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Calorimetric determination of the effect of ammonium-iron(II) phosphate monohydrate on *Rhodic Eutrudox* Brazilian soil

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Abstract

The fertilizer $NH_4FePO_4 \cdot H_2O$ (AIP) was synthesized under mild hydrothermal conditions to be applied on soils to prevent iron deficiencies. The effect of the addition of AIP on soil microbial activity was studied by calorimetry, determining both basal respiration and carbon mineralization by means of the addition of an external carbon source. Thermal analyses (TG and DSC) were also used to provide additional soil properties. The effect of different amounts of AIP on soil microbial activity was quantitatively analyzed by a mass and energy balance performed via the analysis of the power–time curves. These balances allowed determination of the impact of AIP on soil more rapidly than conventional methodologies. The increase in the amount of added AIP leads to a less efficient metabolism, probably due microbial competition for the nitrogen source provided by the AIP and for the carbon source.

Keywords: Microcalorimetry; Thermal analysis; Thermal yield; Soil basal respiration; Ammonium-iron phosphates

1. Introduction

Control of the utilization of soil for economic purposes is of the utmost importance for sustainable development. The Kyoto protocol states that CO_2 emissions due to soil utilization must be controlled and appropriate methodologies introduced that are rational and allow the monitoring of soil activity. The latter directive faces important limitations due to the complexity of the soil system. Most studies focused soil microbial activity employ the CO_2 dissipated and the biomass as indicators. The methodologies to quantify CO_2 and soil biomass are very laborious, and provide results only after very long experimental phases. These studies have only an empirical focus, since it is very difficult to obtain quantitative indicators of soil microbial activity. The most widely used, the metabolic quotient, was seriously criticized [1,2]. The main consequence of the methodological limitations has been inappropriate soil management, which in many cases has been responsible for important losses in soil fertility [3–5].

Thus, there is need for new methodologies to contribute to a better understanding of the biochemical reactions related to the fertility of soil. Methods for the precise estimation of the microbial biomass and its activity, i.e. the metabolic reactions of the soil biomass involved in the carbon cycle, are needed.

Calorimetry appears to be an important option for determination of both biomass and activity. The latest results show that this method can provide qualitative [6–8] and quantitative [9,10] indicators of soil microbial activity that could be used as early warning signals of soil deterioration. Calorimeters are sensitive enough to detect very low heat rates. They can continuously monitor soil microbial activity in terms of dissipated heat, which is a direct product of the degradation of the soil organic matter. Preparation of the samples is clean and easy, avoiding the use of reagents that may affect the results and that may be pollutants [11,12]. The technique therefore has the twofold advantage of being ecological and of rapidly providing results. It has been applied to carry out a diagnosis of the microbial state of soil [13,14], and interesting results have been reported [15,16]. The aim of the present study is to take a further step towards quantitative application of calorimetric methods for the evaluation of the environmental impact of chemical substances on

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soil microbial reactions. A model is suggested for analyzing the power–time curves recorded from soil samples under the effect of different amounts of $NH_4FePO_4{\cdot}H_2O$ (AIP). The synthesis of $NH_4MPO_4{\cdot}H_2O$ [M = Mg, Mn(II), Fe(II), Co(II), or Cu(II)] has been known for more than 100 years [17], and the possibility exist to prepare these compounds by a simple continuous process [18]. Metal ammonium phosphates have been used as pigments for protective paint finishes on metal and as fire retardants in paints and plastics [19,20]. As fertilizers, they can be a source of macro- and micronutrients (P, N, Mg, Fe, Zn, Mn, Cu, and Co) [21]. $NH_4FePO_4{\cdot}H_2O$ has been shown to correct iron deficiency (iron chlorosis) in plants grown in calcareous soils [22]. AIP is hydrothermally synthesized to be applied on soils as a chemical fertilizer and to prevent iron deficiencies in plants. The structure of AIP protects the Fe^{2+} against conversion to Fe^{3+}, which is impossible for plants to assimilate in soils with high pH. It is important to know if the structure of AIP is attacked by soil microorganisms or if it remains intact in the soil and unavailable to plants. Therefore, this study focuses on assessing the effect of AIP on soil microbial reactions and on establishing the role of thermal analysis and calorimetry in the investigation of the soil system. It is believed that this information can be very useful for the agriculture industry and its newly assumed obligations with respect to the Kyoto protocol.

2. Experimental

2.1. Synthesis of AIP

Hydrothermal crystallization of $NH_4FePO_4{\cdot}H_2O$ (AIP) was carried out in a stainless steel (100 cm^3) Teflon-lined vessel under autogenous pressure. $(NH_2)_2CO$ (solid), H_3PO_4 (5.0 mol dm^{-3}), $FeCl_2{\cdot}4H_2O$ (solid) and water were mixed in the molar ratio 2:2:8:67 ($FeCl_2$:H_3PO_4:$(NH_2)_2CO$:H_2O). The autoclave was sealed and heated to 185 °C for 8 days. The obtained solid was filtered off, washed with an excess of distilled water, and dried in air at room temperature. The SEM micrograph (JEOL JSM-6100, 20 kV) shows plates of c.a. 5 μm × 30 μm × 100 μm (see Fig. 1). The phosphorous and iron contents were determined with a Spectra Spectrometer ICP-MS after dissolving a weighed amount of sample in $HF_{(aq)}$. Microanalytical data were obtained with a Perkin-Elmer model 2400B elemental analyzer to give 7.5, 29.6 and 16.7 % for nitrogen, iron and phosphorus, respectively, which correspond to the calculated values 7.49, 29.89 and 16.59%, respectively. Thermogravimetric curves (Mettler TA4000-TG50) was carried out at a rate of 10 °C min^{-1} under a flow of nitrogen. The total weight loss at 600 °C was 22.7% (calculated 23.55%). The weight loss occurs in three steps, with DTG minima at 230, 260, and 500 °C. The final product after thermal decomposition was $Fe_2P_2O_7$.

2.2. AIP solubility in aqueous medium

An excess of AIP was added to buffer solutions (Merck, pH 4.0, 5.0, 6.0, and 8.0 at 20 °C). The P-content in the resulting solutions was analyzed at different intervals of time by UV-spectroscopy [23] with a Perkin-Elmer 200 autosampler.

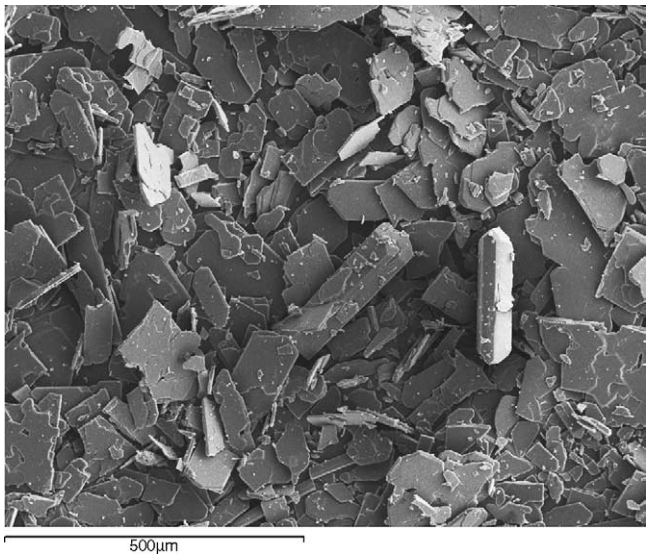

Fig. 1. Scanning electron micrograph of AIP.

The enthalpy of dissolution of AIP was determined by calorimetry with a Setaram Calvet standard 1201. A weighed amount (about 9.5 cm^3) of the buffer solution at pH 4.0, 5.0 or 6.0 was introduced into the calorimetric vessel. Once the heat output was stabilized, 0.10 g of AIP was added and the heat of the endothermic reaction recorded. At the end of the process, the P-concentration in the dissolution was determined by UV-spectroscopy.

2.3. Soil

The soil sample was collected at Campinas University [24,25], in the state of Sao Paulo, Brazil. It corresponds to the *Rhodic Eutrudox* type and was collected at a depth of 5–10 cm after removing the soil surface. The bulk sample was brought to the laboratory, where it was sieved at 2 mm × 2 mm size to remove plants, small insects, small stones and large particles. After this treatment, the sample was kept in polyethylene bags at 4 °C for 3 months before calorimetric experiments.

2.4. Soil organic matter

Microanalytical data (C, H and N, Perkin-Elmer 2400B elemental analyzer) and differential scanning calorimetry (Mettler TA4000-DSC30) were applied to quantify the percentage of soil organic matter (SOM) and the SOM combustion enthalpy, $\Delta_r H_{SOM}$ respectively. DSC experiments were conducted with a heating rate of 10 °C min^{-1} under a flux of air or nitrogen (20 cm^3 s^{-1}).

2.5. Soil biomass

The microbial density of the sample was calculated by the fumigation–extraction method, according to the established method [26]. The sample was kept under refrigeration until the

biomass was measured. It was fumigated with chloroform and incubated for 2 days at 303 ± 3 K. Extraction was performed with 0.50 mol dm^{-3} of K_2SO_4 solution in a proportion of 1:4 (soil:extract) with stirring for 30 min. Simultaneously, a control with K_2SO_4 was also done. The samples were then quantitatively filtered. For the sequential determinations, 4.0 cm^3 of soil extract were amended with 1.0 cm^3 of 0.066 mol dm^{-3} $K_2Cr_2O_7$ solution, 5.0 cm^3 of concentrated H_2SO_4 and 2.5 cm^3 of concentrated H_3PO_4. The solution were heated for 30 min, and after cooling, the volume was brought to 25.0 cm^3 with distilled water. Titration was performed with diphenylamine and 0.033 mol dm^{-3} ammoniacal ferrous sulfate solution.

2.6. Calorimetric measurements

The microbial activity of the soil was continuously recorded with a Setaram Calvet standard 1201 as power–time curves. In a typical experiment, a sample of 0.25 g was introduced into a 9 cm^3 stainless steel ampoule to monitor the basal respiration of the soil. The same mass of an inert substance (α-Al_2O_3) was used as reference. The activity was recorded for 2 days under these conditions. Samples of 0.25 g of soil were assayed: (i) amended with 1.0 cm^3 of a nutrient solution containing 1.0 mg of glucose to measure the microbial activity due to carbon mineralization, (ii) enriched with 1.0 mg of AIP to monitor its effect on basal respiration, and (iii) amended with 1.0 cm^3 of a solution containing glucose and 4.2, 4.5 and 5.0 mg of AIP to study its effect on carbon mineralization in soils. Three replicate were done for each experiment. After the initial experiments, we chose to work with the minimum amount of AIP that provided calorimetric measures with high reproducibility (4–5 mg). All the experiments were performed at 25 °C.

2.7. Analysis of the calorimetric data

The basal respiration of the samples was calculated as the soil mass specific heat rate, $J_{Q/S}$, in joules per gram of soil per day, $J\,g^{-1}\,day^{-1}$, and by the biomass specific heat rate, $J_{Q/X}$, in joules per gram of biomass (X) per day, $J\,g^{-1}\,X\,day^{-1}$ [8].

The power–time curves recorded from the samples amended with glucose and different amounts of AIP were analyzed by means of a combined mass and energy balance [10]. It is assumed that AIP provides the nitrogen that microorganisms need to grow. Therefore, the reaction that takes place in the microcalorimeter under these conditions may be written as follows:

$$a\mathrm{C_6H_{12}O_6} + b\mathrm{NH_4^+} + c\mathrm{O_2} = d\mathrm{CH_{1.8}O_{0.5}N_{0.2}} + e\mathrm{CO_2} + f\mathrm{H_2O} + g\mathrm{H^+};\ \Delta H \quad (1)$$

where $CH_{1.8}O_{0.5}N_{0.2}$ is the formula for the biomass [27].

The energy balance allows calculation of the microbial growth yield of the reaction, $Y_{X/S}$, in mol of biomass per mol of substrate (S) consumed, mol X mol^{-1} S, if the heat yield, $Y_{Q/X}$, in joules per mol of biomass, J mol^{-1} X, and the enthalpy of combustion of the reactants and products are known. The data used were: $\Delta_c H_S = -2803$ kJ mol^{-1} glucose, $\Delta_c H_X = -559$ kJ mol^{-1} X, and $\Delta_c H_N = -296$ kJ mol^{-1} ammonium, representing the enthalpies of combustion of the glucose added, of the biomass and of the nitrogen source, respectively. The values of $Y_{Q/X}$ can be obtained by integrating the power–time curves to quantify the total heat evolution of the process, Q_T, in joules per gram of soil, J g^{-1}, and by then dividing this value by the increment in biomass, ΔX, caused by the addition of the nutrient solution. ΔX is given in micrograms of biomass per gram of soil, μg X g^{-1}. It may also be calculated by calorimetry by applying Sparling's correlation to quantify the biomass that it is activated by the addition of glucose [28] and by the application of the equation that defines the exponential microbial growth [29,30]. Sparling's correlation enables us to calculate the amount of biomass that is activated by the addition of the nutrient solution, X_0, in micrograms of biomass per gram of soil, μg X g^{-1}. The microbial growth rate constant, μ, of the microbial growth reaction may be calculated if exponential microbial growth takes place in the calorimeter, i.e. from the slope of the lines that are obtained when the logarithm of the heat rate is plotted against time.

The $Y_{X/S}$ values yield the enthalpy of the glucose degradation reaction, $\Delta_r H_S$, by means of the equations linked to the energy balance [31]. $\Delta_r H_S$ values can be introduced in Battley's equation [32] to obtain the thermal yield, η, of the reaction taking place in the soil samples.

The mass balance allows obtaining all the stoichiometric coefficients of reaction (1) that provides the amount of CO_2 dissipated by the process.

3. Results

AIP crystallizes in the orthorhombic space group $Pmm2_1$ [33]. The structure consists of approximately square-planar sheets of iron(II) ions, coordinated in a severely distorted octahedron by five phosphate oxygen molecules and one water molecule. The negatively charged layers are bound by hydrogen bonds, with participation of interlayered ammonium cations. The grouping of 5000–6000 individual extended sheets leads to the formation of the particles of AIP shown in Fig. 1. Fig. 2 summarizes the dissolution properties of this compound. The solubility of AIP increases with decreasing pH. Although disso-

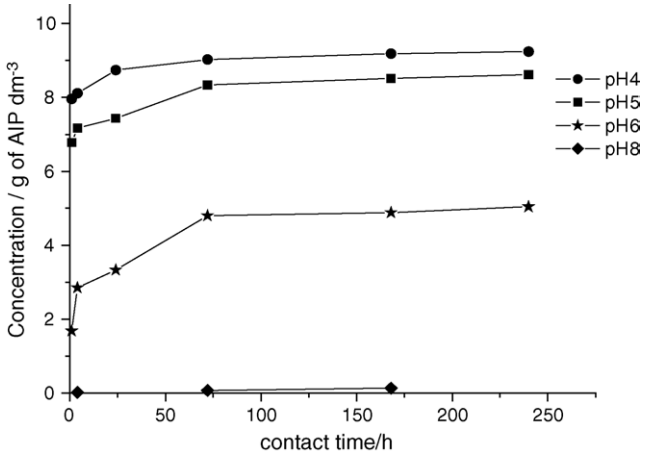

Fig. 2. Dissolution curves for AIP in buffer solutions of different pH: (a) 4.0, (b) 5.0, (c) 6.0, and (d) 8.0 at 25 °C.

Table 1
Physicochemical, biological and thermal properties of the *Rhodic Eutrudox* soil sample, pH, mass percentages (%) of carbon, nitrogen and hydrogen, and soil organic matter (SOM), biomass (BM), water (HM) and the enthalpy of combustion of the SOM ($\Delta_c H_{SOM}$)

pH	5.0
C (%)	1.83 ± 0.24
N (%)	0.16 ± 0.07
SOM (%)	5.40 ± 0.24
BM ($\mu g\, g^{-1}$)	553 ± 21
HM (%)	6.0
$\Delta_c H_{SOM}$ (kJ g^{-1} SOM)	-9.11 ± 0.74

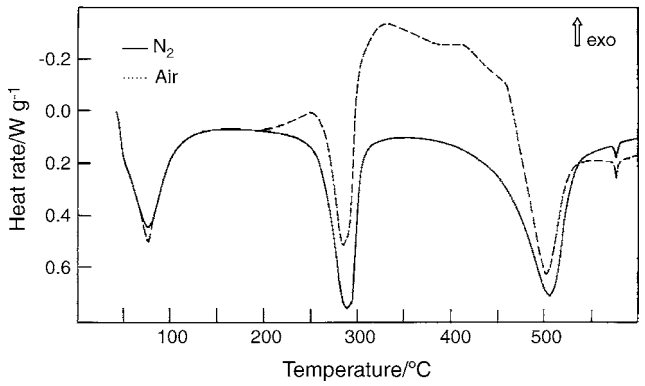

Fig. 3. DSC-curves of the soil in an atmosphere of nitrogen (—) or air (···).

lution occurs rapidly at the beginning of the process, it reaches equilibrium only after 2–4 days. At the pH of the soil sample (pH 5), a large proportion of the AIP added is dissolved at 25 °C.

Some physicochemical, biological and thermal properties of the soil sample used in this study are listed in Table 1. The DSC-curves of the soil sample in both air or nitrogen atmospheres are shown in Fig. 3. These data enable the combustion enthalpy of the soil organic matter, $\Delta_c H_{SOM}$, to be calculated, giving a value of -9.11 kJ g^{-1} SOM.

The heat rate due to basal respiration of the sample and that recorded when 1.0 mg of AIP is added to the soil is shown in Fig. 4. The integration of the plot representing the basal respiration yields a $J_{Q/S}$ value of -0.31 ± 0.10 J g^{-1} day^{-1}. If that value is divided by $\Delta_c H_{SOM}$, a rate for SOM degradation is obtained in micrograms of SOM per gram of soil per day, i.e. 34 μg SOM g^{-1} day^{-1}. As the initial biomass of the sample is known (see Table 1), the specific biomass metabolic heat rate, $J_{Q/X}$, may also be calculated, giving a value of -0.56 kJ g^{-1} X day^{-1}. If this value is also divided by $\Delta_c H_{SOM}$, a specific rate for the degradation of SOM per gram of soil biomass is calculated, giving a value of 62 mg SOM g^{-1} X day^{-1}. Therefore, if the $J_{Q/S}$ data is related to the enthalpy of combustion of the SOM, the rate of SOM degradation by the biomass is obtained. The average percentage of SOM calculated for the soil was 5.40%, as shown in Table 1. Thus, the biomass in our sample would need 1578 days to degrade the SOM of 1.0 g of soil if the given rates were constant over time. Fig. 4 also shows the heat rate obtained when 1.0 mg of AIP is added to the soil. The presence of AIP in the soil modifies the heat rate compared to that from the basal respiration. The latter is an exothermic process, while the addition of AIP induces an initial endothermic activity during the first 15 h after amendment. The reaction becomes exothermic after that time. Integration of the endothermic part of the plot gives a value of $Q_T = 0.20$ J g^{-1}. If this is related to the amount of AIP added, it yields an enthalpy of 201 J g^{-1}AIP. The value obtained for the dissolution enthalpy of AIP was 292 J g^{-1}. Integration of the exothermic part of the plot gives a value of $Q_T = -0.22$ J g^{-1}.

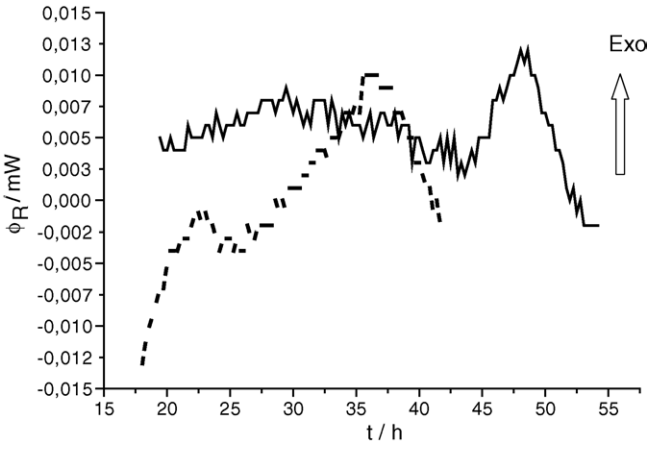

Fig. 4. Plot of the heat flow rate, ϕ_R, against time, of the basal respiration of the soil (solid line) and that recorded when 1.0 mg of AIP is added to the soil sample (dashed line).

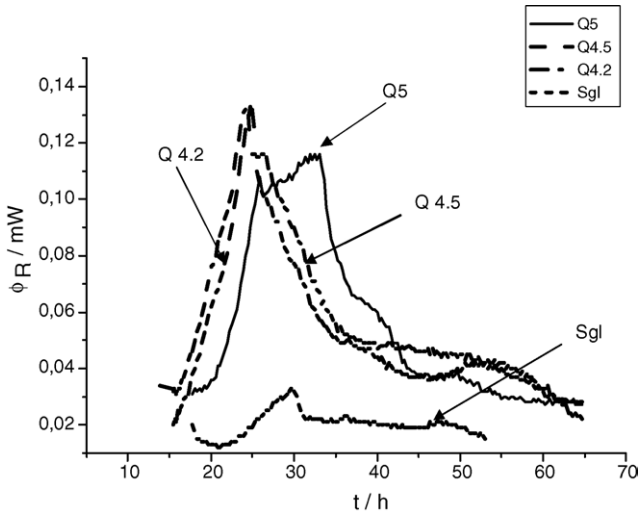

Fig. 5. Power–time curves recorded from soil samples amended only with glucose (Sgl) and with glucose and 4.2, 4.5 and 5 mg of AIP (Q4.2, Q4.5, Q5).

The power–time curves obtained from the samples amended with glucose to study the effect of AIP on carbon mineralization are shown in Fig. 5. Addition of glucose stimulated the exothermic activity of the sample, which is clearly affected by the presence of different amounts of AIP. Qualitative analysis of the curves suggests changes in soil microbial activity that could be attributed to the AIP. To demonstrate this effect in a more quantitative way, a mass and energy balance was developed for the samples enriched with glucose and AIP. Table 2 shows the data quantified from the power–time curves that are necessary for the application of the balances. Comparison of these data

Table 2
Calorimetric data calculated from the power–time curves recorded from the soil samples amended with glucose and different amounts of AIP

Sample	Q_T (J g^{-1})	μ (h^{-1})	PT (h)	$Y_{Q/X}$ (kJ mol^{-1})	X_0 (μg C g^{-1})	ΔX (μg C g^{-1})
Sgl	−2.4 ± 0.5	0.124 ± 0.004	10.3 ± 2.2	−492 ± 73	172 ± 2	120 ± 25
Q4.2	−9.3 ± 0.2	0.180 ± 0.025	17.5 ± 2.5	−501 ± 50	239 ± 2	457 ± 46
Q4.5	−10.4 ± 0.3	0.167 ± 0.052	16.4 ± 5.1	−527 ± 62	256 ± 4	488 ± 57
Q5.0	−8.5 ± 0.3	0.159 ± 0.029	8.3 ± 1.5	−726 ± 94	217 ± 4	289 ± 37

Sgl is the sample enriched with 1.0 mg of glucose, while Q4.2, Q4.5 and Q5.0 are the samples enriched with 1.0 mg of glucose plus 4.2, 4.5, and 5.0 mg of AIP, respectively. PT is the duration of the logarithmic increase of the heat rate.

Table 3
Results of the energy and mass balance. $\Delta_r H_s$ represents the enthalpy of the glucose degradation reaction in kJ/mol of glucose

Sample	$Y_{X/S}$ (mol X mol^{-1} S)	$\Delta_r H_s$ (kJ mol^{-1})	η (%)	C_{kept} (%)	CO_2 (%)
Sgl	2.82	1390	49	47	53
Q4.2	2.80	1403	50	47	53
Q4.5	2.73	1437	51	46	54
Q5.0	2.29	1659	59	31	69

η is the thermal yield of the reactions, calculated as the percentage of the energy from the glucose that is dissipated as heat. C_{kept} shows the percentage of carbon from the glucose that is kept in the soil system as biomass, while CO_2 is the percentage of carbon from the glucose that is lost to the atmosphere through respiration.

suggests that the addition of AIP together with glucose appears to increase the amount of biomass that is activated, X_0, calculated by the Sparling's correlation, when compared with the value of X_0 obtained from the sample enriched only with glucose. The AIP also appears to increase the total heat dissipated by the samples, Q_T, while the values of the microbial growth rate constant, μ, calculated from the power–time curves, appear to decrease with increased values of the amount of added AIP. The values of μ obtained from the samples with the AIP are in all cases higher than that calculated for the sample enriched only with glucose. The results of the energy balance are presented in Table 3. The main reason for applying these balances to study soil microbial activity is the quantification of the enthalpy of the glucose degradation reaction, $\Delta_r H_s$, in order to calculate the thermal yield of the process, η, which in turn provides information on the efficiency of the reaction taking place in the soil. Table 3 shows that the values of $\Delta_r H_s$ and η are higher in samples enriched with AIP than in those obtained for the sample amended only with glucose.

The results of the mass balance are shown in Table 4. The mass balances yield all the stoichiometric coefficients of the reaction stimulated in the soil, which enable us to quantify the CO_2 dissipated by the soil and to determine the percentage of carbon that is lost to the atmosphere through respiration and the amount that remains in the soil. This latter value is also listed in Table 3. The presence of AIP appears to slightly increase the amount of CO_2 dissipated per mol of degraded glucose. This increase appears to be more important when 5.0 mg of AIP is added to the soil together with 1.0 mg of glucose. The stoichiometry of the reactions also shows that if only glucose is added to the soil, the biomass cannot degrade it completely, whereas samples enriched with AIP degrade all the added glucose.

Table 4
Results of the mass balance that yields all the stoichiometric coefficients of the reactions stimulated in the soil samples (see Eq. (1))

Sample	*a*	*b*	*c*	*d*	*e*	*f*	*g*
Sg1	0.35	0.2	1.07	1.0	1.12	1.52	0.2
Q4.2	0.36	0.2	1.09	1.0	1.14	1.54	0.2
Q4.5	0.37	0.2	1.15	1.0	1.20	1.60	0.2
Q5.0	0.44	0.2	1.57	1.0	1.62	2.02	0.2

4. Discussion

The present research demonstrates the important role of different calorimetric methods in soil research. Thermogravimetric (TG) curves are commonly used to provide the percentage of soil organic matter, while differential scanning calorimetry (DSC) is applied in soil research to provide the enthalpy of combustion of the SOM, $\Delta_c H_{SOM,}$ which is especially called for in studies dealing with wild fires [34]. In the present case, DSC is used to quantify $\Delta_c H_{SOM}$ in order to provide a new indicator of soil organic matter degradation. The DSC curve in a nitrogen atmosphere shows four endothermic peaks with minima at 74, 284, 503, and 573 °C. In addition to the quartz polymorphic transformation at 573 °C [35], the curve is typical for a lateritic soil containing halloysite, kaolinite, gibbsite, goethite and lepidocrocite [36]. The endothermic processes that take place in the mineral fraction of the soil conceal the desorption of organic matter, which is also an endothermic process, that should be observed at temperatures below 600 °C [37,38]. The presence of the organic fraction is observed in the DSC curve in an air atmosphere as an exothermic process that extends from 180 up to 500 °C. The difference between the two DSC curves in the two atmospheres should be associated with the heat of combustion of the SOM, since it is considered that SOM desorption heat is negligible in comparison with that associated with the decomposition of the mineral fraction. The calculation of $\Delta_c H_{SOM}$, likewise derived from the curve obtained by DSC, allows us to address the basal respiration of the soil quantitatively. The quotient between the $J_{Q/S}$ values reported by the calorimetric base lines and the $\Delta_c H_{SOM}$ data provide a rate for the decomposi-

tion of organic matter that may be very valuable in comparative studies with ecological goals dealing with the state of the soil. These should be welcomed, since it is very difficult to provide quantitative indicators of soil organic matter degradation. The use of DSC provides valuable information about soil properties.

The results from the calorimetric study of soil microbial activity strongly suggest that the presence of AIP modifies microbial metabolism. The impact on the basal respiration cannot be established, since the addition of AIP without an external carbon source causes a clear initial endothermic effect that affects the analysis of the heat rate. The reason for this may be attributed to the dissolution of the AIP in the soil. The results obtained from the parallel experiments performed to study the dissolution properties of AIP reinforce this hypothesis. Integration of the endothermic part of the power–time curve is very close to the enthalpy of dissolution of this product, and the duration of the endothermic phase, taking around 15 h, agrees with the dissolution times shown in Fig. 2 quite well. It will therefore be necessary to design new experiments to establish the effect of AIP on the basal respiration in soils.

Quantitative analysis of the power–time curves recorded from the samples amended with glucose and AIP strongly suggests that the soil biomass attacks the AIP, probably to use the ammonium as a source of nitrogen for growth. Development of the energy and mass balance reinforces this conclusion, since increased amounts of AIP increase the $\Delta_r H_s$ and η values of the reaction. These data are associated with the efficiency of carbon utilization by the soil biomass and, especially in this case, with the metabolic activity of heterotrophic microorganisms, which are responsible for breaking down the organic matter. It has been stated that a higher value of energy dissipated per unit of carbon source consumed is linked to a less efficient process [39–42]. In line with this behavior, the samples employed in this study appear to follow the same pattern. When the obtained data on $\Delta_r H_s$ are plotted against the microbial growth yield, $Y_{X/S}$, a negative correlation is obtained. The ordinate gives a value of 2800 kJ mol^{-1} S. This is in keeping with the literature, which establishes that at zero biomass yields, the intercept of the dissipation line simply corresponds to the enthalpy of combustion of 1 mol of glucose. When the biomass yield increases, the enthalpy of the glucose degradation and the enthalpy of the overall growth reaction decrease, because some of the energy initially contained in the glucose is now retained in the biomass [42,43]. The slope of that correlation yields a value of about -500 kJ mol^{-1} X, very close to the reported values of the enthalpy of combustion of the biomass [32], which ranges between -562 and -495 kJ mol^{-1} X. The average employed in this study was -559 kJ mol^{-1} X, and that obtained previously for soils was -583 kJ mol^{-1} X [10].

Organic carbon is a key player in how well inorganic substances, including nitrogen and phosphorous components, are used by the soil biomass to maintain carbon–nitrogen–phosphorous (C–N–P) ratios, which are well-established in the literature [44,45]. The development of the energy balances adapted to these processes allows us to study the changes associated with the utilization of carbon by the soil biomass by means of calorimetry, thus providing concomitant information about the assimilation of inorganic sources for microbial growth. The latter information could be applied to assess the right amount of AIP to be used in soils in future experiments. It seems that the increase in the amount of AIP to 5 mg leads microorganisms to metabolize less efficiently. In this respect, recent evidence suggests that when both organic matter and mineral nutrients limit heterotrophs, they have a negative impact on their neighboring biomass [46]. This is not a desirable effect. However, the decrease in metabolic efficiency of the microbial population cannot be attributed to AIP alone, since it may be a consequence of the relationship between the amounts of carbon source and AIP added. The C–N–P ratios are important rulers of soil microbial activity. It has been shown that if only carbon is added to soil, without any inorganic sources, autotrophs are out-competed by heterotrophs for inorganic nutrients, demonstrating a need for the corresponding nitrogen [47,48].

The development of competition between both types of bacteria could explain the differences found between the samples concerning the efficiency of the metabolic reactions that can be attributed to the presence of AIP. The fact is that the mass balance shows that the sample enriched only with glucose was unable to degrade all the added glucose. The reason for this might be that if microorganisms have an easily degradable carbon source, they start to grow very rapidly, until the nitrogen source starts to become a limiting growth factor. The mass balance also shows that when glucose is added together with AIP, it is completely degraded by the soil biomass. If a nitrogen source such as AIP is added at a higher amount than the carbon source, as was the case, on the one hand, the carbon starts to limit the microbial growth of the heterotrophs, and on the other, the presence of nitrogen stimulates the growth of autotrophs, which compete with heterotrophs for the inorganic source. This competition could be responsible for the metabolic changes shown by the calorimetric data, since the calculated parameters are not as variable and as high between the samples so as to suggest stress due to the presence of AIP. In this respect, it has been stated that microorganisms do not seem to be able to retain more than approximately 60% of the carbon atoms available in the substrate for reasons that lie with evolution. Nature would not dissipate more than 50% of the available energy to produce high power, except in stress situations [49,50]. The percentage of dissipated energy ranges from 40 to 59% in the present study, which is not so high as to suggest stress. Future experiments will thus have to be performed that take into account the ratios between carbon, nitrogen and phosphorous so as to avoid the competition phenomena that affects soil metabolism. The obtained results clearly demonstrate the role of calorimetry and thermal analysis in providing relevant information about the soil system.

5. Conclusions

The determination of the basal respiration of the soil by calorimetry and the calculation of the enthalpy of combustion of the soil organic matter by DSC provide a quantitative rate for soil organic matter degradation with important applications in ecological studies. The latter method would allow the CO_2 dissipated by indirect calorimetry to be quantified. Moreover, the

use of DSC may be very helpful in providing more information about soil structure and its mineral composition. Furthermore, the development of both energy and mass balances from the power–time curves provides information about the effect of AIP on microbial metabolism with regard to carbon mineralization. Finally, the addition of increasing amounts of AIP, higher than the amount of the glucose added, leads microorganisms to metabolize less efficiently, which may be caused by competition processes between heterotrophs and autotrophs.

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