

# Monitoring temperature sensitivity of soil organic carbon decomposition under maize–wheat cropping systems in semi-arid India

S. Sandeep • K. M. Manjaiah • M. R. Mayadevi • A. K. Singh

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Abstract Long-term storage of soil organic carbon (SOC) is essential for sustainability of agricultural ecosystems and maintaining overall environment quality as soils contain a significant part of global carbon stocks. In this study, we attempted to explain the carbon mineralization and temperature sensitivity of SOC in maize–wheat systems, a common cropping system in the semi-arid regions of India. Soil samples(0–0.15 m) from long-term experimental plots laid in split plot design with two tillage

K. M. Manjaiah

Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, Indian Council of Agricultural Research, New Delhi 110012, India e-mail: manjaiah@iari.res.in

Present Address: S. Sandeep (🖂)

Department of Soil Science, Kerala Forest Research Institute, Peechi, Thrissur, Kerala 680653, India e-mail: sandeepagri@gmail.com

Present Address: M. R. Mayadevi Department of Soil Science and Agricultural Chemistry, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala 680656, India manthra34@gmail.com

Present Address:

A. K. Singh Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior, Madhya Pradesh 474002, India aks wtc@yahoo.com systems (conventional tillage and bed planting) and six nutrient management treatments ( $T_1 = \text{control}, T_2 = 120 \text{ kg}$ urea—N/ha,  $T_3 = T_2$  (25 % N substituted by farmyard manure (FYM)),  $T_4 = T_2$  (25 % N substituted by sewage sludge),  $T_5 = T_2 + \text{crop residue}$ ,  $T_6 = 100 \%$  recommended doses of N through organic source - 50 % FYM + 25 %biofertilizer + 25 % crop residue) were incubated at different temperatures (25, 30, 35, and 40 °C) to determine the thermal sensitivity parameters associated with carbon mineralization. Earlier reports suggest a selective preservation of C3-derived carbon fractions over C4 in the SOC pool, and this is the first instance where  $\delta^{13}C$  signatures (C<sub>4</sub>derived carbon) were used as a qualitative measure to assess thermal sensitivity of SOC pools in the maizewheat crop rotation systems of semi-arid India. Among the nutrient management treatments, mineral fertilizers were found to add more C<sub>4</sub>-derived carbon to the SOC pool in both the tillage systems but shows less promise in SOC stability as indicated by their lower activation energies (Ea)  $(14.25 \text{ kJ mol}^{-1})$ . Conventional tillage was found to mineralize 18.80 % ( $T_1$ —control at 25 °C) to 29.93 % carbon  $(T_3$ —mineral fertilizer + FYM at 40 °C) during the 150 days of incubation which was significantly higher than bed planting system (14.90 % in  $T_1$ —control at 25 °C and 21.99 % in  $T_6$ —100% organic sources at 40 °C). Organic manures, especially FYM (19.11 kJ mol<sup>-1</sup>) and 100 % organics (19.33 kJ mol<sup>-1</sup>) were more effective in enhancing the Ea of SOC than plots with mineral fertilizers alone (14.25 kJ mol<sup>-1</sup>), but had relatively higher  $Q_{10}$ values thereby corroborating the thermal sensitivity hypothesis of recalcitrant organic compounds in soil. Michaelis–Menten derivatives along with thermal sensitivity indicators such as Ea and  $Q_{10}$  were found to be efficient parameters for explaining carbon mineralization and CO<sub>2</sub> efflux from soils.

Keywords Maize-wheat  $\cdot C_4$ -derived carbon  $\cdot$ Activation energy  $\cdot Q_{10} \cdot$  Michaelis–Menten kinetics  $\cdot$ CO<sub>2</sub> efflux

### Introduction

Soil organic carbon influences various physico-chemical and biological processes and properties; hence, sustaining and improving its levels is essential to ensure soil health and future productivity (Katyal et al. 2001). Besides being a good plant nutrient source, it plays an important role in maintaining overall environmental quality as soil organic carbon (SOC) stores a significant portion of terrestrial carbon stocks (Houghton 2007). Lal et al. (1998) estimates that the annual increments of atmospheric CO<sub>2</sub>-C could easily be offset by a 0.01 % SOC content increase globally. Thus, there is a growing interest in assessing the carbon storage capacity of soil under different cropping systems and agricultural management practices as SOC decomposition is highly sensitive to tillage intensity, nutrient management, crop rotation, and climate (Leite et al. 2003; Verma et al. 2010).

Tracing the origins of organic compounds and their transformation pathways is essential for a qualitative evaluation of SOC dynamics (Sandeep et al. 2016). The differences in photosynthetic pathways lead to variations in the natural isotopic contents of plants and make it possible to trace their origin in the SOC pool (Ryan et al. 1995). In nature, carbon is represented by three isotopes (<sup>12</sup>C, <sup>13</sup>C, and <sup>14</sup>C) of which the stable forms, <sup>12</sup>C and <sup>13</sup>C, account for nearly 98.89 and 1.11 % of the total, respectively (Karlén et al. 1968). The presence of three very distinct photosynthetic pathways among terrestrial plants (C3, C4, and CAM) enables them to show a preferential and varying discrimination towards <sup>13</sup>C during carbon assimilation (Cernusak et al. 2009). Though SOC retains <sup>13</sup>C/<sup>12</sup>C signatures of the parent vegetation, fractionation during decomposition may lead to significant deviations from the original ratio (Sandeep et al. 2016). Analysis techniques that decipher  ${}^{13}C/{}^{12}C$  signatures of C<sub>3</sub>-C<sub>4</sub> plant shifts can thus provide valuable inputs about newly added SOC components from these photosynthetic pathways (Vanhala et al. 2007).

Plants with C<sub>4</sub> photosynthesis predominate the tropical regions due to physiological and ecological reasons (Still et al. 2003). However, data on <sup>13</sup>C/<sup>12</sup>C ratio of SOC from these locales demonstrate a substantially less commitment from C<sub>4</sub> plants to the terrestrial carbon pool (Bird and Pousai 1997). Such significant imbalances between the plant carbon source and its terrestrial sinks may be partially attributable to the stability differences of SOC pools produced by C3 and C4 plants and subsequent loss during carbon mineralization. Wynn and Bird (2007) show that labile fractions of SOM contain more of C<sub>4</sub>-derived materials and decompose faster, while the recalcitrant forms are more biased towards the components derived from C<sub>3</sub> plants. Hence, predominance of carbon fractions in the SOC pool from a specific photosynthetic pathway type can serve as a good indicator of the carbon lability to decomposition (Craine et al. 2010).

Temperature is a key rate deciding factor of SOC decomposition (Kirschbaum 1995). The short-term influences of temperature on SOC decomposition is wellestablished experimentally and is generally modeled by a temperature coefficient,  $Q_{10}$ , or more accurately by the Arrhenius function (Knorr et al. 2005). The Arrhenius equation predicts an increase in thermal sensitivity with stability of organic compounds as recalcitrant substrates have higher Ea, hence, less responsive to decomposition (Reichstein et al. 2005; Davidson et al. 2006; Gillabel et al. 2010; Plante et al. 2010). However, contradictions exist to the above concepts, and it has been suggested that depletion of SOC stocks will be much less than that is projected by most ecosystem models (Giardina and Ryan 2000). Several studies (Bradford et al. 2008; Allison et al. 2010; Wieder et al. 2013; Sihi et al. 2016) that evaluated soil carbon response mechanisms using microbial enzyme models suggest that decline in microbial growth and degradative enzyme activity with warming would lead to attenuated loss in soil carbon emissions. On the other hand, acclimatization of microbial communities to temperature could enhance their carbon use efficiency and accelerate soil carbon decomposition, thereby counteracting the reduction in microbial growth (Allison et al. 2010). In the absence of a general consensus on thermal responsiveness of SOC decomposition, the significance of a positive carbon feedback to global carbon cycle with warming continues to be debated (Davidson et al. 2006; Conant et al. 2011).

Soil organic carbon stocks in the semi-arid regions are highly vulnerable to decomposition due to prevailing environmental conditions (Sandeep and Manjaiah 2014). Proper evaluation of the thermal response of SOC decomposition processes is essential to predict the fate of carbon stocks in these regions (Kirschbaum 1995). The objective of the present study was to ascertain the thermal sensitivity parameters of SOC in maize–wheat system under different agriculture management practices and relate them with carbon mineralization and  $CO_2$  efflux. The study was an attempt to identify the best treatment combination for carbon storage in this cropping sequence.

#### Materials and methods

#### Experimental details

Long-term field experiments were initiated in 2001 at IARI farm (28° 08' N, 77° 12' E, elevation 228.61 m above mean sea level) to study the influence of tillage and nutrient management practices on soil health and crop productivity in maize-wheat cropping systems. The study area receives a mean annual precipitation of about 650 mm, mostly during the monsoon period (July-September). The annual minimum and maximum temperatures are 18 and 35 °C, respectively. Experimental soils have a loamy texture and belong to Holambi series, Typic Haplustepts (USDA). At the start of the experiment, the soils had a pH, oxidizable organic carbon and clay content of 7.7, 0.40, and 25 %, respectively. The experiment was laid out in a split plot with two tillage treatments (main plot), six fertilizer/manurial treatments (subplots), and three replications (Table 1). Recommended doses of phosphorus (26.2 kg ha<sup>-1</sup>) and potassium (50 kg ha<sup>-1</sup>) were being applied through single superphosphate and muriate of potash, respectively, in all the treatments ( $T_1$  to  $T_5$ ), except  $T_6$ . Farmyard manure (FYM) was incorporated to soil 15 days prior to transplanting or sowing of crop. Crop residues after separation of grain were field retained under specific plots and this constituted mineral fertilizer + crop residue treatment  $(T_5)$  for the succeeding crops. For biofertilization, Azotobacter sp. W5 strain and Azospirilum sp. CD strain were applied on the seed at the time of sowing of maize and wheat, respectively, as a carrier-based culture  $@ 10^9$  cells/g.

#### Soil sample collection

Soil samples (0–0.15 m) were collected from the experimental plots during October/November 2006 (after the harvest of maize). From each plot, four representative soil samples were collected and pooled to obtain composite samples. The collected samples were air-dried after removing the crop remnants, ground and sieved (2-mm sieve). These samples were used for subsequent analyses.

Oxidizable organic carbon and C<sub>4</sub>-derived carbon

Wet oxidation by  $K_2Cr_2O_7$  (Walkley and Black 1934) was used to estimate the oxidizable organic carbon content in soil.

The discrimination of  ${}^{13}\text{CO}_2$  during photosynthesis in C<sub>3</sub> and C<sub>4</sub> plants varies and as such produces litter with different  ${}^{13}\text{C}/{}^{12}\text{C}$  ratios (Ehleringer and Osmond 1989). The deviation in  ${}^{13}\text{C}/{}^{12}\text{C}$  molar ratios (*R*) of the sample from an international standard (Vienna-Pee Dee Belemnite, V-PDB) gives the  ${}^{13}\text{C}$  isotope concentration ( $\delta^{13}\text{C}$  ‰) in a sample:

$$\delta^{13}$$
C (‰) = ( $R_{\text{sample}}/R_{\text{standard}} - 1$ )\*1000 (1)

 
 Table 1
 Treatment details of tillage and nutrient management under maize-wheat cropping system

Treatments	Description
Main plot treatment (tillage)	
Bed planting/no tillage (BP)	Bed planting for maize and no tillage for succeeding wheat
Conventional tillage (CT)	For maize and for succeeding wheat
Subplot treatments (manures/fertilizers)	
$T_1$	No N
$T_2 (100 \% \text{ N})^{\text{a}}$	120 kg urea—N/ha
$T_3$	90 kg urea—N + 30 kg N/ha applied through FYM
$T_4$	90 kg urea—N + 30 kg N/ha applied through sewage sludge
T <sub>5</sub>	120 kg urea—N/ha + Crop residues (previous crop)
T <sub>6</sub>	120 kg N/ha (50 % FYM + 25 % biofertilizer + 25 % crop residue)

All the treatments have been receiving recommended dose of P (26.2 kg ha<sup>-1</sup>) and K (50 kg ha<sup>-1</sup>) through mineral fertilizers except  $T_6$ 

 $^a\,100\%$  of the recommended dose of N

Carbon derived from C<sub>4</sub> plants in SOC pool was analyzed using  $\delta^{13}$ C signatures of humic substances. Humic substances were extracted from soil samples using 0.1 mol L<sup>-1</sup> NaOH (soil/extractant ratio of around 1:10 (*m*/*v*) in a nitrogen atmosphere. The extracted humic acids were purified using HCl–HF treatment.

The <sup>13</sup>C measurements in humic substances extracted from soils of experiment plots were carried out with isotope ratio mass spectrometry (Delta plus, Thermo electron, Bremen, Germany). This stable isotope of carbon, <sup>13</sup>C, was used to estimate the amount of C<sub>4</sub>-derived carbon in a C<sub>3</sub>-C<sub>4</sub> cropping sequence (Balesdent and Mariotti 1996). As the two sources (C<sub>3</sub> and C<sub>4</sub>) have different enough  $\delta^{13}$ C values, a two pool mixing model was used to assess the proportional contribution (*f*) of the two different sources to the total SOC mixture (Fry 2006). In the present study, the sources which contributed to the  $\delta^{13}$ C signatures were:

- Source 1  $C_3$  vegetation-derived residues which includes contribution from wheat as well as the  $C_3$  plants prior to the present cropping system
- Source 2  $C_4$  vegetation-derived residues which includes contribution from maize as well as the  $C_4$  plants prior to the present cropping system

The two-pool mixing model based on mass balance mathematically quantifies the proportionate contribution from two different sources to a mixture. The isotopic composition of the mixture being the sum of relative isotopic signatures of the two contributing sources (Biasi et al. 2012), the two pool mixing model can be expressed as

$$\delta_{\text{sample}} = (f_{\text{S1}} \times \delta_{\text{S1}}) + (f_{\text{S2}} \times \delta_{\text{S2}})$$
(2)

$$f_{\rm S1} + f_{\rm S2} = 1 \text{ or} \tag{3}$$

$$f_{S2} = 1 - f_{S1}$$
 (4)

where  $\delta_{\text{sample}}$  is the isotopic signature of the mixture (here:  $\delta^{13}$ C value of humic materials including both C<sub>4</sub> and C<sub>3</sub>-derived fractions);  $\delta_{\text{S1}}$  is the isotopic signature of source 1 (here: theoretical mean  $\delta^{13}$ C value of C<sub>3</sub> plants (-27 ‰)) and  $\delta_{\text{S2}}$  is the isotopic signature of source 2 (here: theoretical mean  $\delta^{13}$ C value of C<sub>4</sub> plants (-12 ‰)). Therefore,

$$f_{S1} = (\delta \text{sample} - \delta_{S2})/(\delta_{S1} - \delta_{S2}) \text{ and } (5)$$

$$f_{S2} = 1 - \left[ (\delta \text{sample} - \delta_{S2}) / (\delta_{S1} - \delta_{S2}) \right]$$
(6)

# Soil respiration and thermal sensitivity of soil organic carbon

Temperature responses (25-40 °C) of processed soils (0-0.15 m) cumulative CO<sub>2</sub> efflux were investigated for 150 days; 100 g soils moistened to field capacity (maintained throughout the experiment) were incubated in wide-mouth bottles at 25, 30, 35, and 40 °C. The CO<sub>2</sub> effluxes from these soils were determined at periodic intervals (3, 6, 9, 12, 15, 18, 30, 45, 60, 70, 90, 105, 120, and 150 days) by alkali trap method (Anderson 1982). The  $CO_2$  evolved from the samples were trapped in 0.1 N NaOH, kept in small vials, and amount of CO<sub>2</sub> (entrapped) was measured by back titration with 0.05 N HCl. The remaining organic carbon in soil was estimated by subtracting the amount lost during the period from the initial organic carbon content (at 0th day) and was used for determining the first-order rate kinetics using the equation:

$$A_t = A_0 e^{-kt} \tag{7}$$

where k is the decomposition rate constant,  $A_0$  and  $A_t$  are the amount of organic carbon at zero and 't' time. Ea was calculated using Arrhenius equation:

$$k = A \exp\left(-\text{Ea}/RT\right) \tag{8}$$

where k is the decomposition rate constant, A is the frequency factor, Ea is the required activation energy in J mol<sup>-1</sup>, R = 8.314 J K<sup>-1</sup> mol<sup>-1</sup>, and T is the temperature (K). Ea was calculated from the slope (Ea/R) obtained by plotting ln k against 1/T (Knorr et al. 2005).

 $Q_{10}$  indicates the responses of biological processes with temperature.  $Q_{10}$  values were calculated as a function:

$$Q_{10} = (k_2/k_1)^{(10/T_2 - T_1)}$$
(9)

where  $k_2$  and  $k_1$  are the reaction rates at temperatures  $T_2$ and  $T_1$ , respectively ( $T_1 = 298$  K and  $T_2 = 308$  K) (Kirschbaum 1995).

Michaelis-Menten kinetics of SOC decomposition

The decomposition rate of SOC is modified by concentration of substrates (Sn) and enzyme affinities for the substrates (Km) as

$$k = V \max \times Sn / Km + Sn$$
(10)

where k is the reaction rate, Sn is the substrate availability (i.e., the concentration of substrate at the active site of the enzyme), Vmax is the maximum reaction rate at a given temperature, and Km is the Michaelis–Menten constant, representing the substrate concentration at which the reaction rate equals Vmax/2 (Davidson et al. 2006).

Respiration responses of the soil were measured at 25 and 40 °C using Michaelis–Menten kinetic model (Eq. 10). The parameters were calculated by plotting carbon dioxide concentration (mmol/100 g soil) against its rate of evolution (mmol/day). It was assumed that none of the product reverts to substrate, and hence, the rate of substrate utilization equals rate of product formation. The non-linear data curve was fitted using solver module with minimum sum of squared errors (SSE) approach in Excel 2007 and the best fit values for Km and *V*max derived. The obtained Km values are in terms of CO<sub>2</sub> which was converted to carbon substrate equivalent stoichiometrically (C + O<sub>2</sub> = CO<sub>2</sub>). The best fit values of Km and *V*max were used to derive concentration of substrates at the active sites of the enzyme, i.e., Sn.

#### Statistical analysis

Analysis of variance (ANOVA) for split-plot design was performed using SAS version 9.1 (SAS Institute Inc.). Treatment means were compared at P < 0.05 level using the least significant difference (LSD) for all the parameters.

#### Results

Oxidizable organic carbon and C<sub>4</sub>-derived carbon

Oxidizable organic carbon content of soil was affected significantly by nutrient treatments whereas tillage and

their interaction were found not to influence this carbon fraction (Fig. 1). Among the different nutrient treatments, lowest oxidizable carbon content was observed in plots receiving primary nutrients through mineral fertilizers alone ( $T_2$ ) which was on par with control ( $T_1$ ). There was 20–24 % enhancement in the oxidizable organic carbon status of soils in plots receiving organic inputs by way of FYM ( $T_3$ ), sewage sludge ( $T_4$ ) and crop residues ( $T_5$ ) compared to treatment with mineral fertilizers alone ( $T_2$ —100 % N through urea) in both bed planting and conventional tillage systems. Between the different organic treatments, highest oxidizable organic carbon content was maintained in soils receiving nutrients through 100 % organics ( $T_6$ ).

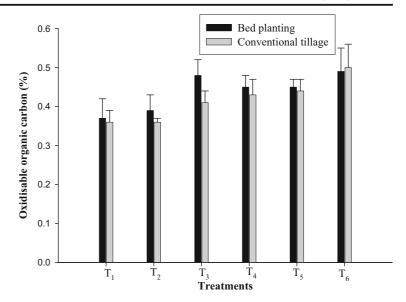
Before initiating the experiment in 2001, the entire area had a uniform C<sub>3</sub>-C<sub>4</sub> plant mix. Hence, the variations in  $\delta^{13}$ C signatures can be assigned entirely to the current cropping and management practices. In general, carbon-derived from C<sub>4</sub> plants was found to range from 7-27 % under various tillage and nutrient treatments (Fig. 2). Integrated  $(T_3-T_5)$  treatments had less than 15 % C<sub>4</sub>-derived carbon and were statistically on par with 100 % organic application ( $T_5$ ). In soils receiving 100 % recommended N through urea  $(T_2)$ , 27 % of the total oxidizable organic carbon was C4 derived and was significantly higher than application of N through any organic treatment  $(T_3-T_5)$ . The results show that bed planting + mineral fertilizer treatment combination was found to contribute maximum C4-derived carbon to the SOC pool and conventional tillage + crop residues made the minimum proportionate enrichment of this fraction.

#### Thermal sensitivity of SOC

The Ea of soil carbon decomposition was effectively modified by tillage and nutrient management treatments (Table 2). Bed planting (18.17 kJ mol<sup>-1</sup>) was found to be more effective in maintaining higher Ea than conventional tillage system (14.88 kJ mol<sup>-1</sup>). 100 % organic treatments ( $T_6$ ) and soil receiving recommended doses of N through urea + FYM ( $T_3$ ) increased Ea significantly over integrated sources ( $T_4$  and  $T_5$ ), mineral fertilizers ( $T_2$ ) and control ( $T_1$ ). Interaction of main and subplot factors show that bed planting along with 100 % organics was the most effective management practice for improving the decomposition resistance of organic carbon in soils of maize–wheat system.

Equation 9 depicts that if the rate of the reaction is completely temperature independent, the resulting  $Q_{10}$ 

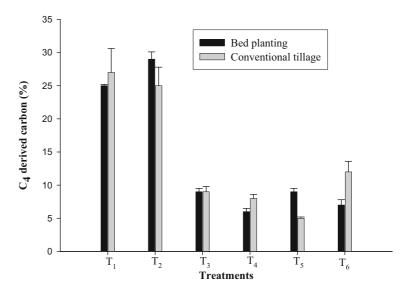
Fig. 1 Oxidizable organic carbon content in soils of bed planting and conventional tillage in maize–wheat cropping systems

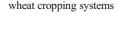


will be 1.0 and the value would increase with increasing thermal dependency of the reaction. Here, we used  $Q_{10}$ as an indicator of temperature dependency of carbon decomposition in soil and also to infer the reaction rate changes over a 10 °C temperature rise (Table 2).  $Q_{10}$ values were significantly affected by tillage with the bed planting system ( $Q_{10} = 1.27$ ) having a significantly higher sensitivity to temperature than conventional tillage ( $Q_{10} = 1.16$ ). Soils receiving organic treatments had  $Q_{10}$  values ranging from 1.20 to 1.31 which was significantly higher than those of the control ( $T_2$ ) and plots treated with urea alone  $(T_2)$ . The interaction of tillage and nutrient management was found non-significant in modifying the  $Q_{10}$  values of soils.

# Soil respiration

The cumulative  $CO_2$  efflux under different nutrient treatments varied from 202.2 mg  $CO_2/100$  g soil in absolute control ( $T_1$ ) to 324.3 mg  $CO_2/100$  g soil in 100 % organics ( $T_6$ ) in bed planting at 25 °C, whereas





**Fig. 2** Proportion of C<sub>4</sub>-derived carbon in soils of bed planting and

conventional tillage in maize-

**Table 2** Thermal sensitivity parameters (Ea and  $Q_{10}$ ) of organic carbon in soils of bed planting and conventional tillage in maize - wheat cropping systems

Treatments	Ea (kJ/mol	)		$Q_{10}$		
	BP	СТ	Mean	BP	СТ	Mean
$T_1$ (control)	14.87	9.63	12.25	1.18	1.11	1.15
$T_2$ (100 % N through urea)	14.99	13.52	14.25	1.2	1.14	1.17
$T_3$ (100 % N through urea and FYM)	20.7	17.53	19.11	1.24	1.17	1.20
$T_4$ (100 % N through urea and sewage sludge)	18.55	15.16	16.86	1.3	1.17	1.23
$T_5$ (100 % N through urea + crop residues)	18.61	16.07	17.34	1.44	1.18	1.31
<i>T</i> <sub>6</sub> (100 % organics)	21.31	17.35	19.33	1.25	1.17	1.21
Mean	18.17	14.88		1.27	1.16	
LSD ( $P = 0.05$ )	(N) = 1.1	,	t management	Tillage (7 (N) = 0 $T \times N = N$	.04;	ent management

*BP* bed planting, *CT* conventional tillage, *A* for the same level of main plot treatment, *B* for the same level of subplot treatment, *NS* non-significant, *LSD* least significant difference

the amounts varied between 248.1 to 419.1 mg CO<sub>2</sub>/ 100 g soil in conventional tillage system. The cumulative CO<sub>2</sub> efflux from conventional tillage was much higher than its bed planting counterpart (Fig. 3a–f). CO<sub>2</sub> evolved from bed planting system even at 40 °C was lesser or at par with the efflux from conventional tillage at 25 °C in all the nutrient managements, except  $T_3$  (mineral fertilizer + FYM). In FYM-treated plots ( $T_3$ ), the ease of carbon decomposition at the study temperatures was found to follow the order: conventional tillage at 40 °C > bed planting at 40 °C > conventional tillage at 25 °C > bed planting at 25 °C.

#### Michaelis-Menten kinetics of SOC decomposition

Soil carbon utilization parameters were estimated from Michaelis–Menten model with a good fit between measured and simulated values. Bed planting system was found to have higher Km values (except  $T_6$ ) and lower substrate availabilities than conventional tillage system (Table 3). Within the main plot treatments (tillage), Km was maximum for  $T_6$  (100 % organics) and minimum for  $T_1$  (control) at both 25 and 40 °C indicating the need for high substrate concentrations in organic matter treated plots to achieve maximum reaction velocity.

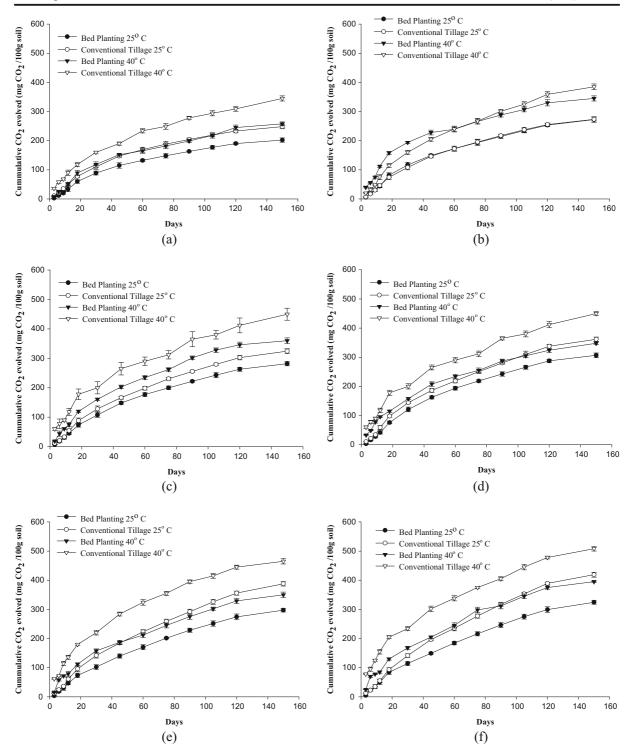
Among the different nutrient treatments, Sn in bed planting was found to be highest in crop residue treated soils ( $T_5$ ) at 25 °C and in FYM + mineral fertilizer ( $T_3$ )treated plots at 40 °C. In conventional tillage, Sn at 25 and 40 °C ranged from 7.20 to 8.70 mg C/g soil/day and 6.57 to 8.75 mg C/g soil/day, respectively, and was maximum in  $T_6$  (100 % organics) plots at both the study temperatures. In organic-treated plots of conventional tillage ( $T_3$ - $T_6$ ) and all nutrient treatments of bed planting ( $T_2$ - $T_6$ ), Sn was found to increase with temperature. *V*max followed a general trend of increase with temperature in all the treatment combinations used in the study.

The carbon utilization parameters derived from Michaelis–Menten kinetics show that conventional tillage system mineralizes significantly higher amounts of carbon in all the treatments at all the studied temperatures (Fig. 4a–f). In bed planting, the carbon mineralization ranged from 14.90 % (C mineralized in  $T_1$  at 25 °C) to 21.99 % (C mineralized in  $T_6$  at 40 °C). In conventional tillage, 18.80 to 29.93 % (C mineralized in  $T_1$  at 25 °C and  $T_3$  at 40 °C, respectively) carbon was lost when the temperatures were increased from 25 to 40 °C. At 40 °C, all organic treatments, i.e.,  $T_3$  to  $T_6$ were equally effective than mineral fertilizers ( $T_2$ ) in maintaining carbon mineralization at a minimum.

#### Discussion

Oxidizable organic carbon and C<sub>4</sub>-derived carbon

Supplement of recommended doses of N through mineral fertilizer alone, i.e.,  $T_2$  was found to be ineffective in significantly improving the oxidizable organic carbon content of soil from that of control ( $T_1$ ), but was successful



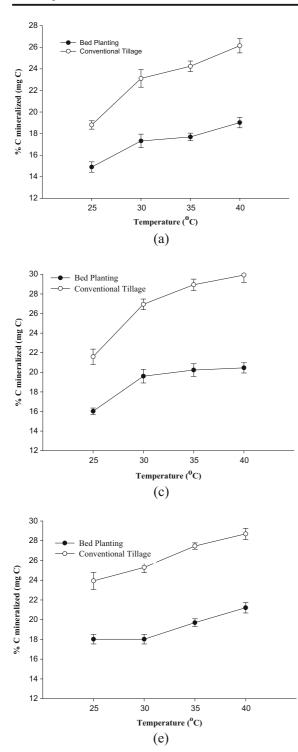
**Fig. 3 a**–**f** Cumulative CO<sub>2</sub> evolved (mg CO<sub>2</sub>/100 g soil) in bed planting and conventional tillage in response to different temperatures over 150 days of incubation (**a**) control plot ( $T_1$ ) soils (**b**) 100 % N through urea treated ( $T_2$ ) soils (**c**) 100 % N through urea

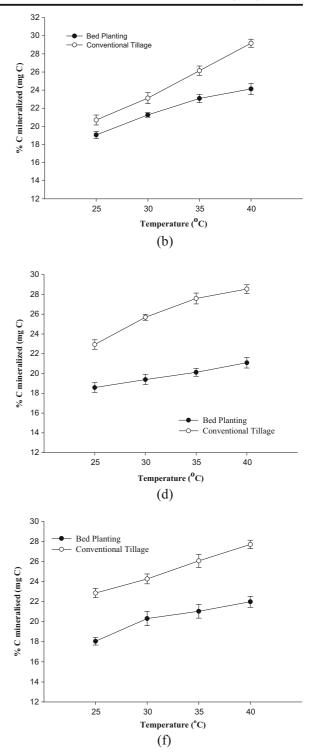
and FYM treated ( $T_3$ ) soils (**d**) 100 % N through urea and sewage sludge treated ( $T_4$ ) soils (**e**) 100 % N through urea + crop residues treated ( $T_5$ ) soils (**f**) 100 % organics treated ( $T_6$ ) soils

Table 3 Michelis-Mente	en parameter va	alues of substrate utiliz	Table 3 Michelis–Menten parameter values of substrate utilization kinetics in soils of bed planting and conventional tillage in maize - wheat cropping systems	planting and conventio	nal tillage in maize -	wheat cropping systems	
		Bed planting			Conventional tillage		
Treatments	Temp. (°C)	Km (mg C/g soil)	$Temp.(\ ^{\circ}C)  \overline{Km}(mgC/gsoil)  \textit{Vmax}(mgCg^{-1}soil/day)  Sn(mgCg^{-1}soil)$	Sn (mg C $g^{-1}$ soil)	Km (mg C/g soil)	Km (mg $C/g$ soil) $V$ max (mg C g <sup>-1</sup> soil/day) Sn (mg C g <sup>-1</sup> soil)	Sn (mg C $g^{-1}$ soil)
$T_1$ (control)	25	369.4 (2.5)	0.05 (0.002)	5.08 (0.16)	360.8 (9.6)	0.07 (0.003)	7.36 (0.20)
	40	370.1 (8.2)	0.07 (0.003)	6.07 (0.13)	366.7 11.4)	0.09 (0.002)	6.57 (0.20)
$T_2$ (100 % N through urea)	25	389.7 15.4)	0.07 (0.003)	4.62 (0.11)	359.9 12.2)	0.07 (0.003)	7.20 (0.05)
	40	397.4 (12.6)	0.10 (0.004)	4.77 (0.09)	362.3 (5.0)	0.09 (0.001)	6.86 (0.15)
$T_3$ (100 % N through urea	25	479.9 (11.6)	0.07 (0.003)	5.05 (0.08)	410.5 (16.2)	0.08 (0.002)	7.76 (0.26)
and FYM)	40	482.1 (8.9)	0.10 (0.003)	6.94 (0.15)	422.6 (4.6)	0.11 (0.001)	8.04 (0.11)
$T_4 (100 \% \text{ N through urea})$	25	449.1 (8.2)	0.07 (0.001)	5.01 (0.12)	430.1 (13.6)	0.08 (0.002)	7.26 (0.25)
and sewage sludge)	40	485.8 (13.9)	0.09 (0.004)	6.85 (0.06)	442.6 (9.6)	0.11 (0.004)	7.99 (0.22)
$T_5$ (100 % N through urea	25	429.3 (10.4)	0.08 (0.002)	5.29 (0.04)	442.4 (2.9)	0.09 (0.003)	7.88 (0.31)
+ crop residues)	40	481.3 (3.7)	0.10 (0.002)	6.18 (0.23)	454.5 (16.7)	0.12 (0.004)	7.76 (0.09)
$T_6 (100 \% \text{ organics})$	25	489.4 (16.6)	0.08 (0.001)	4.58 (0.18)	500.3 (7.5)	0.09 (0.001)	8.70 (0.16)
	40	493.2 (13.6)	0.11 (0.003)	6.18 (0.20)	516.8 (11.4)	0.13 (0.003)	8.75 (0.27)
Values in parenthesis indicate standard error of the	icate standard e	stror of the parameter					

in maintaining the highest proportion of C4-derived carbon. Higher nitrogen availability in plots receiving 100 % recommended doses of N through urea  $(T_2)$  could positively influence the <sup>13</sup>C absorption during photosynthesis. Earlier studies (Medina 1970; Seemann et al. 1987) have shown that treatments supporting higher nitrogen concentrations in plant leaves will also enhance its carbon-fixing enzymes and thereby their photosynthetic capacity. Due to the anatomical and biochemical differences in C<sub>3</sub> and C<sub>4</sub> plants, the latter have a greater nitrogen use efficiency reflected as biomass produced or CO2 fixed per unit of nitrogen in the plant. Brown (1978) corroborates this observation with the hypothesis that nitrogen use efficiency in C<sub>4</sub> plants will be higher than C<sub>3</sub> owing to a lower nitrogen investment in the Rubisco protein and the related  $CO_2$  fixation pathway. Enhanced ability of  $C_4$  plants to capture CO<sub>2</sub> under 100 % N through urea treatment  $(T_1)$ would in turn increase consumption of intercellular CO<sub>2</sub> and lead to a more positive  $\delta^{13}$ C value in below-ground translocated portion of assimilated carbon in a C<sub>4</sub>-C<sub>3</sub> cropping mix (Sandeep et al. 2016). However, the low Ea of the soil carbon in  $T_2$  suggest that these fractions are highly vulnerable to decomposition (discussed later). Studies by Wynn and Bird (2007) also show a selective decomposition of C<sub>4</sub>-derived fraction over C<sub>3</sub> in the SOC pool by way of differences in organic matter quality (arising from differences in the mean particle size or lignin content) or by preservation of C3-derived biomass components in the relatively oxidation resistant stable pool of elemental carbon.

Highest buildup of oxidizable organic carbon was shown in this continuous cropping system managed with 100 % organics ( $T_6$ ). All integrated nutrient management treatments  $(T_3 - T_5)$  used in the study were found to be equal in their effect in enhancing this carbon fraction over control. The high oxidizable organic carbon content in  $T_6$ (100 % organics) may be attributed to the higher amounts of organic inputs added continuously as treatment to these soils. Earlier studies (Singh et al. 2000; Daniel et al. 2002; Majumder et al. 2008) have also reported the superiority of organic inputs over mineral fertilizers in improving the oxidizable organic carbon content of soils. For example, FYM with its higher lignin and phenol contents forms stable complexes with plant proteins making FYM decomposition resistant and thereby promotes carbon accumulation with time (Tian et al. 1992). The results indicate that nutrient management measures such as addition of 100 % recommended N dose through urea alone to optimize plant growth and carbon fixation may lead to a reduction in





**Fig. 4 a**–**f** Carbon mineralization (%) responses to different temperatures of bed planting and conventional tillage after 150 days of incubation (**a**) control plot ( $T_1$ ) soils (**b**) 100 % N through urea treated ( $T_2$ ) soils (**c**) 100 % N through urea and FYM treated ( $T_3$ )

soils (**d**) 100 % N through urea and sewage sludge treated ( $T_4$ ) soils (**e**) 100 % N through urea + crop residues treated ( $T_5$ ) soils (**f**) 100 % organics treated ( $T_6$ ) soils

stability of the SOC and its subsequent depletion under continuous cropping (Kuzyakov and Domanski 2000).

#### Thermal sensitivity of SOC

The Ea of soil carbon decomposition was effectively modified by tillage and nutrient management treatments. Bed planting (18.17 kJ mol<sup>-1</sup>) had an edge over conventional tillage system (14.88 kJ mol<sup>-1</sup>) in improving the Ea of SOC. Tillage systems with lesser soil disturbance generate an in situ temperature insensitive process that alter the release of easily decomposable substrates and thereby reduce the decomposition rate (Von Lützow and Kögel-Knabner 2009). There was an increase of 29.7 % in the Ea of soil carbon when 100 % mineral fertilizers  $(T_2)$  were replaced with 100 % organics  $(T_6)$  in bed planting system, whereas the change was only 22 % for the same substitution in conventional tillage. The substitution effect (organics for mineral fertilizers) on enhancing Ea may be attributed to organic carbon promoted soil aggregation and thereby a physical barrier to carbon decomposition factors in bed planting system. On the other hand, conventional tillage with its greater soil disturbance offers lesser support for aggregation and hence could not generate the effect to same extend even with addition of organic matter. Six et al. (2002, 2004) have also reported that the less disturbed soils provide an enhanced physical protection to SOC and thereby carbon accumulation than soils with continuous disturbance.

The FYM-treated soils were found to be better among different organic manures used in maintaining a higher Ea, which was on par with 100 % organics treated plots. FYM usually contains high amounts of stable organic compounds such as lignin and phenol (Tian et al. 1992) which help in increasing their resistance to decomposition. Hence, Ea as a measure of relative resistance of organic molecules to decomposition suggests that organic treatments especially materials enriched with stable compounds will be an option to improve SOC in maize–wheat continuous cropping systems of semi-arid regions.

 $Q_{10}$ , which explains the change in reaction rate over a 10 °C temperature rise, was used along with Ea to explain the intrinsic temperature sensitivity of SOC decomposition in the system. Both Ea and  $Q_{10}$  values were significantly higher in bed planting than conventional tillage system thereby giving a clear indication of temperature sensitivity of recalcitrant carbon pools in soils of this cropping system. In conventional tillage, the Ea being low, reaction occurs faster even at lower

temperatures exhausting the carbon pools and leaving relatively lesser fraction of substrate molecules to react at higher temperatures, and consequently, the  $Q_{10}$  value decreases. Similar were the observations of Tjoelker et al. (2001) and Davidson et al. (2006) that the relative rate of decomposition of SOC with lower Ea values will be less sensitive to temperature.

 $Q_{10}$  values of crop residue treated plots ( $T_5$ ) were significantly higher than other organic manure-treated plots in both tillage systems. Crop residue decomposition proceeds through a two-step process: decomposition of water-soluble forms during the first phase and recalcitrant and structural carbon components during the second phase (Cogle et al. 1989). As the added crop residues had a wide C:N ratio, they could release sufficient carbon (energy source) from its recalcitrant/structural components and stimulate higher microbial activity with warming, hence higher  $Q_{10}$  values (Lu et al. 2010). Studies have shown that changes in temperature can bring about corresponding fluctuations in soil microbial populations and enzyme activities (Lloyd and Taylor 1994; Wallenstein et al. 2011). Crop residues, through a twophase decomposition and high carbon backup, supply substrates at all levels of enzyme activity and maintain higher reaction rates with temperature. The significantly higher  $Q_{10}$  values of organic treated soils ( $T_3$  to  $T_6$ ) than control plots may be the result of faster desorption of carbon from the adsorption sites or by increased thermal sensitivity of recalcitrant carbon at higher temperatures or due to efficacy of biological decomposition processes at these temperatures or by a combination of these processes supporting each other (Fang et al. 2005; Reichstein et al. 2005; Davidson et al. 2006).

The cumulative CO2 efflux at 25 °C under various treatments ranged from 202.2 to 324.3 mg CO<sub>2</sub>/100 g soil and 248.1 to 419.1 mg CO<sub>2</sub>/100 g soil in bed planting and conventional tillage systems, respectively. The strong physico-chemical protection offered by reduced soil disturbance in bed planting effectively increases Ea and leads to decreased mineralization of soil carbon. However, in conventional tillage system, increased soil disturbance prevents the same level of carbon protection. At a particular temperature, though the CO<sub>2</sub> evolution was more in organic treated plots than control, the percent of carbon mineralized did not vary significantly. Sandeep and Manjaiah (2014) reported that organic matter enhanced water stable aggregates in bed planting system of the experiment plots and pointed out that such aggregations protect SOC physically by restricting enzyme accessibility, control interactions of food web (Shirani et al. 2002) and consequently reduce carbon mineralization.

#### Michaelis-Menten kinetics of SOC decomposition

Michaelis-Menten constant (Km) that represents the concentration of substrate at which the rate of reaction equals Vmax/2 was more in bed planting at both the study temperatures. A higher Km along with lower substrate availability essentially reduces reaction rates in bed planting than conventional tillage system. Management practices such as reduced tillage in bed planting increases aggregation and plays an important role in reducing decomposition losses of SOC, whereas in conventional tillage, greater soil disturbance disrupts physical protection of SOC leading to higher substrate availability for decomposition (Paustian et al. 1998). Studies by Sihi et al. (2016) show that substrate limitations in the form of diminishing marginal return (i.e., a smaller increase in catalysis of SOC by extracellular enzymes than the previous increment) can create breaks in the positive feedback of microbial mediated depolymerization, thereby changing a forward Michaelis-Menten model to a reverse mode. Soil disturbances directly affect carbon substrate availability by influencing their interaction with mineral adsorption sites and cations or indirectly by modifying external factors (e.g., temperature, moisture, aeration, pH, nutrient supply) that control SOC decomposition (Von Lützow et al. 2006). Bed planting system with its lesser soil disturbance can also generate an in situ temperature-insensitive process that restrict release of easily decomposable substrates and thereby reduce carbon mineralization. The results of carbon mineralization (%) support the above deductions with conventional tillage having 21 to 27 % higher mineralization than bed planting system at the study temperatures.

In bed planting system, crop residue-treated plots provided significantly higher amounts of substrates (Sn) for enzyme binding and had lowest Km values among all organic treated plots at 25 °C. A combined effect of these two factors (Km and Sn) was a faster decomposition of carbon in these plots at lower temperatures. However at 40 °C, the Km and Sn values of crop residue-treated plots were on par with other organic matter treatments in bed planting. The higher substrate availability from crop residues even at 25 °C confirms the earlier studies of Trinsoutrot et al. (2000) and Wang et al. (2004) that there are two phases in crop residue decomposition—a rapid initial phase of water soluble components comprising of amino sugars, amino acids, and carbohydrates and a slower second stage comprising of recalcitrant and structural compounds such as cellulose and lignin. Organic materials such as FYM have already passed the first stage before applied to the field and could not provide the same level of available substrates for enzyme action as that of crop residues, hence the lower reaction rates in  $T_3$  (mineral fertilizer + FYM) at lower temperatures.

The Sn and Vmax values of all organic treated soils  $(T_3 \text{ to } T_6)$  were on par at 40 °C indicating the release of recalcitrant compounds for enzyme action from the added organic materials at higher temperatures. We observed an increase in Vmax with temperature which indicates that the microflora can enzymatically acclimatize to decompose stable SOC at higher temperatures in these soils (Boddy et al. 2008; Allison et al. 2010). *V*max being a measure of enzyme activity, its increases with warming can also be due to higher activity of existing enzymes or greater production of enzymes responsible for decomposition reactions. The results are in confirmation with earlier works (Luo et al. 2001; Allison et al. 2010; Sihi et al. 2016) using microbial enzyme models where microbial acclimatization and increases in microbial biomass and degradative enzyme activity were attributed as key reasons for ephemeral increases in soil respiration with warming. These results in turn corroborate the thermal sensitivity hypothesis of recalcitrant soil organic compounds in the maize-wheat cropping systems of semi-arid India.

The present study analyzed the temperature response to carbon decomposition in the temperature range 25 to 40 °C and hence could not explore the  $Q_{10}$  or enzymemediated Vmax beyond this range. The results corroborate our current understanding of the temperature response of SOC decomposition based on the Arrhenius equation,  $Q_{10}$ , and Michaelis-Menten kinetics within this temperature range. Recently, more advanced theories such as macro molecular rate theory-MMRT (Hobbs et al. 2013) establishes a temperature optima for microbial growth and enzyme activity beyond which occur an unusual heat capacity change and denaturation of enzymes (Schipper et al. 2014). Application of a wider temperature range and evaluation of models encompassing thermodynamic principles of enzyme catalysis (e.g., MMRT) may provide new perspectives regarding organic carbon depolymerization in the tropical soils.

#### Conclusions

Bed planting system with its lesser soil disturbance was found to be a better option than conventional tillage for carbon stability in maize-wheat system. In a C<sub>3</sub>-C<sub>4</sub> cropping sequence, though mineral fertilizers increases the proportion of C<sub>4</sub>-derived carbon in SOC, they fail to protect this carbon from rapid decomposition leading to its subsequent depletion under continuous cropping. Organic manure additions, especially materials with high amounts of stable organic compounds (e.g., FYM), was found to have more positive impact on Ea of soil carbon than mineral fertilizers alone, hence can be used as a strategy to enhance SOC reserves in tropical cropping systems. The thermal sensitivity indicators such as Ea,  $Q_{10}$ , and Michaelis–Menten derivatives were efficient parameters in explaining carbon mineralization in soils. Further, higher  $Q_{10}$  values observed for soil carbon with higher Ea corroborates the thermal sensitivity hypothesis of recalcitrant organic compounds in soil.

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