Total and labile pools of organic carbon in relation to soil biological properties under contrasting land-use systems in a dry mountainous region

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ABSTRACT
This study evaluated the effect of contrasting land-use systems on changes in pools of total organic carbon (TOC) and its labile fractions, and identified the sensitivity of soil properties as a minimum data set (MDS) for assessing soil quality change in a dry, mountainous Himalayan region in India. The soils under mono (barley/mustard/oats)- and double (barley-buckwheat/barley-turnip)-cropping systems had significantly \( p < .05 \) lower TOC by \( \sim 34 \) and \( 20\% \), while the less labile C (Fract. 3) concentration was lower by \( \sim 46 \) and \( 48\% \), respectively, compared with the agro-forestry (popular/willow). The stable C pool (Fract. 3 + Fract. 4) comprised \( \sim 74, 77, 86, 74 \) and \( 73\% \) of TOC in soils under mono-cropping, double-cropping, agro-forestry, orchards and vegetable crops, respectively. Land-use significantly impacts the sensitivity of labile C fractions, viz. water extractable organic C (WEOC), microbial biomass C (MBC) and organic C fractions of variable oxidizability (i.e. Fract. 1, Fract. 2, Fract. 3 and Fract. 4). The sensitivity analysis showed a change of \( \sim 21.5\% \)–\( 56.2\% \) in the TOC pool, with the highest change for soils under vegetable crops and the lowest for double-cropping. Soil protein exhibited a significant relationship with TOC and its fractions, enzymatic and biochemical properties, and soils’ fine fraction (silt and clay). The stable C pool exhibited a significant linear relationship with soils’ finer fraction (silt = 0.82**, clay = 0.78**, \( p < .01 \)), indicating that the mineral matrix had a profound influence on C stabilization in soils. The land-use systems with higher soil total glomalin (TG) content had higher moisture retention capacity and stable C pool. The principal component analysis (PCA) identified TG, Fract. 3 and available-K as most important soil quality indicators for discriminating change in soil health in a cold, dry Himalayan region.

KEYWORDS
Stable C pool; biological indicators; soil quality index; dry mountainous; Himalayan region; soil mineral matrix

Introduction
Soils have immense potential and the unique capability to sequester and store large amounts of organic carbon (C). Global estimates indicate that \( \sim 45\% \) soils are under different agricultural land uses such as cropland and grazing land [1], and the majority have lost \( \sim 30\%–50\% \) of the initial total organic C (TOC) stocks in their native environment [2]. The TOC content describes a balance between C input as above- and below-ground biomass vis-à-vis C loss as carbon dioxide (CO\(_2\)) via heterotrophic soil respiration. Within a managed agricultural ecosystem, the C accrual and loss rates are largely impacted by diverse crop production and soil management practices [1,3]; therefore, land-use change and agricultural intensification lead to spectacular change in the terrestrial flow of C within the ecosystem. The croplands are considerably important with immense potential to sequester C because of depletion of the antecedent TOC pool in most cultivated soils [3].

The differences in TOC pool and its fractions among land-use systems generate important information on C build-up and sequestration potential of soils [4]. Land-use change and agricultural intensification due to conversion of natural ecosystems to agricultural croplands increases the rates of decomposition of soil organic matter (SOM) and modifies the quantity and quality of organic residue input and their redistribution [1,5]. In the context of restored engineering under contrasting land-use systems, TOC has high significance compared to the labile C pools which are considered highly sensitive to soil management-induced
changes in soil quality [6]; therefore, alteration in TOC affects soils’ physical, chemical and biological properties which in turn influence soil fertility, crop productivity and C sequestration potential [7–9]. TOC is thought to exhibit unpredictable sensitivity to soil management-induced change within different land-use systems [6,10]. Active C pools are considered more sensitive than the TOC pool to elucidate a transformation in SOM quality; therefore, they are considered sensitive indicators of soil quality change in response to soil management-induced change [11]. These labile and stable organic C pools are widely used to discern the impact of land-use change and associated management on soil health [12,13]. The labile C with greater turnover rates provides the advantage of easy detection of change in quality and quantity of SOM in short- and medium-term impacts of crop production and soil management regimes. Labile C pools are important indicators to discern changes in soil quality, but frequently contradict the evidence that the recalcitrant C pool of TOC could decompose more easily when in contact with the decomposer micro-organisms [9,14]. Therefore, C sequestration and SOM turnover should focus on investigations of C stabilization in the mineral matrix (soils’ fine fraction), microbial ecology and the interactions within the mineral surfaces [3,4]. Soil C stabilization within the mineral matrix is closely associated with soil-related protein (glomalin), which tends to bind the soils’ fine particles together as aggregates [15–17]. Soil C stabilization enhances soil functions like nutrient mineralization and retention, substrate availability for microbial biodiversity and erosion control [18,19]. Soil microbes respond to changes in the soil environment by producing a high microbial cell count, extracellular enzymes and organic acids, which may lead to the modification of the soil environment and nutrient pools to metabolizing C compounds. Soil’s enzymatic activities influence its biological and biochemical properties [20,21], decomposition and nutrient (i.e. C, N and P) cycling to maintain soil quality [22,23]. Hydrolytic enzymes play an important role in the decay of labile C (dehydrogenase; DHA), in P release reactions (alkaline- and acid-phosphatase), and in several other miscellaneous functions (fluorescein diacetate activity; FDA) under different agro-ecological conditions.

Even at high altitudes, a rapid change in temperature due to global warming has disrupted the TOC stocks in soils. A recent meta-analysis revealed reductions in soil C stocks with warming due to increased ratios of ligninase to cellulase activity [24]. The long-term (>5 years) warming leads to a reduction in recalcitrant C pool in soils by ~14%, while short-term warming had a non-significant effect [23]. Although the soils at high altitudes had higher C stocks, nonetheless, hilltop landscapes are highly sensitive to climate change; therefore, the soils at high altitudes play a vital role in the global C cycle. Due to the rising global temperature, the fragile ecosystems of cold Himalayan ranges in Ladakh (northern India) are drastically changing under the influence of natural calamities like flash floods and melting of glaciers [25]. The retreating glaciers and low snowfall have caused a shortage of irrigation water for crop production, reduced crop productivity, altered soil quality and degradation of agricultural land [26]. Therefore, a clear understanding of the impact of climate change, altered soil hydrology, and biological activity that influences the vegetation pattern and the above- and below-ground C input to control SOM quantity and quality is important [27]. The influence of land-use changes on soil C pools and its impact on soil biological properties for sustaining long-term agricultural productivity in highly fragile cold, arid Himalayan regions is less well known. The present study was therefore conducted to assess the impact of land-use systems under mono- and double-cropping, agro-forestry, orchards and vegetable crops on total and labile pools of organic C in relation to biological properties of soils in a dry mountainous region of Ladakh in the northwestern Himalayan range. We hypothesized that cropping intensity influences soil C input due to a change in biological response, which may not vary in the same direction (increase/decrease) due to alteration in SOM quantity and quality. A comprehensive understanding of the distribution of C pools in relation to biological properties in various land-use systems would help in discerning the effect of sustainable agricultural restoration on soil C dynamics. We hypothesized that a comprehensive understanding of the distribution of C pools in relation to soils’ biological properties under various land-use systems would help to discern the effects of sustainable agricultural restoration practices on soil C dynamics. Therefore, the present study was conducted to quantify the effects of restored engineering under contrasting land-use systems (mono- and double-cropping, agro-forestry/orchards vis-
à-vis vegetable crops) in the Ladakh region. More specifically, we studied the change in SOM quality toward more labile or recalcitrant C pools and their sensitivity, mediated by biological properties, and identified a minimum data set (MDS) for evaluating soil quality change in contrasting land-use systems.

Material and methods

Study area

The study region, in Union Territory of Ladakh in India, is a cold, arid tract in the northwest Himalayas (Figure 1). This region (34°09'54.14"N, 77°35'2.47"E; 2300–5000 m above mean sea level), covers an area of ~45,100 km², the second largest district in India. The region is characterized by heavy snowfall, low rainfall (~80–300 mm), dry and harsh winters and a short growing season. The temperature drops extremely low, reaching around 3–35 °C in summer, and the mean minimum temperature ranges from −20 to −35 °C in winter. The region is mainly characterized by light textured soils (sandy to sandy loam in texture) with poor structure and low water holding capacity. Soils are classified as Typic Cryorthents, Typic Orthents, Typic Xerorthent and Typic Hapludoll as per the USDA classification [28]. With a temperature range of −23 °C to −25 °C, the area experiences both seasonal and diurnal temperature fluctuations, which affect not only the plant growth and cropping, but also the livelihood options [28]. The major source of irrigation water is glacier melt. Based on agro-ecological classification, Ladakh has been divided into three divisions: the upper agriculture zone (11,800–14,000 ft) with agri-pastoral system, the central agricultural zone (10,000–11,800 ft) with single-cropped area and the lower agricultural zone (<10,000 ft) with double-cropped area [29]. Mono-cropping is the most prevalent crop production system in most parts of Ladakh, while double-cropping is practiced in

Figure 1. Geographical location of study sites under different land-use systems in a dry mountainous region in northwestern Himalaya, India.
some parts of Khaltsi and Nubra, the administrative blocks in the western and northern parts of Ladakh, respectively.

**Sampling sites**
A total of 35 sites with the same basic characteristics were selected in the study area (Table 1). At each site, three locations were randomly chosen, which were considered pseudo-replicates. These sites were selected to involve mono- and double-cropping systems with a long history (>10 years). Within a mono-cropping system, fields were under cereal crops (barley/mustard/oats; n = 18) and agro-forestry (popular/willow; n = 3). Within the double-cropping system, fields were under cereal crops (barley–buckwheat/barley–turnip n = 5), fruit crops (apple/pear/apricot/grapes/walnut, n = 5) and vegetable crops (peas/potato/onion, n = 4).

**Soil sampling and analyses**
The surface (0–15 cm depth) soil samples were collected from fields under different land-use systems with core sampler (inner diameter = 7.2 cm). At each site, soil samples were collected from three random locations, and were used as pseudo-replicates. Soil samples were collected in July–August (2018) after removing visible plant residue and root debris. One portion of each soil sample was passed through a 2.0 mm stainless steel sieve for basic soil analysis and determination of total and labile C pools, while another portion of each soil sample was retained without sieving and stored immediately at 4 °C to assay enzymatic activities and soil microbial biomass C (MBC).

### Basic soil analysis
Soil samples were analyzed for soil reaction (pH1:2; 1:2; soil:water suspension) using a glass electrode. After, the suspension was settled, and the supernatant was analyzed to determine electrical conductivity (EC1:2) (1:2; soil:water suspension) using a conductivity meter. The particle size distribution was determined by the international pipette method. The available-P (Olsen’s P) in soil samples was determined by extracting the soils with 0.5 M sodium bicarbonate (NaHCO3; pH = 8.5) in 1:20 soil: NaHCO3 solution, followed by determination of available-P concentration in the extract using a colorimeter. Available-K in soil samples was extracted with neutral normal ammonium acetate (1 N, CH₃COONH₄; pH = 7.0) followed by flame photometric determination. The soil water retention characteristics were measured at matric potentials using pressure plate extractors. Soil moisture content at field capacity (FC) was determined at -0.33 bar, and permanent wilting point (PWP) was determined at -15 bar.

### Soil organic carbon and its pools
The TOC in soil was determined by reacting it with 1 N potassium dichromate (K₂Cr₂O₇) solution at 150 °C for 60 min [30]. The time and temperature

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**Table 1. Important properties of the surface (0–15 cm) layer of soils under different land-use systems in a dry mountainous region in northwestern Himalaya, India.**

| Soil property | Mono-cropping (n = 18) | Double-cropping (n = 5) | Agro-forestry (n = 3) | Orchards (n = 5) | Vegetable crops (n = 4) |
|---------------|------------------------|-------------------------|----------------------|----------------|--------------------------|
| pH1:2         | 7.34 ± 0.04            | 7.48 ± 0.04             | 7.78 ± 0.16          | 7.44 ± 0.12    | 7.28 ± 0.60               |
| EC1:2         | 0.48 ± 0.07            | 0.69 ± 0.15             | 0.54 ± 0.19          | 0.83 ± 0.10    | 0.74 ± 0.16               |
| Available-P   | 68.2 ± 3.7             | 52.4 ± 5.2              | 36.1 ± 2.4           | 38.1 ± 3.7     | 94.9 ± 11.6               |
| Available-K   | 509.4 ± 41.6           | 437.8 ± 54.2            | 725.3 ± 73.7         | 709.3 ± 74.6   | 611.4 ± 68.5              |

EC = Electrical conductivity. Values indicate the standard error of the mean. Values across the different land-use systems followed by different letters are significantly (p < 0.05) different by Tukey’s post hoc test.
of heating were standardized in a preliminary experiment conducted with a number of soils from the experimental region. A heating time of 1 h at 150 °C yielded results similar to those obtained with the dry combustion method [10]. The water extractable organic carbon (WEOC) was determined by shaking 10 g of soil with 20 mL of deionized water for 60 min [14]. The four fractions of TOC were determined under a gradient of oxidizability, viz. Fract. 1 (very labile C), Fract. 2 (labile C), Fract. 3 (less labile C) and Fract. 4 (recalcitrant C) fraction (Equations 1–4).

Fract. 1 (very labile C) = Oxidizable organic C under 12N H2SO4 (1)
Fract. 2 (labile C) = Difference in oxidizable C between 18N and 12N H2SO4

\[ (18-12N \text{H}_2\text{SO}_4\text{oxidizable C} ) \] (2)
Fract. 3 (less labile C) = Difference in oxidizable C between 24N and 18N H2SO4

\[ (24-18N \text{H}_2\text{SO}_4\text{oxidizable C} ) \] (3)
Fract. 4 (recalcitrant C) =

TOC – 24N H2SO4 oxidizable C (4)

The active C pool was estimated as the sum of very labile C + labile C (Fract. 1 + Fract. 2), while the stable C pool was computed as the sum of less labile C and recalcitrant C (Fract. 3 + Fract. 4), using Equations (5) and (6).

Active C pool (g kg⁻¹soil)

\[ = \text{Fract. 1 (g kg}^{-1}\text{soil)} + \text{Fract. 2 (g kg}^{-1}\text{soil)} \] (5)

Stable C pool (g kg⁻¹soil)

\[ = \text{Fract. 3 (g kg}^{-1}\text{soil)} + \text{Fract. 4 (g kg}^{-1}\text{soil)} \] (6)

Total organic C (TOC) stocks (Mg ha⁻¹)

\[ = \text{TOC} \times B_D \times \text{depth (m)} \times 100 \] (7)

Soil cores, collected from the 0–7.5 and 7.5–15 cm layers of each sampling site, were oven dried at 105 °C for 24 h, and dry soil weight was recorded. For the determination of soil BD, data for 0–7.5 and 7.5–15 cm was pooled. The BD (Mg m⁻³) was calculated using Equation (8).

\[ B_D = \frac{W_s}{V_t} \] (8)

where \( W_s \) is the weight of the soil (Mg) and \( V_t \) is the volume of the soil sample (m³).

**Carbon management index**

The carbon management index (CMI) was calculated using Equation (9) as per the mathematical procedure described by Blair et al. [11]. Agro-forestry was taken as the reference.

\[ \text{CMI} = \text{CPI} \times \text{LI} \times 100 \] (9)

where CPI is the carbon pool index and LI is the lability index. The CPI and LI were calculated using Equations (10) and (11) [11].

\[ \text{CPI} = \frac{\text{TOC}_{\text{mono-and double-cropping/orchards/vegetable crops}}}{\text{TOC}_{\text{agro-forestry}}} \] (10)

\[ \text{LI} = \left[ \frac{\text{Fract. 1}}{\text{TOC}} \times 3 \right] + \left[ \frac{\text{Fract. 2}}{\text{TOC}} \times 2 \right] + \left[ \frac{\text{Fract. 3}}{\text{TOC}} \times 1 \right] \] (11)

**Soil microbiological properties**

Soil MBC was determined from the field fresh soil samples stored at 4 °C by the chloroform fumigation extraction (CFE) method [31] using a recovery factor \( (K_{EC}) \) of 0.41 [32]. For C mineralization studies and determination of soil microbiological quotients, 50 g of each air-dried soil sample was wetted to FC moisture content about 7 days before the start of the incubation. The rewetted soil samples were placed in a conical flask along with vials containing 10 mL of 1 M sodium hydroxide (NaOH) to trap the amount of CO₂ evolved from microbial respiration [10]. The CO₂ that evolved during ~70 days’ time was absorbed in a known volume and strength of sodium hydroxide (NaOH) in alkali traps. The alkali traps were replaced daily for the initial 7 days, and then on alternate days, for 45 days of incubation. Thereafter, the alkali traps
were replaced at an interval of 2 days until the termination of the incubation study. The excess NaOH in the alkali traps was titrated against standard hydrochloric acid (HCl). The amount of CO₂ released was calculated by measuring the exact volume of NaOH used for CO₂ absorption using barium chloride. The soil respiration rate during the 66th and 70th days of incubation was taken as basal soil respiration (BSR), because during that period the samples reached a relatively constant CO₂ production rate. The mineralization quotient (qm) was calculated as the ratio of Cmic to TOC (µg Cmic / µg TOC⁻¹). The respiratory quotient (qCO₂) was calculated from the ratio of BSR to MBC (µg CO₂ basal-C h⁻¹ × µg biomass C⁻¹) × 10³. The microbial quotient (qmic) was calculated as the ratio of MBC to TOC (µg biomass C / µg TOC⁻¹) [10].

**Soil enzymatic activity**

The acid phosphatase (acid-P) activity in soils was determined using a p-nitrophenyl method [23]. The assay of phosphorus monoesterase activities was based on the colorimetric determination of p-nitrophenol released by phosphatase activity from soil incubated with buffered sodium p-nitrophenyl phosphate solution (pH = 5.4). The DHA was determined as triphenylformazan (TPF) produced by the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) [23]. The total microbial activity potential was measured through FDA hydrolysis assay, which hydrolyzes colorless FDA to release a colored end product fluorescein [33].

**Total and labile glomalin**

Total glomalin (TG) and easily extractable glomalin (EEG) in soil samples were extracted using the procedure described by Wright and Upadhyaya [34]. The EEG was extracted in 20 mM tri-sodium citrate (pH = 7.0) and autoclaved for 30 min (121 °C, 15 psi). It was centrifuged for 15 min at 5000 rpm immediately after extraction. The TG represented the sum of sequential extraction autoclave cycles (5 times) and was obtained by using 50 mM tri-sodium citrate (pH = 8.0) at 121 °C for 60 min and centrifuging at 5000 rpm for 15 min. The protein content in the supernatant was determined following the standard procedure given by Lowry [35].

**Statistical analysis**

The data were statistically analyzed using analysis of variance (ANOVA) in a randomized block design with an unequal number of samples using SPSS software for Windows 21.0 (SPSS Inc., Chicago, USA). At each site, three pseudo-replications were established for each land use (i.e. mono- and double-cropping system, agro-forestry, orchards, and vegetable cultivation). Soil sampling sites were treated as replicates (i.e. the random effects) and land-use systems were considered treatments (i.e. the fixed effects). Means for treatment effects were separated based on Tukey’s post hoc test at p < .05. The selection of appropriate indicators representing the soil chemical, physical or biological properties for the MDSs was achieved by principal component analysis (PCA). The PCA was performed to identify organic C pools and other soil biochemical properties that might contribute to the group separation of different land-use systems. The independent or predictor variables were included stepwise using probability of significance (p < .05) as a criterion for entering a variable. The PCs with high eigenvalues and variables with high factor loading were considered to best represent systems’ attributes. Within each PC, only highly weighted factors were retained for the MDS. Highly weighted factor loadings were defined as having absolute values within 10% of the highest factor loading. When more than one factor was retained under a single PC, the multivariate correlation coefficients (r) were employed to determine whether the variables could be considered redundant and therefore eliminated from the MDSs. The variables with higher contributions toward group separation and the highest factor loading values were considered key predictors of soil quality for the studied land-use systems.

MDSs for developing a soil quality index (SQI) have been widely accepted due to the adequate information for soil quality evaluation [36]. Using a linear scoring method, the MDS indicator scores obtained for each observation were multiplied with the weighted factor obtained. Each PC explained a certain amount (%) of the variation in the total data set. This percentage, when divided by the total percentage of variation explained by all PCs with eigenvectors >1, gave the weighted factors for indicators chosen under a given PC. The score was then multiplied by the weighting factor derived from the PCA to obtain the ultimate index value for soil quality under different land-use systems. The SQI was calculated from the integrated
score and weight factor of each indicator using Equation (12).

$$\text{SQI} = \sum_{i=1}^{n} W_i \times S_i$$  \hspace{1cm} (12)

where $S$ is the indicator score and $W$ is the PC weight factor.

The sensitivity of total and labile C pools was calculated as the percentage change in the ratio of the difference in C fraction in soils under different land-use systems (treatments) and soils under mono-cropping system (control) relative to the reference (control) value, using Equation (13).

$$\text{Percent change in C pool} = \frac{[C \text{ pool}_{\text{treated}} \text{ g kg}^{-1} - C \text{ pool}_{\text{control}} \text{ g kg}^{-1}]}{C \text{ pool}_{\text{control}} \text{ g kg}^{-1}} \times 100$$  \hspace{1cm} (13)

Results

Soil properties and moisture retention characteristics

Soils under mono-cropping systems and vegetable crops had significantly ($p < .05$) lower pH, compared with soils under other investigated land-use systems (Table 1). Soils under agro-forestry had significantly higher pH compared with double-cropping and orchards, which did not differ significantly from each other. The EC was significantly lower for soils under mono-cropping and agro-forestry, compared with other land-use systems. The orchard soils had significantly higher EC than the other investigated land-use systems. Available-P content in soils under agro-forestry and orchards was significantly lower, by 32.1 kg ha$^{-1}$ ($\sim$47%) and 30.1 kg ha$^{-1}$ ($\sim$44%), respectively, compared to mono-cropping systems. The soils under vegetable crops had significantly higher available-P content, of 26.7–58.8 kg ha$^{-1}$ (39.1–163%), compared with other investigated land-use systems. The soils under agro-forestry and orchards had significantly higher available-K, while the soils under double-cropping systems had the lowest. The soils under vegetable crops had significantly lower available-K by 113.9 kg ha$^{-1}$ ($\sim$16%) and 97.9 kg ha$^{-1}$ ($\sim$14%) compared with agro-forestry and orchards, respectively. However, soils under vegetable crops had 102.1 kg ha$^{-1}$ ($\sim$20%) and 173.6 kg ha$^{-1}$ ($\sim$40%) higher available-K, compared with the soils under mono- and double-cropping systems, respectively.

Figure 2 illustrates soil moisture retention characteristics at FC, PWP and available water content (AWC) of soils under different land-use systems. The soils under double-cropping had significantly higher moisture content at FC ($0.33$ bar), compared with that under other investigated land-use systems. The soils under double-cropping had $\sim$13% higher moisture content at FC ($0.33$ bar), compared with that under other investigated land-use systems. The soils under double-cropping had $\sim$13% higher moisture content at FC, compared with mono-cropping system. However, soil moisture at PWP ($-15$ bar) did not differ significantly in soils under different land-use systems, except for soils under double-cropping, where it was significantly lower. The soils under double-cropping
systems had significantly higher AWC, compared with the soils under other investigated land-use systems.

**Total and labile pools of soil organic carbon, and their sensitivity**

Total organic C was significantly lower in monocropping, compared with the soils under other investigated land-use systems (Table 2). The soils under mono- and double-cropping systems had lower TOC by 2.11 g kg\(^{-1}\) (~34%) and 1.23 g kg\(^{-1}\) (~20%), compared with agro-forestry. The TOC concentration in soils under agro-forestry, orchards and vegetable crops did not differ significantly. The WEOC, comprising between 0.30 and 0.76% of TOC, was the smallest organic C fraction in soils under different land-use systems. The WEOC was significantly lower in soils under vegetable crops, respectively (Figure 3).

The sensitivity analysis showed that the percentage change in the TOC pool varied between 21.5 and 56.2%, with the highest change in soils under vegetable crops, and the lowest for soils under double-cropping (Figure 4). Among variable oxidizability TOC fractions, Fract. 1 exhibited the highest change (~52.4–77.4%), while Fract. 4 showed the widest range of change (~36.6–66.8%). The percentage change in MBC was lowest for soils under double-cropping (~38.1%), while the soils under agro-forestry (~82.7%) exhibited the highest change.

### Table 2. Total and labile pools of soil organic carbon, lability index (LI) and carbon pool index (CPI) in surface (0–15 cm) soils under different land-use systems in a dry mountainous region in northwestern Himalaya, India.

| Land-use system | TOC (g kg\(^{-1}\)) | WEOC (mg kg\(^{-1}\)) | Fract. 1 (g kg\(^{-1}\)) | Fract. 2 (g kg\(^{-1}\)) | Fract. 3 (g kg\(^{-1}\)) | Fract. 4 (g kg\(^{-1}\)) | Labile C | Very labile C | Water extractable organic C (WEOC) |
|-----------------|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------|---------------|----------------------------------|
| Mono-cropping   | 4.09 ± 0.24       | 22.8 ± 2.1             | 0.44 ± 0.03            | 0.65 ± 0.03            | 1.01 ± 0.06            | 2.04 ± 0.12            | 1.04 ± 0.06 | 3.05 ± 0.18   | 0.63 ± 0.002                      |
| Double-cropping | 4.97 ± 0.11       | 22.5 ± 2.5             | 0.48 ± 0.01            | 0.65 ± 0.02            | 1.05 ± 0.02            | 2.79 ± 0.06            | 1.13 ± 0.03 | 3.84 ± 0.05   | 0.76 ± 0.064                      |
| Agro-forestry   | 6.20 ± 0.26       | 47.2 ± 4.0             | 0.21 ± 0.01            | 0.63 ± 0.06            | 1.96 ± 0.02            | 3.40 ± 0.24            | 1.56 ± 0.07 | 5.36 ± 0.25   | 1.96 ± 0.024                      |
| Orchards        | 6.01 ± 0.28       | 38.1 ± 8.5             | 0.71 ± 0.03            | 0.85 ± 0.04            | 1.13 ± 0.06            | 3.31 ± 0.16            | 1.75 ± 0.40 | 4.46 ± 0.21   | 0.89 ± 0.001                      |
| Vegetable crops | 6.39 ± 0.78       | 18.9 ± 1.45            | 0.79 ± 0.18            | 0.96 ± 0.22            | 1.42 ± 0.32            | 3.23 ± 0.24            | 1.75 ± 0.40 | 4.65 ± 0.84   | 0.89 ± 0.001                      |

Values indicate the standard error of the mean. Values across the different land-use systems followed by different letters are significantly (p < 0.05) different by Tukey’s post hoc test.

TOC = Total organic C; Fract. 1 = Very labile C; Fract. 2 = Labile C; Fract. 3 = Less labile C; Fract. 4 = Recalcitrant C; Active C = Fract. 1 + Fract. 2; Stable C pool = Fract. 3 + Fract. 4; WEOC = Water extractable organic C.
Figure 3. Relative distribution of total organic carbon (TOC) fractions of varying oxidizability or lability in the surface layer (0–15 cm) of soils under different land-use systems in a dry mountainous region in northwestern Himalaya, India. Bars followed by different letters for a given C fraction are significantly (*p* < .05) different by Tukey’s post hoc test. Fract. 1 = Very labile C; Fract. 2 = Labile C; Fract. 3 = Less labile C; Fract. 4 = Recalcitrant C.

Figure 4. Percent change in total organic carbon (TOC) and various labile and recalcitrant C (Fract. 4) pools over control (mono-cropping system) in the surface (0–15 cm) soils under different land-use systems in a dry mountainous region in northwestern Himalaya, India. Line bars indicate the standard error of the mean. Fract. 1 = Very labile C; Fract. 2 = Labile C; Fract. 3 = Less labile C; MBC = Microbial biomass C; WEOC = Water extractable organic C.
Soil bulk density and total organic carbon stocks in soils

The BD of soils under mono- and double-cropping systems was significantly higher compared with the other investigated land-use systems (Figure 5). The BD in the surface (0–15 cm) soil layer did not differ significantly for soils under agro-forestry and those under orchards and vegetable crops. The soils under mono-cropping had significantly lower TOC stocks, of 4.22 Mg C ha\(^{-1}\) (lower by \(\sim 45\%\)), compared with soils under agro-forestry. The soils under double-cropping had significantly lower TOC stocks (by \(\sim 15\%–18\%\)), compared with soils under orchards and vegetable crops. Data pooled for soils under mono- and double-cropping systems revealed that soil BD decreased with increasing TOC concentration in soils.

Carbon management index

The LI was significantly lower, by \(\sim 49\%\), for soils under mono-cropping compared with agro-forestry (Table 2). Considering soils under agro-forestry with a high proportion of stable C pool (\(\sim 86\%\) of TOC) as a reference, the soils under mono-cropping had the lowest CMI value (41.5) (Figure 6). Conversely, the soils under double-cropping, orchards and vegetable crops had CMI values of 61.2, 80.1 and 91.9\%, respectively. The CPI varied between 0.80 and 1.03, and was significantly lower in soils under double-cropping as compared with orchards and vegetable crops.

Biological activity in soils

The TG concentration varied widely (4.4–22.5 g kg\(^{-1}\)) in soils under different land-use systems (Table 3). It was significantly higher, by 4.7 times, in soils under agro-forestry compared with mono-cropping system. Soils under double-cropping had \(\sim 3.1\) times higher TG than the soils under mono-cropping. Soil under vegetable crops had significantly higher TG compared to soils under double-cropping and orchards. The EEG comprised \(\sim 7–11\%\) of TG concentration in soils under different land-use systems. The agro-forestry system had \(\sim 18–22\%\) higher EEG concentration, compared with the soils under mono- and double-cropping. The MBC was significantly higher under agro-forestry, by 202 mg kg\(^{-1}\) soil (\(\sim 83\%\)) compared with soils under mono-cropping. A significantly higher (2.3 times) respiratory quotient (qCO\(_2\)) for soils under mono-cropping rendered these soils significantly lower in BSR (by \(\sim 32\%\)), compared with the agro-forestry system. The MBC and BSR were also significantly higher in soils under vegetable crops, compared with soils under orchards. The qCO\(_2\) was significantly higher in soils under orchards, compared with double- cropping and vegetable crops, which did not differ significantly from each other. The enzymatic activity (acid-P, DHA and FDA) was significantly higher in soils under agro-forestry, compared to mono- and double-cropping systems. However, the soils under mono- and double-cropping systems had significantly higher acid-P activity than the soils under...
The PCA identified three PCs with eigenvalues greater than 1 (Table 4). The soil variables with the highest variance explained within each PC were considered for MDS. The PCA of the total data set showed that the first PC (PC1) (eigenvalue > 1) explained 45.1% of the total variance in the original data. The TG had the highest loading value (0.992 ***, p < .01) and was significantly correlated with other high-loading indicators. The second PC (PC2) explained 32.7% of the total variance, and Fract. 3 was the most highly loaded indicator (loading value = 0.978 ***, p < .01). In PC3, available-K showed the highest loading value (0.955 ***, p < .01). Therefore, TG in PC1, Fract. 3 in PC2 and available-K in PC3 were included as MDSs for the estimation of SQI. The total variance for each PC ranged between 0.09 and 0.76, and the weighted factor for three distinct MDSs followed PC1 (0.49) > PC2 (0.35) > PC3 (0.16). Bi-plots show the position of different variables and land-use systems in the orthogonal space (Figure 7). The three SQI (TG, Fract. 3 and available-K) were located on the right end of the scoring plot, indicating positive PC scores (Figure 8). The PCA showed that the contribution of TG toward SQI was the highest for mono- and double-cropping systems (0.337 – 0.335), followed by orchards (0.317), and the lowest for agro-forestry (0.243). Fract. 3 made the maximum contribution toward SQI. The relative order of contribution of the selected indicators to SQI was 44.4% for TG, 38.2% for Fract. 3 and 17.4% for available-K in different PCs (Figure 9).

The correlation matrix showed that TOC exhibited a significant relationship with MBC (r = 0.92 ***, p < .01), BSR (r = 0.95 ***, p < .01), Fract. 2 (r = 0.68 **;
Table 4. Loading values and percentage contribution of soil properties on the axis identified by the principal component analysis (PCA).

| Variables | PC1 Loading values | Contribution (%) | PC2 Loading values | Contribution (%) | PC3 Loading values | Contribution (%) |
|-----------|--------------------|------------------|--------------------|------------------|--------------------|------------------|
| MBC       | 0.612*             | 3.07             | 0.735*             | 6.113            | 0.034             | 0.029            |
| BSR       | 0.612*             | 3.07             | 0.779*             | 6.863            | -0.114            | 0.317            |
| pH        | -0.364             | 1.08             | 0.605*             | 4.149            | 0.697*             | 11.9             |
| EC        | 0.940**            | 7.26             | -0.185             | 0.387            | 0.189             | 0.876            |
| Available-P | 0.768*            | 4.85             | -0.177             | 0.354            | -0.300            | 2.216            |
| FC        | 0.749*             | 4.61             | -0.520             | 3.057            | 0.411             | 4.142            |
| PWP       | 0.485              | 1.94             | -0.564*            | 3.606            | 0.372             | 8.046            |
| AWG       | 0.808**            | 5.36             | -0.530             | 1.231            | 0.415             | 4.229            |
| EEG       | 0.954**            | 7.48             | -0.147             | 0.245            | -0.018            | 0.008            |
| TG        | 0.992**            | 8.08             | -0.089             | 0.090            | -0.078            | 0.150            |
| Fract. 1  | 0.825**            | 5.60             | -0.361             | 1.472            | -0.429            | 4.522            |
| Fract. 2  | 0.928**            | 7.07             | 0.100              | 0.113            | -0.355            | 3.102            |
| Fract. 3  | -0.049             | 0.020            | 0.978**            | 10.8             | 0.184             | 0.833            |
| Fract. 4  | 0.654*             | 3.51             | 0.636*             | 4.587            | 0.385             | 3.650            |
| Active C  | 0.892**            | 6.54             | -0.176             | 0.352            | -0.410            | 4.135            |
| Stable C  | 0.401              | 1.32             | 0.852**            | 8.228            | 0.332             | 2.713            |
| WEOC      | -0.370             | 1.12             | 0.566              | 3.632            | 0.352             | 3.054            |
| DHA       | 0.838**            | 5.77             | -0.478             | 2.592            | 0.212             | 1.102            |
| Acid-P    | 0.907**            | 6.75             | -0.410             | 1.907            | 0.047             | 0.053            |
| Available-K| -0.086             | 0.06             | 0.270              | 0.826            | -0.260            | 1.665            |
| FDA       | 0.807**            | 5.35             | 0.501              | 2.644            | -0.402            | 3.963            |
| Sand      | 0.043              | 0.02             | 0.897**            | 9.103            | 0.308             | 3.152            |
| Silt      | -0.296             | 0.72             | -0.835**           | 7.887            | 0.411             | 4.156            |
| Clay      | 0.231              | 0.43             | -0.881**           | 8.783            | -0.576            | 8.150            |
| qCO2      | -0.694*            | 3.96             | -0.377             | 1.608            | -0.195            | 0.644            |
| qmic      | 0.324              | 0.861            | 0.572              | 3.703            | 0.138             | 0.468            |
| TOC       | 0.704              | 4.06             | 0.693*             | 5.446            | 8.83              | 4.07             |
| Eigenvalue| 12.1               |                  | 45.1               |                  | 45.1               |                  |
| Variability| 45.1               |                  |                    |                  |                    |                  |
| Cumulative | 45.1               |                  | 77.8               |                  | 92.9               |                  |

*Significant at Bold values represent a Highest factor loading value, p < .05. **Significant at p < .01.

p < .05), Fract. 3 (r = 0.66*; p < .05), Fract. 4 (r = 0.86**; p < .01) and stable C pool (r = 0.92**; p < .01) (Table 5). The TG (r = 0.62*; p < .05) and EEG (r = 0.55*; p < .05) exhibited a linear significant positive relationship with TOC. The TOC in soils under different land-use systems exhibited a significant linear relationship with soils’ fine fraction – clay (r = 0.79*; p < .05) and silt (r = 0.76*; p < .05) – and a significant negative relationship with sand (r = -0.61*; p < .05). The enzymatic activity, i.e. DHA (r = 0.57*; p < .05) and FDA (r = 0.89**; p < .01), exhibited a significant correlation with TOC. Fract. 4 exhibited a significant relationship with silt (r = 0.63*; p < .05) and clay (r = 0.76*; p < .05). The stable C pool (Fract. 3 + Fract. 4) also exhibited a significant relationship with silt (r = 0.82**; p < .01) and clay (r = 0.78*; p < .05). Conversely, TOC exhibited a negative relationship with qCO2 (r = -0.81**; p < .01). The soil enzymatic activity of DHA showed a significant relationship with the active C pool (Fract. 1 = 0.78*; p < .05, Fract. 2 = 0.66*; p < .05) and FDA activity (Fract. 1 = 0.61*; p < .05, Fract. 2 = 0.90**; p < .01). Conversely, the stable C pool (Fract. 3 + Fract. 4) did not show a significant relationship with enzymatic activity (DHA and FDA). Soil enzymatic activity was also significantly related to TG (DHA = 0.85**; p < .01; FDA = 0.77*; p < .05) and EEG (DHA = 0.83**; p < .01; FDA = 0.66*; p < .05).

Discussion

Land use greatly impacts nutrient availability, especially the soil organic C dynamics due to changes in plant-mediated C input and ecosystem engineering [11,37]. The relatively higher available-P content in soils under cropland ecosystems, i.e. mono- and double-cropping systems and vegetable crops, compared with those under agro-forestry and orchards was ascribed to the continuous application of fertilizer-P. Phosphorus, being a highly immobile nutrient, gets fixed due to large-scale transformations occurring immediately after fertilizer-P application to soils. It is well established that crops often utilize only one-quarter to one-third of applied inorganic fertilizer-P, while large amounts are accumulated in soils. Nonetheless, the nutrient contents in soil and their composition depend on (i) quantity and quality of rhizodeposits, and (ii) substances contained and adsorbed
within land-use systems [6]. Increased soil microbial activity tends to increase P and K availability due to secretion of root exudates and the low molecular weight of organic acids. The lower available-K content in soils under mono- and double-cropping systems was ascribed to the fact that continuous cropping without fertilizer-K application has depleted K reservoirs in soil [38]. A synthesized meta-analysis of 22 long-term field experiments in Asia and results of several long-term studies from South Asia revealed a significant yield decline, besides a large negative K balance in soils due to heavy depletion of soil K [39].

Not only the nutrient dynamics, but also the soil moisture characteristics are significantly influenced by C input due to improved soil physical properties. Nonetheless, soil moisture storage due to improvement in soil properties, such as reduced soil BD and formation of water stable aggregates, has been considered to be influenced by the fine fraction of soil [7,28]. BD affects soils’ hydrological characteristics by controlling the infiltration rate.
and depth, the water holding capacity and the saturated hydraulic conductivity \[40\], which influence biochemical processes and impact C cycling \[8\]. Higher soil B2 has been observed in the torrent-affected areas of the Shivalik (Himalayan) region in India, because of low organic C content and soil aggregation \[41\]. These results revealed that a significantly higher moisture content (at FC and PWP) in soils under double-cropping, compared with soils under other land-use systems, was related to the relatively high fine fraction (silt + clay; 44.8%) and SOM content due to the application of FYM/compost in soils under double-cropping. The soils under mono- and double-cropping received FYM/compost every year, which improves the soil physical properties and leads to increased moisture availability in the soil. Land-use system had a significant impact on soil moisture retention at FC and PWP by influencing SOM and porosity \[42\], although the effect was of higher magnitude at FC than at PWP \[43\]. Manure application and plant-mediated C input increased the soil MBC available for microbial processes and growth, and led to better soil physical conditions and higher water retention \[6,41\]. In the Himalayan region of India, a study showed highest moisture retention at FC in the soils under grasslands, compared with croplands, orchards and forest land \[41\]. The soil moisture retention capacity was higher for soils with a large amount of clay and TOC contents \[44\], which corroborates these results in terms of the effect of higher finer fraction content and C input moisture retention in soil.

Table 5. Correlation matrix (Pearson’s correlation coefficients) depicting relationship between total organic carbon (TOC) and its fractions, and microbial and enzymatic properties of soils in a dry mountainous region in northwestern Himalaya, India.

| Variables | MBC | BSR | EGG | TG | Fract. 1 | Fract. 2 | Fract. 3 | Fract. 4 | Active C | Stable C | DHA | FDA | Sand | Silt | Clay | qCO2 | qmic |
|-----------|-----|-----|-----|----|---------|---------|---------|---------|----------|----------|-----|-----|------|------|------|------|------|
| BSR       | 0.97** |       |     |    |         |         |         |         |          |          |     |     |      |      |      |      |      |
| EGG       | 0.55*  | 0.49 |     |    |         |         |         |         |          |          |     |     |      |      |      |      |      |
| TG        | 0.57*  | 0.55* | 0.97** |    |         |         |         |         |          |          |     |     |      |      |      |      |      |
| Fract. 1  | 0.20  | 0.27 | 0.83** | 0.88** |         |         |         |         |          |          |     |     |      |      |      |      |      |
| Fract. 2  | 0.61* | 0.68* | 0.86** | 0.94** | 0.89**  |         |         |         |          |          |     |     |      |      |      |      |      |
| Fract. 3  | 0.72* | 0.72* | 0.57* | 0.66* | -0.48  | -0.02  |         |         |          |          |     |     |      |      |      |      |      |
| Fract. 4  | 0.84** | 0.84** | 0.59* | 0.65* | 0.15   | 0.54*  | 0.65*  |         |          |          |     |     |      |      |      |      |      |
| Active C  | 0.38  | 0.45 | 0.87** | 0.93** | 0.96**  | 0.96**  | 0.30   | 0.32  |          |          |     |     |      |      |      |      |      |
| Stable C  | 0.87** | 0.87** | 0.24 | 0.29  | -0.12  | 0.34   | 0.87** | 0.94** | 0.07    |          |     |     |      |      |      |      |      |
| DHA       | 0.12  | 0.10 | 0.83** | 0.85** | 0.78**  | 0.66*  | -0.48  | 0.35   | 0.76*   | 0.01    |     |     |      |      |      |      |      |
| FDA       | 0.80** | 0.90** | 0.66* | 0.77* | 0.61*  | 0.90**  | 0.39   | 0.27   | 0.75*   | 0.67*   | 0.41 |     |      |      |      |      |      |
| Sand      | -0.69 | -0.71* | -0.63* | -0.51* | -0.30  | 0.28   | -0.78* | -0.47  | -0.06   | -0.66*  | -0.45 | -0.62 |      |      |      |      |      |
| Silt      | 0.70*  | -0.85** | 0.79* | 0.73* | 0.12   | 0.50   | 0.71*  | 0.63*  | 0.28   | 0.73*   | 0.18 | 0.80* | -0.66* |      |      |      |      |      |
| Clay      | 0.68*  | -0.59* | 0.75* | 0.68* | 0.33   | 0.02   | 0.79*  | 0.76*  | 0.19   | 0.82*   | 0.70* | 0.37 | -0.95** | 0.53* |      |      |      |      |
| qCO2      | -0.78 | -0.67* | -0.65* | -0.62* | -0.17  | -0.47  | -0.46  | -0.89** | -0.30  | 0.78*   | -0.49* | -0.56* | -0.10 | 0.25 | -0.07 |      |      |      |
| qmic      | 0.82** | 0.73* | 0.42 | 0.32 | 0.09   | 0.39   | 0.57*  | 0.40   | 0.22   | 0.52*   | -0.15 | 0.47 | -0.43 | -0.48 | -0.48 |      |      |      |
| TOC       | 0.92** | 0.95** | 0.55* | 0.62* | 0.28   | 0.68*  | 0.66*  | 0.86** | 0.45   | 0.92**  | 0.57* | 0.89** | -0.61* | 0.76* | 0.79* | -0.81** | 0.55* |

*Significant at \( p < 0.05 \). **Significant at \( p < 0.01 \).

Data pooled for soils under different land-use systems. MBC = Microbial biomass C; BSR = Basal soil respiration; EGG = Easily extractable glomalin; TG = Total glomalin; Fract. 1 = Very labile C; Fract. 2 = Labile C; Fract. 3 = Less labile C; Fract. 4 = Recalcitrant C; Active C = Fract. 1 + Fract. 2; Stable C = Fract. 3 + Fract. 4; DHA = Dehydrogenase activity; FDA = Fluorescein diacetate activity; qCO2 = Respiratory quotient; qmic = Microbial quotient; TOC = Total organic carbon.
The differences in organic C storage under different land-use systems are related to plant-mediated C input and plants’ rate of decomposition [8,10]. In addition, the lowest soil disturbance in the present study, due to minimum tillage intensity under agro-forestry and orchards and the retarded rate of humification and mineralization of the organic materials, contributed to lower soil B0. Furthermore, higher TOC content in soils under agro-forestry resulted in decreased soil B0. These results corroborate the research findings of Singh and Benbi [10,22], who reported decreased B0 with an increase in the TOC pool in soils. The crop-land soils under mono- and double-cropping systems had significantly lower TOC, compared with soils under agro-forestry and orchards, which corroborates earlier research [6,13]. The soil C pool is considered a function of above- and below-ground biomass and C input [19,45]. Wheat root biomass of 0.71–1.67 Mg ha\(^{-1}\) year\(^{-1}\) and leaf+ stubble biomass of 0.51–0.83 Mg ha\(^{-1}\) year\(^{-1}\) are reported to be incorporated annually in the soil plow (0–15 cm) layer in sub-tropical regions [7]. The root index for the soil plow layer varied between 10 and 15% of the total above-ground biomass, and of the total plant-mediated C input ∼85–89% occurred at a soil depth of 0–15 cm, while ∼11–15% occurred at 15–30 cm soil depth [5]. The increased C input through root and shoot biomass and rhizodeposition increased soil organic C concentration in a sandy loam soil, as reported by Singh et al. [46], corroborating these results showing that soils under double-cropping had significantly higher TOC, compared with the soils under mono-cropping. The higher root + shoot biomass of crops under double-cropping with differential quality and quantity of C input leads to higher accumulation of TOC in soils, compared with the soils under mono-cropping. Above- and below-ground biomass mediated C input combined with higher soil moisture can result in increased microbial activity and SOC loss, but can increase root biomass as well; this could also impact the SOM turnover because of its contribution toward soils’ stable C pools.

The higher TOC in soils under agro-forestry, compared with mono- and double-cropping systems, was ascribed to higher C input and rhizodeposition under agro-forestry [47]. The popular method of planting under the agro-forestry system adds ∼2.9–3.3 Mg ha\(^{-1}\) year\(^{-1}\) of leaf litter during the winter season and ends up with ∼2.3 Mg C ha\(^{-1}\) year\(^{-1}\), through roots and leaf litter, in the soil plowed layer [48]. Increased root biomass typically releases more C as rhizodeposition [49], leading to increased C storage in soil. Conversely, the lower TOC concentration in soils under mono- and double-cropping systems could be related to more intensified tillage practiced for cereal crop cultivation, compared with soils under agro-forestry and orchards having perennial planting. The more intensive crop rotations contribute to improved soil aggregation through enhanced above- and below-ground C inputs, and greater residue cover on the soil surface that protects soil aggregates from the destructive forces of wind, rain and microbial degradation [4,7]. The polysaccharides and organic acids released during the microbial decomposition of organic matter play a key role in the stabilization of macro-aggregates [6,7]. Since these metabolites do not spread far from the site of production, freshly added residues function as nucleation sites for the growth of fungi and other soil microbes [6,19].

Nonetheless, intensified tillage (as under mono- and double-cropping in the present study) causes breakage of soil aggregates and tends to expose SOM encapsulated within water-stable aggregates against microbial oxidation [4]. Therefore, intense tillage leads to SOM loss due to exposure of soil micro-organisms, which leads to faster decomposition of C input. The breakage of soil macro-aggregates and release of encapsulated C was inversely related to soil protein (TG and EEG). These results indicate that soil protein was significantly lower in soils under mono-cropping. The amount of C received by mycorrhizal fungi from plants is reassimilated and accumulated in the soil as glomalin (∼5–25% of SOC) [15,50]. Glomalin is considered an N-linked glycoprotein and an essential component of soil C sequestration in soil [51]. Glomalin also improves soil moisture retention and leads to greater C accumulation in biomass and more recalcitrant forms of C that act as reservoirs of C: aggregate binding agents [52].

Soil organic matter is considered a continuum spanning the complete array of plant material with varying degrees of oxidizability which leads to highly oxidized C in carboxylic acids. Plant-mediated C inputs are stabilized as microbial by-products of decomposition and are influenced primarily by vegetation type, soil microclimate, substrate quality, and nutrient availability for decomposers. Soil MBC comprised ∼6.1% of TOC in soils under different land-use systems, and was nearly the same (1–5% of TOC) in the present study as that
reported earlier [10]. Soil micro-organisms influence C cycling not only via decomposition, but also because microbial products themselves are important components of SOM [9]. Microbial residues are the dominant source of the stable mineral associated C pool [13,17]. In the present study, higher MBC activities in soils under agro-forestry than mono- and double-cropping systems should increase the chance that below-ground root C inputs are assimilated, metabolized and then transformed into microbial necromass [53].

C stability depends on labile and recalcitrant fractions and their molecular structures [9]. The labile C pool, with its fast turnover rates, is considered a key indicator to discern a rapid change in quality and quantity of SOM with short- and medium-term impacts [10]. Yet this has been debated frequently, with the evidence that the recalcitrant C pool of TOC could decompose more easily, particularly when it comes into contact with the decomposer micro-organisms [9]. These results reveal that the accumulation of microbially processed C within aggregates was potentially responsible for the observed difference in TOC among the land-use systems in the present study. The resulting information, therefore, should be integrated into conceptual and mechanistic models for the purpose of predicting CO₂ emissions from soils under different land-use systems. Singh and Benbi [3] and Lehmann and Kleber [9] reported that most of the models developed to estimate C turnover as a function of soil characteristics, soil and crop management practices, and climatic conditions describe C turnover rates as a sum of multiple and parallel compartments, and each compartment has its own turnover rate. However, the existing large spatial and temporal variability of soil C and the complexity of the soil–plant–atmosphere system demands the integration of simulation models with locally measured data to simulate a change in C storage under different soil management regimes and climatic conditions [3]. Therefore, the models, if well validated, are highly effective tools for predicting and understanding the impact of varying management practices on short-, medium- and long-term C storage [54,55].

In the present study, the active C pool was significantly higher in soils under mono- and double-cropping systems, in contrast to the stable C pool which was higher under agro-forestry. These results reveal that ~86.5% of TOC in soils under agro-forestry was stabilized as the stable C pool (Fract. 3 + Fract. 4). Root-derived C is retained in soils much more efficiently than are above-ground inputs of leaves and needles, and biomarkers have confirmed the dominance of root-derived molecular structures in soil and of root-derived C in soil micro-organisms [56]. In a rice–wheat cropping system in semi-arid, sub-tropical and hot humid conditions, ~72% of TOC was stabilized as the stable C pool [10]. These values are close to those obtained in the present study with an accumulation of ~74–77% of TOC as the stable C pool in soils under mono- and double-cropping systems. The accumulation of C in a stable pool (Fract. 3 + Fract. 4) has significance for C sequestration, because these fractions are very slowly being altered by microbial activities [57].

The C fractions of variable oxidizability were important for C management and indicate soil quality change over a short time frame for the investigated land-use systems. The soils under vegetable crops exhibited a higher CMI value because these soils provide a less oxidative environment for C breakdown [11]. The land-use systems with higher CMI values provide better soil management for C rehabilitation [10,58]. The microbial quotients for soils under contrasting land-use systems were related to soil management-induced alteration in vegetation cover that impacts the quality and quantity of SOM from plant litter and root exudates [59]. Additionally, rhizosphere C incorporated into soil as a continuous, low-volume “drip” (i.e. root exudates) helps to increase the accumulation of microbial residues and stable C formation [60]. Therefore, the role of microbial-derived C in the organic C pool is more conservative since it is less dependent on environmental factors and agricultural management (e.g. fertilization) than the plant residues [61]. These results show that the values for different microbial quotients (MBC:TOC) were within the range of 1–4%, as reported earlier. The higher conversion of plant-mediated C input to microbial biomass suggests better stability of organic C in the investigated land-use systems. A significantly higher qCO₂ (by ~2.3 times) and lower BSR (by ~32%) for soils under mono-cropping, compared to agro-forestry, revealed a greater diversity of organic substrates favoring efficient utilization of SOM by the microbial biomass in soils under agro-forestry [62].

Change in soil management caused by contrasting land-use systems, age and seasonal variation alters the nutrient availability and thus regulates
different enzymatic processes [63]. In the present study, results suggest that DHA, acid-P and FDA activity was significantly higher for soils under double-cropping compared with mono-cropping systems. The above-ground plant biomass derives the differential responses of soil enzymes within a cropping system, because enhanced plant productivity helps in transporting more photosynthetically synthesized C input below ground and leads to increased root exudates and root biomass. The translocation of root exudates and rhizodeposition affect a large number of microbial functions to ultimately influence soil enzymatic activity [45].

The higher available-P in soils under different land-use systems (except for vegetable soils) may be a reason for their lower soil acid-P activity. The higher acid-P activity in soils under vegetable cultivation was ascribed to the fact that up to ~90% of the macroflora and phosphatase-producing bacterial population was increased with fertilizer-P application. Soil quality indicators describe the major ecological processes in soil and assess the basis of deterioration in quality and quantity of SOM, nutrient availability, biological activity and soil physical structure [64]. PCA bi-plots clearly differentiate land-use systems under agro-forestry, mono-cropping and vegetable crops, although there was some overlap for soils under double-cropping and orchards. The glomalin produced by fungal spores and hyphae is considered important because of its multiple ecological functions in soil aggregate formation, C storage within soil aggregates and improvement in soil moisture retention [51,65,66]. Fract. 3, with its large turnover time, plays a significant role in C sequestration [13,67]. Similar to these results, higher turnover time has been observed among the stable C pools under semi-arid land-use and in humid sub-tropical India [19,68]. Available-K is considered important for osmotic regulations by providing the osmotic pull to draw water into plant roots [69]. Agronomic and tillage practices are known to influence K availability by modifying other factors such as oxygen or aeration, temperature and soil moisture, and promoting vigorous plant growth through efficient photosynthesis, contributing 15.1% to the SQI in the present study [27,28].

The significant relationship between TOC and different C fractions signifies a dynamic equilibrium and indicates that they are influenced by soil management practices in a similar way [10]. A significant relationship between TOC and glomalin (TG and EEG) indicates that glomalin concentration was a good predictor of ecosystems’ C budget [68,70]. Similarly, a linear positive relationship between TG and soils’ finer fraction (silt and clay) indicates that soil protein helps in aggregate formation and the protection of SOM against microbial degradation, and is much more sensitive to agricultural restoration. A strong correlation between TOC storage in soils’ silt and clay content in a wide range of soils (uncultivated, grasslands) in tropical, temperate and sub-tropical region has been reported [3,17]. This was attributed to the higher reactive surface area of organo-mineral complexes formed by soils’ fine fraction (silt + clay). Among the C pools, the stable C pool was the most sensitive to soil management-induced changes and exhibited a significant relationship with the silt and clay contents of the soil in the present investigation. Soil organic carbon associated with minerals is considered one of the most fundamental long-term, stable carbon pools [71]. Baldock and Skjemstad [72] reported that the ability of soils to stabilize C depends on the chemical and physical properties of the mineral matrix as well as the morphology and the chemical structure of SOM.

**Conclusions**

These results highlight the overwhelming importance of soil protein (glomalin) due to the multifarious ecological functions it performs in relation to the accumulation of C in the total organic C (TOC) pool, soil moisture storage and increased nutrient availability. Glomalin influenced organic C pools of variable oxidizability due to increased enzymatic activity. The C accrual in the stable C pool (Fract. 3 + Fract. 4) increased significantly with soils’ finer (silt + clay) fraction, indicating the importance of the mineral matrix in C stabilization in soils. The stable C pool in the agro-forestry system comprised the highest TOC fraction (~86%), compared to other land-use systems (~73–77%). As compared with mono-cropping, an increased carbon management index (CMI) (by ~47–121%) indicates C rehabilitation in soils under other investigated land-use systems. Total glomalin, less labile C (Fract. 3) and available-K appeared to be key ecological indicators to discern a soil management-induced change in total and labile C pools and biochemical properties of soils in dry mountainous ecosystems. Available-K in soils under annual (mono- and double-) cropping was significantly lower compared to perennial planting (orchards...
and agro-forestry) and even in soils under vegetable production. These results revealed diligent attention toward regular K application in soils under mono- and double-cropping system. A close nexus between soil glomalin (total glomalin, TG and easily extractable glomalin, EEG) and mineral matrix (silt and clay) emphasized the role of soil protein in binding soils’ fine fraction to store C in TOC pools. We therefore suggest measurement of soil glomalin as a robust and sensitive indicator to discern soil quality change in the context of land-use change and agricultural intensification in the dry mountainous region.

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Conflict of interests

The authors declare that there is no conflict of interests.

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