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A combined mass and energy balance to provide bioindicators of soil microbiological quality

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Abstract

In this work, a method is proposed to quantify the efficiency of carbon utilization by soil microbes. Microcalorimetry was used to compute the heat yield ($Y_{Q/X}$) of six soil samples collected in the Amazon. A combined mass and energy balance is developed to quantify the enthalpy of the glucose oxidation reaction ($\Delta_r H_s$) and the biomass yield ($Y_{X/S}$) from the experimental values of $Y_{Q/X}$. Results were compared by graphical analysis to establish the kinetics of the glucose oxidation and the microbial growth reactions in terms of energy dissipation. The correlations found suggest that the measured values for $Y_{Q/X}$ and $\Delta_r H_s$ are biomass yield dependent. The main environmental factors affecting the kinetics of the glucose oxidation and the microbial growth reactions in soils are the initial microbial population and the percentage of nitrogen of the samples. The comparative study among the samples showed that the deforestation of the Primary forests in the Amazon to establish arable lands, affected the efficiency of the carbon utilization by soil microorganisms.

Keywords: Microcalorimetry; Energy balance; Soil microbial activity; Efficiency

1. Introduction

The potential warming of the global climate, linked to the accumulation of gases (CO_2, N_2O, CH_4) in the atmosphere, and the deterioration of the soil quality through the loss of soil organic carbon, are two of the most pressing global environmental challenges facing the world today. Their impact on human welfare and the sustainability of ecosystems has been the subject of numerous studies [1–3]. In addition to their potential economic and environmental consequences, global warming and loss of soil fertility are connected in many respects. Soil is identified as an important source of atmospheric carbon dioxide [4] contributing 20% to the increment of the CO_2 concentration in the atmosphere. In soils, evolution of CO_2 occurs primarily through respiration which collectively encompasses the microbial decomposition of the soil organic matter (SOM).

For all these reasons, it is important to develop the methodologies that allow one to quantify in some way, the contribution of the land use to the accumulation of gases in the atmosphere and to understand the dynamics of the carbon cycle and how it is affected by deforestation and agriculture

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due to its important role in the conservation of the soil microbiological quality that determines the sustainable fertility status of soil. It can be adjusted through the precise estimation of the microbial biomass and its activity, that is, the metabolic reactions of the soil biomass dealing with the carbon cycling. Information on changes in microbial biomass is valuable, not only because it provides an indication of slower, less easily detectable SOM changes, but also because it represents an important labile pool of plant available nutrients. Studies of carbon dynamics in soils after the addition of glucose have traditionally involved the measurement of respiratory activity either as O_2 uptake [5,6] or CO_2 evolution [7] using simple titrimetric procedures [8] or elaborate instrumentation [9]. These studies have provided bioindicator parameters of soil quality such as the qCO_2 or metabolic quotient which is related to soil development, and indicates the energy needed for maintenance of microbial biomass [10,11]. The problem is that the information provided by the qCO_2 quotient is qualitative, evaluated only in comparative studies and it does not differentiate between development and stress in some cases.

The only method that provides quantitative information about the soil microbial activity is the use of ^{14}C-labelled glucose [12]. This method has greatly facilitated the understanding of the transformations involved in C turnover in soil including its stabilization. However, the assays involved in this methodology are tedious, long and it could derive residues that could be difficult to dispose of. Results obtained are very accurate, for this reason it would be important to be able to obtain the same results with easier methods.

During the last years, microcalorimetry started to be applied as an alternative technique to compute the soil microbial activity. It has found few applications in soil microbiology despite the fact that the rate of heat output is a good measure of overall soil catabolism, and it is largely independent of organism or intermediate reactions. Mortensen and Ljungholm, [13,14] advocated the method for use as an indicator of overall microbial activity and Sparling [15] obtained good correlation with soil respiration and biomass, settling microcalorimetry as an useful additional technique to estimate catabolic activity of the soil population. It has the big advantage to involve non-destructive assays and offers potential for the investigation into the catabolism of the soil biomass. The problem is that results obtained by microcalorimetry are also qualitative, and the limitations are similar to those reported for the metabolic quotient, qCO_2. Therefore, it would be important to find the methodology that allow one to obtain quantitative results dealing with the carbon dynamics as those provided by the ^{14}C-labelled glucose method with the advantages of the microcalorimetric assays which are not tedious and provide results faster than those methods linked to the use of isotopes.

It is possible that microcalorimetry, together with thermodynamics, permits to obtain quantitative data of the efficiency of the carbon utilization by soil microorganisms from the heat released by the glucose oxidation and the microbial growth reactions in soils. That efficiency could be calculated by a combined mass and energy balance. The model developed in this work, is an application of that reported by Heijnen and von Stockar [16,17] to optimize the growth of microorganisms in bioreactors. It is based on the experimental demonstration of the existence of a correlation among the heat evolution rate in soils and the CO_2 evolution and biomass formed [18–20] and, in the conservative nature of the microbial growth reaction in soil [21], that is, the capacity of the soil microbial system to retain part of the energy provided from the carbon source as biomass. The combined mass and energy balances are applied to six soil samples collected in different sites in the Amazon. The results of the efficiency of the reactions stimulated in soil are shown in terms of the enthalpies of the glucose oxidation and microbial growth reactions, $\Delta_r H_s$ and $Y_{Q/X}$, respectively, and in terms of biomass yields $(Y_{X/S})$ and compared among the samples in order to see, on the one hand, if they are affected in some extent by changes in the land-use which could establish those yields as possible quantitative bioindicators of soil microbial quality and, on the other hand, if they permit to establish the kinetics of the glucose degradation in soils in terms of energy.

2. Experimental

2.1. The energy and the mass balances

To develop an energy balance for the microbial growth reactions, it is assumed that the system boundary is defined by the unit of biomass and that cells exchange chemical entities with the environment through the cell surface. The heat dissipation rate (ϕ) as a function of time may be integrated to obtain a curve showing the total heat (Q) generated which parallels the biomass concentration X. Plotting Q as a function of X a straight correlation is obtained. The slope indicates the amount of heat generated per unit biomass formed called heat yield ($Y_{Q/X}$). The heat yield corresponds to the enthalpy changes during growth and it can be called $\Delta_r H_x$.

From measured heat generation rates and heat yields, other conversion rates can be calculated applying energy balances. The formal derivation of the energy balance equation is developed from the models reported by von Stockar [17,22] which permits the calculation of the biomass yield ($Y_{X/S}$) of the microbial growth reaction and the enthalpy of the glucose oxidation reaction in aerobic conditions ($\Delta_r H_s$).

To develop the mass balance it is necessary that the innumerable biochemical reactions accompanying the microbial growth can be lumped into one single overall reaction that can be written by a macrochemical equation. At last it is possible because the microbial growth process has been considered as the net result of several global metabolic reactions of constant stoichiometry such as catabolism, anabolism and maintenance reactions [17]. The reaction induced in the soil samples enriched with glucose can be written as follows:

$$\begin{aligned}&\mathrm{CH_{SH}O_{So}} + Y_{\mathrm{O/S}}\ \mathrm{O_2} + Y_{\mathrm{N/S}}\ \mathrm{NH_4^+}\\&\quad \rightarrow Y_{X/\mathrm{S}}\ \mathrm{CH}_{X\mathrm{H}}\ \mathrm{O_{xo}}\ \mathrm{N}_{X\mathrm{N}} + Y_{\mathrm{C/S}}\ \mathrm{CO_2}\\&\quad + Y_{\mathrm{W/S}}\cdot\mathrm{H_2O} + Y_{\mathrm{N/S}}\ \mathrm{H^+} + \Delta_r H_s^0 \qquad (1)\end{aligned}$$

since it is stated that the glucose added to soils is oxided to CO_2 and water to produce biomass [23,24] and that no intermediate metabolites are formed at 25 °C [25]. $\mathrm{CH_{SH}O_{So}}$ represents the carbon source added to the soil while $\mathrm{CH}_{X\mathrm{H}}\ \mathrm{O_{xo}}\ \mathrm{N}_{X\mathrm{N}}$ is the formulae for the biomass [22]. The $Y_{\mathrm{i/S}}$ values showed in Eq. (1) would have to be known. It is possible since they are not independent from each other because they have to satisfy the elemental balances for C, H, O and N. Therefore, it is sufficient to measure two Y values of the six contained in Eq. (1) to compute the other four [26].

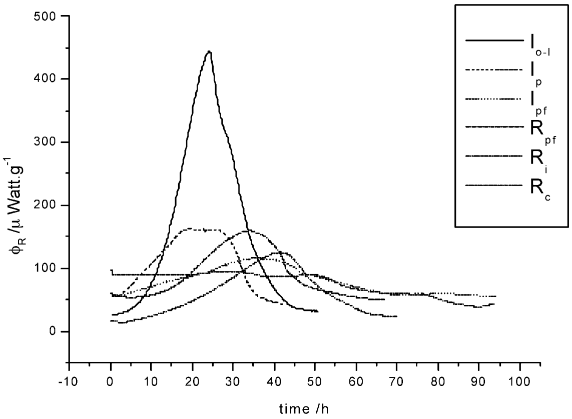

Fig. 1. Power–time curves recorded from the soil samples amended with glucose.

2.2. Calorimetric measurements

Enthalpy changes of microbial growth processes can be determined by microcalorimetry via measuring continuously the heat exchange between the growth system and the environment. The microcalorimeter used for such measurements is a Thermal Activity Monitor. One gram of soil is introduced in a 5-ml stainless steel ampoule amended with a nutrient solution containing 1.5 mg of glucose and 1.5 mg of ammonium sulfate in a volume of 0.2 ml of distilled water to register the energy expenditure of the microbial growth in soil as power–time curves. One gram of soil amended with 0.2 ml of distilled water is introduced in the reference ampoule as blank. The power–time curves obtained by this treatment reflect all the phases of the exponential microbial growth reaction in soil, lag, exponential growth, stationary and death phases [20,27] and the reaction taking place in the microcalorimeter can be written as Eq. (1). The microcalorimetric measurements were performed isothermally at 25 °C. Previously, soil samples were pre-incubated at that temperature during 24 h.

The power–time curves were integrated to compute the total heat dissipation, Q, of the growth process in Joules per gram of soil ($\mathrm{J\,g^{-1}}$). This treatment was done to six soil samples collected in different sites in the Amazon.

2.3. Estimation of the soil biomass

The initial number of microorganisms of the soil samples was calculated by the most probable number method isolating microorganisms in soil extract medium. The specific microbial growth rate constant, μ, can be calculated from the power–time curves. The value of μ, together with the value of the peak-time, allow one to quantify the increment in the number of microorganisms (ΔN) by the equation that defines the exponential microbial growth [27,28]. The problem now is the conversion of the number of bacteria in soil, given by the most probable number method as number of cells per gram of soil, to biomass units (gram of biomass per gram of soil or mole of biomass per gram of soil). This is an essential step to apply the equations of the energy balance. The conversion was done by the equation reported by Sparling [18] that relates the biomass in soil to the heat dissipation rate of the soil amended with glucose:

$$y = 1.025 + 0.856x \tag{2}$$

where $x = \log \phi_R$ in microwatts and $y = \log$ of biomass in microgram. The application of Eq. (2) before and after the addition of glucose gives the increment in biomass (ΔX) after exponential microbial growth. In order to see whether a correlation exists between both methods to assess biomass, graphical regression analysis were performed.

2.4. Calculation of enthalpies and stoichiometric coefficients

The quotient between the experimental values of Q and the values of ΔX computed by Sparling's equation permit to quantify the heat yield, $Y_{Q/X}$, of the microbial growth reactions taking place in soils. The application of the equations reported von Stockar [17] permits to quantify the values of the biomass yield ($Y_{X/\mathrm{S}}$) and the enthalpy of the glucose degradation in soil ($\Delta_r H_s$). The values of the enthalpies of combustion ($\Delta_c H_i^0$) used to estimate $Y_{X/\mathrm{S}}$ and $\Delta_r H_s$ were $-2803\ \mathrm{kJ\,mol^{-1}}$ for the glucose, $-559\ \mathrm{kJ\,mol^{-1}}$ for the biomass and $-296\ \mathrm{kJ\,mol^{-1}}$ for the nitrogen [17]. Eq. (1) and the values of $Y_{X/\mathrm{S}}$ and $\Delta_r H_s$ allow determination of the stoichiometric coefficients dealing with the oxygen consumption ($Y_{\mathrm{O}/X}$), CO_2 production ($Y_{\mathrm{CO_2}/X}$) and water production $Y_{\mathrm{H_2O}}$ together with NH_4^+ consumption and H^+ formation.

Knowing $Y_{X/\mathrm{S}}$ a proper macrochemical reaction equation taking place in soil can be written in a general form, without knowing all the stoichiometric coefficients but one (+1 for biomass) as:

$$a\mathrm{C_6H_{12}O_6} + b\mathrm{O_2} + c\mathrm{NH_4^+} \rightarrow \mathrm{CH_{1.8}O_{0.5}N_{0.2}} + d\mathrm{CO_2} + e\mathrm{H_2O} + f\mathrm{H^+} \tag{3}$$

where $CH_{1.8}O_{0.5}N_{0.2}$ is the formulae reported for the biomass [16].

The following conservation equations can now be written:

C conservation: $6a + 1 + d = 0$
H conservation: $12a + 4c + 1.8 + 2e + f = 0$
O conservation: $6a + 2b + 0.5 + 2d + e = 0$
N conservation: $c + 0.2 = 0$
Charge conservation: $c + f = 0$

There are six unknown stoichiometric coefficients (a–f) that are related by five conservation equations. Having one measured coefficient allows the calculation of all other coefficients. Therefore, the calculated biomass yield from the enthalpy balance permits determination of the stoichiometric coefficients of the previously defined macrochemical equation taking place in soil.

2.5. Soil samples

The combined mass and energy balance was applied to several soil samples collected in different sites in the Amazonian state of Brazil. Properties of the samples and sampling sites can be seen in Table 1. Sampling and manipulation of the soils have been explained in detail in previous papers [29,30]. The sampling place was located in Nova Airao, 200 km far away from Manaus, up Negro River. In Nova Airao, two sampling sites were chosen, location R in the river side and rich in organic matter content, and location I, situated inland and poor in organic matter content. In each location, R and I, three sampling places were chosen supporting Primary forests and arable lands, the last were Cassava plantations, orange and lemon trees and pasture. Details are given in Table 1.

Linear and non-linear graphical analysis were done using the average values computed from microcalorimetric measurements and from the balances to explain the kinetics of the glucose degradation in all samples. We have compared the results among the different samples in order to see which factors could influence the kinetics of the glucose degradation and the efficiency of the carbon mineralization in soil in terms of energy and biomass yields.

3. Results

The properties of the samples and sampling sites can be seen in Table 1.

3.1. Results of the energy balance

Fig. 1 shows the power–time curves obtained from the soil samples. The interpretation of those curves and its correlation to the microbial growth have been established by this group in a previous paper [20]. All of them show the typical pattern of the microbial growth reaction: a lag phase followed by an exponential increase of the heat evolution rate due to the exponential microbial growth, then a stationary phase is reached followed by the decline of the power–time curve due to the microbial death. Fig. 2 shows the correlation obtained between the heat evolution rate and the

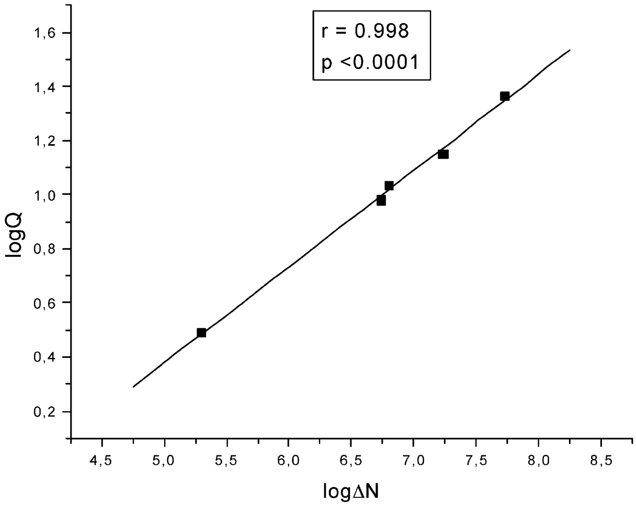

Fig. 2. Plot of the logarithm of the total heat (Q) calculated for the soil samples against the logarithm of the increment in the number of microorganisms after exponential growth (ΔN).

Table 1
Some physical-chemistry and biological properties of the soil samples studied

Samples	Humidity (%)	WHC (%)	SOM (%)	C (%)	N (%)	H (%)	pH	$N_0 \times 10^5$
Rpf	21.84	55.49	6.11	3.9	0.52	0.55	3.4	8.3
Ri	35.04	73.31	12.9	10.89	0.45	1.07	3.9	6.3
Rc	28.95	67.60	5.7	4.21	0.74	0.83	3.7	3
Ipf	6.37	35.45	1.1	1.43	0.05	0.35	3.7	0.52
Io-l	11.64	40.03	2.4	1.55	0.83	0.19	3.7	42.25
Ip	14.11	39.70	3.2	1.56	0.79	0.17	4.52	53.5

WHC (%), it represents the water holding capacity of the soil samples; SOM (%), percentage of soil organic matter; C (%), percentage of carbon; N (%), percentage of nitrogen; H (%), percentage of hydrogen; N_0, number of microorganisms per gram of soil. Soil samples were collected in two locations, location R in the riverside, rich in organic matter content and location I, situated inland, poor in organic matter content. Rpf, samples collected in Primary forests in location R; Ri, samples collected in an 'igarapé' (plot of land seasonally flooded by the Negro River) in location R; Rc, samples collected in a Cassava plantation in location R; Ipf, samples collected in Primary forests in location I; Io-l, samples collected in an orange and lemon arable land in location I; Ip, samples collected in a pasture in location I.

Table 2
Experimental values obtained microcalorimetrically

Samples	ϕ_{Ro} (μW d^{-1})	$\phi_{R(glc)}$ (μW)	Q (J g^{-1})	μ (h^{-1})	PT (h)	$Y_{Q/X}$ (kJ molX^{-1})
Rpf	44.95 ± 10.65	160.79 ± 3.56	10.82 ± 1.33	0.064 ± 0.016	35.68 ± 1.92	490
Ri	40.01 ± 5.42	127.61 ± 3.81	9.51 ± 1.80	0.055 ± 0.015	39.22 ± 1.81	553
Rc	90 ± 2.43	95.105 ± 0.70	3.09 ± 0.72	0.01 ± 0.003	50 ± 2.01	3153
Ipf	38.09 ± 3.54	163.25 ± 9.73	18.67 ± 2.44	0.028 ± 0.005	41.7 ± 6.74	778
Io-l	81.52 ± 0.79	491.05 ± 43.66	23.15 ± 1.59	0.081 ± 0.003	24.87 ± 1.55	340
Ip	49.73 ± 6.73	119.84 ± 4.24	14.12 ± 1.86	0.045 ± 0.017	22.83 ± 0.97	1031

Mean ± S.D. $N=3$. The indirect measurements were done using the mean of the experimental values. ϕ_{Ro} (μW d^{-1}), heat flow rate recorded from soil samples before the addition of glucose in microwatts per day; $\phi_{R(glc)}$ (μW), heat flow rate recorded from soil samples after the addition of glucose in microwatts per gram of soil; Q (J g^{-1}), total heat released per gram of soil samples calculated from the area limited by the power–time curves obtained from soil samples amended with glucose; μm (h^{-1}), microbial growth rate constant calculated from the slope of the lines obtained plotting the logarithm of the heat flow rate against time; PT (h), values of the peak-time, that is, time at which the micro calorimetric signal reaches its maximum amplitude; $Y_{Q/X}$ (kJ molX^{-1}), values of the heat yield computed for each soil sample.

Table 3
Data computed from the energy and mass balances applied to all samples used in this study

Samples	$\Delta_r H_s$ (kJ molS^{-1})	$Y_{X/S}$ (mol$X$ molS^{-1}	η (%)	$Y_{CO_2/S}$	$Y_{O_2/S}$	$Y_{Q/O}$
Rpf	1279	2.61	54.32	3.36	3.24	395
Ri	1360	2.46	51.43	3.5	3.38	402
Rc	2362	0.75	15.64	5.25	5.21	453
Ipf	1595	2.05	43.04	3.96	3.43	465
Io-l	1032	3.03	63.14	2.97	2.96	573
Ip	1787	1.73	36.18	4.28	4.19	426

$\Delta_r H_s$ (kJ molS^{-1}), quantity of heat dissipated per mole of glucose added; $Y_{X/S}$ (mol$X$ molS^{-1}), microbial growth yield in mole of biomass formed per mole of glucose consumed; η (%), percentage of energy that is retained in the system as biomass; $Y_{CO_2/S}$, mole of CO_2 released per mole of glucose consumed; $Y_{O_2/S}$, mole of O_2 consumed per mole of glucose degraded; $Y_{Q/O}$, quantity of heat released in kilojoule per mole of O_2 consumed.

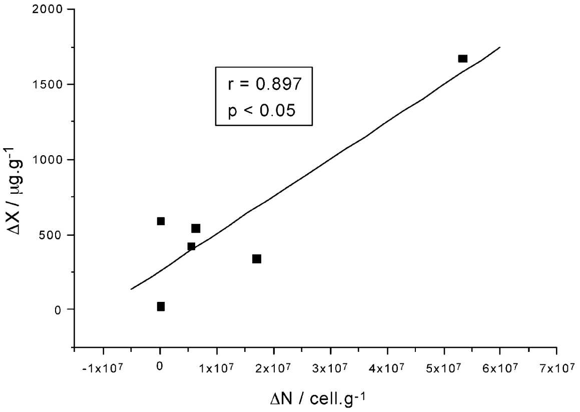

Fig. 3. Plot of the increment in biomass (ΔX) calculated by Eq. (2), against the increment in the number of cells accounted for the samples amended with glucose.

increase in the number of microorganisms in soil. This correlation allows one to apply the enthalpy and mass balances performed.

Values of $Y_{Q/X}$ computed experimentally from the power–time curves are shown in Table 2 together with other microcalorimetric data. Results of the enthalpy and mass balance are shown in Table 3. When data of $Y_{Q/X}$ and $\Delta_r H_s$ are compared among samples it is observed that the highest values correspond to soils supporting Cassava and pasture (samples Rc and Ip, respectively).

Fig. 3 shows the plot of ΔX, obtained from Sparling's equation, vs. ΔN obtained experimentally. It is observed a significant linear correlation between both values. This feature confirms the existence of a relation between the two methods employed to estimate biomass in soil. These data show a significant positive relation between $\ln \Delta_r H_x$ and $\Delta_r H_s$ and a negative logarithmic dependence between $\Delta_r H_x$ and $Y_{X/S}$. There is also a inverse hyperbolic relation between $\Delta_r H_x$ and the microbial growth rate constant (μ) Therefore, values of $\Delta_r H_x$ are biomass yield dependent. In that sense, the graphical analysis establishes that higher dissipation of energy per unit of biomass occurs at lower biomass yields and lower microbial growth rates in soils.

The intercept of the plot of $\ln \Delta_r H_x$ vs. $Y_{X/S}$ is not zero. The value obtained (8.708 kJ $\mathrm{mol}X^{-1}$) could indicate the heat released per unit of biomass at non-growing conditions in soils. It was found a negative linear relation between $\Delta_r H_s$ and $Y_{X/S}$ too.

Results show that if the same quantity of glucose is added to different soil samples, the differences in the values of $\Delta_r H_s$ and $\Delta_r H_x$ found among the samples can be attributed to different biomass yields. Samples with higher values of $Y_{X/S}$ release less energy as heat.

The values of $\Delta_r H_s$, $\Delta_r H_x$ and $Y_{X/S}$ permit to quantify the efficiency of the microbial growth in soils from the Battley's equation [31–33]:

$$\eta_H = \frac{\Delta H_{NC} - \Delta H_C}{\Delta H_{NC}} \qquad (4)$$

which gives the quantity of energy that becomes conserved within the substances of the cells. Therefore, η_H represents the efficiency of the enthalpy conversion which can be called also thermodynamic efficiency. The subscripts NC and C refer to the so-called non-conservative reaction, i.e. the one that would ensue without any biomass formation, and, respectively, to the conservative, i.e. the actual growth reactions in soils. Results are shown in Table 3.

The thermodynamic efficiency, $\eta_{H,}$ is directly related to the biomass yield and negative related to $\Delta_r H_s$ as it was expected. Therefore, higher values of $Y_{X/S}$ implies higher quantities of energy preserved as biomass which is reflected as lower losses of energy as heat in soils. The intercept of the plot of $\Delta_r H_s$ vs. η_H gives the value of the enthalpy of combustion of the glucose (−2800 kJ mol^{-1}), that is, when η_H tends to zero, all the energy from the glucose is dissipated as heat.

Table 4
Values of the glucose degradation rate (r_s) computed for the samples and percentage of the glucose added that was degraded in the soil samples

Samples	r_s (molS h^{-1})	[glucose] (%)
Rpf	2.43×10^{-7}	100
Ri	1.78×10^{-7}	100
Rc	2.60×10^{-8}	16
Ipf	2.22×10^{-7}	100 + SOM
Io-l	3.50×10^{-7}	100 + SOM
Ip	3.67×10^{-7}	100

Values of $\Delta_r H_s$ allow one to quantify the substrate degradation rate, r_s. Results are shown in Table 4 together with the values of the percentage of the glucose added that was degraded in the soil samples. Both values can be calculated directly from the enthalpy balances by the stoichiometry of the microbial growth reaction.

If sample Ip is removed from the regression analysis, linear correlations are found among r_s and μ, $\Delta_r H_s$ and $Y_{X/S}$, therefore, higher substrate degradation rates yield higher biomass yields and less expenditure of energy as heat. Results of r_s and values of $\Delta_r H_s$ show that in samples Ipf and Io-l, the total heat released, Q, is higher than that which should be obtained from the glucose degradation exclusively. This feature suggests that in those samples, microorganisms degrade some other carbon source concomitantly to the glucose consumption.

All these results allow the kinetics of the glucose degradation and microbial growth in soils to be established, which is very important to understand the kinetics of the carbon mineralization and the carbon cycling. In this sense, it seems that samples that show higher rates of glucose degradation and microbial growth dissipate less energy as heat, retain more energy in the system and have higher biomass yields. On the whole, less dissipation yields higher efficiency.

The question now is: which factors have influenced the above kinetics?

If sample Ip is removed from the regression analysis, a linear relation is obtained between the heat flow rate and the initial number of microor-

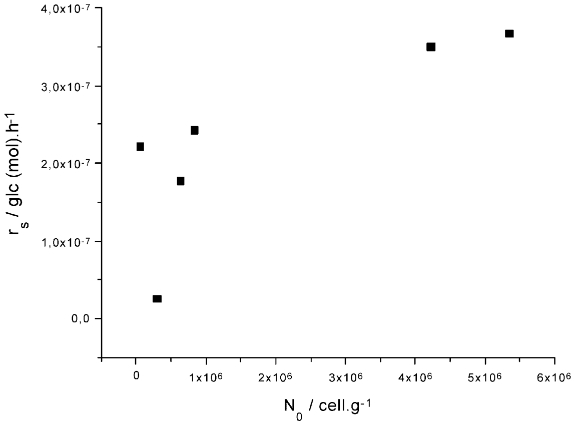

Fig. 4. Plot of the glucose degradation rate (r_s) against the initial number of microorganisms per gram of soil sample (N_0).

ganisms of the samples, N_0. Fig. 4 shows that values of r_s appears to increase with increasing values of N_0. There is also a positive hyperbolic relation between the percentage of nitrogen of the soil samples and N_0. These results suggest that the soil microbial population affects the kinetics of the glucose degradation and that the nitrogen content of the samples is the physico-chemical property of the soil that is engaged with all these processes.

3.2. Results of the mass balances

Table 5 shows the results of the mass balances of the microbial growth reactions in soils. In Table 3 it can be observed the yields of CO_2 production ($Y_{CO_2/S}$) and O_2 consumption ($Y_{O_2/S}$) and the heat released per mole of oxygen respired ($Y_{Q/O}$). The values of $\Delta_r H_s$ correlate positively with the $Y_{CO_2/S}$ values, indicating that samples that release more heat, lose more carbon as CO_2 to the atmosphere through respiration. There is also a significant dependence of $Y_{X/S}$ and η_H with $Y_{CO_2/S}$ and values of $\Delta_r H_s$ also parallel to the oxygen consumption. On the whole it seems that the most efficient processes release less CO_2 and consume less oxygen in soils.

4. Discussion

The positive correlation between the nitrogen percentage of the samples and their initial number of microorganisms is consistent with that published in Ref. [12]. The relation reported between the heat dissipation rate and the initial microbial population is in agreement with that published by Sparling [15]. This feature makes consistent the use of the Sparling's equation to compute the increase in biomass due to exponential microbial growth.

Values of $\Delta_r H_x$ calculated experimentally and values of $Y_{Q/O}$ computed from the mass balance are very close to those published by different authors for different microorganisms, ranging from -385 to -492 kJ molO_2^{-1} for $Y_{Q/O}$ [34,35]. The plot of $\Delta_r H_s$ vs. η_H demonstrates that if growth is so efficient to reduce the need of energy dissipation to zero, the energetic efficiency tends towards unity. This is consistent also with the results obtained plotting $\Delta_r H_s$ against $Y_{X/S}$ that indicates that at zero biomass yield, the intercept of the dissipation line simply corresponds to the enthalpy of combustion of 1 mol of glucose. When increasing biomass yields, the enthalpy of the glucose degradation and the enthalpy of the overall growth reaction decrease because some of the energy initially contained in glucose is now retained in the biomass [16,31,34]. The slope of the line obtained plotting $\Delta_r H_s$ vs. $Y_{X/S}$ gives a value of -583 kJ molX^{-1} which is very close to values of

Table 5
Results of the mass balance applied to the six soil samples collected in the Amazon

$(-0.38)\ C_6H_{12}O_6+(-1.23)\ O_2+(-0.2)\ NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+1.28\ CO_2+1.68\cdot H_2O+0.2\ H^+$
$(-0.40)\ C_6H_{12}O_6+(-1.35)\ O_2+(-0.2)\ NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+1.4\ CO_2+1.8\cdot H_2O+0.2\ H^+$
$(-1.33)\ C_6H_{12}O_6+(-6.93)\ O_2+(-0.2)\ NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+6.98\ CO_2+7.38\cdot H_2O+0.2\ H^+$
$(-0.41)\ C_6H_{12}O_6+(-1.41)\ O_2+(0.2)\ NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+1.46\ CO_2+1.86\cdot H_2O+0.2\ H^+$
$(-0.33)\ C_6H_{12}O_6+(-0.98)\ O_2+(0.2)\ NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+0.98\ CO_2+1.38\cdot H_2O+0.2\ H^+$
$(-0.58)\ C_6H_{12}O_6+(-2.43)\ O_2+(0.2)\ NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+2.48\ CO_2+2.88\cdot H_2O+0.2\ H^+$

the enthalpy of combustion of the biomass reported in Ref. [17] that ranges from -561.6 to -495.1 kJ molX^{-1}. The average used for calculations in this work was -559.4 kJ molX^{-1}.

Values of CO_2 released and O_2 consumption computed from the mass balances are functions of $\Delta_r H_s$ values which parallels the oxygen consumption as it was established for isolated microorganisms. For this reason, the oxygen consumption data permits to compute $\Delta_r H_s$ by multiplying by a factor of -488 kJ molO_2^{-1} for Amazonian soils. The factor reported in the literature is $Y_{Q/S} = Y_{O/S}$ (-460) kJ molO_2^{-1} [34].

Results also show the dependence of the CO_2 production to the biomass and energy yields but that is something well-known and it constitutes the basis of the so-called indirect calorimetry.

Results suggest that in soils, the measured values for biomass yields and heat generation, probably reflect a compromise between two extremes as it has been demonstrated for isolated microorganisms [33]:

- Very high biomass yields but energy dissipation close to zero.
- Very high energy dissipation but growth yields approaching zero.

As a consequence, microorganisms do not seem to be able to retain more than approximately 60% of the carbon atoms available in the substrate for reasons lying in the evolution. Nature would not dissipate more than 50% of the available energy to produce high power, except in stress situations [36,37]. This appears to be truth for soils in this study, since the percentage of energy dissipated as heat ranges from 37 to 57% with the exception of samples Rc and Ip. The percentage of carbon retained in biomass computed from the mass balances ranges from 44 to 13%. No samples show values up 60% which is in agreement with Refs. [26,38,39].

Currently, the only method that permits to compute accurately the percentage of carbon kept in soil as biomass is the use of ^{14}C-labelled glucose. The latest results reported by this method show that the quantity of $^{14}CO_2$ respired in soils accounted for 25–44% of the glucose input after 3 days of experiment while the glucose derived in biomass C amounted to between 18 and 60% at day 3 [12]. These values are closed to those computed by our method.

The efficiency of the carbon and energy utilization varied among the samples and appears to be sensible to changes introduced in the habitat such as the deforestation to establish the agriculture. In this sense, the percentage of the energy dissipation accounted for 84% in sample Rc and 64% in sample Ip, both supporting Cassava and pasture, respectively. On the opposite side, sample Io-l collected in an orange–lemon plantation, appears to optimize the energy from the carbon source since that sample only releases approximately 37% of the energy available in the glucose added. In the Primary forests, the energy dissipated ranges from 46 to 57% that can be considered as normal values. Samples Rc and Ip also show very high values of CO_2 dissipation (~88 and 71%, respectively). Therefore, the deforestation to introduce Cassava and pasture decreases strongly the efficiency of the soil microbial metabolism and probably causes stress. It seems that the parameters reported in this work appear to be sensible to changes in the land-use. This feature would permit to use them as bioindicators of soil quality.

The stoichiometry of the reaction in samples Ipf and Io-l indicates the possibility of the existence of organic matter degradation concomitant to the respiration of the glucose added. Sample Ip releases 18.28 J g^{-1} while the degradation of the glucose added should release 13.28 J g^{-1}. Therefore, this sample releases 5.39 J g^{-1} from the degradation of another energy source. The same effect is observed in sample Io-l. From the stoichiometry of the reaction, 8.58 J g^{-1} are expected from the degradation of glucose but the experimental value accounts for 23.15 J g^{-1}. The quantity of heat derived from the other carbon source was higher in this sample than in sample Ipf. It seems that the introduction of lemon and orange trees increases the initial microbial population and stimulates the SOM degradation which is more available to microbial attack in these samples. As the percentage of SOM in sample Io-l is very low and since the organic matter input in soils under agriculture is lower than in Primary forests, the introduction of agriculture in location I could derive in losses of SOM affecting the soil fertility [40]. This fact introduces an error in the estimation of the efficiency and growth yield but as the formulae for the organic matter can be known yet, its contribution could be included in the equation that defines the reaction taking place in soil. On the opposite side, the organic matter in location R appears to be less available and more protected to microbial attack than samples from location I. Once more, the reason could be attributed to the quality and nature of the SOM [41,42].

The value of $\Delta_r H_x$ computed for sample Ic, -3156 kJ molX^{-1}, is very close to that reported for M. Thermoautotrophicum, -3730 kJ molX^{-1} [43].That microorganism use CO_2/H_2 as carbon source and produces CH_4 in anaerobic conditions. The stoichiometry of that reaction assuming aerobic conditions shows that only 16% of the glucose added was degraded in sample Rc. These data strongly suggest that the heat computed for sample Rc could be reflecting the activity of some anaerobic microorganisms and that the kinetics of the aerobic degradation of the glucose is strongly affected. It seems that the plantations of Cassava affects strongly the nature of the microbial population which could be responsible of the loss of productivity observed in those lands after 4–5 years of culture.

It has been reported that the deforestation to introduce agriculture in tropical soils, causes poor microbial growth and results in low levels of soil microbial population [44]. The deforestation in the Amazon reduces the microbial population in Cassava plantations, causes poor microbial growth in samples Rc and Ip and affected the efficiency of the carbon and energy utilization in all samples under agriculture. On the whole, agriculture in the Amazon could be affecting the soil quality. This fact remarks the importance of the development of studies dealing with the optimization of the land use in the Amazon in order to preserve the soil quality there.

5. Conclusions

The data reported from the combined mass and energy balance permits the kinetics of the aerobic degradation of glucose and microbial growth reactions in Amazonian soils to be established. It can be represented as a coupled irreversible conservative reaction. The differences among values of $\Delta_r H_x$ and $\Delta_r H_s$ computed for the soil samples, lie in the degree of coupling between the oxidative and biomass formation reactions which can be affected by the initial number of microorganisms of the samples, by the percentage of nitrogen on the samples and by the quantity of the glucose added that microorganisms can degrade.

The energy and biomass yields computed appears to be sensible to changes in the land use and for that reason could be considered as bioindicators of soil microbial quality and as an early warming of soil deterioration.

This work demonstrates the powerful role of microcalorimetry and thermodynamics in soil research. Both provides a way to quantify efficiencies of the energy and carbon utilization by soil microorganisms that leads to a better understanding of the carbon cycling in a way that is easier and faster than the current methodologies employed in these kind of studies.

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List of symbols

$Y_{Q/X}$	Heat yield on biomass, kJ mol^{-1}
Q	Total heat, kJ
X	Biomass
X_0	Biomass before the addition of glucose
ϕ_R	Heat flow dissipated by reaction, microwatts per gram of soil, μW g^{-1}
$\Delta_r H_x$	Enthalpy change of the microbial growth reaction, kJ mol^{-1}
$\Delta_r H_s^0$	Standard enthalpy change of the carbon source degradation reaction, kJ mol^{-1}
r_s	Rate of substrate consumption, mol h^{-1}
$Y_{Q/S}$	Heat yield on substrate, kJ mol^{-1}
$\Delta_c H_i^0$	Standard enthalpy of combustion of i,

	kJ (C mol)$^{-1}$ or kJ mol^{-1}
$Y_{i/j}$	Yield or stoichiometric coefficient of i per j, in mole or C mol^{-1}
ϕ	Heat flow rate, W
Subscripts	
S	Carbon substrate
P	Product
O	Oxygen
N	Nitrogen source
H	Hydrogen
W	Water

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