IMPACT OF CARBONATES ON THE MINERALISATION OF SURFACE SOIL ORGANIC CARBON IN RESPONSE TO SHIFT IN TILLAGE PRACTICE

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Highlights

- Overall C mineralisation was higher under CT (20.1%) than NT (9.9%) system
- Tillage shift reduced the hydrophobic SOM components by 19.3% than NT system
- The half-life of soil labile C was 6-12 and 5-7 days at 22 and 37°C, respectively
- Carbonate C started mineralising after interaction with crop residue mulch

Abstract

The inorganic soil C pool is a major source of CO_2 emission into the atmosphere along with the soil respiratory CO_2 fluxes but is comparatively less studied than the organic C mineralisation processes. This study aims to understand how soil available carbonates influence the soil C dynamics under different tillage, mulching and temperature regimes. A 90-day incubation experiment was conducted by adding calcite nodules to soils (10% w/w) collected from an agricultural field maintained with or without 5 t ha^{-1} mulching under no-till (NT) or conventional tillage (CT) systems. Environmental Scanning Electron Microscope (ESEM) examination indicated greater morphological changes in the calcite nodules incubated with CT than NT soils. Soil samples incubated with calcite and mulching recorded 6.3% greater CO_2 evolution than the un-mulched condition. Under the CT system, the overall CO_2 emission rate was higher in the control treatment (43%), followed by a combined treatment of 5 t ha^{-1} mulch + $CaCO_3$ (10% w/w) (29.2 %), 5 t ha^{-1} mulch only treatment (27.9%), and 10% $CaCO_3$ (w/w) (16.5%) treatment, with a rise in incubation temperature from 22°C to 37°C. Kinetic model calculations for CO_2 emission indicated a greater half-life of easily mineralisable C pools in the NT system at 22°C. Microbial biomass carbon (MBC) results further verified that the high temperature and disturbed soil conditions limit the availability of soil MBC under the CT systems, indicating a higher decomposition rate. Eventually, these results indicated that agricultural management practices, including tillage shift, explicitly influence the different functional components of soil organic matter (SOM).

Keywords: Tillage, Mulching, Kinetic decomposition model, Carbonates, Microbial Biomass Carbon, Carbon Sequestration

1. Introduction

More than 48% of Earth's land area contains a significant amount of inorganic carbon (C), mostly found in arid and semi-arid regions (Lal, 2009). This inorganic soil C pool is a major source of CO_2 emission into the atmosphere along with the soil respiratory CO_2 flux (Lal, 2009; Lorenz and Lal 2018; Sanderman, 2012). Many studies estimated that soil respiration is the second largest C flux after the emission due to combustion of fossil fuels (Quéré et al., 2009), contributing about 52–60 Gt of C between the terrestrial ecosystem and atmosphere (Hanson et al., 2000; Kuzyakov, 2006; Lal, 2004). The majority of the studies concerning soil CO_2 fluxes have stated that CO_2 are emitted from soils mainly due to the microbial respiration (Kuzyakov, 2006; Zhang et al., 2010). During this process, organic matter (OM) present in the soil is converted to CO_2 by the microbial action. The gaseous CO_2, thus formed may dissolve in soil water and form carbonic acid (H_2CO_3) (Eq. 1). Later, when H_2CO_3 reacts with soil carbonates ($CaCO_3$), it results in the formation of bicarbonates (HCO_3^-), and then again may dissociate to release CO_2 from the soil system (Eq. 2 and 3).

$$H_2O\,(l) + CO_2\,(g) \rightarrow H_2CO_3\,(aq) \qquad \textbf{……(Eq. 1)}$$

$$H_2CO_3\,(aq) + CaCO_3\,(g) \rightarrow Ca^{2+}(aq) + 2HCO_3^-\,(aq) \qquad \textbf{……(Eq. 2)}$$

$$HCO_3^-(aq) \rightarrow CO_2(g) + OH^-\,(aq) \qquad \textbf{……(Eq. 3)}$$

The CO_2 emission from soil is a continuous process taking place through soil microbial (heterotrophic) and root (autotrophic) respirations, which increases the CO_2 concentration in the soil pores, and ultimately releases into the atmosphere (Bolan et al., 2003). However, calcareous soils (pH>8) contain a large amount of inorganic C including calcium bicarbonate and carbonate. The release of CO_2 from these inorganic C sources may contribute significantly in the total emissions and overestimate the release of CO_2 due to soil organic carbon (SOC) decomposition during the respiration measurement.

Recent investigations revealed that soils under continuous no-tillage (NT) system may be prone to problems such as soil compaction, nutrient stratification, and the emergence of stubble- or soil-borne diseases and herbicide-resistant weeds. Most of these problems are prevalent in the surface layer of soils, and highlight the limitations of NT system (Argent et al., 2013; Walker, 2012). Study conducted by Barbera et al. (2012) indicates that reduced tillage frequency and increased cropping intensity increased the proportion of soil inorganic carbon (SIC) relative to total soil C. The authors also emphasised that such increase in SIC in the reduced tillage system were particularly observed at the surface soil layer, where crop residues accumulate due to reduced soil disturbances (Barbera et al., 2012). The crop residues might lead to increased base cation (Ca and Mg) inputs to the surface soil, consequently could enhance the precipitation of $CaCO_3$ (Barbera et al., 2012). However, the mechanism involved in this processes is not fully clear.

The above situations sometime encourage the farmers to adopt a shift in their tillage system, i.e., from NT to conventional tillage (CT). Under calcareous soil conditions, any shift in the management practices (e.g., tillage shift) may become the probable reason for the redistribution of natural carbonate materials. Therefore, on exposure to the soil organic matter (SOM) the redistributed soil carbonates may undergo different physical, chemical and biological processes. It could be hypothesised that the dissolution of carbonates might occur because of the redistribution of carbonates, which may affect the emission of CO_2 fluxes from such soils. It could also be hypothesised that the dissolution of naturally occurring carbonates in calcareous soils would depend on the prevailing soil temperature, thus would affect the total soil respiration when amended with organic inputs. The variation in temperature would be common during the growth period of a field crop from sowing to the maturity. Therefore, this study aims to determine the effect of naturally occurring carbonates ($CaCO_3$) on CO_2 emission from soils under different tillage management practices. The

specific objectives are: (i) to evaluate the effect of carbonate addition, temperature and mulching on basal respiration and microbial biomass carbon (MBC) in the soil, (ii) to determine the influence of different carbonate addition rates and temperature regimes on the decomposition rate of soil added plant residues as mulching, and (iii) to characterise the components of SOM based upon functional groups (e.g., hydrophobic and hydrophilic organic constituents) as impacted by tillage practices and mulch addition.

2. Materials and Methods

2.1 Site description and experimental design

This study was conducted at the Roseworthy Campus (34°32'15"S 138°41'25"E) of the University of Adelaide from April 2014 to May 2015. The experimental site had a history of more than 14 years of rain-fed and continuous NT cropping system. However, April 2013 onwards, CT was introduced. In the first cropping season (2013-2014), two levels (0 and 5 t ha^{-1}) of mulching were applied manually using barley (Hordeum vulgare L) residues, along with maintaining bare grounds as the control (0 t ha^{-1}) treatment. In the following cropping season (2014), two levels of mulching were attained by leaving a full height and mid-height of standing wheat (Triticum aestivum) crop stubbles after harvesting. These represented a follow-on practice of applying 0 and 5 t ha^{-1} mulching, respectively. Eighteen plots (10 m x 1.6 m) were arranged in a split plot randomised block design with three replications, and tillage systems (NT and CT) were set as the main treatments. No-tillage (NT) treatment was achieved by direct drilling of wheat seeds into unploughed soils, and CT treatment was achieved by using the same seed drill following two full disturbances up to a depth of ~15–20 cm as per local traditional practice.

2.2 Soil sampling

Soil samples from the above-mentioned plots (experiments in the current study involved only 0 and 5 t ha^{-1} mulch treatments) were collected by a hand auger after a week of the tillage

operation in May 2014. Following collection, all the visually available plant materials and debris were discarded. All the collected soil samples were kept at 5°C for the stabilisation of the microbial activity. The experimental field soil was a Brown Chromosol (Isbell, 2016), and characterised as sandy-loam in texture (64.4% sand, 8.1% silt and 27.5% clay) (Jones, 2001). The soil was strongly alkaline in (pH = 8.2–9.5), and slightly to moderately saline (EC = 0.255 dS m^{-1}) in nature (Mahmoud et al., 1978; Carmo et al., 2016) Selected physico-chemical properties of the experimental soil (an Alfisol according to the USDA soil taxonomic classification) are listed in **Table 1 and 2**. The mean annual maximum and minimum temperatures at the site were 23.5°C and 9.6°C, respectively, with an annual precipitation of 371.2 mm (2014).

2.3 Soils carbonate measurement and X-ray diffraction

The-carbonate content in soil samples was determined by the titration method (Horváth et al., 2005), and the naturally occurring carbonate nodules were handpicked from the soil surface of the field site under the respective set of treatments after the tillage operation. These carbonate nodules were later pooled together to make one single carbonate sample. Afterwards, the collected carbonate nodules were cleaned of soil materials, dried, and characterised by using X-ray diffraction (XRD). Finely ground (<50 µm) and well homogenized carbonate material was pressed in a stainless steel sample holder, and XRD patterns were obtained using CuKα radiation (λ = 1.540598 Å) on a PANalytical Empyrean diffractometer equipped with PIXcel^{3D} detector (Malvern Panalytical Ltd., Royston, UK). The diffractometer was operated at 40 kV and 40 mA between 9° to 90° 2θ at a step size of 0.013°.

2.4 Incubation experiment

Emission of C as impacted by carbonates addition was studied by incubating soil samples for 90 days under two different temperature conditions at 22 ± 1 °C and 37 ± 1 °C. These two temperatures represented the average temperature conditions prevailing during the sowing

and maturity stages of the crop (barley or wheat) grown at the experimental site. Field moist soil samples (50 g; passed through a 2 mm sieve) were mixed with two levels (0 and 10 % w/w) of the carbonate nodules (< 2 mm granule size) in Schott bottles. To verify the dissolution of carbonates during the incubation experiment, 50 g of washed sand was also mixed with the carbonate materials having different combinations of the control treatments as listed in **Table 3,** and incubated under similar environmental conditions. At the end of incubation, exchangeable cations (Na, Ca, Mg and K) in the carbonate-amended samples were analysed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Model 5300V, PerkinElmer, Inc., Waltham, MA, USA).

Vials containing 20 ml of 1M NaOH was placed within the Schott bottles of the incubation experiment to trap the CO_2 evolved from each soil sample. The CO_2 evolution was thus monitored for 0, 2, 4, 6, 8, 10, 30, 50, 70, and 90 days of the incubation. Over the incubation period, CO_2 was trapped in the NaOH solution, and the residual amount of alkali was back titrated against 0.5M HCl following adding 2 to 5 drops of 1M $BaCl_2$.

During each of the alkali-replacement occasions, suitable levels of oxygen were maintained in the Schott bottles by opening the stopper briefly. The moisture content of soil samples was maintained constant at 60% water holding capacity (water held in the soil between the field capacity and the permanent wilting point), and deionised water was added where needed. For each set of soil tests, a blank solution of NaOH (without soil as a control) was incubated and titrated. A different set of samples was prepared independently (as control) and in combination with sand, soil (from the CT and NT systems), mulch and carbonates at both the temperatures (22°C and 37°C) (**Table 3**). Each treatment was replicated three times. The total CO_2 produced was calculated using Eq. 4 (Bloem et al., 2005):

$$F_i = \left(\frac{MW * (V_b - V_s) * M * 1000}{DW * 2} \right) \qquad \textbf{……(Eq. 4)}$$

Where, 'F_i ' is the total CO_2 produced (mg CO_2-C kg^{-1}) at different intervals *'i'* (i = 0, 2, 4, 6, 8, 10, 30, 50, 70, 90 days of incubation); 'MW' is the molar mass of C (12 g mol^{-1}); 'V_b' is the volume of HCl for blank titration (L); 'V_s' is the volume of HCl for sample titration (L); 'M' is the concentration of HCl (0.5M); 'DW' is the dry weight of the soils (kg), and '2' is the factor that accounts for the fact that two OH^- are consumed by one CO_2. The converting factor from g to mg is 1000.

The cumulative CO_2 emission (C_{cum}) during the incubation was calculated using Eq. (5), which was calculated for each treatment combination using mean CO_2 mineralisation rates (n=3).

$$C_{cum} = \sum F_i \qquad \text{.....(Eq. 5)}$$

2.5 Kinetic models for CO_2 evolution

First-order (Eq. 6, Murwira et al., 1990) and two-component first-order (Eq. 7, Molina et al., 1980) kinetic models were used to calculate the decomposition rate of soil C affected by carbonate addition.

First-order model: $C_{min} = C_1 (1 - e^{-k_1 t})$ **.....(Eq. 6)**

Two-component first-order model: $C_{min} = C_2 e^{-k_2 t} + C_3 e^{-k_3 t}$ **.....(Eq. 7)**

Where, C_{min} is the cumulative amount of CO_2-C mineralised after time t (mg C kg^{-1} soil); C_1 is the initial easily mineralisable C (mg C kg^{-1} soil); 't' is the incubation period (days); k_1, k_2, k_3 are the rate constants (per day). The parameter C_1 is an initial amount of easily mineralisable C (mg C kg^{-1} soil) recovered at hour 0; C_2 and C_3 represent the readily mineralisable and slowly mineralisable C pools, respectively (mg C kg^{-1} soil).

Eq. (6) and (7) were fitted using the non-linear regression estimation using SigmaPlot® version 12.0 (Systat Software Inc., San Jose, CA, USA). The half-life ($t_{1/2}$) of mineralisation was calculated using Eq. (8):

$$t_{1/2} = \frac{\ln(2)}{k_i} = \frac{0.693}{k_i} \qquad \text{.....(Eq. 8)}$$

193 Where, k_i is the rate of either rapidly (k_1 in Eq. 6 and K_2 in Eq. 7) or slowly (K_3 in Eq. 7)
194 decomposing C fraction. In this study, $t_{1/2}$ was calculated only for the easily mineralisable
195 phase (C_2) because calculating the same for the slower phase (C_3) is subjected to uncertainty.
196 Therefore, $t_{1/2}$ for pool C_3 was not estimated in this study, as there was not enough
197 information about its connectivity to C_2.

198 **2.6 Morphology of carbonate granules**

199 To examine the morphological structure of the carbonate materials, visible carbonate nodules
200 from the incubated soils (after 90 days) were picked with a tweezer, cleaned of soil materials,
201 dried, and scanned using a Quanta 450 FEG Environmental Scanning Electron Microscope
202 (ESEM) (FEI Company, Hillsboro, OR, USA). The ESEM images were taken in high
203 vacuum mode at a 20 kV accelerating voltage using an Everhart–Thornley Detector (ETD).
204 The energy dispersive X-ray analysis (EDXA) spectra were also acquired from the selected
205 area on the samples. A moderately representative analysis was achieved by averaging
206 elemental data obtained by scanning 25 randomly selected carbonate particles.

207 **2.7 Soil C functional groups**

208 After termination of the incubation experiment, functional groups in soils were studied using
209 Fourier transform Infrared (FTIR) spectroscopy (Cary 600 series FTIR spectrometer, Agilent
210 Technologies, Santa Clara, CA, USA). FTIR samples were prepared by mixing the dried soil
211 sample with KBr (FTIR grade 99%, Sigma Aldrich) at a rate of 1% (w/w). Samples were
212 finely ground in an agate mortar and pressed into a sheer slice using a hydraulic press (100
213 KPa for 10 min) to obtain pellets for further analysis. FTIR spectra over the 4,000–400 cm^{-1}
214 range were obtained using KBr background, and each sample was scanned 64 times at a
215 resolution of 4 cm^{-1}. The FTIR spectral interpretation was done for two different absorbance
216 bands based on hydrophobic ($C-H$) and hydrophilic ($C=O$) groups. Previous studies

(Capriel et al., 1995; Cosentino et al., 2006; Šimon et al., 2009) demonstrated that tillage could significantly alter the hydrophobic and hydrophilic components of SOM.

Hydrophilic fractions are represented by carboxyl- and hydroxyl groups, and are affected due to changes in aromatic groups ($C=O$). The $C-H$ bands occur at 3000–2800 cm^{-1}, and the $C=O$ bands occur at 1740–1600 cm^{-1} region in the spectra (Doerr et al., 2000; Ellerbrock et al., 2005). Areas of the absorption bands of the hydrophobic and hydrophilic groups in the FTIR spectra were integrated using Resolutions Pro version 5.0, spectrometer software (Agilent Technologies, Santa Clara, CA, USA), and they were defined as intensities. The amounts of respective groups were calculated from the area beneath the peaks.

2.8 Microbial biomass carbon (MBC)

Microbial biomass carbon (MBC) was estimated by the fumigation-extraction method (Vance et al., 1987). Fumigated and non-fumigated soils were extracted with 50 ml of 0.5 M K_2SO_4 by shaking at 200-revolution min^{-1} for 30 min in an end-over-end shaker. Afterwards, samples were centrifuged at 4500 RPM for 15 min before filtration. The extracted organic C in the clear filtrates was measured by a TOC analyser (Shimadzu Corp. Kyoto, Japan).

2.9 Statistical analysis

The cumulative CO_2 emission was calculated for each treatment (tillage, mulch and carbonates, n = 3). Soil CO_2 emission rates at different temperatures were analysed separately for each soil by using analysis of variance (ANOVA) technique with the help of statistical software CPCS-1 (Cheema and Singh, 1990). When the effects of soil temperature and amount of carbonates on soil C emission were significant ($p<0.05$), the means and interaction effects of treatment were separated using the F-protected least significant differences (LSD) test, at the probability level $p<0.05$ (Webster, 2007).

3. Results and Discussion

3.1 Carbonate characterisation and morphological properties

The carbonate content of the experimental soil was ~17.7% in the 0–10 cm surface layer **(Table 1)**. Visually, the carbonate nodules were of different shapes and sizes, distributed above and below the surface soil layer at the experimental site. The XRD profile of the carbonate nodules indicated that they were dominated by calcite ($CaCO_3$) (60%), followed by quartz (35%), and traces of dolomite and sylvine (**Fig. 1)**. The distinguishing diffraction reflections for calcite appeared at 2θ values 23.11°, 29.46°, 36.04°, 39.48°, 43.24°, 47.6°, 48.58° and 60.14°, respectively, that corresponded for d-values of 3.85, 3.03, 2.49, 2.28, 2.09, 1.91, 1.87 and 1.54 Å, respectively. The most intensive reflection for calcite was found at 2θ = 29.46° (3.03 Å). The XRD results thus confirmed that the naturally occurring carbonate materials present in the experimental soil was dominated by calcite mineral (Hirmas et al., 2012).

Carbonate materials can occur in soils in different shapes of the particles, such as hardpans, nodular or pisolitic layers, and mottled layers (Milnes and Hutton, 1983; Ahmad et al., 2015). A micro-scale observation under ESEM indicated that the carbonate materials collected from the experimental site of this study were mostly needle-shaped (**Fig. 2**). This kind of shape of the calcite micro-particles could be attributed to the occurrence of higher biological activities near the soil surface (Ahmad et al., 2015), and the biological activities of soils are highly responsive to a change in tillage operations. It was also suggested that calcite micro-particles in an alkaline soil, as examined in the current study, would largely influence the surface soil structure, especially near the air-filled pores (Del Campillo et al., 1992; Ramnarine et al., 2012). Therefore, it was further interesting to examine the morphological changes of the calcite materials due to a change in tillage operations at the experimental site. ESEM micrographs indicated that the carbonate nodules found in CT plots were slightly

more porous with smaller and thinner sizes of the needle-like structures than those found in NT plots (**Fig. 2**). These micro-morphological differences in particles could drive variable rates of calcite dissolution, and thus might affect the CO_2 evolution from soils.

The differential dissolution hypothesis was tested by re-examining the carbonate nodules through their EDX elemental analyses following a soil incubation experiment (as described in Section 2.4). The ratio of the intensities of Ca-K_α:Si-K_α spectra was used as the dissolution signature. Results indicated that NT treatment did not enable a significant dissolution of the carbonate nodules at both the temperatures (22° and 37°C) studied (**Fig. 3b & d**). Similarly, CT treatment also did not indicate any considerable carbonate dissolution at 22°C (**Fig. 3a**). In all these three cases as shown in Fig 4a, b, &d, Ca-K_α:Si-K_α ratios were about 9:1. Interestingly, such ratio was reversed with a value of 1:3 in the case of CT treatment (**Fig. 3c**), indicating a significant dissolution of the carbonate nodules in soils. Therefore, micro-morphological features of carbonate nodules found in the CT system (more porous, smaller and thinner needle-like structures), as stated earlier, might have favoured the carbonate dissolution at a semi-arid type temperature (37°C).

A second line of evidence behind the enhanced carbonate dissolution in soils under CT treatment at 37°C, and subsequent accumulation of Ca^{2+} in the incubated soils, was provided by the ICP-MS analysis of exchangeable cations in the incubated soil samples. These incubated soil samples were dominated by Ca^{2+} followed by Mg^{2+}, Na^{2+} and K^{+} in all the treatments (**Table 4**). The incubated soil samples of CT treatment at 37°C temperature showed a greater Ca:Mg ratio than that under NT treatments both for native and added amount of carbonates (**Table 4**). Additionally, higher Ca:Mg ratios at 37°C than 22°C were observed under CT treatment, which was another indication of carbonate dissolution as a result of the positive entropy change in the system. The elevated temperature might have

enhanced the surface reactions and mass transfer that was probably responsible not only for carbonate dissolution, but also for the subsequent C and Ca releases from those soils (Ahmad et al., 2015).

3.2 Effect of temperature and carbonate addition on C mineralisation

The cumulative soil C mineralisation (over 90 days incubation) was significantly ($p<0.05$) influenced by the change in tillage practices, and more pronounced results were observed at an elevated temperature (**Table 5).** The overall C mineralisation was comparatively higher under CT (20.1%) than NT (9.9%) system at 22°C and 37°C, respectively (**Table 5).** Contrarily, the control treatments (sand incubated with or without carbonate materials described as LS_1, LS_2, HS_1, HS_2 in **Table 3**) showed negligible emission of CO_2 at both the temperatures (22° and 37°C), indicating little to no-sign of carbonate dissolution. The difference in emissions from soils added with carbonate and mulch singly and as together, i.e., combined, indicated that carbonates could trigger C mineralisation after interacting with the crop residue when applied as mulch, otherwise carbonate itself might not undergo a significant dissolution. In particular, this interactive effect was found more profound in the soil samples of CT than NT system as shown in **Fig. 5**. The overall impact of temperature and $CaCO_3$ (0% w/w) on the mineralisation of soil C was non-significant. The CO_2 emission increased significantly ($p<0.05$) when the additional carbonate material (10% (w/w)) was added to the soil samples (**Fig. 4b, d**). Therefore, the added carbonates (10% w/w) resulted in a higher emission of CO_2 from the soil at 37°C than 22°C under both CT (47.8% emission) and NT (24% emission) systems **(Fig 5b, d)**. Particularly, the CO_2 emission from the soil samples treated with mulching and carbonates, i.e., (5 t ha^{-1} mulch + 10% w/w carbonates) were 51.3% and 38.4% in the CT and NT systems, respectively, due to the temperature rise (**Fig. 4b, d**).

313 The impact on mineralisation was more notable under the combined effect of mulch (5 t ha^{-1})
314 and carbonate (10% w/w) (**Fig. 4).** Results indicated that the added carbonate itself did not
315 contribute to CO_2 emission by dissolution **(Fig. 5)**, but the extra CO_2 produced from soil in
316 the presence of carbonates compared to its absence suggested a positive priming effect of
317 carbonates on SOM mineralisation. Some studies reported that crop residue return into soil
318 systems, particularly when incorporated in soil surface, has the potential to enhance the
319 decomposition of native SOM ("positive priming") via supporting the microbial activity
320 (Guenet et al., 2010; Qiu et al., 2016). Presumably, the extra CO_2 production could be
321 attributed to the increase in microbial activity and chemical hydrolysis of $CaCO_3$ caused by
322 an increase in carbonate, which might also increase the soil pH. Aye et al. (2017) suggested
323 that higher pH conditions facilitate positive C priming. It was also postulated by
324 Fontaine et al. (2003) that due to the difference in pH levels, there would be a difference in
325 the competition for the nutrient uptake and energy consumption between SOC and fresh
326 organic matter-degrading microorganisms, resulting in a difference in the priming effect. At
327 higher temperature conditions, carbonate dissolution may occur directly through changes
328 either in biotic/abiotic processes or indirectly through the products of SOC decomposition
329 (Ahmad et al., 2015).

330 The results of this study are in strong agreement with the report by Ahmad et al. (2014) who
331 found a significant positive correlation between the soil derived C at different temperature
332 conditions (20° and 40°C). Ahmad et al. (2014) also found that at 40°C the soil derived C
333 was increased by 59% more than the cumulative C release at 20°C. The release of CO_2 due to
334 the dissolution of carbonate sources, may depend on the rate of proton (H^+) addition in soils,
335 which is expected to be faster under intensive agriculture operations such as excessive tillage,
336 irrigation and application of heavy doses of nitrogenous fertilisers (Ahmad et al., 2015;
337 Suarez et al., 2000; Tamir et al., 2011). Ahmad et al. (2015) also reported that soil inorganic

carbon (SIC) stocks are dynamic, which change significantly with time and depend on climate, land-use change and other management practices.

In addition, the soil samples belonging to the CT system without the mulching treatment showed a higher C mineralisation rate (5.7% and 26% greater emission at 22 and 37°C, respectively) than the NT system, which might be due to the addition of carbonates (10% w/w) (**Fig. 4b, d**). These results might be occurred due to the priming effect caused by the addition of carbonates (10% w/w), which might have enhanced the decomposition of native SOM after having been exposed as a result of the CT system. The combined effect of mulching (5 t ha^{-1}) and carbonates (10% w/w) was observed through an increase in CO_2 emissions by 7.3% and 17.4% in the soil of the CT system at 22 and 37°C, respectively. According to Rangel-Castro et al. (2005) the microbial communities are more active in the limed soils resulting in the greater utilization of the plant exuded C compounds in the soil. Statistical analysis indicates a significant ($p < 0.05$) interactions occur only at 37°C between tillage x carbonate; tillage x carbonate x mulching (**Table 5**). The interactive relationship between SOM and carbonate hints about the change in carbonate equilibrium and microbial activity, which were caused due to the alteration in soil pH (Acosta-Martinez and Tabatabai, 2000; Bertrand et al., 2007). Saviozzi et al. (1999) also reported that much larger fraction of mineralized C was found under the CT system than NT, which may occur due to the higher potential of the microbes to oxidise the SOM.

The trends of the C mineralisation curves as shown in **Fig. 4a,b,c**,**d** are almost similar under all the treatments. Particularly, the trend indicated by the soil samples of the CT system treated with mulch (5 t ha^{-1}) and carbonates (10% w/w) at 37°C showed higher (i.e., 2621 mg CO_2-C Kg^{-1}) cumulative emission compared to all other set of treatments even with the samples placed at 22°C. Therefore, the assumption of carbonate dissolution cannot be

ignored, though it is not measured directly in this study, and it demands further investigation. The interaction between carbonates and SOM depends upon the presence of weak and strong acids released by SOM. The dissolution of carbonates by weak acids (carbonic acid) results in the sequestration of 1 mole of CO_2 Eq. (9), whereas dissolution by strong acids (HNO_3) results in the emission of 1 mole of CO_2 for each mole of carbonate Eq. (10) (Page et al., 2009).

$$CaCO_3 + H_2O + CO_2 \rightarrow Ca^{2+} + 2HCO_3^- \quad \textbf{……..(Eq. 9)}$$

$$CaCO_3 + 2HNO_3 \rightarrow Ca^{2+} + 2NO_3^- + H_2O + CO_2 \quad \textbf{……..(Eq. 10)}$$

Previous studies reported similar results and confirmed that soil organic acids are responsible for the dissolution of carbonates (Hamilton et al., 2007; Oh and Raymond, 2006). Some reports also emphasised that CO_2 evolved from soil during such incubation experiments could be caused by the change in biological activities as well as from the acidification effect, i.e., through strong acids (Jia et al., 2006; Wang et al., 2010). Tamir et al. (2011) reported that dissolution of carbonates could contribute up to 30% in the total CO_2 emission. According to the US EPA, carbonate dissolution is the net source of CO_2 emission when applied as lime on agricultural fields (EPA, 2016). US EPA estimated that about 62% of the $CaCO_3$ is dissolved by carbonic acid (H_2CO_3), and 38% of $CaCO_3$ is dissolved by nitric acid (HNO_3).

3.3 Carbon mineralisation dynamics

The C mineralisation kinetics was studied by non-linear regression analyses of the evolved CO_2-C from the different treatment samples using (Eq. 6 and 7). The two-component first-order decay equation gave a better fit for all the C decomposition data compared to the single component first-order decay model **(Table 6)**. The correlation coefficient (R^2) values of model fitting were higher (i.e., R^2>0.98) in all the cases of the two-component model than the single component first-order model (R^2 = 0.97 to 0.98) (**Table 6**). The sizes of both the decomposition pools, i.e., C_2 (easily mineralisable, i.e., labile) and C_3 (recalcitrant) as

387 estimated by Eq. (7), were very different from each other, where easily mineralisable C (C_2)
388 was significantly greater than the slowly decomposing C fraction (C_3) **(Table 6**). Specifically,
389 the decomposing fractions of C_2 from the soil samples of the CT system were higher at 37°C
390 than 22°C. Resulting with an increase of 43.5% in C_2 from the control samples (i.e., C_1 and
391 C_5) at 37°C followed by 29.2% from C_4 and C_8 (combined treatment, i.e., 5 t ha^{-1} + 10%
392 (w/w) $CaCO_3$), 27.9% from C_3 and C_7 (mulch only treatment, i.e., 5 t ha^{-1}) and 16.5% from
393 C_2 and C_6 (added 10% (w/w) carbonate only treatment) than at 22°C (**Table 6 and Fig. 6)**.
394 Notably, the half-life values of the easily mineralisable decomposing fractions as estimated
395 from the two-component kinetic model ranged from 6 to 12 days and 5 to 7 days in both the
396 tillage systems at 22°C and 37°C, respectively (**Table 5**). These results indicated that under
397 the CT system at a higher temperature, microbial attack on C substrates (mulch and
398 carbonates) was more prominent than the NT system. The rate constant (k_2) was
399 proportionally opposite of the predicted half-life value under respective treatments, i.e.,
400 tillage, mulch and temperature **(Table 6).** Soil samples incubated with carbonate (10% w/w)
401 significantly increased the fractions of readily mineralisable organic matter, i.e., C_2, resulting
402 likely from the chemical hydrolysis of carbonate, which increased the microbial activity and
403 C mineralisation (Fuentes et al., 2006; Neale et al., 1997). The half-life values at 22°C under
404 NT system were comparatively higher than CT, indicating that the soils of NT system would
405 sequester more SOC than CT soil despite of different soil environmental conditions.

406 **3.4 Effect of incubation temperature and carbonate addition on microbial biomass**
407 **carbon (MBC)**

408 Microbial biomass C (MBC) was measured after the termination of the incubation, and it was
409 found that MBC decreased significantly ($p < 0.05$) when the temperature of incubation
410 increased from 22°C to 37°C **(Table 7)**. The decrease in MBC in CT (42.3%) system was
411 more than under NT (36.2%) system at 22°C compared to at 37°C. In particular, at 37°C

MBC in the samples incubated with carbonates (0% and 10%) was lower by 26.4% and 46.6%, respectively, than the MBC of samples at 22°C. It was reported by some of the studies that the application of carbonate materials to soil changes the soil microbial biomass, dynamics and diversity (Acosta and Tabatabai, 2000; Sherrod et al., 2005). Statistically, the mulching (5 t ha^{-1}) treatment had significant ($p<0.05$) impact on the soil MBC at both the temperatures compared to the un-mulched treatment (**Table 7** and **Fig. 7).** The overall MBC after termination of the incubation experiment was lower by 47.6 and 30.5% under un-mulched and mulched conditions, respectively, at 37°C than 22°C temperature (**Table 7**). These results apparently suggested that higher temperature conditions would have suppressed the microbial growth. However, the lower MBC at the terminal stage of incubation might occur due to the more rapid substrate depletion, and low substrate availability than at the early stage at higher temperature conditions, which perhaps was not sufficient to support the microbial growth at the terminal stage (Li et al., 2015). Schimel and Mikan (2005) reported that higher temperature conditions were responsible for the shifts in microbial community. Zogg et al. (1997) reported that dominating microbial populations had a greater ability to metabolise the substrates at a higher temperature, but they did not use these substrates at a lower temperature. Previous reports suggested that microbial biomass is the principal source of soil enzymes, which are comparatively higher in the soil from cooler and wetter regions than warmer and dry regions (Allison, 1973; Spain et al., 1983). Therefore, further research is needed to unravel the effect of carbonate addition and mulching on the alternation of soil microbial communities and their substrate use preferences under various temperature and tillage regimes.

A significant ($p<0.05$) interaction (tillage x mulching x carbonate) effect was observed for the MBC only at lower temperature (22°C). No significant interaction effect was observed in the soil samples applied with 10% (w/w) carbonates incubated at 37°C (Table 7). These

results might be attributed to the increased soil pH resulting from the chemical hydrolysis of $CaCO_3$. At a higher soil pH, the proton consumption capacity increases the soil metabolism that creates a favourable condition for prokaryotes to grow, but limits the fungal growth (Bertrand et al., 2007; Haynes and Mokolobate, 2001). Some researchers also reported an increase in MBC due to an increase in the soil carbonate content (Bezdicek et al., 2003; Fornara et al., 2011), while contrasting results were reported by Biasi et al. (2008) indicating no effect of carbonate addition on soil MBC.

3.5 Hydrophobic and hydrophilic components

The FTIR analysis results of the incubated soil samples indicated that functional groups in NT soils were significantly ($p<0.05$) dominated by hydrophobic components as compared to CT soils (**Fig. 8**). The hydrophobic components of SOM were lower by 19.3% in the incubated soils of the CT system than NT system (**Fig. 8a**). The difference in hydrophilic components of SOM from these two tillage systems were statistically non-significant ($P>0.05$) **(Fig. 8).** Capriel (1997) previously reported that poor agricultural management practices, such as extensive tillage practices, might decrease the organic C content accompanied by a decline in hydrophobicity, and cause a decrease in microbial biomass and soil aggregate stability.

In this study, the effect of carbonate addition (10% w/w) to soils on the selected hydrophobic and hydrophilic component bands could not be resolved as such from the respective FTIR spectra. The IR signals of the added carbonate were extremely strong as opposed to the organic component bands, which made the separation of hydrophobic and hydrophilic components challenging in the carbonate-added soils. However, the mulching treatment had a significant ($p<0.05$) effect on the hydrophobic and hydrophilic components of the soil. Results showed that mulched soils had higher intensities of hydrophobic (15%) than hydrophilic (12.8%) components, and both these contents were higher than un-mulched

conditions (**Fig. 8a,b)**. A significant ($p<0.05$) interactive effect of tillage and mulching treatments on the hydrophobic and hydrophilic components of soils was also observed. These results are consistent with previous findings that the applied organic inputs (e.g., cellulose, hemicellulose, proteins, lignin and lipids) in the form of crop residues would predominantly contain hydrophobic components which undergo a lesser microbial decomposition than hydrophilic components (e.g., cellulose, hemicellulose, proteins).

Statistical analysis also indicated a significant ($p<0.05$) interactive effect (tillage x mulch x temperature) among treatments for the hydrophobic components of the soil samples. These results further highlight that, irrespective of the higher hydrophobic components in the crop residue, at lower temperature the hydrophobic component became more sensitive to tillage and mulching practices than higher temperature. Spaccini et al. (2002) concluded that when hydrophobic group-rich substances (aliphatic (C–H) groups) were applied during soil management practices, it improved the biological stability of SOM (Rumpel and Kögel, 2011), and thus mitigated CO_2 emissions from agricultural soils. Results confirmed that the soil of the present study is rich in soil hydrophobic components (aliphatic (C–H) groups) and played an important role in the accumulation of organic material in the soil particles, confirmed the findings of others (Harper et al., 2000; Kubát and Lipavský 2006; McKissock et al., 2003; Piccolo and Mbagwu 1999; Šimon et al., 2009).

3.6 Conclusions

The tillage shift from the NT to CT showed the potential to alter the soil organic C dynamics and morphology of naturally occurring carbonate nodules in soils. Unlike NT, the CT system showed a positive priming effect for the mineralisation of SOM. The overall rate of C mineralisation was higher under the CT than NT system at both 22°C (by 20.1%) and 37°C (by 9.9%) temperatures. Similar trends of C mineralisation in response to temperatures were

observed under both the mulched and un-mulched conditions, and with or without carbonate addition to the soil.

The decomposing pool of SOM under NT system had a higher half-life value than the CT system; these values were higher at 22°C than 37ºC. Therefore, the high temperature condition exacerbated the microbial activity with more prominent effect under the CT than NT system. This concurrently decreased the MBC contents under the CT system by 43.9 and 46.9% than the NT system when soils were treated with mulch (5 t ha^{-1}) and carbonates (10% w/w), respectively. When considering the functional group based characterisation of SOM, only hydrophobic components were significant, and they were found 19% lower in the soils of the CT than NT system.

Future investigation is needed using isotopically labelled lime ($CaCO_3$) in order to distinguish CO_2 emissions either from the organic or inorganic pools of soil C. Furthermore, in-situ experiments at multiple locations are needed to phase out the temporal and spatial variabilities in such research.

Abbreviations

C – Carbon

C_{cum} - Cumulative CO_2 emission

C_{min} - Cumulative amount of CO_2-C mineralised after time t

CT – Conventional tillage

ESEM - Environmental Scanning Electron Microscope

ETD - Everhart–Thornley Detector

EDXA - Energy dispersive X-ray analysis

FTIR - Fourier transform Infrared

ICP-OES - Inductively coupled plasma optical emission spectroscopy

MBC - Microbial biomass carbon

NT – Not-tillage

OM - Organic matter

SOM – Soil Organic matter

SOC - Soil organic carbon

SIC – Soil inorganic Carbon

XRD - X-ray diffraction

Acknowledgements

The first author is thankful to the University of South Australia, Future Industry Institute for providing a scholarship to carry out the PhD (2013-2017) research work. The authors also thank the Commonwealth Government of Australia for providing funding under the Research Training Program (RTP) of the Department of Education and Training in support of PhD research.

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Figures

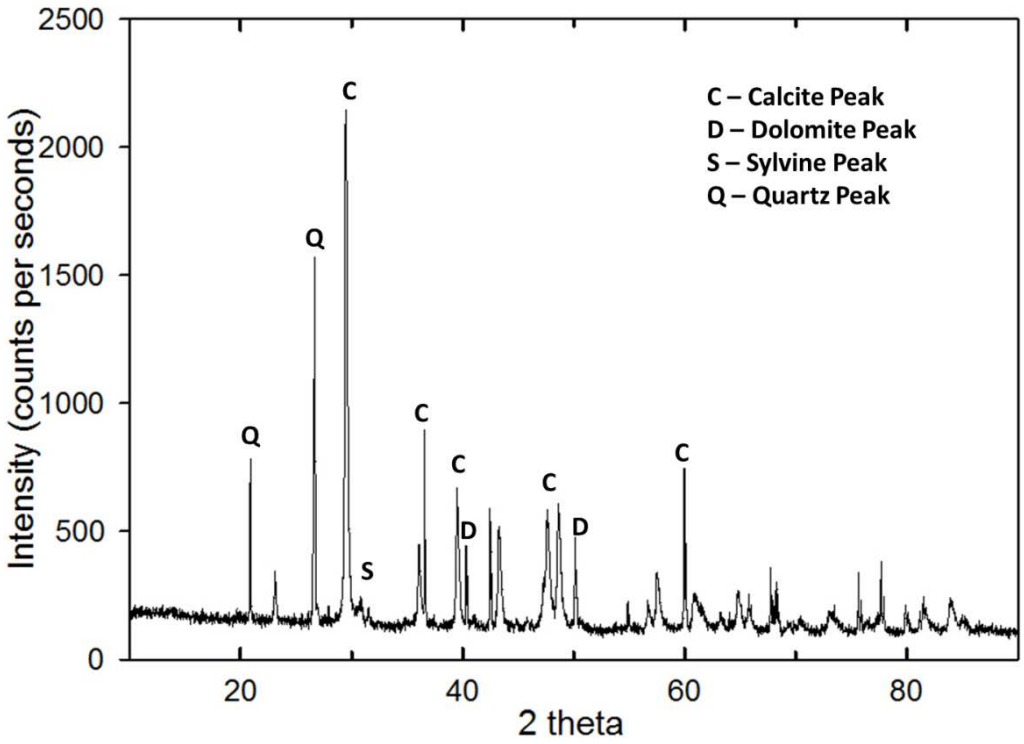


Fig. 1 **X-ray diffraction pattern of naturally available carbonate material identified as predominantly calcite ($CaCO_3$) mineral.**

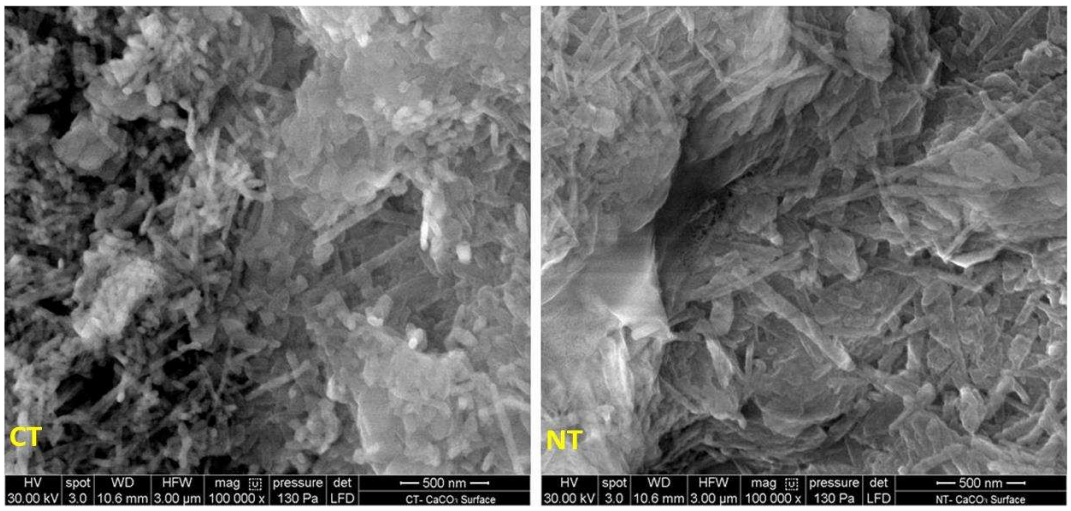


Fig. 2 **ESEM images of incubated carbonate ($CaCO_3$) nodules from mulch-amended soils at 500 nm resolution) under CT and NT systems.**

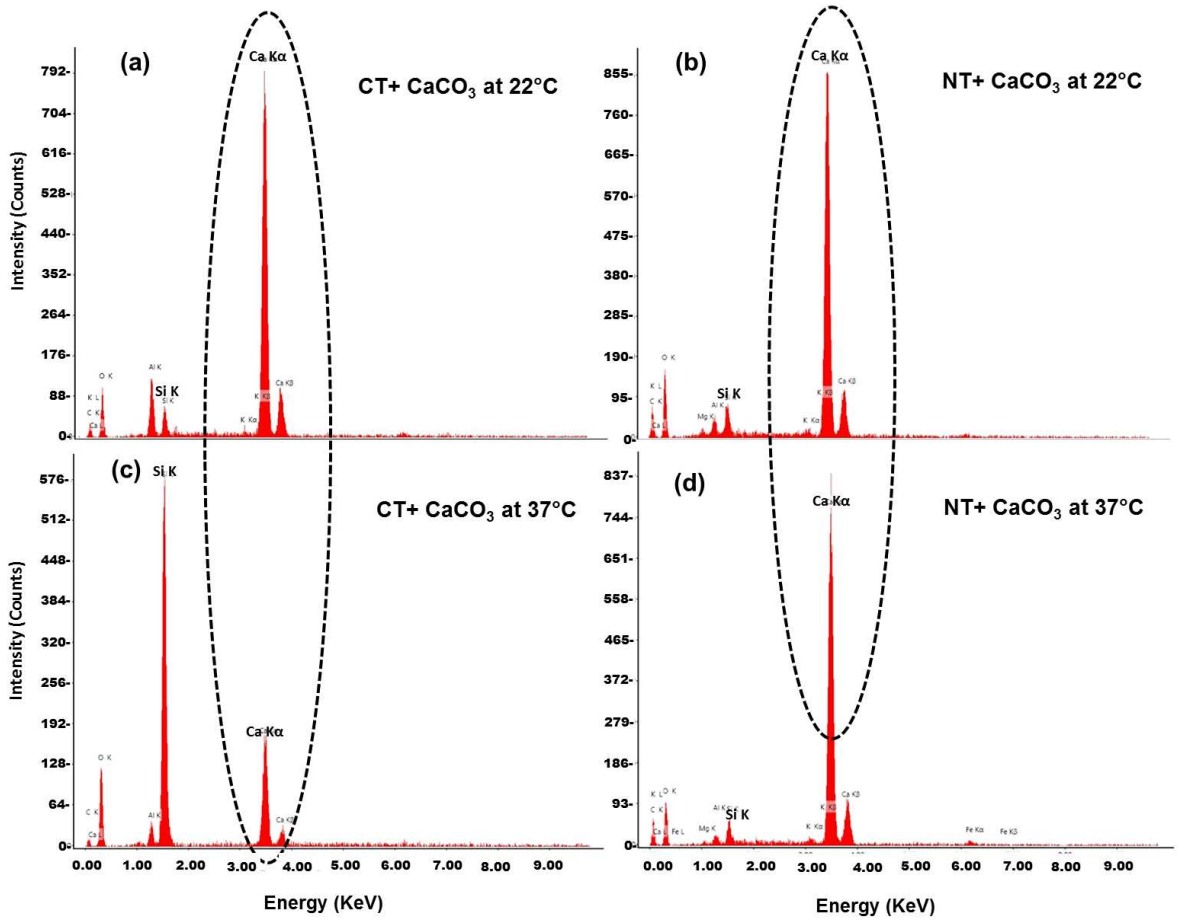


Fig. 3 EDX spectra of carbonate ($CaCO_3$) nodules incubated at 22°C (top row) and 37°C (bottom row) with mulch amended soils under CT and NT systems.

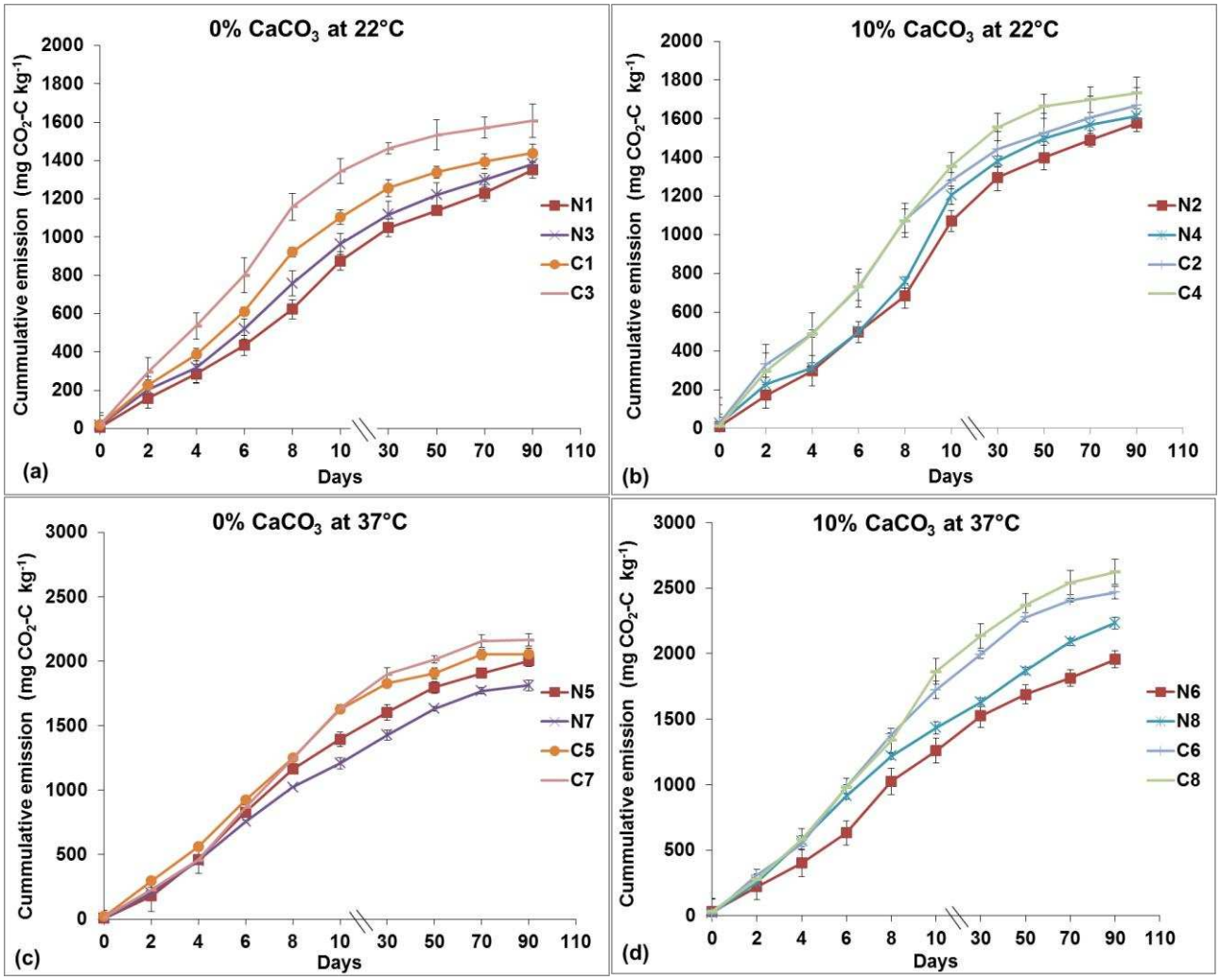
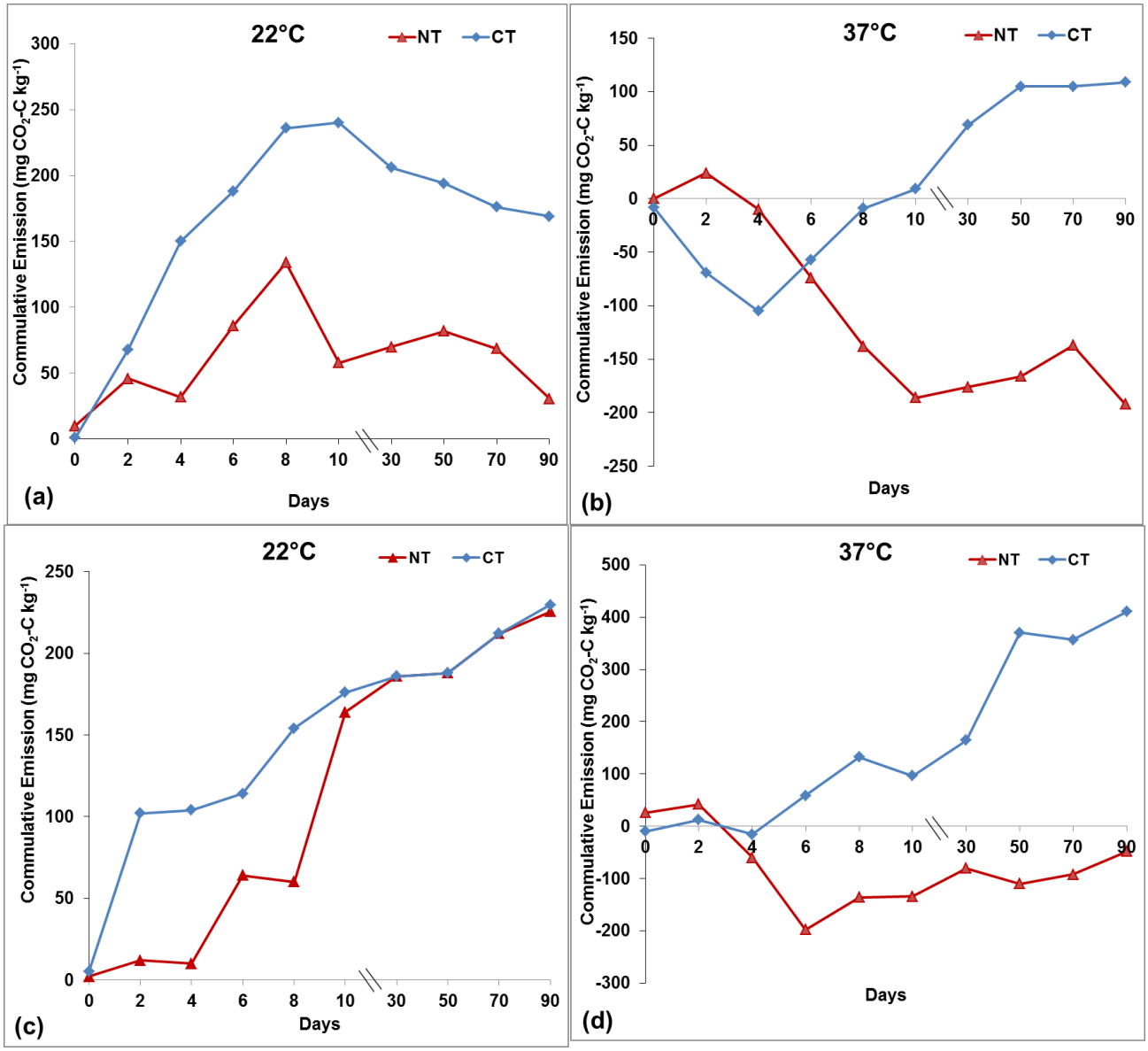


Fig. 4 **Cumulative CO_2-C emissions from soils amended with carbonates (10% w/w) and mulch (5 t ha^{-1}) compared to controlled conditions at different temperatures (22 and 37°C) under different tillage systems. Error bars represent standard errors of means, n = 3 (after subtracting the cumulatively released CO_2–C in respective treatment from the control).** Treatment symbols are described in Table 2.



743

744 **Fig. 5 Differences in cumulative CO_2-C emissions at 22 and 37°C between soils with**
745 **added treatments showing the [(a, b) mulching impact; (c, d) carbonates impact]**
746 **and the control (soil alone)** (NT (no-tillage) and CT (conventional tillage)).

747

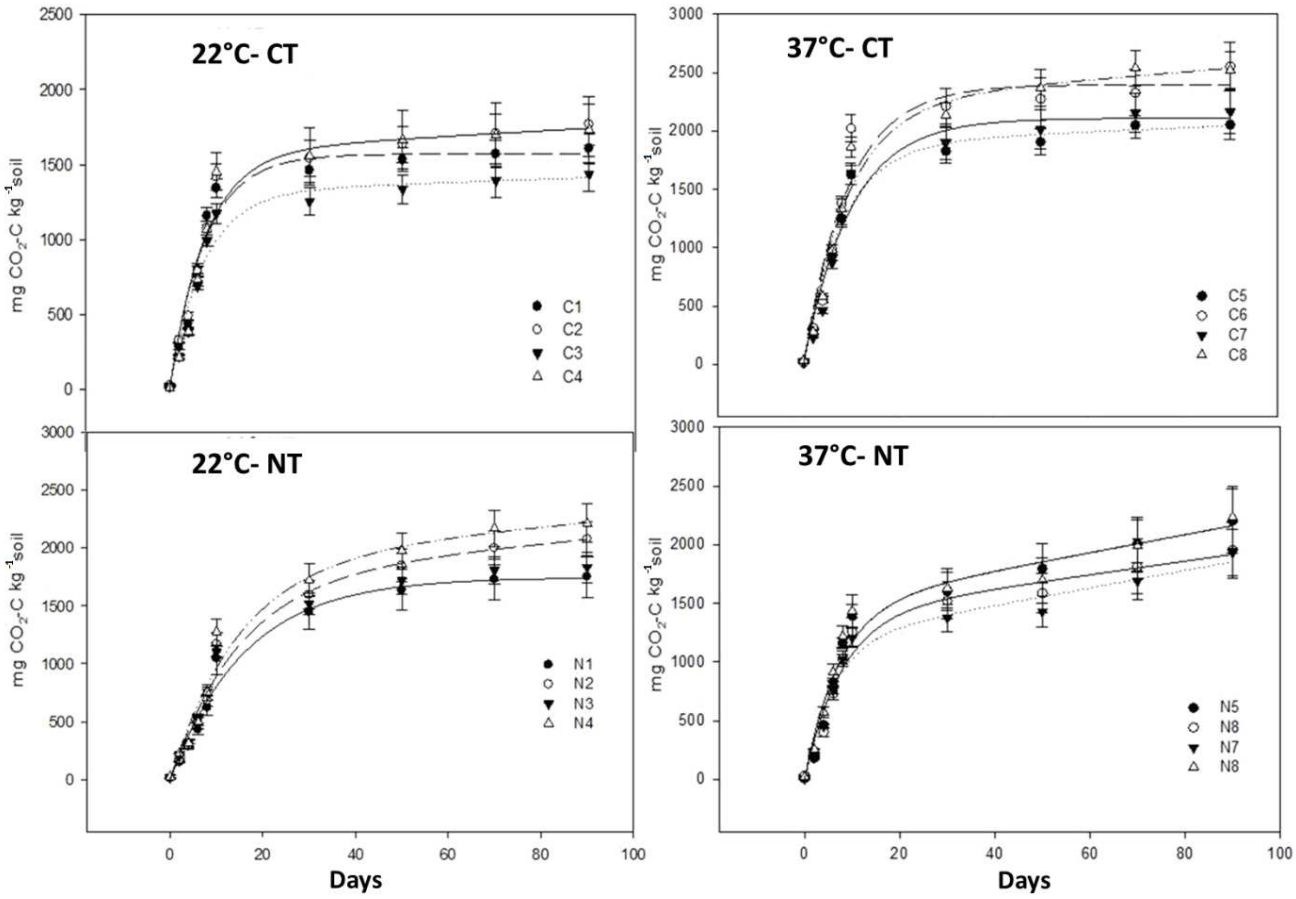


Fig. 6 **The carbonate effect on the cumulative decomposition of SOM present in the soils of different tillage system as best described by two-component first-order exponential (Eq. 7).** (Treatment symbols are described in Table 2 which are under NT (no-tillage) and CT (conventional tillage) system. Vertical bars are standard errors; n = 3)

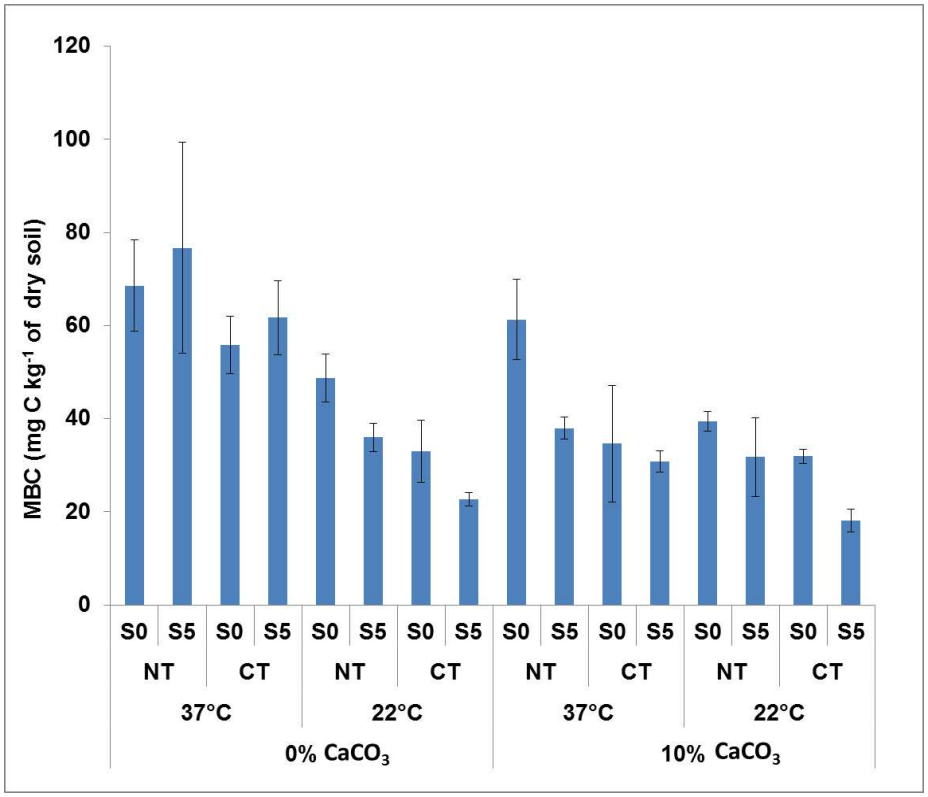
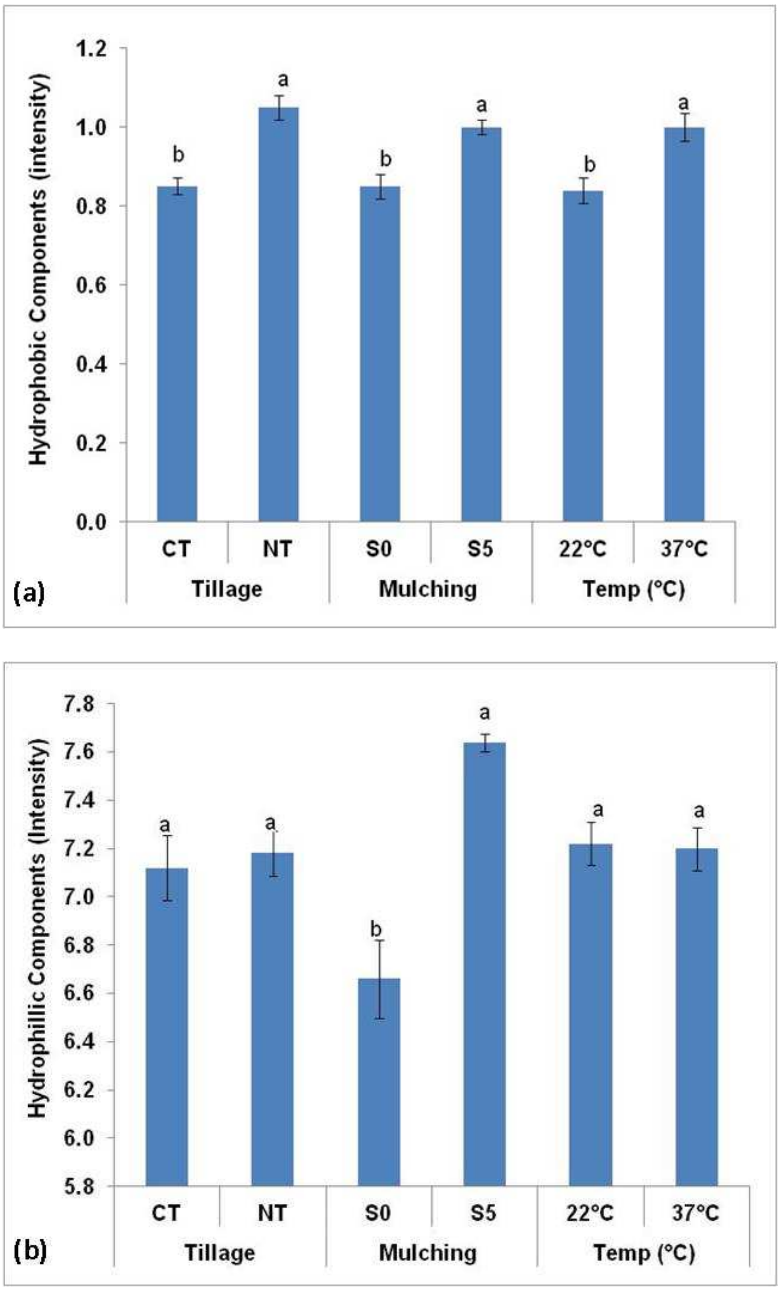


Fig. 7 **Changes in soil microbial biomass carbon (MBC) after 90 days of incubation. Tillage systems (CT= Conventional tillage; NT= No-tillage) treated with different levels of mulch (S0= 0 t ha^{-1}; S5= 5 t ha^{-1}) and carbonates (0% and 10% w/w)** (Vertical bars on columns indicate standard errors).



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775 **Fig. 8** **Average FTIR spectral intensities for (a) hydrophobic and (b) hydrophilic organic**
776 **components in soil samples. Intensities are derived from 3000 to 2800 cm^{-1} and 1740 to**
777 **1600 cm^{-1} area of the IR absorption bands for hydrophobic and hydrophilic groups,**
778 **respectively** (Columns indicated by same letters do not differ significantly ($P<0.05$).
779 Vertical bars on columns indicate standard errors).

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Tables

Table 1. Baseline data (year 2013, before tillage shift) for the soil physical and chemical properties (0–30 cm) of the experimental site

Properties	Unit	Total number of samples (n)	Mean ± SD*
Soil Texture		6	Sandy clay Loam
Sand	%		64.4
Silt	%		8.1
Clay	%		27.5
pH		54	7.74 ± 0.62
Electrical conductivity (EC)	dS m^{-1}	54	0.255 ± 0.91
Bulk density (DB)	gcm^{-3}	54	1.45 ± 0.32
$CaCO_3$	%	6	17.68 ± 0.05
Total N	%	54	0.23 ± 0.28
Total organic carbon (TOC)	%	54	1.68 ± 0.12
Cation exchange capacity (CEC)	cmol kg^{-1}	6	28 ± 0.02

*SD: standard deviation

787 **Table 2 Soil physical and chemical properties of the experimental soil (0–30 cm) during the 2014-2015 experimental year**

Tillage	Depth	Treatment	TC (%)	TOC (%)	BD (gm cm^{-3})	pH	Carbon Stock(t ha^{-1})
CT	0-10	No mulch	1.34 ± 0.14	1.43 ± 0.07	1.18 ± 0.56	6.65 ± 0.03	1.69 ± 0.10
		Mulch[§]	1.38 ± 0.27	1.35 ± 0.18	1.11 ± 0.33	7.46 ± 0.07	1.51 ± 0.28
	10-20	No mulch	1.39 ± 0.16	1.33 ± 0.24	1.42 ± 0.52	7.49 ± 0.07	1.90 ± 0.43
		Mulch	1.38 ± 0.18	1.21 ± 0.17	1.33 ± 0.19	6.96 ± 0.10	1.60 ± 0.11
	20-30	No mulch	1.12 ± 0.07	0.81 ± 0.09	1.55 ± 0.50	7.11 ± 0.06	1.26 ± 0.19
		Mulch	1.12 ± 0.03	1.00 ± 0.02	1.54 ± 0.61	7.97 ± 0.08	1.54 ± 0.07
NT	0-10	No mulch	1.06 ± 0.09	0.83 ± 0.12	1.58 ± 0.95	7.55 ± 0.03	1.31 ± 0.21
		Mulch	1.04 ± 0.06	0.94 ± 0.11	1.57 ± 0.11	7.78 ± 0.03	1.48 ± 0.15
	10-20	No mulch	0.74 ± 0.14	0.85 ± 0.20	1.55 ± 0.07	8.05 ± 0.05	1.32 ± 0.35
		Mulch	0.94 ± 0.04	0.72 ± 0.17	1.52 ± 0.38	8.59 ± 0.06	1.09 ± 0.28
	20-30	No mulch	0.85 ± 0.12	0.78 ± 0.10	1.36 ± 0.03	7.88 ± 0.06	1.06 ± 0.16
		Mulch	0.96 ± 0.04	0.93 ± 0.04	1.32 ± 0.51	8.43 ± 0.02	1.23 ± 0.06

788 *Values *given after* '±' are standard deviation values, (n=3); [§]Mulch was applied at 5 t ha^{-1} rate in all cases.

Table 3 Treatment symbols of samples incubated at different temperatures

Temperature	Symbol	Treatment Combinations
22°C	**LS_1**	Sand (50 g)
	LS_2	Sand + $CaCO_3$ (10%)
	N_1	NT + M (0 t ha^{-1})+ $CaCO_3$ (0% (w/w))
	N_2	NT + M (0 t ha^{-1})+ $CaCO_3$ (10% (w/w))
	N_3	NT + M (5 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	N_4	NT + M (5 t ha^{-1})+ $CaCO_3$ (10% (w/w))
	C_1	CT + M (0 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	C_2	CT + M (0 t ha^{-1}) + $CaCO_3$ (10% (w/w))
	C_3	CT + M (5 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	C_4	CT + M (5 t ha^{-1}) + $CaCO_3$ (10% (w/w))
37°C	**HS_1**	Sand (50 g)
	HS_2	Sand + $CaCO_3$ (10%)
	N_5	NT + M (0 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	N_6	NT + M (0 t ha^{-1}) + $CaCO_3$ (10% (w/w))
	N_7	NT + M (5 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	N_8	NT + M (5 t ha^{-1}) + $CaCO_3$ (10% (w/w))
	C_5	CT + M (0 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	C_6	CT + M (0 t ha^{-1}) + $CaCO_3$ (10% (w/w))
	C_7	CT + M (5 t ha^{-1}) + $CaCO_3$ (0% (w/w))
	C_8	CT + M (5 t ha^{-1}) + $CaCO_3$ (10% (w/w))

LS1– Sand incubated at low temperature (22°C); LS2– Sand incubated with $CaCO_3$ with 10% (w/w) at 22 °C; HS1– Sand incubated at high temperature (37°C); HS2– Sand incubated with $CaCO_3$ with 10% (w/w) at 37°C; NT– No-tillage soil; CT– conventional tillage soil; M – Mulching @ 0 t ha^{-1} (control) and 5 t ha^{-1}; $CaCO_3$ @ 0% and 10% (w/w)

Table 4 Ratio of exchangeable calcium to exchangeable magnesium from the incubated soil samples under different tillage and temperature conditions

Temperature	Tillage	$CaCO_3$ (% w/w)	Exchangeable Cation Percentage*				Exchangeable Ratio Ca:Mg
			Na	Mg	K	Ca	
22°C	CT	0	0.003	0.026	0.009	0.193	7.5 (0.054/0.007)¶
		10	0.002	0.024	0.012	0.125	5.1 (0.035/0.007)
	NT	0	0.003	0.028	0.009	0.197	7.1 (0.055/0.008)
		10	0.003	0.027	0.008	0.182	6.6 (0.051/0.008)
37°C	CT	0	0.003	0.026	0.010	0.212	8.1 (0.060/0.007)
		10	0.002	0.025	0.013	0.147	10.6 (0.074/0.007)
	NT	0	0.003	0.028	0.010	0.266	5.3 (0.041/0.008)
		10	0.003	0.027	0.010	0.224	8.2 (0.063/0.008)

*Exchangeable Cation Percentage is the ratio of exchangeable cation (cmol (p^+) kg^{-1}) divided by CEC of the soil (28 cmol (p^+) kg^{-1}; Table 1).

¶Number in parenthesis are ratio of exchangeable Ca^{2+} and Mg^{2+} in cmol (p^+) kg^{-1}.

Table 5 Cumulative release of CO_2–C during the incubation of soil with and without carbonates at two different temperatures (22 and 37°C) (after subtracting the cumulatively released CO_2–C under treatments from the control)

Description		Cumulative emission (mg CO_2-C kg^{-1})		LSD (P<0.05)	
		22°C	37°C	22°C	37°C
Tillage (A)	CT	1965.0 (14.2)	2286.3 (14.5)	101.1	131.5
	NT	1636.1 (15.8)	2081.3 (24.4)		
Mulching (B) ($t\ ha^{-1}$)	0	1702.2 (14.0)	2154.8 (18.6)	120.5	133.4
	5	1889.9 (16.0)	2212.9 (20.3)		
Carbonate (C) (% w/w)	0	1637.1 (13.1)	2088.7 (19.5)	164.5	142.5
	10	1964.1 (16.9)	2278.9 (19.4)		
A*B				NS	NS
A*C				NS	201.6
B*C				NS	NS
A*B*C				NS	126.5

Numbers in parentheses are the standard errors of the means; n = 3; P<0.05

Table 6 Parameters of single and two components first-order exponential decay equations describing the decomposition data (C_{min} = cumulative CO_2-C mineralised (mg C kg^{-1} soil); C_1 = potentially mineralisable C (mg C kg^{-1} soil) recovered at hour 0; C_2 = easily (rapid) decomposable carbon (mg C kg^{-1} soil); C_3 = the second slower decomposition pool (mg C kg^{-1} soil); k_1, k_2 and k_3 are rate constants (per day), and $t_{1/2}$ = time (days); (Values in parentheses are standard error, n = 3).

Temp (°C)	Treatment code	Single component-first order model		Two component first-order model					
		K_1	R^2	C_2	K_2	C_3	K_3	R^2	$t_{1/2}$ (days)
22°C	N1	0.061 (0.007)	0.978	952.12 (147.92)	0.06 (0.09)	792.82 (167.09)	0.06 (0.22)	0.989	12
	N2	0.064 (0.012)	0.972	1241.18 (221.49)	0.06 (0.06)	317.23 (146.09)	0.18 (0.20)	0.990	12
	N3	0.068 (0.026)	0.979	1102.31 (134.11)	0.06 (0.05)	975.68 (115.45)	0.06 (0.76)	0.989	12
	N4	0.056 (0.011)	0.971	1189.59 (295.13)	0.06 (0.08)	104.29 (106.68)	0.57 (0.24)	0.988	12
	C1	0.129 (0.016)	0.978	1316.33 (409.74)	0.10 (0.07)	126.11 (161.52)	0.59(0.30)	0.980	7
	C2	0.133 (0.014)	0.978	1752.76 (105.36)	0.11 (0.39)	327.84 (105.84)	0.13 (0.21)	0.981	6
	C3	0.120 (0.013)	0.978	1582.77 (174.88)	0.11 (0.06)	305.25 (106.12)	0.81 (0.20)	0.985	6
	C4	0.094 (0.013)	0.981	1681.87 (214.41)	0.12 (0.06)	385.04 (106.05)	0.24 (0.31)	0.985	6
37°C	N5	0.937 (0.011)	0.977	1472.15 (262.84)	0.12 (0.07)	157.71 (173.12)	0.87(0.05)	0.986	6
	N6	0.094 (0.013)	0.981	1502.02 (261.17)	0.13 (0.07)	162.11 (161.06)	0.07(0.06)	0.986	5
	N7	0.093 (0.014)	0.977	1395.14 (304.20)	0.13 (0.07)	368.90 (142.13)	1.17 (0.06)	0.987	5
	N8	0.104 (0.017)	0.970	1586.06 (279.81)	0.16 (0.09)	569.30 (161.03	0.31 (0.03)	0.985	4
	C5	0.115 (0.013)	0.986	1888.25 (345.87)	0.10 (0.05)	159.86 (185.04)	0.09 (0.25)	0.996	7
	C6	0.100 (0.015)	0.982	2041.17 (134.48)	0.12 (0.06)	338.12(159.97)	0.10 (0.19)	0.995	6
	C7	0.095 (0.011)	0.984	2024.45 (145.48)	0.10 (0.06)	373.91(159.21)	0.10(0.08)	0.996	7
	C8	0.106 (0.016)	0.976	2172.59 (208.77)	0.14 (0.06)	437.33(198.40)	0.11(0.04)	0.992	5

Table 7 Effect of temperature on soil microbial biomass carbon at the end of incubation (90 days) as influenced by carbonate and mulching treatments under different tillage systems

Description		Microbial Biomass Carbon (mg C kg^{-1})		LSD ($p<0.05$)	
		22°C	37°C	22°C	37°C
Tillage (A)	CT	45.74 (2.22)[§]	26.41 (2.17)	6.55	8.48
	NT	61.12 (2.50)	38.99 (1.27)		
Mulching (B) ($t\ ha^{-1}$)	0	51.77 (2.45)	27.12 (1.49)	NS[≠]	6.18
	5	55.10 (2.27)	38.28 (1.95)		
Carbonate (C) (% w/w)	0	41.16 (2.71)	30.29 (1.69)	8.07	6.44
	10	65.70 (2.01)	35.11 (1.74)		
Interactions	A*B			4.55	5.64
	A*C			NS	NS
	B*C			3.31	NS
	A*B*C			10.48	NS

[§]Numbers in parentheses are the standard errors of the means (n=3); [≠] NS: Not significant.