formation rate, R_{CO2}) in the soil. Using this method, it is possible to study the turnover of substrates and soil organic matter at the microbial level. By measuring R_q [in W] and R_{CO2} [in mol·s⁻¹], soil-relevant physicochemical quantities can be determined. The current calorespirometric method assumes a purely calorimetric approach, with R_{CO2} calculated from heat flow data.

The calorimetric sample consists of small (≤ 20 mL), airtight ampoule systems filled with the soil sample (≤ 5 g) and a vial filled with NaOH solution (CO₂ trap). The total heat production rate is the sum of the metabolic heat in the soil and the reaction in the CO₂ trap, thus R_{CO2} can be derived from the difference [1]. One major limitation of these measurements is that two separate experiments are necessary: (i) only soil sample and (ii) soil sample + CO₂ trap (vial filled with NaOH). Due to the small size and heterogeneity of soil samples, different effects in R_q cannot be attributed to the additional heat caused by the CO₂ trap and complicate an interpretation of R_q [2]. In addition, the small, airtight ampoule system quickly leads to oxygen starvation and saturation of the CO₂ trap, which on the one hand drastically changes the metabolic response of the microbial community in the soil sample and on the other hand requires frequent change of the CO₂ trap (disturbance of the calorimetric measurement).

In order to overcome these issues, we present in this work an early-engineered, multichannel (up to 18 measuring units) calorespirometer, which addresses the spatial issue of current measurements. The novel calorespirometer has a conductometric measuring cell filled with KOH solution (CO₂ trap, respirometer unit) positioned on the lid of the measuring channel. The measuring channel is placed on a heat sink, which is in contact with the heat flow sensor on which the open sample vessel (> 20 mL) with the soil sample (10 g) is placed (calorimetric unit). Consequently, the heat release is independent of the CO₂ trapping. In addition, oxygen depletion and saturation of the CO₂ trap do not play a role due to the large headspace (> 100 mL) of the experimental setup and a large amount of KOH solution (15 mL).

The development process of this novel calorespirometer is supported by state-of-the-art numerical simulations based on the finite-element method. Such simulations allow predictions about the sensitivity and performance of the instrument. The applicability was already successfully demonstrated on soil samples spiked with glucose as substrate and numerical solutions were validated by experimental data obtained from the custom-built multichannel calorespirometer under laboratory conditions.

[1] Barros, N., Salgado, J., Rodríguez-Añón, J.A. *et al.* Calorimetric approach to metabolic carbon conversion efficiency in soils. *J Therm Anal Calorim* **99**, 771–777 (2010).

[2] Wadsö, L., A method for time-resolved calorespirometry of terrestrial samples. Methods. 2015, 76, 20-26.