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A MASS AND ENERGY BALANCE TO PROVIDE MICROBIAL GROWTH YIELD EFFICIENCY IN SOIL
Sensitivity to metal layering phosphates

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The control on the CO_2 coming from soil handling, makes necessary the introduction of new methodologies that inform about the capacity of the soil as a carbon sink and about the carbon decay. It can be performed through the microbial growth yield efficiency concept by calorimetry and enthalpy balances. Here it is examined the sensitivity of these indicators to two metal layering phosphates, AZP [$(NH)_4Zn_2(PO)_4(HPO)_4$] and AIP [$(NH)_4Fe(PO)_4H_2O$] to assess about their soil impact. Both compounds caused metabolic changes on soil microbial biomass when compared to appropriated references indicating that the proposed methodology is sensitive to different inorganic sources of microbial growth.

Keywords: *AIP, AZP, calorimetry, carbon decay, growth yield efficiency, soil*

Introduction

The deforestation and soil handling is responsible for the 20% of the CO_2 accumulated in the atmosphere [1]. The Kyoto protocol advocates for the control on the CO_2 emissions coming from soil exploitation and for the introduction of new methodologies that permits that control in a rational and ecological way. All these features have given rise to numerous methods to measure soil CO_2 efflux from ecosystems all over the world being one of the most popular the eddy covariance technique to assess ecosystem carbon, C, exchange [2]. But due to some limitations of this model, the classical chamber methods to measure soil respiration are still considered as useful tools. The problem here is that most of these methodologies provide net measures of CO_2 and that is not sufficient information to decide whether the soil is a net source or net sink for atmospheric CO_2 [3]. That is responsible for a flurry of studies and deliberations about building biological carbon (C) banks and for much information about the soil C decay through respiration [4] as they were two independent processes when in fact both together, soil C and respiration are considered as indicators of soil quality [5]. It is truth that the Kyoto protocol contemplates the sequestration of carbon as an alternative to make the atmosphere cooler and the soil better [4, 6], but it is also very well documented, that the organic matter may give highest benefit when it decays [7] through soil microbial respiration. A small change in soil respiration could equal or exceed the annual input of CO_2 to the atmosphere via land use changes and/or fossil fuel combustion, and could significantly exacerbate or mitigate atmospheric increases of CO_2 with consequent feedbacks to climate change [8].

On the whole, it seems that there is a controversy about which is the best way to face the soil manipulation effect on the climate change, is that via carbon sinks or by controlling on respiration? The last reports do not have clear to consider the soil organic matter as a C sink to be the only choice [4]. But if the soil organic C decay option is chosen, then we will find that its understanding is still very limited due to a lack of consensus on methods for measuring soil respiration [8]. The solution could be to show a balance between the capacity of the soil to keep carbon and that to loss it. That is a measure of the efficiency of the soil microbial metabolism that would provide useful information about whether the soil biomass contributes as a net source or a net sink of CO_2, valuable also for the recent carbon balances modeled for the ecosystems. The reality is that there is still an important lack of information about the involvement of the efficiency of the soil microbial metabolism on the C cycle, and about the factors affecting that efficiency. Wardle and Ghani [9] suggested the amount of CO_2–C respired per unit microbial biomass-C, qCO_2, as a useful measure of microbial efficiency. But it does not provide the relation between how much CO_2 was respired per unit new biomass, and without that information it is

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impossible to determine the relative partitioning between biomass production and respiration losses, a necessary requisite for calculating growth yield efficiencies. That makes the last investigations about these topics to claim for quantifying microbial growth yield efficiencies (GYE) for incorporating microbial growth and metabolism parameters into C and nutrient models [10]. The current methodologies providing that information have still some limitations to solve [3, 10], thus we understand that the introduction of new proposals should be welcome nowadays. Calorimetry appears to be a suitable option. It has been applied in soil research successfully [11–13] and it shows some of the reported advantages requested for new methods in soil research: 'have no (or insignificant) disturbance of the ecosystem, be universally applicable to a range of ecosystems, generate reproducible and reliable results and be inexpensive and simple in terms of equipment, maintenance and analysis' [3]. Calorimetry responds well to the mentioned demands since new calorimeters are building right now at lower prices. This methodology registers heat instead of CO_2 but it is well known that both are connected and that correlation between heat and respiration exists in soil [14, 16]. Then, the kinetic of the microbial growth reaction stimulated with glucose in soil, has been very well established in terms of heat [17–19] and that fact has permitted the development of mass and energy balances by calorimetry to determine the microbial GYE of the glucose degradation reaction easily, ecologically and fast from the concept of the enthalpy of the microbial growth reaction [20, 21]. Recent studies also demonstrate calorimetry as an available method to report the soil organic matter degradation kinetics by the registration of the basal metabolism as power–time plots [22, 23]. But information about its application in studies of soil environmental impact based on the GYE is still missing. For that reason here it is tried to test the sensitivity of the calorimetric models to determine GYE when a soil sample is enriched with different chemical sources of ammonium and phosphates with layering structure. These compounds are very popular in chemistry due to their wide range of applications in ionic change reactions, conductivity, nuclear techniques and as catalysts or even as chemical fertilizers [21, 22]. Sooner or later they go through the soil for several reasons. In this paper, two chemical layering phosphate compounds, AZP [$(NH)_4Zn_2(PO)_4(HPO)_4$] and AIP [$(NH)_4Fe(PO)_4H_2O$] were synthesized and their short term effect on soil metabolism is studied by calorimetry and by well known thermodynamic principles in order to see if the GYE of the soil biomass can be affected by inorganic sources of N and P. One of them, the AZP, has Zn on its formulae and it is added to the soil at a toxic level. Then a comparative study is developed to check if the proposed methodology detects metabolic changes associated to the addition of these compounds when compared to adequate references representing the natural activity of the soil sample. The investigation focuses the effect of these compounds on the degradation of the organic matter by the basal microbial metabolism, that is on soil C decay, and on the microbial GYE if glucose is added as an external C source by the development of a mass and energy balance with the calorimetric data.

Experimental

Materials

AIP and AZP synthesis

Hydrothermal crystallization of $NH_4FePO_4{\cdot}H_2O$ (AIP) and $(NH_4)Zn_2(PO_4)(HPO_4)$ (AZP) was carried out in a stainless steel (100 cm^3) Teflon-lined vessel under autogenous pressure. Details about its preparation are reported in detail in a previous paper [23].

Soil sample

The soil is a Rhodic Eutrudox sample collected in Campinas (47° 1′W, 23° 8′S), São Paulo State (Brazil). It was collected from a depth 0–10 cm with an auger to obtain a representative sample of the site. Once the soil had been collected and brought to the laboratory, plant material, animals and stones were removed by hand and it was then passed through a 2 mm mesh sieve. The sieved soil was stored at 4°C in polyethylene bags before analysis.

Methods

Soil characterization

Total soil carbon, C, and nitrogen, N, were determined using a Perkin-Elmer 2400B elemental analyzer. Both yield the C to N ratio of the sample. The soil organic matter content, SOM, was determined indirectly from soil C data [26]. Soil pH was calculated at a 1:2.5 (mass/mass) soil to R.O. water ratio. The humidity is obtained by the mass loss of the sample at 100°C during 24 h. The enthalpy of combustion of the soil, $\Delta_c H_{SOIL}$, was calculated using a differential scanning calorimeter (Mettler TA4000-DSC822). DSC experiments were conducted with a heating rate of 10°C min^{-1} under a flux of air and nitrogen (20 cm^3 s^{-1}) [23, 27]. The enthalpy of combustion of the soil was normalized to the carbon content of the soil.

Soil microbial activity was measured by calorimetry and continuously recorded on a heat conduction

calorimeter: Thermometric TAM 2277. The effect of both AIP and AZP was studied on the soil basal metabolism, on the soil metabolism stimulated with water, and on the soil C mineralization reaction by the addition of glucose and ammonium sulphate as a water-soluble N source. Before the calorimetric measurements, the soil was equilibrated at 25°C for 24 h. Then, 2 g of unamended soil were introduced in 4 mL calorimetric stainless steel ampoules to continuously record basal metabolism during 48 h. Another 2 g of soil were amended with a certain quantity of AIP and AZP providing in both cases the same quantity of N, to study its effect on the basal metabolism. In these cases 2 g of an inert substance (α-Al_2O_3) were used as reference.

1 g of soil was introduced in the calorimetric stainless steel ampoules after 24 h pre-equilibrating at 25°C to register the soil C mineralization reaction. This sample was amended with 0.2 mL of a solution containing 1.5 mg of glucose and 1.5 mg of ammonium sulfate, and 1 g of (α-Al_2O_3) was used as reference. The microbial activity stimulated by the addition of this nutrient solution was used as a reference to be compared to the microbial activity caused by the addition of AIP and AZP to 1 g of the soil amended with 0.2 mL of a solution containing only 1.5 mg of glucose in order to study the effect of both, AIP and AZP, on the glucose degradation reaction. As the water of the nutrient solution can also stimulate the microbial activity in some cases, some experiments were carried out with 1 g of soil amended with 0.2 mL of water. It was used as a reference to compare the activity with that registered from 1 g of soil amended with 0.2 mL of water, AIP and AZP. In all the cases the quantities of AIP and AZP provided the same amount of N as that introduced in the reference experiments. Results obtained with the samples amended with glucose, AIP and AZP were compared with their corresponding references (glucose plus ammonium sulphate) performed in the same period of time (no more than one month) to avoid the effect of storage on the soil biomass [28].

The soil active biomass was also calculated by calorimetry by the Sparling's method [29].

Analysis of the calorimetric data

The integration of the exothermic peak of the curves obtained by DSC yields the enthalpy of combustion of soil, $\Delta_c H_{SOIL}$, in kJ mol^{-1} C.

The soil basal metabolism is recorded by the heat conduction calorimeters as power–time plots. The integral of those plots yields the soil mass specific heat rate, $J_{Q/S}$, in J g^{-1} d^{-1} [22]. It depends on the consumption rate, r_i, of the respective component *i* with the combustion enthalpy $\Delta_c H_i$ (kJ mol^{-1}) [30]. It can be adapted to the soil basal metabolism as follows:

$$J_{Q/S} = -\sum r_C \Delta_c H_{SOIL} \quad (1)$$

Equation (1) gives the degraded C per day, r_C, in μg C g^{-1} d^{-1}, through soil microbial metabolism. This procedure was performed for the unamended samples and for the samples amended with AIP and AZP to show their effect on the soil basal metabolism by the study of quantitative indexes.

The addition of easily degradable C sources to the soil, such as glucose, always stimulates microbial activity. It is recorded by calorimetry as power–time curves. The power–time curves obtained by this treatment reflect a microbial growth reaction that can be written as follows:

$$aC_6H_{12}O_6 + bO_2 + cNH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + dCO_2 + eH_2O + fH^+ \quad (2)$$

where $CH_{1.8}O_{0.5}N_{0.2}$ is the reported formulae for the biomass [31]. The analysis of the curves provides direct and indirect microbial activity indexes. It is possible to calculate the soil active biomass, X_0 [29], the microbial growth rate constant, μ, [12–14], the total heat released, Q_T, the heat yield of the reaction, $\Delta_r H_X$ [32], the duration of the lag phase called latency time, τ [33], and the duration of the exponential heat dissipation or peak time, *PT*, directly from those curves. A mass and energy balance for Eq. (2) can be constructed by the $\Delta_r H_X$ values to obtain the biomass growth yield, $Y_{X/S}$, and the enthalpy of the substrate degradation reaction, $\Delta_r H_S$ [21]. Both provide important information about the stoichiometry of the reaction that takes place in soil. The $\Delta_r H_S$ values permit the calculation of the thermal yield of the reaction, η, by means of Battley's equation [34], that can be considered a measure of the microbial GYE since η gives the capacity of the system to retain the carbon of the glucose added through the mass balance. It yields also the quantification of the calorespirometric ratios and the CO_2 dissipated by the reaction. All these indexes were calculated for the samples amended with the nutrient solution containing glucose and ammonium sulfate, and were compared to those calculated for the samples amended with glucose, AIP and AZP.

Results and discussion

Effect of AIP and AZP on the soil basal metabolism

Table 1 shows the intrinsic and thermal properties of the soil sample. The effect of the AIP and AZP on the soil basal metabolism given by the $J_{Q/S}$ and the r_C data is shown in Table 2. The basal metabolism of our sample needs 0.064% of the total soil C per day. The addition of AZP decreases that value to 0.015%, that could be attributed to an inhibition of the microbial activity due to the Zn of the AZP. Studies about the

Table 1 Carbon, C, nitrogen, N, soil organic matter, SOM, and humidity, H, of the sample, together with the carbon to nitrogen ratio, C/N, pH and enthalpy of combustion of the soil, $\Delta_c H_{SOIL}$

Sample	C/%	N/%	SOM/%	C/N	pH	H/%	$\Delta_c H_{SOIL}$/kJ mol^{-1} C
R. Eutrudox	1.83±0.24	0.16±0.07	5.40±0.24	11±1	5	7	–325±8

Table 2 Values of the heat released by the soil basal metabolism called soil mass heat rate, $J_{Q/S}$, and the amount of soil C that is degraded per day, r_C

Sample	$J_{Q/S}$/J g^{-1} d^{-1}	r_C/μg C g^{-1} d^{-1}
Reference	–0.312±0.100	11.52±1.19
AZP	–0.072±0.001	2.66±0.01
AIP	0.178±0.010	ND

effect of Zn on the soil respiration using enzymatic methodologies report that the Zn depletes the dehydrogenase activity. This inhibitory effect has been shown in the laboratory [35] and in the field [36, 37]. Dehydrogenase activity has been shown to correlate with soil respiratory activity which is also correlated with the heat released by soil metabolism [14, 15]. Therefore, due to the central role that soil microorganisms play in the degradation of the organic matter, the reported decreased activity could have a significant effect of the soil ecosystem [38]. The addition of AIP causes an endothermic effect during the first 15 h after the amendment that could be associated to the interaction of this compound with the organic matter of the sample [23].

Effect of AIP and AZP on carbon mineralization

Figure 1 shows the power–time curves recorded from the soil sample amended with glucose and ammonium sulphate, with glucose-AIP and glucose-AZP. The two last ones were done in May and July, respectively. The

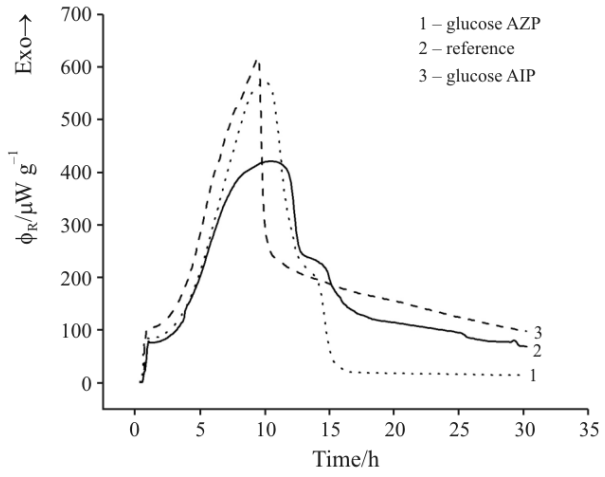

Fig. 1 Power–time curves of the soil samples amended with glucose and ammonium sulphate, glucose–AIP and glucose–AZP

Anova performed with the tabulated data obtained for the six experiments run with glucose and ammonium sulphate used as references, that were performed in May and July too, gave no significant differences at the 0.05 level. Therefore, the storage time of the soil at 4°C did not affect to the kinetics of the glucose degradation reaction. For that reason, Fig. 1 shows the average of the six experiments recorded for the soil sample amended with glucose and ammonium sulphate.

It can be observed that both, AIP and AZP causes a clear increase of the heat flow rate if compared to that recorded for the reference. All the plots show the typical pattern associated to a microbial growth reaction. An initial lag phase of similar duration, an exponential increase of the heat flow rate followed by the decline of the plot until the activity stabilizes again. The plot obtained for the sample amended with glucose and AZP shows the deepest depletion of the activity after the initial reaction, stabilizing under the values of the heat flow rate registered for the sample amended with glucose and AIP and for the reference, both keeping at the same level. When the tabulated data of the plots are compared, the Anova shows significant differences associated to the different treatments of the samples at the 0.05 level. Therefore the power–time curves reflect metabolic differences that could be attributed to the different N sources.

The power–time curves showed in Fig. 1 were analyzed to study the effect of the AZP and AIP on the glucose uptake kinetics quantitatively. Figure 2 shows how τ and *PT* were affected by the treatment of the samples. It seems that these indexes can not show differences associated to the different N sources or to the Zn added with the AZP. Therefore the AIP and AZP do not affect to the lag phase and to the duration of the exponential microbial growth reaction stimulated with glucose. This is a curious result since it has been reported that toxic levels of Zn in soils delay the population growth and that Zn prevented the initial surge in biomass [38]. It seems that effect is not showed by calorimetry through τ and *PT*.

Table 3 shows more quantitative indicators of microbial activity directly calculated from the power–time curves. The comparison of the averages shows no significant differences among those data with the exception of the sample amended with glucose and AIP that show higher values of X_0 than those calculated for the reference. It seems that AIP stimulates a higher fraction of active biomass than the samples amended with ammonium sulphate or AZP. The

Table 3 Values of the total heat dissipated by the microbial growth reaction, Q_T, the initial active biomass, X_0, the apparent microbial growth rate constant, μ, and the heat yield of the reaction, ΔrH_X. Average ±SD. n=3

Samples	Q_T/J g^{-1}	X_0/μg C–X g^{-1}	μ/h^{-1}	$\Delta_r H_X$/kJ mol^{-1} C–X
Reference	–22±6	489±12	0.32±0.02	–242±8
AZP	–18±2	516±30	0.30±0.01	–124±9
Reference	–27±5	564±19	0.29±0.01	–235±18
AIP	–31±1	619±20	0.30±0.01	–206±3

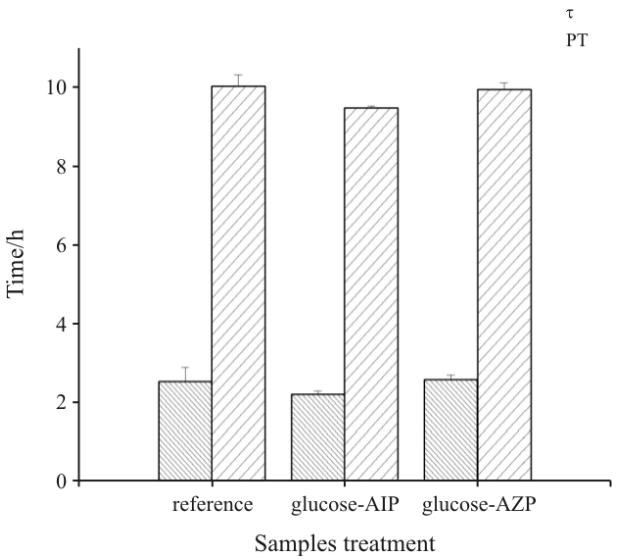

Fig. 2 Comparison of the values for the duration of the lag phase of the microbial growth reaction called also the latency time, τ, and the duration of the exponential microbial growth given as the peak time, *PT*, calculated from the power–time curves recorded for the samples amended with the nutrient solutions containing glucose and ammonium sulphate, AIP and AZP

samples amended with AZP and AIP were compared to their respective references because the active biomass of both references was slightly different. Table 3 also shows that the values of $\Delta_r H_X$ vary with respect to the reference if AZP is used as N source but that effect is not found with AIP. The $\Delta_r H_X$ values correspond to the heat yield of the microbial growth reaction. It is established that microorganisms that dissipate less energy to grow are endowed with a more efficient metabolism [39, 40]. As the AZP could be responsible for changes in soil metabolism, a mass and energy balance is developed to focus the effect of AIP and AZP on the efficiency of the microbial metabolism of the soil sample.

Results of the energy balance are shown in Table 4. Both, AIP and AZP appear to modify the efficiency of the soil metabolism if compared to that observed for the references. That effect is more noticeable when the sample is amended with the AZP than that obtained for the sample enriched with AIP. Both compounds develop a less dissipative metabolism than that recorded when ammonium sulphate is used as N source. That is given by lower values of $\Delta_r H_S$ in the samples amended with AIP and AZP than those calculated for the references, and by higher values of η, that represents the capacity of the soil to retain the energy of the C source and that can be considered as a measure of the capacity of the system to keep the carbon [39]. The η values calculated for the samples amended with AIP and AZP are close to the maximum η value that can be obtained if glucose is used as external carbon source, 80%, given by well known thermodynamic models derived by the calorespirometric ratios and the combustion enthalpies of reactants and products applied to growing and developing systems [30]. It is well known that the oxidation degree of N in substrate affects the stoichiometry of those reactions and that those models are based on the assumption that the N is fully reduced. The last fits well with the results obtained if AZP and AIP are used as N sources, but not with those obtained for samples amended with glucose and ammonium sulphate since the η values of these references are quite lower than the theoretical expected 80%. Therefore, the natural capacity of this soil to keep the assimilated C as biomass is about 68%, the addition of AZP and

Table 4 Results of the energy balance developed for the microbial growth reaction that take place in soil when glucose is added as an external carbon source. It is showed the values of the heat yield, $\Delta_r H_X$, the enthalpy of the glucose degradation reaction, $\Delta_r H_S$, the microbial growth yield, $Y_{X/S}$, and the thermal efficiency, η

Samples	$\Delta_r H_X$/kJ mol^{-1} C–X	$\Delta_r H_S$/kJ mol^{-1} S	$Y_{X/S}$/mol C–X mol^{-1} S	η/%
Ref. AZP	–242±8	–913±18	3.80±0.07	68±1
AZP	–124±9	–556±30	4.49±0.06	80±1
Ref. AIP	–235±19	–894±46	3.81±0.09	68±1
AIP	–206±3	–819±8	3.96±0.02	71±1

AIP together with glucose increases that capacity to 80 and 71%, respectively.

Variations in the efficiency of a microbial growth reaction are attributed to changes in the microbial community structure or to microbial adaptations to environmental changes [41]. It is still difficult to explain these changes since they are not still well understood. Results suggest that both, AIP and AZP are modifying in some way the microbial metabolism. AIP slightly increases the metabolic efficiency if compared to that of its reference. One possible reason could be the presence of the phosphorous together with the ammonium in the AIP that could contribute to the gain in microbial efficiency. In the case of the AZP the measured change is more noticeable than that calculated for the AIP. The reason could be attributed to the presence of Zn. The addition of toxic levels of Zn to the soil alters the microbial structure detected in short and long term experiments [42] as have been reported by PLFA analysis and by thymidine incorporation [43–45]. It seems also that Zn causes losses of bacteria populations as citophaga and stimulates some fungi colonies [46, 47] because they are more resistant to heavy metals than bacteria [48]. It is also well documented that fungi have a more efficient metabolism than bacteria [49]. All these features match well with the significant gain in efficiency of the samples amended with glucose and AZP when compared to that of the references. This effect can not be reported for the Fe of the AIP because it is not added at toxic levels as the Zn is to keep constant the N source. Anyway the calculated data for the thermal efficiency, η, fits well with that reported lately for soil with different fungi to bacteria ratios, F:B, by $^{13}CO_2$–C measurements. Samples amended with labeled glucose have a microbial growth yield efficiency values from 69 to 70%. The addition of N sources together with glucose increased the efficiency values to 78 and 76% [50]. Those values are close to the η values showed in Table 4 as percentages. That paper reports changes in the soil metabolic efficiency that can not be exclusively attributed to the variations in the F:B ratios. It seems that the addition of N sources can decrease the fungal active biomass while active bacterial biomass does not change and that can be accompanied also by enhanced efficiency [51]. From that point of view, the observed variations of η in this work could be attributed too to the more or less availability of the added N sources to microorganisms. Results from aquatic systems suggest too that microbial efficiency can be limited by the C sources or inorganic nutrients [52] supporting our findings. But in general, few studies have reported microbial efficiencies under different N levels in terrestrial ecosystems. For that reason the latest literature recommend more work examining nutrient effects on soil fungal and bacterial efficiencies [50].

The data of the energy balance permits also to study the stoichiometry of the above mentioned reactions. The samples amended with glucose and ammonium sulphate should dissipate 7.60 and 7.45 J g^{-1} if all the glucose added, 1.5 mg, is degraded. The experimental values of Q_T are 22 and 27 J g^{-1}. It is clear this sample shows a priming effect caused by the addition of glucose. That is a very well known phenomena in soil research [53]. Assuming the calculated efficiency the references would degrade about 6% of the total soil C during the microbial growth reaction. The addition of AZP and AIP modifies that percentage to 10 and 12%, respectively. Therefore these compounds increase the priming effect if they are added to the soil together with glucose. One reason could be the increased active biomass, X_0, observed in the samples amended with AIP and AZP. Anyway, the calculated percentages are much lower than those reported for priming effects associated to glucose amendments, that is 23–40% [54], and it is also lower than that reported for a loamy sand amended with labeled Lolium shoots, that was 15% and it was attributed to increased microbial turnover [55]. The fact that our values keep under those published by other authors made we wonder if the observed priming effect in our soil could be associated to microbial turnover processes or to endogenous metabolism.

Results of the mass balance for Eq. (2) are showed in Table 5 and the effect of AZP and AIP on the CO_2 dissipated by the microbial growth reaction can be observed in Fig. 3. The mass and energy balance yield also the calorespirometric ratios for CO_2 dissipation, Rq/RCO_2, that ranged from –411 to –360 kJ mol^{-1} CO_2. The lowest value corresponds to the sample amended

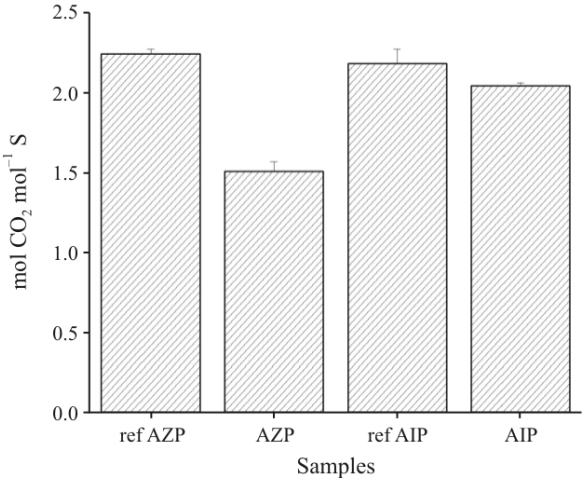

Fig. 3 Comparison of the values of the CO_2 dissipated by the microbial growth reactions stimulated in the samples by the addition of glucose together with ammonium sulphate, AIP and AZP

Table 5 Stoichiometric coefficients obtained for the microbial growth reaction stimulated with glucose and ammonium sulphate, glucose and AZP, and glucose and AIP

Reference AZP	
	$0.27C_6H_{12}O_6+0.55O_2+0.20NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+0.60CO_2+1.00H_2O+0.20H^+$
AZP	
	$0.22C_6H_{12}O_6+0.29O_2+0.20NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+0.34CO_2+0.74H_2O+0.20H^+$
Reference AIP	
	$0.26C_6H_{12}O_6+0.52O_2+0.20NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+0.57CO_2+0.97H_2O+0.20H^+$
AIP	
	$0.25C_6H_{12}O_6+0.46O_2+0.20NH_4^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2}+0.51CO_2+0.91H_2O+0.20H^+$

with glucose and AZP but all of them are in the normal range given for all heterotrophic growth processes [30], $-200<Rq/RCO_2<-455$ kJ mol^{-1} CO_2. Figure 3 shows that both AZP and AIP does not increase the natural levels of CO_2 emissions due to the microbial growth in this sample.

Effect of AIP and AZP on the soil organic matter degradation stimulated with water

The addition of the nutrient solution containing glucose and ammonium sulphate to the soil caused a priming effect that was diagnosed by calorimetry. The reason for that priming effect can not be attributed to microbial turnover or to endogenous metabolism. An explanation could be the effect of the water used to do the nutrient solution on the microbial activity. Figure 4 shows how the addition of water to that soil stimulates also a microbial growth reaction. The heat flow rate was compared to that recorded for the sample when amended with water and AIP and water and AZP. Figure 4 shows that both AIP and AZP increases the heat flow rate if compared to that of the reference but none of them appears to affect the *PT* and τ values. It can be also observed in Fig. 4 that the calorimetric signal of the reference and the sample amended with AZP decay to the same level after the growth reaction while the sample with AIP shows a higher activity than the others after the initial reaction. The values of X_0, μ, Q_T and $\Delta_r H_X$ were also directly calculated from these new plots. Results are shown in Table 6. AIP and AZP caused a clear increase of the X_0 values if compared to that of the reference and both show lower values of μ than the reference. The last could be compatible with

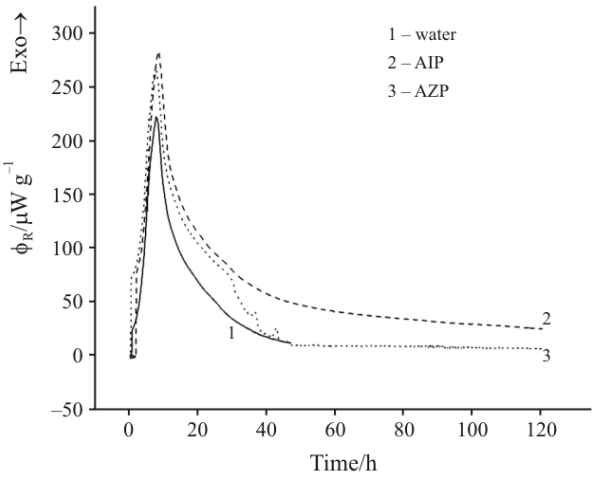

Fig. 4 Power–time curves recorded from the soil samples amended with 0.2 mL of water and 0.2 mL of water together with AIP and AZP

the delay for the microbial growth observed in soil contaminated with Zn [56], but the same effect is found in the sample with AIP that have not contaminant levels of heavy metals. Another reason for the depletion of the μ values could be the competition for the substrate since more active biomass is stimulated with both AIP and AZP than that of the reference. Competition for substrate develops a more dissipative metabolism compatible with lower values of μ [23].

The last is also congruent with the obtained values of $\Delta_r H_X$. The thing is that AIP and AZP modifies the soil metabolism which is less dissipative when AZP is used as N source than that caused by the AIP. In order to get into this topic in some depth, it was developed an energy balance using the soil C as substrate

Table 6 Values of Q_T, μ, X_0 and $\Delta_r H_X$ directly calculated for the power–time curves registered from the soil sample amended with 0.2 mL of water and from those recorded from the samples amended with 0.2 mL of water-AIP and 0.2 mL of water–AZP

Samples	Q_T/J g^{-1}	μ/h^{-1}	X_0/μg C–X g^{-1}	$\Delta_r H_X$/kJ mol^{-1} C–X
Soil water	–10.89±0.22	0.420±0.007	145±3	–151±4
Soil water AIP	–2.40±6.97	0.277±0.006	431±22	–356±112
Soil water AZP	–16.45±2.26	0.273±0.006	447±25	–221±33

Table 7 Results of the energy balance constructed for the microbial growth reaction recorded when 0.2 mL of water is added to the soil, together with those obtained when AIP and AZP are added to the soil with the water

Samples	$\Delta_r H_S$/kJ mol^{-1} C–S	$Y_{X/S}$/mol C–X mol^{-1} C–S	η/%
Soil water	–76±3	0.50±0.01	77±3
Soil water AIP	–135±60	0.38±0.12	58±11
Soil water AZP	–100±22	0.45±0.07	69±4

through the $\Delta_c H_{SOIL}$ data, since these power–time curves show that the addition of water stimulates also a microbial growth reaction, probably caused by labile C sources that are very available to microbial attack. Results can be observed in Table 7. Both AIP and AZP develop a more dissipative metabolism in soil if the organic matter is used as C source. That is reflected in the decay of the η values from 77% calculated for the reference to 58 and 69% for the samples amended with AIP and AZP, respectively. This is congruent with the already mentioned literature that reports the C and N availability as factors influencing the metabolic efficiency. Another explanation for a more dissipative metabolism is the competition for substrate phenomena [23] or the existence of stress [57, 58], but the last should be accompanied by the decay of the active biomass [59] while here the sample shows a clear biomass activation with the AZP and AIP. For that reason we advocate for the competition processes to explain the loss of thermal efficiency observed or for interactions among these compounds and the organic matter that affect to the availability of C and N.

The stimulation of the active biomass after soil rewetting is well documented [60], therefore the water added to the soil can explain the priming effect observed in the experiments made with glucose. That priming effect appears to be caused by microbial C turnover of the organic matter available to microbial attack after rewetting.

Curiously, the η value calculated for the reaction stimulated with water, 77%, is higher than that obtained for the sample amended with glucose and ammonium sulphate, 68%. This result is difficult to explain and even the latest literature is contradictory in that sense. Some investigation reports no differences in the efficiency of the soil metabolism when degrading glucose and the organic matter [61] by the use of qualitative indicators of microbial efficiency calculated by calorimetry, while some other inform about the findings of variations in the metabolic efficiency of the soil biomass when using glucose as an external C source [51] by ^{13}C-respiration as indicator, with respect to that determined for the organic matter. The metabolic indicators of microbial GYE used here appear to fit better with these last ones obtained with the isotopic techniques since our values of efficiency appear to depend on the C source and probably on the availability of the N source also. Anyway, it is truth also, that the sample enriched only with water shows also a lower value of X_0 than that calculated when the sample is amended with glucose and ammonium sulphate, again a higher quantity of active biomass stimulated by the glucose could explain too the development of a more dissipative metabolism than that calculated for the soil C turnover.

Conclusions

The indicators of microbial metabolic efficiency calculated by calorimetry through mass and energy balances appears to be sensitive enough to detect changes linked to the availability of the C and N sources in soil. The last ones also appear to be factors influencing the microbial GYE as it has been timidly suggested in recent papers.

The registration of the basal metabolism in soil by calorimetry also detects metabolic changes that can be associated to the added compounds to the soil.

We think this procedure can be very helpful to understand the contribution of the microbial GYE to the carbon cycle in further studies.

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