

# Temperature responsiveness of soil carbon fractions, microbes, extracellular enzymes and CO<sub>2</sub> emission: Mitigating role of texture

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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 CO<sub>2</sub> 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 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub>-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.

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20 Running Title: **Sensitivity of soil C and biological properties**

21 **ABSTRACT**

22 The interaction of warming and soil texture on responsiveness of the key soil processes i.e.  
23 organic carbon (C) fractions, soil microbes, extracellular enzymes and CO<sub>2</sub> emissions remains  
24 largely unknown. Global warming raises the relevant question of how different soils and their  
25 key processes will respond in near future, and what will be the likely regulatory role of texture?  
26 To bridge this gap, this work applied laboratory incubation method to investigate the effects of  
27 temperature changes (10-50°C) on dynamics of labile, recalcitrant and stable C fractions, soil  
28 microbes, microbial biomass, activities of extracellular enzymes and CO<sub>2</sub> emissions in sandy and  
29 clayey textured soils. The role of texture (sandy and clayey) in the mitigation of temperature  
30 effect was also investigated. The results revealed that the temperature sensitivity of C fractions  
31 and extracellular enzymes was in the order recalcitrant C fractions > stable C fractions > labile C  
32 fractions and oxidative enzymes > hydrolytic enzymes. While temperature sensitivity of soil  
33 microbes and biomass was in the order bacteria > actinomycetes > fungi ≈ microbial biomass C  
34 (MBC) > microbial biomass N (MBN) > microbial biomass P (MBP). Conversely, the  
35 temperature effect and sensitivity of all key soil processes including CO<sub>2</sub> emissions were  
36 significantly ( $P < 0.05$ ) higher in sandy than clayey textured soil. Results confirmed that under  
37 the scenario of global warming and climate change, soils which are sandy in nature are more  
38 susceptible to temperature increase and prone to become the CO<sub>2</sub>-C sources. It was revealed that  
39 clayey texture played an important role in mitigating and easing off the undue temperature  
40 influence, hence, the sensitivity of key soil processes.

41 **Keywords:** Soil C fractions, soil microbes and biomass, extracellular enzymes, CO<sub>2</sub> fluxes,  
42 temperature sensitivity, warming, texture

43 **List of abbreviations:** TOC: total organic C, MBC: microbial biomass C, MBN: microbial  
44 biomass N, MBP: microbial biomass P, RCP: recalcitrant C pool, LCP: labile C pool, SCP:

45 stable C pool, ROC: recalcitrant organic carbon, WHC: water holding capacity, LFOC: light  
46 fraction of organic carbon, RMC: readily mineralizable carbon, DOC: dissolved organic carbon,  
47 POC: particulate organic carbon, EOC: easily oxidizable carbon, ROC: recalcitrant organic  
48 carbon, CFU: colony-forming unit

## 49 INTRODUCTION

50 The world's soils store substantially more carbon (C) than present in the atmosphere ([Badgery et](#)  
51 [al. 2020](#); [Paustian et al. 2019](#)). The estimated global soil C pool at one-meter depth is >1500 GT  
52 and two-meter depth is >2500 GT, which is 3.2 and 4 times the size of combined atmospheric  
53 and biotic C pool ([Zomer et al. 2017](#)). Being a gigantic pool, terrestrial C is receiving increasing  
54 attention both as a potentially large and uncertain source of CO<sub>2</sub> and also as a natural sink to  
55 reduce atmospheric CO<sub>2</sub> ([Badgery et al. 2020](#); [Zomer et al. 2017](#)). It has been estimated that soils  
56 emit  $\geq 11$  times CO<sub>2</sub>-C than fossil fuel combustion which is roughly about 68-100 Pgy<sup>-1</sup> ([Zhang](#)  
57 [et al. 2018](#)). Conversely, even a 0.4% annual increase in soil C has the potential to significantly  
58 halt the yearly atmospheric CO<sub>2</sub> increase ([Amundson and Biardeau, 2019](#)). Therefore, it is of  
59 utmost importance to examine soil C and its divergent fractions, and their likely sensitivity and  
60 response towards temperature increase for future feedbacks and predictions.

61 Due to continuous movement in the soil systems, soil C is constantly disintegrating and  
62 changing into divergent pools ([Yang et al. 2021](#); [Zomer et al. 2017](#)). The three major pools of  
63 soil C, are recalcitrant C pool (RCP), labile C pool (LCP), and stable C pool (SCP) respectively  
64 ([Zhang and Zhou, 2018](#)). The LCP is composed of newly incorporated plant residues, amino  
65 acids, simple carbohydrates, root exudates, and simple C fractions ([Lian et al. 2018](#)). Whereas,  
66 RCP is made of detritus, decomposed plant and microbial byproducts, and C fractions e.g.,  
67 recalcitrant organic carbon (ROC) which is resistant to decomposition ([Zhang and Zhou, 2018](#)).

68 Whereas, the total organic C (TOC) which is a heterogeneous mixture of diverse compounds  
69 (e.g., residues, humin, humic acid, aromatic and hydrophobic compounds) with several hundred  
70 years of a mean age and accounts for 90% of stable fraction is also known as SCP (Lian et al.  
71 2018). These C fractions are of utmost importance, owing to their direct and strong role in soil  
72 structure, C cycling and production and fluxes of CO<sub>2</sub> (Badgery et al. 2020; Zomer et al. 2017).  
73 The C fractions are extremely susceptible to abiotic variables, and multiple earlier studies have  
74 demonstrated that the future C balance of terrestrial ecosystems is highly dependent on the  
75 consequences of global warming (Wang et al. 2016; Qi et al. 2016; Biswas et al. 2018). Qi et al.  
76 (2016) observed a significant reduction in soil labile organic C fractions in response to warming,  
77 while Karhu et al. (2010) reported a decline in stable organic carbon fractions. Nonetheless, the  
78 temperature sensitivity of C fractions is a highly controversial and vague topic to date (Davidson  
79 and Janssens 2006; Sierra et al. 2017). Therefore, it is the need of the day to quantify and  
80 establish the temperature sensitivity of C fractions of labile, recalcitrant, and stable pools.

81 Soil microbes i.e., bacteria, fungi, and actinomycetes, owing to their vast metabolic  
82 diversity play diverse and critical roles in all-major biogeochemical cycles and ecosystem  
83 services (Walker et al. 2018; Nottingham et al. 2019). They also play a key role in regulating the  
84 C decomposition, emission of CO<sub>2</sub>, and overall C cycle of the ecosystem (Qu et al. 2020). Alike,  
85 soil enzymes are major components of biological processes which participate in all biochemical  
86 reactions (Hassan et al. 2013a). Soil enzymes play an important role in the biological catabolism,  
87 decomposition of organic matter and C cycling (Hassan et al. 2013b; Aislabie and Deslippe,  
88 2013). They also perform catalysis of reactions that are necessary for the life processes of  
89 microorganisms (Walker et al. 2018; Hassan et al. 2013b). Soil microbial community and  
90 enzymes respond to changes in soil and environmental factors much faster than do other

91 variables ([Nottingham et al. 2019](#); [Aislabie and Deslippe, 2013](#)). Soil microbial community and  
92 enzymes are sensitive to a number of environmental factors, among them temperature is of  
93 utmost ascendancy ([Walker et al. 2018](#)). Under the scenario of global warming, it is indeed  
94 important to test the temperature sensitivity of the soil microbial communities (bacteria, fungi,  
95 and actinomycetes) and extracellular enzymes (oxidative and hydrolytic).

96         Temperature is rightly known as one of the primary bio-controller, because it influences  
97 soil reactions, biological processes and the inter-spheric gas exchange between the soil and  
98 atmosphere ([Thakur et al. 2016](#); [Fang et al. 2016](#)). Due to its control over energy shifts,  
99 microbial communities, and extracellular enzyme activity, it regulates OM mineralization rates  
100 and storage, and hence the production of CO<sub>2</sub> in soils is also temperature-dependent ([Hassan et](#)  
101 [al. 2014](#); [Thakur et al. 2016](#); [Walker et al. 2018](#)). Therefore, the temperature has a great  
102 influence on the ability of soils to act as a C sink or source ([Walker et al. 2018](#); [Thakur et al.](#)  
103 [2016](#); [Fang et al. 2016](#)). For example, [Zhou et al. \(2013\)](#) found that a six-year warming period  
104 enhanced the activities of  $\beta$ -glucosidase and N-acetylglucosaminidase, which were connected  
105 with changes in microbial biomass C. Under warming conditions, changes in the soil LOC  
106 fractions have been shown to drive changes in soil enzyme activity ([Zhou et al., 2013](#); [Qi et al.](#)  
107 [2016](#)). However, according to [Li et al. \(2018\)](#), microbial responses to climate change may be  
108 influenced by soil properties.

109         The texture is one of the most important properties of soil because it  
110 determines characteristics and biophysical properties that shape and regulate the overall behavior  
111 and response of soils ([Fang et al. 2016](#); [Ding et al. 2014](#); [Hassan et al. 2013a](#)). The texture is  
112 associated with porosity, moisture, gaseous exchange, nutrient cycling, and substrate availability  
113 to microbiota along with other important functions and services in soils ([Oertel et al. 2016](#);

114 [Hobley et al. 2014](#); [Hamarashid et al. 2010](#)). Moreover, it also provides physical protection to  
115 soil microbiota, organic matter, and C from harsh climatic conditions i.e., temperature anomalies  
116 ([Frøseth and Bleken, 2015](#); [Hassan et al. 2013a](#)). Therefore, it affects the microbial and  
117 enzymatic activity, decomposition of organic matter, nutrients and C cycling, and eventually  
118 CO<sub>2</sub> production ([Ding et al. 2014](#); [Feng et al. 2013](#)). Soil texture can modulate the effects of  
119 temperature and climate change and thus production and emission of gases (e.g., CO<sub>2</sub>) through  
120 its strong influence on biochemical processes and C cycling and storage ([Zhang et al. 2015](#); [Feng  
121 et al. 2013](#)). The main reason for the strong influence of texture on key soil processes and  
122 activities is diverse and divergent characteristics of its relative particle's i.e., fine and coarse  
123 ([Frøseth and Bleken, 2015](#); [Hamarashid et al. 2010](#)). The fine particles (i.e., clay) have large  
124 surface areas, numerous reactive sites, strong ligand exchange, and polyvalent cation bridges  
125 than coarse ones i.e., sand ([Fang et al. 2016](#); [Ding et al. 2014](#); [Hassan et al. 2013a](#)). However, the  
126 interaction of warming and soil texture on responsiveness of the key soil processes remains  
127 largely unknown.

128         Therefore, it is indeed important to quantify the role of texture in regulating the  
129 temperature sensitivity of key soil processes for correct future inventories and feedbacks. We  
130 hypothesized that, warming would increase decomposition of recalcitrant and stable soil C pools  
131 via microbial activities and extracellular enzymes. These changes will be more pronounced in  
132 sandy textured soils while clayey soils will mitigate the effects of warming. Thus, the purpose of  
133 this study was (1) to determine the temperature influence and responsiveness of labile,  
134 recalcitrant, and stable C fractions, as well as CO<sub>2</sub> emission from divergent textured soils (2)  
135 quantify the effect of temperature on soil microbial counts (bacteria, fungi, and actinomycetes),  
136 microbial biomass, and extracellular enzymes (oxidative and hydrolytic) activities and their

137 response towards temperature increase in divergent textured soils and (3) identify and establish  
138 the potential role of texture in climate change mitigation.

## 139 **MATERIAL AND METHODS**

### 140 **Soil sampling**

141 The study area has a moderately continental climate, the maximum and minimum mean annual  
142 temperatures are 14.03°C and 6.72°C and average annual precipitation is 24.97 mm. Soil  
143 samples (0-30 cm depth) were collected randomly using a hand auger from ten points within the  
144 selected agricultural fields at Dahlem and Rhinluch, Berlin, Germany (52°27" N and 13°18" E)  
145 in April 2017. Winter wheat and maize was grown in rotation. Soils were Albic Luvisol and  
146 Arenosol with glacial till and periglacial sand parent materials. Samples (field fresh) were sieved  
147 (< 2mm) and separated into two subsamples. One part of the subsample was used to conduct the  
148 incubation experiment while the other was used for microbial and enzymatic analyses. The  
149 remaining soil was used for physicochemical and C fractional analyses after air-drying at room  
150 temperature (25°C) for 7 days by using methods as described by Hassan et al. (2014). The basic  
151 physicochemical properties of experimental soil are given in Table 1A.

### 152 **Experimental layout**

153 For incubation, 400 g dry soil was incubated in 1000 ml glass jars under different temperature  
154 and moisture regimes for 84 days in a randomized block design. Soil samples were wetted to  
155 maintain 60% of water holding capacity (WHC) and equilibrated overnight at 4°C, before being  
156 placed in incubators. The five treatments in triplicate were developed and expressed as T1  
157 (10°C), T2 (20°C), T3 (30°C), T4 (40°C), and T5 (50°C). To keep the soils at their prescribed  
158 WHC, moisture loss in the jars was determined after every 2 days by weighing the jars and the

159 water loss was replenished with distilled water throughout the incubation ([Elliott et al. 1994](#)).  
160 Soil samples were collected from each jar after incubation, for the determination of labile,  
161 recalcitrant and stable C fractions, microbial community and enzymes.

## 162 **Quantification of Carbon dioxide (CO<sub>2</sub>) emission**

163 The emission of CO<sub>2</sub> from the incubated soil (as described above) was estimated by the alkali  
164 trap method as described by [Witkamp, \(1966\)](#). The evolved CO<sub>2</sub> was trapped in 25 ml of 0.1 M  
165 KOH. After exposure, the KOH solution was removed, and any carbonate formed precipitated  
166 with saturated BaCl<sub>2</sub> to form BaCO<sub>3</sub>; the remaining KOH was then titrated with an equivalent  
167 strength of HCl using phenolphthalein as an indicator. A jar without soil, containing the same  
168 amount of KOH, was run simultaneously as a blank. The evolved CO<sub>2</sub> was measured at 7, 21, 42,  
169 63 and 84 days during incubation. The evolved and cumulative CO<sub>2</sub> was calculated, by using the  
170 method of [Hassan et al. \(2014\)](#).

## 171 **Determination of soil C fractions**

### 172 **Total organic carbon**

173 Total soil carbon of the soil before and after the experiment was determined by potassium  
174 dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) oxidation at 170-180°C followed by titration with 0.5 mol L<sup>-1</sup> FeSO<sub>4</sub>  
175 ([Walkley and Black 1934](#)).

### 176 **Light fraction of organic carbon**

177 Light fraction of organic carbon was measured by wet oxidation (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Briefly, 50 ml  
178 NaI solution (1.70 g cm<sup>-3</sup> density) along with soil sample (25 g) was placed into a centrifuge  
179 tube and was shaken (200 rpm) for 15 minutes. The floating material was extracted in triplicate

180 and transferred to a filter paper and rinsed every time with  $\text{CaCl}_2$  (0.01M) and distilled water,  
181 and then dried ( $60^\circ\text{C}$ ) for 48 hours (Gregorich and Ellert, 1993).

### 182 **Readily mineralizable carbon**

183 Readily mineralizable carbon was estimated after extraction with  $\text{K}_2\text{SO}_4$  (0.5 M) followed by wet  
184 digestion with dichromate. Briefly, soil (10 g), after precipitating the  $\text{Fe}^{2+}$  with 1 ml of  
185  $\text{FeCl}_3$  (2.5% solution) and 4 ml of 6 N NaOH, was extracted with 40 ml of  $\text{K}_2\text{SO}_4$  (0.5 M) after  
186 shaking for 1 hour at a rotary shaker. After allowing the precipitate to settle down ( $4^\circ\text{C}$ ) clear  
187 supernatant (aliquots) were titrated with  $\text{FeH}_8\text{N}_2\text{O}_8\text{S}_2$  (0.04 N) by using 2 to 3 drops of  
188 diphenylamine (DPA) indicator after wet digestion with  $\text{H}_2\text{CrO}_4$  (Mishra et al. 1997).

### 189 **Dissolved organic carbon**

190 For dissolved organic carbon fresh soil (10 g) was extracted with the 2.0 M KCl (1:4 soil/water)  
191 after shaking (250 rpm) the soil samples for 30 minutes. The supernatant was then centrifuged  
192 (15,000 rpm) for 10 minutes and filtered (0.45  $\mu\text{m}$  cellulose ester filters) and analyzed at a TOC  
193 (Multi N/C 2100, Germany) analyzer (Zsolnay, 2003).

### 194 **Particulate organic carbon**

195 The particulate organic carbon was quantified after dispersing the soil sample (10 g) with 30 ml  
196 of hexametaphosphate ( $5 \text{ g l}^{-1}$ ) in a reciprocating shaker (90 rpm) for 18 hours. The soil  
197 suspension was transferred into another clean and empty container under a continuous flow of  
198 distilled water over a sieve (53- $\mu\text{m}$ ) to ascertain the separation. The remaining soil on the sieve  
199 was dried at  $55\text{-}60^\circ\text{C}$  for 48 hours after shifting to a glass dish, and ground to powder with a ball  
200 mill, and measured (wet digestion) for POC by using  $\text{K}_2\text{Cr}_2\text{O}_7$  (Camberdella and Elliott, 1992).

**201 Reducing sugar carbon**

202 The content of reducing sugar carbon was determined using a phenol reagent. One ml of soil  
203 extract was mixed with 1 ml of the phenol solution (5% w/v in distilled water), then 5 ml of 18.4  
204 M H<sub>2</sub>SO<sub>4</sub> (1.84 d) was added under continuous shaking. The mixture was left for 10 minutes,  
205 thereafter, incubated in a water bath at 25°C for 20 minutes and the absorbance was read  
206 colorimetrically with a standard curve of glucose at 490 nm by following [Badalucco et al. \(1992\)](#)  
207 with slight modification.

**208 Easily oxidizable carbon**

209 For easily oxidizable carbon soil (3 g) was centrifuged (2000 rpm) for 5 minutes along with 25  
210 ml of KMnO<sub>4</sub> (333 mM) and the absorbance of the supernatant and standards was read  
211 spectrometrically at 565 nm. Likewise, the blank samples (no soil + standard) were also analyzed  
212 in each run. The change in the concentration of KMnO<sub>4</sub> was used to assess the amount of C  
213 oxidized by assuming that 1 mM MnO<sub>4</sub> is consumed for the oxidation of 0.75 mM or 9 g of C  
214 ([Blair et al. 1995](#)).

**215 Recalcitrant organic carbon**

216 The recalcitrant organic carbon was determined by the acid hydrolysis (18 hours) of soil (1 g)  
217 with HCl (6 M). The repeated evaporation and filtration were done in order to remove the HCl  
218 and separate the soluble materials. The residue was washed with de-ionized water (20 ml) and  
219 dried at 55°C. After drying, the residue was ground and passed through a screen (180 mm), and  
220 combusted to CO<sub>2</sub> ([Paul et al. 2001](#)).

**221 Analysis of soil microbial colony counts and biomass**

222 The total number of bacteria, fungus, and actinomycetes was determined using the dilution plate  
223 count technique on nutritional agar, as described previously by Hassan et al (2013b). The  
224 dilution plate technique is based on the assumption that each colony is created by a single cell,  
225 referred to as a colony-forming unit (CFU). In a flask containing 90 ml distilled water and glass  
226 beads, 10 g of fresh soil was added (0.5 mm). For 30 minutes, the flask was shaken at 28°C and  
227 180 rpm. 0.1 ml of the suspension was put to a small tube containing 0.9 ml distilled water after  
228 shaking. The tube was gently shaken and used to perform the remaining dilutions. To count  
229 bacteria, dilutions of  $10^{-1}$ - $10^{-8}$  were utilized. Conversely, a range of  $10^{-1}$ - $10^{-6}$  was used for the  
230 determination of fungi and actinomycete. Each dilution was repeated three times In an incubator,  
231 the plates were incubated at 28°C (301.15 K). Bacteria, fungi, and actinomycetes were identified  
232 four, five, and seven days after plating, respectively (Hassan et al. 2013b). The chloroform  
233 fumigation-extraction method was used to determine the microbial biomass, i.e., MBC, MBN,  
234 and MBP. For this purpose, 10 g fresh soil was fumigated with alcohol-free chloroform for 24  
235 hours at a temperature of 25°C in a desiccator. The soils (fumigated and non-fumigated) were  
236 then extracted for an hour using a horizontal shaker (200 rpm), filtered with Whatman No. 40  
237 filter paper, and finally spectrophotometrically measured and computed (Hassan et al. 2013b).

### 238 **Examination of enzymes activity**

#### 239 **Phenoloxidase and peroxidase activity**

240 The phenoloxidase and peroxidase activity was measured by incubating (25°C) the soil (0.5 g), in  
241 a shaking environment (100 rpm), with acetate buffer (3 ml) and 2 ml of 10 mM L-3,4-  
242 dihydroxyphenylalanine (L-DOPA), followed by centrifugation for 10 minutes at 5°C. For  
243 peroxidase, an addition of 0.3%  $H_2O_2$  (0.2 ml), just before incubation, was made. Then the

244 absorbance of the dopachrome (reaction product) was read at 475 nm spectrophotometrically and  
245 activity of both enzymes was expressed as  $\mu\text{mol dopachrome g}^{-1} \text{ h}^{-1}$  (Dick, 2011).

#### 246 **Catalase activity**

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

#### 250 **Invertase activity**

251 The activity of invertase was determined by incubating (24 hours at  $37^\circ\text{C}$ ) the soil (5 g) with  
252 sucrose solution (15 ml), phosphate buffer (35.6 g  $\text{Na}_2\text{HPO}_4$  + 700 ml distilled  $\text{H}_2\text{O}$  + adjust pH  
253 to 5.5 with HCl + volume to 1 liter) and toluene (4-5 drops), followed by filtration. The activity  
254 (color density) was measured spectrophotometrically at 508 nm, after mixing the filtrate (1 ml)  
255 with 0.5%  $\text{C}_7\text{H}_4\text{N}_2\text{O}_7$  (2 ml), heating (5 minutes) in a boiling water bath and colling (3 minutes)  
256 it down under running water and making the final volume to 25 ml with deionized  $\text{H}_2\text{O}$  and  
257 described as  $\mu\text{mol glucose g}^{-1} \text{ h}^{-1}$  (Dick, 2011).

#### 258 **$\beta$ -glucosidase activity**

259 The  $\beta$ -glucosidase activity was estimated by incubating and treating (1 hour at  $37^\circ\text{C}$ ) the soil (1  
260 g) with 0.25ml toluene, 0.25mn *p*-nitrophenol phosphate (*p*-NPP), 4ml MUB (Modified  
261 universal buffer), 1 ml of glucoside, 1 ml of  $\text{CaCl}_2$  (0.5 M) and 4ml of 0.1 M THAM (Tris-  
262 hydroxymethyl-aminomethane) solution. After filtration (Whatman No. 2V) the activity was  
263 determined through spectrophotometer at 400 nm and described as  $\mu\text{mol } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$   
264 (Eivazi and Tabatabai, 1988).

## 265 **Cellulase activity**

266 The cellulase activity was measured after incubating (24 hours, 50°C), centrifuging (2500xg, 10  
267 min) and treating the soil (10 g) with 5ml acetate buffer (11.2 M, pH 5.5), carboxymethyl  
268 cellulose sodium (7 g) and cellulose substrate (0.7%). After filtration the activity was determined  
269 through spectrophotometer at 690 nm and described as  $\mu\text{mol glucose g}^{-1} \text{h}^{-1}$  (Schinner and Von  
270 Mersi, 1990).

## 271 **Statistical Analysis**

272 The statistical software Statistix 8.1 (Statistix, USA), and Excel 2016 were used for data  
273 analysis. Parametric statistics of ANOVA analysis was carried out to estimate the effect of  
274 temperature on the soil microbes, microbial biomass, enzymes, C fractions and CO<sub>2</sub> production  
275 and emissions under divergent textures. Mean separations were achieved by using the least  
276 significant difference (LSD) test at  $p < 0.05$ . Data presented are means  $\pm$  standard deviation (SD)  
277 of three replicates of each treatment. Correlation coefficients ( $R^2$ ) between soil C fractions of  
278 labile, recalcitrant, and stable pools, CO<sub>2</sub> emissions and cumulative CO<sub>2</sub>, microbial community,  
279 microbial biomass, oxidative and hydrolytic enzymes and temperature were developed by using  
280 the same software.

## 281 **RESULTS**

### 282 **Labile C fractions**

283 The response and decomposition of labile C fractions viz LFOC, DOC, RMC, RSC, POC, and  
284 EOC under a range of elevated temperature (T1-T5) regimes in sandy and clayey soil is  
285 presented in Fig. 1. The response and decomposition of labile C fractions increased significantly  
286 ( $P < 0.05$ ) with the increase in the temperature (per 10°C rise). However, the temperature

287 response and decomposition of labile C fractions were significantly ( $P < 0.05$ ) higher in sandy  
288 than the clayey soil. Therefore, in sandy soil, the maximum increase in the LFOC (2.92-fold),  
289 DOC (3.34-fold), RMC (4.07-fold), RSC (4.54-fold), POC (3.51-fold), and EOC (4.02-fold) was  
290 observed at the highest temperature i.e., T5 compared to lowest temperature (T1). Conversely, in  
291 clayey soil, maximum increase in the LFOC (2.41-fold), DOC (2.05-fold), RMC (3.17-fold),  
292 RSC (2.98-fold), POC (2.71-fold), and EOC (3.03-fold) was observed at the T4 compared to  
293 lowest temperature (T1). Whereas, the minimum sensitivity and decomposition of labile C  
294 fractions were observed at the lowest temperature i.e., T1. Furthermore, owing to higher  
295 temperature impact and decomposition, the sandy soil exhibited significantly lower labile C  
296 fractions i.e., LFOC (1.14-fold), DOC (1.17-fold), RMC (1.14-fold), RSC (1.15-fold), POC  
297 (1.16-fold), and EOC (1.17-fold) compared to the clayey soil. Mainly the effect of temperature  
298 on the labile C fractions in the sandy soil was in the order  $T4 > T5 > T3 > T2 > T1$ . Conversely,  
299 the influence of temperature on the labile C fractions in the clayey soil was in the order  $T5 > T4$   
300  $> T3 > T2 > T1$ .

### 301 **Recalcitrant C fractions**

302 The response and decomposition of recalcitrant C fraction viz ROC under a range of elevated  
303 temperature (T1-T5) regime in sandy and clayey soil is illustrated in Fig. 2. The response and  
304 decomposition of ROC enhanced markedly ( $P < 0.05$ ) with the temperature increase (per  $10^{\circ}\text{C}$   
305 rise) in both textured soils. Unlike the labile C fractions, the increase in temperature caused a  
306 significant and continuous increase in the response and decomposition of ROC under both  
307 textured soils. Therefore, the maximum increase in the ROC (3.16-fold and 3.72-fold) was  
308 observed at the highest temperature i.e., T5 in sandy and clayey soil respectively compared to  
309 lowest temperature (T1). Whereas, the minimum response and decomposition of ROC were

310 observed at the lowest temperature i.e., T1. Due to the higher temperature effect and  
311 decomposition, the decrease in the ROC was higher (1.15-fold) in sandy compared to clayey soil.  
312 In general, the effect of temperature on the ROC in both soils was in the order  $T5 > T4 > T3 >$   
313  $T2 > T1$  endorsing, the fact that ROC likely has higher sensitivity to temperature (T1-T5)  
314 increase than the labile C fractions.

### 315 **Stable C fractions**

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

### 328 **Microbial community**

329 The response of soil microbes i.e., bacteria, fungi, and actinomycetes under a range of elevated  
330 temperature (T1-T5) regimes in sandy and clayey soils is exhibited in Fig. 4. The response of the  
331 soil microbes increased significantly ( $P < 0.05$ ) with the temperature increases (per  $10^{\circ}\text{C}$  rise) in

332 both textured soils. However, the temperature sensitivity and response of the soil microbes were  
333 significantly ( $P < 0.05$ ) variable. The bacteria showed the maximum temperature response (2.22-  
334 fold and 2.57-fold) at the highest temperature (i.e., T5) in sandy and clayey soils correspondingly  
335 compared to lowest temperature (T1). Conversely, the maximum increase in the response of  
336 actinomycetes (2.01-fold and 2.52-fold) and fungi (1.64-fold and 1.73-fold) were found at T4  
337 and T3 in sandy and clayey soils respectively compared to lowest temperature (T1). Indicating  
338 the fact that among soil microbes the temperature sensitivity order is bacteria > actinomycetes >  
339 fungi. Whereas, the minimum sensitivity and response of soil microbes were observed at the  
340 lowest temperature i.e., T1. Besides, owing to higher temperature effect and sensitivity, the  
341 sandy soil showed a markedly lower soil microbes count i.e., bacteria (1.34-fold), fungi (1.12-  
342 fold), and actinomycetes (1.14-fold) compared to clayey soil. In general, the effect of  
343 temperature on the bacterial counts was in the order  $T5 > T4 > T3 > T2 > T1$ . Whereas the  
344 temperature sensitivity of actinomycetes and fungi were in an order of  $T4 > T5 > T3 > T2 > T1$   
345 and  $T3 > T4 > T5 > T2 > T1$  respectively.

#### 346 **Microbial biomass**

347 The response of microbial biomass i.e., MBC, MBN, and MBP under a range of elevated  
348 temperature (T1-T5) regimes in sandy and clayey soils is presented in Fig. 5. The response of  
349 microbial biomass increased significantly ( $P < 0.05$ ) with the temperature surge (per 10°C rise) in  
350 both textured soils. However, like soil microbes colony counts, the temperature sensitivity of  
351 microbial biomass was also significantly ( $P < 0.05$ ) variable. The MBC exhibited the maximum  
352 temperature sensitivity and increase (1.97-fold and 2.21-fold) at the highest temperature (i.e., T5)  
353 in sandy and clayey soils correspondingly compared to lowest temperature (T1). On the contrary,  
354 the maximum increase in the MBN (2.11-fold and 2.22-fold) and MBP (1.84-fold and 2.31-fold)

355 were found at T4 and T3 in sandy and clayey soils respectively compared to lowest temperature  
356 (T1). Indicating the fact that among microbial biomass the temperature sensitivity order is MBC  
357 > MBN > MBP. Whereas, the minimum sensitivity and response of microbial biomass were  
358 observed at the lowest temperature i.e., T1. Moreover, due to the higher temperature effect and  
359 sensitivity the sandy soil exhibited a significantly lower microbial biomass i.e., MBC (1.23-fold),  
360 MBN (1.29-fold), and MBP (1.43-fold compared to the clayey soil. The temperature sensitivity  
361 order for MBC was  $T5 > T4 > T3 > T2 > T1$ . Whereas the temperature sensitivity order for  
362 MBN and MBP was  $T4 > T5 > T3 > T2 > T1$  and  $T3 > T4 > T5 > T2 > T1$  respectively.

### 363 **Oxidative enzymes**

364 The response and activity of oxidative enzymes viz PO, PEO, and CAT under a range of elevated  
365 temperature (T1-T5) regimes in sandy and clayey soil are shown in Fig. 6. However, unlike the  
366 hydrolytic enzymes, a significant ( $P < 0.05$ ) and continuous increase in the activity of oxidative  
367 enzymes was observed with the increase in the temperature (per  $10^{\circ}\text{C}$  rise). As a result, the  
368 maximum increase in the activity of PO (2.61-fold and 4.07-fold), PEO (3.08-fold and 6.77-  
369 fold), and CAT (2.18-fold and 2.71-fold) were found at the highest temperature i.e., T5 in sandy  
370 and clayey soils respectively compared to lowest temperature (T1). Whereas minimum response  
371 and activity of oxidative enzymes were observed at the lowest temperature i.e., T1. Establishing  
372 the fact that oxidative enzymes have decidedly higher responsiveness to temperature increase  
373 than the hydrolytic enzymes. Furthermore, owing to the higher temperature effect, the sandy soil  
374 showed a markedly lower activity and values of oxidative enzymes i.e., PO (1.69-fold), PEO  
375 (1.48-fold), and CAT (1.24-fold) compared to the clayey soil. The overall effect of temperature  
376 on the sensitivity and activity of oxidative enzymes was in the order  $T5 > T4 > T3 > T2 > T1$ .

### 377 **Hydrolytic enzymes**

378 The response and activity of hydrolytic enzymes viz INV, BGL, and CELL under a range of  
379 elevated temperature (T1-T5) regimes in sandy and clayey soils are depicted in Fig. 7. Generally,  
380 an increasing trend was observed in the activity of hydrolytic enzymes under elevated  
381 temperature (per 10°C rise). However, unlike the oxidative enzymes, the maximum increase in  
382 the activity of INV (1.71-fold and 2.01-fold), BGL (1.85-fold and 2.22-fold), and CELL (1.81-  
383 fold and 2.23-fold) were found at T4 in sandy and clayey soils respectively compared to lowest  
384 temperature (T1). After that, an abrupt decrease in the activity of hydrolytic enzymes was  
385 examined at the highest temperature i.e., T5 compared to lowest temperature (T1). Whereas, the  
386 minimum activity of hydrolytic enzymes was observed at the lowest temperature (T1).  
387 Additionally, due to the higher temperature effect, the sandy soil depicted a significantly lower  
388 activity and values of hydrolytic enzymes i.e., INV (1.25-fold), BGL (1.23-fold), and CELL  
389 (1.29-fold) compared to the clayey soil. The overall effect of temperature on the response and  
390 activity of hydrolytic enzymes was in the order  $T4 > T5 > T3 > T2 > T1$ .

### 391 **Emissions and cumulative CO<sub>2</sub>**

392 The response and changes in the emissions and cumulative CO<sub>2</sub> under a range of elevated  
393 temperature (T1-T5) regimes in sandy and clayey soils are illustrated in Fig. 8. Overall, an  
394 increasing trend was found in the emissions and cumulative CO<sub>2</sub> for each 10°C rise in  
395 temperature. However, the temperature responsiveness and changes in emissions and cumulative  
396 CO<sub>2</sub> were significantly ( $P < 0.05$ ) higher in sandy than the clayey soil. Therefore, in sandy soil,  
397 the maximum increase in the emissions (1.84-fold) and cumulative CO<sub>2</sub> (1.81-fold) were  
398 observed at the highest temperature (T5) compared to lowest temperature (T1). Conversely, in  
399 clayey soil, higher emissions (1.45-fold) and cumulative CO<sub>2</sub> (1.36-fold) were observed at the T4  
400 compared to lowest temperature (T1). After that, in clayey soil, a decrease in the response and

401 thus values of emissions and cumulative CO<sub>2</sub> were examined at the highest temperature i.e., T5.  
402 Whereas, the minimum sensitivity and changes in emission and cumulative CO<sub>2</sub> were observed  
403 at the lowest temperature (i.e. T1). Furthermore, unlike other key soil processes, the sandy soil  
404 showed greater increase in the emissions (1.22-fold) and cumulative CO<sub>2</sub> (1.23-fold) owing to  
405 higher temperature effect, decomposition rate and changes than the clayey soil. Underscoring the  
406 fact that CO<sub>2</sub> production and emissions and cumulative CO<sub>2</sub> have positive feedback with the  
407 augmentation in the temperature, and sandy soil are more vulnerable than the clayey ones.  
408 Mainly the effect of temperature on the sensitivity and emissions and cumulative CO<sub>2</sub> in the  
409 sandy soil was in the order T5 > T4 > T3 > T2 > T1. Conversely, the influence of temperature on  
410 the sensitivity and emission and cumulative CO<sub>2</sub> in the clayey soil was in the order T4 > T5 > T3  
411 > T2 >.

#### 412 **Regression analysis**

413 Regression analysis showed that C fractions of labile, recalcitrant, and stable pools, CO<sub>2</sub> fluxes,  
414 and cumulative CO<sub>2</sub> correlated well with the temperature in both sandy and clayey soils (Table  
415 [1B](#)). Nonetheless, overall, temperature accounted for 93% and 79% variability in the C fractions  
416 of labile, recalcitrant, and stable pools in the sandy and clayey soils correspondingly (Table [1B](#)).  
417 Whereas, temperature accounted for 91% and 94% variability in the CO<sub>2</sub> fluxes and cumulative  
418 CO<sub>2</sub> in the sandy soil. Conversely, temperature described for 78% and 75% alterability in the  
419 CO<sub>2</sub> fluxes and cumulative CO<sub>2</sub> in the clayey soil (Table [1B](#)). Furthermore, temperature  
420 accounted variability was significantly higher for C fractions of recalcitrant and stable pools  
421 compared to labile pools in both sandy and clayey soils (Table [1B](#)). Regression analysis showed  
422 that overall, temperature accounted for 85% and 72% variability in the microbial community in  
423 the sandy and clayey soils (Table [2A](#)). The temperature accounted variability was significantly

424 higher for bacteria ( $R^2 = 0.97$  and  $0.91$ ) than actinomycetes ( $R^2 = 0.92$  and  $0.81$ ) and fungi ( $R^2 =$   
425  $0.68$  and  $0.46$ ) in both sandy and clayey soils (Table 2A). Moreover, the temperature described  
426 alterability was markedly higher in sandy soil than clayey soil (Table 2A). Whereas, temperature  
427 designated for 88% and 78% variability in the microbial biomass in the sandy and clayey soils  
428 (Table 2A). The temperature accounted alterability was significantly higher for MBC ( $R^2 = 0.98$   
429 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  
430 clayey soils (Table 2A). Moreover, the temperature designated changeability was markedly  
431 higher in sandy soil than clayey soil (Table 2A). Regression analysis showed that overall,  
432 temperature accounted for 93% and 86% variability in the oxidative enzymes in the sandy and  
433 clayey soils (Table 2B). Whereas, temperature accounted for 73% and 66% variability in the  
434 hydrolytic enzymes in the sandy and clayey soils (Table 2B). The temperature described  
435 alterability was significantly higher for oxidative enzymes than hydrolytic enzymes. Moreover,  
436 the temperature accounted variability was markedly higher in sandy soil than clayey soil (Table  
437 2B).

## 438 DISCUSSION

439 The responsiveness and decomposition of labile C fractions increased significantly ( $P < 0.05$ )  
440 with the temperature increase (per  $10^\circ\text{C}$ ) in both textured soils (Fig. 1). Yang et al. (2021) and Qi  
441 et al. (2016) assessed that temperature increase significantly alters the fractions of soil labile  
442 organic C (RSC, MBC, DOC, and POC) by increasing their response and rate of decomposition.  
443 However, response to the temperature and decomposition of labile C fractions were significantly  
444 ( $P < 0.05$ ) higher in sandy soil than the clayey soil (Fig. 1). Temperature accounted variability  
445 for labile C fractions was significantly higher in sandy soil than clayey soil (Table 1B).  
446 Wankhede et al. (2020), Rittl et al. (2020), Takriti et al. (2018), Ghosh et al. (2016), Frøseth and

447 [Bleken, \(2015\)](#) and [Hobley et al. \(2014\)](#) found that temperature impacts on labile C fractions was  
448 higher in coarse (sandy) than fine (clayey) soils owing to low physical protection, small specific  
449 areas, fewer reactive sites, and weak ligand exchange bridges, where soil C could be sorbed and  
450 protected. In present study, unlike the labile C fractions, the increase in temperature (T1-T5)  
451 caused a significant ( $P < 0.05$ ) and continuous increase in the sensitivity and decomposition of  
452 recalcitrant (ROC) and stable (TOC) C fractions (Figs. 2 and 3) in both soils. Whereas, the  
453 temperature impacts and thus decomposition of ROC (1.15-fold) and TOC (1.14-fold) were  
454 significantly higher in sandy soil at T5 i.e. 50°C (Figs. 2 and 3). [Wankhede et al. \(2020\)](#) and  
455 [Zheng et al. \(2019\)](#) examined that in sandy (coarse) soils the temperature response of recalcitrant  
456 and stable C fractions was much higher due to weak physical protection, fewer cations bridges,  
457 unstable moisture availability, and their low storing ability. The response of C fractions to  
458 temperature was in the order recalcitrant C fractions > stable C fractions > labile C fractions  
459 (Figs. 1-3). In both sandy and clayey soils, temperature accounted variability was significantly  
460 higher for C fractions of recalcitrant and stable pools compared to labile pools (Table 1B). [Zhang](#)  
461 [and Zhou, \(2018\)](#) and [Dai et al. \(2017\)](#) found that recalcitrant and stable C fractions have  
462 decidedly extra sensitivity than the labile fractions to the temperature increase (5°C-30°C) in  
463 divergent coarse and fine textured Chinese soils. The results of current study also endorsed the  
464 fact that recalcitrant and stable C fractions have a higher sensitivity to temperature (T1-T5)  
465 increase than the labile C fractions (Figs. 1-3). Higher  $R^2$  were found for recalcitrant and stable  
466 C fractions than labile ones in both sandy and clayey soils (Table 1B). [Biswas et al. \(2018\)](#), [Lian](#)  
467 [et al. \(2018\)](#), [Fang et al. \(2016\)](#) and [Nguyen et al. \(2010\)](#) confirmed that recalcitrant and stable C  
468 fractions have higher responses to temperature than the labile C fractions in coarse and fine  
469 textured soils.

470 The response of soil microbial counts and microbial biomass increased markedly ( $P <$   
471  $0.05$ ) with the temperature increase (per  $10^{\circ}\text{C}$  rise) in both sandy and clayey textured soils.  
472 However, the temperature responses of microbial colony counts i.e., bacteria (1.34-fold), fungi  
473 (1.12-fold), and actinomycetes (1.14-fold) and biomass i.e., MBC (1.23-fold), MBN (1.29-fold),  
474 and MBP (1.43-fold) were higher in sandy soil (Figs. 4 and 5). Overall, in sandy soil,  
475 temperature accounted for significantly higher variability in microbial population (85%) and  
476 microbial biomass (88%) than in clayey soil (Table 2A). [Qu et al. \(2020\)](#), [Nottingham et al.](#)  
477 [\(2019\)](#), [Hutchins et al. \(2019\)](#), [Zhang et al. \(2016\)](#), [Fang et al. \(2016\)](#), and [Hassan et al. \(2013a\)](#)  
478 examined that microbial counts (bacteria, fungi, and actinomycetes) and biomass (MBC, MBN,  
479 and MBP) had higher sensitivity to temperature increase and their sensitivity increased many  
480 folds in coarse (sandy) soils owing to less favorable conditions, predation, desiccation, and  
481 substrate availability. The results further, revealed that temperature sensitivity and response of  
482 soil microbes colony counts and biomass were significantly variable (Figs. 4 and 5). [Cavicchioli](#)  
483 [et al. \(2019\)](#), [Zhang et al. \(2016\)](#) and [Fang et al. \(2016\)](#) also examined variations in the activity,  
484 behavior, and response of microbial community and biomass towards experimental warming and  
485 temperature increase. The temperature response of soil microbes colony counts and biomass  
486 were in the order bacteria  $>$  actinomycetes  $>$  fungi and MBC  $>$  MBN  $>$  MBP (Table 2A).  
487 Therefore, the maximum activity and response of bacteria, actinomycetes and fungi and MBC,  
488 MBN, and MBP were observed at temperatures T5, T4 and T3 in both soils respectively (Figs. 4  
489 and 5). [Zheng et al. \(2019\)](#), [Dubey et al. \(2019\)](#), [Walker et al. \(2018\)](#), and [Zhang et al. \(2016\)](#)  
490 found a strong association between temperature increase and responses of soil microbes colony  
491 counts and biomass and stated that temperature sensitivity of bacteria and MBC is much higher  
492 followed by actinomycetes and fungi and MBN and MBP in diverse textured soils (coarse and

493 fine). The temperature accounted variability was significantly higher for bacteria ( $R^2 = 0.97$  and  
494  $R^2 = 0.91$ ) than actinomycetes and fungi in both sandy and clayey soils (Table 2A). [Romero-](#)  
495 [Olivares et al. \(2017\)](#), [Zhang et al. \(2016\)](#), [García-Palacios et al. \(2015\)](#), and [Wang et al. \(2014\)](#)  
496 also stated that among microbes and biomass, bacterial community and MBC have decidedly  
497 higher sensitivity, contrarily, fungi are less sensitive to changes in temperature owing to the  
498 chitinous cell walls that make them highly resilient. The temperature accounted variability was  
499 significantly higher for MBC ( $R^2 = 0.98$  and  $R^2 = 0.92$ ) than MBN and MBP in both sandy and  
500 clayey soils (Table 2A). [Melillo et al. \(2017\)](#), and [Crowther et al. \(2016\)](#) also found a significant  
501 association between the increase in temperature (warming), temperature sensitivity, and  
502 reduction in the microbial biomass and stated that temperature sensitivity of MBC is markedly  
503 higher.

504         The extracellular enzymes (i.e., oxidative and hydrolytic) response and activity increased  
505 significantly ( $P < 0.05$ ) with the temperature increase (per  $10^\circ\text{C}$  rise) in both textured soils.  
506 However, the temperature response of oxidative enzymes i.e., PO (1.69-fold), PEO (1.48-fold),  
507 and CAT (1.24-fold) and hydrolytic enzymes i.e., INV (1.25-fold), BGL (1.23-fold), and CELL  
508 (1.29-fold) were markedly higher in sandy soil (Figs. 6 and 7). The temperature accounted  
509 variability for oxidative and hydrolytic enzymes was markedly higher in sandy soil than clayey  
510 soil (Table 2B). [Wankhede et al. \(2020\)](#), [Cavicchioli et al. \(2019\)](#), [Zheng et al. \(2019\)](#), [Thakur et](#)  
511 [al. \(2016\)](#), and [Fang et al. \(2016\)](#) assessed a significant increase in the sensitivity and response of  
512 extracellular enzymes i.e., oxidative and hydrolytic with the temperature increase and stated that  
513 temperature sensitivity increases strongly in sandy (coarse) soils due to less favorable conditions,  
514 unstable moisture, and substrate availability. The results of present study further revealed that the  
515 temperature sensitivity of extracellular enzymes was in the order oxidative enzymes > hydrolytic

516 enzymes (Figs. 6 and 7). The temperature accounted variability was significantly higher for  
517 oxidative enzymes (93% and 86%) than hydrolytic enzymes (73% and 66%) in both sandy and  
518 clayey soils (Table 2B). Establishing the fact that oxidative enzymes have higher temperature  
519 sensitivity than the hydrolytic enzymes in both sandy and clayey soils. Meng et al. (2020), Tang  
520 et al. (2019), Walker et al. (2018), Allison et al. (2018), Cheng et al. (2017), and Fang et al.  
521 (2016) examined a strong synergistic association between extracellular enzymes sensitivity and  
522 temperature and revealed that oxidative enzymes (e.g., PO, PEO, and CAT) have decidedly  
523 higher temperature sensitivity than the hydrolytic (e.g., DEH, URE, INV, BGL, and PHP)  
524 enzymes.

525 Overall, an increasing trend was found in the emissions and cumulative CO<sub>2</sub> under  
526 elevated i.e., each 10°C rise in temperature in both sandy and clayey soils (Fig. 8). However, the  
527 temperature effect and changes in emissions and cumulative CO<sub>2</sub> were significantly ( $P < 0.05$ )  
528 higher in sandy than the clayey soil. Temperature accounted for 91% and 94% variability in the  
529 CO<sub>2</sub> emissions and cumulative CO<sub>2</sub> in the sandy soil (Table 1B). Sánchez-Cañete et al. (2018),  
530 Zomer et al. (2017), Ekwurzel et al. (2017), Fang et al. (2016) and Frøseth and Bleken, (2015)  
531 examined a significant increase in the emissions and cumulative CO<sub>2</sub> with the temperature rise  
532 and stated that coarse i.e., sandy soils have much higher temperature effect thus emissions and  
533 cumulative CO<sub>2</sub> than the fine (clayey or silty) soils. Therefore, in sandy soil, the maximum  
534 increase in the CO emissions (1.84-fold) and cumulative CO<sub>2</sub> (1.81-fold) was observed at the  
535 highest temperature (T5). Furthermore, the sandy soil showed significantly ( $P < 0.05$ ) higher  
536 CO<sub>2</sub> emissions (1.22-fold) and cumulative CO<sub>2</sub> (1.23-fold) owing to higher temperature effect,  
537 response and decomposition rate than the clayey soil (Fig. 8). Significantly higher correlation  
538 coefficients were observed between CO<sub>2</sub> emissions ( $R^2 = 0.91$ ) and cumulative CO<sub>2</sub> ( $R^2 = 0.94$ )

539 and temperature in sandy soil than clayey soil (Table 1B). [Wachiye et al. \(2020\)](#), [Badagliacca et](#)  
540 [al. \(2017\)](#), [Frøseth and Bleken, \(2015\)](#) and [Ding et al. \(2014\)](#) found a significantly higher  
541 responsiveness of CO<sub>2</sub> emissions and cumulative CO<sub>2</sub> to temperature in the sandy soils and  
542 established that this was due to high rate of C decomposition, low humification, and small  
543 specific areas in sandy soils, where soil C could be sorbed, secured and stored. Underscoring the  
544 fact that CO<sub>2</sub> production and emissions and cumulative CO<sub>2</sub> have a strong synergistic association  
545 with the temperature augmentation, and sandy soils have much higher temperature sensitivity  
546 and vulnerability to become CO<sub>2</sub>-C sources than the clayey ones (Fig. 8 and Table 1B).  
547 Temperature accounted for significantly lower variability for the CO<sub>2</sub> fluxes (78%) and  
548 cumulative CO<sub>2</sub> (75%) in clayey soil compared to sandy soil (Table 2). [Oertel et al. \(2016\)](#),  
549 [Frøseth and Bleken, \(2015\)](#), [Zhang et al. \(2015\)](#) and [Six and Paustian, \(2014\)](#) inspected that the  
550 sandy soils are more sensitive to temperature increase and the main reason of sandy/coarse soils  
551 to foster higher CO<sub>2</sub> production and emissions and cumulative CO<sub>2</sub> is high decomposition rate,  
552 low humification and availability of C sorption, and attachment sites.

553

## 554 CONCLUSION

555 The study concluded that the temperature sensitivity of soil C fractions, microbial colony counts,  
556 microbial biomass, extracellular enzymes, and CO<sub>2</sub> fluxes increased with the upsurge in  
557 temperature. However, the recalcitrant and stable C fractions have decidedly higher responses  
558 than labile C fractions. Alike, among microbes, microbial biomass, and extracellular enzymes,  
559 bacteria, MBC, and oxidative enzymes (PO, PEO, and CAT) have markedly higher sensitivity. It  
560 was concluded that the temperature effect and variability for all measured key soil processes  
561 along with CO<sub>2</sub> fluxes were markedly higher in sandy textured soil. Conversely, clayey texture  
562 performed a significant role in the mitigation of undue temperature influence, hence, the

563 sensitivity of key soil processes and CO<sub>2</sub> fluxes. The study also suggests between sandy and  
564 clayey textured soils, the soils which are sandy in nature under the scenario of global warming,  
565 are more vulnerable to become CO<sub>2</sub>-C sources therefore must be managed and treated wisely.  
566 Furthermore, in future research and models instead of generalizing effects of global warming,  
567 temperature sensitivity of individual key soil processes must also be considered carefully. The  
568 findings of the study will be helpful in alleviating the controversy of the temperature sensitivity  
569 of key soil processes in sandy and clayey soils. And enabling the scientists and environmentalists  
570 to formulate measures and devise recommendations to reduce the excessive increase in CO<sub>2</sub>-C  
571 fluxes from divergent textured soils.

#### 572 **CONFLICT OF INTEREST**

573 The authors have no conflict of interest to declare.

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#### 579 **Author contribution**

580 WH conceptualized and conducted the study and led the writing. TS analyzed the data and  
581 prepared figures and tables under WH's supervision. LY, and JW reviewed and improved the  
582 manuscript. All authors contributed to this work and approved the final manuscript prior to  
583 submission.

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#### 764 **Figure captions**

765 **Fig 1.** Effect of temperature on labile C fractions under sandy and clayey texture

766 LFOC, light fraction of organic carbon; DOC, Dissolve organic carbon; RMC, readily  
767 mineralizable carbon; RSC, reducing sugar carbon; POC, Particulate organic carbon; EOC,  
768 easily oxidizable carbon

769 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

770 **Fig 2.** Effect of temperature on recalcitrant C fraction under sandy and clayey texture

771 ROC, recalcitrant organic carbon

772 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

773 **Fig 3.** Effect of temperature on stable C fraction under sandy and clayey texture

774 TOC, total organic carbon

775 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

776 **Fig 4.** Effect of temperature on microbial community under sandy and clayey texture

777 Units: Bacteria, CFU $\times 10^6$  g $^{-1}$ ; Fungi, CFU $\times 10^4$  g $^{-1}$ ; Actinomycetes, CFU $\times 10^5$  g $^{-1}$

778 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

779 **Fig 5.** Effect of temperature on microbial biomass under sandy and clayey texture

780 MBC, microbial biomass carbon, MBN, microbial biomass nitrogen; MBP, microbial biomass

781 phosphorous

782 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

783 **Fig 6.** Effect of temperature on oxidative enzymes activity under sandy and clayey texture

784 PO, phenol oxidase; PEO, peroxidase; CAT, Catalase

785 Units: PO and PEO,  $\mu\text{mol dopachrome g}^{-1} \text{ h}^{-1}$ ; CAT,  $\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$

786 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

787 **Fig 7.** Effect of temperature on hydrolytic enzymes activity under sandy and clayey texture

788 INV, Invertase; BGL,  $\beta$ -glucosidase; CELL, cellulose

789 Units: INV,  $\mu\text{mol glucose g}^{-1} \text{ h}^{-1}$ ; BGL,  $\mu\text{mol } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ ; CELL,  $\mu\text{mol glucose g}^{-1} \text{ h}^{-1}$

790 Vertical bars represent means  $\pm$  SD (n = 3). ANOVA significant at  $p \leq 0.05$

791 **Fig 8.** Effect of temperature on CO<sub>2</sub> emissions and cumulative CO<sub>2</sub> under sandy and clayey

792 texture

793 Unit: CO<sub>2</sub> emission, mg kg<sup>-1</sup> h<sup>-1</sup>; Cumulative CO<sub>2</sub>, mg kg<sup>-1</sup>

794 Vertical bars represent means ± SD (n = 3). ANOVA significant at p ≤ 0.05

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# Figure 1

Effect of temperature on labile C fractions under sandy and clayey texture

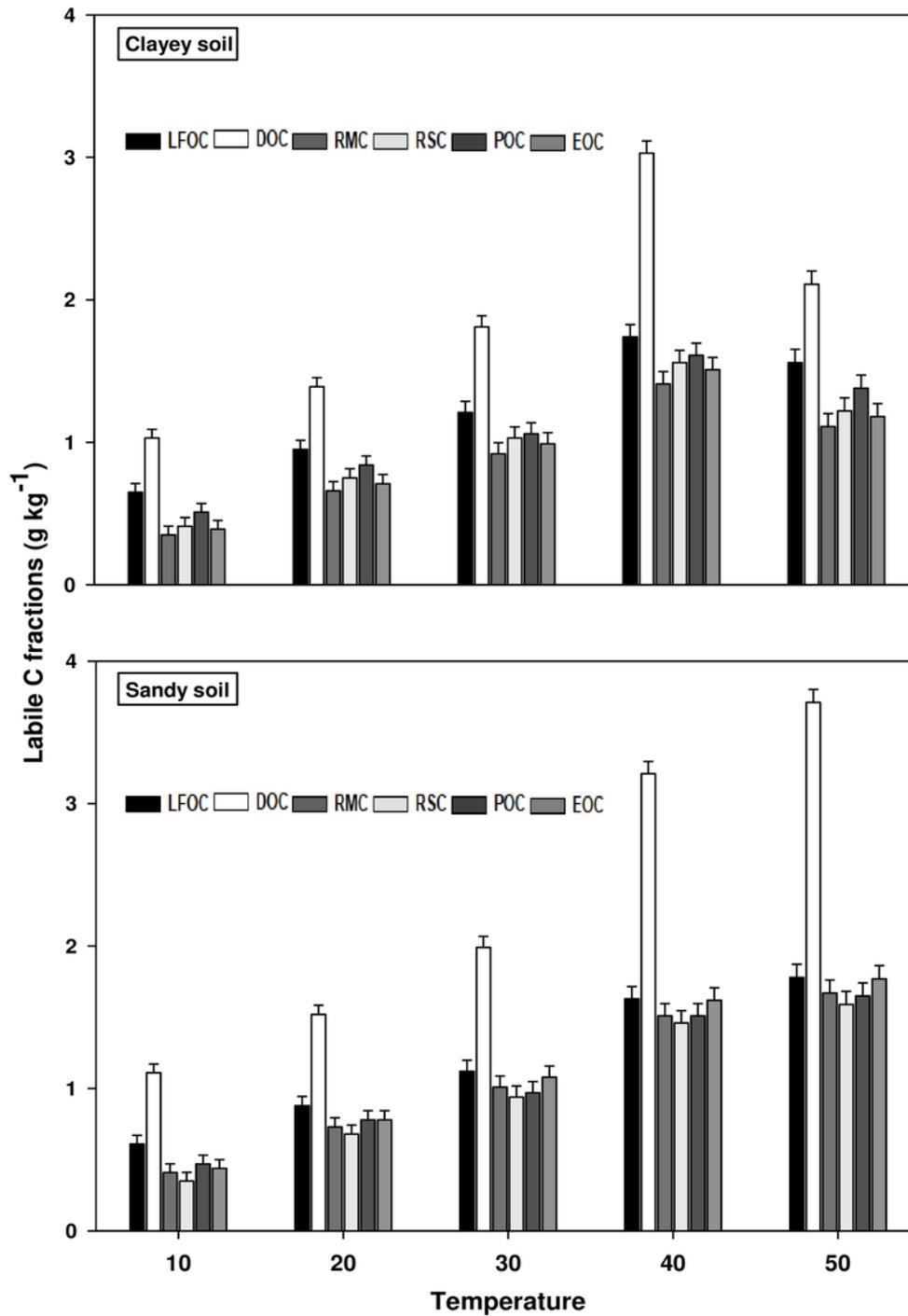


Fig. 1

## Figure 2

Effect of temperature on recalcitrant C fraction under sandy and clayey texture

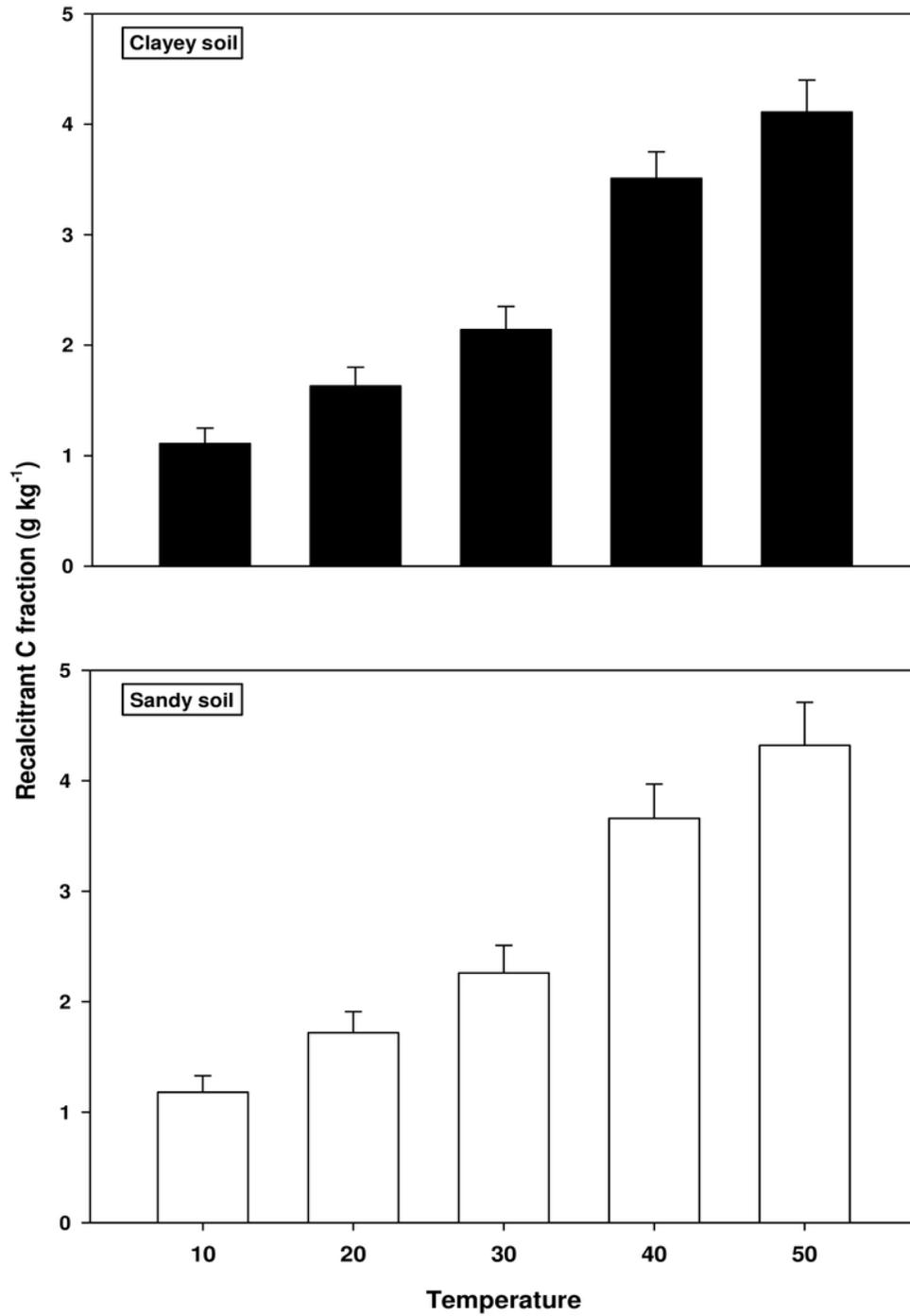


Fig. 2

## Figure 3

Effect of temperature on stable C fraction under sandy and clayey texture

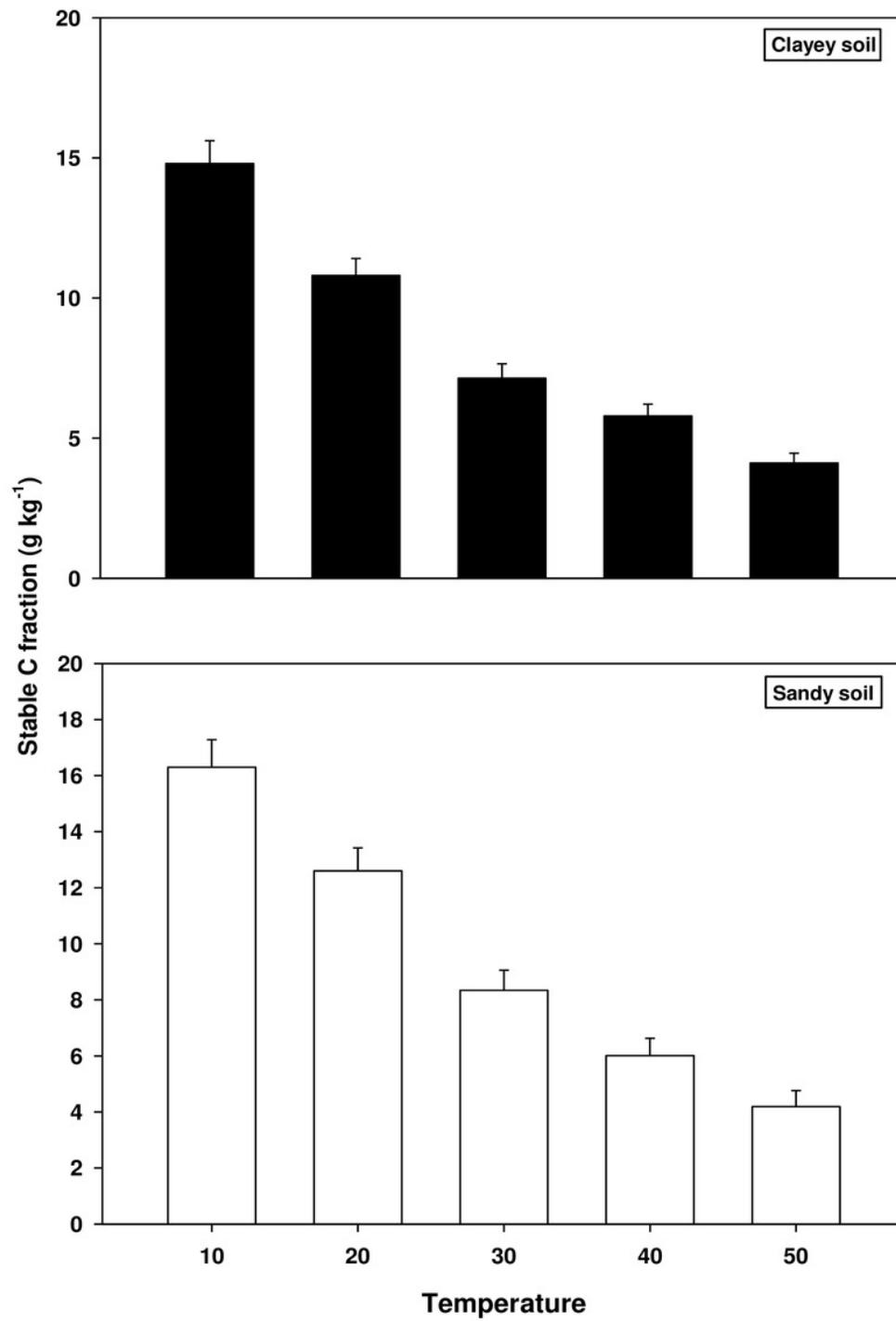


Fig. 3

## Figure 4

Effect of temperature on microbial community under sandy and clayey texture

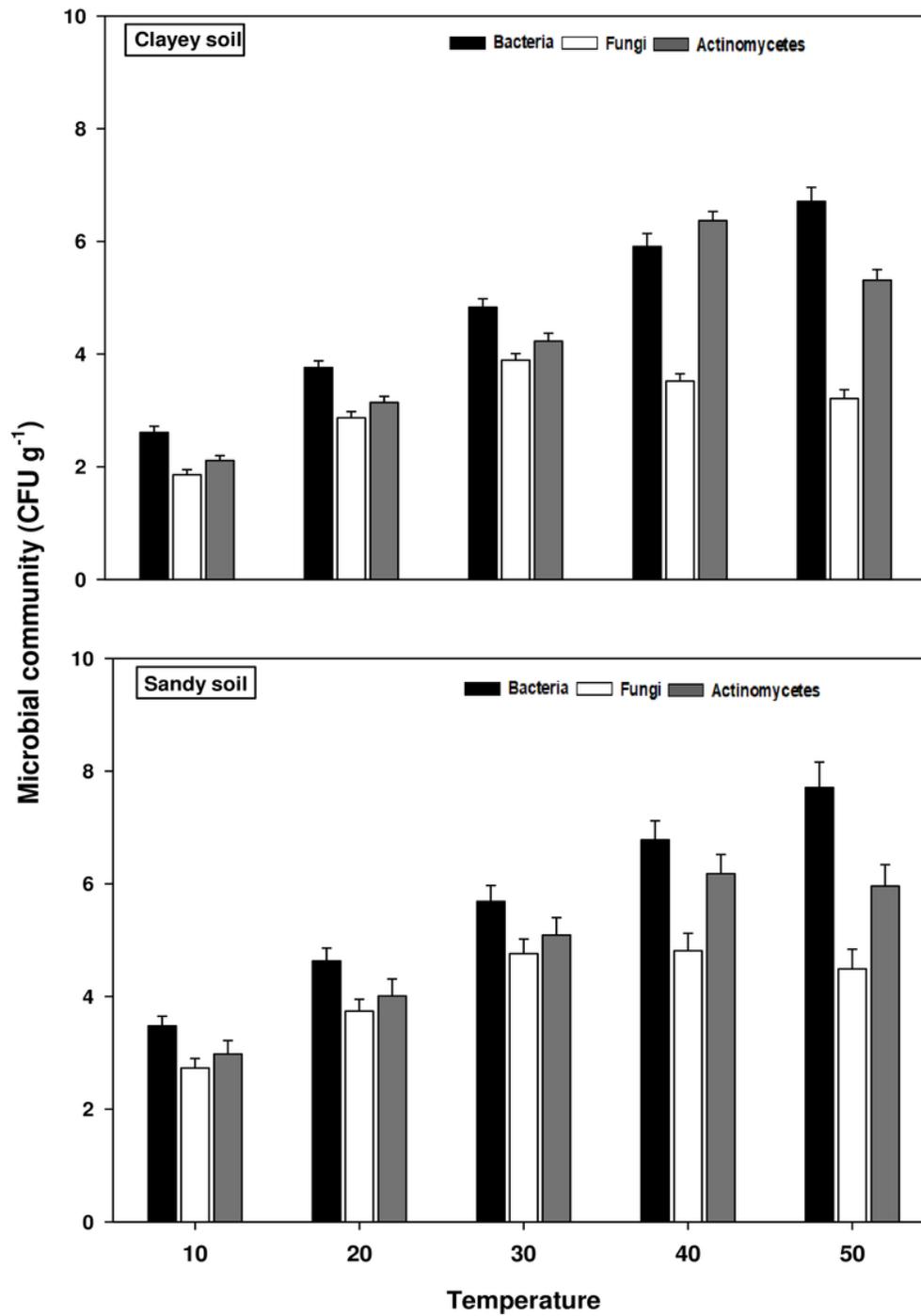


Fig. 4

## Figure 5

Effect of temperature on microbial biomass under sandy and clayey texture

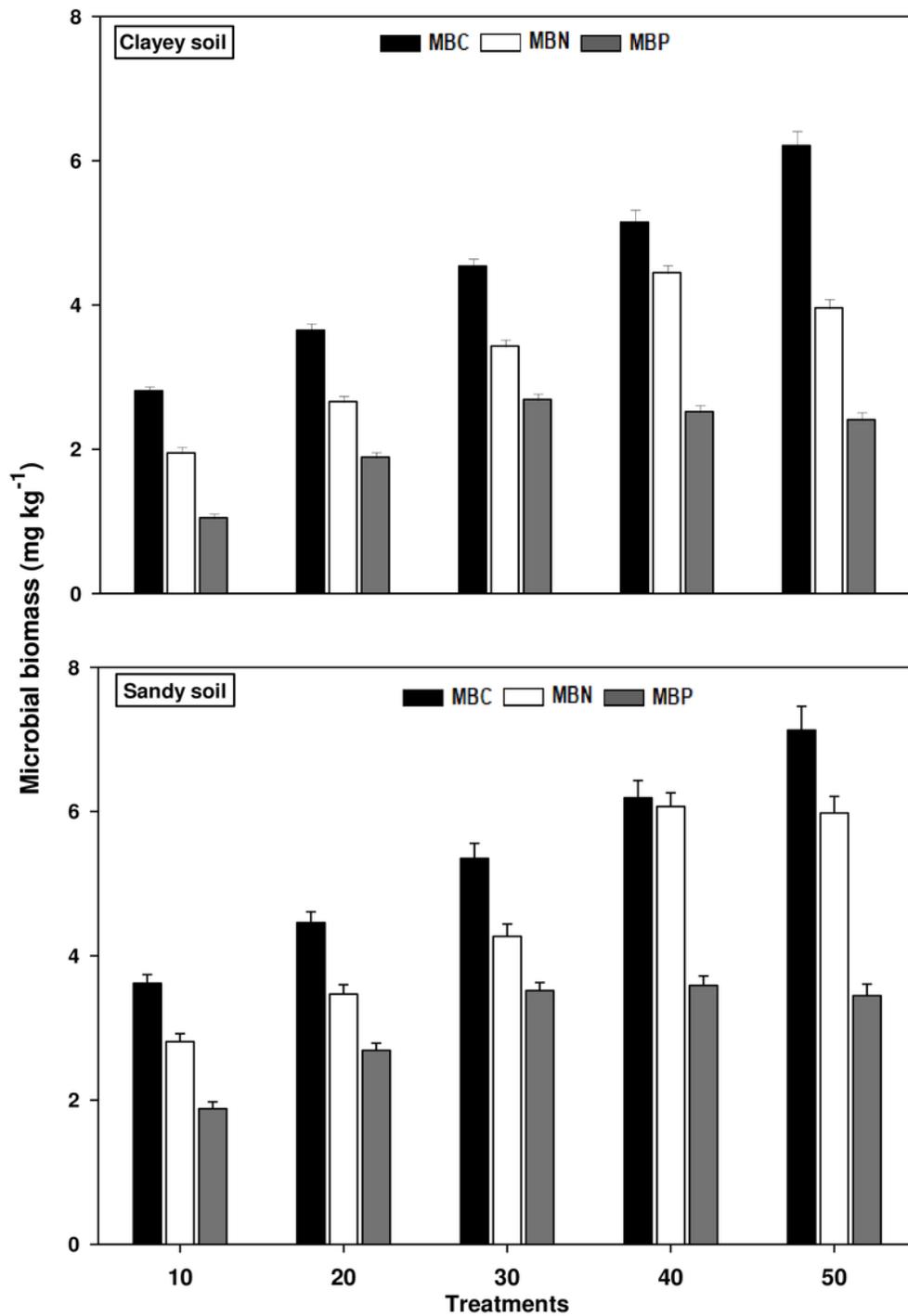


Fig. 5

## Figure 6

Effect of temperature on oxidative enzymes activity under sandy and clayey texture

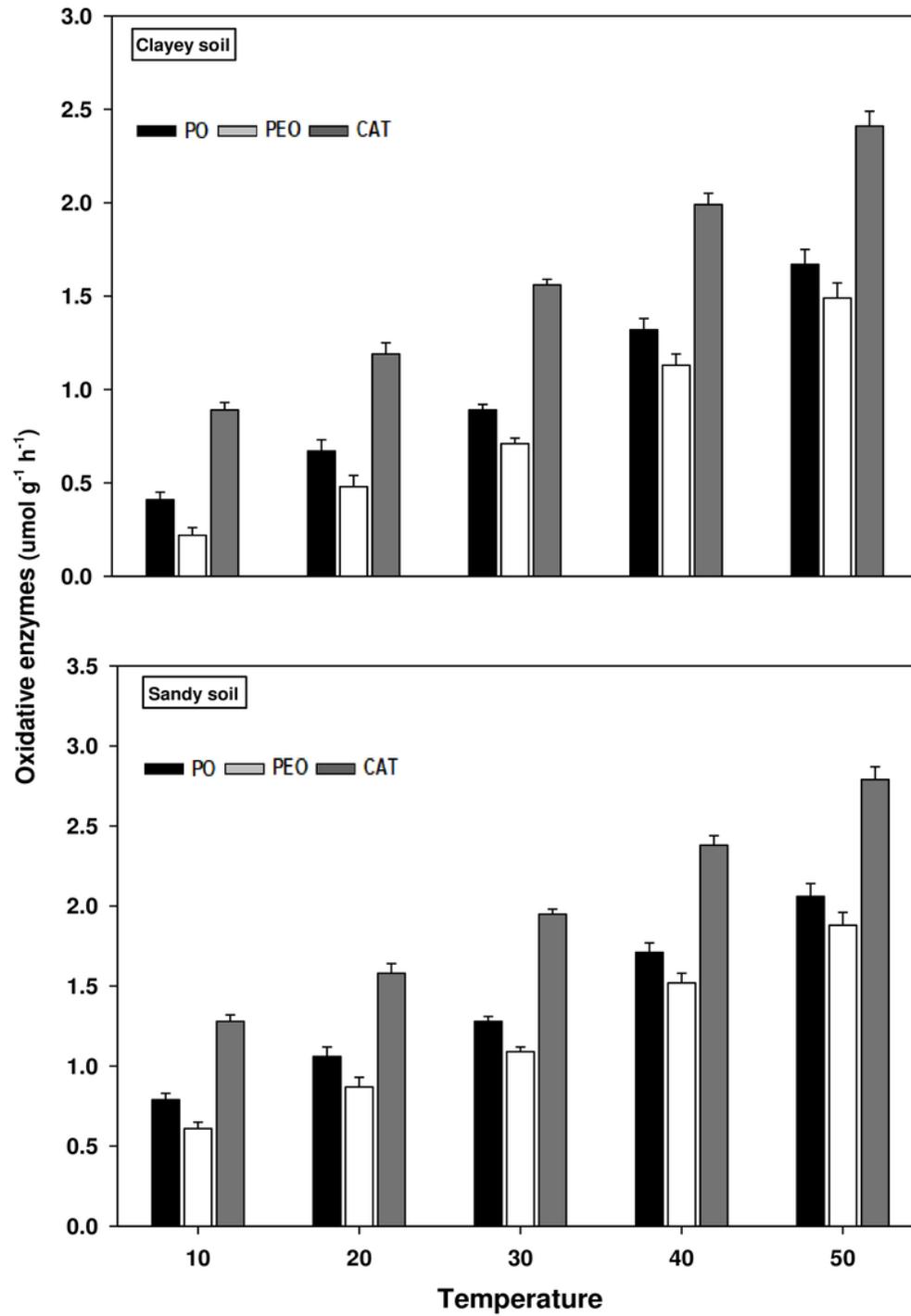


Fig. 6

## Figure 7

Effect of temperature on hydrolytic enzymes activity under sandy and clayey texture

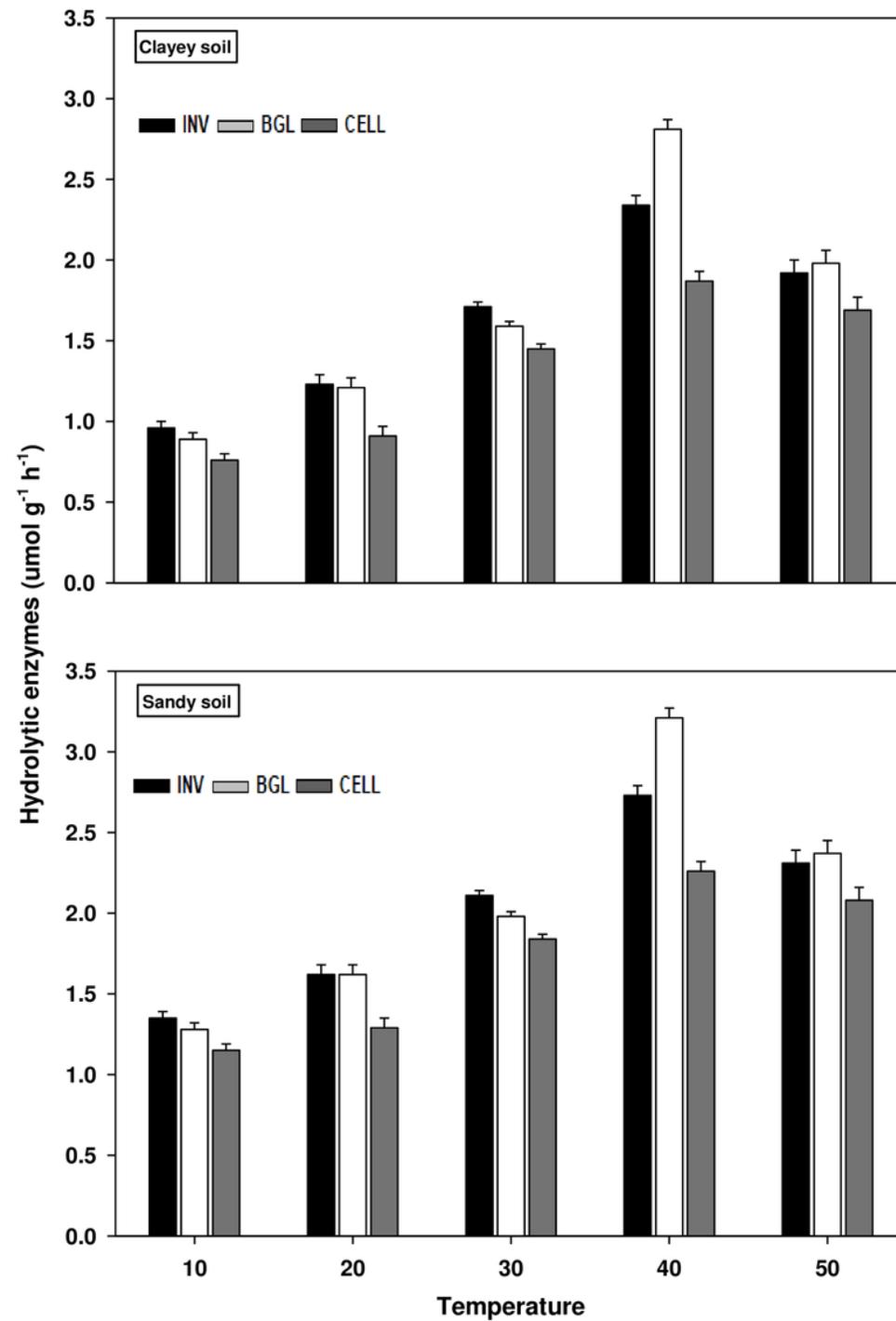


Fig. 7

## Figure 8

Effect of temperature on CO<sub>2</sub> emissions and cumulative CO<sub>2</sub> under sandy and clayey texture

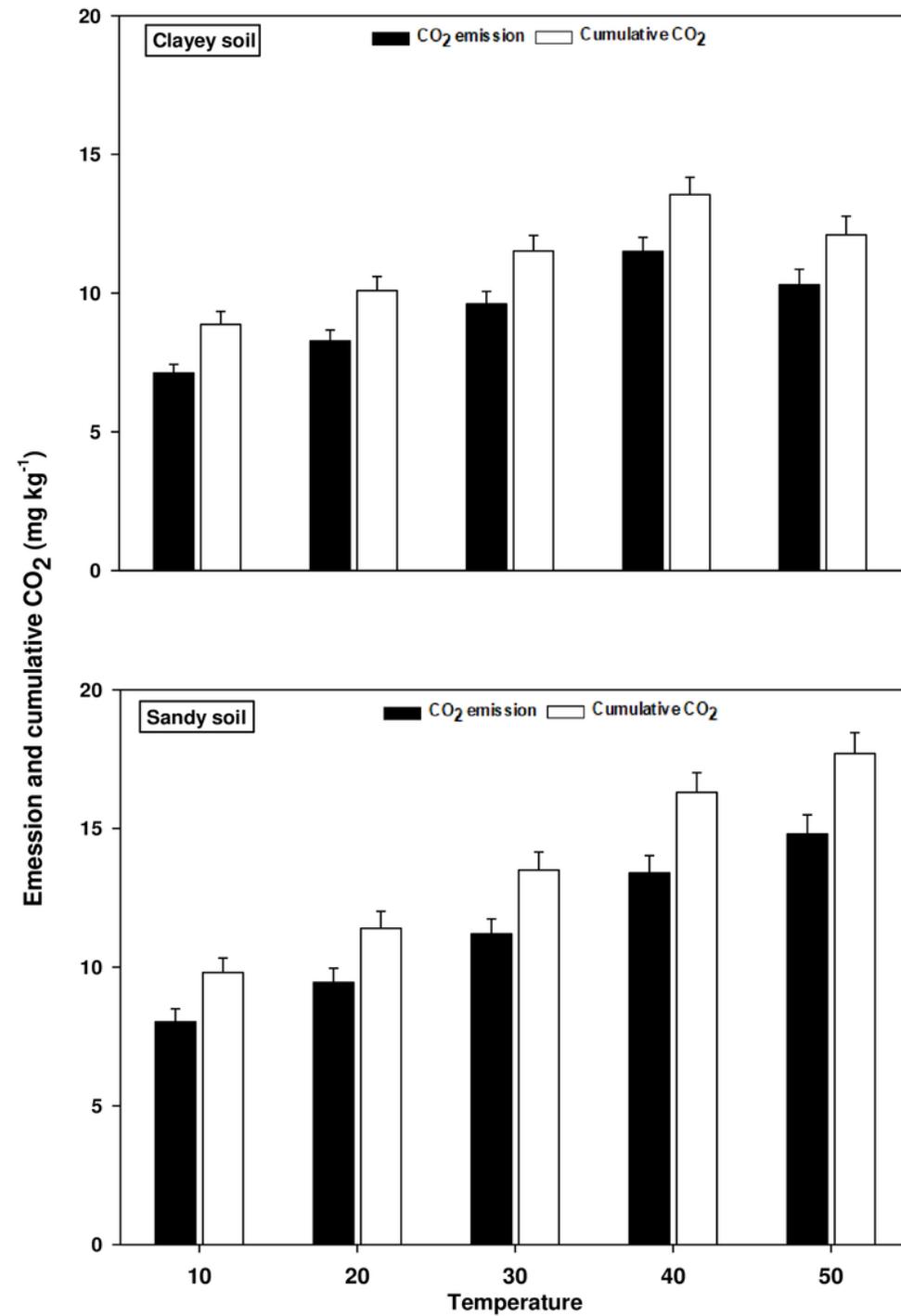


Fig. 8

**Table 1** (on next page)

Table 1A and 1B

**Table 1A.** Physico-chemical properties of experimental soils; **1B.** Correlation coefficient ( $R^2$ ) between C fractions, CO<sub>2</sub> emissions, cumulative CO<sub>2</sub> and temperature

- 1 **Table 1A.** Physico-chemical properties of experimental soils; **1B.** Correlation coefficient ( $R^2$ )  
 2 between C fractions, CO<sub>2</sub> emissions, cumulative CO<sub>2</sub> and temperature

Properties	Textural class	
	Sandy loam	Clay loam
Sand	539.3	232.4
Silt	251.2	286.3
Clay	209.5	481.3
pH (1:2.5)	7.11	7.24
EC ( $\mu\text{S cm}^{-1}$ )	149.8	169.5
CEC ( $\text{C mol}_c \text{ kg}^{-1}$ )	7.32	8.35
Bulk density ( $\text{g cm}^3$ )	1.58	1.28
Available N ( $\text{mg kg}^{-1}$ )	5.61	6.91
Available P ( $\text{mg kg}^{-1}$ )	4.63	6.49
Available K ( $\text{mg kg}^{-1}$ )	170.5	201.8
Total P ( $\text{g kg}^{-1}$ )	0.18	0.32
Total N ( $\text{g kg}^{-1}$ )	0.37	0.53

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- 4 **Table 1B.**

Parameters	$R^2$ (Sandy soil)	$R^2$ (Clayey soil)
LFOC	0.93**	0.85**
DOC	0.94**	0.62*
RMC	0.92**	0.75*
RSC	0.91**	0.76*
POC	0.92**	0.71*
EOC	0.93**	0.84**
<b><sup>a</sup>Total R<sup>2</sup></b>	<b>0.92**</b>	<b>0.75*</b>
ROC	0.97**	0.91**
TOC	0.96**	0.89**
<b><sup>b</sup>Total R<sup>2</sup></b>	<b>0.97**</b>	<b>0.90*</b>
CO <sub>2</sub> emission	0.91**	0.78*
Cumulative CO <sub>2</sub>	0.94**	0.75*

- 5 <sup>a</sup>Total R<sup>2</sup>, Correlation coefficient from all labile C fractions, <sup>b</sup>Total R<sup>2</sup>, Correlation coefficient  
 6 from recalcitrant and stable C fractions, \* Significant at P < 0.05. \*\* Significant at P < 0.01.

7 <sup>b</sup>Total R<sup>2</sup>, Correlation coefficient from all microbial biomass

- 8 \* Significant at P < 0.05. \*\* Significant at P < 0.01.

**Table 2** (on next page)

Table 2A and 2B

**Table 2A.** Correlation coefficient ( $R^2$ ) between microbial community and biomass and temperature; **2B.** Correlation coefficient ( $R^2$ ) between oxidative and hydrolytic enzymes and temperature

1 **Table 2A.** Correlation coefficient ( $R^2$ ) between microbial community and biomass and  
 2 temperature; **2B.** Correlation coefficient ( $R^2$ ) between oxidative and hydrolytic enzymes and  
 3 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*
<b><sup>a</sup>Total <math>R^2</math></b>	<b>0.85**</b>	<b>0.72*</b>
MBC	0.98**	0.92**
MBN	0.91**	0.80*
MBP	0.75*	0.63*
<b><sup>b</sup>Total <math>R^2</math></b>	<b>0.88**</b>	<b>0.78*</b>

4 <sup>a</sup>Total  $R^2$ , Correlation coefficient from all microbial community

5 <sup>b</sup>Total  $R^2$ , Correlation coefficient from all microbial biomass

6 \* Significant at  $P < 0.05$ . \*\* Significant at  $P < 0.01$ .

7 **Table 2B.**

Parameters	$R^2$ (Sandy soil)	$R^2$ (Clayey soil)
PO	0.96**	0.88**
PEO	0.93**	0.86**
CAT	0.91**	0.84**
<b><sup>a</sup>Total <math>R^2</math></b>	<b>0.93**</b>	<b>0.86**</b>
INV	0.76*	0.69*
BGL	0.64*	0.58*
CELL	0.81*	0.73*
<b><sup>b</sup>Total <math>R^2</math></b>	<b>0.73*</b>	<b>0.66*</b>

8 <sup>a</sup>Total  $R^2$ , Correlation coefficient from all oxidative enzymes

9 <sup>b</sup>Total  $R^2$ , Correlation coefficient from all hydrolytic enzymes

10 \* Significant at  $P < 0.05$ . \*\* Significant at  $P < 0.01$ .

**Table 3** (on next page)

Methods Graphical Representation

Graphical Representation of methods

