Temperature responsiveness of soil carbon fractions, microbes, extracellular enzymes and CO₂ emission: mitigating role of texture

Waseem Hassan¹², Yu’e Li², Tahseen Saba³, Jianshuang Wu², Safdar Bashir⁴, Saqib Bashir⁴, Mansour K. Gatasheh⁵, Zeng-Hui Diao⁶ and Zhongbing Chen⁷

¹ Landwirtschaftlich-Gärtnerischen, Humboldt-Universität zu Berlin, Berlin, Germany
² Institute of Environment and Sustainable Development in Agriculture/Laboratory for Agricultural Environment, Ministry of Agriculture and Rural Affairs, Chinese Academy of Agricultural Sciences, Beijing, China
³ College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan, China
⁴ Department of Soil and Environmental Sciences, Ghazi University, Dera Ghazi Khan, Dera Ghazi Khan, Pakistan
⁵ Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia
⁶ School of Environmental Science and Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou, China
⁷ Department of Applied Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Praha-Suchdol, Prague, Czech Republic

ABSTRACT

The interaction of warming and soil texture on responsiveness of the key soil processes i.e. organic carbon (C) fractions, soil microbes, extracellular enzymes and CO₂ emissions remains largely unknown. Global warming raises the relevant question of how different soil processes will respond in near future, and what will be the likely regulatory role of texture? To bridge this gap, this work applied the laboratory incubation method to investigate the effects of temperature changes (10–50 °C) on dynamics of labile, recalcitrant and stable C fractions, soil microbes, microbial biomass, activities of extracellular enzymes and CO₂ emissions in sandy and clayey textured soils. The role of texture (sandy and clayey) in the mitigation of temperature effect was also investigated. The results revealed that the temperature sensitivity of C fractions and extracellular enzymes was in the order recalcitrant C fractions > stable C fractions > labile C fractions and oxidative enzymes > hydrolytic enzymes. While temperature sensitivity of soil microbes and biomass was in the order bacteria > actinomycetes > fungi ≈ microbial biomass C (MBC) > microbial biomass N (MBN) > microbial biomass N (MBP). Conversely, the temperature effect and sensitivity of all key soil processes including CO₂ emissions were significantly (P < 0.05) higher in sandy than clayey textured soil. Results confirmed that under the scenario of global warming and climate change, soils which are sandy in nature are more susceptible to temperature increase and prone to become the CO₂-C sources. It was revealed that clayey texture played an important role in mitigating and easing off the undue temperature influence, hence, the sensitivity of key soil processes.
INTRODUCTION

The world’s soils store substantially more carbon (C) than is present in the atmosphere (Badgery et al., 2020; Paustian et al., 2019). The estimated global soil C pool at one-meter depth is >1,500 GT and two-meter depth is >2,500 GT, which is 3.2 and four times the size of combined atmospheric and biotic C pool (Zomer et al., 2017). Being a gigantic pool, terrestrial C is receiving increasing attention both as a potentially large and uncertain source of CO₂ and also as a natural sink to reduce atmospheric CO₂ (Badgery et al., 2020; Zomer et al., 2017). It has been estimated that soils emit ≥11 times CO₂-C than fossil fuel combustion which is roughly about 68–100 Pg y⁻¹ (Zhang & Zhou, 2018). Conversely, even a 0.4% annual increase in soil C has the potential to significantly halt the yearly atmospheric CO₂ increase (Amundson & Biardeau, 2019). Therefore, it is of utmost importance to examine soil C and its divergent fractions, and their likely sensitivity and response towards temperature increase for future feedbacks and predictions.

Due to continuous movement in the soil systems, soil C is constantly disintegrating and changing into divergent pools (Yang et al., 2021; Zomer et al., 2017). The three major pools of soil C are recalcitrant C pool (RCP), labile C pool (LCP), and stable C pool (SCP) respectively (Zhang & Zhou, 2018). The LCP is composed of newly incorporated plant residues, amino acids, simple carbohydrates, root exudates, and simple C fractions (Lian et al., 2018). Whereas, RCP is made of detritus, decomposed plant and microbial byproducts, and C fractions e.g., recalcitrant organic carbon (ROC) which is resistant to decomposition (Zhang & Zhou, 2018). Whereas, the total organic C (TOC) which is a heterogeneous mixture of diverse compounds (e.g., residues, humin, humic acid, aromatic and hydrophobic compounds) with several hundred years of a mean age and accounts for 90% of stable fraction is also known as SCP (Lian et al., 2018). These C fractions are of utmost importance, owing to their direct and strong role in soil structure, C cycling and production and fluxes of CO₂ (Badgery et al., 2020; Zomer et al., 2017). The C fractions are extremely susceptible to abiotic variables, and multiple earlier studies have demonstrated that the future C balance of terrestrial ecosystems is highly dependent on the consequences of global warming (Wang et al., 2016; Qi et al., 2016; Biswas et al., 2018). Qi et al. (2016) observed a significant reduction in soil labile organic C fractions in response to warming, while Karhu et al. (2010) reported a decline in stable organic carbon fractions. Nonetheless, the temperature sensitivity of C fractions is a highly controversial and vague topic to date (Davidson & Janssens, 2006; Sierra, Malghani & Loescher, 2017). Therefore, it is the need of the day to quantify and establish the temperature sensitivity of C fractions of labile, recalcitrant, and stable pools.

Soil microbes i.e., bacteria, fungi, and actinomycetes, owing to their vast metabolic diversity play diverse and critical roles in all-major biogeochemical cycles and ecosystem services (Walker et al., 2018; Nottingham et al., 2019). They also play a key role in
regulating the C decomposition, emission of CO\textsubscript{2}, and overall C cycle of the ecosystem (Qu et al., 2020). Alike, soil enzymes are major components of biological processes which participate in all biochemical reactions (Hassan et al., 2013a). Soil enzymes play an important role in the biological catabolism, decomposition of organic matter and C cycling (Hassan et al., 2013b; Aislabie & Deslippe, 2013). They also perform catalysis of reactions that are necessary for the life processes of microorganisms (Walker et al., 2018; Hassan et al., 2013b). Soil microbial community and enzymes respond to changes in soil and environmental factors much faster than do other variables (Nottingham et al., 2019; Aislabie & Deslippe, 2013). Soil microbial community and enzymes are sensitive to a number of environmental factors, among them temperature is of utmost ascendancy (Walker et al., 2018). Under the scenario of global warming, it is indeed important to test the temperature sensitivity of the soil microbial communities (bacteria, fungi, and actinomycetes) and extracellular enzymes (oxidative and hydrolytic).

Temperature is rightly known as one of the primary bio-controller, because it influences soil reactions, biological processes and the inter-spheric gas exchange between the soil and atmosphere (Thakur et al., 2017; Fang et al., 2016). Due to its control over energy shifts, microbial communities, and extracellular enzyme activity, it regulates OM mineralization rates and storage, and hence the production of CO\textsubscript{2} in soils is also temperature-dependent (Hassan, David & Abbas, 2014; Thakur et al., 2017; Walker et al., 2018). Therefore, the temperature has a great influence on the ability of soils to act as a C sink or source (Walker et al., 2018; Thakur et al., 2017; Fang et al., 2016). For example, Zhou et al. (2013) found that a 6-year warming period enhanced the activities of \(\beta\)-glucosidase and N-acetylglucosaminidase, which were connected with changes in microbial biomass C. Under warming conditions, changes in the soil LOC fractions have been shown to drive changes in soil enzyme activity (Zhou et al., 2013; Qi et al., 2016). However, according to Li et al. (2014), microbial responses to climate change may be influenced by soil properties.

The texture is one of the most important properties of soil because it determines characteristics and biophysical properties that shape and regulate the overall behavior and response of soils (Fang et al., 2016; Ding et al., 2014; Hassan et al., 2013a). The texture is associated with porosity, moisture, gaseous exchange, nutrient cycling, and substrate availability to microbiota along with other important functions and services in soils (Oertel et al., 2016; Hobley et al., 2014; Hamarashid, Othman & Hussain, 2010). Moreover, it also provides physical protection to soil microbiota, organic matter, and C from harsh climatic conditions i.e., temperature anomalies (Frøseth & Bleken, 2015; Hassan et al., 2013a). Therefore, it affects the microbial and enzymatic activity, decomposition of organic matter, nutrients and C cycling, and eventually CO\textsubscript{2} production (Ding et al., 2014; Feng, Plante & Six, 2013). Soil texture can modulate the effects of temperature and climate change and thus production and emission of gases (e.g., CO\textsubscript{2}) through its strong influence on biochemical processes and C cycling and storage (Zhang et al., 2015; Feng, Plante & Six, 2013). The main reason for the strong influence of texture on key soil processes and activities is diverse and divergent characteristics of its relative particle’s i.e., fine and coarse (Frøseth & Bleken, 2015; Hamarashid, Othman & Hussain, 2010). The fine
particles (*i.e.*, clay) have large surface areas, numerous reactive sites, strong ligand exchange, and polyvalent cation bridges than coarse ones *i.e.*, sand (*Fang et al., 2016; Ding et al., 2014; Hassan et al., 2013a*). However, the interaction of warming and soil texture on responsiveness of the key soil processes remains largely unknown.

Therefore, it is indeed important to quantify the role of texture in regulating the temperature sensitivity of key soil processes for correct future inventories and feedbacks. We hypothesized that, warming would increase decomposition of recalcitrant and stable soil C pools *via* microbial activities and extracellular enzymes. These changes will be more pronounced in sandy textured soils while clayey soils will mitigate the effects of warming. Thus, the purpose of this study was (1) to determine the temperature influence and responsiveness of labile, recalcitrant, and stable C fractions, as well as CO₂ emission from divergent textured soils (2) quantify the effect of temperature on soil microbial counts (bacteria, fungi, and actinomycetes), microbial biomass, and extracellular enzymes (oxidative and hydrolytic) activities and their response towards temperature increase in divergent textured soils and (3) identify and establish the potential role of texture in climate change mitigation.

**MATERIAL AND METHODS**

**Soil sampling**

The study area has a moderately continental climate, the maximum and minimum mean annual temperatures are 14.03 °C and 6.72 °C and average annual precipitation is 24.97 mm. Soil samples (0–30 cm depth) were collected randomly using a hand auger from 10 points within the selected agricultural fields at Dahlem and Rhinluch, Berlin, Germany (52°27″ N and 13°18″ E) in April 2017. Winter wheat and maize was grown in rotation. Soils were Albic Luvisol and Arenosol with glacial till and periglacial sand parent materials. Samples (field fresh) were sieved (<2 mm) and separated into two subsamples. One part of the subsample was used to conduct the incubation experiment while the other was used for microbial and enzymatic analyses. The remaining soil was used for physicochemical and C fractional analyses after air-drying at room temperature (25 °C) for 7 days by using methods as described by *Hassan, David & Abbas (2014)*. The basic physicochemical properties of experimental soil are given in Table 1A.

**Experimental layout**

For incubation, 400 g dry soil was incubated in 1,000 ml glass jars under different temperature and moisture regimes for 84 days in a randomized block design. Soil samples were wetted to maintain 60% of water holding capacity (WHC) and equilibrated overnight at 4 °C, before being placed in incubators. The five treatments in triplicate were developed and expressed as T1 (10 °C), T2 (20 °C), T3 (30 °C), T4 (40 °C), and T5 (50 °C). To keep the soils at their prescribed WHC, moisture loss in the jars was determined after every 2 days by weighing the jars and the water loss was replenished with distilled water throughout the incubation (*Elliott et al., 1994*). Soil samples were collected from each jar after incubation, for the determination of labile, recalcitrant and stable C fractions, microbial community and enzymes.
Quantification of Carbon dioxide (CO₂) emission

The emission of CO₂ from the incubated soil (as described above) was estimated by the alkali trap method as described by Witkamp (1966). The evolved CO₂ was trapped in 25 ml of 0.1 M KOH. After exposure, the KOH solution was removed, and any carbonate formed precipitated with saturated BaCl₂ to form BaCO₃; the remaining KOH was then titrated with an equivalent strength of HCl using phenolphthalein as an indicator. A jar without soil, containing the same amount of KOH, was run simultaneously as a blank. The evolved CO₂ was measured at 7, 21, 42, 63 and 84 days during incubation. The evolved and cumulative CO₂ was calculated, by using the method of Hassan, David & Abbas (2014).

Determination of soil C fractions

Total organic carbon

Total soil carbon of the soil before and after the experiment was determined by potassium dichromate (K₂Cr₂O₇) oxidation at 170–180 °C followed by titration with 0.5 mol L⁻¹ FeSO₄ (Walkley & Black 1934).

Light fraction of organic carbon

Light fraction of organic carbon was measured by wet oxidation (K₂Cr₂O₇). Fleeting, 50 ml NaI solution (1.70 g cm⁻³ density) along with soil sample (25 g) was placed into a centrifuge tube and was shaken (200 rpm) for 15 min. The floating material was extracted in triplicate and transferred to a filter paper and rinsed every time with CaCl₂ (0.01 M) and distilled water, and then dried (60 °C) for 48 h (Gregorich & Ellert, 1993).

Readily mineralizable carbon

Readily mineralizable carbon was estimated after extraction with K₂SO₄ (0.5 M) followed by wet digestion with dichromate. Briefly, soil (10 g), after precipitating the Fe²⁺ with 1 ml
of FeCl₃ (2.5% solution) and 4 ml of 6 N NaOH, was extracted with 40 ml of K₂SO₄ (0.5 M) after shaking for 1 h at a rotary shaker. After allowing the precipitate to settle down (4 °C) clear supernatant (aliquots) were titrated with FeH₈N₂O₈S₂ (0.04 N) by using 2 to 3 drops of diphenylamine (DPA) indicator after wet digestion with H₂CrO₄ (Mishra et al., 1997).

**Dissolved organic carbon**

For dissolved organic carbon fresh soil (10 g) was extracted with the 2.0 M KCl (1:4 soil/water) after shaking (250 rpm) the soil samples for 30 min. The supernatant was then centrifuged (15,000 rpm) for 10 min and filtered (0.45 µm cellulose ester filters) and analyzed at a TOC (Multi N/C 2100, Germany) analyzer (Zsolnay, 2003).

**Particulate organic carbon**

The particulate organic carbon was quantified after dispersing the soil sample (10 g) with 30 ml of hexametaphosphate (5 g l⁻¹) in a reciprocating shaker (90 rpm) for 18 h. The soil suspension was transferred into another clean and empty container under a continuous flow of distilled water over a sieve (53-µm) to ascertain the separation. The remaining soil on the sieve was dried at 55–60 °C for 48 h after shifting to a glass dish, and ground to powder with a ball mill, and measured (wet digestion) for POC by using K₂Cr₂O₇ (Cambardella & Elliott, 1992).

**Reducing sugar carbon**

The content of reducing sugar carbon was determined using a phenol reagent. One ml of soil extract was mixed with 1 ml of the phenol solution (5% w/v in distilled water), then 5 ml of 18.4 M H₂SO₄ (1.84 d) was added under continuous shaking. The mixture was left for 10 min, thereafter, incubated in a water bath at 25 °C for 20 min and the absorbance was read colorimetrically with a standard curve of glucose at 490 nm by following Badalucco et al. (1992) with slight modification.

**Easily oxidizable carbon**

For easily oxidizable carbon soil (3 g) was centrifuged (2,000 rpm) for 5 min along with 25 ml of KMnO₄ (333 mM) and the absorbance of the supernatant and standards was read spectrometrically at 565 nm. Likewise, the blank samples (no soil + standard) were also analyzed in each run. The change in the concentration of KMnO₄ was used to assess the amount of C oxidized by assuming that 1 mM MnO₄ is consumed for the oxidation of 0.75 mM or 9 g of C (Blair, Lefory & Lise, 1995).

**Recalcitrant organic carbon**

The recalcitrant organic carbon was determined by the acid hydrolysis (18 h) of soil (1 g) with HCl (6 M). The repeated evaporation and filtration were done in order to remove the HCl and separate the soluble materials. The residue was washed with de-ionized water (20 ml) and dried at 55 °C. After drying, the residue was ground and passed through a screen (180 mm), and combusted to CO₂ (Paul, Morris & Bohm, 2001).
Analysis of soil microbial colony counts and biomass

The total number of bacteria, fungus, and actinomycetes was determined using the dilution plate count technique on nutritional agar, as described previously by Hassan et al. (2013b). The dilution plate technique is based on the assumption that each colony is created by a single cell, referred to as a colony-forming unit (CFU). In a flask containing 90 ml distilled water and glass beads, 10 g of fresh soil was added (0.5 mm). For 30 min, the flask was shaken at 28 °C and 180 rpm. A total of 0.1 ml of the suspension was put to a small tube containing 0.9 ml distilled water after shaking. The tube was gently shaken and used to perform the remaining dilutions. To count bacteria, dilutions of $10^{-1}$–$10^{-8}$ were utilized. Conversely, a range of $10^{-1}$–$10^{-6}$ was used for the determination of fungi and actinomycete. Each dilution was repeated three times. In an incubator, the plates were incubated at 28 °C (301.15 K). Bacteria, fungi, and actinomycetes were identified 4, 5, and 7 days after plating, respectively (Hassan et al., 2013b). The chloroform fumigation-extraction method was used to determine the microbial biomass, i.e., MBC, MBN, and MBP. For this purpose, 10 g fresh soil was fumigated with alcohol-free chloroform for 24 h at a temperature of 25 °C in a desiccator. The soils (fumigated and non-fumigated) were then extracted for an hour using a horizontal shaker (200 rpm), filtered with Whatman No. 40 filter paper, and finally spectrophotometrically measured and computed (Hassan et al., 2013b).

Examination of enzymes activity

**Phenoloxidase and peroxidase activity**

The phenoloxidase and peroxidase activity was measured by incubating (25 °C) the soil (0.5 g), in a shaking environment (100 rpm), with acetate buffer (3 ml) and 2 ml of 10 mM L-3,4-dihydroxyphenylalanine (L-DOPA), followed by centrifugation for 10 min at 5 °C. For peroxidase, an addition of 0.3% H$_2$O$_2$ (0.2 ml), just before incubation, was made. Then the absorbance of the dopachrome (reaction product) was read at 475 nm spectrophotometrically and activity of both enzymes was expressed as µmol dopachrome g$^{-1}$ h$^{-1}$ (Dick, 2011).

**Catalase activity**

The catalase activity was measured by titrating residual H$_2$O$_2$ in the filtrate with KMnO$_4$ (0.1 N), after mixing the soil (1 g) with 3% H$_2$O$_2$ (1 ml) and H$_2$SO$_4$ (5 ml) after shaking (20 min), followed by filtration. The activity was expressed as µmol H$_2$O$_2$ g$^{-1}$ h$^{-1}$ (Roberge, 1978).

**Invertase activity**

The activity of invertase was determined by incubating (24 h at 37 °C) the soil (5 g) with sucrose solution (15 ml), phosphate buffer (35.6 g Na$_2$HPO$_4$ + 700 ml distilled H$_2$O + adjust pH to 5.5 with HCl + volume to 1 l) and toluene (4–5 drops), followed by filtration. The activity (color density) was measured spectrophotometrically at 508 nm, after mixing the filtrate (1 ml) with 0.5% C$_7$H$_4$N$_2$O$_7$ (2 ml), heating (5 min) in a boiling water bath and colling (3 min) it down under running water and making the final volume to 25 ml with deionized H$_2$O and described as µmol glucose g$^{-1}$ h$^{-1}$ (Dick, 2011).
**β-glucosidase activity**

The β-glucosidase activity was estimated by incubating and treating (1 h at 37 °C) the soil (1 g) with 0.25 ml toluene, 0.25 nm p-nitrophenol phosphate (p-NPP), 4 ml MUB (Modified universal buffer), 1 ml of glucoside, 1 ml of CaCl₂ (0.5 M) and 4 ml of 0.1 M THAM (Tris-hydroxymethyl-aminomethane) solution. After filtration (Whatman No. 2V) the activity was determined through spectrophotometer at 400 nm and described as µmol p-nitrophenol g⁻¹ h⁻¹ (Eivazi & Tabatabai, 1988).

**Cellulase activity**

The cellulase activity was measured after incubating (24 h, 50 °C), centrifuging (2,500g, 10 min) and treating the soil (10 g) with 5 ml acetate buffer (11.2 M, pH 5.5), carboxymethyl cellulose sodium (7 g) and cellulose substrate (0.7%). After filtration the activity was determined through spectrophotometer at 690 nm and described as µmol glucose g⁻¹ h⁻¹ (Schinner & Von Mersi, 1990).

**Statistical analysis**

The statistical software Statistix 8.1 (Statistix, Tallahassee, FL, USA), and Excel 2016 were used for data analysis. Parametric statistics of ANOVA analysis was carried out to estimate the effect of temperature on the soil microbes, microbial biomass, enzymes, C fractions and CO₂ production and emissions under divergent textures. Mean separations were achieved by using the least significant difference (LSD) test at P < 0.05. Data presented are means ± standard deviation (SD) of three replicates of each treatment. Correlation coefficients (R²) between soil C fractions of labile, recalcitrant, and stable pools, CO₂ emissions and cumulative CO₂, microbial community, microbial biomass, oxidative and hydrolytic enzymes and temperature were developed by using the same software.

**RESULTS**

**Labile C fractions**

The response and decomposition of labile C fractions viz LFOC, DOC, RMC, RSC, POC, and EOC under a range of elevated temperature (T1–T5) regimes in sandy and clayey soil is presented in Fig. 1. The response and decomposition of labile C fractions increased significantly (P < 0.05) with the increase in the temperature (per 10 °C rise). However, the temperature response and decomposition of labile C fractions were significantly (P < 0.05) higher in sandy than the clayey soil. Therefore, in sandy soil, the maximum increase in the LFOC (2.92-fold), DOC (3.34-fold), RMC (4.07-fold), RSC (4.54-fold), POC (3.51-fold), and EOC (4.02-fold) was observed at the highest temperature i.e., T5 compared to lowest temperature (T1). Conversely, in clayey soil, maximum increase in the LFOC (2.41-fold), DOC (2.05-fold), RMC (3.17-fold), RSC (2.98-fold), POC (2.71-fold), and EOC (3.03-fold) was observed at the T4 compared to lowest temperature (T1). Whereas, the minimum sensitivity and decomposition of labile C fractions were observed at the lowest temperature i.e., T1. Furthermore, owing to higher temperature impact and decomposition, the sandy soil exhibited significantly lower labile C fractions i.e., LFOC.
(1.14-fold), DOC (1.17-fold), RMC (1.14-fold), RSC (1.15-fold), POC (1.16-fold), and EOC (1.17-fold) compared to the clayey soil. Mainly the effect of temperature on the labile C fractions in the sandy soil was in the order T4 > T5 > T3 > T2 > T1. Conversely, the influence of temperature on the labile C fractions in the clayey soil was in the order T5 > T4 > T3 > T2 > T1.

**Recalcitrant C fractions**

The response and decomposition of recalcitrant C fraction viz ROC under a range of elevated temperature (T1–T5) regime in sandy and clayey soil is illustrated in Fig. 2. The response and decomposition of ROC enhanced markedly (P < 0.05) with the temperature increase (per 10 °C rise) in both textured soils. Unlike the labile C fractions, the increase in temperature caused a significant and continuous increase in the response and decomposition of ROC under both textured soils. Therefore, the maximum increase in the ROC (3.16-fold and 3.72-fold) was observed at the highest temperature i.e., T5 in sandy and clayey soil respectively compared to lowest temperature (T1). Whereas, the minimum response and decomposition of ROC were observed at the lowest temperature i.e., T1. Due to the higher temperature effect and decomposition, the decrease in the ROC was higher (1.15-fold) in sandy compared to clayey soil. In general, the effect of temperature on the ROC in both soils was in the order T5 > T4 > T3 > T2 > T1 endorsing, the fact that ROC likely has higher sensitivity to temperature (T1–T5) increase than the labile C fractions.

**Stable C fractions**

The response and decomposition of stable C fractions viz TOC under a range of elevated temperature (T1–T5) regime in sandy and clayey soil is presented in Fig. 3. The response and decomposition of TOC enhanced greatly (P < 0.05) with the temperature increase (per 10 °C rise) in both textured soils. Unlike the labile and stable C fractions the increase in temperature significantly (P < 0.05) decreased the TOC. Therefore, the maximum decrease in the TOC (3.89-fold and 3.60-fold) was observed at the highest temperature (T5) in both sandy and clayey soil respectively compared to lowest temperature (T1). Highlighting that TOC has a strong (P < 0.05) antagonistic association to the temperature increase (T1–T5). Whereas, the minimum response and decomposition of TOC were observed at the lowest temperature i.e., T1. Owing to the higher temperature effect and decomposition, the decrease in the TOC was higher (1.14-fold) in sandy compared to the clayey soil. In general, the effect of temperature on the TOC decomposition and sensitivity was in the order T5 > T4 > T3 > T2 > T1.

**Microbial community**

The response of soil microbes i.e., bacteria, fungi, and actinomycetes under a range of elevated temperature (T1–T5) regimes in sandy and clayey soils is exhibited in Fig. 4. The response of the soil microbes increased significantly (P < 0.05) with the temperature increases (per 10 °C rise) in both textured soils. However, the temperature sensitivity and response of the soil microbes were significantly (P < 0.05) variable. The bacteria
showed the maximum temperature response (2.22-fold and 2.57-fold) at the highest temperature (i.e., T5) in sandy and clayey soils correspondingly compared to lowest temperature (T1). Conversely, the maximum increase in the response of actinomycetes (2.01-fold and 2.52-fold) and fungi (1.64-fold and 1.73-fold) were found at T4 and T3 in sandy and clayey soils respectively compared to lowest temperature (T1). Indicating the fact that among soil microbes the temperature sensitivity order is bacteria > actinomycetes > fungi. Whereas, the minimum sensitivity and response of soil microbes
were observed at the lowest temperature i.e., T1. Besides, owing to higher temperature effect and sensitivity, the sandy soil showed a markedly lower soil microbes count i.e., bacteria (1.34-fold), fungi (1.12-fold), and actinomycetes (1.14-fold) compared to clayey soil. In general, the effect of temperature on the bacterial counts was in the order T5 > T4 > T3 > T2 > T1. Whereas the temperature sensitivity of actinomycetes and fungi were in an order of T4 > T5 > T3 > T2 > T1 and T3 > T4 > T5 > T2 > T1 respectively.
**Microbial biomass**

The response of microbial biomass \( i.e., \) MBC, MBN, and MBP under a range of elevated temperature (T1–T5) regimes in sandy and clayey soils is presented in Fig. 5. The response of microbial biomass increased significantly \( (P < 0.05) \) with the temperature surge (per 10 °C rise) in both textured soils. However, like soil microbes colony counts, the temperature sensitivity of microbial biomass was also significantly \( (P < 0.05) \) variable. The MBC exhibited the maximum temperature sensitivity and increase (1.97-fold and 2.21-fold) at the highest temperature \( (i.e., \) T5) in sandy and clayey soils correspondingly.

![Figure 3] Effect of temperature on stable C fraction under sandy and clayey texture. TOC, total organic carbon. Vertical bars represent means ± SD \((n = 3)\). ANOVA significant at \( P \leq 0.05\).
compared to lowest temperature (T1). On the contrary, the maximum increase in the MBN (2.11-fold and 2.22-fold) and MBP (1.84-fold and 2.31-fold) were found at T4 and T3 in sandy and clayey soils respectively compared to lowest temperature (T1). Indicating the fact that among microbial biomass the temperature sensitivity order is MBC > MBN > MBP. Whereas, the minimum sensitivity and response of microbial biomass were observed at the lowest temperature i.e., T1. Moreover, due to the higher temperature effect and sensitivity the sandy soil exhibited a significantly lower microbial biomass i.e., MBC (1.23-fold), MBN (1.29-fold), and MBP (1.43-fold) compared to the clayey soil.

The temperature sensitivity order for MBC was T5 > T4 > T3 > T2 > T1. Whereas the

Figure 4 Effect of temperature on microbial community under sandy and clayey texture. Units: bacteria, CFU × 10^6 g⁻¹; fungi, CFU × 10^4 g⁻¹; Actinomycetes, CFU × 10^5 g⁻¹. Vertical bars represent means ± SD (n = 3). ANOVA significant at P ≤ 0.05.  

DOI: 10.7717/peerj.13151/fig-4
temperature sensitivity order for MBN and MBP was T4 > T5 > T3 > T2 > T1 and T3 > T4 > T5 > T2 > T1 respectively.

**Oxidative enzymes**

The response and activity of oxidative enzymes viz PO, PEO, and CAT under a range of elevated temperature (T1–T5) regimes in sandy and clayey soil are shown in Fig. 6. However, unlike the hydrolytic enzymes, a significant \( P < 0.05 \) and continuous increase in the activity of oxidative enzymes was observed with the increase in the temperature.
As a result, the maximum increase in the activity of PO (2.61-fold and 4.07-fold), PEO (3.08-fold and 6.77-fold), and CAT (2.18-fold and 2.71-fold) were found at the highest temperature i.e., T5 in sandy and clayey soils respectively compared to the lowest temperature (T1). Whereas minimum response and activity of oxidative enzymes were observed at the lowest temperature i.e., T1. Establishing the fact that oxidative enzymes have decidedly higher responsiveness to temperature increase than the hydrolytic enzymes. Furthermore, owing to the higher temperature effect, the sandy soil showed a markedly lower activity and values of oxidative enzymes i.e., PO (1.69-fold), PEO...
(1.48-fold), and CAT (1.24-fold) compared to the clayey soil. The overall effect of temperature on the sensitivity and activity of oxidative enzymes was in the order T5 > T4 > T3 > T2 > T1.

**Hydrolytic enzymes**

The response and activity of hydrolytic enzymes viz INV, BGL, and CELL under a range of elevated temperature (T1–T5) regimes in sandy and clayey soils are depicted in Fig. 7. Generally, an increasing trend was observed in the activity of hydrolytic enzymes under
elevated temperature (per 10 °C rise). However, unlike the oxidative enzymes, the maximum increase in the activity of INV (1.71-fold and 2.01-fold), BGL (1.85-fold and 2.22-fold), and CELL (1.81-fold and 2.23-fold) were found at T4 in sandy and clayey soils respectively compared to lowest temperature (T1). After that, an abrupt decrease in the activity of hydrolytic enzymes was examined at the highest temperature i.e., T5 compared to lowest temperature (T1). Whereas, the minimum activity of hydrolytic enzymes was observed at the lowest temperature (T1). Additionally, due to the higher temperature effect, the sandy soil depicted a significantly lower activity and values of hydrolytic enzymes i.e., INV (1.25-fold), BGL (1.23-fold), and CELL (1.29-fold) compared to the clayey soil. The overall effect of temperature on the response and activity of hydrolytic enzymes was in the order T4 > T5 > T3 > T2 > T1.

**Emissions and cumulative CO\(_2\)**

The response and changes in the emissions and cumulative CO\(_2\) under a range of elevated temperature (T1–T5) regimes in sandy and clayey soils and a graphical summary of methodology are illustrated in Figs. 8 and 9. Overall, an increasing trend was found in the emissions and cumulative CO\(_2\) for each 10 °C rise in temperature. However, the temperature responsiveness and changes in emissions and cumulative CO\(_2\) were significantly (\(P < 0.05\)) higher in sandy than the clayey soil. Therefore, in sandy soil, the maximum increase in the emissions (1.84-fold) and cumulative CO\(_2\) (1.81-fold) were observed at the highest temperature (T5) compared to lowest temperature (T1). Conversely, in clayey soil, higher emissions (1.45-fold) and cumulative CO\(_2\) (1.36-fold) were observed at the T4 compared to lowest temperature (T1). After that, in clayey soil, a decrease in the response and thus values of emissions and cumulative CO\(_2\) were examined at the highest temperature i.e., T5. Whereas, the minimum sensitivity and changes in emission and cumulative CO\(_2\) were observed at the lowest temperature (i.e. T1).

Furthermore, unlike other key soil processes, the sandy soil showed greater increase in the emissions (1.22-fold) and cumulative CO\(_2\) (1.23-fold) owing to higher temperature effect, decomposition rate and changes than the clayey soil. Underscoring the fact that CO\(_2\) production and emissions and cumulative CO\(_2\) have positive feedback with the augmentation in the temperature, and sandy soil are more vulnerable than the clayey ones. Mainly the effect of temperature on the sensitivity and emissions and cumulative CO\(_2\) in the sandy soil was in the order T5 > T4 > T3 > T2 > T1. Conversely, the influence of temperature on the sensitivity and emission and cumulative CO\(_2\) in the clayey soil was in the order T4 > T5 > T3 > T2 >.

**Regression analysis**

Regression analysis showed that C fractions of labile, recalcitrant, and stable pools, CO\(_2\) fluxes, and cumulative CO\(_2\) correlated well with the temperature in both sandy and clayey soils (Table 1B). Nonetheless, overall, temperature accounted for 93% and 79% variability in the C fractions of labile, recalcitrant, and stable pools in the sandy and clayey soils correspondingly (Table 1B). Whereas, temperature accounted for 91% and 94% variability in the CO\(_2\) fluxes and cumulative CO\(_2\) in the sandy soil. Conversely, temperature described
for 78% and 75% alterability in the CO₂ fluxes and cumulative CO₂ in the clayey soil (Table 1B). Furthermore, temperature accounted variability was significantly higher for C fractions of recalcitrant and stable pools compared to labile pools in both sandy and clayey soils (Table 1B). Regression analysis showed that overall, temperature accounted for 85% and 72% variability in the microbial community in the sandy and clayey soils (Table 2A). The temperature accounted variability was significantly higher for bacteria (R² = 0.97 and 0.91) than actinomycetes (R² = 0.92 and 0.81) and fungi (R² = 0.68 and 0.46) in both sandy and clayey soils (Table 2A). Moreover, the temperature described

Figure 8 Effect of temperature on CO₂ emissions and cumulative CO₂ under sandy and clayey texture. Unit: CO₂ emission, mg kg⁻¹ h⁻¹; Cumulative CO₂, mg kg⁻¹. Vertical bars represent means ± SD (n = 3). ANOVA significant at P ≤ 0.05. DOI: 10.7717/peerj.13151/fg-8
alterability was markedly higher in sandy soil than clayey soil (Table 2A). Whereas, temperature designated for 88% and 78% variability in the microbial biomass in the sandy and clayey soils (Table 2A). The temperature accounted alterability was significantly higher for MBC ($R^2 = 0.98$ and 0.92) than MBN ($R^2 = 0.91$ and 0.80) and MBP ($R^2 = 0.75$)
and 0.63) in both sandy and clayey soils (Table 2A). Moreover, the temperature designated changeability was markedly higher in sandy soil than clayey soil (Table 2A). Regression analysis showed that overall, temperature accounted for 93% and 86% variability in the oxidative enzymes in the sandy and clayey soils (Table 2B). Whereas, temperature accounted for 73% and 66% variability in the hydrolytic enzymes in the sandy and clayey soils (Table 2B). The temperature described alterability was significantly higher for oxidative enzymes than hydrolytic enzymes. Moreover, the temperature accounted variability was markedly higher in sandy soil than clayey soil (Table 2B).

**DISCUSSION**

The responsiveness and decomposition of labile C fractions increased significantly ($P < 0.05$) with the temperature increase (per 10 °C) in both textured soils (Fig. 1). Yang *et al.* (2021) and Qi *et al.* (2016) assessed that temperature increase significantly alters the fractions of soil labile organic C (RSC, MBC, DOC, and POC) by increasing their response and rate of decomposition. However, response to the temperature and

| Table 2A Correlation coefficient ($R^2$) between microbial community and biomass and temperature. |
|-----------------------------------------------|-----------------------------------------------|
| Parameters | $R^2$ (Sandy soil) | $R^2$ (Clayey soil) |
|-------------------|-------------------|-------------------|
| Bacteria | 0.97** | 0.91** |
| Fungi | 0.68* | 0.46* |
| Actinomycetes | 0.92* | 0.81* |
| aTotal $R^2$ | 0.85** | 0.72* |
| MBC | 0.98** | 0.92** |
| MBN | 0.91** | 0.80* |
| MBP | 0.75* | 0.63* |
| bTotal $R^2$ | 0.88** | 0.78* |

Notes:
* Total $R^2$, Correlation coefficient from all microbial community.
+ Total $R^2$, Correlation coefficient from all microbial biomass.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

| Table 2B Correlation coefficient ($R^2$) between oxidative and hydrolytic enzymes and temperature. |
|-----------------------------------------------|-----------------------------------------------|
| Parameters | $R^2$ (Sandy soil) | $R^2$ (Clayey soil) |
|-------------------|-------------------|-------------------|
| PO | 0.96** | 0.88** |
| PEO | 0.93** | 0.86** |
| CAT | 0.91** | 0.84** |
| aTotal $R^2$ | 0.93** | 0.86** |
| INV | 0.76* | 0.69* |
| BGL | 0.64* | 0.58* |
| CELL | 0.81* | 0.73* |
| bTotal $R^2$ | 0.73* | 0.66* |

Notes:
* Total $R^2$, Correlation coefficient from all oxidative enzymes.
+ Total $R^2$, Correlation coefficient from all hydrolytic enzymes.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$. 

Hassan et al. (2022), PeerJ, DOI 10.7717/peerj.13151
decomposition of labile C fractions were significantly ($P < 0.05$) higher in sandy soil than the clayey soil (Fig. 1). Temperature accounted variability for labile C fractions was significantly higher in sandy soil than clayey soil (Table 1B). Wankhede et al. (2020), Rittl et al. (2020), Takrit et al. (2018), Ghosh et al. (2016), Frøseth & Bleken (2015) and Hobley et al. (2014) found that temperature impacts on labile C fractions was higher in coarse (sandy) than fine (clayey) soils owing to low physical protection, small specific areas, fewer reactive sites, and weak ligand exchange bridges, where soil C could be sorbed and protected. In present study, unlike the labile C fractions, the increase in temperature ($T1$–$T5$) caused a significant ($P < 0.05$) and continuous increase in the sensitivity and decomposition of recalcitrant (ROC) and stable (TOC) C fractions (Figs. 2 and 3) in both soils. Whereas, the temperature impacts and thus decomposition of ROC (1.15-fold) and TOC (1.14-fold) were significantly higher in sandy soil at $T5$ i.e. 50 °C (Figs. 2 and 3). Wankhede et al. (2020) and Zheng et al. (2019) examined that in sandy (coarse) soils the temperature response of recalcitrant and stable C fractions was much higher due to weak physical protection, fewer cations bridges, unstable moisture availability, and their low storing ability. The response of C fractions to temperature was in the order recalcitrant C fractions > stable C fractions > labile C fractions (Figs. 1–3). In both sandy and clayey soils, temperature accounted variability was significantly higher for C fractions of recalcitrant and stable pools compared to labile pools (Table 1B). Zhang & Zhou (2018) and Dai et al. (2017) found that recalcitrant and stable C fractions have decidedly extra sensitivity than the labile fractions to the temperature increase (5 °C to 30 °C) in divergent coarse and fine textured Chinese soils. The results of current study also endorsed the fact that recalcitrant and stable C fractions have a higher sensitivity to temperature ($T1$–$T5$) increase than the labile C fractions (Figs. 1–3). Higher $R^2$ were found for recalcitrant and stable C fractions than labile ones in both sandy and clayey soils (Table 1B). Biswas et al. (2018), Lian et al. (2018), Fang et al. (2016) and Nguyen et al. (2010) confirmed that recalcitrant and stable C fractions have higher responses to temperature than the labile C fractions in coarse and fine textured soils.

The response of soil microbial counts and microbial biomass increased markedly ($P < 0.05$) with the temperature increase (per 10 °C rise) in both sandy and clayey textured soils. However, the temperature responses of microbial colony counts i.e., bacteria (1.34-fold), fungi (1.12-fold), and actinomycetes (1.14-fold) and biomass i.e., MBC (1.23-fold), MBN (1.29-fold), and MBP (1.43-fold) were higher in sandy soil (Figs. 4 and 5). Overall, in sandy soil, temperature accounted for significantly higher variability in microbial population (85%) and microbial biomass (88%) than in clayey soil (Table 2A). Qu et al. (2020), Nottingham et al. (2019), Hutchins et al. (2019), Zhang et al. (2016), Fang et al. (2016), and Hassan et al. (2013a) examined that microbial counts (bacteria, fungi, and actinomycetes) and biomass (MBC, MBN, and MBP) had higher sensitivity to temperature increase and their sensitivity increased many folds in coarse (sandy) soils owing to less favorable conditions, predation, desiccation, and substrate availability. The results further, revealed that temperature sensitivity and response of soil microbes colony counts and biomass were significantly variable (Figs. 4 and 5). Cavicchioli et al. (2019), Zhang et al. (2016) and Fang et al. (2016) also examined variations in the activity,
behavior, and response of microbial community and biomass towards experimental warming and temperature increase. The temperature response of soil microbes colony counts and biomass were in the order bacteria > actinomycetes > fungi and MBC > MBN > MBP (Table 2A). Therefore, the maximum activity and response of bacteria, actinomycetes and fungi and MBC, MBN, and MBP were observed at temperatures T5, T4 and T3 in both soils respectively (Figs. 4 and 5). Zheng et al. (2019), Dubey et al. (2019), Walker et al. (2018), and Zhang et al. (2016) found a strong association between temperature increase and responses of soil microbes colony counts and biomass and stated that temperature sensitivity of bacteria and MBC is much higher followed by actinomycetes and fungi and MBN and MBP in diverse textured soils (coarse and fine). The temperature accounted variability was significantly higher for bacteria ($R^2 = 0.97$ and $R^2 = 0.91$) than actinomycetes and fungi in both sandy and clayey soils (Table 2A). Romero-Olivares, Alisson & Trescedar (2017), Zhang et al. (2016), Garcia-Palacios et al. (2015), and Wang et al. (2014) also stated that among microbes and biomass, bacterial community and MBC have decidedly higher sensitivity, contrarily, fungi are less sensitive to changes in temperature owing to the chitinous cell walls that make them highly resilient. The temperature accounted variability was significantly higher for MBC ($R^2 = 0.98$ and $R^2 = 0.92$) than MBN and MBP in both sandy and clayey soils (Table 2A). Melillo et al. (2017), and Crowther et al. (2016) also found a significant association between the increase in temperature (warming), temperature sensitivity, and reduction in the microbial biomass and stated that temperature sensitivity of MBC is markedly higher.

The extracellular enzymes (i.e., oxidative and hydrolytic) response and activity increased significantly ($P < 0.05$) with the temperature increase (per 10 °C rise) in both textured soils. However, the temperature response of oxidative enzymes i.e., PO (1.69-fold), PEO (1.48-fold), and CAT (1.24-fold) and hydrolytic enzymes i.e., INV (1.25-fold), BGL (1.23-fold), and CELL (1.29-fold) were markedly higher in sandy soil (Figs. 6 and 7). The temperature accounted variability for oxidative and hydrolytic enzymes was markedly higher in sandy soil than clayey soil (Table 2B). Wankhede et al. (2020), Cavicchioli et al. (2019), Zheng et al. (2019), Thakur et al. (2017), and Fang et al. (2016) assessed a significant increase in the sensitivity and response of extracellular enzymes i.e., oxidative and hydrolytic with the temperature increase and stated that temperature sensitivity increases strongly in sandy (coarse) soils due to less favorable conditions, unstable moisture, and substrate availability. The results of present study further revealed that the temperature sensitivity of extracellular enzymes was in the order oxidative enzymes > hydrolytic enzymes (Figs. 6 and 7). The temperature accounted variability was significantly higher for oxidative enzymes (93% and 86%) than hydrolytic enzymes (73% and 66%) in both sandy and clayey soils (Table 2B). Establishing the fact that oxidative enzymes have higher temperature sensitivity than the hydrolytic enzymes in both sandy and clayey soils. Meng et al. (2020), Tang et al. (2019), Walker et al. (2018), Allison et al. (2018), Cheng et al. (2017), and Fang et al. (2016) examined a strong synergistic association between extracellular enzymes sensitivity and temperature and revealed that oxidative enzymes (e.g., PO, PEO, and CAT) have decidedly higher
temperature sensitivity than the hydrolytic (e.g., DEH, URE, INV, BGL, and PHP) enzymes.

Overall, an increasing trend was found in the emissions and cumulative CO$_2$ under elevated i.e., each 10 °C rise in temperature in both sandy and clayey soils (Fig. 8). However, the temperature effect and changes in emissions and cumulative CO$_2$ were significantly ($P < 0.05$) higher in sandy than the clayey soil. Temperature accounted for 91% and 94% variability in the CO$_2$ emissions and cumulative CO$_2$ in the sandy soil (Table 1B). Sánchez-Cañete, Barron-Gaford & Chorover (2018), Zomer et al. (2017), Ekwurzel et al. (2017), Fang et al. (2016) and Frøseth & Bleken (2015) examined a significant increase in the emissions and cumulative CO$_2$ with the temperature rise and stated that coarse i.e., sandy soils have much higher temperature effect thus emissions and cumulative CO$_2$ than the fine (clayey or silty) soils. Therefore, in sandy soil, the maximum increase in the CO emissions (1.84-fold) and cumulative CO$_2$ (1.81-fold) was observed at the highest temperature (T5). Furthermore, the sandy soil showed significantly ($P < 0.05$) higher CO$_2$ emissions (1.22-fold) and cumulative CO$_2$ (1.23-fold) owing to higher temperature effect, response and decomposition rate than the clayey soil (Fig. 8). Significantly higher correlation coefficients were observed between CO$_2$ emissions ($R^2 = 0.91$) and cumulative CO$_2$ ($R^2 = 0.94$) and temperature in sandy soil than clayey soil (Table 1B). Wachiye et al. (2019), Badagliacca et al. (2017), Frøseth & Bleken (2015) and Ding et al. (2014) found a significantly higher responsiveness of CO$_2$ emissions and cumulative CO$_2$ to temperature in the sandy soils and established that this was due to high rate of C decomposition, low humification, and small specific areas in sandy soils, where soil C could be sorbed, secured and stored. Underscoring the fact that CO$_2$ production and emissions and cumulative CO$_2$ have a strong synergistic association with the temperature augmentation, and sandy soils have much higher temperature sensitivity and vulnerability to become CO$_2$-C sources than the clayey ones (Fig. 8 and Table 1B). Temperature accounted for significantly lower variability for the CO$_2$ fluxes (78%) and cumulative CO$_2$ (75%) in clayey soil compared to sandy soil (Tables 2A and 2B). Oertel et al. (2016), Frøseth & Bleken (2015), Zhang et al. (2015) and Six & Paustian (2014) inspected that the sandy soils are more sensitive to temperature increase and the main reason of sandy/coarse soils to foster higher CO$_2$ production and emissions and cumulative CO$_2$ is high decomposition rate, low humification and availability of C sorption, and attachment sites.

**CONCLUSION**

The study concluded that the temperature sensitivity of soil C fractions, microbial colony counts, microbial biomass, extracellular enzymes, and CO$_2$ fluxes increased with the upsurge in temperature. However, the recalcitrant and stable C fractions have decidedly higher responses than labile C fractions. Alike, among microbes, microbial biomass, and extracellular enzymes, bacteria, MBC, and oxidative enzymes (PO, PEO, and CAT) have markedly higher sensitivity. It was concluded that the temperature effect and variability for all measured key soil processes along with CO$_2$ fluxes were markedly higher in sandy textured soil. Conversely, clayey texture performed a significant role in the
mitigation of undue temperature influence, hence, the sensitivity of key soil processes and CO₂ fluxes. The study also suggests between sandy and clayey textured soils, the soils which are sandy in nature under the scenario of global warming, are more vulnerable to become CO₂-C sources therefore must be managed and treated wisely. Furthermore, in future research and models instead of generalizing effects of global warming, temperature sensitivity of individual key soil processes must also be considered carefully. The findings of the study will be helpful in alleviating the controversy of the temperature sensitivity of key soil processes in sandy and clayey soils. And enabling the scientists and environmentalists to formulate measures and devise recommendations to reduce the excessive increase in CO₂-C fluxes from divergent textured soils.

**LIST OF ABBREVIATIONS**

- TOC: total organic C
- MBC: microbial biomass C
- MBN: microbial biomass N
- MBP: microbial biomass P
- RCP: recalcitrant C pool
- LCP: labile C pool
- SCP: stable C pool
- ROC: recalcitrant organic carbon
- WHC: water holding capacity
- LFOC: light fraction of organic carbon
- RMC: readily mineralizable carbon
- DOC: dissolved organic carbon
- POC: particulate organic carbon
- EOC: easily oxidizable carbon
- ROC: recalcitrant organic carbon
- CFU: colony-forming unit

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**

The present study was conducted with the support of the China Postdoctoral Council and the Institute of Environment and Sustainable Development in Agriculture (Grant No. NNSFC 42007073 and MARA, PRC 13210352). This work was also supported by the Special project in key areas of Guangdong Province Ordinary Universities (No. 2020ZDZX1003), the Guangdong Provincial Special Fund for Modern Agriculture Industry Technology Innovation Teams (No. 2019KI140), the Key Real R&D Program of Guangdong Province (2020B1111350002 and 2020B0202080002), and the National Natural Science Foundation of China (No. 21407155). Support was also provided by the Researchers Supporting Project number (RSP-2021/393), King Saud University, Riyadh,
Saudi Arabia. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**
The following grant information was disclosed by the authors:
China Postdoctoral Council and Institute of Environment and Sustainable Development in Agriculture: 42007073 and 13210352.
Guangdong Province Ordinary Universities: 2020ZDZX1003.
Modern Agriculture Industry Technology Innovation Teams: 2019KJ140.
Key Real R&D Program of Guangdong Province: 2020B1111350002 and 2020B0202080002.
National Natural Science Foundation of China: 21407155.
Researchers Supporting Project number: RSP-2021/393.

**Competing Interests**
The authors declare that they have no competing interests.

**Author Contributions**
- Waseem Hassan conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Yue Li conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Tahseen Saba analyzed the data, prepared figures and/or tables, and approved the final draft.
- Jianshuang Wu conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Safdar Bashir performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Saqib Bashir performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Mansour K. Gatasheh performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Zeng-Hui Diao conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Zhongbing Chen conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

**Data Availability**
The following information was supplied regarding data availability:
The raw data is available in the Supplemental File.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13151#supplemental-information.
REFERENCES

Aislabie J, Deslippe JR. 2013. Soil microbes and their contribution to soil services. In: Dymond JR, ed. Ecosystem Services in New Zealand—Conditions and Trends. Lincoln: Manaaki Whenua Press.

Allison SD, Romero-Olivares AL, Ying Lu, Taylor JW. 2018. Temperature sensitivities of extracellular enzyme V max and K m across thermal environments. Global Change Biology 24:2884–2897 DOI 10.1111/gcb.14045.

Amundson R, Biardeau L. 2019. Soil carbon sequestration is an elusive climate mitigation tool. Proceedings of the National Academy of Sciences of the United States of America 115:11652–11656 DOI 10.1073/pnas.1815901115.

Badagliacca G, Ruisi P, Rees RM, Saia S. 2017. An assessment of factors controlling N2O and CO2 emissions from crop residues using different measurement approaches. Biology and Fertility of Soils 53:547–561 DOI 10.1007/s00374-017-1195-z.

Badalucco L, Gelsonimo A, Del’Orco S, Greco S, Nannipieri P. 1992. Biochemical characterization of soil organic compounds extracted by 0.5 M K2SO4 before and after chloroform fumigation. Soil Biology and Biochemistry 24:569–578 DOI 10.1016/0038-0717(92)90082-9.

Badgery W, Murphy B, Cowie A, Orgill S, Rawson A, Simmons A, Crean J. 2020. Soil carbon market-based instrument pilot—the sequestration of soil organic carbon for the purpose of obtaining carbon credits. Soil Research 59:12–23 DOI 10.1071/SR19331.

Biswas DR, Ghosh A, Ramachandran S, Basak BB, Moharana PC. 2018. Dependence of thermal and moisture sensitivity of soil organic carbon decomposition on manure composition in an incertisol under a 5-year-old maize-wheat cropping system. Journal of Geophysical Research: Biogeosciences 123(5):1637–1650 DOI 10.1029/2017JG004329.

Blair GJ, Lefory RDB, Lise L. 1995. Soil carbon fractions based on their degree of oxidation and the development of a carbon management index for agricultural system. Australian Journal of Agricultural Research 46(7):1459–1466 DOI 10.1071/AR9951459.

Cambardella C, Elliott E. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56:777–783 DOI 10.2136/sssaj1992.03615995005600030017x.

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken JR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW, Danovaro R, Foreman CM, Huisman J, Hutchins DA, Jansson JK, Karl QM, Koskella B, Welch DBM, Martiny JBH, Moran MA, Orphan VJ, Reay DS, Remais JV, Rich VI, Singh BK, Stein LY, Stewart FJ, Sullivan MB, von Oppen MHH, Weaver SC, Webb EA, Webster NS. 2019. Scientists’ warning to humanity: microorganisms and climate change. Nature Reviews 17(9):569–586 DOI 10.1038/s41579-019-0222-5.

Cheng L, Zhang N, Yuan M, Xiao J, Qin Y, Deng Y, Tu Q, Xue K, Nostrand JDV, Wu L, He Z, Zhou X, Leigh MB, Konstantinidis KT, Schuur EAG, Luo Y, Tiedje JM, Zhou J. 2017. Warming enhances old organic carbon decomposition through altering functional microbial communities. The ISME Journal 11(8):1–11 DOI 10.1038/ismej.2017.48.

Crowther TW, Todd-Brown KEO, Rowe CW, Wieder WR, Carey JC, Machmuller MB, Snoek BL, Fang S, Zhou G, Allsomsd SD, Blair JM, Bridgham SD, Burton AJ, Carrillo Y, Reich PB, Clark JS, Classen AT, Dijkstra FA, Elberling B, Emmett BA, Estiarte M, Frey SD, Guo J, Harte J, Jiang L, Johnson BR. 2016. Quantifying global soil carbon losses in response to warming. Ecological Letters 104(7631):104–108 DOI 10.1038/nature20150.
Dai SS, Li LJ, Ye R, Zhu-Barker X, Horwath WR. 2017. The temperature sensitivity of organic carbon mineralization is affected by exogenous carbon inputs and soil organic carbon content. *European Journal of Soil Biology* **81**:69–75 DOI 10.1016/j.ejsobi.2017.06.010.

Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**:165–173 DOI 10.1038/nature04514.

Dick RP. 2011. *Methods of soil enzymology*. Madison: Soil Science Society of America.

Ding F, Huang Y, Sun W, Jiang G, Chen Y. 2014. Decomposition of organic carbon in fine soil particles is likely more sensitive to warming than in coarse particles: an incubation study with temperate grassland and forest soils in northern China. *PLOS ONE* **9**:e103801 DOI 10.1371/journal.pone.0103801.

Dubey A, Malla MA, Khan F, Chowdhary K, Yadav S, Kumar A, Sharma S, Khare PK, Khan ML. 2019. Soil microbiome: a key player for conservation of soil health under changing climate. *Biodiversity and Conservation* **28**:2405–2429 DOI 10.1007/s10531-019-01760-5.

Eivazi F, Tabatabai MA. 1988. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* **20**:601–606 DOI 10.1016/0038-0717(88)90141-1.

Ekwurzel B, Boneham J, Dalton MW, Heede R, Mera RJ, Allen MR, Frumhoff PC. 2017. The rise in global atmospheric CO2, surface temperature, and sea level from emissions traced to major carbon producers. *Climatic Change* **144**:579–590 DOI 10.1007/s10584-017-1978-0.

Elliott ET, Burke IC, Monz CA, Frey SD, Paustian KH, Collins HP, Paul EA, Cole CA, Blevins RL, Frye WW, Lyon DW, Halvorson AD, Huggins DR, Turco RF, Hickman MV. 1994. Terrestrial carbon pools. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA, eds. *Preliminary data from the Corn Belt and Great Plains regions. Defining soil quality for a sustainable environment*. Madison: American Society of Agronomy, 179–191.

Fang X, Zhou G, Li Y, Liu S, Chu G, Xu Z, Liu J. 2016. Warming effects on biomass and composition of microbial communities and enzyme activities within soil aggregates in subtropical forest. *Biology and Fertility of Soils* **52**:353–365 DOI 10.1007/s00374-015-1081-5.

Feng W, Plante AF, Six J. 2013. Improving estimates of maximal organic carbon stabilization by fine soil particles. *Biogeochemistry* **112**:1–13 DOI 10.1007/s10533-011-9679-7.

Frøseth RB, Bleken MA. 2015. Effect of low temperature and soil type on the decomposition rate of soil organic carbon and clover leaves, and related priming effect. *Soil Biology and Biochemistry* **80**:156–166 DOI 10.1016/j.soilbio.2014.10.004.

García-Palacios P, Vandegehuchte ML, Shaw EA, Dam M, Post KH, Ramirez KS, Sylvain ZA, de Tomasel CM, Wall DH. 2015. Are there links between responses of soil microbes and ecosystem functioning to elevated CO2, N deposition and warming? A global perspective. *Global Change Biology* **21**:1590–1600 DOI 10.1111/gcb.12788.

Ghosh A, Bhattacharyya R, Dwivedi BS, Meena MC, Agarwal BK, Mahapatra P, Shahi DK, Salwani R, Agnihorti R. 2016. Temperature sensitivity of soil organic carbon decomposition as affected by long-term fertilization under a soybean based cropping system in a sub-tropical Alfisol. *Agriculture, Ecosystem and Environment* **233**:202–213 DOI 10.1016/j.agee.2016.09.010.

Gregorich EG, Ellert BH. 1993. Light fraction and macroorganic matter in mineral soils. In: Carter MR, ed. *Soil Sampling Methods and Analysis*. Boca Raton: Canadian Society of Soil Science.

Hamarashid N, Othman M, Hussain M. 2010. Effects of soil texture on chemical compositions, microbial populations and carbon mineralization in soil. *Egyptian Journal of Experimental Biology* **6**:59–64.
Hassan W, David J, Abbas F. 2014. Effect of type and quality of two contrasting crop residues on CO₂ emission potential of Ultisol soil: implications for indirect influence of temperature and moisture. *CATENA* 114:90–96 DOI 10.1016/j.catena.2013.11.001.

Hassan W, Akmal M, Muhammad I, Ali F, Younas M, Zahaid KR. 2013a. Response of soil microbial biomass and enzymes activity to cadmium toxicity under different soil textures and incubation times. *Australian Journal of Crop Science* 7:674–680 DOI 10.3316/informit.364836133250432.

Hassan W, Chen W, Huang Q, Mohamed I. 2013b. Microcalorimetric evaluation of soil microbiological properties under plant residues and dogmatic water gradients in Red soil. *Soil Science and Plant Nutrition* 59:858–870 DOI 10.1080/00380768.2013.845735.

Hobley E, Willgoose GR, Frisia S, Jacobsen G. 2014. Stability and storage of soil organic carbon in a heavy-textured Karst soil from south-eastern Australia. *Soil Research* 52:476–482 DOI 10.1071/SR13296.

Hutchins DA, Jansson JK, Remais JV, Rich Singh VI, Trivedi BK, P. 2019. Climate change microbiology-problems and perspectives. *Nature Reviews Microbiology* 17:391–396 DOI 10.1038/s41579-019-0178-5.

Karhu K, Fritze H, Hämäläinen K, Vanhala P, Jungner H, Oinonen M, Sonninen E, Tuomi M, Spetz P, Kitunen V, Liski J. 2010. Temperature sensitivity of soil carbon fractions in boreal forest soil. *Ecology* 91:370–376 DOI 10.1890/09-0478.1.

Lian Z, Jiang Z, Huang X, Liu S, Zhang J, Wu Y. 2018. Labile and recalcitrant sediment organic carbon pools in the Pearl River Estuary, southern China. *Science of the Total Environment* 640-641:1302–1311 DOI 10.1016/j.scitotenv.2018.05.389.

Li Y, Liu YH, Wang YL, Niu L, Xu X, Tian YQ. 2014. Interactive effects of soil temperature and moisture on soil N mineralization in a Stipa krylovii grassland in inner Mongolia, China. *Journal of Arid Land* 6:571–580 DOI 10.1007/s40333-014-0025-5.

Melillo JM, Frey SD, DeAngelis KM, Werner WJ, Bernard MJ, Bowles FP, Pold G, Knorr A, Grandy AS. 2017. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science* 358:101–105 DOI 10.1126/science.aan2874.

Meng C, Tian D, Zeng H, Li Z, Chen HYH, Niu S. 2020. Global meta-analysis on the responses of soil extracellular enzyme activities to warming. *Science of the Total Environment* 705:135992 DOI 10.1016/j.scitotenv.2019.135992.

Mishra S, Rath AK, Adhya TK, Rao VR, Sethunathan N. 1997. Effect of continuous and alternate water regimes on methane efflux from rice under greenhouse conditions. *Biology and Fertility of Soils* 24:399–405 DOI 10.1007/s003740050264.

Nguyen BT, Lehmann J, Hockaday WC, Joseph S, Masiello CA. 2010. Temperature sensitivity of black carbon decomposition and oxidation. *Environmental Science and Technology* 44(9):3324–3331 DOI 10.1021/es903016y.

Nottingham AT, Bååth E, Reischke S, Salinas N, Meir P. 2019. Adaptation of soil microbial growth to temperature: using a tropical elevation gradient to predict future changes. *Global Change Biology* 25(3):827–838 DOI 10.1111/gcb.14502.

Oertel C, Matschullat J, Zurba K, Zimmermann F, Erasmi S. 2016. Greenhouse gas emissions from soils: a review. *Geochemistry* 76(3):327–352 DOI 10.1016/j.chemer.2016.04.002.

Paul EA, Morris SJ, Bohm S. 2001. The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers, in assessment methods for soil C pools. In: Lal R, Kimble JM, Follett RF, eds. *The Determination of Soil C Pool Sizes and Turnover Rates: Biophysical Fractionation and Tracers, in Assessment Methods for Soil C Pools*. Boca Raton: CRC Press.
Paustian K, Larson E, Kent J, Marx E, Swan A. 2019. Soil C sequestration as a biological negative emission strategy. *Frontiers in Climate* 1:8 DOI 10.3389/fclim.2019.00008.

Qi R, Li J, Lin Z, Li Z, Li Y, Yang X, Zhang J, Zhao B. 2016. Temperature effects on soil organic carbon, soil labile organic carbon fractions, and soil enzyme activities under long-term fertilization regimes. *Applied Soil Ecology* 102:36–45 DOI 10.1016/j.apsoil.2016.02.004.

Qu Y, Tang J, Li Z, Zhou Z, Wang J, Wang S, Cao Y. 2020. Soil enzyme activity and microbial metabolic function diversity in soda saline-alkali rice paddy fields of Northeast China. *Sustainability* 12:10095 DOI 10.3390/su122310095.

Rittl TF, Canisares L, Sagrilo E, Butterbach-Bahl K, Dannenmann M, Cerri CEP. 2020. Temperature sensitivity of soil organic matter decomposition varies with biochar application and soil type. *Pedosphere* 30:336–342 DOI 10.1016/S1002-0160(20)60013-3.

Roberge MR. 1978. Methodology of enzymes determination and extraction. In: Burns RG, ed. *Soil Enzymes*. New York: Academic Press, 341–373.

Romero-Olivares AL, Alisson SD, Trescedar KK. 2017. Soil microbes and their response to experimental warming over time: a meta-analysis of field studies. *Soil Biology and Biochemistry* 107:32–40 DOI 10.1016/j.soilbio.2016.12.026.

Schinner F, Von Mersi W. 1990. Xylanase, CM-cellulase and invertase activity in soil: an improved method. *Soil Biology and Biochemistry* 22:511–515 DOI 10.1016/0038-0717(90)90187-5.

Sánchez-Cañete EP, Barron-Gaford GA, Chorover J. 2018. A considerable fraction of soil respired CO$_2$ is not emitted directly to the atmosphere. *Scientific Reports* 8:13518 DOI 10.1038/s41598-018-29803-x.

Sierra CA, Malghani S, Loescher HW. 2017. Interactions among temperature, moisture, and oxygen concentrations in controlling decomposition rates in a boreal forest soil. *Biogeosciences* 14(3):703–710 DOI 10.5194/bg-14-703-2017.

Six J, Paustian K. 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biology and Biochemistry* 68:A4–A9 DOI 10.1016/j.soilbio.2013.06.014.

Takriti M, Wild B, Schnecker J, Moosjammer M, Knoltsch A, Lashchinskiy N, Alves RJE, Gentsch N, Giteel A, Mikutta R, Wanek W, Richter A. 2018. Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect. *Soil Biology and Biochemistry* 121:212–2020 DOI 10.1016/j.soilbio.2018.02.022.

Tang L, Zhong L, Xue K, Wang S, Xu Z, Lin Q, Luo C, Rui Y, Li X, Li M, Liu W, Yang Y, Zhou J, Wang Y. 2019. Warming counteracts grazing effects on the functional structure of the soil microbial community in a Tibetan grassland. *Soil Biology and Biochemistry* 134:113–121 DOI 10.1016/j.soilbio.2019.02.018.

Thakur MP, reich PB, Wagg C, Fischelli NA, Ciobanu M, Hobbie SE, Rich RL, Stefanski A. 2017. Effects of soil warming history on the performances of congeneric temperate and boreal herbaceous plant species and their associations with soil biota. *Journal of Plant Ecology* 10:670–680 DOI 10.1093/jpe/rtw066.

Wachiye S, Merbold L, Vesala T, Rinne J, Räsänen M, Leitner S, Pellikka P. 2019. Soil greenhouse gas emissions under different land-use types in savanna ecosystems of Kenya. *Biogeosciences* 17(8):2149–2167 DOI 10.5194/bg-17-2149-2020.

Wang X, Dong S, Gao Q, Zhou H, Liu S, Su X, Li Y. 2014. Effects of short-term and long-term warming on soil nutrients, microbial biomass and enzyme activities in an alpine meadow on the Qinghai-Tibet Plateau of China. *Soil Biology and Biochemistry* 76:140–142 DOI 10.1016/j.soilbio.2014.05.014.
Wang Y, Gao S, Li C, Zhang J, Wang L. 2016. Effects of temperature on soil organic carbon fractions contents, aggregate stability and structural characteristics of humic substances in a Mollisol. *Journal of Soils and Sediments* 16:1849–1857 DOI 10.1007/s11368-016-1379-4.

Walker TWN, Kaiser C, Strasser F, Herbold CW, Leblans NIK, Woebken D, Janssens IA, Sigurdsson BD, Richter A. 2018. Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. *Nature Climate Change* 8(10):885–889 DOI 10.1038/s41558-018-0259-x.

Walkley A, Black IA. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* 37:29–38 DOI 10.1097/00010694-193401000-00003.

Wankhede M, Ghosh A, Manna MC, Misra S, Sirothia P, Rahman MM, Bhattacharyya P, Singh M, Bhattacharyya R, Patra AK. 2020. Does soil organic carbon quality or quantity govern relative temperature sensitivity in soil aggregates? *Biogeochemistry* 148(2):191–206 DOI 10.1007/s10533-020-00653-y.

Witkamp M. 1966. Decomposition of leaf litter in relation to environment, microflora and microbial respiration. *Ecology* 47:194–201 DOI 10.2307/1933765.

Yang F, Wei X, Huang M, Li C, Zhao X, Zhang Z. 2021. Spatiotemporal variability of soil organic carbon for different topographic and land use types in a gully watershed on the Chinese Loess Plateau. *Soil Research* 59(4):383–395 DOI 10.1071/SR19317.

Zhang H, Zhou Z. 2018. Recalcitrant carbon controls the magnitude of soil organic matter mineralization in temperate forests of northern China. *Forest Ecosystems* 5(1):1 DOI 10.1186/s40663-018-0137-z.

Zhang Q, Wu J, Yang F, Lei Y, Zhang Q, Cheng X. 2016. Alterations in soil microbial community composition and biomass following agricultural land use change. *Scientific Reports* 6(1):36587 DOI 10.1038/srep36587.

Zhang ZS, Dong XJ, Xu BX, Chen YL, Zhao Y, Gao YH, Hu YM, Huang L. 2015. Soil respiration sensitivities to water and temperature in a revegetated desert. *Journal Geophysical Research Biogeosciences* 120(4):773–787 DOI 10.1002/2014JG002805.

Zheng Q, Hu Y, Zhang S, Noll L, Böckle T, Richter A, Wanek W. 2019. Growth explains microbial carbon use efficiency across soils differing in land use and geology. *Soil Biology and Biochemistry* 128(6):45–55 DOI 10.1016/j.soilbio.2018.10.006.

Zhou XQ, Chen CR, Wang YF, Xu ZH, Han HY, Li LH, Wan SQ. 2013. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. *Science of Total Environment* 444:552–558 DOI 10.1016/j.scitotenv.2012.12.023.

Zomer RJ, Bossio DA, Sommer R, Verchot LV. 2017. Global sequestration potential of increased organic carbon in cropland soils. *Scientific Reports* 7(1):15554 DOI 10.1038/s41598-017-15794-8.

Zsolnay A. 2003. Dissolved organic matter (DOM): artefacts, definitions, and functions. *Geoderma* 113(3–4):187–209 DOI 10.1016/S0016-7061(02)00361-0.