J Therm Anal Calorim (2011) 104:23–29
DOI 10.1007/s10973-010-1091-3

A new approach to determine soil microbial biomass by calorimetry

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ISBC XVI Conference Special Issue

Abstract A method to determine soil microbial biomass (SMB) by isothermal calorimetry is reported. Soil samples ranging in pH from 6.2 to 9.4 and different textures were used to develop the method. Soil at 60% of its field capacity humidity was amended with a previously determined amount of glucose as to give the maximal response of CO_2 evolution. Then, an aliquot was weighed in the calorimeter ampoule and specific thermal power (p)–time (t) curves were obtained at 25 °C. After 1–2 h, a vial containing a 0.5 M NaOH solution was introduced to determine the specific thermal power due to CO_2 evolution, pCO_2 during 1–2 h. Then, the vial was removed and the experiment continued for 1–2 h. Specific thermal power due to CO_2 evolution was converted to rate (CO_2-C/mm^3 g^{-1} h^{-1}) by using the heat of reaction of CO_2 with NaOH and the molar volume. This value was further converted into SMB/μg g^{-1} by using a conversion factor of 32.4. A guide to perform the calculations is given. Values of log SMB were linearly related with values of log p giving a similar relation to a previously reported where SMB was determined by conventional methods.

Keywords Calorimetry · Soil · Microbial biomass

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Introduction

Microbial biomass carbon is an important indicator of soil fertility and its determination is essential in ecological research and agriculture. The first method to be developed to determine soil microbial biomass (SMB) was the fumigation with $CHCl_3$-incubation (CFI) [1]. In this method, equal aliquots of fumigated and not fumigated soil are inoculated with a suspension of untreated soil in water and incubated during 10 days in presence of a NaOH solution that acts as trap of CO_2. The difference in CO_2 evolved between the fumigated and the not fumigated samples divided by a factor (0.45 for 25 °C) gives the SMB. Since then, several methods were developed based on this first one. However, the substrate-induced respiration, SIR [2] and the $CHCl_3$ fumigation–extraction, CFE [3] methods developed later, are the most commonly used nowadays because they are not much influenced by soil conditions such as pH [4].

The SIR method is based in the determination of CO_2 evolution from soil during a pre-determined incubation period, after the addition of a carbon source—currently glucose—that triggers it [2, 5]. This CO_2-C (μl g^{-1} soil h^{-1}) flush was correlated to soil microbial biomass (SMB/μg g^{-1} soil) by the expression: SMB = 40.04 × CO_2-C [2] after calibration against the CFE method at 22 °C. This method is based on the activity of SMB [5]. However, a proportionality factor of 30 (22 °C) was obtained to convert CO_2-C to SMB by calibration of SIR with CFI method [6] and it is this factor that was recommended later to be used for this conversion [7]. This factor of 30 was corrected for the effect of temperature becoming 25.3 for determinations of SIR-SMB at 25 °C [4]. It seems that 1 °C increase in temperature between 20 and 25 °C produces 7% increase in respiration rate.

Calorimetry could be a suitable technique to measure CO_2 evolution from glucose amended soils. A method was developed to measure CO_2 evolution from plant tissues [8, 9] and consists in the introduction of a vial containing a solution of NaOH in the calorimeter vessel to trap the CO_2 evolved. This approach was also used to determine rate of CO_2 evolution from not amended soils [10, 11]. Simultaneous measurements of thermal power due to basal metabolism and of CO_2 evolution allowed the calculation of the calorespirometric ratio which is an index to assess the mineralizing activity of soil microorganisms. However, to our knowledge, no attempts have been made to use this approach to determine SMB by calorimetry. It is well documented that there is a linear relation between the logarithmic values of SMB determined by the CFE method and those of the thermal power values determined in the calorimeter from amended soils [12]. The reported equations are: $\log \text{SMB} = 1.352 + 0.913 \log p$ for stored mineral soils and $\log \text{SMB} = 1.025 + 0.856 \log p$ for fresh mineral soils.

In view of this background, we hypothesized that if simultaneous measurements of p and of CO_2-C were performed by calorimetry, a similar equation to those reported [12] should be obtained when plotting the $\log_{10}$ values of p and those of SMB as determined by calorimetry. Thus, in this article we report the results obtained.

Materials and methods

Soil samples

In order to develop the method soil (5 samples: M1–M5) was collected after removal of the very top layer, up to a depth of 10 cm in the fields of the Catamarca INTA (National Institute of Agricultural Technology), Provincial Road N° 34, km N° 4, Sumalao, Valle Viejo Department, Catamarca province, Argentina in May 2009. Soil samples were sieved (2 × 2 mm) to eliminate coarse material and root residues and stored in polyethylene bags at 4 °C until used. To validate the method, the following soil samples were used: DMSO, soil incubated during 2 months with 0.1% (*v/w*) dimethylsulphoxide; Brz, soil incubated during 2 months with 100 mg kg^{-1} ethyl acetate extract of roots of the weed *Brachiaria plathyphylla*; *B*IS and *B*NIS, soils collected from a field either invaded or not yet invaded with the weed *B. plathyphylla*, respectively.

Chemical and microbiological analysis

Water content (WC) was determined by drying an aliquot of soil (2×) until constant weight at 105 °C [13]. Field capacity humidity (FCH) was determined by the graduated cylinder method [14]. The pH was measured with a glass electrode on a suspension of soil in deionised water (1:1) [15]. Organic carbon (OC) was determined by wet oxidation with $K_2Cr_2O_7/H_2SO_4$ [16]. Extractable phosphorus (P) was photometrically determined after soil extraction with the Olsen method [16]. Extractable Nitrogen, as the sum of NO_3^-, NO_2^-, and NH_4^+ was determined by the diffusion method [17]. Colony formation units (CFU g^{-1}) were determined by the serial dilution method by using trypteine soy agar (TSA) as culture media.

Calorimetry

A twin heat conduction type calorimeter (Lund University, Sweden) was used [8, 18, 19]. An aliquot of soil was pre-incubated with a WC of 50% its FCH in polyethylene bags at 25 °C. In some cases (see "Results and discussion" section), Nitrogen as $(NH_4)_2SO_4$ was added (0.2 mg g^{-1}) during the pre-incubation period. After 24 h, WC of soil was adjusted either to its FCH or to 60% of FCH with water containing glucose and thoroughly mixed. Then 1.0–1.5 g soil dry weight equivalent (d.w.) was weighed in the calorimeter vessel (8 cm^3). In all determinations, thermal power, P_1, was measured during certain time before a vial containing 0.2 cm^3 of a 0.5 M NaOH solution was introduced in the calorimeter vessel to record the thermal power, P_2, due to CO_2 evolution. After a determined period of time, the vial was removed and thermal power, P_3, was measured for 1–2 more hours. To determine the appropriate time to introduce the vial containing NaOH, *p–t* curves of microbial growth were recorded during 8 h with soil samples at FCH amended with different glucose concentrations.

Analysis of calorimetric curves

Thermal power (*P*)–time (*t*) curves obtained were analyzed with the Origin 6.0 computer program (Microcal, Inc. 1991–1999). First, they were converted into specific thermal power (*p*)–time (*t*) curves by dividing the values of *P* by the mass of soil (dry weight basis). A typical *p–t* curve is shown in Fig. 1a. The values of p_1 were averaged and represent the p value before the exponential microbial growth starts. Then, the following question arises: how to calculate from these curves the heat due to CO_2 evolution (q)? The ideal case would be that the lag phase of the microbial growth curve is long enough so p_3 values are close to those of p_1. Then, q due to the evolution of CO_2 during 1 h can be calculated by the following expression: $q/\text{mJ g}^{-1} = \{p_2–[(p_1 + p_3)/2]\} \times 3{,}600 \text{ s h}^{-1}$. However, this is not always the case, and therefore, a different approach must be used to calculate q. The data set of the original *p–t* curve must be pasted again in two new columns

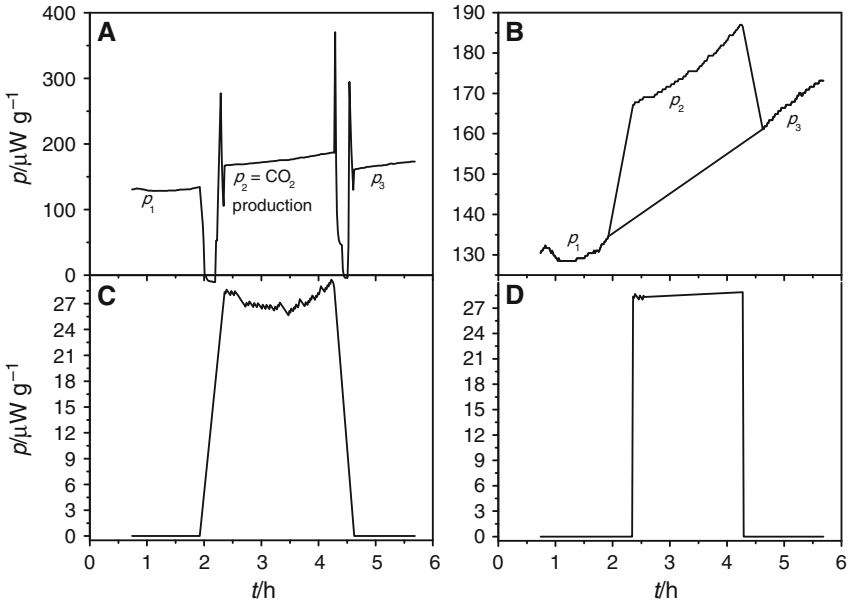

Fig. 1 Analysis of specific thermal power (p)–time (t) curves: **a** original curve; **b** Plot of the original curve and the modified one; **c** Resulting p–t curve after subtraction of the modified curve from the original one; **d** Resulting p–t curve after deleting the lower values of p and time adjustment to the period of CO_2 evolution measurement. This latter curve is integrated to obtain the heat released due to CO_2 (q)

of the computer graph program worksheet and the p_2 values due to the CO_2 production must be deleted. Also, data due to the ampoule openings must be removed from both data sets. Then, if both curves (the original without ampoule openings and the new one without ampoule openings plus p_2 values) are plotted together Fig. 1b is obtained. Then, the new p–t curve can be subtracted from the original one to obtain Fig. 1c. Note that in Fig. 1b, p_1 and p_3 belong to both plotted curves and p_2 only to the original curve. Thus, the values of p_1 and p_3 in the new curve are connected through a straight line which is not the real pattern that p values would follow during that period of time if not vial was introduced. Therefore, after subtraction the lower values of the data set representing Fig. 1c can be deleted to obtain Fig. 1d. Also note that the time values should be adjusted to the period of CO_2 evolution measurements. Then, the area under the resulting p–t curve times 3,600 s h^{-1} gives the heat (q/mJ g^{-1}) due to CO_2 evolution during that period of time. The calculated q value must be then divided by the time of measurement and by the heat of reaction of CO_2 with NaOH (108.5 kJ mol^{-1}) and multiplied by the molar volume at 25 °C (24.5 dm^3 mol^{-1}) to obtain the rate of CO_2 evolution in mm^3 g^{-1} h^{-1}. Then, by using a determined factor, the calorimetric soil microbial biomass (CAL-SMB/μg g^{-1} soil) is calculated. Results are reported as the mean of 2–4 measurements ± SD.

Determination of SIR-SMB

In order to determine SIR-SMB the SIR3 (AA) method [4] was used with modifications according with Nakamoto and Wakahara [20]. Soil (10 g d.w.), mixed with 2 mg g^{-1} glucose at 60% of its FCH and previously stabilized at 25 °C during 24 h, was placed in a Petri dish after 2 h of glucose addition. Then, it was inserted in a hermetically closed jar containing 20 cm^3 of a 0.05 M NaOH solution. A control experiment was performed without soil. After 2 h, the dish was removed and the absorbed CO_2 was precipitated with 2 cm^3 of a 0.5 M of $BaCl_2$ solution. The remaining NaOH was back-titrated with a 0.05 M HCl solution. The values of CO_2-C were calculated from the expression: $(V_c - V_s)/2tm$ where V_c is the remaining volume of NaOH for control and V_s is the remaining volume of NaOH for the sample. SIR-SMB was calculated by using a coefficient of 25.3 as previously reported for 25 °C [4].

Results and discussion

Table 1 shows the characteristic of the studied soils. Figure 2 shows a typical p–t curve of microbial growth during the first 8 h since the addition of glucose. Curves like this should be obtained for each soil to be studied to determine when the vial with NaOH should be introduced to quantify CO_2 evolution. It was reported that the rate of CO_2 evolution decreases during the first 2 h then, is stable from 2 to 4 h to increase in some cases after 4 h [20]. It was recommended that one should start measurements of CO_2 when the initial decline ceased and stop when the increase starts because it indicates new biomass synthesis [2, 20]. Therefore, measurements should be performed during the time period shown between the arrows in Fig. 2. Then, the minimum glucose concentration as to obtain the maximum response of CO_2 production must be determined. Table 2

Table 1 Values of pH, organic carbon (OC), available phosphorus (P), nitrogen as $NO_3^- + NO_2^- + NH_4^+$ (N), colony formation units (CFU g^{-1}) soil type and field management

Soil	pH	OC/g kg^{-1}	P/mg kg^{-1}	N/mg kg^{-1}	10^{-6} CFU g^{-1}	Soil type	Crop-2008	Crop-2009
M 1	9.38	7.93 ± 0.4	32.9 ± 0.3	60.9	8.26 ± 0.23	Loam	Anise	Rape
M 2	9.00	12.7 ± 0.2	29.2 ± 0.1	60.0	7.67 ± 0.22	Silt loam	Cumin	Anise
M 3	8.36	13.1 ± 0.2	32.7 ± 0.3	53.9	7.18 ± 0.21	Silt loam	Rape	Cumin
M 4	7.83	19.5 ± 0.1	26.5 ± 0.5	46.4	5.14 ± 0.62	Loam	Citrus	Citrus
M 5	8.50	17.9 ± 0.5	28.8 ± 0.4	53.5	6.25 ± 0.35	Loam	Olives	Olives
DMSO	7.50	24.6 ± 0.4	31.1 ± 1.8	55.5	5.51 ± 1.03	Sandy loam		Garden yard
Brz	6.22	14.3 ± 0.1	22.0 ± 0.6	54.6	1.68 ± 0.05	Sandy loam	Forest + extract of grass	
*B*IS	7.51	17.9 ± 0.1	40.8 ± 0.7	51.1	1.55 ± 0.01	Sandy loam	Citrus	Citrus
*B*NIS	6.29	16.1 ± 0.1	33.3 ± 0.3	57.5	0.91 ± 0.01	Sandy loam	Citrus	Citrus

shows the results obtained with soil at FCH. Note that all five samples produced either more, or the same volume of CO_2-C with 2 mg g^{-1} glucose than with 4 mg g^{-1}. Soils (M3 and M4) amended with 1 mg g^{-1} glucose produced 30–35% less CO_2-C than when 2 mg g^{-1} glucose was used. Soil M1 was also tested with 6 mg g^{-1} glucose and both: *p* and CO_2-C gave values that could be averaged with those obtained with 4 mg g^{-1}. Thus, all further experiments were performed by using 2 mg g^{-1} glucose. Something important to remark here is that soil was much disturbed by the introduction of the vial, and therefore, a vial holder was introduced together with the soil in the calorimeter vessel to avoid this problem.

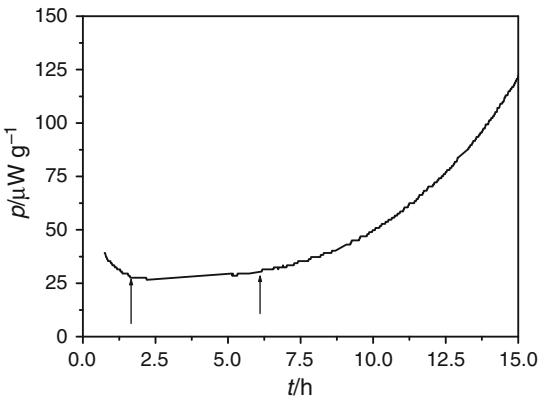

Fig. 2 Specific thermal power–time curve of microbial growth during 15 h. The *arrows* indicate the period when the experiment should be performed

Also, it was important to evaluate the stability of soils stored at 4 °C with time. One way to assess the heterotrophic function of soil microbial community—often called community level physiological profile (CLPP)—is to provide soil with different carbon sources and to observe its ability to utilize them. In experiments performed to test the stability of CLPP during storage at 4 °C, it was found that the profiles obtained varied considerably during the first 24 days. Then, they were stable until 101 days which was the extent of the experiments [21]. As our hypothesis of

Table 2 Values of specific thermal power (*p*) before microbial growth starts; heat released due to CO_2 evolution during 1 h (*q*); CO_2 evolved (CO_2-C); calorimetric soil microbial biomass (CAL-SMB) and concentration of glucose used for the experiment (G)

Soil	*p*/μW g^{-1}	−*q*/mJ g^{-1}	CO_2-C/mm^3 $g^{-1}h^{-1}$	CAL-SMB/μg g^{-1}	G/mg g^{-1}
M1	99.9 ± 5.0	60.5 ± 2.4	13.7 ± 0.5	449 ± 18	4 and 6
M1	93.3 ± 6.1	80.5 ± 1.4	18.2 ± 0.3	598 ± 10	2
M2	80.3 ± 9.8	44.6 ± 2.9	10.1 ± 0.7	332 ± 22	4
M2	92.6 ± 10.9	81.3 ± 8.7	18.4 ± 1.9	604 ± 64	2
M3	112.5 ± 5.2	41.6 ± 2.9	9.3 ± 0.8	309 ± 21	4
M3	126.2 ± 14.3	45.0 ± 2.1	10.2 ± 0.5	334 ± 16	2
M3	111.3 ± 1.8	28.9 ± 1.7	6.5 ± 0.4	214 ± 12	1
M4	106.7 ± 12.3	54.1 ± 0.1	7.2 ± 0.0	236 ± 1	4
M4	144.4 ± 6.2	38.5 ± 4.3	8.7 ± 1.0	286 ± 32	2
M4	96.1 ± 2.7	27.1 ± 1.7	6.1 ± 0.4	201 ± 12	1
M5	117.1 ± 9.1	57.2 ± 3.7	12.9 ± 0.8	425 ± 27	4
M5	96.2 ± 4.2	52.1 ± 1.5	11.8 ± 0.3	387 ± 11	2

Table 3 Values of specific thermal power (p) before microbial growth starts; heat released due to CO_2 evolution during 1 h (q); CO_2 evolved (CO_2-C); calorimetric soil microbial biomass (CAL-SMB); concentration of glucose and Nitrogen used for the experiment (G-N); water content of soil sample (WC) and soil microbial biomass determined by SIR method (SIR-SMB)

Soil	p/μW g^{-1}	$-q$/mJ g^{-1}	CO_2-C/mm^3 $g^{-1}h^{-1}$	CAL-SMB/μg g^{-1}	G-N/mg g^{-1}	%WC	SIR-SMB/μg g^{-1}
M1	72.1 ± 3.0	59.5 ± 1.0	13.4 ± 0.1	434 ± 5	2-0	FCH	
M1[a]	136.5 ± 5.2	90.8 ± 2.4	20.5 ± 0.5	664 ± 18	2-0	60%	637 ± 7
M1[b]	139.4 ± 5.5	94.8 ± 2.7	21.4 ± 0.6	693 ± 20	2-0	60%	
M1	109.3 ± 8.6	90.0 ± 1.4	20.3 ± 0.3	658 ± 9	2-0.2	60%	
M2	139.4 ± 8.1	65.0 ± 2.2	14.7 ± 0.5	476 ± 17	2-0	FCH	
M2	132.5 ± 0.8	91.7 ± 0.1	20.7 ± 0.0	671 ± 1	2-0	60%	672 ± 41
M3	133.5 ± 9.0	31.5 ± 1.1	7.1 ± 0.2	230 ± 8	2-0	FCH	
M3	116.7 ± 10.6	82.2 ± 3.0	18.6 ± 0.7	602 ± 22	2-0	60%	610 ± 29
M4	97.3 ± 8.3	43.2 ± 2.2	9.7 ± 0.5	316 ± 16	2-0	FCH	
M4	124.7 ± 9.1	49.6 ± 6.1	11.2 ± 1.4	363 ± 44	2-0	60%	403 ± 30
M4	124.8 ± 6.6	74.5 ± 3.9	16.8 ± 0.0	545 ± 0	2-0.2	60%	
M4[c]	105.9 ± 3.9	72.9 ± 2.4	16.5 ± 0.5	533 ± 18	2-0.2	60%	558 ± 46
M5	123.0 ± 4.9	90.2 ± 2.5	20.4 ± 0.5	660 ± 18	2-0	60%	671 ± 15

[a] Experiments performed in August 2009

[b] Experiments performed in November 2009

[c] N was added during pre-incubation period

work was that we should obtain a similar equation to that previously reported [12] when plotting the $\log_{10}$ SMB as a function of the $\log_{10}$ p and no correlation was found with the recently collected soils, we left the soil samples to stand for 2 more months at 4 °C.

After 3 months since collection, we continued our experiments first, with soil at its FCH. This was because we thought that the same experiment could be used to determine SMB and the thermodynamic parameters of microbial growth. However, this is a limitation when CO_2 evolution should be determined. If WC is at FCH or near it some of the CO_2 evolved dissolves in water, and therefore, can not react with the alkaline solution. Table 3 shows the results

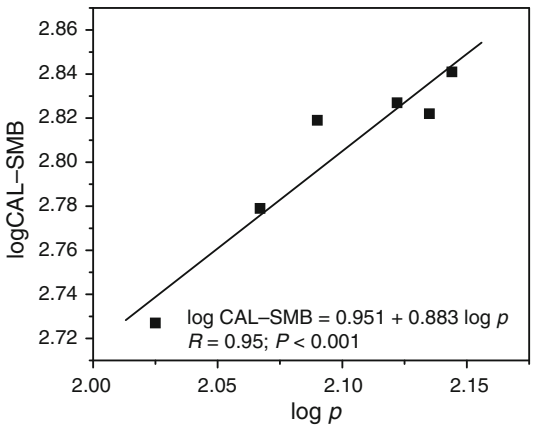

Fig. 3 Plot of log CAL-SMB vs. p for soil samples M1, M1[b]–M5

obtained. Note that the higher values of CO_2-C obtained at 60% FCH. Also, the average ratio between p and CO_2-C at 60% FCH was 22.7 ± 1.5 J $cm^{-3}$for all samples except for M4 that was two times this value (40.5 ± 5.5). The heat output of 22.7 J cm^{-3} gas respired was the same previously reported (22.1–23.0 J cm^{-3}) for microorganisms under aerobiosis [12]. The question was: why the much higher value for M4? It is known that soil microbial community dynamics is closely linked to C and N transformations and often varies in response to substrate quantity and quality [20]. Looking into Table 1, this soil is the poorest in N content, and therefore, we amended it with 0.2 mg g^{-1} $(NH_4)_2SO_4$ besides glucose either during glucose amendment or during the pre-incubation period 24 h before the experiment.

To our surprise, a heat output of 23.2 J cm^{-3} gas respired was obtained for the sample where N was added during the pre-incubation period. Note in Table 3 that amendment of M4 with a glucose—$(NH_4)_2SO_4$ solution gave the same value of p as when only glucose was added, whereas CO_2-C was higher with N addition. On the other hand, when $(NH_4)_2SO_4$ was added 24 h before glucose (see M4[c]), p was lower, and CO_2-C was the same as when N was added together with glucose. This experience with M4 would suggest that it could be possible to detect the nutritional status of soil by calorimetry.

Once we had all five samples giving the same value of heat output per volume of gas respired, we plotted the $\log_{10}$ mm^3 CO_2-C kg^{-1} h^{-1} as a function of $\log_{10}$ p. A

Table 4 Values of specific thermal power (p) before microbial growth starts; heat released due to CO_2 evolution during 1 h (q); CO_2 evolved (CO_2-C); calorimetric soil microbial biomass (CAL-SMB) and soil microbial biomass determined by SIR method (SIR-SMB)

Soil	p/μW g^{-1}	$-q$/mJ g^{-1}	CO_2-C/mm^3 $g^{-1}h^{-1}$	CAL-SMB/μg g^{-1}	SIR-SMB/μg g^{-1}
M1	136.5 ± 5.2	90.8 ± 2.4	20.5 ± 0.5	664 ± 18	637 ± 7
M1[b]	139.4 ± 5.5	94.8 ± 2.7	21.4 ± 0.6	693 ± 20	ND
M2	132.5 ± 0.8	91.7 ± 0.1	20.7 ± 0.0	671 ± 1	672 ± 41
M3	116.7 ± 10.6	82.2 ± 3.0	18.6 ± 0.7	602 ± 22	607 ± 21
M4	105.9 ± 3.9	72.9 ± 2.4	16.5 ± 0.5	533 ± 18	558 ± 46
M5	123.0 ± 4.9	90.2 ± 2.5	20.4 ± 0.5	660 ± 18	671 ± 15
Brz	77.1 ± 3.7	66.1 ± 1.5	14.9 ± 0.3	483 ± 12	ND
DMSO	61.8 ± 3.3	48.2 ± 0.0	10.9 ± 0.0	353 ± 0.0	ND
*B*IS	119.8 ± 12	87.0 ± 5.4	19.6 ± 1.2	636 ± 39	650 ± 57
*B*NIS	82.7 ± 0.8	59.7 ± 6.6	13.5 ± 1.5	461 ± 51	432 ± 7

straight line was obtained giving the equation: $\log_{10}$ CO_2-C = 2.408 + 0.899 $\log_{10}$ p ($R = 0.95$; $P = 0.02$). This equation was very similar to those previously reported [12] for soils under different conditions of storage or with or without glucose amendment where the slope was always 0.858 and the intercept varied between 2.238 and 2.823. At this point, we were very optimistic with the results because the hypothesis was working.

The next question was to convert the values of CO_2-C into SMB. Which conversion factor to use? In the first place, SIR-SMB was determined for the five soils studied by using the conventional method [4, 20]. The factor of 25.3 reported for 25 °C [4] was used to convert the determined CO_2-C into SIR-SMB. The values obtained are shown in Table 3. By using the same factor to convert the calorimetric determined CO_2-C into CAL-SMB, values consistently lower than those determined by the SIR method by an average factor of 1.28 ± 0.14 were obtained. Thus, a factor of 32.4 that emerges from multiplying 25.3 × 1.28 was used to calculate CAL-SMB. Strikingly, if one considers a 7% increase in respiration per 1 °C increase in temperature between 20 and 25 °C [4], this factor would approximately be calculated when the reported value of 40.04 [2] for 22 °C is corrected for measurements at 25 °C. It is worth to note here that calorimetric measurements are much more reliable than those obtained by SIR in routine analysis due to temperature control of the system. The calorimeter is exactly at 25 °C whereas a culture oven varies ± 2 °C. Figure 3 shows the plot between the $\log_{10}$ values of the measured parameters. The resulting equation found was: log CAL-SMB = 0.951 + 0.883 log p, $R = 0.95$, $P < 0.01$. The slope of this equation takes an intermediate value between those reported for stored and fresh soils (0.913 and 0856, respectively) [12]. This excellent correlation found with these five soil samples gave us the impulse to continue these studies with soils

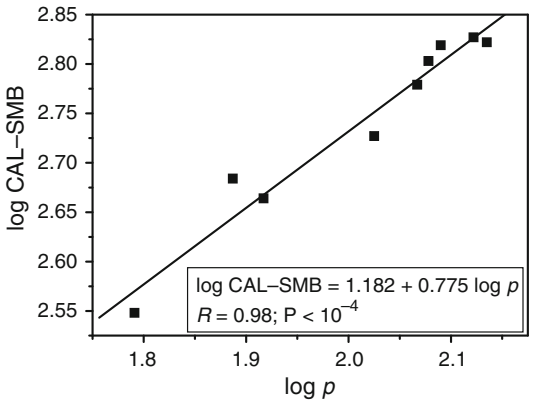

Fig. 4 Plot of log CAL-SMB vs. p for soil samples M1–M5, DMSO, Brz, *B*IS, and *B*NIS

that we already had in our laboratory. Something to remark is that after 6 month from recollection we had not significantly different results with soil M1 (see Table 3, M1[b] and Fig. 3).

Table 4 shows the results obtained and Fig. 4 the plot between log CAL-SMB and log p. Linear fitting of this plot gave an intercept of: 1.182 ± 0.114 and a slope of: 0.775 ± 0.056. These values are not significantly different from those previously reported for fresh soil [12].

Conclusions

In this study, calorimetry has proven to be a very useful tool to determine soil microbial biomass carbon. To produce reliable results it is important (1) to determine the time at which microbial growth starts in the samples to be studied so as to know the time at which to introduce the vial with NaOH solution in the calorimeter vessel. In cases where the lag phase of the exponential growth curve is

short, the period of CO_2 measurement can be shortened; (2) to determine the optimum concentration of glucose as to produce the maximum response of CO_2 respired; (3) Water content of the soil sample should be always 60% of its FCH. Our experience at FCH is that the measured CO_2-C is too low resulting in a heat output that doubles or triplicates the calculated value of 22.7 ± 1.7 J cm^{-3} as calculated when soil is at 60% of FCH; (4) In cases where these previous conditions are accomplished and the heat output per volume of gas respired is still high $(NH_4)_2SO_4$ and/or KH_2PO_4 might be added to the soil during the incubation period to improve the microbial dynamics. In this sense the method could be also used to determine the nutrient status of the soil.

Acknowledgements The authors thank al Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, project PIP N° 5176 and the Consejo de Investigaciones de la Universidad Nacional de Tucumán, CIUNT, project 26/D450 for providing founds for research as well as CONICET for the grant of M.E. Sesto Cabral.

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