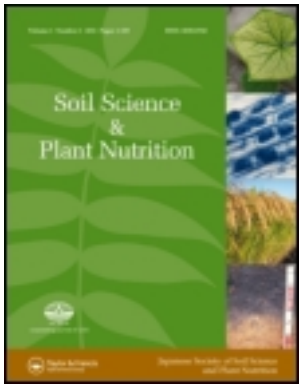

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Microcalorimetric evaluation of soil microbiological properties under plant residues and dogmatic water gradients in Red soil

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ORIGINAL ARTICLE

Microcalorimetric evaluation of soil microbiological properties under plant residues and dogmatic water gradients in Red soil

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Abstract

A study of 6 months duration was carried out to investigate the effect of water regimes and organic amendments on the soil microbial biomass and microbial population under Red soil collected from Hunan Providence, China. The soil microbial biomass and population were measured with traditional methods and results obtained by conventional methods, corroborated with microcalorimetry. The incorporation of rice (*Oryza sativa* L.) straw (RS) and green manure (GM), especially at high rates, enhanced the soil microbial activities. We observed that the use of GM exhibited more significant stimulating effects on microbial activities than RS. Similarly, water regimes, 25% (W1) and 200% (W2) of water holding capacity, also had significant effects on microbial activities. Comparing the effects of water levels, we noticed that W2 had a significant negative influence on soil microbial biomass and population. To compare the results of conventional methods and to check the sensitivity of microcalorimetry, the thermodynamic parameters, microbial growth rate constant (k), total heat evolution (Q), peak height (P_{max}) and peak time (t_{max}) were calculated. Highest $P_{max,}$ k and Q were observed in GM treatments at water regime W1, while highest t_{max} values were recorded in CK (control) and RS treatments at W2. The microcalorimetric parameters, $P_{max,}$ k and Q were positively correlated, whereas t_{max} negatively linked with microbial biomass and population at $p < 0.01$. Our results suggest that microcalorimetry successfully verified the results obtained from customary methods and microcalorimetric parameters $P_{max,}$ $t_{max,}$ Q and k proved that they are highly sensitive to microbial properties and could be used as indices of microbial community shifts and activities in soil ecosystems.

Key words: Microcalorimetry, microbial biomass, microbial population, organic amendments, water regimes.

INTRODUCTION

Soil microorganisms are vital to agro-ecosystem health through their roles in residue decomposition, nutrient cycling and their associations with other organisms (Ge *et al.* 2008). In agricultural soils, microorganisms are known to impact profoundly the status of soil quality and health, especially the availability of soil nutrients (Li *et al.* 2008a). Soil microbial properties such as microbial biomass and population activities have strong correlations with soil health and quality indices (Li *et al.* 2008b). Due to the complex dynamics of soil ecosystems, understanding the microbial community in the soil environment, especially in Red soils, has still not been completed and it is necessary to examine it through assiduous techniques.

Organic sources, generally increase soil microbial biomass, carbon dioxide evolution and enzyme activities, whereas inorganic fertilizers have relatively less effect on these soil properties (Chu *et al.* 2007). The impact of organic matter inputs, such as green manure and rice (*Oryza sativa* L.) straw, either alone or in combination,

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on soil biological health is an important area of investigation for assessing soil sustainability (Tu *et al.* 2006; Tejada *et al.* 2009). This area is of particular importance for the sustainable management of degraded Red soils [Ultisols and Oxisols according to United States Department of Agriculture (USDA) Soil Taxonomy]. Red soils are clayey, kaolinitic thermic Typic Plinthudults with over 2 m deep profile derived from quaternary red clay, subjected to severe erosion (FAO/UNESCO 1974). These soils contained huge amounts of polyvalent aluminium (Al^{3+}) and iron (Fe^{3+}) cations and their oxides or hydroxides. This diminished the availability of carbon (C), nitrogen (N) and phosphorus (P) in the soils, which served as substrate, and ultimately microbe activity through cationic bridging and formation of organo-metallic compounds and gels (Amezketa 1999). Overall, Red soils cover about 1.13 million km^2 or 11.8% of the country land surface, and supporting 22.5% of the population (Zhao 2002). Therefore, this is of great concern for understanding the behavior and microbial ecology of these soils.

Water plays a key role to sustain the stability of soil biota, and the change of water level drastically influenced the soil microbial properties (microbial population) and active C, i.e. microbial biomass carbon, and dissolved organic carbon (Van Bodegom *et al.* 2005). Global warming is evolving a worldwide environmental change and disturbing the delicate soil-air-water balance; both drought and waterlogged conditions ultimately lead to soil degradation. Feyen and Dankers (2009) pointed out that global change is likely to increase drought periods. Drought directly affects the soil microorganisms by creating osmotic stress, which leads to microbial death and cell lysis (Turner *et al.* 2003). Similarly prolonged anoxic conditions decreased the soil microbial biomass and microbial population (Wang and Lu 2006; Tripathy *et al.* 2008; Zhongmei *et al.* 2008). Scant information is available on how submerged and dry conditions influence the microbial and biochemical properties of soils (Van Bodegom *et al.* 2005). This emerging field of exploration will improve our understanding about soil abiotic and biotic influences on microbial ecosystems and enhance the success of soil quality and health restoring efforts.

Different microbiologic and classical analytical methods are used to investigate microbial activity; however, these methods destroyed the soil sub-samples in each experiment and the conditions of investigation are very different from that of the soil environment (Sposito 1989). Moreover, these methods are of low precision, and are time- and labor-consuming. This problem is overcome by microcalorimetry that quantifies the microbial and enzymes activity in real time and actual soil conditions, without disturbing the system (Gustafsson 1991; Critter *et al.* 2001). This highly sensitive tool can be applied for estimating the influence of agricultural practices on soil microbial activity and the connections between soil microbial properties, and microcalorimetric parameters could elucidate which microcalorimetric parameter best indicates microbial activity in soils (Laor *et al.* 2004; Zheng *et al.* 2007; Ahamadou et al. 2009). It would be very useful to study these questions; previously published studies lack the sensitivity of calorimetric indices to detect microbial and metabolic activity changes caused by organic amendments and harsh water contents. To our knowledge, no study has shown the sensitivity and working potential of microcalorimetry under rigorous water conditions. Therefore the present study was conducted with the following objectives: (1) to better understand the comparative effectiveness of organic amendments (rice straw and green manure), at extreme water contents (25% or 200%), on microbial biomass and microbial ecology of degraded Red soils; (2) to assess the relationship of soil microbial properties with microcalorimetric parameters for establishing the sensitivity of the different organic amendments and water levels under Red (Ultisols) soils.

MATERIALS AND METHODS

An incubation experiment was conducted in the greenhouse of Huazhong Agricultural University, Wuhan, China. Soil samples (0–20 cm depth) of Red soil (Ultisols and Oxisols) collected from Ganshan Village, Changsha County, Changsha city of Hunan province (113°12′ E, 28°09′ N) were used in this study. The soil has been under the cultivation of tea (*Camellia sinensis*) for 10 years.

The study was laid out in a randomized complete block design with 10 treatments in triplicates. Rice straw (RS) and green residues of peanut (*Arachis hypogaea*) plants, as a green manure (GM), were obtained from a farm at Huazhong Agricultural University, Wuhan, China. Before the incorporation of organic amendments in the soil, RS and GM were chopped properly (0.5–1 cm). The sieved soil (500 g air-dried for few hours in complete shade, 2 mm) was transferred into 1-kg capacity pots (20 × 15 cm). The RS and GM were put into pots and mixed thoroughly at two rates (5 and 25 mg g^{-1} soil). The soil was maintained at two water regimes 25% (W1) and 200% (W2) of water holding capacity with deionized water. The soil water was kept at the predetermined level throughout the experimental period with deionized water by weighing periodically. Similarly, the temperature of the green house was maintained at 25°C (298.15 K) throughout the incubation period. Soil samples were collected from each pot after 1, 7, 13, 19 and 25 weeks, then the homogenized samples were sieved through a 2-mm mesh and separated into two parts. The first part was

Table 1 Treatments of the incubation experiment

Treatments	Amount of organic inputs (mg g^{-1})	Water contents (%)
RS1W1	5	25
RS2W1	25	25
RS1W2	5	200
RS2W2	25	200
GM1W1	5	25
GM2W1	25	25
GM1W2	5	200
GM2W2	25	200
CK1	0	25
CK2	0	200

RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25 and 200% WHC. WHC, water holding capacity; CK1 and CK2, controls at W1 and W2.

Table 2 Physico-chemical properties of experimental soil

Properties	Red soil
Clay (g kg^{-1})	213.8
Silt (g kg^{-1})	481.7
Sand (g kg^{-1})	304.5
Textural class	loam
pH (1:2)	4.09
electrical conductivity (EC) (μS cm^{-1})	88
Soil redox potential (Eh) (mV)	405
Organic matter (g kg^{-1})	31.44
Soluble organic carbon (mg kg^{-1})	96.59
Available nitrogen (mg kg^{-1})	36.53
Available phosphorus (mg kg^{-1})	3.82

Table 3 Chemical properties of crop residues used in the experiment

Properties	Rice straw	Green manure
Organic matter (g kg^{-1})	634.70	569.10
Total nitrogen (g kg^{-1})	8.87	22.5
Total phosphorus (g kg^{-1})	3.10	14.6
carbon/nitrogen ratio	71.55:1	25.29:1

air dried for 1 week for the physical and chemical analyses, while the second part was stored at 4°C (277.15 K) for the measurement of microbial and biochemical properties. The treatments of our study are listed in Table 1.

Soil particle size distribution was evaluated by the international pipette method (Klut 1986). Soil pH and electrical conductivity (EC) were measured using soil/water ratio [weight/volume (w/v)] of 1:2 (Thomas 1996). Soil redox potential (Eh) was measured using soil/water ratio (w/v) of 1:1, by using portable pH-redoxmeter (APW PH-EH, France). Soil organic matter content (SOM) was estimated according to the method of Nelson and Sommers (1982). The organic matter contents of the organic substances (RS and GM) were obtained by ashing duplicate samples of each batch in muffle furnace at 540°C (813.15 K) for 6 h. The change in the dry weight of these organic wastes before and after ashing was used to calculate the OM content. Soluble organic C was determined by a modified version of the method of Gregorich *et al.* (2003) and Sparling *et al.* (1998). One gram of soil was shaken for 30 min (30 rpm) in plastic tubes. The tubes were then capped and placed in a hot water bath at 80°C for 16 h. At the end of this period, each tube was centrifuged (20 min at 8000 rpm), the supernatant was filtered (0.7-μm filter membranes), and the filtrate was analyzed for soluble organic C using a TOC/TN analyzer (multi N/C 2100, Analytic Jena, Germany). Available N was measured by the sodium hydroxide (NaOH) pervasion method (Bao 2000). Soil available P was assayed by the sodium bicarbonate ($NaHCO_3$) method (Hesse 1972). Total P in soil and organic amendments samples were analyzed by NaOH fusion and colorimetric procedures (Olsen and Somers 1982), while total N contents were quantified by sample digestion and the Kjeldahl method (Zellweger Analytics Inc 1996). Some pertinent characteristics of the soil and organic amendments are shown in Tables 2 and 3.

The chloroform fumigation-extraction method was used to measure soil microbial biomass carbon (MBC). A soil sample equivalent to 10 g (fresh soil) was fumigated for 24 h at 25°C (298.15 K) with alcohol-free chloroform ($CHCl_3$) in a vacuum desiccator containing soda-lime. The fumigated soil was then transferred into a clean, empty desiccator and residual $CHCl_3$ was removed from the fumigated soil by repeated evacuations. The fumigated soil was extracted immediately for 30 min by using horizontal shaking at 200 rpm with 50 mL 0.5 M potassium sulphate (K_2SO_4) and filtered through a filter paper (Whatman No. 40). The non-fumigated control soil (10 g fresh soil) was extracted similarly at the time when fumigation commenced. Total organic C in the extracts was determined using a multi N/C analyzer (multi N/C 2100, Analytic Jena, Germany). The MBC was calculated as $(Ct_1–Ct_0) \times 2.22$, where Ct_1 is the extracted carbon (mg kg^{-1}) from fumigated samples, Ct_0 is the extracted carbon (mg kg^{-1}) from unfumigated samples and 2.22 is the factor, calculated by 0.45 i.e. 100/45 = 2.22; where 0.45 is the extractable part of microbial C after fumigation (Wu *et al.* 1990). For microbial biomass nitrogen (MBN) total N in the K_2SO_4 extract was measured after Kajeldahl digestion. The MBN was calculated as $(Nt_1–Nt_0) \times 1.85$, where Nt_1 is the extracted nitrogen (mg kg^{-1}) in fumigated samples, Nt_0 is the nitrogen (mg kg^{-1}) in unfumigated samples and 1.85 is a factor which is obtained via 0.54 (i.e. 100/54 = 1.85) which is the extractable part of

microbial N after fumigation (Brookes *et al.* 1985). For microbial biomass phosphorus (MBP) the fumigated and the non-fumigated soil samples were extracted by 0.5 M $NaHCO_3$ (pH 8.5) for 30 min. The concentrations of P were determined using a spectrophotometer at 882 nm wavelength. The MBP was calculated as $(Pt_1–Pt_0) \times 2.5$, where Pt_1 is the phosphorus (mg kg^{-1}) in fumigated samples, Pt_0 is the phosphorus (mg kg^{-1}) from unfumigated samples and 2.5 is a factor, computed by 0.4 (e.g. 100/40 = 2.5), while 0.4 is the extractable part of microbial P after fumigation (Öhlinger 1996).

The total bacteria, fungi and actinomycetes were determined by the dilution plate count technique on nutrient agar. Dilution plate technique assumes that every colony is founded by a single cell CFU (Colony Forming Unit). In brief, 10 g of fresh soil samples was placed into flask contained 90 mL distilled water and glass beads (0.5 mm). The flask was shaken at 28°C (301.15 K) and 180 rpm for 30 min. 0.1 mL from the suspension was added to small tube contained 0.9 mL distilled water. The tube was shaken carefully and used to perform the other dilutions. For bacterial enumerations, dilutions of 10^{-1}–10^{-8} were used. Conversely, a range of 10^{-1}–10^{-6} was used for the determination of fungi and actinomycetes. Each dilution was repeated three times. The plates were incubated at 28°C (301.15 K) in an incubation machine. Bacteria, fungi and actinomycetes were accounted 4, 5 and 7 d after the planting process, respectively.

A TAM III thermal activity monitor (Thermometric AB, Sweden) was used for all heat-effect measurements. The calorimetry has precise control of the isothermal conditions in the thermostated bath and of the detection of the thermal events in the system (Barros *et al.* 2000). All living systems produce heat and therefore can be measured by some type of isothermal calorimeter, provided its detection limit is sufficient (Wadsö 2009). Soil samples were incubated at 25°C (298.15 K) for 24 h and their moisture maintained at 35% (water-holding capacity) to maximize microbial activity (Prado and Airoldi 2001). All determinations were performed in 4-mL stainless-steel ampoules at 25°C (298.15 K). The ampoules were sterilized by rinsing in 75% ethanol and sterile deionized water for 10 min and dried under a laminar flow hood, before the experiment. One gram of soil was placed into the sterile ampoule and 0.2 mL of a solution containing 1.5 mg glucose and 1.5 mg ammonium sulfate was added immediately. The ampoules were simultaneously introduced into the multichannel of the microcalorimeter. They were lowered to a preheating position for 15 min and then to the measuring position. Once the baseline was stable, data and growth power-time curves were monitored and recorded by a computer until the signal was back to baseline again. Each measurement lasted for about 48 h. All the experiments were performed in triplicate. The final value was calculated by comparing the integrated area of the power time curves, which corresponds to the thermal effect of the experiment (Critter *et al.* 2001). The power-time curves from every experiment were analyzed, and from these analyses characteristic parameters, such as growth rate constant (k) and total thermal effect (Q) which can reflect the biochemical reactions were determined (Wang *et al.* 2009). The total heat output, Q, was obtained through the integration of each curve. The value of peak height (P_{max}) and corresponding time (t_{max}) of each curve were picked through the TAM assistant software kit (Thermometric AB). The microbial growth rate constant (k) determined by microcalorimetry is based on the assumption that the heat evolved from metabolism in the vegetative stage is proportional to the rate of cell division (Boling *et al.* 1973). This parameter was calculated by fitting a logarithmic growth model based on data of the power time curve in the logarithmic growth stage. Thus, if the cell number is n_0 at time 0, and n_t at time t,

$$n_t = n_0 \exp(kt) \tag{1}$$

where k is the growth rate constant. If the power output of each cell is w, then

$$n_t w = n_0 w \exp(kt) \tag{2}$$

if the heat output power is p_0 at time 0 and p_t at time t, then

$$p_0 = n_0 w$$

and

$$p_t = n_t w$$

giving

$$p_t = p_0 \exp(kt) \text{ or } \ln pt = \ln p_0 + kt \tag{3}$$

The growth power-time curves of the log phase correspond to Eq. 3. So, using the data ln Pt and t taken from the curves to fit a linear equation, the thermokinetic equation for the soil microbial activity and the correlation coefficients can be obtained.

The data were interpreted using Statistical Package of M-STATC 98 program for Windows, and the differences between the treatment means were calculated using the least significance difference test.

RESULTS

Soil microbial biomass and its links with microcalorimetric parameters

The influences of the organic amendments and water levels on soil microbial biomass are shown in Fig. 2. The contents of MBC, MBN and MBP were markedly increased due to the addition of organic amendments as compared to the control. These increments became more obvious with the increase of the amendment application rate. The lowest values of MBC, MBN and MBP were noticed in CK2 and ranged from 53.9 mg kg^{-1}, 21.94 mg kg^{-1} and 0.773 mg kg^{-1} respectively, whereas the highest values were noticed in GM2 at water level W1, ranging from 265.86 mg kg^{-1}, 237.74 mg kg^{-1} and 10.72 mg kg^{-1} respectively. On the other hand, less microbial biomass was found under submerged conditions at water gradient W2. Substantial correlations ($p < 0.01$) were observed between microbial biomass and microcalorimetric parameters (P_{max}, t_{max}, k, and Q) in the inspected soil (Table 4).

Soil microbial communities and their correlations with microcalorimetric parameters

Microbial population (fungi, actinomycetes and bacteria) aligned from 0.48–11.2 10^3 CFU g^{-1}, 0.64–9.6 10^5 CFU g^{-1} and 0.83–26.88 10^6 CFU g^{-1} (minimum and maximum range) in the tested soil respectively (Fig. 4). Maximum microbial population was observed in GM treatments at W1, whereas minimum was noticed in CK2 at W2. Treatments amended with RS showed long lasting positive impact on microbial population with the incubation time. Higher water regime (W2) demonstrated significant negative effect on microbial population. Data regarding microcalorimetry clearly indicate that all the microcalorimetric parameters P_{max}, t_{max}, k and Q were strikingly correlated ($p < 0.01$) with microbial communities (Table 4).

Microcalorimetric parameters under organic manipulation and stringent water regimes

The data of calorimetric parameters P_{max} (peak height), t_{max} (peak time), k (growth rate constant) and Q (total

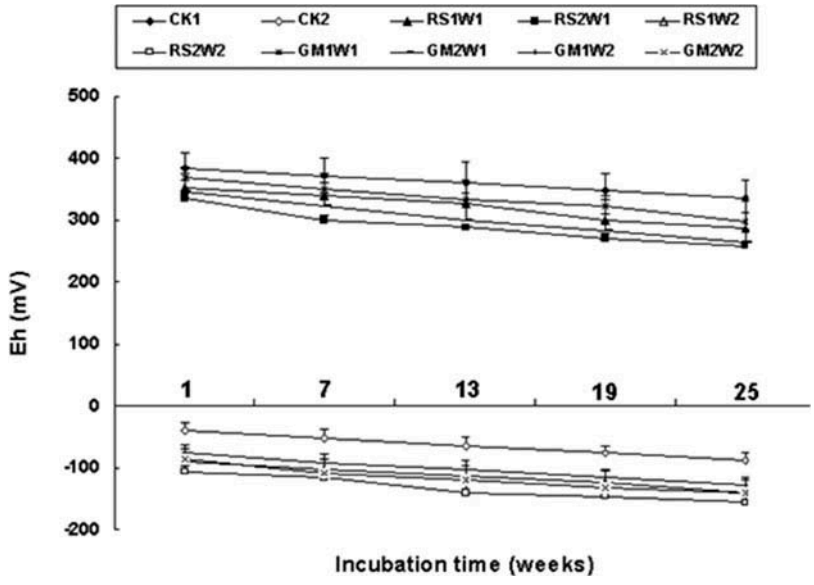

Figure 1 Effect of organic amendments on soil redox potential (Eh). CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25 and 200% WHC. WHC, water holding capacity. Bars represent standard errors.

Table 4 Correlative coefficient between microcalorimetric parameters and microbial properties in Red soil

Parameters	MBC	MBN	MBP	Bact	Fung	Actino
P_{max}	0.968**	0.961**	0.941**	0.981**	0.971**	0.967**
t_{max}	−0.858**	−0.861**	−0.904**	−0.840**	−0.826**	−0.821**
Q	0.697**	0.722**	0.612**	0.665**	0.650**	0.671**
k	0.978**	0.968**	0.937**	0.982**	0.985**	0.979**

MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; Bact, bacteria; Fung, fungi; Actino, actinomycetes. P_{max}, peak height; t_{max}, peak time; k, growth rate constant; Q, total thermal effect. ** is significant at $p < 0.01$.

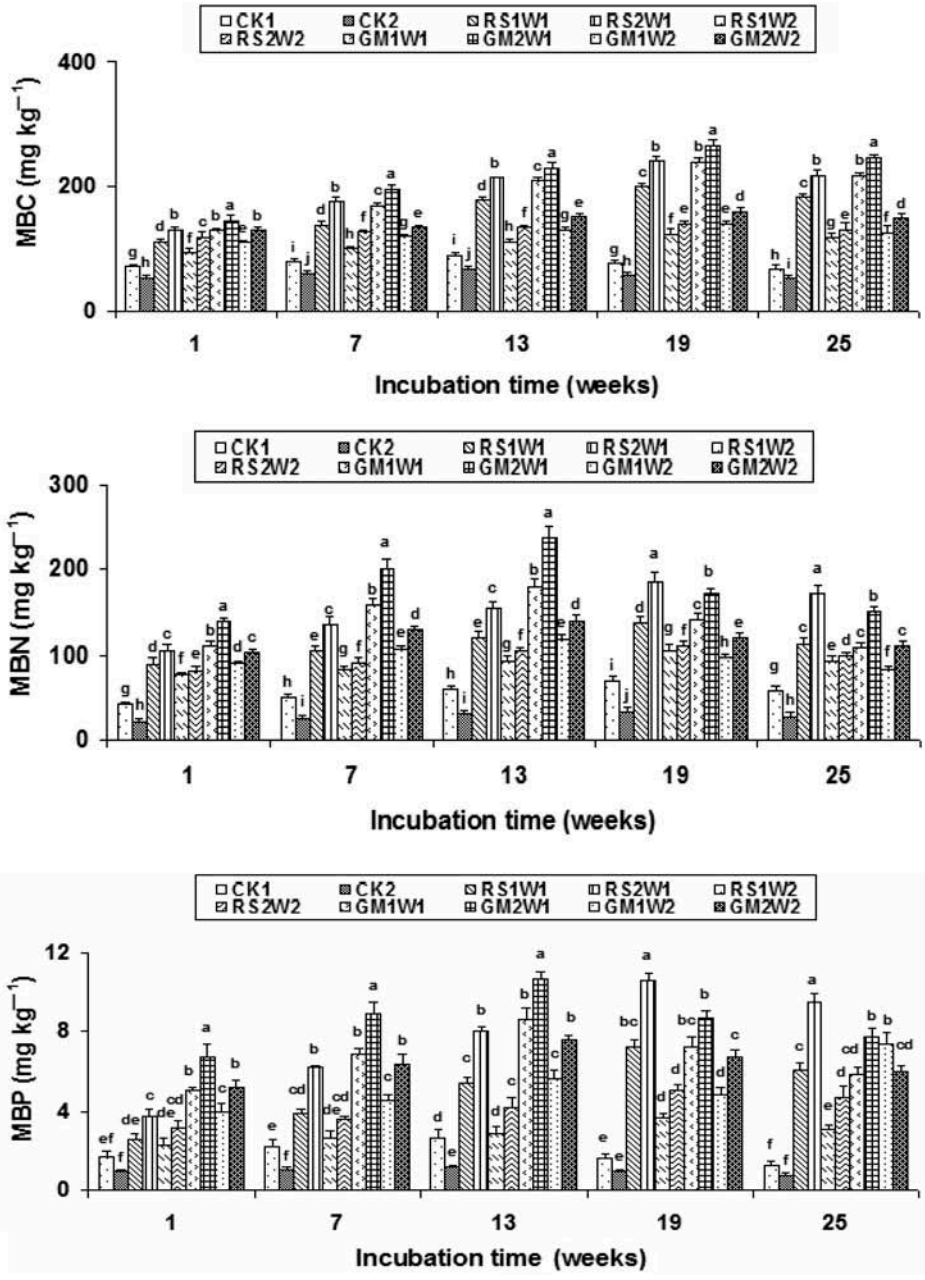

Figure 2 Effect of organic amendments on soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP). CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25 and 200% WHC. WHC, water holding capacity. Different letters (a–j, a–j and a–f) on bars indicate significant differences of mean values for MBC, MBN and MBP respectively. Bars represent standard errors.

thermal effect) after 1, 13 and 25 weeks of incubation are presented in Tables 5, 6 and 7 respectively. The results indicate that both organic inputs (RS or GM) and water contents (W1 or W2) have significant effects on all measured calorimetric parameters. As a consequence of this, highest P_{max} (μW) and k (min^{-1}), and lowest t_{max} (min), were observed in the treatment amended with GM2 and water level W1, during incubation. The effect of other GM treatments, mingled with water gradient, W1 or W2, on calorimetric parameters (P_{max}, t_{max}, k and Q) was in the order of GM1W1 > GM2W2 > GM1W2, in comparison to controls. Conversely, treatment amended with RS at water gradient W1 was the second best, with maximum P_{max} (μW) and k (min^{-1}), whereas the lowest t_{max} (min) was observed at that treatment after GM2, correspondingly.

Table 5 Microcalorimetric parameters as influenced by organic amendments and water gradients after 1 week of incubation

Treatments	P_{max} (μW)	t_{max} (min)	Q (Jg^{-1})	k (min^{-1})
CK1	93.70 g	1770.32 e	10.96 e	4.03×10^{-6} f
CK2	54.46 h	2618.23 b	7.99 f	2.29×10^{-6} g
RS1W1	208.89 c	1371.56 g	15.27 c	1.24×10^{-5} c
RS2W1	254.02 b	1687.26 e	14.88 c	1.51×10^{-5} b
RS1W2	110.01 f	1878.62 d	16.05 b	5.41×10^{-6} e
RS2W2	164.44 d	2819.12 a	13.69 d	9.98×10^{-6} d
GM1W1	231.46 b	992.32 h	15.36 c	1.44×10^{-5} b
GM2W1	300.94 a	1562.06 f	29.88 a	1.79×10^{-5} a
GM1W2	134.84 e	1293.55 g	13.49 d	9.22×10^{-6} d
GM2W2	165.94 d	2134.16 c	16.96 b	1.17×10^{-5} c

P_{max}, peak height; t_{max}, peak time; k, growth rate constant; Q, total thermal effect. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25% and 200% WHC. WHC, water holding capacity. Different letters (a–h) within a same column indicate significant differences of mean values.

Table 6 Microcalorimetric parameters as influenced by organic amendments and water gradients after 13 weeks of incubation

Treatments	P_{max} (μW)	t_{max} (min)	Q (Jg^{-1})	k (min^{-1})
CK1	129.46 h	2372.5 a	13.37 c	9.5×10^{-6} e
CK2	105.53 i	1677.7 b	9.07 f	4.4×10^{-6} f
RS1W1	255.25 d	716.89 c	15.01 b	2.1×10^{-5} c
RS2W1	387.54 b	196.88 f	11.78 d	2.8×10^{-5} a
RS1W2	145.40 g	2364.1 a	13.85 c	1.1×10^{-5} d
RS2W2	205.75 e	578.12 d	26.92 a	1.3×10^{-5} d
GM1W1	290.37 c	745.66 c	14.20 c	2.6×10^{-5} b
GM2W1	429.62 a	196.88 f	11.78 d	3.2×10^{-5} a
GM1W2	179.98 f	538.81 d	10.92 e	1.2×10^{-5} d
GM2W2	236.34 d	233.48 e	11.88 d	1.4×10^{-5} d

P_{max}, peak height; t_{max}, peak time; k, growth rate constant; Q, total thermal effect. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25 and 200% WHC. WHC, water holding capacity. Different letters (a–i) within a same column indicate significant differences of mean values.

The influential tendency of other RS treatments at water levels W1 or W2 followed the subsequent order of RS1W1 > RS2W2 > RS1W2, in comparison to controls. The highest values of Q (Jg^{-1}), after 1, 13 and 25 weeks of incubation, were found in the order of GM2W1 > RS2W2 > RS1W1.

Table 7 Microcalorimetric parameters as influenced by organic amendments and water gradients after 25 weeks of incubation

Treatments	P_{max} (μW)	t_{max} (min)	Q (Jg^{-1})	k (min^{-1})
CK1	117.66 f	1043.7 f	15.26 c	8.9×10^{-6} e
CK2	34.00 g	3496.8 a	5.70 e	3.4×10^{-6} f
RS1W1	225.18 d	2867.1 b	24.32 a	1.4×10^{-5} c
RS2W1	375.61 a	767.99 h	15.25 c	1.8×10^{-5} b
RS1W2	129.46 f	2372.5 c	13.37 d	9.6×10^{-6} d
RS2W2	195.98 d	1657.6 e	14.96 c	1.1×10^{-5} c
GM1W1	260.80 b	935.09 g	15.14 c	1.8×10^{-5} b
GM2W1	400.28 a	1074.7 f	21.32 b	2.9×10^{-5} a
GM1W2	162.74 e	1821.2 d	13.50 d	1.1×10^{-5} c
GM2W2	220.47 d	1910.6 d	14.86 c	1.4×10^{-5} c

P_{max}, peak height; t_{max}, peak time; k, growth rate constant; Q, total thermal effect. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25 and 200% WHC. WHC, water holding capacity. Different letters (a–h) within a same column indicate significant differences of mean values.

DISCUSSION

Microcalorimetric parameters as indices of soil microbial properties

Naturally, soil is a balanced but complex ecosystem, having a significant population of a variety of microorganisms; any slight external change can disrupt the whole balance and ultimately decline the soil quality. Due to the significant decline in soil quality occurring worldwide by adverse changes in its physical, chemical and biological properties, and by the contamination caused by inorganic and organic chemicals (Arshad et al. 2002), it is necessary to use more sensitive and accurate techniques to figure out new indices, and to understand what constitutes an indicator of soil quality and of soil state. The application of calorimetry to study soil properties and soil metabolism is of great worth. Calorimetry constitutes a very suitable method to face the main topics related to soil quality and activity (Barros *et al.* 2007). Calorimetry indices are sensitive to the main biological and physico-chemical properties of soil (Barros *et al.* 2003). Our observations agreed well with these results. P_{max}, t_{max}, k and Q were significantly correlated with soil microbial properties of Red soils.

In this study, P_{max} and t_{max} designate significant correlations ($p < 0.01$) to microbial biomass C (0.968, –0.858), N (0.961, –0.861), P (0.941, –0.904), bacteria (0.981, –0.840), fungi (0.971, –0.826) and actinomycetes (0.967 and –0.821 respectively) in Red soil (Table 4). These outcomes suggested that microcalorimetric indices are highly sensitive for soil biological activities and can be used for precise measurement of soil microbial parameters. Metabolic enthalpy, microbial growth rate constant, total heat evolution, mass specific heat rate and MBC could be used to assess soil quality as real-time indicators (Barros *et al.* 2006). The kinetic parameters of glucose degradation in soils show that the thermal effect and the active cell populations were estimated by the quantification of the kinetic parameters of the microbial growth in the soil (Barros *et al.* 2010).

The total heat evolution and the microbial growth rate constant seem to be the most widely employed calorimetric indices in soil research. They fit well with parallel carbon dioxide (CO_2) measurements and are sensitive

enough to soil perturbation. They also have the advantage of being easily determined by calorimetry, providing an easy interpretation of the results (Barros *et al.* 2007). This was agreed by our observations; Q and k were significantly associated ($p < 0.01$) to microbial biomass C (0.697, 0.978), N (0.722, 0.968), P (0.612, 0.937), bacteria (0.655, 0.982), fungi (0.650, 0.985) and actinomycetes (0.671 and 0.979 respectively) in Red soil (Table 4). Critter *et al.* (2002) studied the comparison between microorganism counting (bacteria and fungi) and a calorimetric method applied to tropical soils of Brazil; the soils were amended with a range of organic materials (cattle manure, municipal refuse compost, earthworm casts, agrochemicals and trifluralin) during incubations of 85 days. They indicated that stimulation was detected by calorimetry as an increase in the heat flow rate, correlated with the increase in biomass measured by fumigation and by the most probable number method. To see the effect of soil moisture on soil microbial activity in soil collected from Santiago de Compostela, Spain, using microcalorimetry, results showed that different moisture regimes produce changes in heat flow recorded for soil samples, affecting also certain parameters such as the total heat evolution (Q_{tot}), the microbial growth rate constant (μ), and growth yield (Y) (Critter *et al.* 2001). Wang *et al.* (2010) conducted an experiment to measure the inhibitory effects of the pesticide Chlorpyrifos on soil microbial activity, in orchard soils collected from Hubei, China. They found linear correlations between the growth rate constant (k) and soil MBC and concluded that k is a microbial growth yield-dependent parameter. Prado *et al.* (2002) studied the toxic impact of the immobilized and free pesticide diuron on microbial activity of Red Brazilian Latosol soil by microcalorimetry. They deduced that the increasing amounts of diuron, either free or immobilized, caused a significant decrease in the original thermal effect and microbial growth rate constant. Moreover, calorimetric data also showed that the immobilized pesticide presented a much lower toxic effect on soil microbial activity than did free diuron. These investigations stated that microcalorimetry can be a useful method to study the way in which the biological activity of soils is affected by environmental factors.

Microcalorimetric parameters as influenced by organic treatments and water gradients

Calorimetry appears to be a useful tool for calculating the latency time, together with the total heat and the kinetics of microbial growth, without any disruption (Barros *et al.* 2007). Our results are in parallel with these findings; in the present study, the microbial activity presented by higher growth rate (k), more peak height (P_{max}), shorter peak time (t_{max}) and longer heat dissipation (Q) per cell unit (Tables 5, 6 and 7), indicates that microorganisms under organic treatments (RS or GM) and aerobic conditions at water gradient W1 had more efficient metabolism and growth, while less microbial activity and growth under submerged conditions (W2) and in control treatments were due to lack of aeration, substrate availability and nutrient deficiency. Barros *et al.* (2001) used the microcalorimetric method to calculate the metabolic enthalpy change per mol of glucose degraded by soil microorganisms and revealed changes in the values of enthalpy, suggesting a dependence of this value with the microbial growth rate constant, with the percentage of growth, with the initial number of microorganisms of soil samples and with the strain of bacteria growing in soil. Microcalorimetric techniques have been used to study the influence of different physicochemical parameters on microbial growth in different soils in Galicia (northwestern Spain); the influence of different environmental parameters, temperature (ambience and soil), moisture content (sample and residual), pH in water, and C/N ratio were considered. Parameters such as peak time (t_{max}), peak height (P_{max}) and microbial growth rate constants (k) were determined. Results disclosed that microcalorimetric technique is a suitable indicator that informs us about the soil state and the soil disruption (Núñez-Regueira *et al.* 2002). This suggests that microcalorimetric parameters P_{max}, t_{max}, Q and k are highly sensitive to many intrinsic soil properties, and could be used as indices of biomass and microbial community properties under harsh water gradients.

Impact of organic amendments and water regimes on soil microbial properties

It is widely believed that the return of legume and other crop residues improve the soil microbial biomass and soil quality (Biederbeck *et al.* 2005; Bakht *et al.* 2009). Applying organic amendments has been shown to increase soil microbial activity, microbial diversity and bacterial densities (Van Bruggen and Semenov 2000; Girvan *et al.* 2004). However, the influence of organic matter on soil properties depends on amount, type, and size of added organic materials (Barzegar *et al.* 2002). Flooding of soils might cause some shift in the population of aerobic and facultative anaerobes (Gaind et al. 2010).

As shown in Fig. 1, in general the Eh values tended to decrease after the incorporation of plant residues (RS and GM) under both water regimes, i.e., W1 and W2. Comparing the effect of water regimes, the lowest and negative Eh values and reducing or anaerobic conditions were found in water regime W2 (submerged conditions). Conversely, high and positive Eh values were observed in water regime W1. Our results are in great agreement with the findings of Taboada (2006), who revealed that

in strongly oxidizing systems the Eh is positive and high, while in strongly reducing systems the Eh is negative and low. The soil moisture regime controls both soil redox potential and biological activity in soils, and, under saturated conditions, low and negative redox potential was observed in an experiment conducted in Mississippi, USA (Han *et al.* 2001). Comparing the impact of treatments, maximum Eh values were observed in controls under both water regimes W1 and W2, whereas incorporations of RS showed lower Eh values than GM, and these Eh values decreased with the passage of incubation time. Variations in soil Eh depend on the amount of bioavailable organic C, and low amounts of bio-available organic C diminish the microbial activity and cause the redox potentials to suddenly stop decreasing (Wheeler *et al.* 1999). Low oxygen and decay-resistant characteristics of the decayed organic material markedly decrease the values of redox potential (Hogg 1993).

In this study, the application of GM and RS significantly enhanced the MBC (5.03- and 4.56-fold), MBN (10.83- and 8.51-fold) and MBP (13.96- and 13.83-fold) in Red experimental soil, respectively (Fig. 2). Moreover, higher rates of organic inputs had more positive influence than lower rates did. Consequently, maximum microbial biomass C, N and P were quantified at GM2W1, while the minimum levels occurred at CK2. The effect of RS was also substantial; as a result, RS2W1 was the best treatment after GM2W1. The trend of other treatments was in the order GM1W1>RS1W1>GM2W2>RS2W2>GM1W2>RS1W2. Stimulation of microbes by different constituents of organic residues may result in peak MBC (Mukherjee *et al.* 1990). Examining the effect of legume green manure on microbial populations and activities in a Canadian silt loam (Aridic Haploboroll) soil, Biederbeck *et al.* (2005) reported that MBC and MBN were significantly increased by the legume green manure application in soil under a rice-wheat (*Triticum aestivum*) system. By contrast, the average improvement gained from legume green manure was 107% for MBC and 191% for MBN compared with fallow-wheat cropping. Chirinda *et al.* (2008) also obtained higher MBN and nitrification rate in cropping systems involving legume green manure compared with those reliant on inputs from animal manure and mineral fertilizer.

Water regimes also had a significant effect; microbial biomass tends to decline with the increase of water levels. For this reason, W2 had an obvious suppressing impact. Changes in moisture levels affect microbial and biotic activity (Bronick et al. 2005). Treatments having RS as an organic amendment showed less sudden decreases with the passage of incubation time than did those with GM, since organic amendments with higher rates of decomposition caused rapid but transient effects (Kay 1998) while slower decomposing organic inputs had subtler but longer-lived effects on soil properties (Martens 2000).

The incorporation of plant residue (GM and RS) generally decreased the *C*/N ratio of soil microbial biomass under both water regimes WI and W2, although the differences were not significant (Fig. 3). It has been reported that the plant residue incorporation reduces the C/N ratio of the microbial biomass (Kushwaha *et al.* 2000). The decrease and different C/N ratios occur as a result of changes in microbial population during the decomposition of different incorporated residue (Tate *et al.* 1988). Similarly, Singh *et al.* (1993) conducted an experiment to observe the effect of residue placement on microbial biomass in Inceptisol soils of Varanasi, India; they found that the incorporation of straw reduced the overall C/N ratio of the microbial biomass.

Significant increases in bacterial (32.38- and 22.21-fold), fungal (23.33- and 19.77-fold) and actinomycete (30.62- and 20.04-fold) populations were observed after the addition of GM and RS in Red soil, respectively (Fig. 4). All the treatments showed an upsurge in

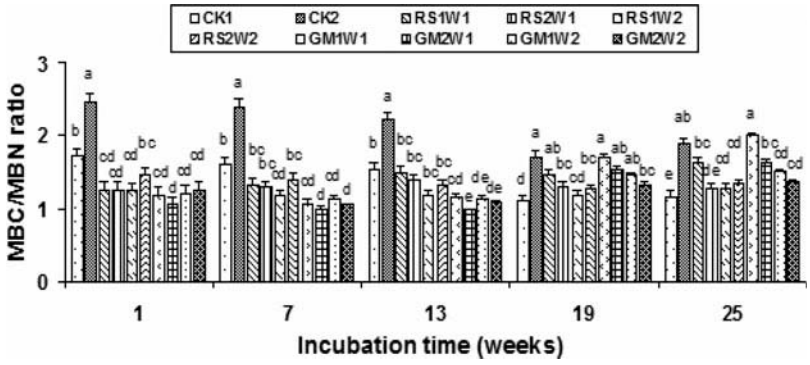

Figure 3 Effect of organic amendments on soil microbial biomass carbon (MBC)/microbial biomass nitrogen (MBN) ratio. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25 and 200% WHC. WHC, water holding capacity. Different letters (a–e) on bars indicate significant differences of mean values for MBC/MBN ratio. Bars represent standard errors.

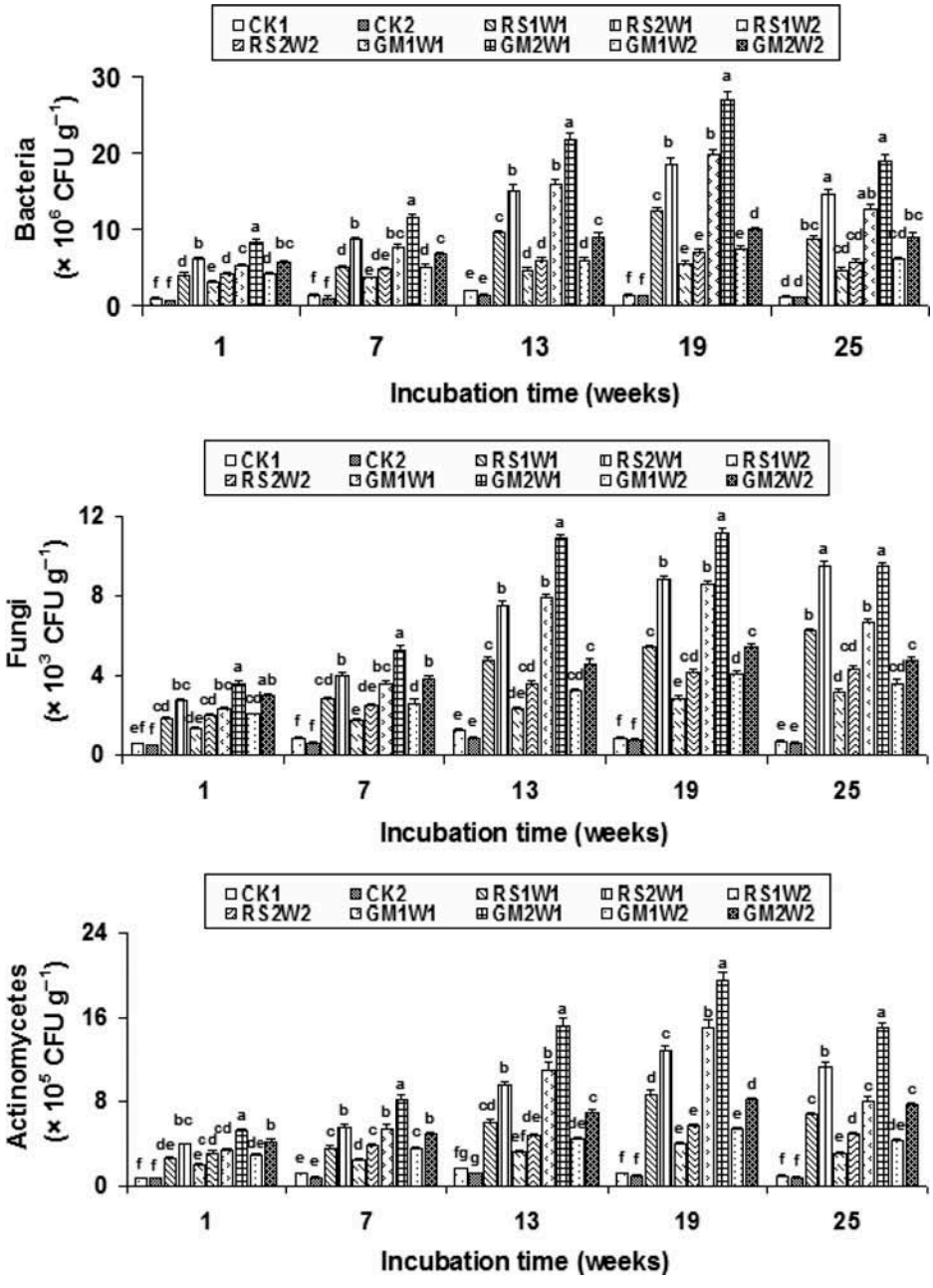

Figure 4 Effect of organic amendments on soil bacteria, fungi and actinomycetes. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice (*Oryza sativa* L.) straw at 5 and 25 mg g^{-1} soil; GM1 and GM2, green manure at 5 and 25 mg g^{-1} soil; W1 and W2, water gradients at 25% and 200% WHC. WHC, water holding capacity. Different letters (a–f, a–f and a–g) on bars indicate significant differences of mean values for bacteria, fungi and actinomycetes respectively. Bars represent standard errors.

microbial population, but treatments amended with GM were more influential than RS. GM2W1 and RS2W1 were the most effective treatments, while GM1W1 and RS1W1 were the second best. Conversely, treatments amended with GM and RS, at W2, were not very effective. This could be due to the negative impact of submerged conditions, which eventually caused the anoxic environment. The general effectual tendency of organic treatments at W2 was GM2W2 > RS2W2 > GM1W2 > RS1W2. It has been documented that the addition of organic amendments increased the CFU values of microbial populations. Increased microbial population in soil could be due to enhanced organic matter inputs from the legume green manure and crop

residues. The fertility build-up in organic cropping systems has consequences for soil biological properties including microbial populations (Mader *et al.* 2002; Stromberger *et al.* 2007; Vineela *et al.* 2008). In a study conducted in Hangzhou, China, to figure out the effect of straws (Bt and non-Bt) on selected biological activities in water-flooded yellow loamy soil, results demonstrated that the amendment of the rice straw altered some important biological properties in water-flooded soil, indicating a shift in microbial populations or a change in the metabolic abilities of the microbial community as a result of substrate availability in soil (Wu *et al.* 2004). Data related to controls lucidly explain that water contents had significant effect on microbial communities; consequently, a lower microbial population was obtained in CK2 than in CK1. The waterlogged soil shows the lowest microbial population (Critter *et al.* 2001).

CONCLUSIONS

The results demonstrated that microcalorimetry is a reliable technique to study the way in which the microbiological properties of soils are affected by soil reforms. The microcalorimetric parameters P_{max}, t_{max}, Q and k quantitatively reflect the influence of organic inputs and water gradients on microbial properties, and can be used as indices of microbial biomass and community in soils. This also can be the basis for future studies on the thermodynamics and kinetics of soil physico-chemical and biological interactions through a combination of the microcalorimetric method and other analytical techniques, focusing on precise quantification of underlying phenomena and their careful interpretation. This undoubtedly reiterates that further studies are crucial in improving our understanding of soil microbial ecology.

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