J Therm Anal Calorim (2017) 127:1457–1465
DOI 10.1007/s10973-016-5952-2

Exploring the potential of microcalorimetry to study soil microbial metabolic diversity

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Received: 18 February 2016 / Accepted: 8 November 2016

Abstract Microcalorimetry and BIOLOG are common tools in the study of soil microbial metabolism. When used combined, they may reveal further details about soil microbial metabolic diversity than individually. Through this study, we demonstrated the advantages of such a combinatorial methodology by comparing soil samples from two locations in China, each with (OM samples) and without (control) organic fertilization. We used BIOLOG and microcalorimetry to study soil microbes' ability to metabolize different C substrates. Microcalorimetric measurements helped us further reveal the differences in the microbial growth kinetics under different BIOLOG-identified C substrates. Results showed that soils differed in the preferred C substrates, as denoted by the thermodynamic parameters. Some C substrates stimulated the active microbial biomass, while some stimulated microbial growth rate. Most interestingly, certain C substrates (e.g., L-arginine for Shandong soil and glycogen for Henan soil) showed stimulating effects on both OM and control soils, which could be attributed to the pH value and P availability in soil. Hence, we believe microcalorimetry could be potentially used to explore the soil microbial metabolic diversity by combining BIOLOG measurement, especially in determining how microbes assimilate different nutrient sources.

Keywords BIOLOG · C substrate · Fertilization · Microbial metabolism

Introduction

Soil microorganisms are the critical components of terrestrial ecosystems. Their vital functions in cycling mineral and organic compounds are indispensable to a healthy and sustainable environment. Disturbances in environment will influence the microbial structure as well as the microbial metabolic diversity in soils, which could in return alter the soil characteristics [1]. The soil microbial metabolism involves a vast variety of biochemical reactions [2]. Based on these reactions, many methods have been developed to measure different soil microbial properties. Examples include basal respiration rates, substrate-induced respiration to quantify microbial biomass, dehydrogenase activity, heat output of the soil microbial metabolism and community-level physiological profile [2].

In the past two decades, a physiological method, viz. BIOLOG approach, has been widely used to determine the metabolic profile of heterotrophic function at the community level [1, 3–5]. However, BIOLOG could bring artifacts into data interpretation, which makes it controversial in identifying exogenous interruption on soil microbial metabolic activity. In particular, BIOLOG results are influenced by the inoculum density and by the inability of some uncultured

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bacteria and all fungi to reduce the tetrazolium dye [2, 6]. Hence, BIOLOG technique shows an incomplete picture of the function diversity of microbial community and does not inform about the community metabolic kinetics either. Because of those shortcomings, results from alternative methods are necessary for a more complete understanding of the metabolic activity profiles in soils.

Isothermal microcalorimetry is often used to monitor microbial activity in various types of samples ranging from soil to liquid cultures [7–9]. This technique is highly sensitive and easily conducted, which makes it receive more and more attentions for determining the metabolic characteristics in the complex environment such as soil [10, 11]. Furthermore, it allows a continuous and real-time monitoring of the metabolic process over a prolonged period, without disturbing the system [9, 11]. It provides data for calculating multiparameters that can better define the microbial growth kinetics [12]. However, limitations also exist for microcalorimetry in the aspect of interpreting the microcalorimetric results in complex reaction systems, such as the soil environment [13]. Scientists argue that this might be ascribed to the non-specificity of calorimetric signal, and that the specific analytical techniques might be necessary to better interpret the calorimetric data [14]. Thus, in applied soil biology, it is pertinent to combine the microcalorimetric technique with other measurements like BIOLOG to assess the impacts of specific C substrates, which has not yet been reported so far.

Many studies have elaborated the responses of microbial metabolism to exogenous factors in various ecosystems by using microcalorimetry or BIOLOG independently [1, 15]. In this investigation, we set an exploratory example to combine these two approaches to assess soil microbial metabolic diversity. Our approach is to apply BIOLOG first to identify C substrates that affect the microbial metabolism in different soil samples, followed by microcalorimetric measurements to further characterize and to detect the details of the metabolic response of such substrates in specific soils. Two types of soils in China were chosen based on pH and P content, each of which included the same fertilization strategies. Those results can lead to a more complete understanding of how microbial metabolic processes respond to exogenous influences, together with quantitative data of the kinetics of microbial utilization of specific substrates.

Materials and methods

Site description

Two upland soils were selected for this investigation. One is vertisol (nearly neutral pH) from Shandong Province, representing the coastal arable soil in Southeast China (36°04″N, 119°34′E) [16]. This region has an average annual precipitation of 750 mm and a mean annual temperature of 12.1 °C. The soil had been cropped with *Stevia rebaudiana* (Bertoni) for more than 3 years before sample collection. Another is aquic inceptisol (alkaline soil) from Henan Province, representing the typical semiarid regions in North China (35°00′N, 114°24′E). This region had a temperate monsoon climate, with a mean annual precipitation of 615 mm and a mean annual temperature of 13.9 °C. This soil has been subjected to the crop succession of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) for more than 20 years.

Fertilization treatments

Both the experiments at the Shandong and Henan provinces consisted of three treatments, each with three replications. The treatments were as follows: no fertilization (control); chemical N, P, and K fertilizer treatment (NPK); and organic fertilizer plus NPK treatment (OM).

The detail information of Shandong experiment was described in a previous report [17]. Briefly, in the NPK treatment, N, P, and K were applied in the form of urea (108 kg N ha^{-1}), superphosphate (60 kg P_2O_5 ha^{-1}), and potassium sulfate (130 kg K_2O ha^{-1}), respectively. Organic fertilizer was made up of the matured compost derived from the residue of *Stevia rebaudiana* (Bertoni) after sweetener extraction, the properties of which were previously described [17]. For the OM treatment, 15-t ha^{-1} organic fertilizers were applied in early March 2011, and the N, P, and K fertilizers were added to equate the nutrient amounts with the NPK treatment. The soil samples were collected after the *Stevia* harvest at the end of August in 2011.

The long-term experiment in Henan Province was established in 1989, located at Fengqiu Agro-ecological Experimental Station of Chinese Academy of Sciences. Detailed information was described by Meng et al. [18]. Briefly, for the NPK treatment, N, P, and K were applied in the form of urea (150 kg N ha^{-1}), superphosphate (60 kg P_2O_5 ha^{-1}), and potassium sulfate (150 kg K_2O ha^{-1}), respectively. The organic fertilizer was the compost produced from wheat straw, oil cake, and cotton cake in a ratio of 100:40:45. In the OM treatment, organic fertilizer was applied as basal fertilizer in October 2011. By considering the amounts of N, P, K contents in the organic fertilizer, mineral fertilizers were applied to equate the amount in the NPK treatment. The soil samples were collected after wheat harvest in June 2012.

Soil sampling

After harvesting, surface soil samples (0–20 cm depth) were collected randomly from the two arable soils. The samples were packed on-site into sealed polythene bags and then transported to the laboratory. Large roots, macrofauna, and stones were removed from the samples, and then, the remainder was crushed and sieved (2 mm). Each sample was divided into two portions: the air-dried one for physicochemical determinations and the other stored at 4 °C that would be used for microcalorimetric and BIOLOG assays within one month.

Soil physicochemical properties

Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. Soil organic C and total N (TN) were determined by dichromate oxidation [19] and Kjeldahl digestion [20], respectively. Soil total P (TP) was determined by HF–$HClO_4$ digestion and then molybdenum blue spectrophotometry and flame photometry, respectively. Soil available P (AP) was determined by extraction with sodium bicarbonate followed by the molybdenum blue method [21].

BIOLOG assay

BIOLOG® Eco microplates (BIOLOG, Hayward, CA, USA), containing 31 C substrates, were used to determine community-level physiological profiles of soil samples. The method used for the inoculum preparation was adapted from Zak et al. [22] and Staddon et al. [23]. The fresh soil equivalent to 10 g dry soil of each soil was added to 90 mL sterile NaCl solution (0.85%, w/v) and shaken at 200 rpm for 30 min. One milliliter of each suspension was diluted 100 times in a tenfold dilution series. Then, 150 µl of the soil suspensions was added to each well microplate. The microplates were incubated at 25 °C, and color development in each well was recorded as optical density (OD) at 590 nm with a microplate reader (BIOLOG, Hayward, CA, USA) every 12 h and up to 168 h. Metabolic activity of microbial community, expressed as average well color development (AWCD),was determined as follows [24]:

$$\mathrm{AWCD} = \sum \mathrm{OD_i}/31 \tag{1}$$

where OD_i is the optical density value from each well, corrected by subtracting the blank well value from each microplate well.

Microcalorimetric assay

An isothermal multichannel microcalorimeter TAM III (TA Instruments, Delaware, USA) was used for microcalorimetric measurements. The procedure was adopted from Zheng et al. [25]. The 4.0-mL new purchased glass ampoules were used for calorimetric measurements. Soil samples were equilibrated at 28 °C for 24 h before being introduced in the calorimeter. Then, 1.2-g soil samples were weighted into the glass ampoules and amended with 0.2 mL of a nutrient solution containing glucose (5.0 mg) and ammonium sulfate (5.0 mg). To evaluate the effect of specific substrate giving positive results from BIOLOG, the compositions of the introduced nutrient solution (0.2 mL) were composed of each substrate (5.0 mg) and ammonium sulfate (5.0 mg). Then, the soil microbial metabolism was continuously monitored in the microcalorimeter as power–time curves recorded by computer monitoring. The temperature in the microcalorimeter for measurements was set at 28 °C. The measurement was terminated until the heat dissipation remained steadily.

At these conditions, it is possible to quantify from power–time curves, the growth rate constant (k) of the microbial growth reaction, the maximum thermal power in microwatts ($P_{\max}$), commonly defined as the peak height of the curves, the time to reach the maximum peak height ($T_{\max}$). The total heat dissipation of the metabolic reaction (Q), involved in the degradation of the substrate added, was obtained by direct integration of the power–time curves, and reported in the unit of Joules per gram of soil (J/g).

The microbial growth rate constant (k) was calculated by fitting an exponential growth model based on the power–time curve data that represent the microbial growth reaction by the following thermodynamic equation:

$$\ln P_{\mathrm{t}} = \ln P_0 + kt \tag{2}$$

where t is the time, P_{t} is the power output at time t in microwatts (µW), and P_0 is the power at the beginning of the exponential growth phase.

The thermodynamic parameter, X_0, is associated with the quantity of initial active soil biomass in micrograms (µg C_{mic} g^{-1} soil). It is determined in the lag phase of the power–time curve by Sparling's correlation: 1 g biomass C produces about 180 mW [26, 27]. This method approaches the quantity of microbial biomass that is activated by an external C source in the calorimeter. That is, we assume that the increment in the heat rate caused by the addition of a C source is the main consequence of the activation of the microbial biomass and that the extent of that increase may be more or less proportional to the quantity of microbial biomass activated.

Statistical analysis

All data were tested for normal distribution by Shapiro–Wilk test, as well as for homogeneity of variances by Levene's test. If the data were normally distributed, then one-way analysis of variance (ANOVA) was performed to compare means among all measured variables followed by Tukey's multiple range test. If necessary, variables were transformed to meet the requirement of normality of variance among treatments. A nonparametric test (Kruskal–Wallis H) followed by pairwise comparisons was performed when variables could not be transformed. This was done for the following variables: TP and AP in Henan soil, the parameters derived from the power–time curves of C substrates amendments. AWCD at exponential period was analyzed by principal component analysis (PCA) using a covariance matrix, and the subsequent results of substrates with high Pearson's correlation coefficients ($r > 0.5$) to PC1 were summarized. All statistical analyses were performed using IBM SPSS19.0 (IBM Corporation, NY, USA), and the statistical significance for all analyses was accepted at a level of $P < 0.05$.

Results

Soil physicochemical properties

Differences were observed in the physicochemical properties of all soil samples (Table 1). Taking control soils as example, Shandong soil was almost neutral (pH = 7.38), while Henan soil was much more alkaline (pH = 8.90). Shandong soil had higher C/N ratio than Henan soils. The available P contents in Henan (0.75 mg kg^{-1}) were distinguishably lower than that in Shandong soil (58.38 mg kg^{-1}). Nevertheless, organic fertilization treatments resulted in similar effects on the soils. For both regions, OM led to increases in the C/N ratio. OM also caused an increase in P content, especially in Henan sample.

Changes in microbial community-level physiological profiles

AWCD data showed the average utilization intensity of 31 different types of C substrates, which characterized metabolic profiles of the microbial communities in these samples. AWCD increased rapidly after 36 h in all soil samples, and the microbial exponential growth phases ranged from 36 h to 108 h during the incubation period. Pairwise comparison of results revealed that samples under OM treatments had higher AWCD values than control soils (Table 2). Statistically significant differences in AWCD were found between OM and control treatments ($P < 0.05$). Principal component analysis (PCA) of AWCD showed that the soil metabolic profiles varied with fertilization practices (Fig. 1). The first two principal components (PCs) for Shandong and Henan soils accounted for 42.5 and 36.7%, respectively, of the variations in AWCD. OM treatments differentiated from controls in both soils along the PC1 axis, suggesting that OM fertilization influenced soil microbial metabolism largely.

To identify C substrates that differentiated the soil microbial metabolic diversity between OM and control, those with high Pearson's correlation coefficients ($r > 0.5$) to PC1 were listed in Table 3. Specifically, L-arginine was positively correlated with PC1 in both soils ($r > 0.88$). Glycogen was associated with PC1 in Henan soils ($r > 0.58$).The α-D-lactose was related to PC1 in Shandong

Table 2 AWCD values of soils under different fertilization treatments ($n = 3$)

Treatment	AWCD	
	Shandong	Henan
control	0.72 ± 0.08b	0.78 ± 0.03b
NPK	0.72 ± 0.06b	0.82 ± 0.10ab
OM	0.90 ± 0.02a	0.87 ± 0.03a

AWCD values at the exponential growth phases (84 h and 72 h of incubation for Shangdong and Henan soils, respectively) were compared. Different letters within the same column indicated significant differences between the means (Tukey, $P < 0.05$). Refer to Table 1 for control, NPK, and OM

Table 1 Physiochemical properties of the tested soils ($n = 3$)

Sample site/soil type	Treatment	pH	Organic C/g kg^{-1}	TN/g kg^{-1}	TP/g kg^{-1}	C/N	AP/mg kg^{-1}
Shandong/vertisols	control	7.38 ± 0.16a	8.45 ± 0.29a	0.74 ± 0.11a	0.82 ± 0.13a	11.42	58.38 ± 3.27a
	NPK	7.26 ± 0.45a	8.45 ± 0.64a	0.75 ± 0.08a	0.85 ± 0.10a	11.26	55.67 ± 2.84a
	OM	7.75 ± 0.17a	9.70 ± 0.74a	0.78 ± 0.12a	0.91 ± 0.16a	12.44	61.90 ± 5.68a
Henan/aquic inceptisol	control	8.92 ± 0.09A	3.59 ± 0.25A	0.46 ± 0.03C	0.53 ± 0.02B#	7.80	0.74 ± 0.37B#
	NPK	8.65 ± 0.09B	5.37 ± 0.02B	0.62 ± 0.09B	0.73 ± 0.01AB#	8.67	11.15 ± 1.69AB#
	OM	8.72 ± 0.07B	9.21 ± 0.61B	0.96 ± 0.04A	0.72 ± 0.03A#	9.49	14.63 ± 2.60A#

control no fertilization; *NPK* chemical fertilization treatment; *OM* organic fertilizer plus NPK treatment. *TN* total nitrogen; *TP* total phosphate; *AP* available phosphate

A nonparametric test was performed because of normality among the treatments. Different letters within the same column indicated significant differences at $P < 0.05$ among treatments according to Tukey's test or Kruskal–Wallis H test (indicated by #)

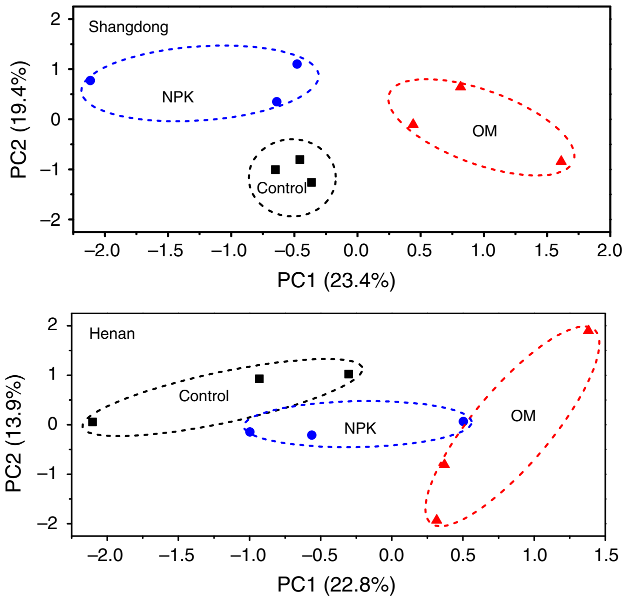

Fig. 1 Principle component analysis (PCA) for C utilization of soil microbial community under different fertilizations in Shandong (*top*) and Henan (*bottom*) provinces of China. Refer to Table 1 for control, NPK, and OM

Table 3 Substrates with high Pearson's correlation coefficients ($r > 0.5$) for PC1 in the PCA of substrate utilization patterns of soil microbial community ($n = 3$)

Sample site	Substrate	r
Shandong	L-arginine	0.88
	L-phenylalanine	0.83
	α-D-lactose	0.75
Henan	L-arginine	0.94
	Glycogen	0.58
	α-D-lactose	0.53

and Henan soils ($r > 0.53$). Such different microbial metabolic patterns suggested that soils from different sites preferred different C substrates.

Changes in heat dissipation profiles

Addition of glucose and ammonium sulfate stimulated microbial growth in all samples as shown in Fig. 2a1 and b1. The registered power–time curves after glucose and ammonium sulfate amendment showed the different phases of a microbial growth reaction. Table 4 illustrated the thermodynamic parameters determined from the power–time curves in Fig. 2. The active microbial biomass, X_0, stimulated by glucose was higher in Shandong than in Henan. Microbial population in Shandong degraded the glucose added at a higher rate than Henan, too. Similar to BIOLOG results (Table 2), OM treatments yielded higher microbial activities in both soils, indicated by shorter $T_{\max}$, and larger k values than controls. Moreover, long-term fertilization in Henan soil posed amount of active microbial biomass (X_0) in OM than that in control, indicating a higher overall soil microbial activity.

Heat dissipation patterns after amendments with additional C substrates highlighted by BIOLOG

In order to evaluate the effect of the C substrates detected by BIOLOG on soil microbial metabolism, the ones with higher correlation coefficients were added to Shandong and Henan soils to monitor the microbial response by microcalorimetry and to compare it with that obtained with glucose (Fig. 2). L-arginine and α-D-lactose were added to both soils, individually. Besides, L-phenylalanine was supplemented to Shandong soil, while glycogen was added to Henan soil. Power–time curves showed control soils responded differently to various amendments (Fig. 2a2 and b2). The microbial response to L-arginine was weaker in Henan than in Shandong. α-D-lactose stimulated a microbial reaction in Henan but not in Shandong. For the third different substrate, Shandong did not respond to L-phenylalanine, while Henan showed a remarkable microbial growth reaction caused by glycogen. Therefore, both samples showed different metabolic functions and different abilities to utilize the added nutrients, suggesting differences in the microbial population in both soils. With microcalorimetry, we were able to differentiate the microbial responses to the same BIOLOG-identified substrate.

The thermodynamic parameters, i.e., k, X_0, and P_t (Table 5), characterized the microbial reaction caused by each of the substrates highlighted by BIOLOG. They gave a more descriptive picture about the differences in the ability of the soil microbial population to metabolize the substrate added in the control samples. X_0 approaches the active microbial biomass, and k indicates the extent of the soil microbial response to a nutrient source by microbial growth. In both soils, addition of amino acids (L-arginine) activated less microbial population (X_0) and led to lower microbial growth rates (k), in comparison with the addition of glucose. Shandong soil had higher microbial active biomass to metabolize L-arginine than Henan soil. Both soils differed in their responses to α-D-lactose too, with higher microbial biomass observed in Henan than in Shandong soil. In Shandong soil, the low Q values for α-D-lactose amendment suggested that lactose was not utilized by soil microorganisms for growing. Glycogen was utilized in Henan soil at a slightly higher rate than other substrates, but with a lower activation of heat assigned to microbial active biomass than glucose.

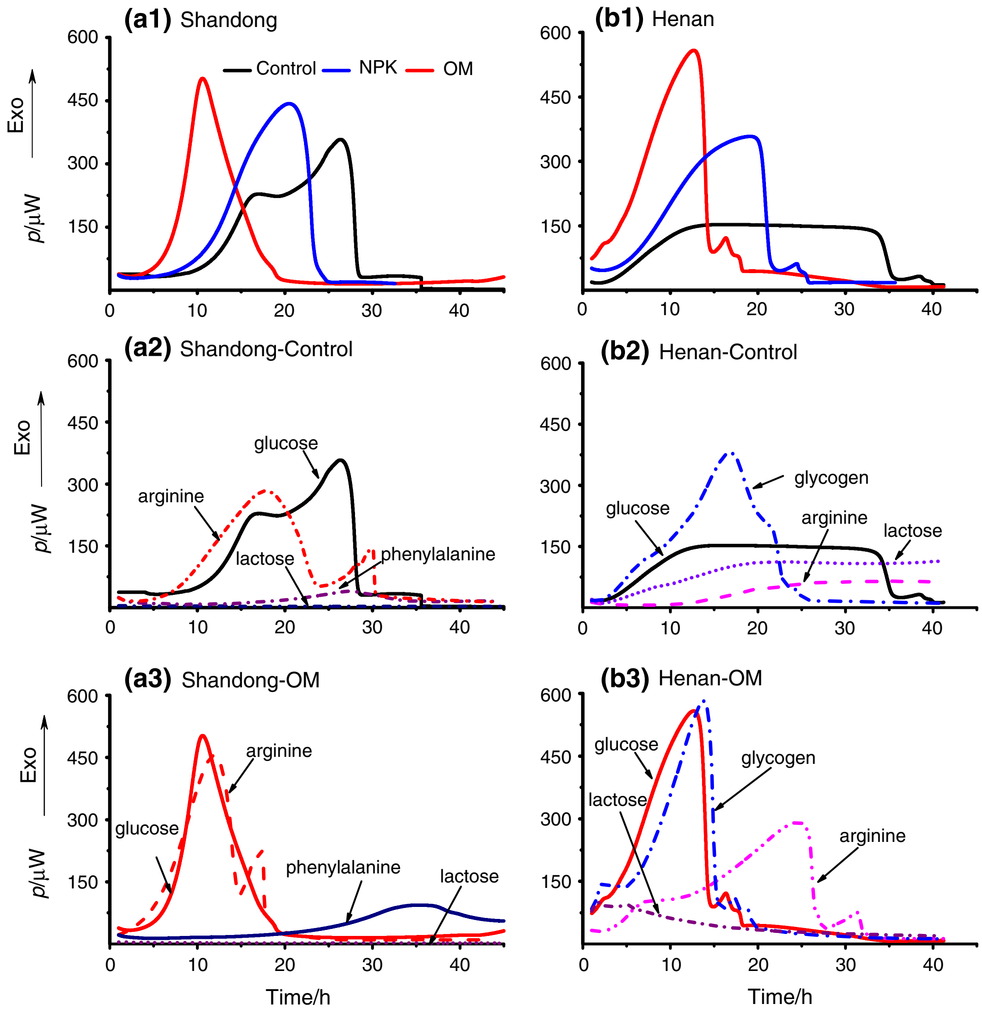

Fig. 2 Power–time curve profiles for control and OM soils amended with glucose and ammonium sulfate (a1 and b1), control soils amended with discerned C substrates and ammonium sulfate (a2 and b2), OM soils amended with discerned C substrates and ammonium sulfate (a3 and b3). Refer to Table 1 for control and OM

Table 4 Thermodynamic parameters from power–time curve profiles measured by microcalorimetric method ($n = 3$)

Treatment	P_{max}/μW	T_{max}/h	k/h^{-1}	Q/J g^{-1}	X_0/μg C_{mic} g^{-1}
Shandong					
control	357.3 ± 21.6b	26.2 ± 0.35a	0.30 ± 0.05b	13.20 ± 0.86a	209.9 ± 15.1a
NPK	461.0 ± 18.2a	19.56 ± 0.32b	0.28 ± 0.02b	15.08 ± 1.44a	200.0 ± 10.9a
OM	502.7 ± 37.9a	10.59 ± 0.32c	0.49 ± 0.04a	14.17 ± 0.58a	213.3 ± 16.6a
Henan					
control	152.7 ± 22.6C	15.95 ± 0.64B	0.11 ± 0.00B	15.54 ± 1.09A	103.9 ± 7.2C
NPK	358.0 ± 19.4B	19.09 ± 0.23A	0.12 ± 0.00B	16.82 ± 1.59A	277.1 ± 13.1B
OM	558.1 ± 12.4A	12.66 ± 0.35C	0.17 ± 0.01A	18.54 ± 1.97A	406.2 ± 18.5A

The data obtained from power–time curves with 1.2-g soil samples supplemented with 0.2 mL solution containing 5.0 mg of glucose and 5.0 mg of ammonium sulfate

Values within the same column not followed by the same letter differed significantly (Tukey, $P < 0.05$). P_{max}, value of thermal power at the maximum of the peak; T_{max}, value of peak time, that is the time to reach the maximum peak height; k, the microbial growth rate constant; Q, value of total heat released by the microbial growth reaction; X_0, the initial active soil microbial biomass. Refer to Table 1 for control, NPK, and OM

In order to check the sensitivity of the method, results obtained with these control samples (Fig. 2a2 and b2) were compared with those obtained with the same soils after organic fertilization (OM samples) (Fig. 2a3 and b3). The OM soils appeared to be more sensitive to some C substrates (i.e., L-arginine, α-D-lactose, and glycogen), in

Table 5 Thermodynamic parameters calculated from power–time curve profiles recorded from soil samples amended with relevant C substrates discerned by BIOLOG ($n = 3$)

Amendment	P_{max}/μW	T_{max}/h	k/h^{-1}	Q/J g^{-1}	X_0/μg Cmic g^{-1}
Shandong control+					
Glucose	357.3 ± 21.6a	26.21 ± 0.35ab	0.30 ± 0.05a	13.2 ± 0.86ab	209.9 ± 15.1a
L-arginine	283.0 ± 17.5ab	17.83 ± 0.56ab	0.23 ± 0.04ab	14.16 ± 0.35ab	121.5 ± 7.6ab
α-D-lactose	4.8 ± 1.4b	1.01 ± 0.17b	0.01 ± 1.22b	0.54 ± 0.03b	26.6 ± 2.2b
L-phenylalanine	52.6 ± 2.5ab	70.31 ± 1.30a	0.06 ± 0.01ab	2.90 ± 0.20ab	136.8 ± 12.1ab
Shandong OM+					
Glucose	502.7 ± 37.9a	10.59 ± 0.32ab	0.49 ± 0.04a	14.17 ± 0.58a	213.3 ± 16.6a
L-arginine	454.8 ± 52.5ab	10.91 ± 0.41ab	0.42 ± 0.05ab	14.08 ± 0.34a	147.9 ± 15.3ab
α-D-lactose	4.8 ± 1.0b	1.01 ± 0.15b	0.01 ± 0.01b	1.43 ± 0.12a	26.8 ± 2.7b
L-phenylalanine	93.6 ± 8.4ab	34.99 ± 0.86a	0.13 ± 0.01ab	7.07 ± 0.26a	120.6 ± 4.2ab
Henan control+					
Glucose	152.7 ± 22.6AB	15.95 ± 0.64B	0.11 ± 0.00A	15.54 ± 1.09AB	103.9 ± 7.2AB
L-arginine	64.6 ± 11.6B	35.24 ± 0.24AB	0.03 ± 0.01A	5.68 ± 0.09B	74.9 ± 8.7AB
L-glycogen	380.3 ± 28.3A	16.77 ± 0.35AB	0.13 ± 0.01A	15.91 ± 0.27A	37.0 ± 11.3B
α-D-lactose	114.8 ± 17.2AB	42.62 ± 0.43A	0.03 ± 0.01A	12.62 ± 0.24AB	110.8 ± 8.2A
Henan OM+					
Glucose	558.1 ± 12.4AB	12.66 ± 0.35AB	0.17 ± 0.01AB	18.54 ± 1.97A	406.2 ± 18.5A
L-arginine	290.7 ± 14.9AB	24.57 ± 0.71A	0.16 ± 0.02AB	15.47 ± 0.41AB	182.1 ± 15.4A
Glycogen	581.9 ± 8.9A	13.76 ± 0.19AB	0.66 ± 0.02A	17.73 ± 0.52AB	466.9 ± 54.7A
α-D-lactose	92.0 ± 13.1B	4.61 ± 0.62B	0.01 ± 0.00B	6.21 ± 0.20B	437.1 ± 23.6A

Data were obtained from power–time curves with 1.2-g soil samples supplemented with 0.2 mL solution containing 5.0 mg of relevant C source or glucose, and 5.0 mg of ammonium sulfate. Different letters within the same column indicated a significant differences at $P < 0.05$ among treatments according to nonparametric Kruskal–Wallis H test

respect of the heat assigned to active microbial biomass (X_0) and growth rate (k) than the control soils. The existing microbial population in Shandong OM samples utilized L-arginine and phenylalanine faster than the control samples, while Henan OM samples utilized L-arginine and glycogen faster than control samples. k values were insensitive to α-D-lactose in spite of the activation observed in the X_0 values in Henan. This effect indicated that lactose activated the microbial metabolism in Henan. However, the activated microbial population could not use the lactose for growing due to some unknown reason in both soils. Therefore, addition of C sources (such as glucose or organic fertilizers) stimulated the metabolism of proteins in both Shandong and Henan soils, as well as the metabolism of glycogen in Henan.

Discussion

Our results showed that both BIOLOG and microcalorimetry are useful approaches in assessing the soil microbial metabolic profiles. BIOLOG results obtained from Shandong and Henan soils clearly revealed microbial changes in response to organic fertilizations, which meanwhile allowed us to identify the difference in C sources utilized by soil microorganisms between different fertilization treatments. A great variety of C sources, including organic acids and carbohydrates, are expected to be released to soil through root exudation and exogenous organic matters importation (such as crop litter, crop residue, and organic fertilizer). These C sources are important factors that shape the soil microbial communities in their specific environment [28]. However, due to the complexity of soil environment and the technical bias of BIOLOG, it is hard to confirm effectively the C sources that lead to the changes in soil microbial metabolism by BIOLOG alone. Based on those prospective, followed by microcalorimetry analysis, our investigation successfully discriminated microbial metabolic responses to utilization of certain C sources detected by BIOLOG and some C sources with relative low impacts. In this sense, it could be feasible to figure out the different microbial metabolisms between different treated soils by combining these two technologies, and more information could be unraveled in comparison with each of them individually.

In this investigation, we found that OM treatments in both soils had the higher microbial activity than NPK and control soils (Table 4), supporting the literatures [25, 29]. Moreover, the results from the same sites of this study show that both OM treatments significantly stimulate the crop productions [17, 18]. Although a set of quantifiable criteria can assess soil quality, in the case of agricultural soil, the production of crops is a much important aspect, and the positive correlation between microbial activity and crop production is also reported [18, 25, 30]. However, the underlying reason for the elevated microbial activity remains elusive. In this investigation, when BIOLOG and microcalorimetry approaches were combined, it was easier and faster to pinpoint impacts caused by different C substrate amendments in soil, such as the capacity to metabolize the L-phenylalanine and L-arginine in Shandong soil and that for glycogen in Henan soil. Also, such combination enabled us to differentiate the microbial responses to the same substrate (glucose, α-D-lactose, and L-arginine). In the case of α-D-lactose, microcalorimetric measurement showed that it was highlighted by BIOLOG because of an initial activation of the heat associated with the microbial biomass. However, it was not accompanied by the exponential increase in the heat rate associated with microbial growth, suggesting that it could not be used by the microbial population in all samples. Therefore, microcalorimetry could be applied to detect the authentic impacts of certain C substrate on soil microbial metabolism, by taking as reference the extent of the microbial growth by the heat rate.

Furthermore, by combining BIOLOG and microcalorimetry, we can provide kinetic parameters for the microbial metabolism of various substrates; hence, it is now possible to rank the preferences of C substrates utilizations in the specific soils. Specifically, we found some C substrates (L-arginine and glycogen) could stimulate microbial metabolism of both control and OM soils in a similar manner. For example, L-arginine amendment changed the heat dissipation patterns in Shandong and Henan soils, indicating that it was metabolized by the microbial population. However, depending on the soil type and OM treatments, different kinetic profiles were observed by the microcalorimetric analysis, showing that this method is highly sensitive to how microbes metabolize the same substrate in different soils or under different soil managements. These differences may be associated with environmental or chemical properties of soils. For instance, it is known that the L-arginine metabolism is more active in a neutral to acid environment [31]. This might explain the higher response to L-arginine in Shandong with neutral pH than in Henan with alkaline pH. Likewise, glycogen amendment also stimulated the heat metabolic diversity in Henan soils, in both control and OM samples. We believe the underlying reason is the crucial role of glycogen in P cycling that is the limiting factor for the Henan soils [32]. Reports show glycogen could serve as the C source and energy source for phosphate-accumulating microorganisms [33, 34], relating to the P availability in the soil. The noticeable stimulating effects of glycogen were in line with the positive effect of P amendment to the same soil by microcalorimetry in our previous report [25]. Further investigations are needed to figure out the exact roles of L-arginine and glycogen on the tested soil. It is certain that the combination of those two techniques can broaden our knowledge about the effects of anthropogenic and/or environmental perturbations on soil microbial metabolism.

Conclusions

To sum up, we have demonstrated here that the combination of microcalorimetry and BIOLOG can be a robust tool to study how a specific C substrate affects soil microbial metabolism that would allow us to improve knowledge about the relations among soil, soil management, soil environment, and C cycle with the soil microbial structure and metabolic profile.

Acknowledgements This work was supported jointly by National Natural Science Foundation of China (Grants Nos. 41501264, 41271256 and 41371253), Natural Science Foundation of Jiangsu Province (BK20140991), CAS Strategic Priority Research Program Grant (XDB15020103 and XDB15010103), the Research Fund of State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Science (Y412201441), and Startup Foundation for Advanced Talents of Nanjing University of Information Science and Technology (NUIST) and the foundation from China Scholarship Council. It was also developed in collaboration with the University of Santiago de Compostela (Spain) by the project of Xunta de Galicia (10PXIB291027PR).

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