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Grass root litter and soil carbon quality paradoxically control Q_{10} of labile and recalcitrant carbon pools in a semi-arid Inceptisol

Running head: Soil carbon mineralization and carbon quality

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ABSTRACT

Aim: The temperature sensitivity of labile (Q_{10L}) and recalcitrant (Q_{10R}) pools of soil organic carbon (SOC) decomposition is a critical for predicting soil carbon (C) fluxes under changing climatic conditions.

Methods: Soils under six grass covers, namely, *Cenchrus ciliaris*, *Chrysopogon fulvus*, *Panicum maximum*, *Sehima nervosum*, *Heteropogon contortus*, and *Vetiveria zizanioides* from semi-arid India were evaluated for Q_{10L} and Q_{10R} of bulk soil, macroaggregates, microaggregates, and silt+clay associated SOC. Soil fractions (from 0-20 and 21-40 cm depths) were incubated at 25, 32, and 37 °C for 100 days, and cumulative C mineralization was measured. The Q_{10L} and Q_{10R} were calculated using a two-pool decay model. The quality of root litter C and SOC was assessed through FTIR spectroscopy.

Results: Q_{10L} and Q_{10R} of microaggregate-C was significantly higher (22-64% and 22-24%, respectively) than macroaggregate-C and silt+clay-C. Among grasses, Q_{10L} and Q_{10R} values under *C.ciliaris*, *H.contortus*, and *S.nervosum* were lower than other grasses, indicating their

capability to store SOC under global warming scenarios. Q_{10L} at topsoil layer was correlated with root litter C quality and at the sub-surface soil layer, it was influenced by labile C concentration. The Q_{10R} was correlated with the recalcitrant C concentration and SOC quality in both soil layers, indicating that quality and availability of recalcitrant SOC had pivotal roles in governing Q_{10R} in restored land.

Conclusions: Soil C and litter C quality should be potentially incorporated into the biogeochemical models to better predict SOC dynamics in managed ecosystems in the context of global warming and land use changes.

Keywords: Carbon mineralization; Carbon-quality-temperature hypothesis; Soil organic matter quality; Grass root litter; Grasslands;

List of abbreviations:

C.cil: *Cenchrus ciliaris*; *C.ful*: *Chrysopogon fulvus*; *P.max*: *Panicum maximum*; *S.ner*: *Sehima nervosum*; *H.con*: *Heteropogon contortus*; *V.ziz*: *Vetiveria zizanioides*; URC: Unrestored control; TSL: topsoil layer; SSL: sub-surface soil layer; C_L : labile C; C_R : recalcitrant C; k_L : decay rate of labile C; k_R : decay rate of recalcitrant C; Q_{10L} : temperature sensitivity of labile C; Q_{10R} : temperature sensitivity of recalcitrant C; HI: humification index; DDI: degree of decomposition index; MA: macroaggregates; MI: microaggregates; SC: silt+clay; MI-OC: MI associated total soil organic carbon; MA-OC: MA associated total soil organic carbon; SC-OC: SC associated total soil organic carbon; DHA: dehydrogenase; GLU: β -glucosidase; PHOX: phenoloxidase; PEROX: peroxidase; Ct : Cumulative C mineralization; SOM: Soil organic matter; SOC: Soil organic carbon

46 **Introduction**

47 Grass-based land restoration can help to restore deteriorated soil health (Patidar et al., 2023).
48 In restored areas, researchers have found that grasses can improve the soil microbial population
49 (Perkins and Nowak, 2013), soil aggregation (Deng et al., 2018), and the storage of soil organic
50 carbon (SOC) (Singhal et al., 2025). To investigate impacts of grasses on SOC storage, these
51 aspects have often been taken into account separately (Liu et al., 2015). Although microbial
52 carbon mineralisation determines SOC storage in soil under global warming scenarios, little
53 research has been done to examine how grasses affect the kinetics of SOC mineralisation
54 during vegetation restoration. In fact, because microbial activity and temperature are positively
55 correlated, global warming would promote the microbial degradation of SOC (Crowther et al.,
56 2016). Therefore, it is imperative to identify grasses that can enhance soil organic carbon
57 storage following the restoration of damaged lands by counteracting soil organic carbon
58 mineralisation. The temperature sensitivity (Q_{10}) of SOC decay, which indicates that the rate
59 of SOC decay increases by 10 °C as the ambient temperature rises, is a major determinant of
60 the amplitude of this stimulation (Davidson and Janssens, 2006; Friedlingstein et al., 2006).
61 Depending on the kind of grass cover, the soil organic matter (SOM) quality of ecologically
62 restored land varies due to presence of a variety of organic C compounds; slowly decomposable
63 stable recalcitrant pool and quickly decomposable reactive labile pool (Davidson and Janssens,
64 2006). SOM consists of a variety of organic C pools with differences in physical structure,
65 combination with soil particles and chemical recalcitrance that determines their stability in soil.
66 Soil temperature has significant impacts on the rate of decomposition of labile and recalcitrant
67 pools of SOM (Craine and Gelderman, 2011). In addition to temperature, the two most
68 important factors determining Q_{10} of labile pool (Q_{10L}) and recalcitrant pools (Q_{10R}) are quality
69 and availability these substrates (Fissore et al. 2013).

The carbon-quality-temperature (CQT) hypothesis predicts that the intrinsic Q_{10} rises with increasing recalcitrance of the SOM (Davidson and Janssens, 2006); however, apparent Q_{10} is regulated mostly by environmental conditions, such as, temperature, moisture (Liu et al., 2021). Henceforth, kinetics theory foretells that recalcitrant C will be more susceptible at high temperatures than labile C. The relationship between SOM stability and its temperature sensitivity is still up for debate, despite the fact that numerous laboratory incubation studies have empirically supported CQT theory (Bracho et al., 2016; Ghosh et al., 2020). However, other studies have found conflicting results depicting greater Q_{10} for labile soil organic carbon pool (Pang et al., 2015; Liu et al., 2021).

The impact of labile C inputs on Q_{10} has not been taken into account in many previous studies, despite the fact that substrate availability is a key driver of Q_{10} (Xu et al., 2014). Although breakdown of litter is a major source of labile C substrates in soil, the effect of litter C quality on Q_{10} has not been taken into account in previous studies. Ironically, it is anticipated that labile C inputs from decomposing roots would affect labile C mineralisation while also influencing the breakdown of resistant C. Further research is required to monitor the impact of root litter on SOC quality on Q_{10L} and Q_{10R} to understand their impact on carbon sequestration (Lavallee et al., 2020).

Root litter inputs also make the carbon mineralization kinetics more complicated by incorporating new organic materials. The quantity of root litter, its ratios of labile to recalcitrant compounds, affect microbial activity and enzyme production thus regulating the rate of decomposition of both labile and recalcitrant SOC pools. Also, the proportionality between the availability of the substrate and the quality of the substrate can change over time as litter subsides. Right after litter addition, substrate availability would most probably take centre stage in the responses of the microbes, but later periods may be due to substrate quality and recalcitrance. The physical protection of SOC that is being mediated by the soil aggregation

and organo-mineral associations could further interplay with the chemical composition and microbial process to establish the effective temperature sensitivity of each carbon fraction. Although there have been advances in the knowledge of SOC decomposition, there is a critical gap between availability of substrates, quality of substrates and SOC fraction-specific Q_{10} responses in realistic litter input conditions. The process of decoupling these interactions is crucial to the proper prediction of carbon cycle feedbacks in a warming climate, especially in ecosystems where root-derived contributions make up a large proportion of SOC. Furthermore, variable chemical recalcitrance, physical protection, and enzymatic breakdown processes of SOM could lead to variations in Q_{10L} and Q_{10R} of SOM considerably. Regretfully, Q_{10L} and Q_{10R} of SOM has not been independently measured in many investigations.

The Q_{10} of SOC varies largely in grasslands. In Chinese grasslands, the Q_{10} values ranged from 1.03 to 8.13 (mean 2.60 ± 0.08). Moreover, the Q_{10} values varied largely within and among grassland types and soil temperature measurement depths (Feng et al., 2018). Among grassland types, the highest Q_{10} occurred in alpine grasslands. They suggested that the combined factors of soil temperature and moisture would better predict soil respiration in arid and semi-arid regions, and implied that alpine grasslands in China might release more carbon dioxide to the atmosphere under climate warming. However, grass type-controlled soil carbon decomposition processes in snowy mountains region of NSW, Australia, with potential implications for feedbacks under warming conditions—especially via increased sensitivity of recalcitrant carbon (Jenkins and Adams, 2011). A global review by Wang and Fang (2009) revealed that the Q_{10} values of grassland varied from 0.9 to 4.6. The modal Q_{10} values ranged from 1.5 to 2.0 with an overall mean of 2.13. The mean Q_{10} values were 2.23 and 1.94 in temperate and tropical grasslands, respectively. They also described that root and soil respiration were majorly responsible for the variability of the Q_{10} value.

We conducted a laboratory incubation under three temperatures (25, 32, and 37 °C) to monitor CO₂ respiration from bulk soil, macroaggregates, microaggregates, and silt+clay fractions of six grass covers in restored semi-arid lands of India, and quantified Q_{10L} and Q_{10R}. We further examined the simultaneous effects of root litter C quality and SOM quality on these temperature sensitivities. In semi-arid ecologically restored degraded land, our primary targets were to: 1) evaluate the effects of different grass covers on Q_{10L} and Q_{10R} and 2) determine whether the CQT hypothesis applies to ecologically restored degraded lands (which originally had a low C content) with a constant supply of labile C from root litter. We posit that: 1) labile substrate availability would increase the Q_{10L} due to the cancelling effect according to Michaelis–Menten kinetics, whereas, Q_{10R} will be enhanced by the SOM recalcitrance; 2) after root litter decomposition, the effect of substrate availability on Q_{10L} and Q_{10R} would be more important than that of substrate quality; and 3) the changes in Q_{10L} and Q_{10R} would be differently influenced by root litter C and soil C quality due to their variable chemical recalcitrance, physical protection, and enzymatic decomposition mechanisms (Fig. 1a and 1b).

Materials and methods

Experimental strategies

The experimental site was located at a degraded landscape in Jhansi, Uttar Pradesh, India (Fig. 1). The primary drivers of degradation were soil erosion, poor soil fertility, low organic matter content, and low rainfall. The properties of soil prior to restoration were measured during Pre Monsson season of 2015. The soil properties before restoration and climatic variables during the study period are tabulated (Table S1). The URC had low (1.00 g kg⁻¹), poor soil available phosphorus (P; 9.1 kg ha⁻¹), available nitrogen (N; 182 kg ha⁻¹), and available potassium (K; 252 kg ha⁻¹). The URC had high soil erosion (>10 Mg ha⁻¹). The restoration programme was introduced in rainy season of 2015 (July 2015) and is continuing to date. The high temperature during summer, erratic monsoon, high aridity index, and insufficient profile moisture

categorize the region as semi-arid. From the start of the restoration programme, an unrestored fallow control land (URC) was maintained with identical slope, topography, texture, and parent material for future assessment of possible changes in soil properties. Three replicated strips for each grass were identified after randomization (Fig. 1c). Barren land was converted to grasslands with six tropical perennial grasses, namely *Cenchrus ciliaris* (C.cil), *Chrysopogon fulvus* (C.ful), *Panicum maximum* (P.max), *Sehima nervosum* (S.ner), *Heteropogon contortus* (H.con), and *Vetiveria zizanioides* (V.ziz). During mid-July, 2015, seedlings of each grass was planted at a rate of 10 seedlings m⁻². For every grass, a model plot (10 m × 15 m) was created. They were watered two to three times throughout the first two years to ensure a consistent plant population of each grass for improved establishment. The grasses were then acclimated to surviving solely on rainwater, and no irrigation was used. The restored land was not allowed for grazing. Every year, during mid-November, the aboveground biomass of grasses is taken, allowing the tussock to flourish throughout the upcoming monsoon season. The triplicates of 2 m × 2 m sub-plots, randomly distributed in the model 10 m × 15 m plots, were identified for measuring pasture productivity and soil properties. The biomass productivity of grasses in field was estimated in three replicates of uniform area of 2 m × 2 m. The soil of the whole experimental area had similar SOC, texture, slope, pH, water holding capacity, and nutrient availability.

Measurements of above ground biomass, root biomass, and root C quality

Samples of plant and soil were collected after harvesting of grasses from regions with consistent population density of grasses and soil physiography. For every grass, three sampling points were spotted within a model plot. Soil samples were collected using a core from top (TSL: 0–20 cm) and sub-surface (SSL: 21–30 cm) layers for restored area and URC. These soil samples were quickly taken to the laboratory and divided into two splits: one was stored at 4°C to evaluate microbiological characteristics, while the other was allowed to air dry and was used

to examine physical and chemical data. The grasses were acquired, tagged, and kept in paper bags before being dried in an oven ($65 \pm 2^{\circ}\text{C}$) until they reached a constant weight. The dry mass was determined and reported in as dry matter production (g m^{-2}). The dead roots were collected using cores from 0-20 and 21-40 cm soil layers and were washed with water and ethyl alcohol and their functional groups of roots were studied using a FTIR spectrometer (Bruker; Alpha II). The abundance of carboxylate and polysaccharides were detected by the peak absorbance at 1410 and 1050 cm^{-1} , respectively. Similarly, the abundance of aromatic + phenolic (by peak absorbance at 1520 and 1610 cm^{-1}) and aliphatic (2983 and 2921 cm^{-1}) compounds were also identified using FTIR spectra. The morphological parameters of roots were studied using a root analyzer.

Root decomposition kinetics

Roots were collected by excavating one grass tussock for each grass. The soil burying approach was also used to determine the rate of root degradation. Air dried roots of every grass (100 g) was placed in a paper bag and covered with soil. To retrieve the undecomposed root, each paper bag was put into a 30 cm $\times$ 15 cm nylon-mesh litter bag. Total 10 litter bags for each grass (each bag containing 100 g root of a grass) was placed in soil. On each sampling day (i.e. 30th, 60th, 90th, 120th, and 180th days after burial (DAB)), two samples of each grass were taken out (Magh et al., 2024). The dry root mass was noted on each sampling date. The decay kinetic of root was assessed with an exponential equation:

$$M_t = M_0(1 - \exp(-rt)) \dots (1)$$

where, M_t is the mass of decomposed roots after t DAB, M_0 is the mass of potentially decomposable root, r is decomposition of root (RDR)

Separation of soil aggregate and estimation of soil organic carbon

Aggregates from bulk soil (passed through 4.75 mm sieve) were separated using a yoder apparatus in wet sieving and classified as macroaggregate fraction (MA; >250-4750 μm), microaggregate fraction (MI; 53–250 μm), and silt+clay (SC; <53 μm) (Six et al., 2000). Total SOC concentrations in bulk soil, MA, MI, and SC were measured through dry combustion-oxidation method using an enviro TOC analyzer (enviro TOC analyzer; ELEMENTAR). Labile and recalcitrant pools of SOM were estimated for bulk soil, MA, MI, and SC (Rovira and Vallejo, 2002). In first step, 500 g samples were hydrolyzed with 20 ml of H_2SO_4 (2.5 M) at 105 °C for half an hour. The hydrolysed substances were collected through centrifugation. In second step, the remaining part was cleansed with ion free water and oven dried at 40 °C. Thereafter, the samples were shaken for overnight after adding 2 ml of H_2SO_4 (13M). The strength of acid was diluted to 1M by adding water. The samples were then hydrolyzed for 3 h at 105 °C. The hydrolysed substances were collected through centrifugation. The hydrolysed substances collected in two steps constituted the labile pool of SOM. The residue was cleansed again, oven-dried at 65 °C. It constituted the recalcitrant pool of SOM (Rovira and Vallejo, 2002). The labile and recalcitrant pools were analysed for labile C and recalcitrant C, respectively, using an enviro TOC analyzer.

Furthermore, absolute activities of extracellular soil enzymes in bulk soils, namely, dehydrogenase (DHA), glucosidase (GLU), phenoloxidase (PHOX), and peroxidase (PEROX) were assessed using substrate-based incubation methods, where, triphenyl tetrazolium chloride, p-nitrophenol glucoside, and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) were used as substrate (Dick, 2011). Specific activities of soil enzymes were estimated by dividing absolute activities of extracellular soil enzymes with their respective concentration of SOC.

Measurements of C mineralization kinetics and temperature sensitivity

Five hundred grams of sieved and air-dried samples from bulk soil, MA, MI, and SC was taken into 100 mL glass jar. The samples were subjected to preincubation at 50% water holding

capacity at 27 °C for 14 days to overcome the impact of rewetting and sieving on microbial respiration (Zacháry et al., 2018). The preincubated soils from bulk soils, MA, MI, and SC were divided into three parts for incubation at three temperatures, i.e. 25, 32, and 37 °C. Three replicates of soil (each weighing 50 g) from bulk soils, MA, MI, and SC were taken in a 250 mL glass jar for incubation at a specific temperature. Three blank sample without soil was used for the whole incubation experiment at each specific temperature. The samples were kept in a dark incubator at specified temperatures for 100 days at 70% WHC to avoid disintegration of soil aggregates during handling (Frøseth and Bleken, 2015). The CO₂ was trapped in glass vials containing 12 ml of 1 M NaOH, placed in the air-tight incubation jars. The NaOH traps were replaced on days 2, 7, 14, 21, 49, 70 and 100. The amount of CO₂ evolved was measured by titrating the NaOH with 1 M HCl in the presence of BaCl₂ (Anderson, 1982). The cumulative carbon production was fitted to the following two-pool model

$$C_t = C_L(1 - e^{-k_L \times t}) + C_R(1 - e^{-k_R \times t}) \dots (2)$$

C_t is mineralized carbon during t days of incubation. C_L is labile C pool, k_L is labile C pool mineralization constant, C_R is recalcitrant C pool, k_R is recalcitrant C pool mineralization constant.

A non-linear exponential equation was employed to relate k_L and k_R with temperatures (Eqs. 3 and 5) and the Q_{10L} and Q_{10R} were estimated by Eqs. 4 and 6 (Fierer et al., 2006; Ghosh et al., 2020).

$$k_L = M_{0L}e^{pt} \dots (3)$$

$$Q_{10L} = e^{10p} \dots (4)$$

$$k_R = M_{0R}e^{qt} \dots (5)$$

$$Q_{10R} = e^{10q} \dots (6)$$

240 M_{0L} and M_{0R} signifies the mineralization rate of C_L and C_R at 0 °C temperature, p and q are
 241 temperature coefficients for C_L and C_R , respectively. Q_{10L} and Q_{10R} are temperature sensitivity
 242 for C_L and C_R , respectively.

243 **Estimation of soil organic matter quality**

244 The air-dried samples from bulk soils, MA, MI, and SC were taken to a 100 mL plastic
 245 centrifuge tube. Hydrogen fluoride (HF) solution (10% v/v) was added to the soil at a ratio soil
 246 to solution of 1:10. The resultant mixed solution was shaken for an hour and centrifuged for
 247 10 min (3000 rpm), and the residue was collected. Successively, the same procedure was
 248 repeated with residual part of sample for eight times with different period of shaking (4 repeats
 249 with 1 h shaking, 3 repeats with 12 h shaking, and 1 repeat with 24 h shaking, serially). At last,
 250 the residual sample was washed with ion-free water for 6 times to discard any residual HF in
 251 the sample adding 50 mL water, followed by 10 min shaking and centrifugation (at 3000 rpm)
 252 for 10 min. The purified samples were oven-dried at 45 °C, ground through a 250 μ sieve, and
 253 stored in a polythene bag for FTIR spectroscopy. The SOM from bulk soils, MA, MI, and SC
 254 were spectrally characterised with a scan range of 4000-400 cm^{-1} and a resolution of 4 cm^{-1} . 50
 255 scans were performed for each sample. The OPUS 2023 was used for processing spectral data
 256 to correct the baseline, smoothing, and normalisation. The peak intensity (by height) was
 257 recorded to assess the magnitude of infrared absorption of the selected peaks. The SOM was
 258 characterized by estimating the following quality parameters using spectral absorption (Obeng
 259 et al., 2023).

$$260 \quad \text{Humification index-a (HI)} = \frac{CC \text{ (Absorption at } 1410 \text{ } cm^{-1})}{PC \text{ (Absorption at } 1050 \text{ } cm^{-1})} \dots (7)$$

261 Where CC is carboxylate compounds and PC is polysaccharide compounds

$$262 \quad \text{Degree of decomposition index (DDI)} = \frac{HC \text{ (Absorption at } 1720 \text{ } cm^{-1})}{ALC \text{ (Absorption } 2983 + 2921 \text{ } cm^{-1})} \dots (8)$$

263 Where HC represents hydrophilic compounds and ALC represents aliphatic compounds.

264 Aromaticity index = $\frac{\text{ARC (Absorption at } 1620 + 1520 \text{ cm}^{-1})}{\text{ALC (Absorption } 2983 + 2921 \text{ cm}^{-1})} \dots (9)$

265 Where, ARC represents aromatic compounds.

266 **Statistical analysis**

267 The data was analyzed for variance analysis using one-way ANOVA to test for variations across
268 the grasses. We assume that a) soils are randomly sampled and independently chosen from the
269 populations; b) variances of each sample are equal; c) the residuals are normally distributed.
270 The Tukey test was conducted at $P < 0.05$ to differentiate the means. The Pearson correlation
271 was used to interpret the interrelations among the variables.

272 We used a path model (i.e., structural equation model) depicting impact of labile and
273 recalcitrant C pools (C_L and C_R), soil organic matter quality, and root litter C quality on
274 temperature sensitivity of labile and recalcitrant C pools (Q_{10L} and Q_{10R}) at top and sub-surface
275 soil layers, respectively. The suitability of the model was verified by χ^2 -test, goodness of fit
276 (GFI) index, and root mean squared error of approximation (RMSEA). Favourable model fits
277 were indicated by no significant difference on the χ^2 -test ($P > 0.05$), high GFI (> 0.60), and
278 low RMSEA (< 0.075).

279 **Results**

280 **Above and below ground biomass production and bulk soil carbon concentration**

281 Among the grasses, P.max had the highest above ground biomass yield, followed by C.ful,
282 C.cil, and H.con (Table S2). However, the root biomass was significantly higher for C.cil. The
283 root biomass of P.max, C.ful, and H.con were similar. The decay rates of roots of C.cil, S.ner,
284 and H.con were significantly higher than other grasses. The SOC concentrations in bulk soils

were the highest under C.cil in both soil layers. Despite, P.max having higher above ground biomass, C.cil had greater SOC than P.max at the TSL and SSL (Table S2). The H.con and S.ner had significantly higher ($p<0.05$) SOC at the TSL and SSL than V.ziz. At TSL, bulk soil C_L values under C.cil and S.ner were significantly higher ($p<0.05$) than P.max, whereas, at the SSL, C.cil had only significantly greater bulk soil C_L than P.max (Table 1). The C_L was significantly higher under all grasses over URC. The bulk soil C_R values under C.cil and S.ner were significantly higher ($p<0.05$) at TSL and SSL than P.max.

Soil aggregation, aggregate associated C, and carbon pools

The mass of MA and MI increased under all grasses as compared to URC (Fig. 2A). However, in the TSL, the mass of MA was ~14-38% greater under different grasses and that of MI was ~20-55% higher under those grasses over URC. In the SSL, the mass of MA increased by ~11-54% under those grasses over URC. The mass of SC declined by 1.62-2.23 times at the TSL and 1.50-2.31 times at the SSL in restored land compared to URC.

The C.cil, H.con, and S.ner had significantly higher ($p<0.05$) MA-OC at the TSL and SSL compared to P.max (Fig. 2B). The C.cil and S.ner also had significantly greater ($p<0.05$) MI-OC at the TSL compared to P.max. The C.cil, H.con, and S.ner had significantly greater ($p<0.05$) MI-OC at the SSL compared to P.max. These three grasses resulted in significantly greater ($p<0.05$) SC-OC at the TSL and SSL compared to P.max. The mean (of six grass types) MI-OC values were ~26-55 and 11-16% greater than the mean MA-OC and SC-OC, respectively (Fig. 2B). The MA- C_L values under C.cil, H.con, and S.ner were also significantly higher ($p<0.05$) than P.max at the TSL. Although, the MA- C_L values were significantly higher ($p<0.05$) under C.cil and S.ner than P.max, at the SSL; P.max and H.con had similar MA- C_L at the SSL (Table 1). Contradictorily, the MI- C_L under C.cil was significantly higher ($p<0.05$) than P.max; H.con and S.ner had identical MI- C_L to P.max at the TSL. However, the MI- C_L under C.cil, H.con, and S.ner were significantly higher ($p<0.05$) than P.max at SSL. The

SC-C_L followed the similar trends to MA-C_L at the TSL. The MA-C_L, MI-C_L, and SC-C_L were significantly lower under C.ful and V.ziz than other grasses, although they had greater MA-C_L, MI-C_L, and SC-C_L than URC at the TSL and SSL (Table 1). The MA-C_R values under C.cil, H.con, and S.ner were also significantly higher ($p<0.05$) at the TSL and SSL than P.max. The MI-C_R and SC-C_R followed the similar trends. The MA-C_R, MI-C_R, and SC-C_R values were significantly lower under C.ful and V.ziz than other grasses, although they had greater MA-C_R, MI-C_R, and SC-C_R values than URC at the TSL and SSL (Table 1).

Cumulative C mineralization

The cumulative carbon mineralization (C_t) values of bulk soil, MA, MI, and SC were significantly higher under P.max than C.cil, H.con, and S.ner at all temperatures and soil layers (Fig. 3a-h; Fig. S1). However, the C_t values of bulk soil, MA, MI, and SC at TSL were significantly higher than SSL. For bulk soils, C_t values at 32 and 37 °C were similar to that at 25 °C. For MA, MI, and SC, C_t values at 32 and 37 °C were significantly ($p<0.05$) higher than that at 25 °C, respectively. Overall, mean (of six grass types) C_t values of MA were ~6-20% lower than that of bulk soil. However, mean (of six grass types) C_t of MI were ~16-30% greater than that of MA, whereas, mean (of six grass types) C_t values of SC were ~29-36% lower than that of MA (Fig. 3a-h). The proportion of C_t from bulk soils and aggregate fractions at both soil layers were higher for P.max and C.ful compared to other grasses (Fig. S1). The proportion of C_t were significantly higher for bulk soils than aggregates. Among the aggregates, the proportion of C_t for MA was significantly greater than that of MI and SC at all soil layers.

C mineralization kinetics

The decay rates of labile and recalcitrant C in bulk soil, MA, MI, and SC were significantly lower under all grass cover compared to URC (Table 2). Among the grasses, V.ziz had higher k_L and k_R than other grasses for bulk soil and aggregates. The k_L values under C.cil, H.con, and

S.ner were significantly ($p<0.05$) lower than V.ziz in bulk soils, MA, MI, and SC at all temperatures and soil layers. However, at the SSL, the bulk soil k_L data under C.cil and S.ner were significantly lower than V.ziz at all temperatures, whereas, k_L of MA, MI, and SC under C.cil, H.con, and S.ner were significantly lower than V.ziz. The k_R values under C.cil, H.con, and S.ner were significantly ($p<0.05$) lower than V.ziz in bulk soils, MA, MI, and SC at all temperatures and soil layers. At the SSL, the bulk soil k_R values under C.cil, H.con, and S.ner were similar; however, the k_R values of MA, MI, and SC under C.cil was significantly lower than that of S.ner and H.con at all temperatures (Table 2).

The mean (of six grass types) k_L at 32 and 37 °C increased by 1.07 – 3.16 times at TSL and 1.02 – 2.04 times in the SSL over that at 25 °C across soil fractions. The mean (of six grass types) k_R at 32 and 37 °C increased by 1.09 – 1.47 times at TSL and 1.11 – 1.36 times at the SSL over that at 25 °C across soil fractions. The mean (of six grass types) k_L values for bulk soils and MA at SSL were significantly higher than that at the TSL at all temperatures. Contrarily, the mean (of six grass types) k_R at the TSL was significantly higher than that at the SSL for all soil fractions at all temperatures (Table 2).

Temperature sensitivity

The temperature sensitivity of labile C (Q_{10L}) was significantly influenced by grass type. For bulk soil, the Q_{10L} values under C.cil, H.con, and S.ner were significantly ($p<0.05$) lower at the TSL and SSL than V.ziz (Table 3). For MA, MI, and SC, the Q_{10L} under C.cil and H.con were significantly ($p<0.05$) lower than V.ziz at the TSL and SSL than C.ful. The temperature sensitivity data of recalcitrant C (Q_{10R}) of MA, MI, and SC under C.cil, H.con, and S.ner were significantly ($p<0.05$) lower at the TSL and SSL than V.ziz. The Q_{10L} and Q_{10R} of bulk soils were similar at the TSL and SSL. The means (of six grass types) Q_{10L} and Q_{10} values for MA, MI, and SC at the TSL were significantly ($p<0.05$) higher than SSL, respectively. The means

(of six grass types) Q_{10L} and Q_{10} values for MA, MI, and SC at the TSL and SSL were significantly ($p<0.05$) higher than those of bulk soils (Table 3).

Activities of soil enzymes

The activities of DHA, GLU, PHOX, and PEROX were significantly higher under C.cil, H.con and S.ner compared to C.ful, P.max, and V.ziz at both soil layers (Fig. 4a). However, specific activities of these enzymes were significantly lower under C.cil, H.con, and S.ner than P.max at both soil layers (Fig. 4b).

The root litter carbon and soil carbon quality

The abundance of carboxylate groups was significantly higher in roots of C.cil and S.ner over other grasses. The polysaccharide compounds were more profound in roots of C.cil, H.con, and S.ner over other grasses. The distribution of aromatic+phenolic compounds was higher under roots of C.cil and S.ner over other grasses. The abundance of aliphatic compounds was higher in roots of C.cil, P.max, and H.con over other grasses (Fig. 5a-b). The HI values of SOC in bulk soils, MA, MI, and SC were significantly higher under C.cil, H.con, and S.ner than other grasses at both soil layers (Table 4; Fig. S2 and S3). The HI(a) values of SOM in MA, MI, and SC were significantly ($p<0.05$) higher than bulk soils at the TSL. The aromaticity of SOM in MA, MI, and SC was significantly ($p<0.05$) greater than bulk soils at the TSL. Similar results stand true at SSL also (Table 4; Fig. S2 and S3).

Impact of root litter carbon and soil carbon quality on Q_{10}

At the TSL, the Q_{10L} values of bulk soils, MA, and MI were positively impacted by root C quality parameters, such as, carboxylate and polysaccharide content, whereas, the Q_{10L} of SC was positively impacted by aliphatic groups in roots (Table 5). The Q_{10L} of bulk soils, MA, MI, and SC was impacted by the abundance of aromatic+phenolic compounds in roots.

Interestingly, at the SSL, the root C quality had no significant influence on Q_{10L} and Q_{10R} . The Q_{10L} of bulk soils was directly related to C_L concentration and inversely related to aromaticity at the TSL, whereas, the Q_{10L} data of MA and MI were influenced by aromaticity of SOC only. However, the Q_{10L} of SC was impacted by its C_L at the TSL (Table 5). The Q_{10R} data of bulk soils, MA, MI, and SC were impacted by humification indices, aromaticity (except SC), and concentration of C_R at the TSL. At the SSL, the Q_{10L} values of bulk soils, MA, SC, and MI were not impacted by root C quality parameters. The Q_{10L} values of bulk soils, MA, SC, and MI were positively impacted by C_L and inversely related to aromaticity (except SC). The Q_{10R} data of bulk soils, MA, MI, and SC were influenced by humification indices, aromaticity (except SC), and concentration of C_R at SSL. Overall, the Q_{10L} values of bulk soils, MA, and MI were influenced by root C quality, whereas, Q_{10R} of bulk soils, MA, MI, and SC were significantly influenced by root C quality, soil C quality and quantity at the TSL. At the SSL, Q_{10L} values of bulk soils, MA, MI, and SC were impacted by C_L quantity only and Q_{10R} of bulk soils, MA, MI, and SC were impacted by C_R quality and quantity (Table 5).

Discussion

Grasses for improving soil aggregation and C pools

The grasses had a major effect on the aggregate size distribution. The considerable decline in mass of MI (aggregates < 250 μm) and significant increase in mass of MA after grass cover establishment implied that the MI transformed to MA and consequently stabilized the soil structure (Fig. 2A). In this study, the greater mass of MI in URC indicated its further deterioration than grass cover (Zhu et al., 2017). Furthermore, the results revealed that there were a greater mass of macroaggregates for the C.cil, H.con, and S.ner compared with URC, which corresponded with the results of the earlier study (Zhong et al., 2021).

The C_L is vital for soil aggregation. This study also found the significant correlation of C_L with MA ($R^2 = 0.73$; $p < 0.05$). The linkage of C_L to soil minerals or MI might be responsible for MA

formation (Bucka et al., 2019). Additionally, the root decomposition products of different grasses enacted as active binding agents. Adsorption of organic decomposition products (such as polysaccharides; organic acids, etc) to mineral surfaces might happen through hydrogen bonding, van der Waals interaction, and long-range electrostatic interaction (Bucka et al., 2019). Additionally, the occurrence of hydroxy group, methylene C-H asymmetric stretch, open-chain azo group, open chain imino group, cyclohexane ring, and secondary amine with CN stretch at the root surface of *C.cil*, *H.con*, and *S.ner* (Fig. 5) could assist in ligand formation to stabilize MA (Sarkar et al., 2018) (Fig. 2A and 2B). The greater stability of aggregates of *S.ner* and *C.cil* might be linked to their corresponding enzyme activities, root decay rates, and C_L contents (Fig. 4A and 4B; Tables 2 and 3). Of late, researchers have also revealed that improvements in soil structural stability are connected with gains in SOC throughout the plant establishment phase because SOC functions as a cementing and consolidating agent to enhance soil aggregate synthesis and stability (Peng et al., 2023).

Among the soil layers, the SOC under all grasses had a decreasing trend at the SSL (Table 1). The reason could be that the majority of root biomass was accumulated at the TSL, the greater microbial activity facilitating decomposition organic matter and promoting C at the TSL over SSL (Xiao et al., 2020). The SOC content varied with the decrease in the soil aggregate size and supports the findings of Fang et al. (2015). This might have occurred due to short span of restoration and it might have restricted the transfer of SOC from the MI and SC to the MA.

This could have happened as a result of the study's inadequate vegetation restoration period and the failure to transfer the SOC from the MI and SC into the MA. This is due to the fact that SOC eventually moves to the macro aggregates after first accumulating in the SC and MI (Yang et al., 2024). The influences of MI grew and the SOC of MA progressively declined as soil depth increased. This suggests that the deeper is the soil layer, the stronger are the impacts of MI on SOC and the weaker the physical effects of MA on SOC. Zhong et al. (2021)

hypothesised that a lower degree of stable MA formation is caused by a lower soil clay content linked to a relatively small aggregate size (Hontoria et al., 2016). Previous research has clearly shown that, along with this portion of the soil nutrients, MAs are readily lost from deteriorated soil due to wind erosion and runoff (Qiu et al., 2015). As a result, SOC in recovered regions is vulnerable to loss. This might be the cause of MA's lower SOC compared with MI. In particular, due to their considerably larger root biomass and root degradation rate, *C. ful*, *H. con*, and *S. ner* exhibited higher SOC content than other grasses (Singhal et al., 2025).

Grasses control over C mineralization kinetics

The greater C_t from bulk soil and macroaggregate of URC was due to lower protection of SOC and the greater proportion of labile C in URC (Ma et al., 2019). The greater C_t under P.max at all temperatures and soil layers compared to other grasses was due to poor soil aggregation (Fig. 2A and 2B), higher specific activities of enzymes (Fig. 4A and 4B), and lower humification ratio (Table 4) under P.max (Wagai et al., 2013). The greater C_t at the TSL than SSL could possibly be linked to higher substrate availability and greater activities of soil enzymes (Ghosh et al., 2025). The non-significant impact of temperature rise on bulk soil C_t could be related to greater C_L to SOC ratio than C_R to SOC at both soil layers. The higher proportions of C_L ensured its mineralization at low temperature; with increase in temperature substrate deficiency in bulk soils restricted its C_t , hence a non-significant significant impact of temperature was observed (Canisares et al., 2023). Despite this, the significant increase in C_t of MA, MI, and SC with increase in temperatures could be related to greater proportions of MA- C_R , MI- C_R , SC- C_R than bulk soil C_R (Table 1) and also secondary supply of labile compounds by decomposition of recalcitrant substrates (Larionova et al., 2007). The solubility and reactivity of recalcitrant substrates and substrate affinity of enzymes increased with increasing temperatures, leading to a significant rise in C_t at higher temperature (Ma et al., 2019). The lower C_t in aggregates than bulk soils could be due to higher humification ratio,

lower DDI (Table 4), lower proportion of C_L in aggregates than bulk soil as well as physico-chemical protection of C_L and C_R within aggregates. The lowest C_t in SC could be due to strong chemical bonding of C_L and C_R with clay and higher humification ratio than that in MA, MI, and bulk soils. Over the course of 100 days of incubation at SSL, mineralisation caused the loss of approximately 62-79% of the potentially mineralised C at 25 °C. Therefore, depletion of potentially mineralizable C resulted in a nonsignificant rise in carbon mineralisation at SSL even after raising the incubation temperature to 37 °C from 32 °C. Oligotrophic bacteria, which are better suited to mineralise the residual, more complex organic substances (cellulose, lignin) of the roots, outcompeted copiotrophs when the majority of the potentially mineralizable C was depleted (Potthast et al., 2010). Furthermore, there was a nonsignificant rise in carbon mineralisation at the SSL layer due to the reduced amount of labile C not being able to supply enough substrate for mineralisation at 35 °C.

The greater k_L and k_R under V.ziz at all temperatures and soil layers over other grasses could be related to poor soil aggregation, low humification ratio, and higher specific activities of enzymes (Wagai et al., 2013). On the other hand, lower k_L and k_R under C.cil, S.ner, and H.con at all temperatures and soil layers could be associated with improved soil aggregation (Fig. 2A) and lower specific activities of enzymes (Fig. 4B). The non-significant impact of temperature rise on k_L and k_R of bulk soil could be related to similar proportion of C_t to SOC (~22%) at all temperatures. Despite this, the significant increase in k_L and k_R of MA, MI, and SC at higher temperatures could be related to increasing C_t at higher temperatures. The greater solubility and reactivity of labile and recalcitrant compounds as well as substrate affinity of enzymes within aggregates at higher temperatures might have caused significant rises in k_L and k_R at higher temperatures. The greater k_L at the TSL than SSL in bulk soil and aggregates could be related to greater C_L and activities of DHA and GLU (Fig. 2A) in addition to the influence of root C quality (Canisares et al., 2023). The greater k_R in bulk soils and aggregates at the TSL than SSL could be related to greater C_R and activities of PHOX and PEROX (Fig. 2A) in

addition to the influence of soil C quality. The lower k_L in aggregates compared with bulk soils could be related to higher humification ratio, lower DDI (Table 4), lower proportion of C_L in aggregates than bulk soils as well as physico-chemical protection of C_L within aggregates (Fig. 2A). The greater k_R in aggregates than bulk soils at the TSL could be related to greater proportion of C_R in aggregates than bulk soils. The decay rate of SOC in the subsurface layer increased significantly over the surface layer due to the higher specific activities of the enzymes, which resulted in the degradation of a greater quantity of SOC for the same enzyme activity.

Grasses for governing Q_{10} of semi-arid restored land

The greater Q_{10L} in bulk soils, MA, MI, and SC of V.ziz, C.ful, and P.max than other grasses could be due to poor soil aggregation (Ghosh et al., 2020), greater specific activities of DHA and GLU, and lower humification ratio (Wagai et al., 2013). The lower mean Q_{10L} in MA over MI and SC in restored land could possibly be linked with greater physical protection of C within MA due to their greater mass and lower specific surface area. Initially, the degraded area was SOC-limited (Table S1). In accordance to our first hypothesis, the highest Q_{10L} in MI could be related to the highest proportion of C_L in MI over other aggregates after restoration, ensuring substrate availability at higher temperatures. The greater Q_{10L} at the TSL than SSL under all grasses was associated with higher C_L concentrations as well as specific activities of DHA and GLU at the TSL. The similar Q_{10L} of bulk soils at the TSL and SSL could be ascribed to the similar proportion of C_L (~63-65%) at both soil layers, leading to consistent substrate availability at both soil layers. However, lower Q_{10L} of bulk soils compared with aggregates could be related to lower concentration of C_L in bulk soils than aggregates (Fig. 2B)

The greater Q_{10R} values in MA, MI, and SC of V.ziz, C.ful, and P.max than other grasses could also be linked to poor soil aggregation, greater specific activities of PHOX and PEROX, and higher degree of decomposition of SOM. The higher Q_{10R} values in MI over MA and SC could

be ascribed to MI formation mechanisms and greater concentration of C_R within MI. Recalcitrant substrates requiring high activation energy will decompose more readily at higher temperatures than labile substrates requiring low activation energy (Conant et al., 2011) because fine particles associated with MI have the ability to strongly adsorb labile organic substances through a variety of mechanisms (e.g. ligand exchange, polyvalent cation bridges, and large surface areas) (Wankhede et al., 2020). Resultantly, MI had a higher Q_{10R} than SC and MA. Higher C_R concentrations were linked to Q_{10R} of MA, MI, and SC being higher at the TSL than SSL (Liu et al., 2015). Thus, our first hypothesis was accepted as labile carbon concentration had direct control on the Q_{10L} , whereas, Q_{10R} was governed by the quantity of recalcitrant carbon.

Generally speaking, organic matter becomes more chemically resistant as soil depth increases. Thus, according to theory, Q_{10} should rise with depth (Karhu et al., 2010). Our findings, however, contradicted this hypothesis, indicating that processes other than chemical recalcitrance are required to account for our findings. According to published literatures, there are two potential reasons of declining Q_{10} at the SSL: (1) the greater protection of SOC at the SSL restricts its availability to microbes, and (2) the nature of the microbial community changes with depth. Firstly, the proportion of physically protected SOC rises at the SSL due to association with soil minerals, specifically with SC (Schrumpf et al. 2013), creating a physical disconnection between microbes and substrates, leading to a lesser dependence of microbial respiration on temperature in subsoils (Conant et al. 2011). The C_R contents within soil aggregates may reveal the degree of physically protected SOC against microbial breakdown. The proportion of C_R within MA was 10% higher at the SSL than TSL (Table 1). Consequently, one of the reasons causing the declining Q_{10} with depth may be the increased substrate constraint for microbes brought on by the physical protection of SOC. Secondly, the temperature sensitivity of microbial respiration at different depths may be partially explained by the different microbial activities at different depths, as indicated by the changes in specific

activities of enzymes (Fig. 4A and 4B) (Stone et al. 2014). According to the correlation study, C_L and C_R had a significant role in influencing Q_{10L} and Q_{10R} at various depths, respectively (Table 5). The variation in Q_{10} values may be directly caused by changes in community composition, which are probably linked to variations in C_L and C_R at two depths; as indicated by the differential specific activity of DHA, GLU, PHOX, and PEROX.

We found that Q_{10L} values were higher than Q_{10R} within aggregates due to strong influence of Q_{10R} on Q_{10L} as indicated by SEM (Fig. 6). This could possibly be due to increase of C_L at higher temperature due to a boost in the enzymatic activity accelerating decay of recalcitrant pools of SOM. Two major groups of enzymes, namely, oxidative and hydrolytic enzymes play pivotal roles in decomposing SOM. Oxidative enzymes transform aromatic and phenolic polymers to relatively labile substances, which are further decomposed by hydrolytic enzymes. Greater activities of oxidative enzymes (PHOX and PEROX) at high temperature assisted in providing greater microbial access to the recalcitrant SOM, and, therefore, resulting in an increased C_L (Waldrop and Firestone, 2004). This mechanism might have ensured the labile substrate supply by offsetting its depletion in substrate-limited degraded land at higher temperatures and resulted in greater increase of k_L over k_R . Consequently, Q_{10L} values were higher than Q_{10R} within aggregates.

The higher Q_{10L} over Q_{10R} was also reported by previous researchers (Zhu and Cheng 2011). It was demonstrated by Larionova et al. (2007) that the concentration of rapidly decomposable carbon affects how temperature affects microbial respiration. In addition, Fissore et al. (2013) found that labile substrate-depleted soils with prolonged incubation had a reduced temperature dependency of SOC decomposition compared to non-depleted soils or substrate-depleted soils treated with saturated glucose. The function of C_L in regulating the temperature sensitivity of microbiological respiration in soils is strongly supported by all of these investigations. Additionally, environmental restrictions on decomposition can decrease substrate availability

and lessen the intrinsic temperature sensitivity of SOC decomposition, according to recent syntheses (Davidson and Janssens 2006; Conant et al., 2011). By demonstrating higher Q_{10L} than Q_{10R} within aggregates at the TSL and SSL in ecologically restored grass covers, our current work advanced the understanding of Q_{10} in restored land. This study demonstrated how C_L concentration in bulk soils and aggregates affects SOC mineralization in response to warming. All of these factors would contribute to the growing understanding of the mechanisms that could play a major role in determining its persistence in a warming environment (Conant et al., 2011).

Root C quality and soil C quality or quantity: The governing factor behind temperature sensitivity of soil C in semi-arid restored land

Our results indicated that root and soil C quality exerted contrasting controls on the Q_{10} of SOC fractions. At the topsoil, root litter carboxylates and polysaccharides enhanced Q_{10} of labile C, consistent with the view that readily available substrates stimulated microbial activities and amplified temperature responses (Figs. 3 and 4) (Blagodatskaya et al., 2016; Qin et al., 2019). In contrast, SOM aromaticity was negatively associated with labile-C Q_{10} , reflecting the chemical recalcitrance and mineral protection of aromatic compounds that limit microbial access (Leifeld et al., 2014). By comparison, Q_{10} of recalcitrant SOC was positively related to the humification index, decomposition degree of SOM, and aromatic/phenolic compounds of roots (Fig. 5), in line with the higher activation energy required to decompose chemically complex substrates (Qin et al., 2019). At the subsoil, however, root litter chemistry had little effect, likely due to lower root inputs and microbial activity, while SOM quality and pool size had the dominant control on Q_{10} (Blagodatskaya et al., 2016). Together, these findings suggested that labile inputs might increase the short-term temperature sensitivity of SOC, whereas chemically complex substrates could lead to higher Q_{10} in recalcitrant pools, with depth-dependent shifts in the relative importance of root- versus soil-derived controls.

At the TSL, quantity of root litter is greater than that at the SSL, leading to higher supply of labile compounds. In other words, labile compound supply at the TSL is less limiting than that at the SSL. Hence, at the TSL, quality of root litter played an influential role on Q_{10L} and Q_{10R} . However, a theoretical relationship between labile substrate availability and the temperature sensitivity of SOC decomposition could be derived using the Michaelis–Menten model. In this model, both V_{max} (maximal rate of enzymatic activity) and K_m (dissociation constant or half-saturation constant) are positively temperature dependent. Therefore, the relative importance of K_m for estimation of soil C mineralization rate is reduced when labile substrate is added in initially labile substrate limited soil. The lower concentration of C_L at the SSL and the reduced relative importance of K_m with slight increase in C_L the Q_{10L} becomes solely dependent upon the C_L at the SSL (Fissore et al., 2013; Pang et al., 2014). The higher amount of root litter at the TSL assured greater supply of carboxylates, polysaccharides, aromatic and phenolic compounds, resulting in a greater influence of K_m on Q_{10L} and Q_{10R} at the TSL than that at the SSL. This led to a combined influence of labile substrate quality and quantity on Q_{10L} at the TSL only. Thus, our second hypothesis—that availability of labile substrate has a greater impact on Q_{10L} after root litter decomposition than substrate quality, with substrate quality alone not being able to explain variation in Q_{10L} —was supported by the positive correlation of Q_{10L} with carboxylates and polysaccharides of root litter and its negative correlation with aromaticity of SOM (Fig. 7).

The structural equation model clearly revealed that influence of C_L and C_R was significantly higher on Q_{10L} and Q_{10R} , respectively, at the TSL and SSL. It also indicated that at the TSL, Q_{10L} was influenced by C_L and root C quality parameters; whereas, at the SSL, it was only impacted by C_L , although Q_{10R} continued to be influenced by C_R and SOC quality parameters (Fig. 6). The question if Q_{10L} and Q_{10R} change with substrate quality is one that is hotly contested in SOC decomposition. According to the CQT theory, Q_{10} should rise as recalcitrance increases (Davidson and Janssens, 2006). Our results generally contradicted the theory. This

disparity might result from failing to consider how labile substrate supply influences C mineralization reactions in previous studies (Gershenson et al., 2009). The kinetic theory of decomposition and the CQT hypothesis were both supported by the significant correlations found in our study between Q_{10R} and the HI and aromaticity of SOM and the aromatic and phenolic substances of root litter (Table 5). Thus, the observed difference in Q_{10R} can be explained exclusively by substrate quality (Craine et al., 2010; Eberwein et al., 2015). The dependence of Q_{10R} on substrate quality in addition to C_R could be linked to substrate chemistry. The recalcitrant pools of SOM comprised of lignin-derived compounds as exhibited by the stronger peaks at 1512 cm^{-1} (Kostyukov et al., 2023). Furthermore, the FTIR analysis in this work (Fig. S2 and S3) also indicated, similar to previous researches, that the primary chemical elements contributing to aromatic carbons in litter and SOM can actually be quite different depending on the type of vegetation (Nierop et al., 2006; Obeng et al., 2023). Both relatively high-quality molecules, such as hydrolysable tannins (1610 , 1510 , and 1450 cm^{-1}) or aromatic amino acids (1340 – 1250 cm^{-1}), and lower-quality structural components, like lignin, condensed tannins, and suberin (921 , 2852 , 1737 , 1242 , 1158 and 724 cm^{-1}), might compose the aromatic compounds (Fig. S2 and S3). The distinct link between aromatic compounds and Q_{10L} and Q_{10R} may thus be due in part to differences in the amount and composition of aromatic amino acids between litter and SOM. According to the FTIR absorbance spectroscopy, the average concentration of carboxylate and polysaccharide in root litter was higher than that in SOM; the content of these compounds was clearly declining as the litter degraded. In spite of this, the C fraction was dominated by carboxylate and polysaccharide, suggesting that a sizable part of the carbons in carboxylate and polysaccharide make up a rather labile carbon pool. This would also account for a rising Q_{10L} when the contents of polysaccharides and carboxylates increase. The lower Q_{10R} than Q_{10L} in aggregates could also be caused by the rapid depletion of readily available labile compounds within aromatic substrate at higher temperature. The increased amount of lignin, tannins, suberin, etc. could indicate a shift towards decomposition of more

complex plant structural compounds (ligno-cellulose), resulting in the dependence of Q_{10R} on substrate quality parameters. Thus, our third hypothesis—that changes in Q_{10L} and Q_{10R} would be differently influenced by root litter C and soil C quality due to their variable chemical recalcitrance, physical protection, and enzymatic decomposition mechanisms—is supported by the variable correlations of C_L and C_R with Q_{10L} and Q_{10R} , respectively (Fig. 7) (Davidson and Janssens, 2006).

Limitations and implications of the study

The generalisation of our findings should be done with care because (i) the duration of incubation was most likely insufficient for thermal adaptation of microbial community; (ii) the study lacks the influence of soil moisture fluctuation; and (iii) our research had a small sample size as well as soil type. Therefore, it is necessary to validate the association between SOM and root C chemistry, as well as Q_{10L} and Q_{10R} , utilising diverse soils and methodologies. Most significantly, the conclusion drawn from the present approach is restricted to the ecologically restored degraded land.

The results from this study indicated that changes in SOC stability in ecologically restored land under grasses with regular litter input and higher temperatures will be highly dependent on labile C content in bulk soils and aggregates. Thus, soil C cycling models that explicitly incorporate factors that influences the protection and accessibility of C as well as microbial acclimation in the soil matrix should improve prediction of SOC dynamics in response to enhanced aboveground litter input and elevated temperature.

Conclusions

In consequence to the first objective this study demonstrated the effect grass covers on Q_{10} of SOC decomposition in restored land under warming environment due to of variations substrate availability. The Q_{10L} and Q_{10R} was also impacted by soil aggregation and enzyme activity. This

led to a lower Q_{10L} and Q_{10R} under C.cil, H.con, and S.ner over other grasses, indicating that these grass covers could contribute to greater SOC storage under warming situations. In relation to the second objective, we found that Q_{10L} and Q_{10R} of macroaggregate was lower than microaggregate, indicating that long-term restoration activity that promote macroaggregate formation would facilitate greater macroaggregate formation and consequently C sequestration under a rising temperature scenario. The Q_{10L} and Q_{10R} was significantly influenced by labile and recalcitrant C contents under all grass covers at both soil layers. Furthermore, Q_{10L} and Q_{10R} was impacted by root litter C quality at the topsoil only, indicating that the Q_{10} of SOC decomposition in sub-surface soil layer with low substrate availability might be enhanced more by labile C input. Furthermore, the positive relationship between Q_{10L} and Q_{10R} with labile and recalcitrant C contradicted the CQT hypothesis in restored degraded soils, which had low initial C content. Therefore, C-climate models based on the CQT hypothesis should be used cautiously when predicting soil C dynamics in future studies. The observed variations in Q_{10L} and Q_{10R} highlight the important role of substrate availability in soil C cycles, and future earth system model should take this into consideration because labile C is continuously added into soil via root exudates or plant litter in the field.

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Conflict of Interest

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Author Contributions

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Vikas K Singhal, Deepak Ojha and Sourav Kumar.

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Data Availability

“The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.”

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References

- Anderson, J.P., 1982. Soil respiration. *Methods of soil analysis: part 2 chemical and microbiological properties*, 9, pp.831-871.
- Blagodatskaya, E., Blagodatsky, S., Anderson, T. H., & Kuzyakov, Y. (2016). Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. *PLoS ONE*, 11(8), e0163457. <https://doi.org/10.1371/journal.pone.0163457>
- Qin, S., Chen, L., Fang, K., Zhang, Q., Wang, J., Guo, X., ... & Yang, Y. (2019). Temperature sensitivity of SOM decomposition governed by aggregate protection and microbial communities. *Global Change Biology*, 25(12), 3926–3940. <https://doi.org/10.1111/gcb.14850>
- Leifeld, J., Von Lützow, M., Kögel-Knabner, I., & Ekschmitt, K. (2014). A conceptual approach to the quality–temperature relationship of organic matter decomposition. *Soil Biology and Biochemistry*, 74, 60–68. <https://doi.org/10.1016/j.soilbio.2014.02.021>
- Bracho, R., Natali, S., Pegoraro, E., Crummer, K.G., Schädel, C., Celis, G., Hale, L., Wu, L., Yin, H., Tiedje, J.M. and Konstantinidis, K.T., 2016. Temperature sensitivity of organic matter decomposition of permafrost-region soils during laboratory incubations. *Soil Biology and Biochemistry*, 97, pp.1-14.
- Bucka, F.B., Kölbl, A., Uteau, D., Peth, S. and Kögel-Knabner, I., 2019. Organic matter input determines structure development and aggregate formation in artificial soils. *Geoderma*, 354, p.113881.

714 Canisares, L.P., Banet, T., Rinehart, B., McNear, D. and Poffenbarger, H., 2023. Litter quality
715 and living roots affected the formation of new mineral-associated organic carbon but did
716 not affect total mineral-associated organic carbon in a short-term
717 incubation. *Geoderma*, 430, p.116302.

718 Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E.,
719 Frey, S.D., Giardina, C.P., Hopkins, F.M. and Hyvönen, R., 2011. Temperature and soil
720 organic matter decomposition rates—synthesis of current knowledge and a way
721 forward. *Global change biology*, 17(11), pp.3392-3404.

722 Craine, J.M. and Gelderman, T.M., 2011. Soil moisture controls on temperature sensitivity of
723 soil organic carbon decomposition for a mesic grassland. *Soil Biology and*
724 *Biochemistry*, 43(2), pp.455-457.

725 Crowther, T.W., Todd-Brown, K.E., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B.,
726 Snoek, B.L., Fang, S., Zhou, G., Allison, S.D. and Blair, J.M., 2016. Quantifying global
727 soil carbon losses in response to warming. *Nature*, 540(7631), pp.104-108.

728 Davidson, E.A. and Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition
729 and feedbacks to climate change. *Nature*, 440(7081), pp.165-173.

730 Deng, L., Kim, D.G., Peng, C. and Shangguan, Z., 2018. Controls of soil and aggregate-
731 associated organic carbon variations following natural vegetation restoration on the L oess
732 P plateau in China. *Land Degradation & Development*, 29(11), pp.3974-3984.

733 Dick, R.P., 2011, September. Methods of soil enzymology. Madison: Soil Science Society of
734 America.

735 Fang, X.M., Chen, F.S., Wan, S.Z., Yang, Q.P. and Shi, J.M., 2015. Topsoil and deep soil
736 organic carbon concentration and stability vary with aggregate size and vegetation type in
737 subtropical China. *PLoS One*, 10(9), p.e0139380.

738 Fierer, N., Colman, B. P., Schimel, J. P., & Jackson, R. B., 2006. Predicting the temperature
739 dependence of microbial respiration in soil: A continental-scale analysis. *Global*
740 *Biogeochemical Cycles*, 20(3).

741 Fissore, C., Giardina, C.P. and Kolka, R.K., 2013. Reduced substrate supply limits the
742 temperature response of soil organic carbon decomposition. *Soil Biology and*
743 *Biochemistry*, 67, pp.306-311.

744 Fissore, C., Giardina, C.P. and Kolka, R.K., 2013. Reduced substrate supply limits the
745 temperature response of soil organic carbon decomposition. *Soil Biology and*
746 *Biochemistry*, 67, pp.306-311.

747 Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., Cadule, P., Doney,
748 S., Eby, M., Fung, I. and Bala, G., 2006. Climate–carbon cycle feedback analysis: results
749 from the C4MIP model intercomparison. *Journal of climate*, 19(14), pp.3337-3353.

750 Frøseth, R.B. and Bleken, M.A., 2015. Effect of low temperature and soil type on the
751 decomposition rate of soil organic carbon and clover leaves, and related priming effect. *Soil*
752 *Biology and Biochemistry*, 80, pp.156-166.

753 Ghosh, A., Das, A., Das, D., Ray, P., Bhattacharyya, R., Biswas, D.R. and Biswas, S.S., 2020.
754 Contrasting land use systems and soil organic matter quality and temperature sensitivity in
755 North Eastern India. *Soil and Tillage Research*, 199, p.104573.

756 Ghosh, A., Kumar, S., Kushwaha, B.P., Lal, R., Singh, A.K., Yadav, V.K. and Chandra, A.,
757 2025. Impact of short-term pasture development on soil aggregation, temperature
758 sensitivity of soil organic carbon, and functional diversity in semi-arid ecosystem. *Arid*
759 *Land Research and Management*, pp.1-27.

760 Hontoria, C., Gómez-Paccard, C., Mariscal-Sancho, I., Benito, M., Pérez, J. and Espejo, R.,
761 2016. Aggregate size distribution and associated organic C and N under different tillage
762 systems and Ca-amendment in a degraded Ultisol. *Soil and Tillage Research*, 160, pp.42-
763 52.

764 Jackson, O., Quilliam, R.S., Stott, A., Grant, H. and Subke, J.A., 2019. Rhizosphere carbon
765 supply accelerates soil organic matter decomposition in the presence of fresh organic
766 substrates. *Plant and Soil*, 440, pp.473-490.

767 Karhu, K., Fritze, H., Tuomi, M., Vanhala, P., Spetz, P., Kitunen, V. and Liski, J., 2010.
768 Temperature sensitivity of organic matter decomposition in two boreal forest soil
769 profiles. *Soil Biology and Biochemistry*, 42(1), pp.72-82.

770 Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C. and Sollins, P., 2011.
771 Old and stable soil organic matter is not necessarily chemically recalcitrant: implications
772 for modeling concepts and temperature sensitivity. *Global change biology*, 17(2), pp.1097-
773 1107.

774 Kostyukov, S.G., Matyakubov, H.B., Masterova, Y.Y., Kozlov, A.S., Pryanichnikova, M.K.,
775 Pynenkov, A.A. and Khluchina, N.A., 2023. Determination of lignin, cellulose, and
776 hemicellulose in plant materials by FTIR spectroscopy. *Journal of Analytical*
777 *Chemistry*, 78(6), pp.718-727.

778 Larionova, A.A., Yevdokimov, I.V. and Bykhovets, S.S., 2007. Temperature response of soil
779 respiration is dependent on concentration of readily decomposable C. *Biogeosciences*, 4(6),
780 pp.1073-1081.

781 Lavallee, J.M., Soong, J.L. and Cotrufo, M.F., 2020. Conceptualizing soil organic matter into
782 particulate and mineral-associated forms to address global change in the 21st
783 century. *Global change biology*, 26(1), pp.261-273.

784 Liu, N., Kan, H.M., Yang, G.W. and Zhang, Y.J., 2015. Changes in plant, soil, and microbes in
785 a typical steppe from simulated grazing: explaining potential change in soil C. *Ecological*
786 *Monographs*, 85(2), pp.269-286.

787 Liu, Y., Xu, L., Zheng, S., Chen, Z., Cao, Y., Wen, X. and He, N., 2021. Temperature sensitivity
788 of soil microbial respiration in soils with lower substrate availability is enhanced more by
789 labile carbon input. *Soil Biology and Biochemistry*, 154, p.108148.

790 Ma, Y., McCormick, M.K., Szlavecz, K. and Filley, T.R., 2019. Controls on soil organic carbon
791 stability and temperature sensitivity with increased aboveground litter input in deciduous
792 forests of different forest ages. *Soil Biology and Biochemistry*, 134, pp.90-99.

793 Magh, T., Mozhui, L., Kakati, L.N., Ao, B., Lemtur, T. and Jing, L., 2024. Litter decomposition
794 and nutrient dynamics in a subtropical ecosystem: A comparison of natural and plantation
795 forests (*Duabanga grandiflora*) in Nagaland, North-East India. *Global Ecology and*
796 *Conservation*, 56, p.e03321.

797 Nierop, K.G., Verstraten, J.M., Tietema, A., Westerveld, J.W. and Wartenbergh, P.E., 2006.
798 Short-and long-term tannin induced carbon, nitrogen and phosphorus dynamics in Corsican
799 pine litter. *Biogeochemistry*, 79, pp.275-296.

800 Obeng, A.S., Dunne, J., Giltrap, M. and Tian, F., 2023. Soil organic matter carbon chemistry
801 signatures, hydrophobicity and humification index following land use change in temperate
802 peat soils. *Heliyon*, 9(9).

803 Pang, X., Zhu, B., Lü, X. and Cheng, W., 2015. Labile substrate availability controls
804 temperature sensitivity of organic carbon decomposition at different soil
805 depths. *Biogeochemistry*, 126, pp.85-98.

806 Patidar, P., Sannagoudar, M.S., Ghosh, A., Singh, A.K., Misra, S., Khandibagur, V., Ojha, D.,
807 Casini, R., Elansary, H.O. and Chandra, A., 2023. Tropical range grasses can sustain soil
808 functions despite nutrient depletion in semiarid degraded land. *Frontiers in Sustainable*
809 *Food Systems*, 7, p.1230156.

810 Peng, X., Huang, Y., Duan, X., Yang, H. and Liu, J., 2023. Particulate and mineral-associated
811 organic carbon fractions reveal the roles of soil aggregates under different land-use types
812 in a karst faulted basin of China. *Catena*, 220, p.106721.

813 Perkins, L.B. and Nowak, R.S., 2013. Native and non-native grasses generate common types
814 of plant–soil feedbacks by altering soil nutrients and microbial
815 communities. *Oikos*, 122(2), pp.199-208.

816 Phillips, R.P., Finzi, A.C. and Bernhardt, E.S., 2011. Enhanced root exudation induces
817 microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation. *Ecology*
818 *letters*, 14(2), pp.187-194.

819 Qiu, L., Wei, X., Gao, J. and Zhang, X., 2015. Dynamics of soil aggregate-associated organic
820 carbon along an afforestation chronosequence. *Plant and Soil*, 391, pp.237-251.

821 Rovira, P. and Vallejo, V.R., 2002. Labile and recalcitrant pools of carbon and nitrogen in
822 organic matter decomposing at different depths in soil: an acid hydrolysis
823 approach. *Geoderma*, 107(1-2), pp.109-141.

824 Sarkar, B., Singh, M., Mandal, S., Churchman, G.J. and Bolan, N.S., 2018. Clay minerals—
825 Organic matter interactions in relation to carbon stabilization in soils. In *The future of soil*
826 *carbon* (pp. 71-86). Academic Press.

827 Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I. and Schulze, E.D.,
828 2013. Storage and stability of organic carbon in soils as related to depth, occlusion within
829 aggregates, and attachment to minerals. *Biogeosciences*, 10(3), pp.1675-1691.

830 Singhal, V.K., Ghosh, A., Singh, A.K., Singh, Y., Biswas, S.S., Ojha, D. and Bhattacharyya, R.,
831 2025. How grasses stabilize soil organic carbon in aggregates of semi-arid ecologically
832 restored land: Evidence from ¹³C natural abundance. *Catena*, 249, p.108627.

- Six, J., Paustian, K., Elliott, E.T. and Combrink, C., 2000. Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Science Society of America Journal*, 64(2), pp.681-689.
- Stone, M.M., DeForest, J.L. and Plante, A.F., 2014. Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biology and Biochemistry*, 75, pp.237-247.
- Wagai, R., Kishimoto-Mo, A.W., Yonemura, S., Shirato, Y., Hiradate, S. and Yagasaki, Y., 2013. Linking temperature sensitivity of soil organic matter decomposition to its molecular structure, accessibility, and microbial physiology. *Global Change Biology*, 19(4), pp.1114-1125.
- Wankhede, M., Ghosh, A., Manna, M.C., Misra, S., Sirothia, P., Rahman, M.M., Bhattacharyya, P., Singh, M., Bhattacharyya, R. and Patra, A.K., 2020. Does soil organic carbon quality or quantity govern relative temperature sensitivity in soil aggregates?. *Biogeochemistry*, 148, pp.191-206.
- Xiao, L., Yao, K., Li, P., Liu, Y., Chang, E., Zhang, Y. and Zhu, T., 2020. Increased soil aggregate stability is strongly correlated with root and soil properties along a gradient of secondary succession on the Loess Plateau. *Ecological Engineering*, 143, p.105671.
- Xu, W., Li, W., Jiang, P., Wang, H. and Bai, E., 2014. Distinct temperature sensitivity of soil carbon decomposition in forest organic layer and mineral soil. *Scientific Reports*, 4(1), p.6512.
- Yang, H., Long, H., Li, X., Luo, X., Liao, Y., Wang, C., Cai, H. and Shu, Y., 2024. Vegetation restoration improved aggregation stability and aggregated-associated carbon preservation in the karst areas of Guizhou Province, southwest China. *PeerJ*, 12, p.e16699.
- Zacháry, D., Filep, T., Jakab, G., Varga, G., Ringer, M. and Szalai, Z., 2018. Kinetic parameters of soil organic matter decomposition in soils under forest in Hungary. *Geoderma regional*, 14, p.e00187.
- Zhong, Z., Wu, S., Lu, X., Ren, Z., Wu, Q., Xu, M., Ren, C., Yang, G. and Han, X., 2021. Organic carbon, nitrogen accumulation, and soil aggregate dynamics as affected by vegetation restoration patterns in the Loess Plateau of China. *Catena*, 196, p.104867.
- Zhu, B. and Cheng, W., 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology*, 17(6), pp.2172-2183.
- Zhu, G.Y., Shangguan, Z.P. and Deng, L., 2017. Soil aggregate stability and aggregate-associated carbon and nitrogen in natural restoration grassland and Chinese red pine plantation on the Loess Plateau. *Catena*, 149, pp.253-260.

Table 1. Impact of grass covers on bulk soil and aggregate associated labile and recalcitrant carbon (C_L and C_R) at top (TSL) and sub-surface (SSL) soil layers in semi-arid restored land

	BS		MA		MI		SC	
	C _L (mg 100g ⁻¹)							
	TSL	SSL	TSL	SSL	TSL	SSL	TSL	SSL
<i>C.cil</i> [#]	114.1a ^s	92.5a	244.1a	129.6ab	297.0a	217.4a	233.2a	142.0a
<i>P.max</i>	88.8c	83.9a	226.6b	120.6b	271.6b	176.9c	203.3b	94.4c
<i>C.ful</i>	88.2c	43.8c	217.0b	71.4d	246.7c	179.6c	165.8c	92.7c
<i>H.con</i>	90.1c	61.7b	248.2a	109.6c	243.4c	200.3b	201.2b	123.8b
<i>S.ner</i>	106.1b	87.0a	255.8a	137.5a	272.3b	220.8a	232.2a	148.9a
<i>V.ziz</i>	83.9c	52.4b	146.1c	56.3e	254.5c	119.6d	146.4d	72.9d
URC	63.5d	36.4c	91.3d	82.8d	86.4d	67.6e	40.4e	52.3e
	C _R (mg 100g ⁻¹)							
	TSL	SSL	TSL	SSL	TSL	SSL	TSL	SSL
<i>C.cil</i>	67.9a	54.5a	216.3a	114.8a	267.6b	138.6a	292.9a	172.3a
<i>P.max</i>	52.2b	49.1b	154.8c	70.4d	220.5c	111.0bc	245.7c	155.0b
<i>C.ful</i>	51.8b	24.2d	136.3d	57.9e	181.9d	108.4c	232.4c	133.6c
<i>H.con</i>	52.9b	35.3c	187.6b	119.5b	257.7b	119.5b	274.6b	161.9a
<i>S.ner</i>	62.9a	51.0ab	180.1b	106.1c	292.3a	135.2a	281.7ab	165.0a
<i>V.ziz</i>	49.1b	29.6cd	88.4e	70.3d	133.3e	127.5ab	180.6d	127.5c
URC	36.5c	19.6e	45.4f	36.5f	53.3f	61.7d	33.1e	70.1d

[#]See materials and methods for details

^sStatistical inference according to Tukey's test is shown by lowercase letters.

874 Table 2 Impact of grass covers on decay rates of labile ($k_L \times 10^{-2}$ mg C day⁻¹) and recalcitrant ($k_R \times 10^{-2}$ mg C day⁻¹) pools of soil organic carbon from
875 bulk soils, macroaggregate (MA), microaggregate (MI), and silt plus clay (SC) at top (TSL) and sub-surface (SSL) soil layers in semi-arid restored
876 land.

TSL																								
Soil fraction	Bulk soil			MA			MI			SC			Bulk soil			MA			MI			SC		
Decay rate	k_L			k_L			k_L			k_L			k_R			k_R			k_R			k_R		
Temperature (°C)	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37
<i>C.cil</i> [#]	1.42e ^s	1.77f	1.86g	0.24c	0.24c	0.45c	0.19d	0.39d	0.97e	0.24e	0.42f	0.81e	0.25b	0.31b	0.42b	0.23b	0.29b	0.40b	0.27b	0.36b	0.51b	0.25b	0.37c	0.47c
<i>P.max</i>	2.95c	4.16c	4.52d	0.40b	0.56b	1.51b	0.30c	0.79c	2.49c	0.41d	0.91d	2.19c	0.31a	0.34ab	0.43b	0.29a	0.36a	0.50a	0.34a	0.44a	0.63a	0.31a	0.46b	0.58b
<i>C.ful</i>	2.95c	4.32c	5.08c	0.44b	0.63b	1.73b	0.38b	1.01b	3.28b	0.56c	1.12c	2.45c	0.31a	0.36a	0.46ab	0.28a	0.36a	0.49a	0.34a	0.44a	0.62a	0.31a	0.45b	0.58b
<i>H.con</i>	2.36b	3.08d	3.38e	0.26c	0.28c	0.60cd	0.27cd	0.52c	1.23de	0.32e	0.55e	1.03d	0.26b	0.32ab	0.43ab	0.23b	0.30b	0.41b	0.28b	0.37b	0.53b	0.26b	0.38c	0.49c
<i>S.ner</i>	1.78d	2.36e	2.60f	0.26c	0.31c	0.72d	0.23d	0.45cd	1.09d	0.27e	0.51e	1.08d	0.27b	0.33ab	0.44ab	0.25ab	0.31b	0.43b	0.29b	0.39b	0.55ab	0.27b	0.40c	0.51c
<i>V.ziz</i>	3.24a	4.80b	5.78b	0.68a	0.97a	1.70b	0.40b	1.22a	4.49a	0.76b	1.63b	3.84b	0.30a	0.36a	0.48a	0.28a	0.35a	0.49a	0.33a	0.44a	0.61a	0.34a	0.51a	0.64a
URC	5.35a	5.72a	7.01a	0.69a	1.06a	3.18a	0.53a	0.93b	3.25b	2.36a	3.82a	4.77a	0.29a	0.35a	0.48a	0.28a	0.36a	0.49a	0.33a	0.44a	0.62a	0.31a	0.45b	0.58b

SSL																								
Soil fraction	Bulk soil			MA			MI			SC			Bulk soil			MA			MI			SC		
Decay rate	k_L			k_L			k_L			k_L			k_R			k_R			k_R			k_R		
Temperature (°C)	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37	25	32	37
<i>C.cil</i>	2.12d	2.89d	2.98f	0.64e	1.28d	1.65e	0.31d	0.49c	0.91e	0.49e	0.59e	0.83f	0.24a	0.33b	0.35b	0.17b	0.20b	0.27b	0.20b	0.25b	0.33b	0.19b	0.25b	0.34b
<i>P.max</i>	3.80c	5.63c	6.31d	1.10c	1.88b	3.55b	0.58bc	1.12a	2.49a	1.16c	1.42c	2.03d	0.26a	0.36a	0.38a	0.21a	0.25a	0.33a	0.25a	0.30a	0.40a	0.23a	0.31a	0.41a
<i>C.ful</i>	4.60b	6.95a	8.13a	1.70b	1.62c	2.64c	0.56c	1.09a	2.44a	1.28c	1.68c	2.57c	0.27a	0.36a	0.39a	0.21a	0.25a	0.32a	0.24a	0.29a	0.39a	0.23a	0.30a	0.41a
<i>H.con</i>	4.82b	6.63a	6.95c	0.83d	1.18d	1.42f	0.38d	0.67b	1.41c	0.64d	0.77d	1.12e	0.24a	0.33b	0.36b	0.18b	0.21b	0.27b	0.21b	0.26b	0.37ab	0.19b	0.26a	0.37ab
<i>S.ner</i>	2.44d	3.40e	3.58e	0.65e	1.46cd	2.15d	0.32d	0.57bc	1.18d	0.51d	0.69de	1.10e	0.25a	0.34b	0.36b	0.18b	0.22b	0.28b	0.21b	0.27ab	0.36ab	0.20b	0.27ab	0.37ab
<i>V.ziz</i>	5.39a	6.17b	7.18c	1.08c	1.30d	1.69e	0.73a	1.10a	1.90b	1.83b	2.11b	2.86b	0.27a	0.36a	0.39a	0.21a	0.25a	0.32a	0.24a	0.29a	0.39a	0.23a	0.30a	0.41a
URC	5.54a	6.61a	7.72b	2.51a	2.79a	4.00a	0.63b	0.64b	1.25d	3.19a	3.54a	3.77a	0.27a	0.37a	0.39a	0.21a	0.25a	0.32a	0.24a	0.30a	0.41a	0.23a	0.30a	0.42a

877 [#]See materials and methods for details

878 ^sStatistical inference according to Tukey's test is shown by lowercase letters.

879 Table 3 Impact of grass covers on temperature sensitivity of labile (Q_{10L}) and recalcitrant (Q_{10R})
880 pools of soil organic carbon in bulk soils (BS), macroaggregate (MA), microaggregate (MI),
881 and silt plus clay (SC) at top (TSL) and sub-surface (SSL) soil layers in semi-arid restored land.

	Q _{10L}							
	BS		MA		MI		SC	
	TSL	SSL	TSL	SSL	TSL	SSL	TSL	SSL
<i>C.cil</i> [#]	1.26c ^s	1.34bc	1.65d	2.24c	2.73cd	2.07c	2.69ab	1.53c
<i>P.max</i>	1.44b	1.54a	2.92b	2.62a	3.06b	2.98s	2.81a	1.58c
<i>C.ful</i>	1.58a	1.62a	3.00b	2.40b	2.91c	2.60b	2.54b	1.76b
<i>H.con</i>	1.36bc	1.37b	1.9d	1.57d	2.41e	2.56b	2.61b	1.57c
<i>S.ner</i>	1.38bc	1.39b	2.23c	1.73d	2.60c	2.53b	2.64b	1.89a
<i>V.ziz</i>	1.63a	1.27c	3.04b	2.45b	3.28a	1.84d	2.78a	1.44cd
URC	1.24	1.32bc	3.43a	2.45b	3.33a	1.36e	1.81c	1.38d
	Q _{10R}							
	BS		MA		MI		SC	
	TSL	SSL	TSL	SSL	TSL	SSL	TSL	SSL
<i>C.cil</i>	1.53a	1.38d	1.59c	1.65c	1.68c	1.50cd	1.71c	1.45b
<i>P.max</i>	1.30c	1.68b	1.89b	1.42d	2.65b	1.65b	1.98a	1.62a
<i>C.ful</i>	1.40b	1.69b	1.97b	1.62c	2.65b	1.68b	1.83b	1.63a
<i>H.con</i>	1.54a	1.44cd	1.58c	1.44d	1.67c	1.46d	1.70c	1.41b
<i>S.ner</i>	1.49ab	1.48c	1.57c	1.43d	1.66c	1.54c	1.69c	1.47b
<i>V.ziz</i>	1.44b	1.39d	2.01b	1.74b	2.65b	1.79a	1.84b	1.63a
URC	1.53a	1.79a	2.40a	1.84a	2.96a	1.68b	2.07a	1.66a

882 [#]See materials and methods for details

883 ^sStatistical inference according to Tukey's test is shown by lowercase letters.

884

885

886 Table 4 Impact of grass covers on quality parameters (humification index: HI; aromaticity; and
887 degree of decomposition: DDI) of soil organic matter in bulk soils (BS), macroaggregate (MA),
888 microaggregate (MI), and silt plus clay (SC) at top (TSL) and sub-surface (SSL) soil layers in
889 semi-arid restored land.

TSL												
BS			MA			MI			SC			
	HI	Aromaticity	DDI	HI	Aromaticity	DDI	HI	Aromaticity	DDI	HI	Aromaticity	DDI
<i>C.cil</i> [#]	0.891a [§]	0.292a	0.335d	1.847a	1.095a	0.635d	1.605a	1.327a	0.828b	2.194a	1.646a	0.524c
<i>P.max</i>	0.478c	0.305a	0.634c	1.428b	1.096a	0.768c	1.238b	1.328a	1.074a	1.456c	1.147bc	0.789b
<i>C.ful</i>	0.415d	0.314a	0.745b	1.347b	1.107a	0.825b	1.166b	1.341a	1.154a	1.326c	1.259b	0.877a
<i>H.con</i>	0.802b	0.302a	0.381d	1.765a	1.110a	0.629d	1.533a	1.344a	0.877b	2.054b	1.621a	0.566c
<i>S.ner</i>	0.904a	0.286a	0.325d	1.862a	1.082a	0.585e	1.618a	1.310a	0.815b	2.212a	1.532a	0.515c
<i>V.ziz</i>	0.457c	0.314a	0.682c	1.410b	1.111a	0.792c	1.222b	1.346a	1.107a	1.406c	1.064c	0.831a
URC	0.284e	0.261b	0.842a	1.125c	1.111a	1.095a	1.023c	0.954b	0.856b	1.015d	0.984d	0.760b
SSL												
<i>C.cil</i>	0.894b	0.275a	0.149c	1.850a	1.060a	0.573d	1.608a	1.284a	0.799b	2.198a	1.609a	0.505c
<i>P.max</i>	0.472c	0.305a	0.215b	1.419b	1.095a	0.772c	1.230b	1.327a	1.079a	1.447b	1.147b	0.793b
<i>C.ful</i>	0.418c	0.309a	0.229b	1.351b	1.097a	0.813b	1.170b	1.330a	1.137a	1.329b	1.049c	0.865a
<i>H.con</i>	0.899b	0.301a	0.162c	1.856a	1.112a	0.599d	1.613a	1.347a	0.836b	2.204a	1.614a	0.529c
<i>S.ner</i>	0.935a	0.295a	0.156c	1.894a	1.102a	0.583d	1.646a	1.336a	0.812b	2.250a	1.542a	0.514c
<i>V.ziz</i>	0.451c	0.309a	0.221b	1.400b	1.101a	0.788c	1.213b	1.334a	1.102a	1.396b	1.053c	0.827a
URC	0.291d	0.268b	0.850a	1.141c	1.127a	1.095a	1.037c	0.967b	0.856b	1.029c	0.998d	0.760b

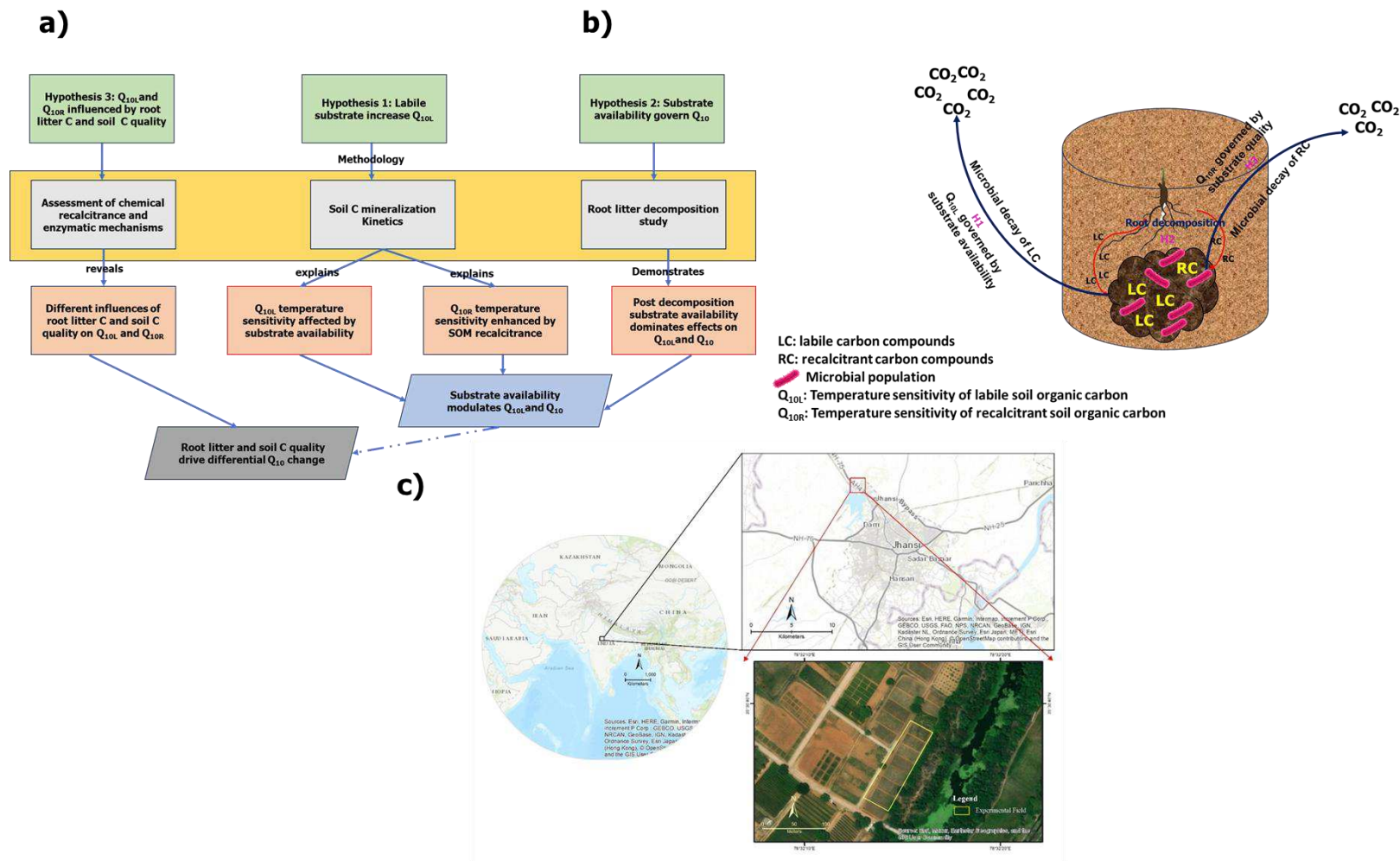
890 [#]See materials and methods for details

891 [§]Statistical inference according to Tukey's test is shown by lowercase letters.

Table 5 Correlations of temperature sensitivity of labile and recalcitrant C (Q_{10L} and Q_{10R}) with quality parameters (humification index: HI; aromaticity; and degree of decomposition: DDI) of soil organic matter, concentration of labile and recalcitrant C (C_L and C_R), and root litter carbon quality in bulk soils (BS), macroaggregate (MA), microaggregate (MI), and silt plus clay (SC) at top (TSL) and sub-surface (SSL) soil layers in semi-arid restored land.

		Soil carbon quality				Root litter carbon quality			Soil carbon quantity	
	TSL	HI	DDI	Aromaticity	Carboxylate	Polysaccharide	Aromatic+Phenolic	Aliphatic	C _L	C _R
BS	Q _{10L}	-0.287 ^{ns}	-0.187 ^{ns}	-0.833	0.834	0.659	-0.150 ^{ns}	-0.476 ^{ns}	0.800	-0.100 ^{ns}
	Q _{10R}	0.769	0.771	0.804	0.456 ^{ns}	-0.029 ^{ns}	0.657	0.400 ^{ns}	0.511 ^{ns}	0.751
MA	Q _{10L}	-0.326 ^{ns}	-0.374 ^{ns}	-0.634	0.787	0.651	-0.311 ^{ns}	-0.485 ^{ns}	0.490 ^{ns}	-0.098 ^{ns}
	Q _{10R}	0.758	0.782	0.799	-0.446 ^{ns}	0.098 ^{ns}	0.633	-0.614 ^{ns}	-0.113 ^{ns}	0.894
MI	Q _{10L}	-0.009 ^{ns}	-0.339 ^{ns}	-6.471	0.706	0.833	-0.141 ^{ns}	-0.513 ^{ns}	-0.300 ^{ns}	0.094 ^{ns}
	Q _{10R}	0.845	0.856	0.745	-0.512 ^{ns}	0.089 ^{ns}	0.661	0.323 ^{ns}	-0.359 ^{ns}	0.868
SC	Q _{10L}	-0.097 ^{ns}	-0.128 ^{ns}	0.079 ^{ns}	-0.332 ^{ns}	-0.198 ^{ns}	-0.290 ^{ns}	0.694	0.644	-0.074 ^{ns}
	Q _{10R}	0.827	0.783	0.213 ^{ns}	-0.607 ^{ns}	-0.110 ^{ns}	0.662	-0.681	-0.464 ^{ns}	0.827
		HI	DDI	Aromaticity	Carboxylate	Polysaccharide	Aromatic+Phenolic	Aliphatic	C _L	C _R
BS	Q _{10L}	-0.463 ^{ns}	-0.474 ^{ns}	-0.636	-0.351 ^{ns}	0.269 ^{ns}	-0.326 ^{ns}	-0.406 ^{ns}	0.768	-0.198 ^{ns}
	Q _{10R}	0.659	0.686	0.719	-0.436 ^{ns}	0.503 ^{ns}	-0.427 ^{ns}	-0.428 ^{ns}	-0.208 ^{ns}	0.721
MA	Q _{10L}	-0.301 ^{ns}	-0.291 ^{ns}	-0.635	-0.252 ^{ns}	0.124 ^{ns}	-0.119 ^{ns}	-0.424 ^{ns}	0.679	-0.380 ^{ns}
	Q _{10R}	0.819	0.837	0.738	0.187 ^{ns}	0.151 ^{ns}	0.428 ^{ns}	-0.178 ^{ns}	-0.009 ^{ns}	0.730
MI	Q _{10L}	-0.262 ^{ns}	-0.261 ^{ns}	-0.731	-0.425 ^{ns}	0.491 ^{ns}	-0.485 ^{ns}	-0.331 ^{ns}	0.637	-0.277 ^{ns}
	Q _{10R}	0.890	0.891	0.737	-0.214 ^{ns}	-0.061 ^{ns}	-0.049 ^{ns}	-0.362 ^{ns}	-0.189 ^{ns}	0.734
SC	Q _{10L}	0.234 ^{ns}	0.233 ^{ns}	0.238 ^{ns}	0.431 ^{ns}	0.523 ^{ns}	0.338 ^{ns}	0.480 ^{ns}	0.685	0.197 ^{ns}
	Q _{10R}	0.976	0.764	0.131 ^{ns}	-0.357 ^{ns}	0.118 ^{ns}	-0.215 ^{ns}	-0.485 ^{ns}	-0.050 ^{ns}	0.820

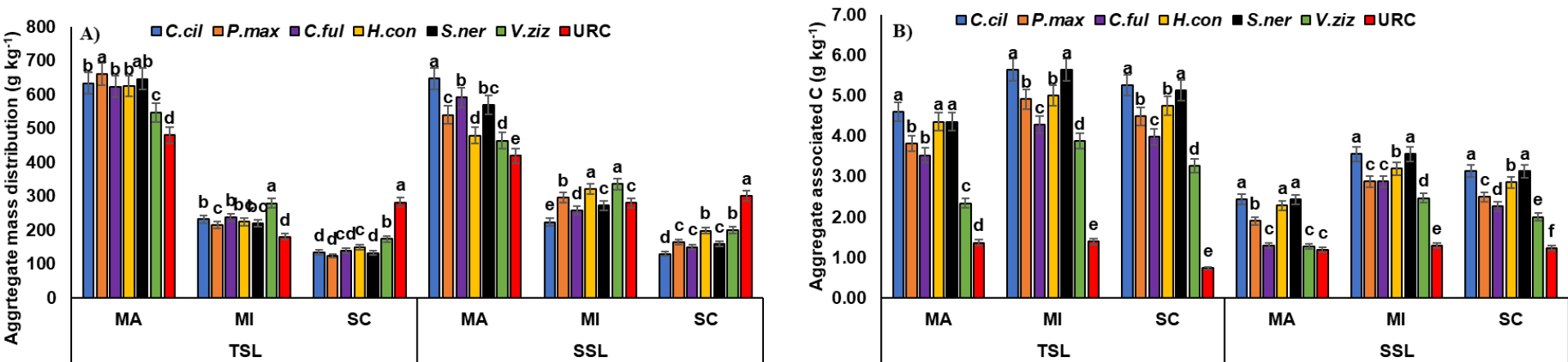
Green and red coloured cell indicated significant positive and negative corelations at $p \leq 0.05$, respectively; ns: non-significant relations at $p \leq 0.05$.



899

900 Figure 1: a) Schematic representation of three hypotheses b) Pictorial display of the hypotheses; c) The geographic location of the experimental site
 901 and lay out of experiment.
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904

905 Figure 2. Impact of grass covers on A) soil aggregate mass distribution and B) aggregate associated soil organic carbon (C) concentration at top (TSL)
906 and sub-surface (SSL) soil layers in semi-arid restored land. Error bars signify standard error of mean. The columns with same lowercase letters within
907 an aggregate size fraction are not statistically different following Tukey's HSD test.;

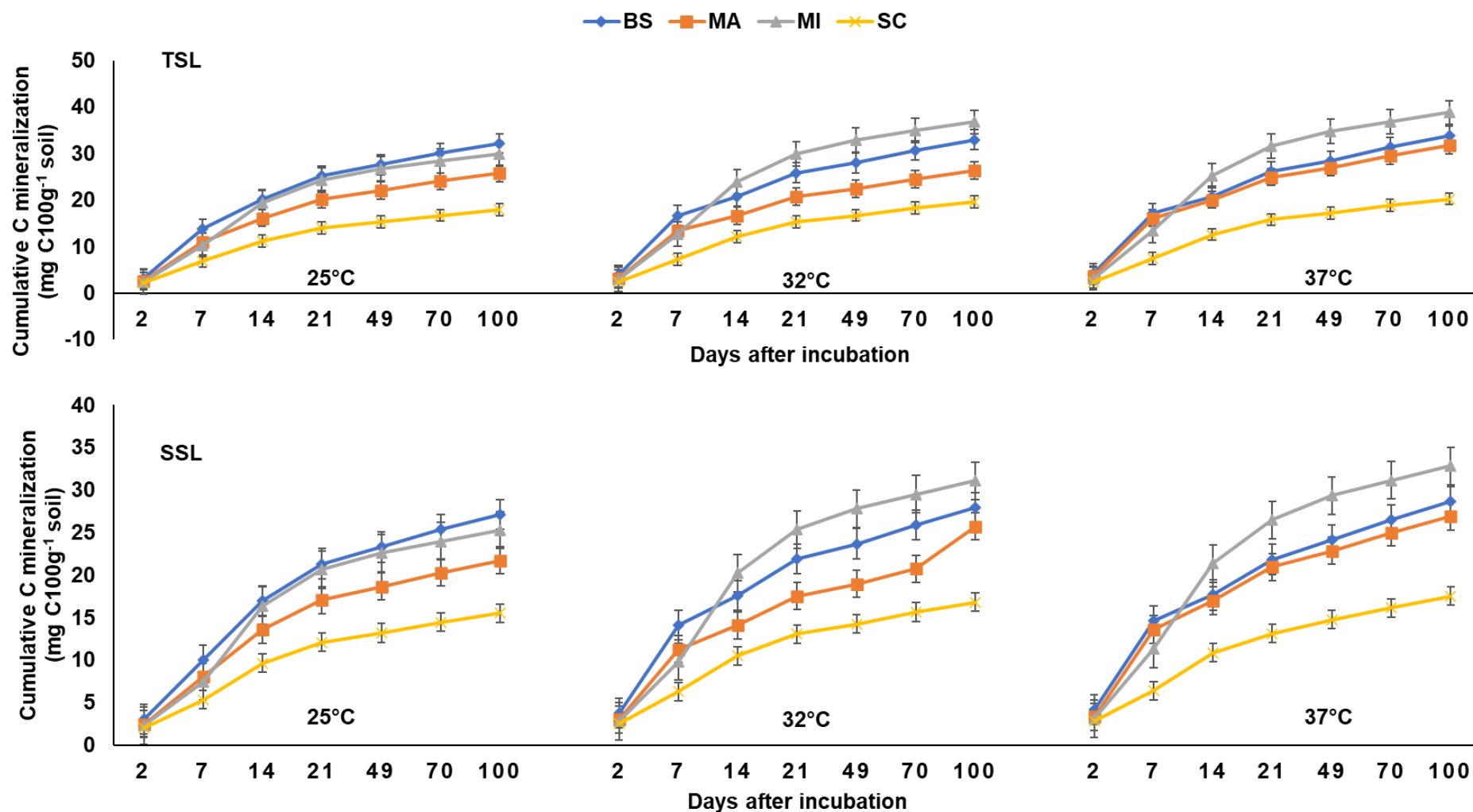
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MA: Macroaggregates; MI: Microaggregates; SC: silt plus clay.

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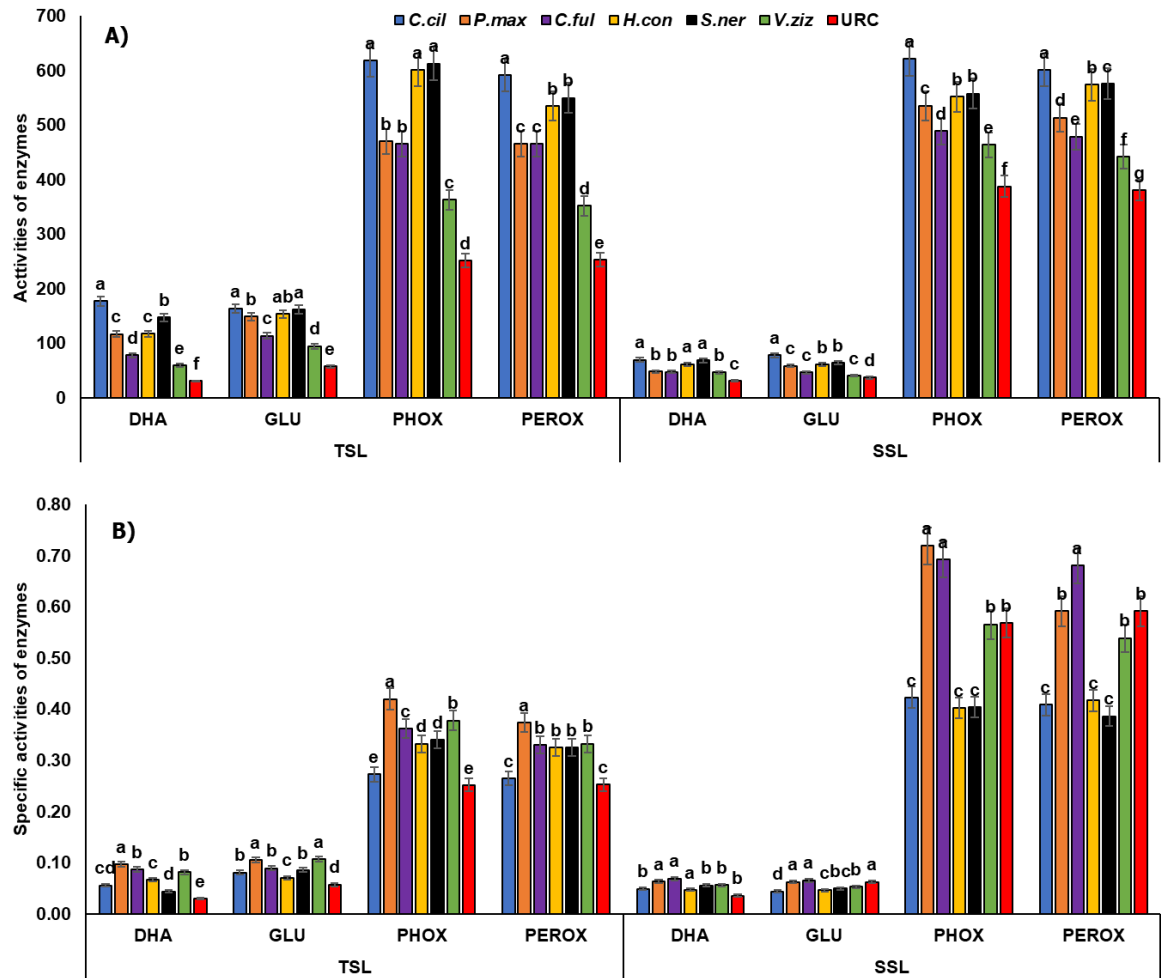
C.cil: *Cenchrus ciliaris*; C.ful: *Chrysopogon fulvus*; P.max: *Panicum maximum*; S.ner: *Setaria nervosa*; H.con: *Heteropogon contortus*; V.ziz: *Vetiveria zizanioides*; URC: Unrestored control;

910



911

912 Figure 3. Impact of incubation temperatures (at 25, 32, and 37 °C) on cumulative carbon (C) mineralization from bulk soil (BS), macroaggregate (MA),
 913 microaggregate (MI), and silt plus clay (SC) temperatures at top (TSL) and sub-surface (SSL) soil layers in semi-arid restored land. Error bars signify
 914 statistical difference following Tukey's HSD test.



916

917 Figure 4. Impact of grass covers on A) absolute activities and B) specific activities
918 dehydrogenase (DHA; $\mu\text{g TPF g}^{-1} 24\text{h}^{-1}$), β -glucosidase (GLU; $\mu\text{g PNG g}^{-1} 2\text{h}^{-1}$),
919 phenoloxidase (PHOX; $\mu\text{g ABTS g}^{-1} \text{h}^{-1}$), and peroxidase (PEROX; $\mu\text{g ABTS g}^{-1} \text{h}^{-1}$)
920 activities at top (TSL) and sub-surface (SSL) soil layers in semi-arid restored land. Error bars
921 signify standard error of mean. The columns with same lowercase letters within an aggregate
922 size fraction are not statistically different following Tukey's HSD test.

923 C.cil: *Cenchrus ciliaris*; C.ful: *Chrysopogon fulvus*; P.max: *Panicum maximum*; S.ner: *Setaria nervosa*; H.con: *Heteropogon contortus*;
924 V.ziz: *Vetiveria zizanioides*; URC: Unrestored control

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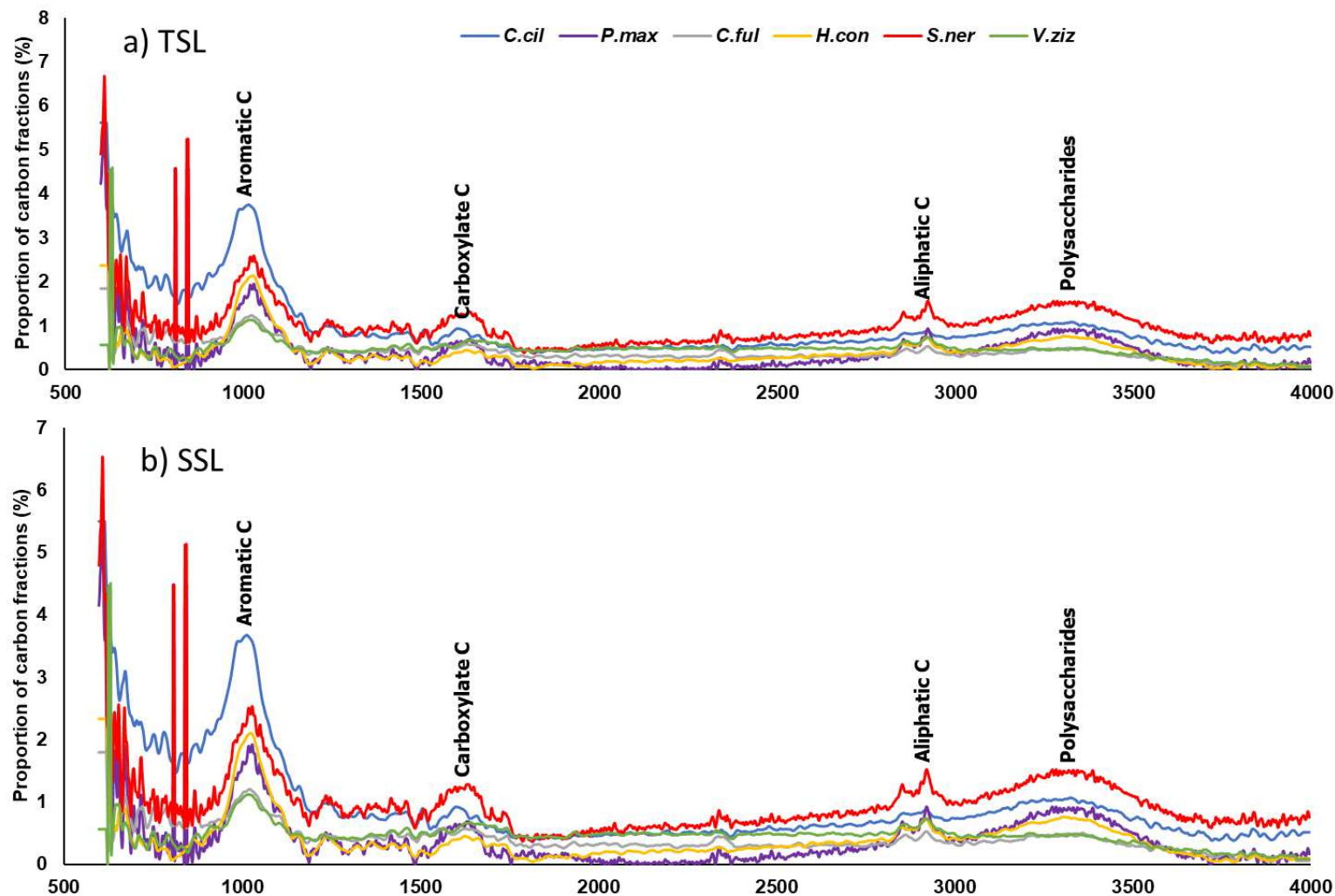
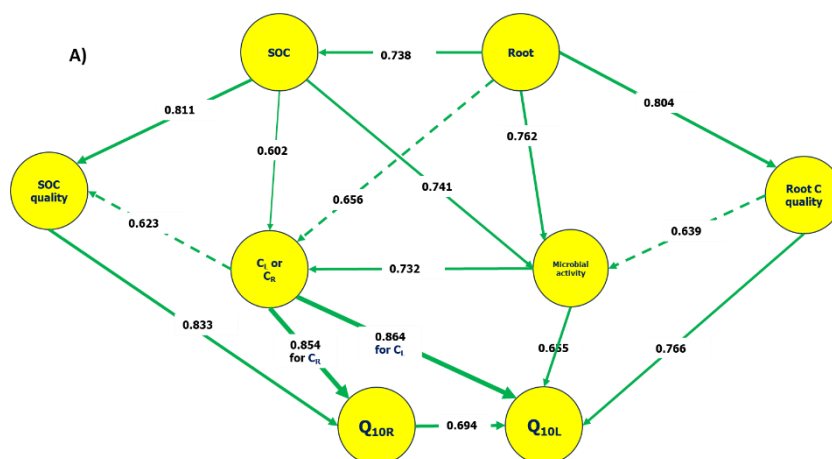


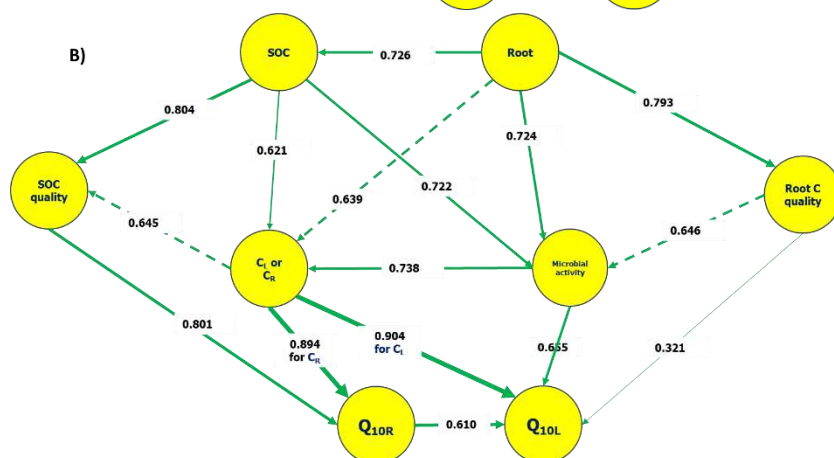
Figure 5. The proportions of different organic C fractions in root litters of different grasses at A) top (TSL) and B) sub-surface (SSL) soil layers in semi-arid restored land.

C.cil: *Cenchrus ciliaris*; C.ful: *Chrysopogon fulvus*; P.max: *Panicum maximum*; S.ner: *Sehima nervosum*; H.con: *Heteropogon contortus*; V.ziz: *Vetiveria zizanioides*;

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935 Figure 6. Path analysis depicting impact of labile and recalcitrant C pools (C_L and C_R), soil
 936 organic matter quality, and root litter C quality on temperature sensitivity of labile and
 937 recalcitrant C pools (Q_{10L} and Q_{10R}) at A) top and B) sub-surface soil layers. Models
 938 satisfactorily fitted to data based on χ^2 and RMSEA analyses [$\chi^2 = 112.56$, GFI= 0.69, RMSEA
 939 < 0.001]. Widths of the arrows indicate the strength of the casual relationship. Dashed arrows
 940 represent indirect effects, solid arrows indicate direct relationships.

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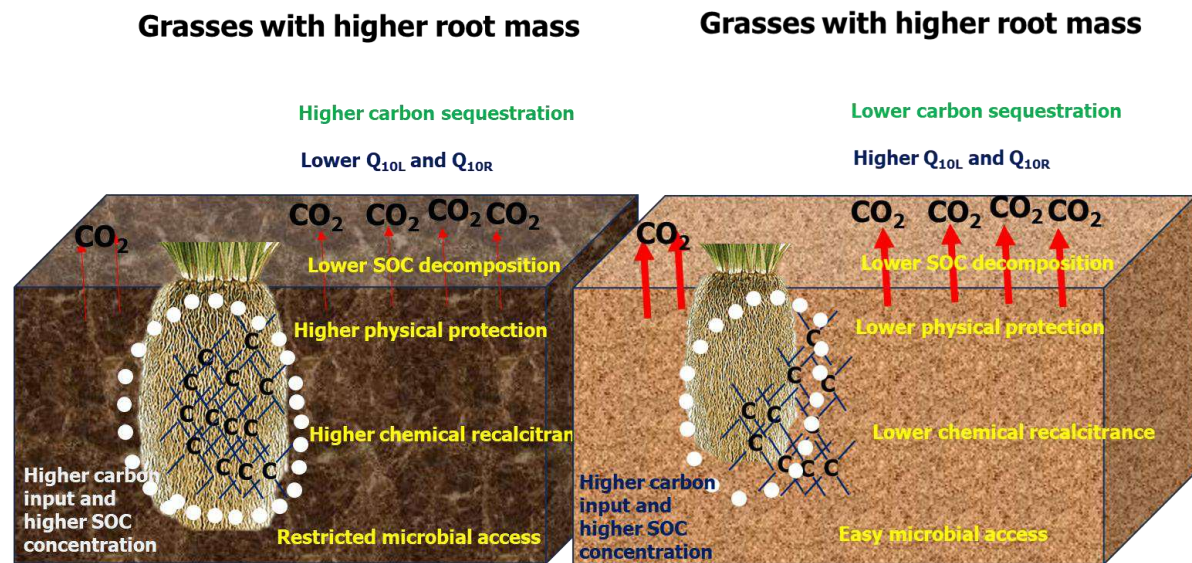


Figure 7. Schematic representation of roles of root litter traits on temperature sensitivity (Q_{10}) and soil carbon sequestration.

Supplementary Files

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- [Supplementary040425.docx](#)