Hot Holobionts! Using Calorimetry to Characterize These Relationships

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ABSTRACT

Isothermal calorimetry is a viable technique for determining the quality of soil through respiration measurements. Substrate induced respiration follows the carbon in the soil and can be used to determine carbon incorporation. Additional experimental conditions can further characterize the soil through the idea of calorespirometry. The findings via calorimetry in the references show a direct correlation between calorimetry results and soil quality.

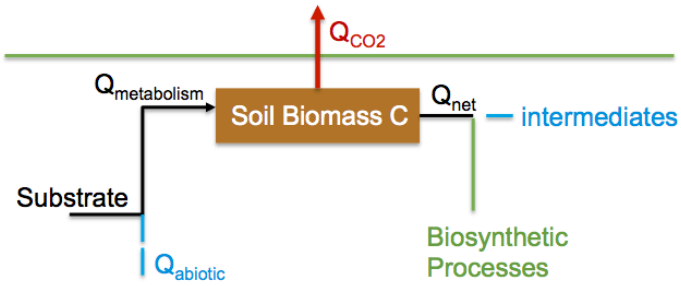
INTRODUCTION

METABOLISM AND THE CARBON CYCLE

Plants are a holobiont that include the rhizome and endophytes. This portion of the entire entity largely interacts with the soil environment which includes nutrients and biomass existing in the soil. Evaluation of the soil quality gives insight to the possibility of plant quality or survival in a particular system. In a recent Science article these microbes and endophytes are described as little farmhands and their symbiotic relationship with plants is likened to the microbiome of the gut (de Vrieze, J Science, 2015). As the gut microbiome is currently being exploited, potential lies in exploration of the plant microbiome.

Environmental quality is linked to carbon in the soil and in particular, areas with low soil organic matter (SOM) may have an increased risk of desertification (Barros, N. et al. Thermochimica Acta 2000). SOM contains the largest pool of carbon in the terrestrial carbon cycle and accounting for this carbon and tracking its use has strong environmental implications as well as plant longevity (Herrman, A. et al. Environmental Science & Technology). A first approach would be to understand the current environment and the next step would be to tune the system to increase crop production, resistivity to pesticides, and investigate crops with specific endophytes. Initial lab investigations could be an effective compliment to the "field first approach" in understanding and designing a combination of microbes, and biopesticides, and temperatures. All of these objectives can be probed via calorimetry.

This paper discusses the use of metabolic measurements to describe microbial soil activity. This activity is associated with particular environments such as deforestation that is known for poor organic matter because there is a connection between biomass and activity in terms of thermal efficiency. Although metabolism consists of thousands of processes, the net process can be simplified to the heat effects shown in the scheme below. The metabolic heat measured comes from two processes, catabolism that releases energy as heat (Q) and anabolism that requires energy. Abiotic processes usually produce negligible heat since these are very slow processes.



Scheme 1: Terrestrial Carbon Cycling. Interpreted from: Herrmann, A., Coucheney, E. and Nunan, N. "Isothermal Microcalorimetry Provides New Insight into Terrestrial Carbon Cycling" Environmental Science & Technology. 2014, 48, 4344-4352

ISOTHERMAL CALORIMETRY

Isothermal calorimeters measure heat released or consumed by a sample at a constant temperature. Heat is not used to induce these processes and the duration and temperature are the variables that the user can control. For typical metabolic studies, a TAM Air, TAM III/IV or Multi Cell DSC can all be used in isothermal mode. The calorimeter choice depends on the magnitude of the heat signal. For metabolic studies on unamended soil, the TAM III/IV is the best choice. If the heat measured is from substrate-induced metabolism then the TAM Air and Multi Cell DSC will have sufficient sensitivity.

EXPERIMENTAL

A common consideration for sample preparation is to have sufficient head space in the ampoule to provide enough oxygen to support metabolism. For a 20 mL ampoules, 5 g dry weight of sample is a good starting point – this ratio can be scaled for smaller ampoules. The soil must be brought to a known fraction of the water holding capacity with 60% being a good place to begin. The nutrient or substrate is delivered

as a solution and as part of the water so it is evenly distributed through the sample.

Typical experimental time for substrate-induced experiments is 48 hours; the long duration allows for all the substrate to be metabolized. A control experiment of the basal metabolism is also performed where DI water is added instead of the nutrient. To take the control one step further to account for abiotic processes, the system can be irradiated to kill all microorganisms. This step is recommended only for measurements of the native metabolism. For substrate-induced studies this step can be considered optional because of the much larger metabolic heats.

The study temperature depends on the purpose of the experiment. An isothermal study at multiple temperatures enables determination of the optimal temperature and temperature dependence of metabolism. All three instruments listed previously can operate over a wide, biologically relevant temperature range.

Events occurring within a few minutes of lowering the sample into the calorimeter should be ignored. If the event of interest occurs in the first 20 minutes or less, then the substrate should be added after the sample is in the measuring position. This type of addition can be completed with an additional accessory such as an admix, titration, or perfusion accessory.

DISCUSSION

SUBSTRATE INDUCED METABOLIC STUDIES

Four general periods are typically observed in a microbial growth thermogram: lag, exponential growth, stationary, and death phases (Barros, N. Feijóo, S. Biophysical Chemistry 2003). Figure 1 is an example of soil metabolism that was measured internally using a TAM AIR instrument. Two samples, A and B were examined in the figure below. Controls A1 and B1 are compared to the substrate-induced heat rate data, A2 and B4. A2 can be described as having a short lag and steep exponential growth indicating that it quickly incorporated the substrate. In contrast, B4 has a long lag period and shallow exponential growth. The native metabolism for A1 is atypically large and the significant heat rate of basal metabolism should be subtracted from A2 before integration to obtain the total heat effect. Note that a second growth process begins in A2 after the first growth curve ends.

When investigating soil, the first question is: What are the sources of heat (Q)? The four major sources of heat (Q) generated are: 1. Respiration 2. Microbial maintenance and growth, 3. Production of secondary metabolites, 4. Substrate interaction – abiotic processes such as complexation with iron aluminum oxide ($Fe_2Al_2O_3$). The

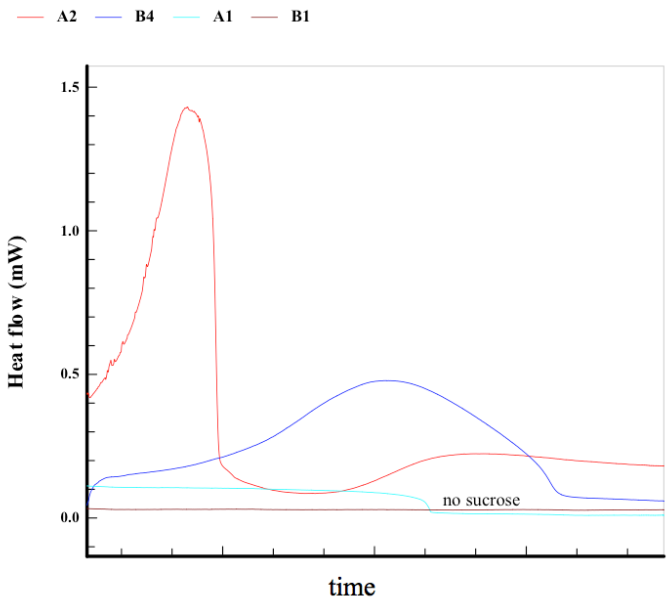


Figure 1. Thermogram of substrate induced soil metabolic growth observed in a TAM Air. Initial perturbation of the calorimeter is not shown.

control experiment accounts for basal metabolism and abiotic processes and can be subtracted from the total heat. The remaining overall metabolism, comprised of catabolic and anabolic processes, is then normalized to the total moles of substrate (Eqns 1 and 2) (Barros, N. et. al. Thermochimica Acta. 2000).

$$\frac{Q_T}{S_0} = \Delta H_{metabolism} \quad (1)$$

$$\Delta H_{metabolism} = \Delta H_{catabolic} + \Delta H_{anabolic} \quad (2)$$

A simple reaction describes the catabolic process of aerobic respiration (Eqn 3.)

$$O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O \quad (3)$$

Equation 3 enables the interpretation of whether the biomass is respiring at steady-state or if some of the substrate is being incorporated into growth. For a stationary system with no growth, the total heat generated will be close to the heat of combustion (ΔH°_c= -2814 kJ/mol), the enthalpy associated solely with respiration. If some of the substrate is being incorporated into the system by growth, there will be an anabolic component that is endothermic and will thereby decrease the total heat ($\Delta H_{metabolism}$) generated.

Multiple types of substrates can be investigated, although glucose is the most common. Equation three can be written for other substrates such as citric acid, L-alanine and phenols.

ASSIGNING ENTHALPY

Substrate-induced samples that have higher rates of glucose degradation and microbial growth dissipate less energy as heat, retain more energy in the system, and have higher biomass yield. If no biomass is forming, i.e. there is no growth, then all the heat is lost to combustion/respiration.

The von Stockar energy balance can be used to describe the system (Eqn 4) (von Stockar, U., Liu, J. Biochim. Biophys Acta. 1999).

$$aC_6H_{12}O_2 + bO_2 + cNH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + dCO_2 + eH_2O + fH^+ \quad (4)$$

After this mass balance equation, the conservation of glucose can be described by the following relationships (Eqn 5-7, Barros, N. Feijóo, S., Alvarez, J. International Journal of Low Carbon Technologies). $\Delta_r H_X^0$ is the enthalpy change for the microbial growth reaction, $Y_{X/S}$ is the growth yield where X is moles of biomass and S is moles of glucose. Finally, $\Delta_r H_S^0$ is the enthalpy change for the conservation glucose degradation reaction and $\Delta_c H_N^0$ is the combustion of the nitrogen source, if applicable. It is added in the case of Equation 4 and in other cases where the soil has been stimulated by the addition of ammonium. If this compound has not been added then this portion of the equation can be ignored.

$$\Delta_r H_X^0 = \frac{Q}{\Delta X} \quad (5)$$

$$\Delta_r H_X^0 = \frac{\Delta H_S^0}{Y_{X/S}} - \Delta_c H_X^0 + x_3 \Delta_c H_N^0 \quad (6)$$

$$\Delta_r H_x^0 = \frac{1}{Y_{X/S}} - \Delta_r H_S^0 \quad (7)$$

Determination of the soil biomass (X) can be determined via a few different methods. Two common ways the mass of living bacteria (X) can be determined is by the colony forming units from extracted soil or by Sparling's method (Sparling, G. Soil Biol. Biochem 1981 and Sparling, G.P. J. Soil Sci. 1983). The normalized enthalpy and related values from the equations above can be further applied to determine the thermal yield and the value $Y_{X/S}$ is correlated to the efficiency of the system in degrading soil organic matter.

CALORESPIROMETRY

Calorespirometry is an approach for assessing microbial efficiency in soils. It couples the simultaneous measurement of CO_2 (R_{CO2}) and heat (R_q) production rates by isothermal calorimetry. The additional information on CO_2 increases understanding and description of the soil system (Figure 2).

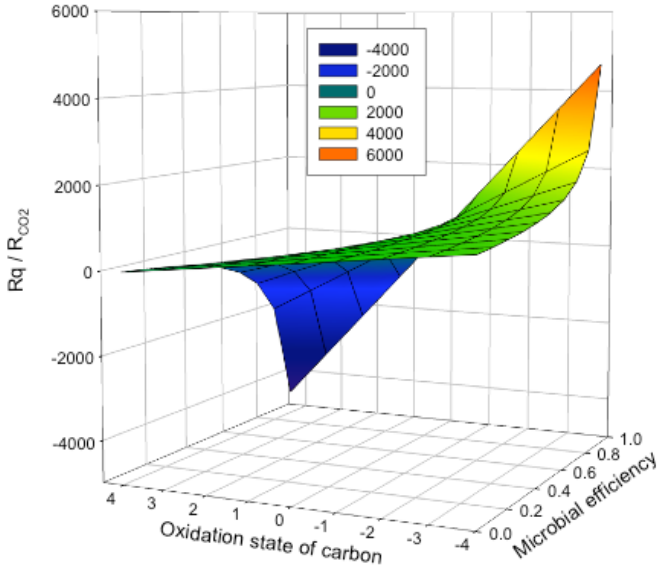


Figure 2. Multi-dimensional plot displacing a relationship between variables of soil metabolism. This figure was created by Serita Frey (UNH), unpublished data.

The ratio of heat to CO_2 production is related to metabolic efficiency (Eqn 8, Barros, N. et al J. Therm Anal Calorim. 2010).

$$\frac{R_q}{R_{CO_2}} = -\Delta H_{O_2}\left(1 - \frac{\gamma_S}{4}\right) - \Delta H_B\left[\frac{\varepsilon}{(1-\varepsilon)}\right] \quad (8)$$

ε is the metabolic efficiency, γ_S is the oxidation state of substrate C and γ_B is the oxidation state of microbial biomass, which is assumed to be -0.3. ΔH_{O_2}) is Thornton's constant (-455±15 kJ/mol) and ΔH_B is the difference in the heat of combustion of the biomass and glucose (-559 and -469 kJ/Cmol, respectively). There are a few ways that have been used to obtain the CO_2 (g) released and these include, but are not limited to, soda lime capture, gel plate detector, and portable infrared gas analyzer. In addition to these ancillary methods CO_2 rates can be quantified via calorimetry. A solution of fresh concentrated, sodium hydroxide solution can be added to the sample vial (Picture 1). The excess heat released in the presence of sodium hydroxide is directly related to the rate of CO_2(g) release; the heat of this reaction is -108.5 kJ/mol. To perform this type of analysis, the respiration rate should be taken with and without the NaOH present.

Picture 1. A 20 mL reaction vial with a solution of NaOH placed into the chamber for calorespiration determinations.

The difference in the heat rates with NaOH and without NaOH gives the rate of production of CO_2 (Eqn 9).

$$\frac{Q_{(NaOH-CO_2)}}{\Delta H_{(NaOH-CO_2)}} = R_{CO_2} \quad (9)$$

The integral of the plot of R_{CO2} versus time gives the moles of CO_2. After this both the heat and gas exchange is measured and the metabolic efficiency of the biomass is better understood.

CONCLUSION

Calorimetry provides a direct qualitative and quantitative assessment of soil condition and carbon cycling. In addition to heat measurements, simultaneous measurements of heat and CO_2 production increase understanding of soil processes. Well-designed control experiments allow separation of measured heat into different sources. Trends in calorimetric evaluation of soil have emerged that are consistent with soil conditions. This technique and the analysis methods applied to soil can also be applied to plants, insects, and other organisms.

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