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Calorimetric approach to metabolic carbon conversion efficiency in soils

Comparison of experimental and theoretical models

N. Barros · J. Salgado · J. A. Rodríguez-Añón · J. Proupín · M. Villanueva · L. D. Hansen

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Abstract Soil carbon is the largest reservoir of organic carbon on the planet and CO_2 production by soil thus has potentially large effects on atmospheric CO_2. Carbon sequestration in soil is determined by the metabolic efficiency (substrate carbon conversion efficiency) of soil micro-organisms. That could be measured by calorespirometric methodology (parallel measurement of metabolic heat rate and CO_2 production rate) and by theoretical thermodynamic models. Carbon conversion efficiency of the glucose degradation reaction in soil is calculated from both the calorespirometric ratio of heat rate to CO_2 rate and from energy and mass balance models combined with calorimetric heat rates. Results obtained, 0.77 and 0.75, are in good agreement.

Keywords Metabolic efficiency · Calorespirometry · Soils · Energy balance

List of symbols

Rq/R_{CO_2}	Ratio of the heat rate to the CO_2 rate in kJ mol^{-1} CO_2
R_{CO_2}	CO_2 rate in mol s^{-1}
Rq	Heat rate in J s^{-1}
ϕ_R	Heat flow rate in μW
Q_T	Total heat released by the microbial growth reaction stimulated with glucose in J g^{-1} of soil
μ	Apparent microbial growth rate constant
ΔX	Increment in microbial biomass in micrograms of biomass C
X_0	Initial active soil biomass in micrograms of biomass C g^{-1} of soil
$\Delta_r H_S$	Enthalpy of the glucose microbial degradation reaction in kJ mol^{-1} of substrate
η	Thermal yield
ε	Carbon conversion efficiency
γs	Oxidation number
$\triangle H_{O_2}$	Thorton's constant in kJ mol^{-1} of oxygen
ΔH_B	Difference between the heat of combustion of the biomass and that of the substrate in kJ mol^{-1} of C

N. Barros (✉) · J. Salgado · J. A. Rodríguez-Añón · J. Proupín · M. Villanueva
Department of Applied Physics, University of Santiago de Compostela, Santiago de Compostela, Spain
e-mail: nieves.barros@usc.es

L. D. Hansen
Brigham Young University, Provo, UT, USA
e-mail: Lee_Hansen@byu.edu

Introduction

Calorespirometry is the combined measure of heat and gas exchange rates produced in respiration [1], and it can be applied to study the metabolic efficiency of microorganisms, cells and tissues. The metabolic efficiency can be defined as the fraction of substrate carbon converted into new biomass [2] or the fraction of heat energy contained in the substrate that is retained as new biomass [3]. The latter can be calculated from calorimetric measurements of heat and thermodynamic models based on energy and mass balances that have been developed for microbial growth reactions [4]. The metabolic efficiency of a given metabolic process can be quantified by Battley's equation [5], which defines efficiency of a microbial growth reaction by the enthalpy conservation, or in terms of the calorespirometric ratio, Rq/R_{CO_2}, in the models of Criddle and Hansen [6]. The

concept of efficiency given by Battley has been widely applied to the energetics of microbial metabolism [7], and it could be useful in soil research [8, 9]. The ratio Rq/R_{CO_2} has been determined for a wide range of living systems and metabolic pathways [10, 11], but no data have been published for soils.

Rq/R_{CO_2} can be directly determined by calorimetry by measuring the heat flow rate of a biological sample in a closed ampoule before and after a CO_2 absorbent solution is placed in the ampoule with the sample [12]. The methodology is widely applied for many biological systems, but curiously enough, it has not been developed to study soil microbial metabolism in closed ampoules. Application to soils contributes to study the metabolic efficiency of the biochemical reactions linked to the *C*-cycle, easily, ecologically and fast. In this sense, the only previous related works involving calorimetry, used the concept of metabolic efficiency calculated by energy and mass balances applied to microbial growth reactions [8, 9] stimulated in soil by the addition of glucose [13], and it is based on the direct measure of the enthalpy change of the induced reaction. That could be useful to study the microbial activity of basic soils [14] where the metabolic CO_2 determinations are strongly limited by the methodology. In general, the study of the metabolic efficiency of the soil reactions is still very restricted because most of the procedures provide results after tedious and long experimental phases. Calorimetry has the advantage to give that information quickly and easily.

One of the disadvantages affecting the application of these thermodynamic models is that they have been never experimentally compared to other methodologies in soil research. In that sense, direct measure of the CO_2 rate, R_{CO_2}, and heat rate, Rq, in soil by calorespirometry to provide the Rq/R_{CO_2} ratios could be an attractive option, because Rq/R_{CO_2} ratios are directly related to metabolic efficiency [6]. Thus, on the one hand, it would enable the direct calculation of metabolic efficiency to a wider range of reactions in soils than those of the enthalpy models, and on the other hand, it is an option for testing theoretical and experimental data dealing with metabolic efficiency in soil. The problem is that calorespirometry has not been applied to soils yet, and for that reason, it is necessary to develop first the right methodology for R_{CO_2} and Rq R_{CO_2} determinations. With that goal in mind, this article describes methodology for simultaneous determination of the heat and CO_2 rates in soils, and a procedure to quantify R_{CO_2} and Rq/R_{CO_2} for microbial growth reactions in soils amended with glucose. Then, metabolic efficiency parameters experimentally obtained by this method are compared with those obtained by calorimetry and theoretical thermodynamic models to reinforce their application in soil research.

Materials and methods

The soil sample collected in Galicia (NW Spain) is a forest soil under Eucalyptus. The sample is sieved through 2 × 2 mm screen, then kept at 4 °C in polyethylene bags. Before calorimetric measurements, it is pre-equilibrated at the calorimeter temperature (25 °C) for 24 h. The calorimeter is a TAM 2277 (TA Instruments, Lindon, Utah, USA) with three measuring channels. The calorimeter was statically calibrated with constant electrical power at 300 μW. A calorimeter base line was recorded before every experiment. Three experiments are run simultaneously in parallel in the three calorimetric channels. Each channel has two 4-mL stainless steal ampoules, one containing 1 g of soil amended with 0.2 mL of a solution made with 1.5 mg of glucose and 1.5 mg of Ammonium sulphate, the other, reference ampoule, contains 1 g of Al_2O_3. A small vial of 0.4 M NaOH is placed in the ampoules with the soil in two of the measuring channels. The third channel has only the amended soil. In one of the channels, the ampoule is removed from the calorimeter to insert/remove the NaOH vial at different intervals of time. In the other channel, the NaOH is left in place until throughout the experiment. The channel with only the soil sample registers the power–time curve from the exothermic metabolic reaction caused by the glucose. The channels with the soil and the NaOH register the sum of the power–time curves of two exothermic reactions: metabolism of the glucose and reaction of the NaOH with CO_2 released by the soil. The enthalpy change of reaction of CO_2 with NaOH at this concentration is -108.5 kJ mol^{-1} [15].

Addition of glucose to the soil usually stimulates a microbial growth reaction that can be written as follows [7]:

$$aC_6H_{12}O_6 + bO_2 + cNH_4^+ = CH_{1.8}O_{0.5}N_{0.2} + dCO_2 + eH_2O + fH^+ \quad (1)$$

where $CH_{1.8}O_{0.5}N_{0.2}$ is the reported formula for bacterial biomass [16]. Integration of the power–time curves with respect to zero yields the total heat dissipated, Q_T, in joules per gram of soil, J g^{-1}. The slope of a linear fit of a plot of ln (heat flow rate) against time gives the apparent microbial growth rate constant, μ [17, 18]. μ permits the calculation of the increment in biomass, ΔX, through the exponential equation that describes the microbial growth and calculation of the initial activated biomass, X_0, that can be determined from the power–time curve by Sparling's correlation; 1 g biomass *C* produces 180 mW [19]. X_0 is measured in the lag phase of the power–time curve before the exponential microbial growth. The ratio between, $Q_T/\Delta X$, can be used with the equations for the energy balance of reaction (1) [4] to calculate the enthalpy of the microbial glucose degradation reaction, $\Delta_r H_S$, to obtain the thermal yield, η, through Battley's equation [3, 5].

The thermal yield is the fraction of substrate energy kept in the biomass, and $1 - \eta$ is then the fraction of energy in the substrate released to the environment as heat. The mass balance provides the stoichiometric coefficients of reaction (1) that give the theoretical CO_2 yield and the theoretical calorespirometric ratio, Rq/R_{CO_2} [15]. Both η and Rq/R_{CO_2} permit calculation of the carbon conversion efficiency of the reaction (1) in the soil, that is the percentage of carbon from the glucose that is kept as biomass and the percentage that is lost through respiration. The CO_2 yield and Rq/R_{CO_2} were calculated theoretically by the energy and mass balance and experimentally by measurement of the CO_2 absorbed by the NaOH. The difference in heat flow rate simultaneously measured in the channel with soil and NaOH and in the channel with only soil gives the CO_2 production rate, R_{CO_2}. R_{CO_2} values obtained by this way were compared with those calculated from the differences in heat flow rate in the channel where the NaOH was inserted/removed. The R_{CO_2} and Rq/R_{CO_2} values calculated by this procedure were compared to those determined from the energy and mass balance method.

The Rq/R_{CO_2} values allow calculation of the carbon conversion efficiency, ε by the following equation applied to reaction (1) [2]:

$$Rq/R_{CO_2} = -(1 - \gamma s/4)\Delta H_{O_2} - \Delta H_B(\varepsilon/1 - \varepsilon) \qquad (2)$$

where γs is the oxidation number of the C source, $\gamma s = 0$ for glucose; $\triangle H_{O_2}$ is Thornton's constant [20], -455 ± 15 kJ mol^{-1} O_2, and ΔH_B is the difference in the heat of combustion of the biomass, -559 kJ mol^{-1} C [4, 7] and that of the glucose, -467 kJ mol^{-1} C. The ε values calculated by Eq. 2 where compared to those obtained by the mass balance applied to reaction (1) and to the η values calculated by the Battley's equation.

Results and discussion

Figure 1 shows the heat flow rates measured simultaneously in the three calorimetric channels under the given experimental conditions. Channel 4 does not have NaOH and shows only the microbial growth reaction in the soil after glucose addition. Channel 3 has NaOH inside continuously and shows the rates of soil metabolic activity and the reaction of the CO_2 with NaOH. NaOH is inserted in channel 2 at the beginning of the experiment and is removed and replaced at time intervals. The heat flow rate in this channel reaches that of channel 4 when the NaOH is removed and reaches the channel 3 signal when NaOH is inside, demonstrating the good reproducibility of the reactions taking place in the three channels. The power–time curve recorded in channel 4 is typical for microbial growth [17, 18].

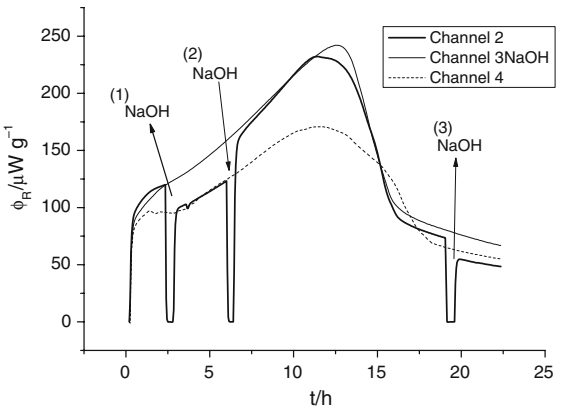

Fig. 1 Power–time curves of the soil sample amended with glucose and those with the NaOH vial inside. The *arrow keys* show the points on the curves were the NaOH vial was removed and reinserted in the sample running in channel 2. The NaOH was inserted in channel 3 for the whole experiment. Channel 4 has the soil sample only

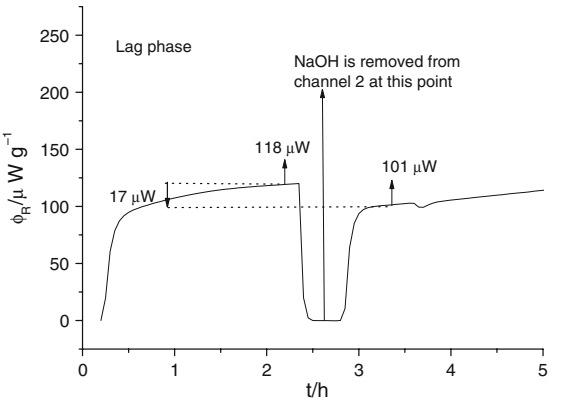

Fig. 2 Power–time curve of the lag phase of the soil in channel 2 after glucose amendment. It can be observed as the difference in the heat flow rate after removing NaOH from the calorimetric ampoule

The R_{CO_2} is calculated for the lag phase in channel 2 by the difference in heat flow rate in the curve where the NaOH is removed and reinserted, as shown in Fig. 2. The time interval, Δt, between the two values that are subtracted (-118 and -101 μW g^{-1}) is 1.17 h, the time needed to remove the NaOH from the calorimetric ampoule, let the ampoule pre-equilibrate and let the calorimeter signal recover after replacing the ampoule with the soil and without the NaOH. The R_{CO_2} and Rq/R_{CO_2} values obtained by this procedure in channel 2 are $1.57 \cdot 10^{-10}$ mol CO_2 s^{-1} and -643 kJ mol^{-1} CO_2. The same procedure applied to the heat flow rate of the sample in channel 4 (without the NaOH) and the sample in channel 3 with NaOH inside, both running continuously (see Fig. 3), give R_{CO_2} and Rq/R_{CO_2} values of $2.03 \cdot 10^{-10}$ mol CO_2 s^{-1} and -473 kJ mol^{-1} CO_2 respectively, at 2.19 h, and $2.76 \cdot 10^{-10}$ mol CO_2 s^{-1} and -354 kJ mol^{-1} CO_2 at 3.36 h. The differences found between the continuous measurement and the punctual

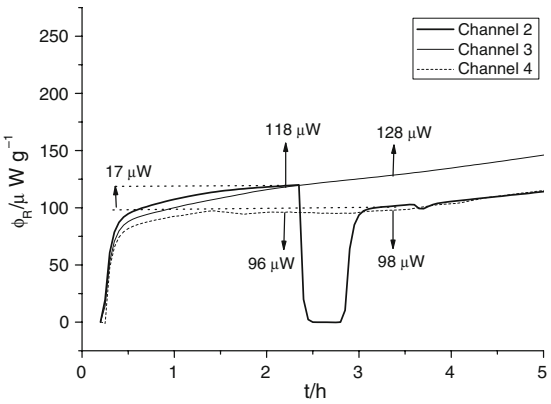

Fig. 3 Power–time curve of the lag phase of the soil sample after glucose amendment in channel 4, that of the soil with the NaOH in the channel 3 and that of the soil where the NaOH is removed, in channel 2

model in Fig. 2 are caused by the Δt between when the NaOH is inserted/removed in the ampoule, apparently because R_{CO_2} is not constant during this time.

The same procedure was performed for the exponential growth phase, and for the period of basal metabolism after the growth reaction, indicated in the Fig. 1 by the arrows as points 2 and 3. The quantitative results are shown in Table 1. The differences associated with the different procedures used to measure R_{CO_2} strongly affected the calculated Rq/R_{CO_2} values in all the phases, suggesting that the results obtained by subtracting punctual heat rates when the NaOH is inserted/removed in the calorimeter are not accurate. For that reason, a method to determine R_{CO_2} and Rq/R_{CO_2} at the different phases of the growth process based on the continuous registration of the heat flow rate was developed. It is possible to check the reproducibility of the microbial growth reaction in the soil samples if power–time curves of two soil aliquots are continuously recorded and compared to the power–time curve of a third soil sample with NaOH that can be removed at the end of the microbial growth reaction to check the heat flow rate due to microbial metabolism in that channel. By this procedure, the tabulated data from curves can be easily processed to show the continuous R_{CO_2} and Rq/R_{CO_2} evolution during the reaction as shown in Figs. 4 and 5. These curves are obtained by direct subtraction of the tabulated heat flow rate in the channels with the soil and in the channel with the soil plus NaOH. The R_{CO_2} is calculated by dividing these heat flow rates by the enthalpy change for reaction of CO_2 with NaOH. Then, the heat flow rate from the soil is divided by the R_{CO_2} values to yield Rq/R_{CO_2} values.

Data from this procedure clearly shows that Rq/R_{CO_2} is not constant in the different growth phases as shown in Fig. 5. The tabulated Rq/R_{CO_2} data give average values for the different microbial growth phases together with the standard deviation, *SD*. *SD* gives the variance of the Rq/R_{CO_2} values over a period of time. The Rq/R_{CO_2} values obtained are (-490 ± 117 kJ mol^{-1}) for the lag phase, (-387 ± 23 kJ mol^{-1}) for the exponential growth phase, and (-486 ± 15 kJ mol^{-1}) for basal metabolism after the growth process. As the *SD* shows, Rq/R_{CO_2} values are highly variable in the lag phase and constant during exponential growth and basal metabolism. Rq/R_{CO_2} are close to those for carbohydrates substrates [20] for different living systems [2, 21], indicating the consumption of the glucose added.

The CO_2 evolution rate can be assessed similarly in the phases where it is constant over a certain period of time. Figure 4 shows R_{CO_2} remains constant in the lag phase, increases during the exponential growth phase, decreases in the stationary phase before increasing to a new basal metabolic state characterized by a slow decline of R_{CO_2}. Total CO_2 released in the lag phase is easily obtained by multiplying the R_{CO_2} value by the duration of that phase, but it is necessary to model the growth phase to quantify total CO_2. If R_{CO_2} increases exponentially during the growth phase, then a plot of *ln* (R_{CO_2}) values against time during that phase should be linear. Results shown in Fig. 6 show this to be the case.

Total CO_2 and Rq/R_{CO_2} values for the microbial growth reaction can theoretically be calculated by a mass and energy balance. This procedure is experimentally easier than the process with the NaOH, and it was developed for a kinetic that was usually observed in the soil when glucose is added as *C* source [13]. We cannot confirm that the CO_2

Table 1 Data of the CO_2 evolution rate, R_{CO_2} and the calorespirometric ratios, Rq/R_{CO_2} calculated for the soil microbial growth reaction stimulated by glucose addition

	Punctual model R_{CO_2}/nano mol s^{-1}	Continuous model R_{CO_2}/nano mol s^{-1}	Punctual model Rq/R_{CO_2} kJ mol^{-1}	Continuous model Rq/R_{CO_2} kJ mol^{-1}
Lag phase	0.157	0.240	−608	−414
Growth	0.405	0.336	−299	−376
Basal	0.194	0.216	−273	−441

The above values were determined by the subtraction of the punctual heat flow rate data in two chosen points of the power–time curve where the heat flow rate is recorded continuously, and at the points where the NaOH was inserted/removed from the calorimeter. Different values are obtained depending on the applied model

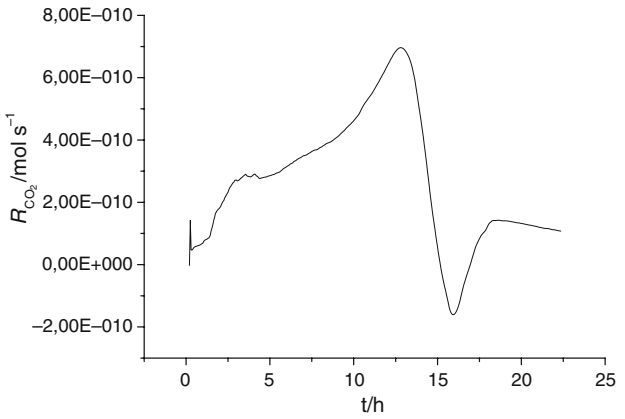

Fig. 4 Plot of the CO_2 evolution rate of the microbial growth reaction

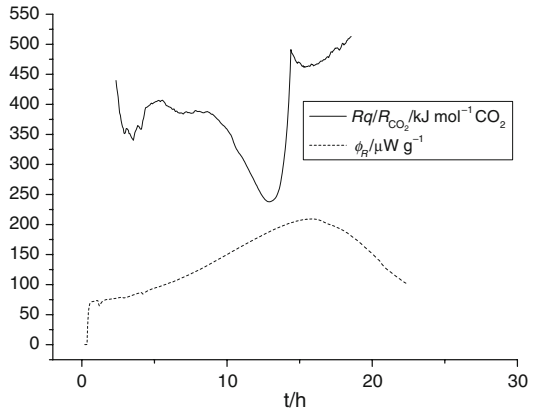

Fig. 5 Plot of the heat flow rate (ϕ_R) and the calculated calorespirometric ratio (Rq/R_{CO_2}) of the microbial growth reaction

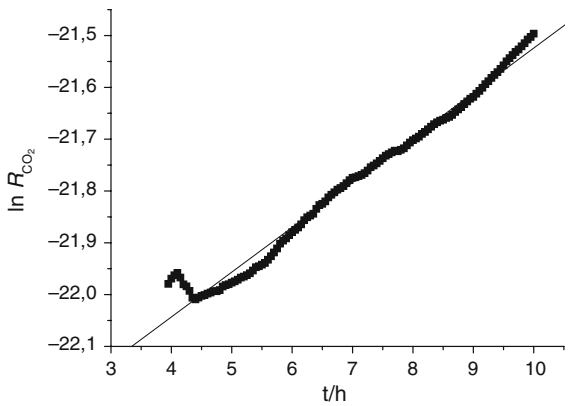

Fig. 6 Plot of the ln(CO_2 evolution rate) versus time. The exponential kinetics takes place from $t = 4$ to 10 h. It is significant ($p < 0.0001$), it has a correlation index of $r = 0.99$, and follows the equation: $y = 0.0865x - 22$

kinetics shown here can be globally applied to every soil under the same experimental conditions. Then, the mass and energy balance is applicable to desert soil because it is based exclusively on the heat flow rate. The direct determination of R_{CO_2} with NaOH is not possible in desert and basic soils due to the CO_2 released by inorganic reactions with carbonates [14].

Comparison of the methods is also interesting if the goal is to determine the metabolic efficiency of microbial growth reactions in soil. That is an important indicator of the soil fertility status requested by the Kyoto protocol [22] since it is directly connected with the carbon sequestration and the impact of the soil CO_2 rate on the atmosphere [23]. It is also sensitive to the soil exploitation and management permitting the quantitative evaluation of environmental impact on soil [24, 25].

The enthalpy and mass balance was applied to the power–time curves registered in this experiment. The experimental data involved in the energy balance directly calculated from the power–time curves given in Fig. 1 are shown in Table 2 together with the results of the energy balance. The mass balance gives the stoichiometric coefficients of reaction (1):

$$0.25C_6H_{12}O_6 + 0.47O_2 + 0.20NH_4^+ = CH_{1.8}O_{0.5}N_{0.2} + 0.53CO_2 + 0.92H_2O + 0.20H^+ \quad (3)$$

The stoichiometric coefficients give the CO_2 yield in mol CO_2 mol^{-1} glucose allowing calculation of the total CO_2 dissipated through the $\Delta_r H_S$ value determined by the energy balance equations [3, 4] and given in Table 2. The total CO_2 obtained is 22.83 μmol. The total CO_2 absorbed by the NaOH, calculated by integration of Fig. 4, is 21.22 μmol CO_2, very close to the value given by the mass balance. The theoretical Rq/R_{CO_2} value obtained by the energy and mass balance, −403 kJ mol^{-1}is close to the experimental value during the exponential growth phase, −387 kJ mol^{-1}. The energy and mass balance calculation agrees quite well with experiment and indicates Eq. 3 approaches well to the reaction taking place in soil amended with the glucose. The dynamics of the glucose degradation reaction in soil is very well established by literature and by different methodologies. It is well known there is not partial glucose oxidation in soil unless it takes place under 4 °C. In that case, glucose is partially degraded to Malate [26]. We work at 25 °C. If Eq. 3 did not approach to the glucose degradation in soil, the experimental and theoretical $Rq\ R_{CO_2}$ values obtained should be different. Another feature influencing the $Rq\ R_{CO_2}$ values could be respiration with acceptor/donor electrons different from O_2, but that should affect to the experimental values of $Rq\ R_{CO_2}$ too. Then, we are proposing a method to continuously monitor the variations of the $Rq\ R_{CO_2}$ with time. A change of the kinetics to anaerobic processes or to other electron acceptors would be reflected in the curve of the $Rq\ R_{CO_2}$ evolution as depletion to lower values than those on the

Table 2 Results of the total heat dissipated by the microbial growth reaction, Q_T, the microbial growth rate constant, μ the duration of the exponential microbial growth, PT, and the initial activated biomass, X_0

Q_T/J g^{-1}	μ/h^{-1}	PT/h	X_0/μg C g^{-1}	ΔX/μg C g^{-1}
−9.20	0.076	9	539	529
$\Delta_r H_x$/kJ mol^{-1} C	$Y_{X/S}$/molX mol^{-1} S	$\Delta_r H_s$/kJ mol^{-1}S	η	Rq/R_{CO_2}/kJ mol^{-1}
−209	3.95	−826	0.70	−403

The above values are directly calculated from the power–time curves. The increment in biomass, ΔX, is determined by the exponential equation that defines microbial growth. $\Delta_r H_x$ is the quotient between Q_T and ΔX, which is launched in the energy balance equations to obtain the microbial yield, $Y_{X/S,}$ in mol of carbon biomass per mol of glucose. $\Delta_r H_s$, is the enthalpy of the glucose degradation reaction, the thermal yield, η, and the calorespirometric ratio Rq/R_{CO_2}

range −250 to −460 kJ mol^{-1} associated to aerobic degradation of carbohydrates via respiration.

When the theoretical and experimental Rq/R_{CO_2} values are used in Eq. 2, the obtained ε values are 0.77 and 0.75, very close to the maximum value, 0.80, expected for glucose and agree quite well with the η value obtained from Battley's equation, 0.70. The proposed models appear to describe the metabolic efficiency of the system quite well.

Conclusions

Direct measurement of the rate of CO_2 production in acidic soils can be done by calorespirometry. R_{CO_2} is not constant during microbial growth and thus punctual subtraction of heat flow rates after removing/inserting NaOH in the calorimeter is not an accurate method for measuring R_{CO_2}. Simultaneous and continuous measurement of the heat flow rate of the soil reaction and of the reaction of CO_2 with NaOH, appear to give more accurate information about the evolution of the R_{CO_2} and Rq/R_{CO_2} during the microbial growth reaction. Directly measured values for Rq/R_{CO_2} and R_{CO_2} agree with those calculated by energy and mass balances applied to the power–time curves, reinforcing applicability of the energy and mass balances to give data about the energy and carbon conversion efficiency as requested by the Kyoto protocol. The energy and mass balance method is also useful when applied to basic soils such as those commonly found in deserts since calorimetry is based exclusively on the heat dissipated by the soil microbial metabolism. The proposed methodology permits to study the soil in terms of metabolic efficiency very quickly, ecologically and by a non-invasive way. This can be very useful to give more knowledge about the metabolic efficiency responses to different environmental impacts and to give further insight in the thermodynamics of the biochemical reactions linked to the C cycle.

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References

1. Gnaiger E, Shick JM, Widdows J. Metabolic microcalorimetry and respirometry of aquatic animals. Soc. exp. bio. seminar series. Cambridge: Cambridge University Press; 1989.
2. Hansen LD, Macfarlane C, McKinnon N, Smith BN, Criddle RS. Use of calorespirometric ratios, heat per CO_2 and heat per O_2, tonquantify metabolic paths and energetics of growing cell. Thermochim Acta. 2004;422:55–61.
3. Von Stockar U, Marison IW. The definition of energetic growth efficiencies for aerobic and anaerobic microbial growth and their determination by calorimetry and by other means. Thermochim Acta. 1993;229:157–72.
4. Von Stockar U, Gustafsson L, Larsson C, Marison I, Tissot P, Gnaiger E. Thermodynamic considerations in constructing energy balances for cellular growth. Biochim Biophys Acta. 1993;1183:221–40.
5. Battley EH. The development of direct and indirect methods for the study of the thermodynamics of microbial growth. Thermochim Acta. 1998;309:17–37.
6. Criddle RS, Hansen LD. Calorimetric methods for analysis of plant metabolism. In: Kemp RB, editor. Handbook of thermal analysis and calorimetry, vol. 4. Amsterdamm: Elsevier; 1999. p. 711–63.
7. Battley EH. Energetics of microbial growth. New York: J. Wiley; 1987.
8. Barros N, Feijóo S. A combined mass and energy balance to provide bioindicators of soil microbiological quality. Biophys Chem. 2003;104:561–72.
9. Barros N, Feijóo S, Simoni JA, Airoldi C, Ramajo B, Espina A, et al. A mass and energy balance to provide microbial growth yield efficiency in soil: sensitivity to metal layering phosphates. J Thermal Anal Calorim. 2008;93:657–65.
10. Summers HA, Smith BN, Hansen LD. Comparison of respiratory and growth characteristics of two co-occurring shrubs from a cold desert, *Coleogyne ramosissima* (blackbrush) and *Atriplex confertifolia* (shadscale). J Arid Environ. 2009;73:1–6.
11. Kemp RB. Fire burn and cauldron bubble (W. Shakespeare): what the calorimetric–respirometric (CR) ratio does for our understanding of cells? Thermochim Acta. 2000;355:115–24.
12. Criddle RS, Breidenbach RW, Rank DR, Hopkin MS, Hansen LD. Simultaneous calorimetric and respirometric measurements on plant tissues. Thermochim Acta. 1990;172:213–21.
13. Barros N, Salgado J, Feijóo S. Calorimetry and soil. Thermochim Acta. 2007;458:11–7.

14. Barros N, Feijóo S, Salgado J, Ramajo B, García JR, Hansen LD. The dry limit of microbial life in the Atacama Desert revealed by calorimetric approaches. Eng Life Sci. 2008;5:477–86.
15. Criddle RS, Fontana AJ, Rank DR, Paige D, Hansen LD, Breidenbach RW. Simultaneous measurement of metabolic heat rate, CO_2 production, and O_2 consumption by microcalorimetry. Anal Biochem. 1991;194:413–7.
16. Van Gulik WM, Heijnen JJ. A metabolic network stoichiometry analysis of microbial growth and product formation. Biotechnol Bioeng. 1995;48:681–98.
17. Nuñez L, Barros N, Barja I. A kinetic analysis of the degradation of glucose by soil microorganisms studied by microcalorimetry. Thermochim Acta. 1994;237:73–81.
18. Liu JS, Marison IW, von Stockar U. Anaerobic calorimetry of the growth of Lactobacillus helveticus using a highly sensitive Bio-RCl. J Thermal Anal Calorim. 1999;56:1191–5.
19. Sparling GP. Estimation of microbial biomass and activity in soil using microcalorimetry. J Soil Sci. 1983;34:381–90.
20. Thornton WM. The relation of oxygen to the heat of combustion of organic compounds. Philos Mag. 1917;33:196–203.
21. Wadsö L, Li Y, Bjurman J. Measurements on two mould fungi with a calorespirometric method. Thermochim Acta. 2004;422:63–8.
22. Kyoto protocol. Kyoto protocol to the United Nations framework convention on climate change. Kyoto: UNEP/WMO; 1997.
23. Kuzyakov Y. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol Biochem. 2006;38:425–48.
24. Barros N, Airoldi C, Simoni JA, Ramajo B, Espina A, García JR. Calorimetric determination of the effect of ammonium-iron(II) phosphate monohydrate on *Rhodic Eutrudox* Brazilian soil. Thermochim Acta. 2006;441:89–95.
25. Frey SD, Knorr N, Parrent JJ, Simpson RT. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecol Manag. 2004;196:159–71.
26. Coody PN, Sommers LE, Nelson DW. Kinetics of glucose uptake by soil microorganisms. Soil Biol Biochem. 1986;18:283–9.