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Estimation of enzymatic, microbial, and chemical properties in Brown soil by microcalorimetry

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Abstract The study was conducted with the objective to assess soil enzymatic, microbial, and chemical properties by customary methods and results obtained by conventional methods, corroborated with microcalorimetry. The experiment was laid out in a randomized complete block design with ten treatments in triplicates. The RS and GM were used at three rates (0, 5, and 25 mg g^{-1} soil, respectively). The soils were maintained at two water levels 25 % (W1) and 200 % (W2) of soil water-holding capacity. All soil enzymatic, microbial, and chemical properties were measured by standard methods. The incorporation of GM and RS, especially at high rates and water levels, 25 % (W1) and 200 % (W2) significantly ($p < 0.05$) affected the soil enzymatic, microbial, and chemical properties compared to controls. The microcalorimetric parameters P_{max} and k were positively correlated, whereas t_{max} negatively linked with the results of enzymatic, microbial, and chemical properties at $p < 0.01$. Conversely, Q elucidated non-significant correlation ($p < 0.05$) to urease (0.248), neutral phosphatase (0.281), dehydrogenase (0.291), MBC (0.283), MBP (0.277), DOC (0.269), DON (0.190), SOM (0.284), and pH (0.047). Our results suggested that calorimetric parameters P_{max}, t_{max}, and k are highly sensitive and could be used as indices of soil enzymatic, microbial, and chemical properties, while Q is an indigent indicator.

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Abbreviations

MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MBP	Microbial biomass Phosphorous
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
SOM	Soil organic matter
OM	Organic matter
CFU	Colony-forming unit
RS	Rice straw
GM	Green manure
RCBD	Randomized complete block design
WHC	Water-holding capacity
rpm	Revolutions per minute
TOC	Total organic carbon

Introduction

Ecological prominence of soil enzymes have been progressively expanded since the first report on soil enzymes about a century ago [1]. Soil enzymes, once used as descriptive parameters, have now been appreciated for their multifaceted functions in microbial activities, soil processes, and ecosystem responses to management and global environmental change [2]. Microorganisms synthesize different extracellular enzymes, among which hydrolytic and oxidative enzymes are particularly important; hydrolytic enzymes catalyze the hydrolysis process and played a vital

role in the biodegradation of labile OM [3]. However, soil oxidative enzymes have been considered as a proximate control for the decomposition of OM [4]. Soil enzymes activities have been suggested as suitable indicators of soil quality and health, they rapidly respond to the changes caused by both natural and anthropogenic factors [5], being thus well suited to measure the impact of organic amendments and water levels on the quality and health of soil.

Soil microorganisms are vital to agro-ecosystem health through their crucial roles in the initial comminution and mixing of residues into the soil, and with their suite of enzymes for chemical breakdown of organic materials [6]. Soil microbial properties such as microbial biomass and population activities have strong correlations with soil health and quality indices [7]. Soil microbial biomass regulates many critical processes in ecosystems, such as the biophysical integration of organic matter (OM) with soil solid, aqueous, and gaseous phases, the quantity and quality of components in the hydrologic cycle and in greenhouse gas emissions, biomass turnover [8]. In contrast to other species, microorganisms have the ability to physiologically adapt quickly which allows them to survive and remain active in the face of differing environmental stresses [9]. Based on these properties, differences in the structures and functional processes of microbes could emerge simultaneously if circumstances changed, so microbes can be used as a sensitive indicator of soil environmental quality. Due to the complex dynamics of soil ecosystems, understanding the microbial community in the soil environment, especially in Brown soils, has still not been unanimously determined and it is necessary to examine them through assiduous techniques.

Organic residues are able to improve the enzyme activity and the diversity of soil microbial populations, and increase the OM content; however, the influence of organic matter on soil microbial and chemical properties depends on the amount, type, and size of the added organic materials [10]. The rice crop produces large quantities of straw, as an agricultural waste, ranging from 2 to 9 t/ha (tones per hectare) worldwide, and in many countries the traditional management practice of this voluminous post-harvest rice residues is open-air burning, many drawbacks are related to this practice, the utmost important is release of greenhouse gases [11]. Therefore, the impact of OM inputs, such as green manure and rice straw, either alone or in combination, on soil biological health is an important area of investigation for assessing soil sustainability [12]. This area is of particular importance for the sustainable management of extensively cropped Brown soils. Brown soils also known as Alfisol (according to USDA Soil Taxonomy) are important soils in China, these soils occupy about 1.25 million km^2, or about 13 % of the land area [13]. Brown soils in China are generally found in broad climatic zones from cool temperate, warm temperate to north subtropical [13]. Brown soils in China are mostly under cultivation for a long time, and some are under conifer and broad-leaved forests [14]. Therefore, this is of great concern to know the enzymatic, microbial, chemical, and carbon dynamics of these soils, in the presence of two contrasting plant residues and to assess the working potential of these plant residues under different moisture conditions.

Moisture plays a key role to sustain the stability of soil biota and the change of water level drastically influenced the soil enzymatic, microbial, and chemical properties [15]. Global warming evolving a worldwide environmental change and disturbing the delicate soil air–water balance, both drought and waterlogged conditions ultimately lead to soil degradation [16]. The fluctuation in moisture modified the temporal pattern of enzymes activities, by altering substrate availability and aeration [17]. Scant information is available on how submerged and dry conditions influence the microbial and chemical properties of soils [15]. This emerging field of exploration will improve our understanding about soil abiotic influences on enzymatic, microbial, and chemical properties in soil ecosystem and enhance success of soil quality and health-restoring efforts.

During the development of the microbial activity stimulated by the presence of nutrients, a flow of thermal effect is generated, and can be monitored by microcalorimetric technique [18]. The microcalorimetric study can be complemented through a deep analysis of the most important soil physical (temperature, moisture, texture, and field capacity), chemical (pH), and biological properties (OM content and the most probable number of microorganisms) together with a study of the environmental properties e.g., rainfall, moisture, and different bioclimatic intensities in soils as it is independent of the types of microorganisms and their form of evolution, and permits the continuous monitoring of the activity of a living process in situ over a prolonged period without disturbing the system [19]. The connections between soil microbial properties and microcalorimetric parameters could elucidate which microcalorimetric parameter best indicates microbial activity in soils [20]. It would be very useful to study these questions; publications are missing the sensitivity of calorimetric indices to detect enzymatic, microbial, chemical, and metabolic activity changes caused by organic amendments and water contents, especially in Brown soil. This is important since any new indicator must be sensitive to the activity and biomass changes related to soil properties [21]. To our knowledge, no study has shown the sensitivity and working potential of microcalorimetry in Brown soils under two contrasting organic amendments and water contents, to measure enzymatic, microbiological, and chemical properties of these soils. Therefore, the present

study was conducted with the objectives: (1) to better understand the comparative effectiveness of organic amendments (Rice Straw or Green Manure), at two water levels (25 or 200 %), on enzymatic, microbial, and chemical properties of Brown soil; (2) to analyze the connections between soil enzymatic, microbial, and chemical properties and microcalorimetric parameters for establishing the sensitivity of the latter indices to different organic amendments and water contents under Brown soil.

Materials and methods

Soil sampling

Samples (0–20 cm depth) of Brown soil (Alfisol) collected from Guxing town, Xingyang county, and Zhengzhou city of Henan province (113.5°E, 34.9°N) were used in this study. The soil was under wheat–corn alternative crop rotation.

Experimental design

The study was laid out in a randomized complete block design with ten treatments in triplicates. Rice straw (RS) and green residues of peanut 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, 2 mm) was transferred into 1-kg-capacity pots (18 × 13 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 levels 25 % (W1) and 200 % (W2) of water-holding capacity (WHC) with deionized water. The soil water was maintained at the desired WHC throughout the incubation. Water loss in the pots was monitored by mass and replenished after opening, with deionized water. The WHC was estimated by volumetric soil water method [22]. 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 5, 10, and 15 weeks, then the homogenized samples were sieved through a 2 mm mesh and separated into two parts. The first part was 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 microbial and biochemical properties. The treatments of our study are listed in Table 1.

Table 1 Treatments of the incubation experiment

Treatments	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

WHC water-holding capacity

RS1 and RS2, rice 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 levels at 25 and 200 % WHC; CK1 and CK2, controls at W1 and W2

Analyses of soil and organic amendments

Soil particle size distribution was evaluated by the international pipette method [23]. Soil pH and electrical conductivity (EC) were measured using soil/water ratio (w/v) of 1:2 [24]. 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 [25]. The organic matter (OM) 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 mass of these organic wastes before and after ashing was used to calculate the OM content. Soil bulk density was determined by soil coring; samples of undisturbed cores of known volume were subsequently oven-dried at 105 °C until constant mass was reached and each measurement was replicated three times [26]. Soluble organic carbon was determined by a modified version of the method of Gregorich et al. [27] and Sparling et al. [28]. 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 8,000 rpm), the supernatant was filtered (0.7–l m-filter membranes), and the filtrate was analyzed for soluble organic carbon using a TOC/TN analyzer (multi N/C 2100, Analytic Jena, Germany). Available N was measured by NaOH pervasion method [29]. Soil available P was assayed by $NaHCO_3$ method [30]. Total P in soil and organic amendments samples were analyzed by NaOH fusion and colorimetric procedures [31], while total N contents were quantified by sample digestion and Kjeldahl method [32]. The amounts of cellulose, hemicellulose, and lignin were measured using the procedure of Goering and van-Soest [33]. Some pertinent characteristics of the soil and organic amendments are shown in Tables 2 and 3.

Table 2 Physico-chemical properties of experimental soil

Properties	Brown soil
Clay/g kg^{-1}	98.6
Silt/g kg^{-1}	192.4
Sand/g kg^{-1}	709
Textural class	Sandy loam
pH/1:2	7.86
EC/μS cm^{-1}	195
Eh/mV	356
Organic matter/g kg^{-1}	16.7
Bulk density/g cm^3	1.28
Soluble organic carbon/mg kg^{-1}	101.5
Available N/mg kg^{-1}	21.71
Available P/mg kg^{-1}	6.16

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 N/g kg^{-1}	8.87	22.5
Total P/g kg^{-1}	3.10	14.6
C:N ratio	71.55:1	25.29:1
Cellulose/g kg^{-1}	356.6	245.8
Hemicellulose/g kg^{-1}	171.5	119.9
Lignin/g kg^{-1}	132.7	105.8

Soil enzyme activities

Catalase activity was determined using 2 g of fresh soil with 40 mL of distilled water and 5 mL of 0.3 % H_2O_2, shaken for 20 min (at 150 rpm) and then filtered (Whatman No. 2 V) immediately. The filtrate was titrated with 0.1 mol L^{-1} $KMnO_4$ in the presence of sulfuric acid and the results were expressed as mol $KMnO_4$ g^{-1} soil h^{-1}. Phenol oxidase activity was measured using 5 g of fresh soil, incubated for 2 min in a water bath at 30.8 °C, with 10 mL of distilled water, 6 mL of 0.1 % ascorbic acid, and 10 mL of 0.02 mol L^{-1} catechol. 3 mL of 10 % phosphoric acid was then added to the suspension and the filtrate was titrated with 0.01 mol L^{-1} iodine. Results were expressed as mL 0.01 mol L^{-1} I_2 g^{-1} h^{-1}. Urease activity was assessed using 5 g of fresh soil, 5 mL of citrate solution at pH 6.7, and 5 mL of 10 % urea solution. The samples were incubated at 37.8 °C for 3 h and then diluted to 50 mL with distilled water. The suspension was filtered and a 1 mL aliquot was treated with 4 mL of sodium phenol solution (a mixture of 100 mL of 6.6 M phenol solution and 100 mL of 6.8 M NaOH) and 3 mL of 0.9 % sodium hypochlorite solution. The released ammonium was quantified by atomic absorption spectrophotometer (Hitachi, UV2300) at 578 nm wavelength. Results were expressed as mg NH_4^+–N g^{-1} soil 24 h^{-1} [34]. Alkaline phosphatase and neutral phosphatase activities were computed by measuring the release of p-nitrophenol by incubating 1 g soil at 37 °C for 1 h with 0.2 mL toluene, 4 mL universal buffer (pH 11.0 for alkaline phosphatase and pH 7.0 for neutral phosphatase), and 1 mL 50 mmol p-nitrophenyl phosphate. Enzymes activity was expressed as mg PNP kg^{-1} soil h^{-1} [35]. Dehydrogenase activity was determined by the method of Öhlinger [36]. Fleetingly, 20 g of air-dried soil was mixed with 0.2 g of $CaCO_3$ and then 6 g of this mixture was placed in three different test tubes. Samples were incubated at 37 °C for 24 h after adding 1 mL of 3 % aqueous solution of Triphenyl Tetrazolium Chloride (TTC) and 2.5 mL of distilled water. Then 10 mL of methanol was added and filtered after shaking. The red color intensity was measured using a spectrophotometer at a wavelength of 546 nm. Soil dehydrogenase activity was expressed as mg TPF (Triphenyl formazan) kg^{-1} dry soil 24 h^{-1}.

Soil microbial biomass

The chloroform fumigation–extraction method was used to measure soil microbial biomass carbon (MBC). 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 soils by repeated evacuations. The fumigated soil was extracted immediately for 30 min by horizontal shaking at 200 rpm with 50 mL 0.5 M 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 carbon (TOC) in the extracts was determined using a TOC/TN 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 non-fumigated samples, and 2.22 is the factor, calculated by 0.45, i.e., $100/45 = 2.22$, here 0.45 is the extractable part of microbial C after fumigation [37]. For microbial biomass nitrogen (MBN), total N in the K_2SO_4 extract was measured after Kjeldahl digestion. The soil 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 non-fumigated samples, and 1.85 is a factor which is obtained via 0.54 (i.e., $100/54 = 1.85$) which is an extractable part of microbial N after fumigation [38]. 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 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 non-fumigated 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 [36].

Soil microbial population

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. Briefly, 10 g of fresh soil samples was placed into flask containing 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 into small tube containing 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 incubator. Bacteria, fungi, and actinomycetes were accounted 4, 5, and 7 days after the planting process, respectively [39].

Soil C and N mineralization

A 21-d laboratory incubation experiment was conducted to evaluate the effects of organic amendments (RS and GM) and water levels (25 and 200 %) on C and N mineralization of SOM. Carbon mineralization was measured as CO_2 evolution after 1, 7, 14, and 21 days of incubation. Briefly, 50 g soil (dry mass equivalent) was treated with one of the ten treatments given in Table 1. Soils were then transferred to 1 l Mason jars along with vials containing 10 mL 0.5 M NaOH, and incubated at 20 °C. After the incubation, the glass vials were removed and the CO_2 trapped in the NaOH was immediately determined using a TOC/TN analyzer (Multi N/C 2100, Analytic Jena, Germany). Simultaneously, soil samples were collected from each jar after 1, 7, 14, and 21 days of incubation from the start of the incubation for the determination of N mineralization. For soil N mineralization, samples were extracted with 1 M KCl for the colorimetric determination of soil inorganic N (i.e., NH_4^+ and NO_3^+). Nitrogen mineralization was calculated as the difference in soil inorganic N after and before the incubation [40].

Soil dissolved organic matter

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted by distilled water for 1 h and filtered (Whatman No. 42). The DOC was measured using a TOC/TN analyzer (Multi N/C 2100, Analytic Jena, Germany), while DON was determined by measuring the difference between total dissolved nitrogen and inorganic nitrogen (NH_4^+–N and NO_3^-–N) as described by [41, 42].

Microcalorimetry

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 [43]. All living systems produce heat and, therefore, can be measured by some type of isothermal calorimeter, provided its detection limit is sufficient [44]. Soil samples were incubated at 25 °C (298.15 K) for 24 h and their moisture was maintained at 35 % (water-holding capacity) to maximize microbial activity [45]. 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 [46]. 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 [47]. The total heat output, Q, was obtained through the integration of each curve. The value of peak height (P_{max}) and the 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 [48]. 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,

$$n_t = n_0 \exp(kt), \quad (1)$$

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

$$n_t w = n_0 w \exp(kt) \quad (2)$$

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

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

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.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), using the Statistix 8.1 (Michigan, USA). The means and standard deviations for triplicates were calculated. Significant differences of means for all treatments were judged by the least significant difference (LSD) and expressed at $p < 0.05$. Pearson correlation analysis was conducted to evaluate the relationships between soil enzymatic, microbial, and chemical properties with microcalorimetric parameters.

Results

Soil enzymatic and microbial activities under organic manipulation and water levels

The application of GM and RS significantly ($p < 0.05$) enhanced the soil enzymatic and biological activities. Soil oxidative enzymes-namely catalase and phenol oxidase (Fig. 1), and hydrolytic enzymes—alkaline phosphatase, neutral phosphatase (Fig. 2) urease, and dehydrogenase (Fig. 3) activity significantly ($p < 0.05$) increased by 21.1-fold, 19.1-fold, 14.8-fold, 18.8-fold, 11.4-fold, and 10.1-fold due to the addition of higher rate of GM-residue (GM2) at water level, W1, respectively. Conversely, the addition of RS-residue at higher dose i.e., RS2 and water level, W1, was the second best treatment and significantly ($p < 0.05$) increased the soil catalase, phenol oxidase, alkaline phosphatase, neutral phosphatase, urease and dehydrogenase activity by 16.8-fold, 15.7-fold, 11.55-fold, 16.32-fold, 10-fold, and 8.56-fold, respectively, in comparison to control i.e., CK2.

The effect of the organic amendments (GM and RS) and water levels i.e., W1 and W2, on soil microbial biomass is shown in Figs. 4 and 5. The MBC, MBN, and MBP increased by 32.6-fold, 75.8-fold, and 29.6-fold, respectively, due to the application of higher dose of GM-residue i.e., GM2, at water level, W1. Similarly, due to the addition of increased rate of RS-residue (RS2) at water level, W1 significantly ($p < 0.05$) increased the MBC, MBN, and MBP by 29.8-fold, 65.61-fold, and 24.8-fold, respectively, as compared to the control with submerged water level, W2, i.e., CK2. Conversely, in general the incorporation of residue (GM and RS) decreased the C/N ratio of soil microbial biomass under both water levels i.e., WI or W2, although the differences were not significant (Fig. 6).

Microbial population i.e., fungi, actinomycetes, and bacteria in the tested soil increased from 0.11 to 11.2 $\times$ 10^3 CFU g^{-1}, 0.14 to 15.5 $\times$ 10^5 CFU g^{-1}, and 0.31 to 33.6 $\times$ 10^6 CFU g^{-1} in control to green manure-mixed treatment, GM2 at water level, W1, respectively. On the other hand, the increase in microbial population (fungi, actinomycetes, and bacteria) due the addition of RS-residue at higher dose and water level, W1 was from 0.11 to 8.2 $\times$ 10^3 CFU g^{-1}, 0.14 to 12.5 $\times$ 10^5 CFU g^{-1}, and 0.31 to 27.4 $\times$ 10^6 CFU g^{-1}, in comparison to control with submerged water level, i.e., CK2 (Figs. 7, 8).

The soil enzymatic and microbial activities markedly increased due to the addition of organic amendments (GM or RS) as compared to the controls. The treatments, GM2W1 and RS2W1, were the most effective amendments, while GM1W1 and RS1W1 were the second best. Conversely, treatments amended with GM and RS, at water level, W2, were not much effective, the general effectual tendency of treatments at water level W2 was: GM2W2 > RS2W2 > GM1W2 > RS1W2. All enzymatic and microbial properties were significantly ($p < 0.01$) correlated to soil chemical properties viz. pH, EC, SOM, DOM, and C and N mineralization (data not shown).

Relationship of soil enzymatic and microbial activities with microcalorimetric parameters

The microcalorimetric parameters, P_{max}, and k, were positively linked ($p < 0.01$), whereas t_{max} was negatively correlated ($p < 0.01$) to the results of soil enzymatic and microbial activities (Table 4). Conversely, Q had no relationship with urease, neutral phosphatase, dehydrogenase, MBC, and MBP. The highest P_{max}, k, and Q were observed in organic-amended treatments at water level W1, whereas t_{max} in control and organic amendments with water level, W2.

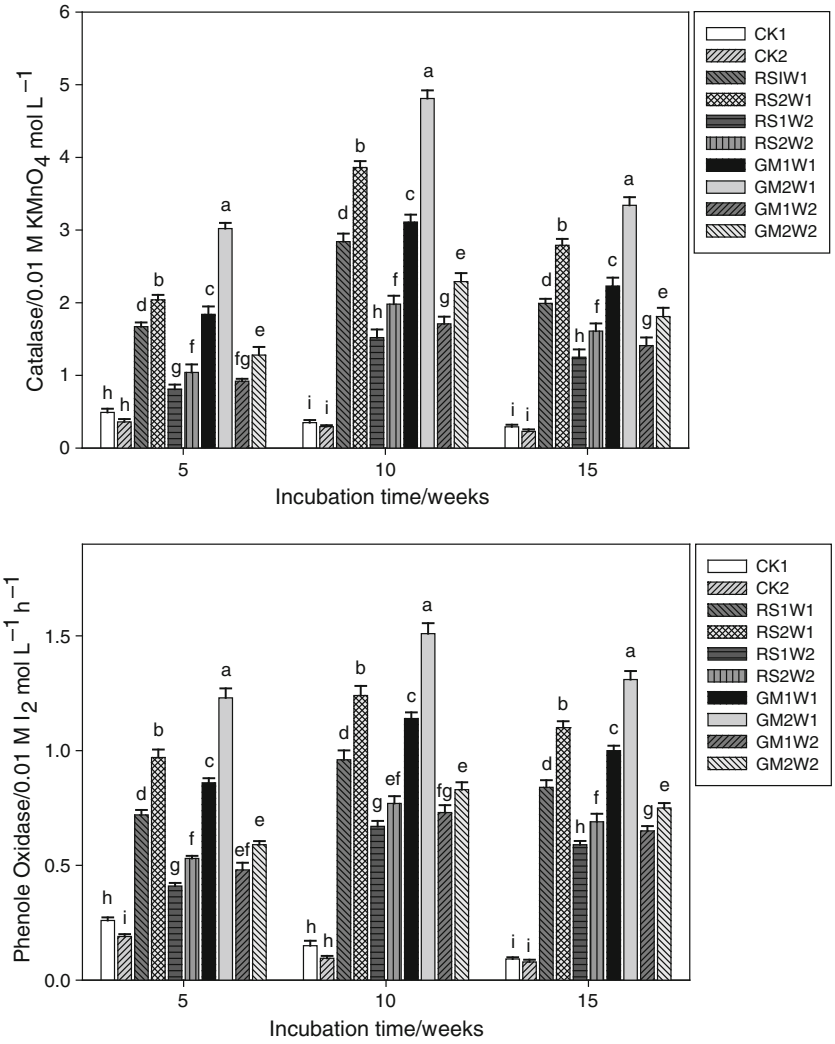


Fig. 1 Oxidative enzymes activity (catalase and phenol oxidase) in Brown soil under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–i and a–i) on *bars* indicate significant differences of mean values for catalase and phenol oxidase, respectively. *Bars* represent standard errors

Soil chemical properties under organic manipulation and water levels

The effect of organic amendments i.e., GM or RS and water levels, W1 or W2, on SOM is shown in Fig. 9. The addition of GM and RS caused significant ($p < 0.05$) increase in SOM, showing the patronage and fecundity of organic materials. The RS-mixed treatment, RS2, under water level, W2, significantly ($p < 0.05$) increased the amount of SOM (7.64-fold), in comparison to controls (CK2 > CK1). The effect of RS-residue-mixed treatments, at both water levels, on SOM was in the order of RS2W2 > RS2W1 > RS1W2 > RS1W1. Similarly, GM-residue-mixed treatment, GM2, at water level W2, also increased the SOM (5.6-fold), but this increase was significantly ($p < 0.05$) low than RS-residue-amended treatments. The effect of GM-mixed treatments on SOM, under both water levels, was in the order of GM2W2 > GM2W1 > WM1W2 > GM1W1, in comparison to controls. The trend of controls was CK2 > CK1.

The increase in the C and N mineralization due to the addition of GM- and RS-residues under water level, W1 or W2 is shown in Fig. 10. The C and N mineralization in the investigated soil increased from 7.12 to 95.5 mg C kg^{-1} soil and 4.12 to 81.8 mg N kg^{-1} soil in control (CK2) to treatments mixed with higher dose of green manure i.e., GM2. Similarly, the addition of higher rate of RS-residue also increased the C and N mineralization from 7.12 to 89.5 mg C kg^{-1} soil and 4.12 to 75.9 mg N kg^{-1} soil.

The effect of the organic amendments (GM or RS) and water levels (W1 or W1) on DOM i.e., DOC and DON is presented in Fig. 11. The DOC and DON in the experimental soil increased by 20.7- and 16-fold, respectively, in GM-amended treatment with higher dose i.e., GM2, in comparison to control i.e., CK2. Conversely, the addition of RS-residue at higher dose i.e., RS2 and water level, W1,

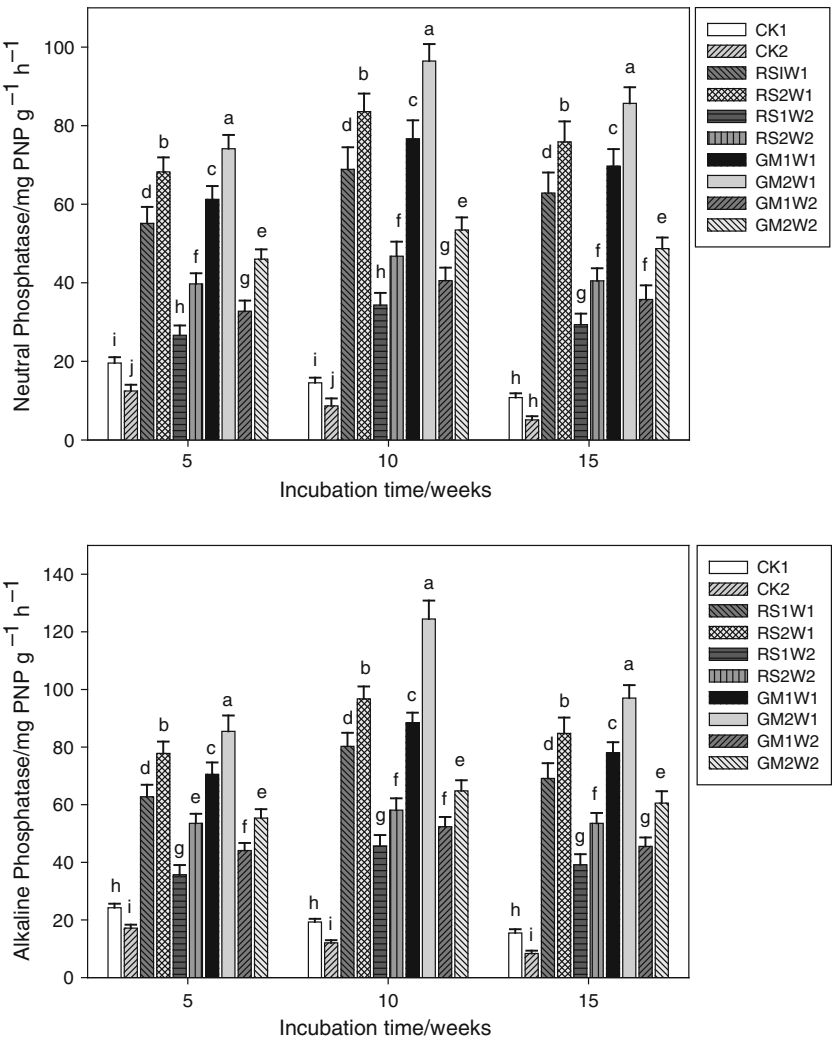


Fig. 2 Hydrolytic enzymes activity (alkaline phosphatase and neutral phosphatase) in Brown soil under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–i and a–j) on *bars* indicate significant differences of mean values for alkaline phosphatase and neutral phosphatase, respectively. *Bars* represent standard errors

increased the DOC and DON by 18.8- and 14.5-fold, respectively.

Soil pH and EC in experimental soil increased from 7.65 to 8.48 and 162 to 732 μScm^{-1} in control to treatment amended with GM2 residues, at water level, W1. Whereas, the increase in soil pH and EC in control to treatment mixed with RS2, at water level, W1, ranged from 7.65 to 8.11 and 162 to 540 μScm^{-1} (data not shown).

The GM-residue-mixed treatments showed significant ($p < 0.05$) increase in all chemical properties (except in SOM) than the RS-residue. Water levels had strong effect regardless of the type of organic amendments, under flooding water level, W2, significant ($p < 0.05$) decline was observed. Among GM-residue-mixed treatments at both water levels, W1 or W2, following influential tendency was acquired: GM2W1 > GM1W1 > GM2W2 > GM1W2, while trend of RS-residue-mixed treatments was: RS2W1 > RS1W1 > RS2W2 > RS1W2. All chemical properties were significantly ($p < 0.01$) associated with soil enzymatic activities, microbial biomass, and microbial population. Nevertheless, there was a non-significant correlation ($r = 0.217$, $p < 0.05$) between SOM and N mineralization (data not shown).

Soil chemical properties and their association to microcalorimetric parameters

Significant ($p < 0.01$) relationship was noticed between microcalorimetric parameters, P_{max}, t_{max}, and k, and soil chemical properties i.e., SOM, DOC, DON, C and N mineralization, pH, and EC (Table 4). Conversely, Q had no relationship with SOM, DOC, DON, and pH (Table 4).

Microcalorimetric parameters under organic manipulation and water levels

The data of calorimetric parameters P_{max}, t_{max}, k, and Q after 5, 10, and 15 weeks of incubation, are presented in

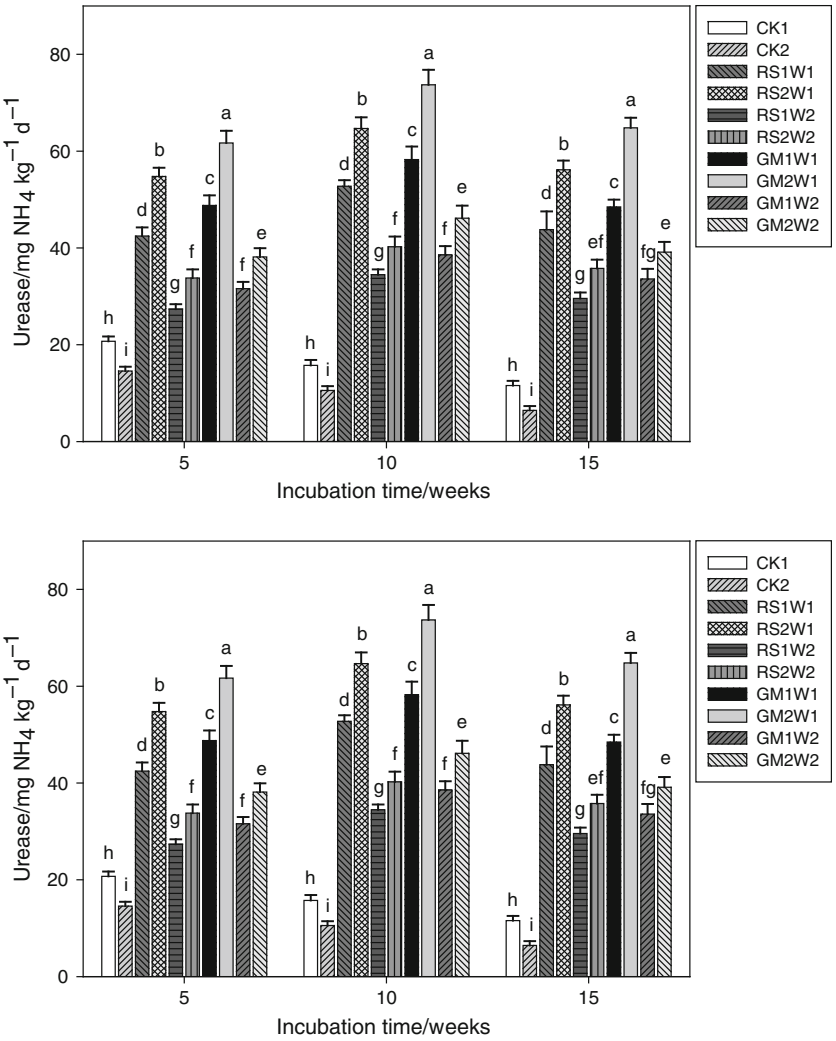


Fig. 3 Hydrolytic enzymes activity (urease and dehydrogenase) in Brown soil under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–i and a–j) on *bars* indicate significant differences of mean values for urease and dehydrogenase, respectively. *Bars* represent standard errors

Tables 6, 7, and 8, respectively. The results showed that organic inputs (GM or RS) and water levels (W1 or W2) had significant ($p < 0.05$) effects on all measured calorimetric parameters. The highest $P_{max}/\mu w^{-1}$ and k/min^{-1} were observed at treatment amended with higher rate of green manure i.e., GM2 and water level, W1, respectively. The effect of other GM-residue-mixed treatments, at water level, 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 higher rate of rice straw residues i.e., RS2 at water level, W1, was the second best; maximum P_{max} and k were observed at that treatment, after GM2. The influential tendency of other RS-residue-mixed treatments at water levels, W1 or W2, followed subsequent order of: RS1W1 > RS2W2 > RS1W2, in comparison to controls. The highest values of t_{max}/min and Q/Jg^{-1} were found in control at water level W1 i.e., CK1, and treatment amended with higher dose of green manure residue (GM2) and water level, W1, respectively.

Discussion

Microcalorimetric parameters as indices of soil enzymatic, microbial, and chemical properties

In this study, the P_{max} and t_{max} exhibited highly significant ($p < 0.01$) correlation to catalase (0.944, −0.731), phenol oxidase (0.944, −0.731), alkaline phosphatase (0.972, −0.738), neutral phosphatase (0.923, −0.815), urease (0.871, −0.858), and dehydrogenase (0.951, −0.811) enzymes activities (Table 4). Similarly, the k also showed significant ($p < 0.01$) correlation with all measured enzymes, whereas Q demonstrated non-significant ($p < 0.01$) correlation with urease (0.248), neutral phosphatase (0.281), and dehydrogenase (0.291). These outcomes argued that microcalorimetric indices are highly sensitive for soil biological activities and can be used for precise measurement of enzymatic activity in soils. Cenciani et al. [49] set an experiment to measure the enzymatic activity by microcalorimetry in clayey soils of São Paulo, (Brazil) amended

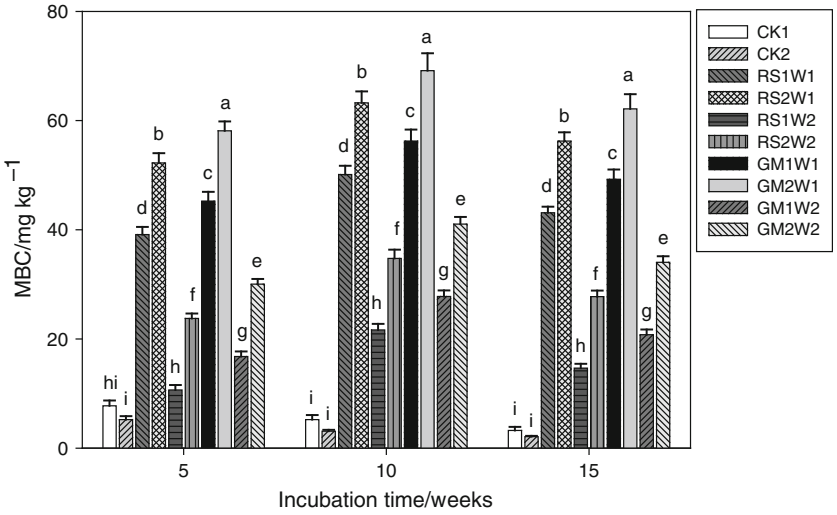
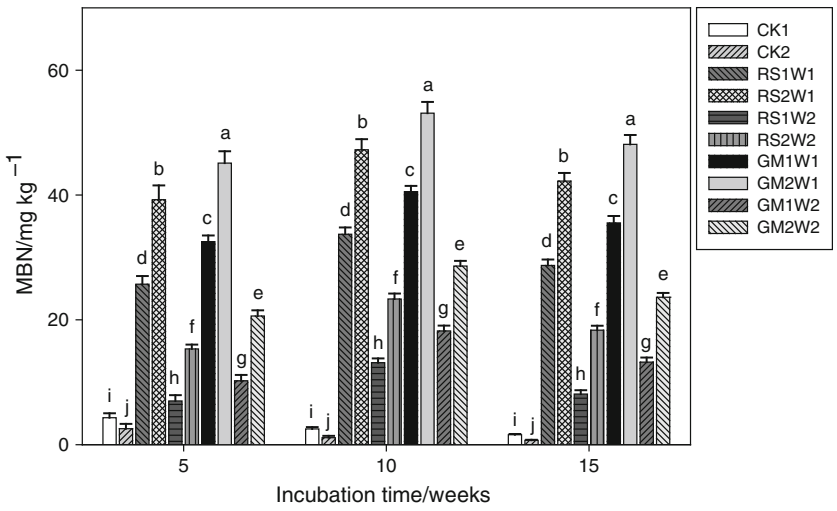


Fig. 4 Microbial biomass (MBC and MBN) in Brown soil under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–i and a–j) on *bars* indicate significant differences of mean values for MBC and MBN, respectively. *Bars* represent standard errors



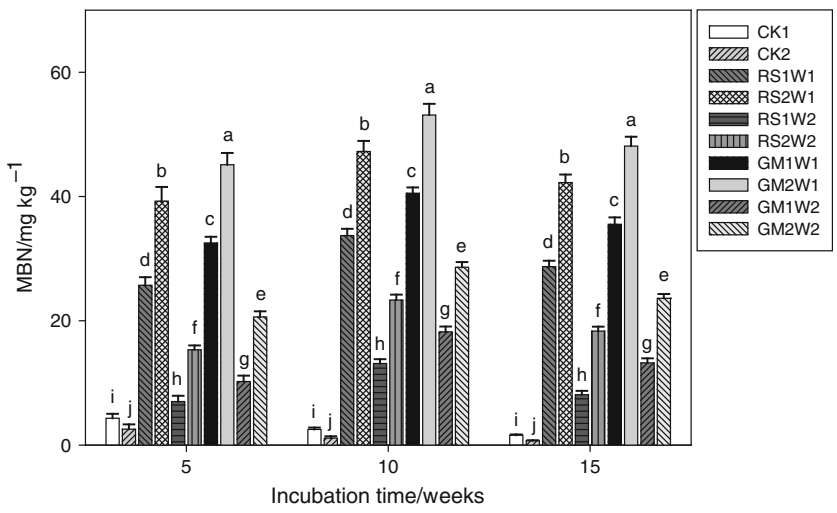


Fig. 5 Microbial biomass phosphorous (MBP) in Brown soil under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–j) on *bars* indicate significant differences of mean values for MBP. *Bars* represent standard errors

with organic residues (cattle manure, earthworm casts, barueri sludge, and franca sludge), and they concluded that microcalorimetry is a powerful tool for analyzing microbial or enzymatic activity in the soil. An isothermal microcalorimetric technique and enzyme assay have been used to see the effect of β-cypermethrin on soil enzyme activities

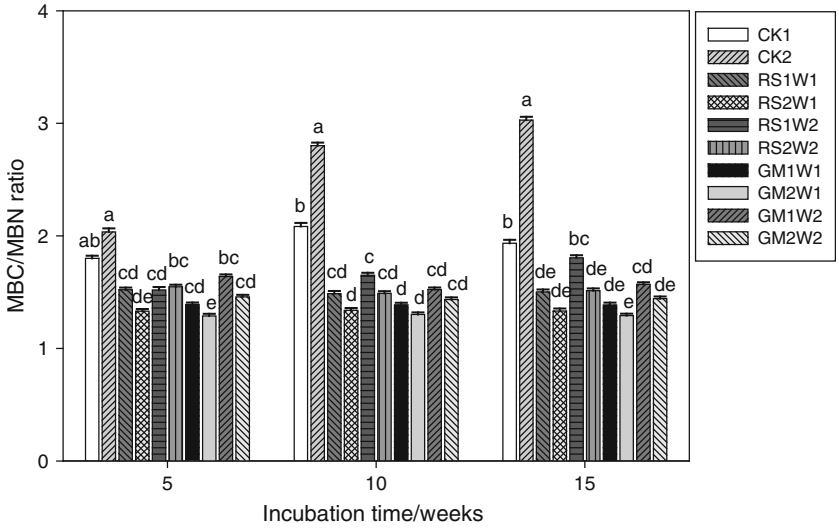


Fig. 6 MBC/MBN ratio in Brown soil under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Same *letters* on *bars* indicate non-significant differences of mean values for MBC/MBN. *Bars* represent standard errors

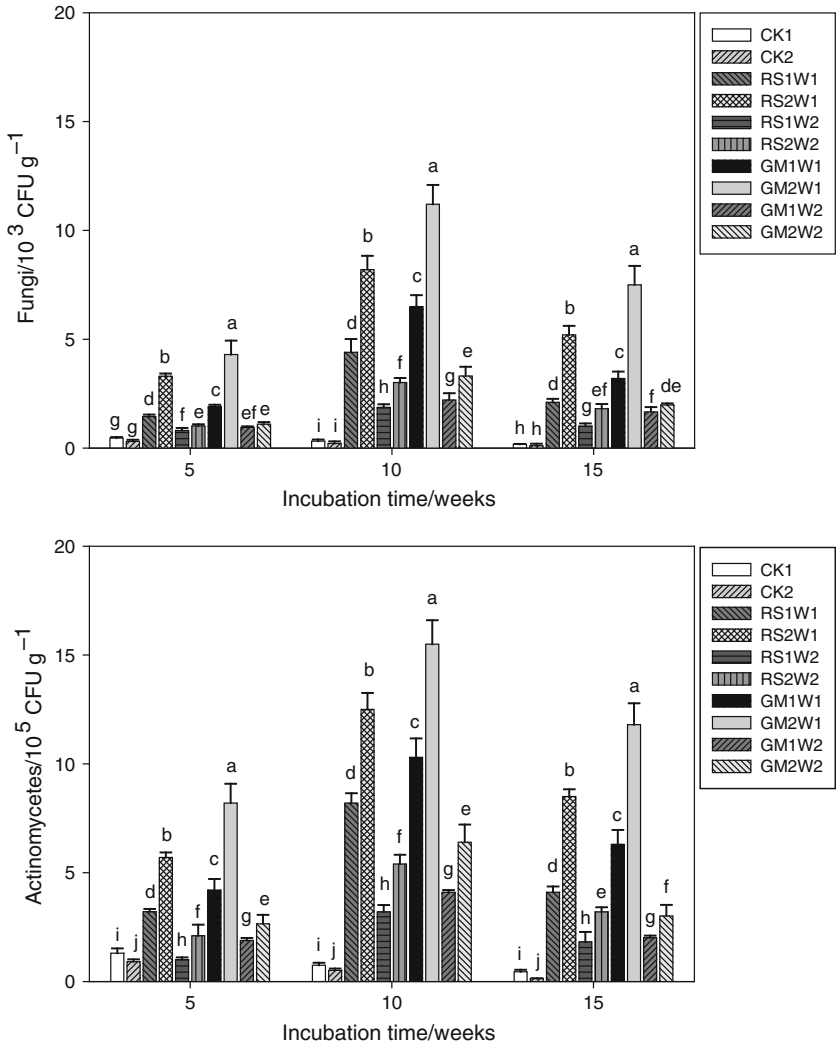


Fig. 7 Microbial population (fungi and actinomycetes) in Brown soils under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different letters (a–i and a–j) on *bars* indicate significant differences of mean values for fungi and actinomycetes, respectively. *Bars* represent standard errors

such as urease, acid phosphatase, and dehydrogenase, results revealed that the thermokinetic parameters obtained by microcalorimetry are in good agreement with the activities of the soil enzymes measured through conventional methods [50]. To study the enzymatic hydrolysis of butyrylcholine, catalyzed by horse serum

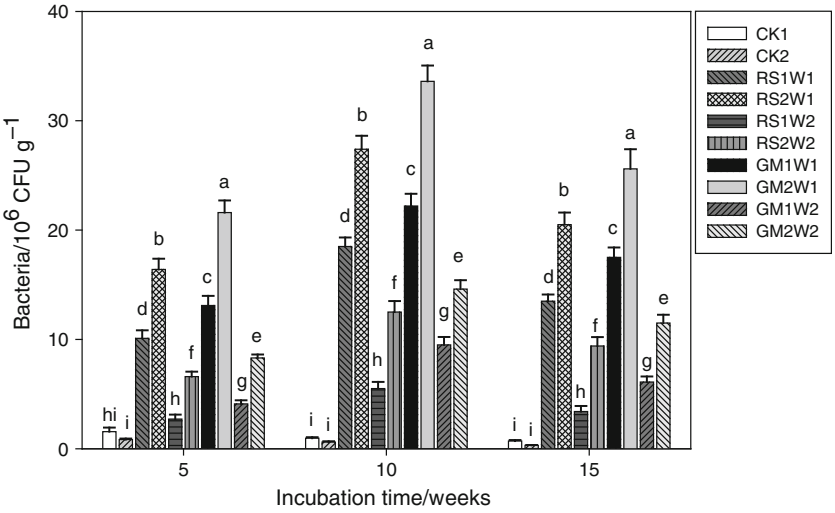


Fig. 8 Microbial population (bacteria) in Brown soils under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–i) on *bars* indicate significant differences of mean values for bacteria. *Bars* represent standard errors

butyrylcholinesterase, at 37 °C in Tris buffer (pH 7.5), thermodynamic parameters such as, k, P_{max}, t_{max}, and Q were calculated; results revealed that microcalorimetry is a very useful technique to study the kinetic of enzymes and their inhibitors [51]. A significant ($p < 0.01$) positive and negative linear correlation between alkaline phosphatase and urease activities and k and t_{max} was observed in an experiment conducted near Shanghai, China [52].

The P_{max} (positively correlated) and t_{max} (negatively correlated) showed significant ($p < 0.01$) correlations with MBC (0.919, −0.806), MBN (0.931, −0.756), and MBP (0.872, −0.842), and microbial population i.e., bacteria (0.983, −0.717), fungi (0.945, −0.728), and actinomycetes (0.969, −0.747) (Table 4). Data related to Q and k also expressed considerable relatedness ($p < 0.01$) to microbial biomass and population, except Q had no correlation with MBC (0.283) and MBP (0.277). These results advocate that microcalorimetric indices can be used for accurate evaluation of soil microbial parameters. Metabolic enthalpy (ΔH), k, and t_{max} could be used to assess soil quality as real-time indicators [53]. Ye et al. [54] conducted a microcalorimetric study on the microbial activity of permafrost on the Tibetan plateau of China, and they observed a significant ($p < 0.01$) positive linear correlation between microbial population i.e., bacteria (CFU) and k. A significant ($p < 0.01$) relationship between t_{max}, and the time of glucose consumption of microbial population i.e., bacteria and fungi under toxic effect of heavy metals (As, Cu, Cd, Cr, Co, Pb, and Zn) was observed in an experiment conducted in an orchard soil in Wuhan, China [55]. Studying the comparison between microbial counting (Bacteria and fungi) and a calorimetric method applied to tropical soils of Brazil; significant correlation (with $r = 0.8181$ and $p = 0.0131$ for bacteria and $r = 0.8134$ and $p = 0.014$ for fungi) was found between microbial counting through the most probable number method i.e., CFU and calorimetric method [56].

Soil research accepts the usefulness of glucose to study the capacity to mineralize external C sources, but its connection to SOM and C and N mineralization is not yet well understood [57]. Keeping in view all these facts, and to fill this gap, in this study we correlated crucial intrinsic soil chemical properties to microcalorimetric indices. P_{max}, and t_{max} were significantly ($p < 0.01$) correlated with SOM (0.387, −0.707), DOC (0.862, −0.847), DON (0.954, −0.850), C mineralization (0.921, - 0.731), N mineralization (0.921, −0.604), pH (0.416, −0.607), and EC (0.902, and −0.701) (Table 5). Similarly, higher links of k ($p < 0.01$) to SOM (0.417), DOC (0.881), DON (0.930), C mineralization (0.947), N mineralization (0.950), pH (0.542), and EC (0.934) were observed (Table 5). Conversely, Q elucidated non-significant correlation ($p < 0.05$) to SOM (0.284), DOC (0.269), DON (0.190), and pH (0.047); and significant correlation ($p < 0.01$) to C mineralization (0.415), N mineralization (0.520), and EC (0.436) (Table 5). These investigations stated that microcalorimetry can be a useful method to study the way in which the biochemical activity of soils is affected by management and environmental factors [21]. A study was performed in a humic Cambisol soil after reforestation over one year in Viveiro (Galicia, NW Spain) and results revealed that microcalorimetric parameters—peak time, peak height, and the growth rate constant can be successfully used to study the soil chemical and physical properties e.g., moisture, pH, OM content, and C-to-N ratio [19]. To study the influence of different environmental parameters, temperature, moisture content, pH, and C/N ratio, parameters such as t_{max}, P_{max}, and k were determined. Results showed that microcalorimetric technique is a suitable indicator that informs us about the soil state and the soil disruption under

Table 4 Correlative coefficient of microcalorimetric parameters between soil enzymatic and microbial properties in Brown soil

	Cata	Ure	A Phos	N Phos	PO	Dehy	MBC	MBN	MBP	Bact	Fung	Actino
P_{max}	0.944**	0.871**	0.972**	0.923**	0.944**	0.951**	0.919**	0.931**	0.872**	0.983**	0.945**	0.969**
t_{max}	−0.731**	−0.858**	−0.738**	−0.815**	−0.731**	−0.811**	−0.806**	−0.756**	−0.842**	−0.717**	−0.728**	−0.747**
Q	0.398**	0.248	0.376**	0.281	0.398**	0.291	0.283	0.337**	0.277	0.386**	0.401**	0.374*
K	0.955**	0.901**	0.975**	0.935**	0.951**	0.901**	0.916**	0.948**	0.905**	0.977**	0.955**	0.954**

Cata catalase, *Ure* urease, *A Phos* alkaline phosphatase, *N Phos* neutral phosphatase, *PO* phenol oxidase, *Dehy* dehydrogenase, *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

* Significant at $p < 0.05$; ** significant at $p < 0.01$

different management and land uses [58]. In a study conducted near Shanghai, China in soils of organic and conventional horticultural systems a strong positive correlation ($p < 0.05$) between P_{max}, and k values ($p < 0.01$) and chemical properties i.e., TOC, total N, MBC, MBN, and MBP was observed [52].

The absence of any connection between Q and urease (0.248), neutral phosphatase (0.281), dehydrogenase (0.291), MBC (0.283), MBP (0.277), DOC (0.269), DON (0.190), SOM (0.284), and pH (0.047) implied that Q is not a good indicator. It has also been mentioned that Q was not correlated with soil MBC and the number of microorganisms because the higher dissipation of the heat per unit of cell is linked to a less efficient metabolism [21]. Ahamadou et al. [20] conducted a microcalorimetric assessment to measure the microbial activity in long-term fertilized Red soils collected from Hunan, China, and they stated that the absence of any connection between Q and soil enzymatic activities i.e., phosphatase, urease, invertase, protease, and dehydrogenase suggests that Q is not a good indicator of soil microbial properties. Wang et al. [59] applied a microcalorimetric technique to a series of experiments to follow the toxic effect caused by the trivalent iron on the single and mixed microbes in sterilized soil, the microcalorimetric parameters viz. k and Q were determined, results showed that the mixed-species have moderate tolerance to the iron overload, comparing with single species and exhibit synergistic interaction in exponential growth phase to k, meanwhile, not much difference in the Q per gram soil sample for the single and mixed culture was observed. A lower to no correlation was found between OM and microbial quantity and Q_t in an experiment conducted near Wuhan, China [60]. Therefore, we propound that P_{max}, t_{max}, and k could be used as the indices of soil enzymatic, microbial, biological, and chemical properties, whereas total heat evolution, Q, is a poor indicator.

Microcalorimetric parameters affected by organic treatments and water levels

Calorimetry appears to be a useful tool by calculating the latency time, together with the total heat and the kinetics of microbial growth without any disruption [21]. Our results are in parallel with these findings; in the present study, the microbial activity presented by higher k, more P_{max}, shorter t_{max}, and longer Q per cell unit (Table 6, 7, 8), indicated that microorganisms under organic treatments (GM or RS) and aerobic conditions at water level, W1, had more efficient metabolism and growth, while less microbial activity and growth under submerged conditions (W2) and in controls was due to lack of aeration, substrate availability, and nutrient deficiency. Microcalorimetric technique was used to investigate the effects of balanced versus nutrient-

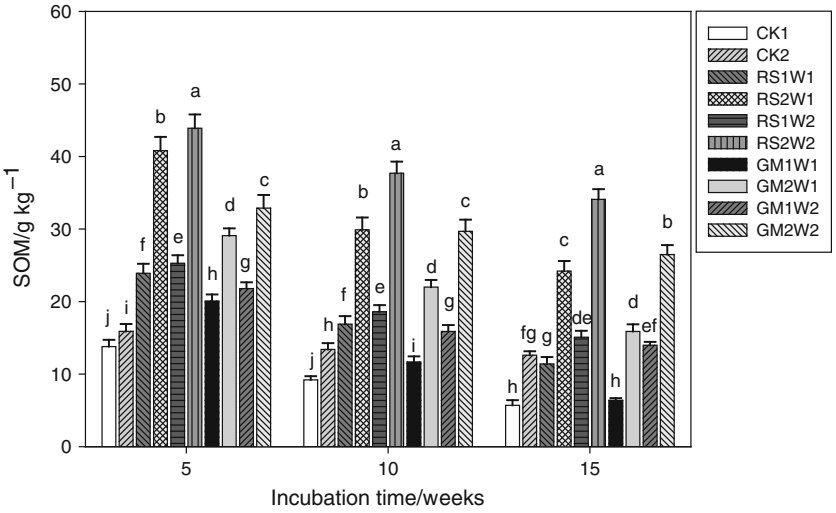


Fig. 9 Soil organic matter (SOM) in Brown soils under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–j) on *bars* indicate significant differences of mean values for SOM. *Bars* represent standard errors

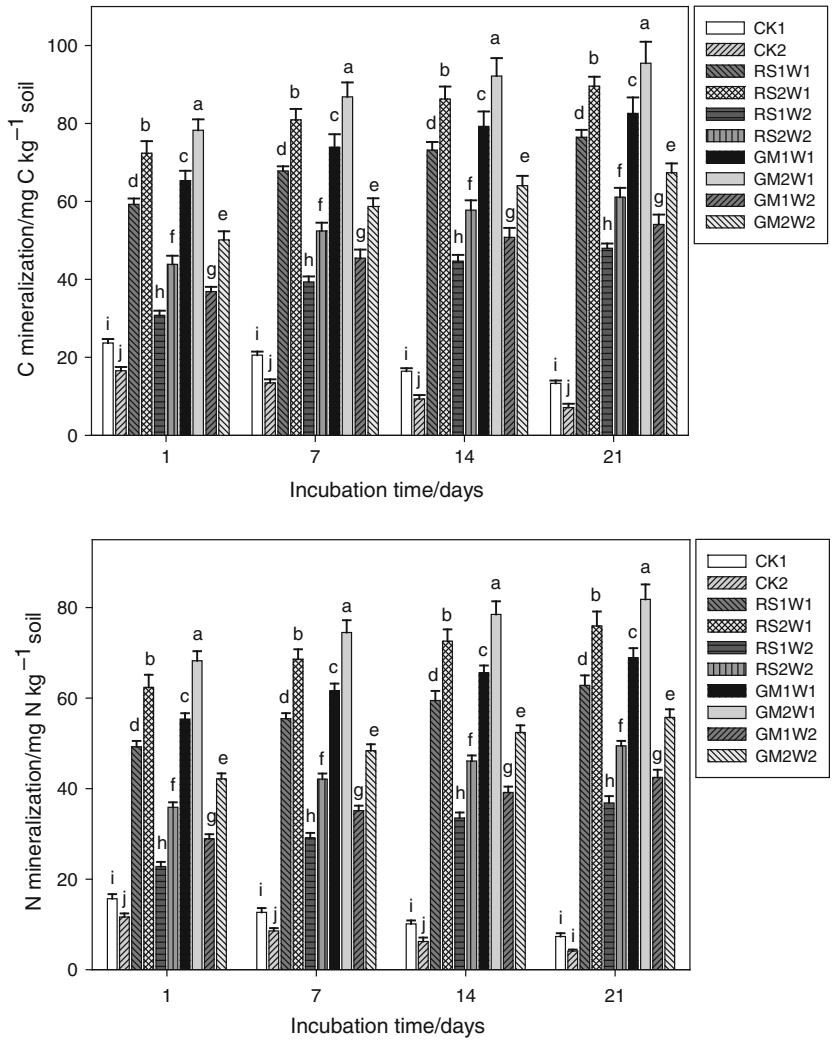


Fig. 10 C and N mineralization in Brown soils under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–j and a–j) on *bars* indicate significant differences of mean values for C and N mineralization, respectively. *Bars* represent standard errors

deficiency fertilization on soil microbial activity; in an experiment in Henan, China, the number of microorganisms in soils was measured by viable cell count, and the power–time curves, the microcalorimetric results agreed very well with the results of other traditional microbiological assessment, the lower k, less P_{max}, and longer t_{max} all indicated the

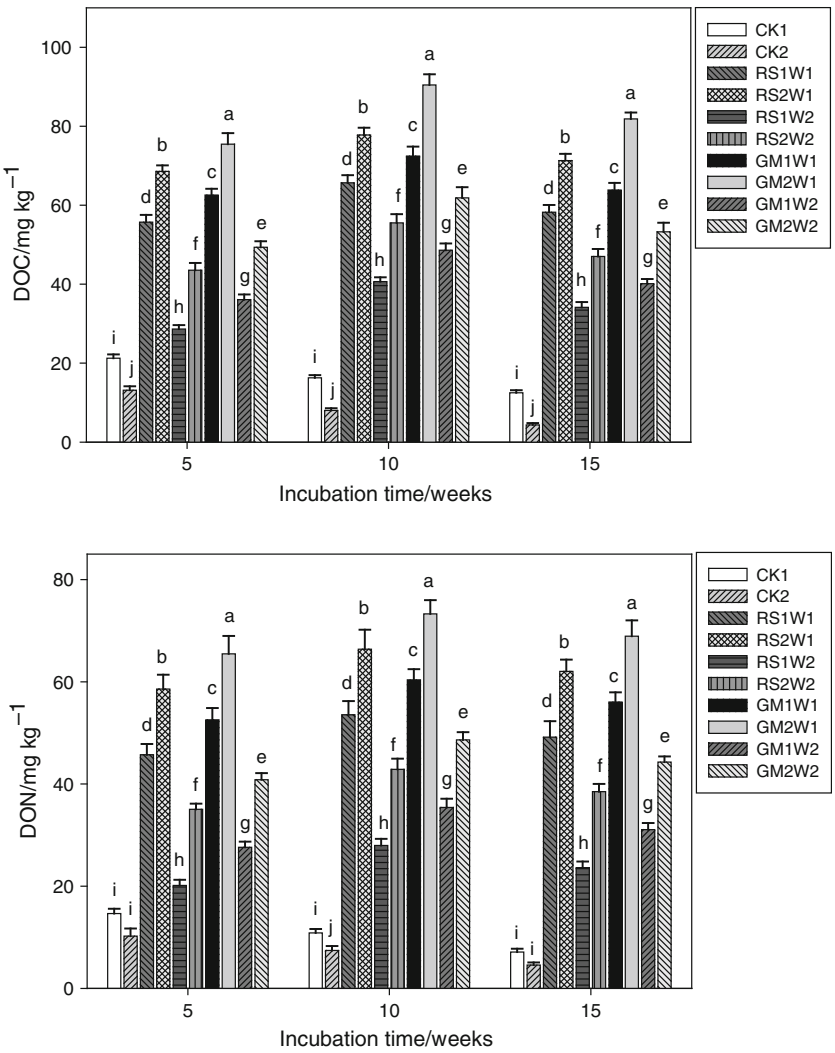


Fig. 11 Dissolved organic matter (DOC and DON) in Brown soils under organic inputs and water levels, RS1 rice straw at 5 mg g^{-1} soil, RS2 rice straw at 25 mg g^{-1} soil, GM1 green manure at 5 mg g^{-1} soil, GM2 green manure at 25 mg g^{-1} soil, W1 water level 25 %, W2 water level 200 %, CK1 control at W1, and CK2 control at W2. Different *letters* (a–j and a–j) on *bars* indicate significant differences of mean values for DOC and DON, respectively. *Bars* represent standard errors

Table 5 Correlative coefficient between microcalorimetric parameters and soil chemical properties in Brown soil

	SOM	DOC	DON	C min	N min	pH	EC
P_{max}	0.387*	0.862**	0.954**	0.901**	0.921**	0.416**	0.902**
t_{max}	−0.707**	−0.847**	−0.850**	−0.731**	−0.604**	−0.607**	−0.701**
Q	0.284	0.269	0.190	0.415**	0.520**	0.047	0.436**
K	0.417**	0.881**	0.930**	0.947**	0.950**	0.542**	0.934**

SOM soil organic matter, *DOC* dissolved organic carbon, *DON* dissolved organic nitrogen, *C min* carbon mineralization, *N min* nitrogen mineralization, p_{max} peak height, t_{max} peak time, k growth rate constant, Q total thermal effect

* Significant at $p < 0.05$; ** significant at $p < 0.01$

low activity of soil microorganisms in nutrient-deficiency fertilization [61]. Microbial growth in soils under conventional management displayed lower k, lower P_{max}, and longer t_{max}, all of which indicated lower activity of soil microorganisms compared with organic management [52]. Núñez-Regueira et al. [58] used microcalorimetric techniques to study the influence of different physicochemical parameters on microbial growth in different soils in Galicia (NW Spain), the influence of different environmental parameters, temperature (ambience and soil), moisture content (sample and residual), pH in water, and C/N ratio. Microcalorimetric parameters namely t_{max}, P_{max}, and k were determined. Results revealed that microcalorimetric technique is a suitable indicator that informs us about the soil state i.e., physiochemical properties and the soil disruption. Due to the limitations of traditional microbiological

Table 6 Microcalorimetric parameters as influenced by organic amendments and water levels after 5 weeks of incubation

Treatments	P_{max}/μw	t_{max}/min	Q/J g^{-1}	k/min^{-1}
CK1	114.8 i	1649.1 a	16.72 c	8.24×10^{-6} e
CK2	69.30 j	1394.6 c	12.88 f	7.74×10^{-6} e
RS1W1	232.7 d	389.59 g	12.86 f	1.36×10^{-5} cd
RS2W1	321.9 b	324.45 h	10.46 g	2.22×10^{-5} b
RS1W2	119.2 h	1564.0 b	14.47 d	1.20×10^{-5} d
RS2W2	193.5 f	389.59 g	9.410 h	1.32×10^{-5} cd
GM1W1	235.9 c	577.16 f	17.62 b	1.55×10^{-5} c
GM2W1	407.5 a	616.31 d	21.53 a	4.22×10^{-5} a
GM1W2	147.1 g	583.04 e	13.93 e	1.26×10^{-5} cd
GM2W2	201.0 e	232.97 i	10.53 g	1.35×10^{-5} cd

P_{max} peak height, t_{max} peak time, k growth rate constant, Q total thermal effect, *WHC* water-holding capacity. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice 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 levels at 25 and 200 % WHC. Different letters (a–j) within a same column indicate significant differences of mean values at $p < 0.05$

Table 7 Microcalorimetric parameters as influenced by organic amendments and water levels after 10 weeks of incubation

Treatments	P_{max}/μw	t_{max}/min	Q/J g^{-1}	k/min^{-1}
CK1	175.0 h	781.04 a	15.04 b	2.30×10^{-5} f
CK2	127.2 i	697.16 c	12.09 d	8.70×10^{-6} g
RS1W1	288.9 c	417.86 f	7.730 fg	4.70×10^{-5} de
RS2W1	558.0 a	232.97 j	14.03 c	7.20×10^{-5} b
RS1W2	178.4 g	706.54 b	7.510 g	2.60×10^{-5} f
RS2W2	207.2 e	628.31 d	11.20 e	4.40×10^{-5} e
GM1W1	345.5 b	324.45 i	8.490 f	5.90×10^{-5} c
GM2W1	558.0 a	388.18 h	18.22 a	9.70×10^{-5} a
GM1W2	183.9 f	415.38 g	8.340 fg	3.00×10^{-5} f
GM2W2	257.7 d	559.07 e	14.48 bc	5.50×10^{-5} cd

P_{max} peak height, t_{max} peak time, k growth rate constant, Q total thermal effect, *WHC* water-holding capacity. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice 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 levels at 25 and 200 % WHC. Different letters (a–j) within a same column indicate significant differences of mean values at $p < 0.05$

methods, the use of microcalorimetric techniques to measure soil microbial and chemical properties is reliable and advantageous [62]. This inferred 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 enzymatic, microbial, biological, and chemical properties, but their relative importance in terms of specific soil function or ecosystem service may vary.

Table 8 Microcalorimetric parameters as influenced by organic amendments and water levels after 15 weeks of incubation

Treatments	P_{max}/μw	t_{max}/min	Q/J g^{-1}	k/min^{-1}
CK1	126.7 i	1394.5 a	14.60 a	4.10×10^{-6} fg
CK2	88.30 j	1032.9 b	5.470 i	2.60×10^{-6} g
RS1W1	160.2 d	493.59 g	8.020 f	1.10×10^{-5} d
RS2W1	280.2 b	231.16 j	8.430 e	2.50×10^{-5} b
RS1W2	135.4 h	416.09 h	6.100 h	5.70×10^{-6} ef
RS2W2	146.7 f	382.63 i	6.360 g	6.50×10^{-6} e
GM1W1	257.5 c	942.64 c	12.30 c	1.40×10^{-5} c
GM2W1	282.0 a	541.79 f	14.48 a	3.10×10^{-5} a
GM1W2	144.5 g	866.86 e	11.31 d	9.40×10^{-6} d
GM2W2	153.9 e	909.35 d	14.26 b	1.00×10^{-5} d

P_{max} peak height, t_{max} peak time, k growth rate constant, Q total thermal effect, *WHC* water-holding capacity. CK1 and CK2, controls at W1 and W2; RS1 and RS2, rice 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 levels at 25 and 200 % WHC. Different letters (a–j) within a same column indicate significant differences of mean values at $p < 0.05$

Impact of organic amendments and water levels on soil enzymatic, microbial, and chemical properties

In this study the incorporation of GM and RS significantly ($p < 0.05$) enhanced the catalase, phenol oxidase, alkaline phosphatase, neutral phosphatase, urease, and dehydrogenase activity (Figs. 1, 2, 3). Liang et al. [63] narrated that the placement of organic manures (rice straw, pig manure, and their mixture), substantially increased the activity of urease, alkaline phosphatase, and dehydrogenase in paddy soil collected form Dafeng, China. Wu et al. [64] observed significant increases ($p < 0.05$) in soil dehydrogenase and soil neutral phosphatase activity in soils amended with rice straw compared to soil without added straw. The water levels (W2 and W1) had significant ($p < 0.05$) effect on enzymes activity, and enzymes activity decreased with the increase of water level. It may be possible that water logging of soils markedly affected the reaction rates of these enzymes, impeded the decomposition and possibly, substrates cycling, enzymatic potential tied to substrate availability [4]. The change in moisture level modified the temporal pattern of enzymes release by inhibiting the transport of soil substrates, resulting in the reduction of extracellular enzyme activity [17].

We observed that microbial biomass i.e., MBC, MBN, and MBP was significantly ($p < 0.05$) higher in the GM- and RS-amended soils than in the control soils (Figs. 4 and 5). This suggested that the incorporation of plant residues capable of activating the soil's innate biomass. Chirinda et al. [65] obtained higher MBN in cropping systems involving green manure legumes compared with those reliant on inputs from animal manure and mineral

fertilizer. Biederbeck et al. [66] reported that a gain of 107 % for MBC and 191 % for MBN was obtained after the incorporation of organic residues in a Canadian silt loam soil. The water levels had strong ($p < 0.05$) impact on the microbial biomass, the treatments amended with higher water level (GM2W2, RS2W2, GM1W2, and RS1W2) presented lower microbial biomass than those recorded in lower water level (GM2W1, RS2W1, GM1W1, and RS1W1). This could be because there is equilibrium between water and soil particles; superfluous water contents distort this balance which leads to microbial death and as a result less microbial biomass [67]. On the other hand, generally, the incorporation of plant residue (GM and RS) decreased the C/N ratio of soil microbial biomass under both water levels i.e., WI or W2, although the differences were not significant (Fig. 6). It has been reported that the plant residue incorporation reduces the C/N ratio of the microbial biomass [68]. The decrease and different C/N ratios occur as a result of changes in microbial population during the decomposition of incorporated residue [69].

Significant ($p < 0.05$) improvement in microbial population i.e., bacterial, fungal, and actinomycetes by the addition of plant residues (GM or RS) at lower water level, W1, was observed (Figs. 7 and 8). The fertility buildup in organic cropping system has consequences for soil biological properties including microbial population [70]. Wu et al. [64] observed that amendment of the soils with rice straw improve the soil microbial activity as a result of substrate availability in soil. At the end of the incubation period the soil mixed with the higher rate of plant residues showed higher population than those of the controls and soils treated with the lower rate. The least differentiated soils in this study were controls which amended with higher water level, which are least biologically active [46].

Comparing the effect of two contrasting plant residues on SOM, we found that RS-residue-mixed treatments showed significantly ($p < 0.05$) higher OM than GM-mixed treatments (Fig. 9). This varied behavior of two plant residues was because straw and manure represented the low and high lignin and cellulose compounds and C/N ratios. The use of plant residues with higher lignin, secondary metabolites, and C/N ratios increase the soil C due to high C/N ratios and by decreasing the C leaching [71]. Moreover, high OM was noticed under submerged conditions (W2) compared to dry condition (W1). This maybe due to higher moisture that impeded the diffusion of air, as a result of microbial and enzymes activity, which decreased the rate of decomposition of OM. The anoxic conditions decrease the microbial and enzymes activity, and ultimately OM decomposition [72].

In the current study significant ($p < 0.05$) increase in the C and N mineralization after plant residues incorporation was observed, during whole incubation, compared to controls (Fig. 10). The maximum C and N mineralization was noticed in soil amended with higher doses of green manure (GM2) and rice straw (RS2). Biederbeck et al. [66] observed 205 % increase in C mineralization after green manuring legumes compared with the control. The enhanced rate of C and N mineralization after green manuring legumes was as a result of increased inputs of OM [73]. The C and N mineralization was inversely correlated with water levels, for this reason all the treatments with water level, W2, had less C and N mineralization than W1. There is equilibrium between water and soil particles; high water contents disturb this balance which leads to slow microbial activity and decrease in the OM mineralization and decomposition [74].

The significant ($p < 0.05$) improvement was found in DOM i.e., DOC and DON, after the incorporation of RS and GM (Fig. 11). Many studies have found immediate increases in soil DOM content upon amendment with plant residues [75]. Water contents had strong ($p < 0.05$) effects on the DOM; therefore, treatments with water level, W2, have less DOM than treatments amended with water level, W1. Change in moisture levels affects microbial and biotic activity, which alter decomposition rates of organic inputs and the level of organic carbon and nitrogen in soils [67].

The organic amendments had significant ($p < 0.05$) positive effect on soil pH and EC. Low pH and EC were obtained in control, with water level, W2 (CK2), while their maximum values were observed in treatments amended with higher rates of green manure and rice straw i.e., GM2 and RS2 at lower water level W1 (data not shown), respectively. The increase in soil pH and EC when organic inputs were added to soil was reported by many researchers [76, 77]. The decomposition of organic materials released acids or acid-forming compounds that reacted with the sparingly soluble salts already present in the soil and either converted them into soluble salts or at least increased their solubility; hence, the EC of soil was increased [78].

Conclusions

Microcalorimetry is a reliable technique to study the way in which the enzymatic, microbiological, and chemical properties of soils are affected by soil reforms. Microcalorimetric parameters k, P_{max}, and t_{max} quantitatively reflect the influence of organic inputs and water levels on enzymatic, microbial, and chemical properties much better than Q does. Therefore, we propose that k, P_{max}, and t_{max} can be used as the indices of enzymatic, microbiological, and chemical properties in soils. This also can be the basis for future studies on the thermodynamics and kinetics of soil physico-chemo and biological interactions through a combination of the microcalorimetric method and other

analytical techniques, focusing on precise quantification of underlying phenomenons and their careful interpretation. All measured soil enzymatic, microbiological, and chemical properties were significantly higher under GM and RS at both water levels compared to controls. This suggested not only GM but also cellulase-, hemicellulose-, and lignin-rich RS can be successfully used as an organic amendment in the soils, instead of burning. This undoubted reiterates that future in-depth studies involving microcalorimetry and more enzymatic, microbiological, and chemical properties of soils are rudimental in improving our understanding about the change in soil ecology and enzymology under different soil reforms and management practices.

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