https://doi.org/10.17221/168/2018-PSE

Comparison of heat output and CO_2 respiration to assess soil microbial activity: a case of ultisol soil

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ABSTRACT

Jia X.H., Cao H.C., Jiang L.L., Yuan J.H., Zheng S.X. (2018): Comparison of heat output and CO_2 respiration to assess soil microbial activity: a case of ultisol soil. Plant Soil Environ., 64: 470–478.

Glucose-induced microcalorimetry and carbon dioxide (CO_2) production are two widely applied methods to assess microbial activity in soil. However, the links among them, microbial communities and soil chemical properties based on large number of soil samples are still not fully understood. Seventy-two soil samples of different land uses were collected from an ultisol soil area in south China. The best correlation between the rate of heat output and the rate of CO_2 respiration occurred in 8–16 h reaction (R^2 = 0.64), followed by 0–8 h (R^2 = 0.50) (P < 0.001). However, the correlations decreased sharply after 16 h. The heat output per biomass unit (Q_T/MBC) was well correlated with the total phospholipid fatty acids (PLFAs) (R^2 = 0.56) and bacterial PLFAs (R^2 = 0.53) (P < 0.001). In contrast, these links were not apparent between soil respiratory quotient (qCO_2) and the total PLFAs and microbial communities. Redundancy analysis further confirmed that Q_T/MBC was a more comprehensive indicator to assess soil microbial activity and soil quality than qCO_2, showing a good negative correlation to soil organic carbon, total nitrogen (N) and mineral N, and pH. This work is very helpful to better guide the application of calorimetry and CO_2 respiration in assessing microbial activity in soils.

Keywords: soil microorganism; microbial community; nutrient; calorespirometric ratio; metabolic efficiency

Soil microorganisms are the dominant engines to drive the biogeochemical cycles, and higher organisms such as plants and animals are intimately dependent on microbial activities for key nutrients recycle and for organic matter degradation (Falkowski et al. 2008, Madigan et al. 2015). There is a continuous interest to understand the microbial community structure, activities and their relationships (Talbot et al. 2014). In recent years, DNA sequence and phospholipid fatty acid (PLFA) analyses have been widely applied in research of community structure (Talbot et al. 2014, Cao et al. 2016). Meanwhile, many approaches encompassing soil proteins/enzymes, soil basal respiration, substrate-induced respiration and isothermal microcalorimetry have been applied to study soil microbial activity (Bobille et al. 2016, Cao et al. 2016, Xu et al. 2016).

In particular, carbon dioxide (CO_2) is an indicator of microbial degradation of organic matter and assessment of the impact of soil management on CO_2 emission in soil (Barros et al. 2016). Besides, microcalorimetry is a highly sensitive method to measure a heat-dissipating process of cell growth

Xiuhong Jia and Haichuan Cao contributed equally. Supported by the Natural Science Foundation of China, Grant No. NSFC 41771283, and by the Fundamental Research Funds for the Central Universities, Grant No. 2662018JC038.

https://doi.org/10.17221/168/2018-PSE

in a continuous and real-time way during a long period of time (Barros et al. 2007, Wadsö 2009). It provides quantitative indicators with respect to microbial growth rate constant, heat yield of microbial growth, and efficiency of carbon utilization by soil microorganisms (Barros et al. 2007, Zheng et al. 2009). A few studies have reported that the heat output is well correlated with the CO_2 respiration (Sparling 1983, Critter et al. 2004). However, biological significance of CO_2 respiration was different from that of thermal dissipation. CO_2 measurements mainly contain the aerobic metabolism and anaerobic fermentation of soil microorganisms (Vor et al. 2002, Dyckmans et al. 2006, Wadsö 2009), and autotrophic microorganisms transform CO_2 into organic matter of cells. In contrast, calorimetry quantifies all microbial metabolic processes in soils (i.e. oxic and anoxic reactions, catabolic and anabolic processes, heterotrophic and autotrophic reactions) (Wadsö 2009, Herrmann et al. 2014). Recently, calorespirometric ratio (R_q/R_{CO_2}) was applied to evaluate microbial carbon use efficiency in soil systems (Barros et al. 2011, 2016, Herrmann and Bolscher 2015, Wadsö and Hansen 2015). This ratio varied among soils with different land uses, and was affected by soil organic matter, soil moisture, soil particle size, and soil management (Herrmann and Bolscher 2015, Barros et al. 2016). Therefore, a more comprehensive understanding based on numerous soil samples is needed to help to elucidate the links between CO_2 respiration, heat output, microbial groups and soil nutrition level. In this study, 72 ultisol soil samples from south China were performed to compare the heat output and CO_2 respiration during a long period of time. PLFA analysis was also combined to indicate the microbial biomass and groups in soils. The aim of this study is to better understand the relationship between PLFA, heat output and CO_2 respiration, and to compare the differences of calorespirometric ratio, soil metabolic quotient (qCO_2 and Q_T/MBC) in assessing soil microbial activities.

MATERIAL AND METHODS

Soil sampling. Seventy-two soil samples were collected from an ultisol area in south China, encompassing Yujiang in the Jiangxi province and Xianning in the Hubei province (Table 1). At each plot, soil was collected at a depth of 1–15 cm from 5–10 locations within a given plot of 100 m^2 and composited into a single bulk sample. They were air-dried, homogenized by sieving to less than 2 mm to separate roots and large objects, and stored in polyethylene bags at 4°C.

Chemical analyses of soils. Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. Soil organic carbon (C) and total nitrogen (N) were determined by dichromate oxidation (Mebius 1960) and Kjeldahl digestion (Bremner 1965), respectively. Soil total phosphorus (P) was digested by HF-$HClO_4$ (Jackson 1958), and determined by molybdenum-blue colorimetry. Soil

Table 1. Main chemical properties of soil samples

Samples No.		Vegetation, land use and sites	pH	SOC	TN	TP	TK	MN	AP	AK
				(g/kg)				(mg/kg)		
1–18	P	peanuts, upland soils, Yujiang	4.3–5.5	7.1–10.5	0.7–1.0	0.5–0.7	4.3–7.8	8.0–23.5	15.2–45.0	133.5–226.2
19–36	R	rice, perpetual paddy soils, Yujiang	5.0–5.6	7.9–13.8	0.6–1.2	0.2–0.6	2.6–5.4	10.9–35.5	0.1–2.7	93.2–149.6
37–54	F	forest, upland soils, Yujiang	4.2–5.1	6.3–14.1	0.6–1.3	0.3–0.5	2.7–7.3	4.1–14.2	1.6–176.3	56.9–185.9
55–63	W	wheat, upland-paddy rotation, Xianning	7.6–7.9	15.1–18.8	1.1–1.6	0.6–0.8	0.5–1.6	95.9–106.4	8.4–16.8	106.5–133.1
64–72	RC	rice, upland-paddy rotation, Xianning	7.6–7.8	12.6–16.4	1.4–1.7	0.6–0.7	0.5–1.8	83.0–100.5	7.9–13.3	91.7–115.4

SOC – soil organic carbon; TN – total nitrogen; TP – total phosphorus; TK – total potassium; MN – mineral nitrogen; AP – available phosphorus; AK – available potassium; P – peanut soils; R – perpetual paddy soils; F – forest soils; W – wheat soils; RC – rotational paddy soils

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mineral N was extracted with 2 mol/L KCl in a 1:4 soil-to-solution ratio for 1 h, and then determined by an automated procedure (Skalar SANplus segmented flow analyser, Breda, Netherlands) (Chu et al. 2007). Available P was extracted by sodium bicarbonate and determined using the molybdenum blue method (Olsen et al. 1954). Available potassium (K) was extracted by ammonium acetate and determined by flame photometry (Carson 1980). Microbial biomass C was determined with the chloroform fumigation extraction method as reported by Jenkinson and Ladd (1981).

Microcalorimetric measurement. Substrate-induced metabolic activities of soil microorganisms were evaluated with the third-generation thermal activity monitor (TAMIII, Järfälla, Sweden). All soil samples were firstly placed at 28°C for 6 h and then submitted to microcalorimetric measurements (Sparling 1983, Cao et al. 2016). A solution containing 5.0 mg of glucose and 5.0 mg of ammonium sulphate was added to 1.0 g soil sample in a 4-mL sterilized steel ampoule. Soil moisture was kept at 30% and 35% of upland soils and paddy soils, respectively. The temperature of the calorimeter system was controlled at 28°C. The thermodynamic parameters, namely peak power (P_{max}) and peak time (t_{max}), were obtained directly from the power-time curve monitored and recorded on a computer. The P_{max} and t_{max} are the power and time to reach the maximum of the peak, respectively. The k was the constant of growth rate and was calculated from the slope of semi-logarithm of the exponential phase. Total heat output (Q_T) was obtained by integrating the power-time curve, indicating the total heat output during organic material consumption and it reflects the activities of soil microorganisms.

Carbon dioxide detection. Emission of carbon dioxide (CO_2) was detected by substrate-induced respiration. All soil samples were first placed at 28°C for 6 h and then submitted to CO_2 detection under the same conditions such as substrate addition and moisture at microcalorimetric measurement. Then, CO_2 content was detected by using the gas chromatography (Agilent 7890A, Palo Alto, USA) at 8, 16, 24, 32 and 40 h, respectively.

Phospholipid fatty acid analysis. PLFAs were detected by gas chromatography-mass spectrometer (6890-5973 N, Agilent, Palo Alto, USA) as described (Wu et al. 2009, Cao et al. 2016). The total quantity of individual fatty acid methyl esters was determined using methyl nonadecanoate (19:0) as an internal standard. The fatty acids 15:0, 16:0, cy17:0, 17:0, 18:0, cy19:0, 16:1ω7, and 16:1ω5 were used as bacterial identification markers. The methylated, branched, saturated fatty acids of 10Me18:0 and 10Me19:0 represented actinomycetes. The fatty acids of 18:2ω6 and 9c were used as indicators for fungi.

Statistical analysis. All results were expressed on a soil oven-dry weight basis (105°C, 24 h). The rate of CO_2 respiration was calculated based on the amount of carbon dioxide released per hour in an eight-hour interval. Likewise, the rate of heat output was the amount of dissipated thermal power per hour in an eight-hour interval. Statistical analyses were carried out with SPSS 17.0 (SPSS Inc., Chicago, USA). Correlations among soil CO_2 respiration, microcalorimetric parameters and phospholipid fatty acid composition were examined by using regression analysis. Redundancy analysis (RDA), a multivariate direct gradient analysis method, was calculated by Canoco version 4.5 (Microcomputer Power, Ithaca, USA) to elucidate the relationships among microcalorimetric indices, metabolic quotient (qCO_2 and Q_T/MBC), calorespirometric ratio (R_q/R_{CO_2}), soil chemical properties and sampling sites.

RESULTS AND DISCUSSION

Main chemical properties of soil. A summary of the chemical properties of soils is presented in Table 1. In order to make sure that soil samples have a good representation, they were collected from multiple land uses including peanut soil, wheat soil, forest soil, rotational and perpetual paddy soil. Soil pH in Yujiang was lower than that in Xianning. The soil organic C (6.3–18.8 g/kg), total N (0.6–1.7 g/kg), total P (0.2–0.8 g/kg), total K (0.5–7.8 g/kg), mineral N (4.1–106.4 mg/kg), available P (0.1–176.3 mg/kg) and available K (56.9–226.2 mg/kg) shifted greatly in different soils.

Soil microbial growth indicated by calorimetric profiles. Recorded power-time curves were obtained from incubated soils with added glucose and ammonia, showing a growing process of microorganisms and substrate-induced soil microbial activity (Figure 1). The thermal flow presented a typical microbial growth curve, i.e. microbial growth increased exponentially after the lag phase, followed by the stationary phase and then the decline phase. The

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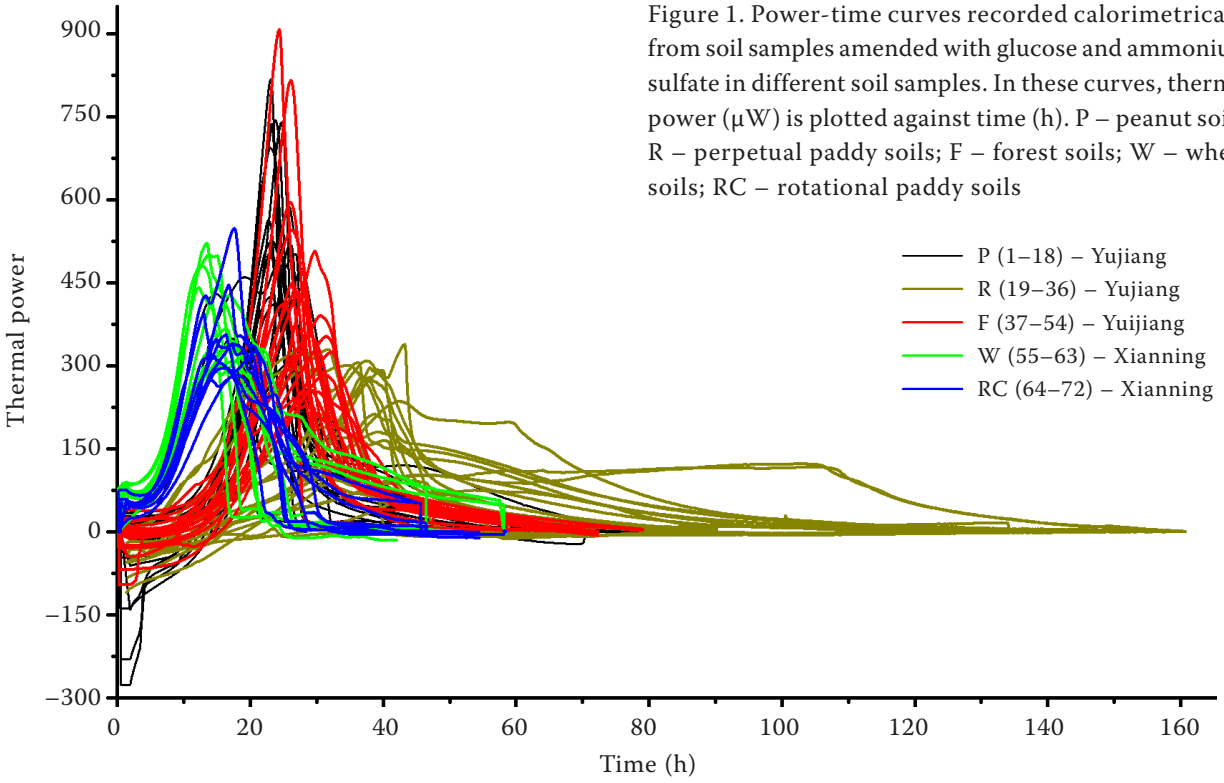

Figure 1. Power-time curves recorded calorimetrically from soil samples amended with glucose and ammonium sulfate in different soil samples. In these curves, thermal power (μW) is plotted against time (h). P – peanut soils; R – perpetual paddy soils; F – forest soils; W – wheat soils; RC – rotational paddy soils

calorimetric curves showed three overlapped clusters. The first cluster, rotational paddy soils (RC) and wheat soils (W) from Xianning showed the shortest lag phase and the lowest peak time (t_{max}), indicating the fastest response to glucose and ammonia addition. In contrast, the third cluster, perpetual paddy soils (R) from Yujiang, had the longest peak time. The second group was peanut (P) and forest soils (F) from Yujiang, showing the middle level of peak time and the highest peak power (P_{max}). In general, shorter lag phase, lower t_{max}, and higher P_{max} indicated a high microbial activity in a calorimetric analysis (Barros et al. 2007, Wadsö 2009, Zheng et al. 2016). Accordingly, it suggested that soils from Xianning had the highest microbial activity, but perpetual paddy soils from Yujiang showed the lowest microbial activity.

Correlation between the rate of heat output and carbon dioxide respiration. In previous studies, the heat output was well correlated with CO_2 respiration during 24 h measured by the substrate-induced method (Sparling 1983) and during 98 days under non-substrate induced conditions (Critter et al. 2004). It seems that their correlation was not affected by substrate supplement. As different biological significance of CO_2 respiration and heat output, the correlations of their rates (CO_2 emission and heat output per hour in an eight-hour interval during 40 h) were analysed (Figure 2). The rate of heat output was not always consistent with the rate of CO_2 respiration. The best correlation occurred in the time frame 8–16 h (R^2 = 0.64, P < 0.001), followed by 0–8 h (R^2 = 0.50, P < 0.001). However, the correlations decreased sharply after 16 h. One possible reason leading to the deviation of correlations is that the calorespirometric ratio was affected by many factors such as land use, soil size fraction and organic matter (Barros et al. 2016). Another possible reason is that added glucose was quickly consumed by active microorganisms, which resulted in O_2 depletion or CO_2 excess in some soil samples such as W and RC after 16 h (Figure 1). After that, the relation between heat and CO_2 is ruled by the oxidation degree of organic substrates that microorganisms used in soil (Wadsö and Hansen 2015, Barros et al. 2016). If so, the correlations would be affected by soil factors such as organic matter and the microbial structure or diversity. Consequently, the correlations of PLFAs and soil metabolic quotient (qCO_2 and Q_T/MBC) were necessary to be analysed. RDA was also performed to understand the links between soil chemical properties, thermal parameters, metabolic quotient and calorespirometric ratio.

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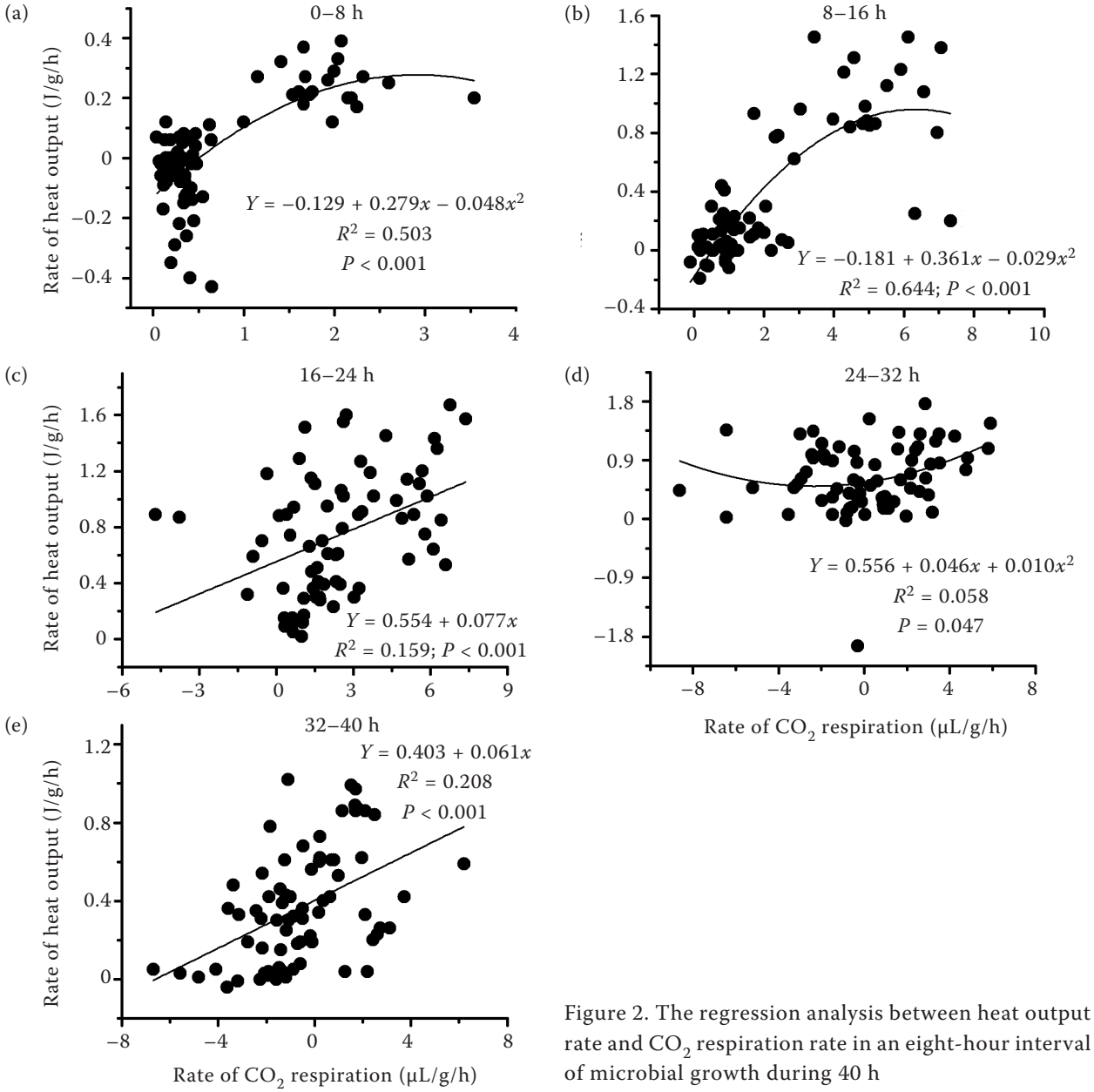

Figure 2. The regression analysis between heat output rate and CO_2 respiration rate in an eight-hour interval of microbial growth during 40 h

Correlations of PLFAs and soil metabolic quotient. In previous studies, heat output per biomass unit (Q_T/MBC, one of the metabolic quotients) is a good index to indicate metabolic efficiency, the lower Q_T/MBC indicating the higher metabolic efficiency and carbon conversion of soil microorganisms (Barros et al. 2003, 2007, Zheng et al. 2009, 2016). The regression analysis showed that Q_T/MBC was well correlated with microbial biomass indicating by total PLFAs (R^2 = 0.56, P < 0.001) (Figure 3). Among the various correlations of Q_T/MBC with microbial groups, the best correlation occurred in soil bacteria (R^2 = 0.53, P < 0.001). In contrast, Q_T/MBC had lower correlations with soil fungi (R^2 = 0.32), actinomycetes (R^2 = 0.13), bacteria:fungi ratio (R^2 = 0.21), and bacteria:actinomycetes ratio than with bacteria. Therefore, bacteria were major predictors to drive the metabolic efficiency of soil microorganisms.

Likewise, regression analysis was also performed to show correlations between soil respiration quotient (qCO_2) and PLFAs (Figure 4). Compared to Q_T/MBC, the link of qCO_2 was not apparent with total PLFAs (R^2 = 0.06, P < 0.001) and biomass of other microbial groups encompassing bacteria (R^2 = 0.12,), fungi (R^2 = 0.14), actinomycetes (R^2 = 0.008), bacteria:fungi ratio (R^2 = 0.15), and bacteria:actinomycetes ratio (R^2 = 0.039) (P < 0.001).

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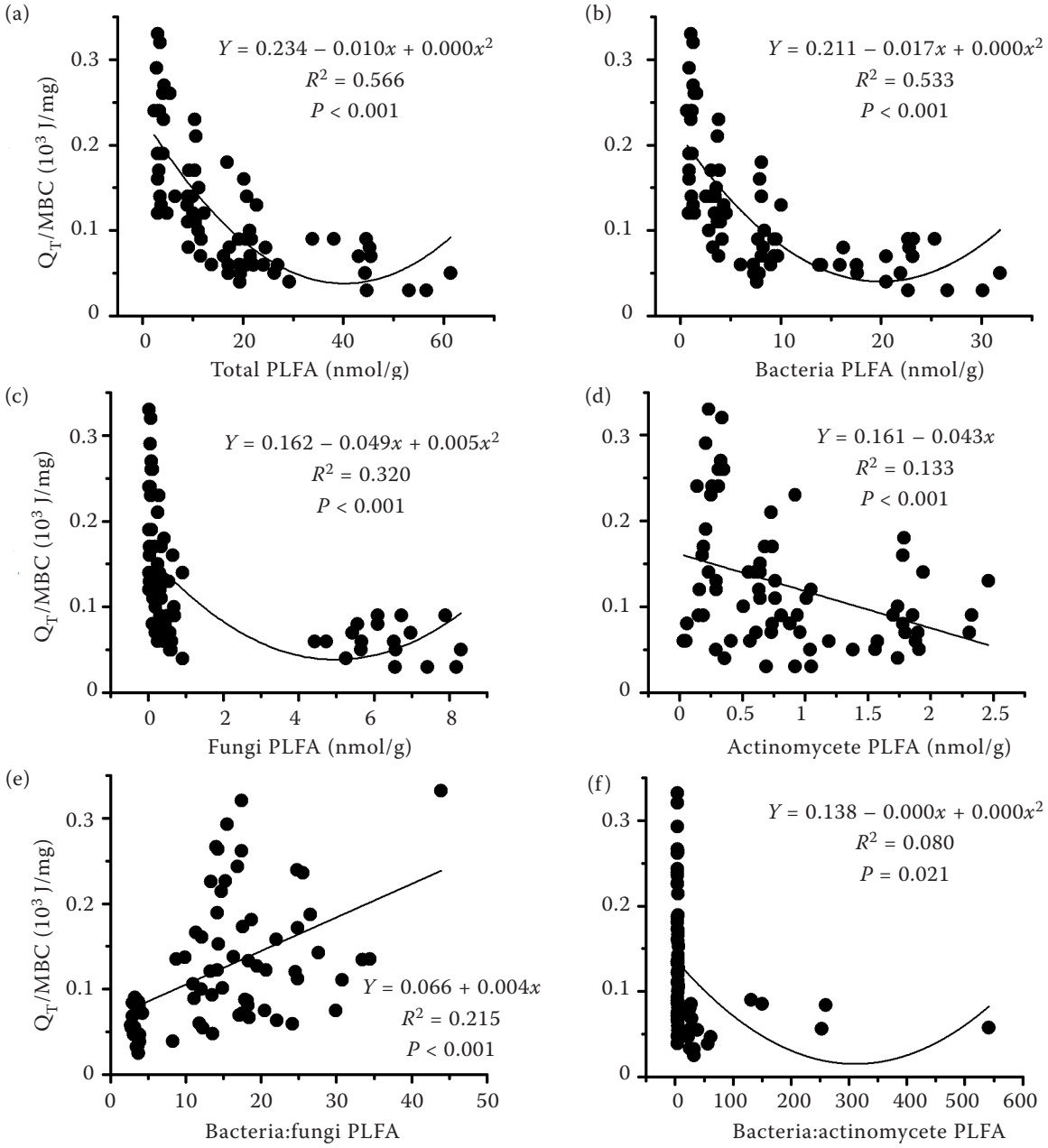

Figure 3. The regression analysis between metabolic quotient of heat (Q_T/MBC) and bacteria, fungi, actinomycetes and total phospholipid fatty acids (PLFAs) in different soil samples. Q_T – total heat output; MBC – microbial biomass carbon

This result is consistent with previous reports that soil microbial communities are functionally linked with thermal flows but not with CO_2 emission (Herrmann et al. 2014). Therefore, microcalorimetric method provides a more complete description of microbial processes than that of microbial CO_2 production in soils (Herrmann et al. 2014).

Assessment of soil microbial activity. In order to better understand the links among soil chemical properties, thermal parameters, metabolic quotient and calorespirometric ratio, redundancy analysis was performed (Figure 5). The metabolic quotient (Q_T/MBC and qCO_2) and t_{max} are all good indices to indicate metabolic efficiency, the lower value indicating the higher metabolic efficiency and carbon conversion of soil microorganisms and higher soil quality (Barros et al. 2003, 2007, Wadsö and Hansen 2015, Zheng et al. 2009, 2016).

In this case, Q_T/MBC, qCO_2 and t_{max} had the opposite pattern to soil pH, SOC, TN and MN,

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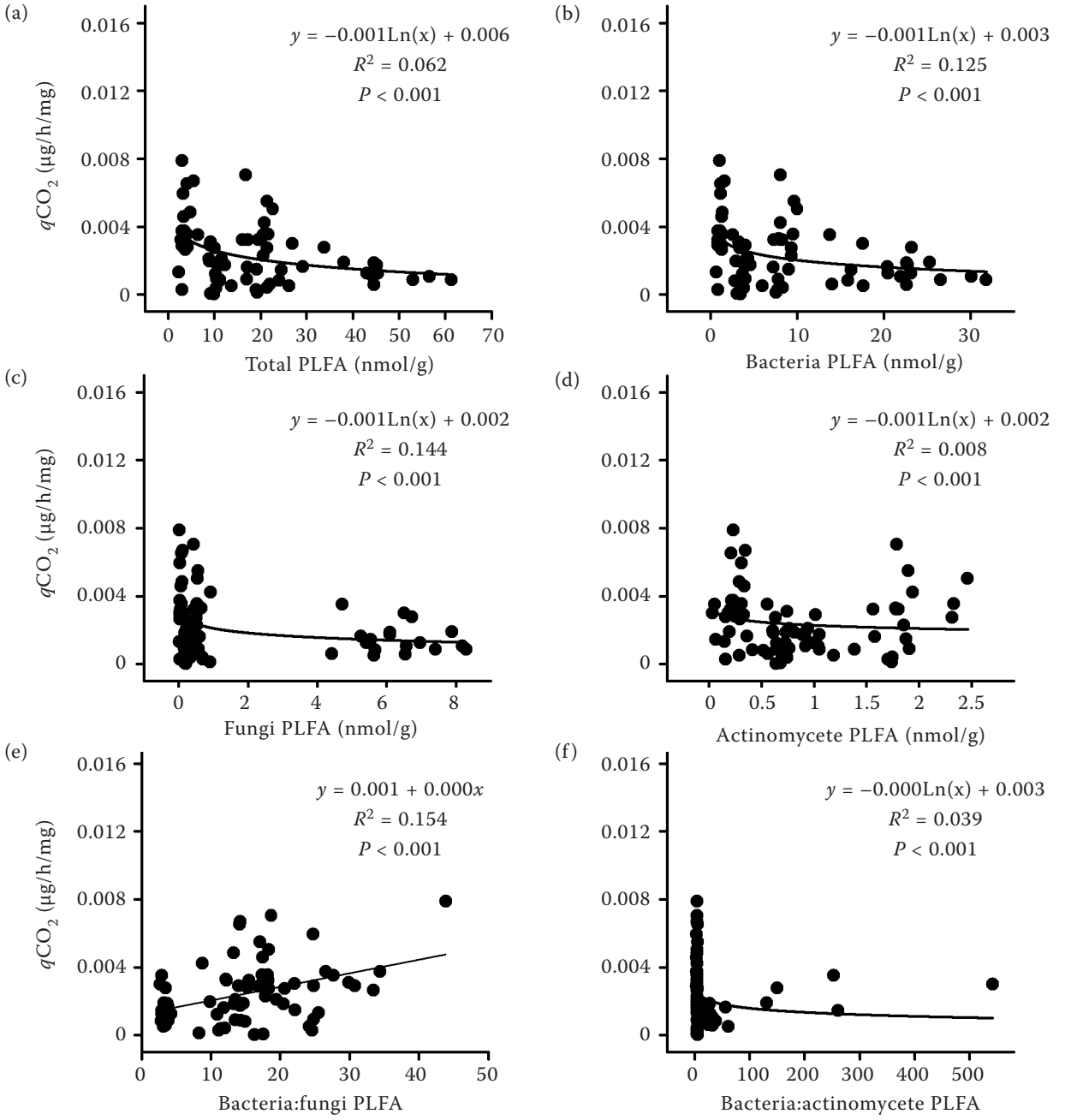

Figure 4. The regression analysis of soil respiratory quotient (qCO_2) and bacteria, fungi, actinomycetes and total phospholipid fatty acids (PLFAs) in different soil samples

indicating higher activity in soils (W and RC) from Xianning than that in other soils (P, R and F) from Yujiang. In particular, Q_T/MBC had the best correlations with SOC, TN and MN (the essential indicators of soil fertility). This result suggested that Q_T/MBC is a more comprehensive indicator in comparison with qCO_2 to assess soil microbial activity and soil quality (Figure 5).

It is interesting that calorespirometric ratio (R_q/R_{CO_2}) had a similar pattern to k and P_{max}. A low value of R_q/R_{CO_2} indicates that relatively little energy is lost from catabolism (Wadsö and Hansen 2015). In this case, the higher value of R_q/R_{CO_2} occurred in upland P soils, which was probably due to fast growth of soil microorganisms (larger k) (Figures 1 and 5) and there was a higher rate of heat output, which led to the higher value of this ratio. The opposite was appeared in R soils. On the other hand, these three parameters were negatively correlated with SOC/TN and SOC/TP. It seems that bacteria

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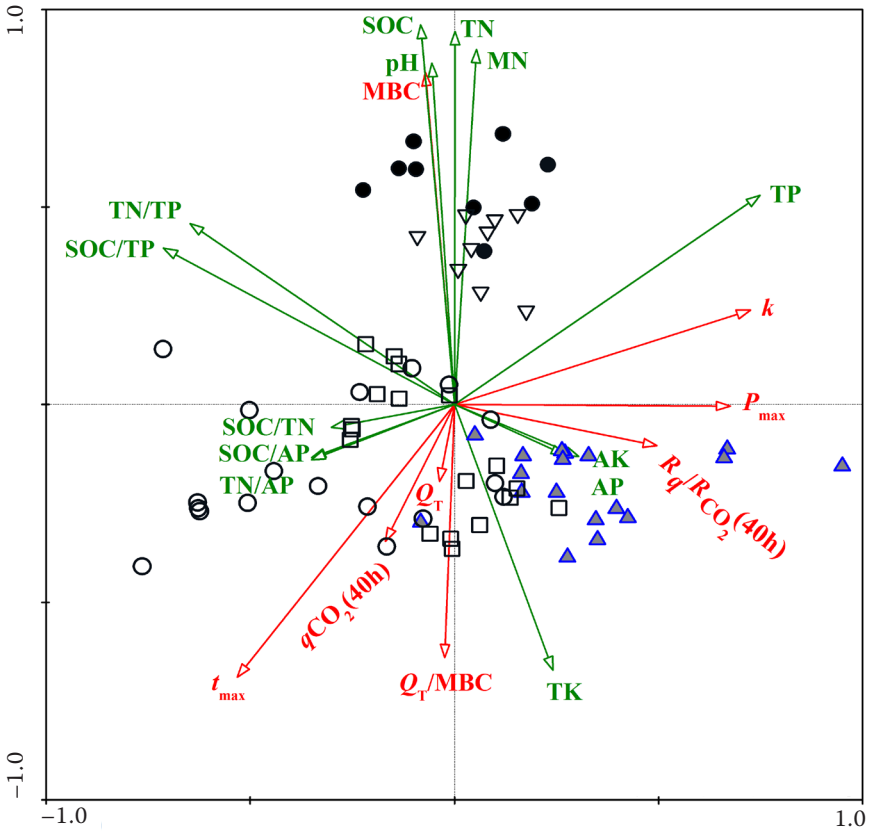

Figure 5. Redundancy analysis of the relationships among soil chemical properties, thermal parameters, metabolic quotient (qCO_2 and Q_T/MBC), and calorespirometric ratio (R_q/R_{CO_2}). MBC – microbial biomass carbon; SOC – soil organic carbon; TN – total nitrogen; MN – mineral nitrogen; TP – total phosphorus; AP – available phosphorus; AK – available potassium; Q_T – total heat output; P_{max}, t_{max} – power and time to reach the maximum of the peak, respectively; k – growth rate constant; P – peanut soils; R – perpetual paddy soils; F – forest soils; W – wheat soils; RC – rotational paddy soils

- P (1–18) – Yujiang
- R (19–36) – Yujiang
- F (37–54) – Yuijiang
- W (55–63) – Xianning
- RC (64–72) – Xianning

quickly responded to the added glucose because bacterial growth needs a lower C/N ratio than fungi (Madigan et al. 2015). It also could be led by more aerobic microorganisms living in peanut soils (P) than in perpetual paddy soils (R). Altogether, these results reveal the complex interactions between nutrients and microbial activity in soils, and calorespirometric ratio needs more comprehensive elucidation.

Acknowledgements

We sincerely acknowledge two anonymous reviewers for their insightful comments in improving this paper.

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Received on March 14, 2018
Accepted on September 3, 2018
Published online on September 30, 2018